PHARMACOGNOSTICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL SCREENING OF *NYMPHAEA* SPECIES LINN. (NYMPHAEACEAE)

Thesis submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI-600032.

For the partial fulfilment for the award of the Degree of

DOCTOR OF PHILOSOPHY

In

PHARMACY

Submitted By

T.KARTHIYAYINI, M.Pharm.,

Rc.No.ACAD-I(2)/33745/2008

Under the guidance of

Dr. NAGESH R. SANDU, M.Pharm., Ph.D.,

Department of Pharmacognosy

Padmavathi College of Pharmacy and Research Institute

Dharmapuri – 635205, Tamil Nadu, India.

SEPTEMBER-2014

CERTIFICATE

This is to certify that the investigation in the Thesis entitled **"PHARMACOGNOSTICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL SCREENING OF NYMPHAEA SPECIES LINN. (NYMPHAEACEAE)"** submitted to the Tamilnadu Dr.M.G.R.Medical University, Chennai, for the partial fulfillment of the award of the Degree of **DOCTOR OF PHILOSOPHY** in Pharmacy, was carried out by **Tmt. T. Karthiyayini, M.Pharm., Rc.No.ACAD-I(2)/33745/2008** at Padmavathi College of Pharmacy and Research Institute, Dharmapuri - 635205, under the guidance and supervision of **Dr. Nagesh R Sandu, M.Pharm., Ph.D.,** Professor, Department of Pharmaceutics, Padmavathi College of Pharmacy and Research Institute, Dharmapuri - 635 205.

This work is original and has not been submitted in part or full to any other Degree or Diploma of this or any other University.

Place: Dharmapuri
Date:

Dr. K.L.SENTHIL KUMAR, M.Pharm, Ph.D.,
PRINCIPAL

Prof. Dr. Nagesh R. Sandu, M.Pharm, Ph.D.,

Head, Department of Pharmaceutics,

Padmavathi College of Pharmacy and Research Institute,

Periyanahalli (Post), Dharmapuri – 635 205.

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This work is original and has not been submitted in part or full to any other Degree or Diploma of this or any other University.

Place: Dharmapuri **Prof. Dr. Nagesh R. Sandu, M.Pharm, Ph.D.,**

Date:

DECLARATION

I hereby declare that the Thesis entitled **"PHARMACOGNOSTICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL SCREENING OF NYMPHAEA SPECIES LINN. (NYMPHAEACEAE)"** was submitted to The Tamilnadu Dr. M.G.R Medical University, Chennai, as partial fulfillment for the award of the Degree of **DOCTOR OF PHILOSOPHY** in Pharmacy was carried out by me at Padmavathi College of Pharmacy and Research Institute, Dharmapuri, under the guidance of **Prof. Dr. Nagesh R. Sandu, M.Pharm., Ph.D.,** Padmavathi College of Pharmacy and Research Institute, Dharmapuri-635205.

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Place: Dharmapuri **T. KARTHIYAYINI**

Date:


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CONTENTS

Dedicated

To my

Beloved parents

And

My son

CHAPTER-I

CHAPTER – I

1. INTRODUCTION

India is a country known for ancient scripts. Indian traditional medicines are used by about 60% of the world's population. These are used for primary health care not only in developing countries but also in developed countries, where modern medicines are used predominantly. While the traditional medicines are derived from medicinal plants, minerals, and organic matters; these herbal drugs are prepared from medicinal plants only **(Raju Ilavarasana, 2005).**

People on all continents have used thousands of indigenous plants for treatment of ailments since pre-historic times. Pharmacognosy is the study of medicines derived from natural sources. The substances are used to maintain human health and other animals derived from many plants. Similarly, number of herbs is thought to be likely to cause adverse effects. Furthermore adulteration, inappropriate formulation or lack of understanding of plant and drug interactions have lead to adverse reactions that are sometimes life threatening.

Natural products serve as a precursor for synthesis of several medicinally important components. Some isolated compounds from plants were made into different formulations and used as drugs. Now people have realized the utility of drugs from natural sources which are not only economical but also safer. Active constituents from plant sources have lead to rapid developments in pharmacognosy and phytochemistry. World Health Organization (**WHO**) emphasized the utilization of indigenous systems of medicine based on folklore and locally available plant materials **(www.en.wikipedia.org).**

Until now, 6000 plants constituents have been isolated and studied. The flora on this earth representing an inexhaustible source of medicinal plants remains unexplored. Undoubtedly the plant kingdom still holds many species of plants containing substances of medicinal value which are yet to be discovered. Large number of plants is constantly

being screened for their pharmacological value. The plants used in traditional system of medicine of India and China are now receiving much scientific attention. Another fascinating area of research which has proved rewarding in the examination of plants, used for medicinal narcotics and other purposes by tribal societies **(William Charles Evans, 1997).**

1.1. The History of Herbal Medicine

Over the past decades, people relied on herbal remedies derived from herbs and species which find place in day to day uses. Many of these are used as herbal remedies. Herbal remedies can be used in many forms. In Infusions the parts like leaves and flowers of herbs are steeped into boiling water for some time and used. The Essential oils of herbs and spices are also used as herbal remedies. Action of herbal remedies varies from man to man. Herbalists, herbal medicine practitioners, traditional medicine practitioners, ayurvedic, homeopathic, and naturopathic practitioners are all use herbal remedies in their practices **(www.herbsnspicesinfo.com).**

The study of herbs over 5,000 years to the Sumerians, who described well, established medicinal uses for such plants as laurel, caraway, and thyme. Ancient **Egyptian** medicine of 1000 B.C. are known to have used garlic, opium, castor oil, coriander, mint, indigo, and other herbs as medicine and the Old Testament also mentions herbal cultivation and use, including mandrake, vetch, caraway, wheat, barley, and rye.

Man has been using herbs and plants products for combating diseases since times immemorial. Earliest mention of the use of medicinal plants is to be found in the Rig-Veda which was written between 4500 – 1600 B.C. A detailed account of the world's first symposium, on medicinal plant is given in ***Vrihat Samhita***. A continued search for medicinal plants during the last several centuries has given rise to a long list of plants which are of great use in the treatment of diseases and for promoting health. Plants have been one of the most important sources of medicines since civilization, for instance Chinese drug ***Ma-Huang*** was in use for over 5000 years for the treatment of different types of fever and respiratory disorders. In Peru, Cinchona is in use from 1825 in controlling malaria.

With regard to the history of the ***Materia Medica*** of ancient Egypt is **"papyri"** discovered in 1873. The most important document Papyrus which might be considered as one of the most Ancient Pharmacopeia's and principle source of information on Egyptian medicines.

Folk medicine, Temple medicine and Hippocratic tradition form three main themes of ancient Greek medicine. Hippocrates used singularly few and mostly simple drugs.

Theophrastus produces the remarkable ***"De Historia Plantarum"***, the first extensive and accurate description of 455 herbal plants. During the period of **Dark Ages,** learning continued in Muslim west. The medical texts of **Greece** were translated as they were carrying eastward by the rise of the Neostorian Church and then into the possession of Mohammedan Physicians. In the mean time new exotic drugs made their appearance in Europe.

The follow of **Byzantine** to the Turks in 1453, and the flight of the Byzantine Scholars with their precious manuscripts to Italy and centres of learning in Europe, was one of the chief means of the Renaissance. The Renaissance is still promoting plant medicines and this interest lead to the vast growth of herbals.

In India, **Ayurveda** is a holistic system of medicine whose principle of therapeutics is applicable universally. Ayurveda evolved over 5000 years ago. The **Unani** system of medicine owes its origin to Greece. The **Siddha** system is one of the oldest systems of medicine in India. The fundamentals and applications of the principle and doctrines of this system have close similarity to Ayurveda.

The first **Chinese** herbal book, the ***Shennong Bencao Jing***, compiled during the Han Dynasty but dating back to a much earlier date, possibly 2700 B.C lists 365 medicinal plants and their uses. The ancient **Greeks** and **Romans** made medicinal use of plants. Greek and Roman medicinal practices, as preserved in the writings of **Hippocrates** and especially by **Galen**, provided the pattern for later western medicine. Hippocrates advocated the use of a few simple herbal drugs - along with fresh air, rest, and proper diet.

The **Arabs** venerated Greco-Roman culture and learning, and translated tens of thousands of texts into Arabic for further study. As a trading culture, the Arab travelers had access to plant material from distant places such as China and India. During 15^{th} to 17^{th} centuries there were the great age of herbals, many of them available for the first time in English and other languages rather than in Latin and Greek **(Agarwal, S.S. 2007).**

1.2. Herbalism

Herbal Medicine also referred to as **"Herbalism"** or "**Botanical Medicine"** is the study and use of herbs for their therapeutic or medicinal value. An herb is a whole plant or part of a plant valued for its medicinal, aromatic and savoury qualities. Herbal plants contain a variety of chemical substances that act upon the body. The scope of herbal medicine is sometimes extended to include fungal and its by-products, as well as minerals, shells and certain animal parts. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Many of these phytochemicals have beneficial effects on long-term health when consumed by human, and can be used to effectively treat human diseases. More than 12,000 of such compounds have been isolated so far, i.e. a number estimated to be less than 10% of the total **(Tapsell, LC., 2006).**

Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines not only as effective as conventional medicines, but also gives them the same potential to cause harmful side effects **(Tapsell, LC., 2006, Lai, PK., 2004).**

Herbal medicine is a major component in all indigenous traditional systems of medicine and a common element in **Ayurvedic, Homeopathic, Naturopathic, Traditional oriental and Native American Indian Medicine**. The **World Health Organization (WHO)** notes that of 119 plant-derived pharmaceutical medicines, about 74 percent are used in modern medical methods that are correlated directly with their traditional uses as plant medicines by native cultures.

The **WHO** estimates that 4 billion people, i.e. 80 percent of the world population, presently use herbal medicine for some aspect of primary health care. Use of plants as a source of medicine has been inherited and is an important component of the health care system in India. Hence this requires proper documentation and research. In western countries also, the use of herbal medicines are gradually growing with approximately 40% of population reporting use of herbs to treat human ailment. Public, Academic and Government's interest in traditional medicines are growing exponentially due to the increased incidences of the adverse drug reactions and economic burden of the modern system of medicine.

In 20th century much of the pharmacopoeia of scientific medicine was derived from the herbal folklore of native peoples. Many drugs commonly used today are of the herbal origin. Indeed, about 25 percent of the prescribed drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound **(Thorat, S. 1991).**

1.3. Timeline of Herbal Medicine

No one knows, for sure, when humans began using herbs for medicinal purposes? The first written record of herbal medicine use was shown in 2800 B.C. in China. Since then, the use of herbs has gained, and fallen out of favor many times in the medical field. The timeline that follows shows some of the key dates and major points in the history of herbal medicine.

2800 B.C. - The first written record of herbal medicine use showed up. (Titled the Pen Ts'ao by Shen Nung)

400 B.C. - The Greeks joined the herbal medicine game. Hippocrates stressed the ideas that diet, exercise and overall happiness formed the foundation of wellness.

50 A.D. - The Roman Empire spread herbal medicine around the Empire and began the commerce of cultivated herbs.

200 A.D. - The first classification system that paired common illnesses with their herbal remedy appeared. This was prepared by the herbal practitioner Galen.

800 A.D. - Monks took over the herbal fields with herbal gardens at most Monasteries and Infirmaries for the sick and injured.

1100 A.D. - The Arab world became a centre of medicinal influence. Physician Avicenna wrote the Canon of Medicine, which gave mention to herbal medicines.

1200 A.D. - Black Death spread across Europe and herbal medicines were used alongside "modern" methods such as bleeding, purging, arsenic and mercury with equal, or better, results.

1500 A.D. - Herbal medicine and herbalists were promoted and supported by Henry VII and the Parliament, due to the large number of untrained apothecaries giving substandard care.

1600 A.D. - Herbs were used in treating the poor, while extracts of plant, minerals, and animals (the "drugs"), were used for the rich.

"The English Physician", an herbal treatise explaining the practice of herbal medicine, was written during this time.

1700 A.D. - Herbal medicine got another high profile endorsement from Preacher Charles Wesley. He advocated for sensible eating, good hygiene and herbal treatments for healthy living.

1800 A.D. - Pharmaceuticals began to hit the scene and herbal treatments took a back seat. As side effects from the drugs began to be documented, herbal remedies came into favor again. The National Association of Medical Herbalists was formed, and later renamed the **National Institute of Medical Herbalists (NIMH)**.

1900 A.D. - lack of availability of drugs during **World War I** increased the use of herbal medicines again. After the World War pharmaceutical production increased and penicillin was discovered. Herbal practitioners had their rights to dispense their

medications taken away and then reinstated. People began to express the concern over the large number of side effects and environmental impact of the drugs of the 1950's.

2000 A.D. - EU took action on regulation and testing of herbal medicines similar to those used for pharmaceuticals.

Herbal medicines have been documented during 4000 years. These medicines have survived real world testing and thousands of years of human testing. Some medicines have been discontinued due to their toxicity, while others have been modified or combined with additional herbs to reduce side effects. Many herbs have been undergone changes in their uses. Studies conducted on the herbs and their effects keep changing their potential uses **(www.herbal-supplement-resource.com).**

1.4. Herbal Medicine Today

The World Health Organization estimate that about 80% of population living in the developing countries rely almost exclusively on traditional medicines for their primary health care needs. Herbal medicines are of great importance to the health of individuals and communities. But their quality assurance needs to be developed.

The herbal basis of at least 50% of modern drugs is indisputable. In many cases chemical synthetics have been developed with mimic effects of the active constituents in herbs. With an advent powerful antibiotics once heralded as **'Miracle Drugs',** the use of herbs declined dramatically for decades. In a surprising turnaround recent problems with increasing the resistant and adoptable bacteria and viruses have led to renewed interest in herbal medicines as viable alternative to antibiotics. In contrast with conventional antibiotics the problems with resistant bacteria $1/10^{th}$ avoided in the herbal approach. Because, herbs usually act to strengthen the body rather than directly attack the infecting organisms **(Agarwal, S.S., 2007).**

The use of medicinal plants for healing purpose predates human history and forms the origin of much modern medicines. Many conventional drugs originate from plant sources. A century ago, most of the few effective drugs were plants based. Examples

include **Aspirin** (from willow bark), **Digoxin** (from fox glove), **Quinine** (from cinchona bark) and **Morphine** (from the opium poppy).

The leads of **Central Nervous System** active medicinal plants which have emerged besides ***Rawoulfia serpentina***, ***Mucuna pruriens*** for Parkinson's disease, ***Ocimum santum*** as an Antistress agent, ***Withania somnifera*** as Anxiolytic. The study related to epilepsy is focused towards the traditional medicine. The recent trends in the pharmacological studies are based on the biochemical and molecular mechanism which leads to the development of CNS active principles from the herbal drugs **(Sharma, PC., 2002).**

1.5. Modern Prescription Drugs

Although herbalism waned in the eighteenth and nineteenth centuries, many of the remedies employed by the herbalists provided effective treatment. Some of these became useful prescriptions as physician began experimenting with therapeutic agent. William Withering was the first in the medical field to scientifically investigate a folk remedy. His studies (1775-1785) on **foxglove** as a treatment for dropsy **(congestive heart failure)** set the standard for pharmaceutical chemistry

In the nineteenth century, scientists began purifying the active extracts from medicinal plants, one breakthrough in pharmaceutical chemistry came when **Friedrich Serturner** isolate morphine from the opium poppy **(*Papaver somniferum*)** in 1806. Continuing this progress, **Justus von lieberg**, a **German Scientist** became a leader in pioneering the field of Pharmacology. With increased knowledge of active chemical ingredients, the first purely synthetic drugs based on natural products were formulated in the middle of the nineteenth century.

Although the direct use of plants extracts continued to decrease in the late nineteenth and early twentieth century's, medicinal plants still contribute significantly to prescription drugs. It is estimated that 25% of prescriptions written in the United States contain plant-derived active ingredients (close to 50%, if fungal products are included). An even greater percentage is based on semi-synthetic or wholly synthetic ingredients originally isolated from plants. Today there is a renewed interest in investigating plants for

medically useful compounds, with some of the leading pharmaceutical and research institutions involved in this search **(Kong, *et al.,* 2003)**.

1.6. Standardization of Herbal Drugs

India leads a major role in the global market for herbal product based medicines. Exports of herbal materials and medicines can be jumped from just Rs. 456 crores to Rs. 3,000 crores by 2005 and with a 'grand strategic plan' to export of Rs.10,000 crores by 2010 **(Gupta, AK., and Chitime, HR., 2000).**

The single and most important factor, which stands in the way of wider acceptance of traditional herbal medicines, is the non-availability or inadequate availability of standards for checking their quality by chemical or bioassay methods. This also prevents modernization or modification of the methods of their preparation or production, as there is a need to establish the equivalence of the product made by the modified method with the original product. This standardized drug of well-defined consistent quality is needed for reliable clinical trials and therapeutic use. A major reason for the difficulty in developing quality control standards is that these products use whole plants or parts of plants or their total extracts.

In some cases, even a mixture of number of plants is used. It is thus challenging to develop suitable standards because a vegetable drug or a preparation thereof, is not just an analytical operation, it does not end with the identification and assay of an active principle rather it embodies total information and controls which are necessary to guarantee composition. Standardization of plant drugs has also been stressed by the WHO **(Samantha, *et al.*, 1999, Spree man and Gaedcke., 2000).**

There are primary reasons for the standardization of herbs into phytochemical drug. The reasons are-

- ♣ In pharmacological toxicological and clinical studies with herbal drug, their compositions need to be well documented in order to obtain reproducible results. The WHO has recognized this problem and has published guidelines to ensure reliability and reproducibility of research on herbal medicines.

- ♣ Phytopharmaceuticals want to be regarded as rational drugs, they need to be standardized and pharmaceutical quality must be approved.

- ♣ This concept should be followed not only in research, but also in the production and therapeutic application of phytopharmaceuticals.

Standardization of herbal products has defined standards as to how and when to harvest the medicinal herbs as well as how they may be dried, powdered and extracted in water or alcohol, its packing and storage conditions.

1.7. Strategies in Natural Product Research

There have been several strategies used in the past to expedite natural products research. Traditional medicine is a major indicator of activity and the isolation of active compounds in traditional medicines, for which the efficacy has been proven, might result in interesting products, although it cannot be excluded that the activity could be due to the combination effects of certain compounds. There is also a degree of predictability in the distribution of natural products in nature. Chemotaxonomy offers certain advantages, in that it may be used to search for new and richer sources of known compounds or related structures. It can also be used as **'negative indicators'**, for example, if a cytotoxic compound with no value as lead has been found in several related species, then other related species may be omitted from the screening. Understanding of the important role of secondary metabolites to plants especially in terms of resistance to pests and diseases, if not completely understood, have improved over the years and tapping a plant natural defence could also be a fruitful approach especially in the search for natural antibiotics.

In the search for bioactive compounds from plants, the choice and availability of plants for evaluation are important and criteria such as traditional uses or ethno pharmacology, chemotaxonomy and plant ecological observations may be used either alone or complementary to each other. Nevertheless preliminary screening of a random collection of plants can also yield fruitful results. In this approach, the usual strategy involves an initial step of putting the plant extracts through a selected biological assay or panel of assays. The bioassay will be decided upon various factors such as therapeutic interest, simplicity, costs and speed. Extracts satisfying a defined criterion of bioactivity will then

be selected for further work. The plants may undergo bioassay-guided isolation of active constituents or may go through a straightforward phytochemical work up to isolate as many compounds as possible. This is then followed by the usual structural elucidation by spectroscopic methods and biological testing of the isolated compounds.

1.8. Plant Selection Criteria

The research work was carried out in connection with the ***Preservation of Forest Environment and Protecting the Natural Resources of India***. The objective of this project was to promote the conservation, sustainable use and cultivation of rare and holistic medicinal plants in India, by demonstrating effective models at the local level, and developing a legal framework for the conservation, sustainable use, and equitable sharing of benefits from medicinal plants.

The baseline course of action will see increasing use of indigenous medicinal plants by local people and traditional healers as an effective complement to modern medicines. The thesis was focused on adding value to commonly-used and threatened traditional medicinal plants being used to treat common ailments in India.

More than 27 tribal practising medicinal plants reports were obtained from about seven different places of two districts of Krishnagiri and Salem in Tamil Nadu in India. After these were reviewed, the plants with antidiabetic, hepatoprotective, antiviral and antioxidant activities were noted and the literature available on background knowledge of these plants was collected and filed as a part of value addition process.

Two plants of greater interest with Genus ***Nymphaea*** belonging to the same family Nymphaeaceae were chosen from these reports and were compiled into '***plant monographs***' consisting of names of plants (vernacular, Latin, synonyms), description, cultivation, ethno botany, tested pharmacological activities, known chemical constituents, toxicology and marketing status given in **Table No-1**. The anthology of the plants chosen for the study with the added information obtained from the project can be read in the **"conclusion"** part on page 311.

Table No. 1: Plants chosen for the Study and their Ethnobotany

No	Synonym	Botanical Name & Family	Vernacular Name	Ethnobotanical Information	Parts Used	Parts Collected	Studies Already Done
1	*Nymphaea lotus var. Pubescens* Willdenow *Hook. f. Thomson*	***Nymphaea pubescens*** *Willdenow* Nymphaeaceae	E: Indian red water lily H: Kanval, Kokka T: □□□□□□□□□□□□ Vellambel T: Tella-kalava M:Periambal, Neerambal K: Nyadale hurvu, Kannaidile	Astringent, Cardiotonic Diarrhoea, Dysentery, Dyspepsia	Leaf Flower Root Rhizome Seed Pistil	Flower	Whole plant for hepatoprotective activity
2	*N.lotus* Hook., *N.stellata* Willdenow, *N.rubra* Roxb.Ex salisb	***Nymphaea nouchali*** Burmann. F. Nymphaeaceae	E: Indian white water lily H: Kanval, Kokka T: □□□□□□□□□□□□ Allittamarai T: Allikada M:Periambal, Neerambal K: Kannaidile, Bilenaydilie	Astringent, Cardio tonic, Diabetic, Palpitation of the heart, Liver damage, Diseases of Brain, Antidote in Snake Poison	Leaf Flower Root Rhizome Seed Pistil	Flower	Only identification of taxonomy of the plant

E-English, H-Hindi, T-Tamil, T-Telugu, M-Malayalam, K-Kannada

CHAPTER-II

CHAPTER II

AIM AND OBJECTIVES

2.1. Aim of the study

The present study was aimed to evaluate the pharmacognostical characteristics, various phytochemical parameters and pharmacological screening efficacy of the flower extracts of ***Nymphaea pubescens*** Willdenow and ***Nymphaea nouchali*** Burmann. F. in varying dose levels.

The potential utility of the flowers of both the species remain untouched for most of its phytochemical standard parameters and pharmacological activity. Hence, it is necessary to evaluate the phyto-constituents and their quantification by spectral chromatography and acute toxicity, antioxidant activity, alloxan induced antidiabetic activity, paracetamol induced hepatoprotective activity and Dalton's method of anticancer screening of the flower extracts of ***Nymphaea pubescens*** Willdenow and ***Nymphaea nouchali*** Burmann. F.

Medicinal plants are rich sources of therapeutic agents without serious side effects, for prevention and cure of various ailments of human being. Herbal medicines are generally perceived as safe products, the immense use of medicinal plants and their utility in various medicinal systems of the world in general and India in particular, has prompted this research work on known plant source for certain drugs.

The demonstration of the presence of natural products viz., polyphenols, alkaloids, triterpenoids, flavonoids and other secondary metabolites in medicinal plants will provide a scientific validation for their popular use and serve as guides which may help in the selection of the plants with hepatoprotective, antidiabetic and anticarcinogenic activity. So far, the hepatoprotective, antidiabetic and anti-tumour properties of the medicinal plant namely ***Nymphaea pubescens*** Willdenow and ***Nymphaea nouchali*** Burmann. F. has not been reported.

2.2. Objectives of the study

2.2.1. Primary objective

- To extract the various extracts from ***Nymphaea pubescens*** Willdenow and ***Nymphaea nouchali*** Burmann. F.
- To isolate the desired active components from ***Nymphaea pubescens*** Willdenow and ***Nymphaea nouchali*** Burmann. F.

2.2.2. Secondary objective

- To screen the various extracts of the flowers of ***Nymphaea pubescens*** Willdenow and ***Nymphaea nouchali*** Burmann. F. for its pharmacognostical, phytochemical and pharmacological studies.
- To isolate and screen the active components for various spectral studies.

2.3. Ethanobotanical Information (The Wealth of India., 1988)

2.3.1. Nutrition Value

- ♣ The seeds, long stalk of flowers and rhizomes are edible; eaten boiled or roasted.
- ♣ Roots and rhizomes often eaten raw.
- ♣ The tender leaves and flower peduncles of the red and blue-water lily are also valued as food.
- ♣ Flowers stalks considered an excellent source of iron and a fair source of calcium.

2.3.2. Folkloric Medicine

- ♣ Juice is astringent; decoction of the juice used as injection for gonorrhoea.
- ♣ Plant juice considered mildly narcotic, rubbed on the forehead and temples to induce sleep.
- ♣ Powdered roots used as demulcent for piles; also for dysentery and dyspepsia.
- ♣ Flowers used as astringent and cardiotonic.
- ♣ In Bangladesh, the roots used by the traditional healers of the Tripura, Marma and Murong tribes to treat dysuria, urinary tract infections and leucorrhoea. Also, used for indigestion, heart diseases, stomach aches, cancer, and as anti Hemorrhagic.
- ♣ In Nepal and India flowers used in treatment of diabetes.
- ♣ In Ayurveda and Siddha systems of medicines, used for diabetes, liver disorders, urinary problems, menorrhagia, blenorrhagia; also used as tonic and aphrodisiac **(Yan Yao shui lian., 2013, Shui lian shu., 2001).**

CHAPTER-III

PLANT PROFILES AND REVIEW OF LITERATURE

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PLANT PROFILES AND REVIEW OF LITERATURE

3.1. Plant Profiles

Botanical Information

3.1.1. *Nymphaea pubescens* Willdenow (Nymphaeaceae)

Botanical name	**:**	*Nymphaea pubescens* Willdenow
Family	**:**	*Nymphaeaceae* (Waterlily family)
Vernacular Name	**:**	**Tamil name -** □□□□□□□□□□□□-Vellambel
Common Name	**:**	Pink Lotus, Pink Water Lilly
Parts Used	**:**	Flowers, Roots, Leaves, Stem
Synonym	**:**	***Nymphaea lotus var. pubescens*** (Willdenow) *Hook. f. & Thomson* ***Nymphaea spontanea*** *K. C. Landon, nom. inval.* ***Nymphaea pubescens*** (Willdenow) **(The Wealth of India., 1988)**

i). Taxonomy

Kingdom	-	**Plantae**	-	Plants
Subkingdom	-	Tracheobionta	-	Vascular plants
Super division	-	Spermatophyta	-	Seed plants
Division	-	Magnoliophyta	-	Flowering plants
Class	-	Magnoliopsida	-	Monocotyledons
Family	-	***Nymphaeaceae***	-	Water Lily Family
Genus	-	***Nymphaea***		
Species	-	***Nymphaea pubescens*** **(Willdenow)**		

ii). Vernacular Names

Common name	:	Pink Water Lily, Pink Lotus
English	:	Indian red water lily
Hindi	:	Kanval, Kokka, Koi, Neel kamal, Kumudinee

Tamil	:	□□□□□□□□□□□ Vellambel, Alli-tamarai
Telugu	:	Allitamara, Tella-kalava
Malayalam	:	Periambal, Neerambal, Aampal
Kannada	:	Nyadale hurvu, Kannaidile
Manipuri	:	Tharo angouba
Sanskrit	:	□□□□□ Kumuda, Kamala, Indeevaram, Padma, Ulpalam
Gujarati	:	Kanval, Nilophal
Punjabi	:	Neel kamal, Kamalini
Bengali	:	Shaluk, Rakta-kamal, Sundi
Assamese	:	Boga bhet, Seluk, Nal

(QAISER, M., Flora of Pakistan)

Occurrence : Grow gregariously on water places like ponds, lakes, ditches etc.

iii). Distribution / Habitat

Throughout warmer parts of India, in tanks, ponds & ditches upto 500 m altitudes. Ponds and hills. [Bangladesh, India, Indonesia, Myanmar, New Guinea, Pakistan, Philippines, Sikkim, Sri Lanka, Thailand, Vietnam] **(Shui lian shu., 2001).**

iv). Description

Rhizomes	-	Erect, producing slender stolons
Leaf blade	-	Ovate-elliptic to sub orbicular, 15–26(–50) cm, papery, abaxially densely pubescent, peltate more than 5 mm from base of sinus, base deeply cordate and basal lobes sub parallel, margin dentate and teeth acute to subspinose
Flowers	-	Emergent, (2–) 5–8(–15) cm in diameter
Calyx	-	Insertion on receptacle circular
Sepals	-	Oblong, 5–8 cm, conspicuously veined, caducous or decaying after anthesis
Petals	-	12–14(–30), white, red, or pink, oblong, 5–9 cm, transition to stamens abrupt. Filament of inner stamens only slightly wider than anther; connective apically unappendaged
Carpels	-	Completely united walls between locules of ovary single

Stigma	-	Rays 12–15(–30); carpellary appendages linear
Fruit	-	Ovoid to subglobose, 3.5–5 cm in diam
Seeds	-	Ellipsoid to globose, 1–2 mm, with longitudinal ridges
Flowering	-	Aug–Oct. 2*n* = 84

v). Uses

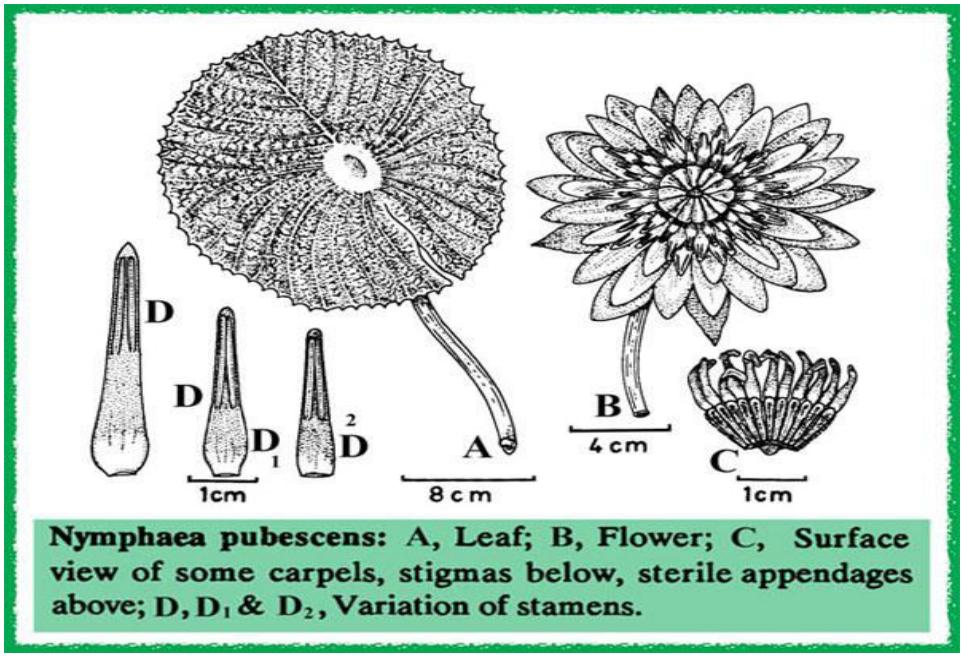
Rhizome	-	Diarrhoea, Dysentery, Dyspepsia and General debility
Flowers	-	Astringent and Cardiotonic
Seeds	-	Constipating, Aphrodisiac, Dyspepsia, Diarrhoea and Dermatopathy **(Shui lian shu., 2001, The Wealth of India., 1988)**

vi). Ethanomedical Information

All parts of the plant are eaten in times of scarcity, the starchy rhizomes are eaten raw or boiled; they are sometimes baked.

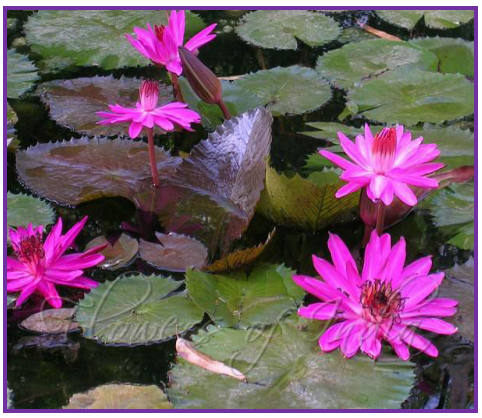
The seeds are edible and can be eaten raw after parching; they may also be ground into flour and made into a kind of bread or cooked into kanji with water. They produce toxic effects when consumed in excessive quantities. The rhizome is considered demulcent and used for dysentery, diarrhoea and dyspepsia. Flowers are astringent and cardiotonic. Some preparations, known as Ghilad, Gulkand etc. are reported to be prepared from the flowers. Seeds are used as a cooling medicine in cutaneous diseases **(The Wealth of India., 1988, Nair, NC., 1983).**

Fig No: 1 Leaf, Flower and its Floral Parts of *Nymphaea pubescens* Willdenow



Nymphaea pubescens: A, Leaf; B, Flower; C, Surface view of some carpels, stigmas below, sterile appendages above; D, D_1 & D_2, Variation of stamens.

Fig No: 2 a, b Habitat - Flower of *Nymphaea pubescens* Willdenow



(a)

(b)

Fig No: 3 Dorsal surface of Leaf with Flower **Fig No: 4** Ventral surface of Leaf

showing Venation

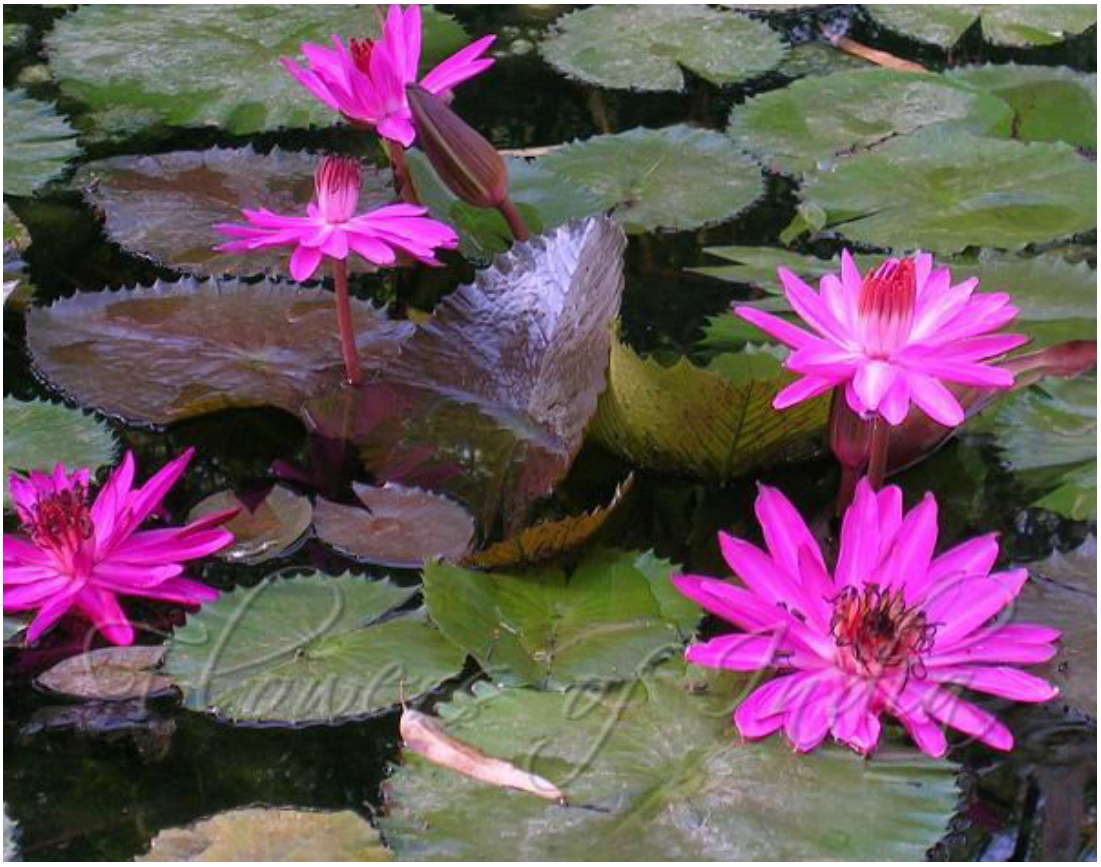
Fig No: 5 Exomorphic features of the flower *Nymphaea pubescens* Willdenow Sepal Inner and Outer view

a) Entire Flower front view

b) Sepal Inner and Outer view, Petal and Stamen

Plate No: 1

Habitat of the flower *Nymphaea pubescens* Willdenow



3.1.2. *Nymphaea nouchali* (Nymphaeaceae) Burmann. F.

Botanical name	**:**	*Nymphaea nouchali*
Family	**:**	*Nymphaeaceae* (Waterlily family)
Vernacular Name	**:**	**Tamil name -** □□□□□□□□□□□ Vellambel
Common Name	**:**	White Lotus, White Water Lilly
Parts Used	**:**	Flowers, Roots, Leaves, Stem
Synonym	**:**	***N. lotus*** Hook., ***N. stellata*** Willdenow, ***N. rubra*** Roxb. Ex. salisb **(**Vellambel, Indian Waterlily)

i). Taxonomy

Kingdom	-	Plantae	-	Plants
Subkingdom	-	Tracheobionta	-	Vascular plants
Super division	-	Spermatophyta	-	Seed plants
Division	-	Magnoliophyta	-	Flowering plants
Class	-	Magnoliopsida	-	Monocotyledons
Family	-	***Nymphaeaceae***	-	Water Lily Family
Genus	-	***Nymphaea***		
Species	-	***Nymphaea nouchali* Burmann. F.**		

ii). Vernacular Names – (Variers, PS., 1995)

Common name	:	White Water Lily, White Lotus
English	:	Indian white water-lily
Hindi	:	Kanval, Kokka, Koi
Tamil	:	□□□□□□□□□□□ Vellambel, Allittamarai
Telugu	:	Allikada, Tella-kaluva
Malayalam	:	Periambal, Neerambal, Ampal
Kannada	:	Kannaidile, Bilenaydilie
Manipuri	:	Tharo angouba
Sanskrit	:	□□□□□ Kumudam
Gujarati	:	Kanval, Nilophal
Bengali	:	Shaluk, kamal
Assamese	:	Nal
Occurrence	:	Grow gregariously on water places like ponds, lakes, ditches etc.

iii). Distribution / Habitat

Throughout warmer parts of India, in tanks, ponds & ditches **(Variers, PS., 1995).** In shallow lakes and ponds, often locally abundant, from northern Luzon to Mindanao. Usually cultivated for its attractive flowers. Also occurs in tropical Asia to Malaya, **(Yan Yao shui lian., June 2013)**.

iv). Description

Rhizomes	-	Erect, unbranched
Leaf blade	-	Elliptic-orbicular to orbicular, 7–15(–45) cm in diam. papery, abaxially glabrous, peltate a few mm from base of sinus, base cordate, basal lobes parallel to spreading, margin sub entire to deeply crenate
Flower	-	Slightly emergent, 3–15 cm in diameter
Calyx	-	Insertion on receptacle circular; sepals lanceolate to oblong-lanceolate, 2.5–8 cm, slightly veined, persistent
Petals	-	10–30, white tinged with purple, blue, or purple-red, linear-oblong to lanceolate, 4.5–5 cm, transition to stamens gradual filament of inner stamens ± as wide as anther; connective apically appendaged
Carpels	-	Only partially united walls between locules of ovary double
Stigma	-	Rays (8–) 10–30; carpellary appendages triangular-tapered
Fruit	-	Globose, 1.5–4.5 cm in diameter
Seeds	-	Ellipsoid-globose, 0.5–1.3 mm, with longitudinal rows of hairs
Flowering	-	Jul–Dec. $2n = 28, 56, 84$

v). Uses

Flowers	-	Astringent, Cardio tonic, Diabetic, Palpitation of the heart, Liver damage, Biliousness, Vomiting, Antidote in Snake poison (Cobra bite, and in scorpion sting), Dyspepsia, Diarrhoea, Diuretic, Leprosy, Astringent, Inflammatory Diseases of Brain and Dysuria **(Shui lian shu, 2001, Qaiser, M., Flora of Pakistan)**

vi). Ethanomedical Information

It is a beautiful white water-lily, native to India and South-east Asia. The toothed floating leaves vary from being elliptic-oval to almost circular, and are 15-26 cm across. The leaves are papery and have fine hair on them. White flowers are 5-15 cm in diameter, with 12-14 shiny petals, and rise above the water. There are red and pink varieties too. This flowered is revered in ancient Hindu literature. Rhizome powder is taken orally in case of dyspepsia and dysentery. Rhizome paste is used locally on piles. Seed powder is used against leucorrhoea, body heat and as tonic.

Fig No: 6 Root, Leaf, Flower & its Floral parts of *Nymphaea nouchali* Burmann. F.

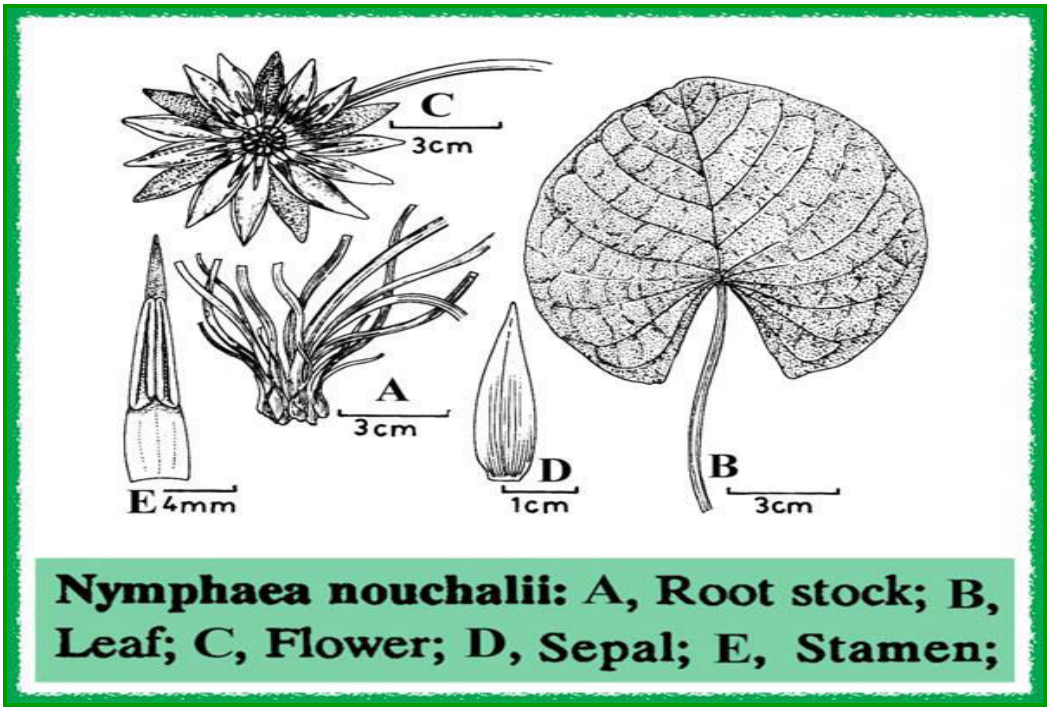


Fig No: 7 Exomorphic feature of Plant *Nymphaea nouchali* Burmann. F.

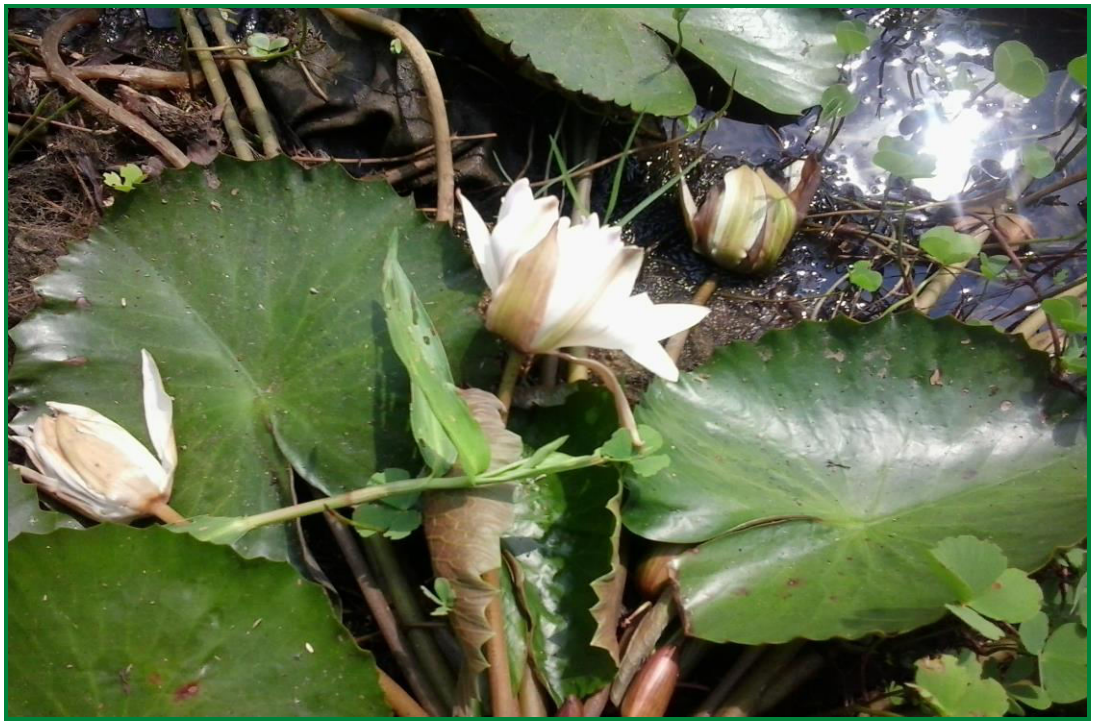


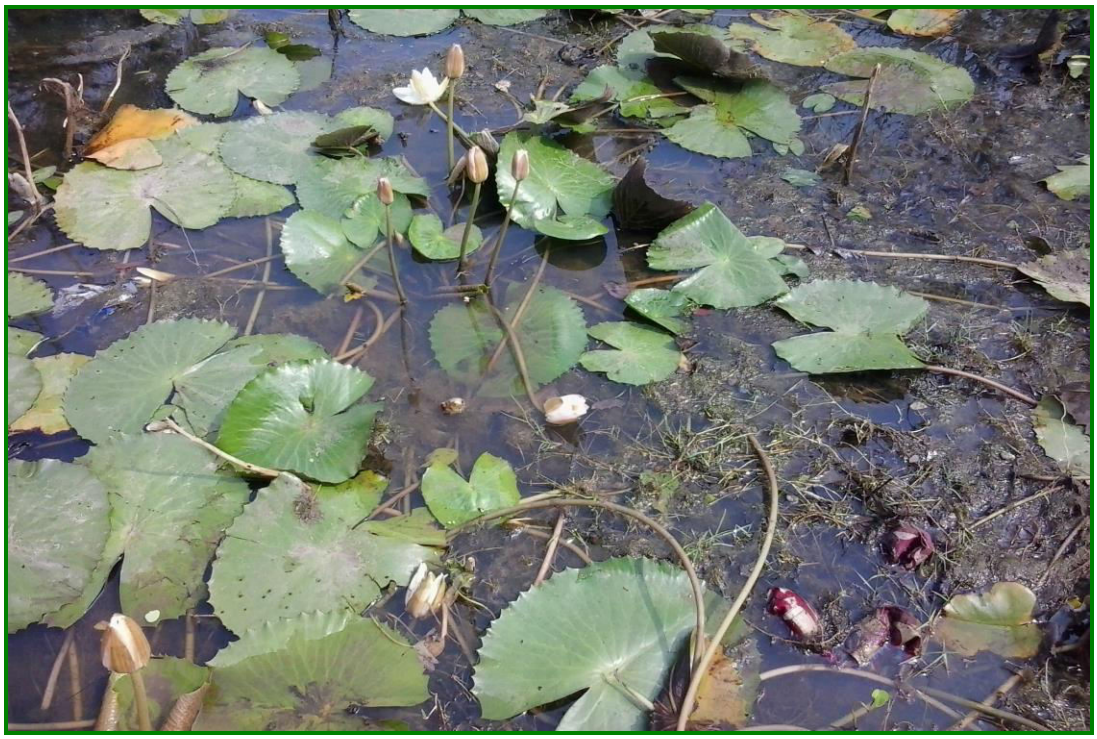
Fig No: 8 Exomorphic features of the Flower *Nymphaea nouchali* Burmann. F.

Sepal Inner and Outer view

a) Entire flower front view b) Sepal inner and outer view, petal and stamen

Plate No. 2

Exomorphic feature of Plant *Nymphaea nouchali* Burmann. F.



3.2. Review of Literature

Systemic literature survey is the main basis for the planning of any scientific work and due to the same reasons here the review of literature regarding the ***Nymphaea*** species has been done.

Nymphaea species are known to provide extracts which have been used in traditional medicine such as dysentery, dyspepsia, diarrhoea and piles, emollient, diuretic, blennorrhagia and diseases of the urinary tract. Alcoholic extract of the rhizome (containing the alkaloid) have mild sedative and spasmolytic action; they do not depress the heart; in larger doses, they have a paralysing action on the medulla **(Irvine & Trickett, Kew Bull., 1953, 363; Henry, 758; Chem. Abstr., 1945, 39, 5327**).

The rhizome is considered demulcent and used for dysentery and dyspepsia. Flowers are astringent and cardiotonic. Seeds are used as a cooling medicine in cutaneous diseases **(Krit. & Basu, I, 112: Fl. Delhi, 54).** Leaves are applied topically in erysipelas and in eruptive fevers. A decoction of flowers is considered narcotic. Seeds are stomachic and restorative **(Krit. & Basu, I, 114: Fl. Delhi, 54).** Nymphaeaceae family is known as a source of folk medicine for treatment to nervous disorders.

Preliminary phytochemical screening of the species revealed the presence of flavanoids and phenolic compounds and tannins. Flavanoids have been reported to exert multiple biological effects due to their antidiabetic, antioxidant and free radical scavenging activity. High levels of enzymatic antioxidants were found in the fresh flower extracts **(Nagavani, V., 2010).**

3.2.1. *Nymphaea pubescens* Willdenow (Nymphaeaceae)

- **Almeida, (1926)** compiled notes on the structure and life history of ***Nymphaea pubescens*** Willdenow.

- **McCann, C., (1935)** reviewed some pharmacognostical & taxonomical observations on ***Nymphaea pubescens*** Willdenow.

- **Selvakumari, E., Sreenathkumar**, **Shantha Arcot., (2010)** reported antidiabetic activity of ***Nymphaea pubescens*** Willdenow - A plant drug of Aquatic flora interest. The ethanolic extract was orally administered to

diabetic rats at 200 and 400mg/kg doses daily for 14 consecutive days to determine antidiabetic activity. The ethanolic extract at 400mg/kg body weight showed significant reduction in blood glucose level upto 99.28mg/dl.

- **Selvakumari, E., Shantha, S., Purushoth Prabu, T., Sreenathkumar, C., (2012).** Antiproliferative activity of Ethanolic flower extract from ***Nymphaea pubescens*** Willdenow against Human cervical and breast carcinoma *In-vitro*.

- **Shajeela, P. S., Kalpanadevi, V., and Mohan, V. R., (2012).** Potential antidiabetic, hypolipidaemic and antioxidant effects of ***Nymphaea pubescens*** extract in alloxan induced diabetic rats. The ethanol extract of *Nymphaea pubescens* tuber at a dose of 200 mg/kg and 500 mg/kg body weight were administered at single dose per day to diabetes induced rats for a period of 14 days. The extract also caused significant increase in plasma insulin ($p<0.05$) in the diabetic rats.

3.2.2. *Nymphaea nouchali* Burmann. F. (Nymphaeaceae)

- **Isabelle Orban and Jules Bouharmont, (1998).** Megagametophyte development of ***Nymphaea nouchali*** Burm. f. (Nymphaeaceae). Megasporogenesis results in a triad of one micropylar cell and two innermost megaspores. Embryo sacs derive from a single functional megaspore and are tetranucleate. Megasporogenesis is monosporic and of the *Polygonum* type, but megagametogenesis is of *Oenothera* type.

- **Shui lian shu, (2001).** NYMPHAEA Linnaeus, Many species of the genus ***Nymphaea*** are cultivated as ornamentals, in China. About 50 species: widespread in temperate and tropical regions; five species in China. Many species of the genus *Nymphaea* are cultivated as ornamentals. In China, in addition to the native species, both *Nymphaea mexicana* Zuccarini and *N. alba* Linnaeus var. *rubra* Lönnroth are cultivated.

- **Tetali, P., Shrikant Sutar and Sujata Tetali., (2004)** reported on Selective insectivory in ***Nymphaea nouchali*** Burm. F. Carnivorous plants are generally insectivorous, and carnivory in flowering plants is generally found in taxa that are adapted to nutrient-deficient habitats. ***Nymphaea nouchali*** **Burmann. F.**

(a cultivar of var. cernua), a large aquatic member of the family Nymphaeaceae, indulges in a primitive form of insectivory and represents the missing evolutionary link.

- **Kiran Kumar, A., Sai Babu and K. Ammani., (2008).** Antimicrobial and phytochemical analysis of ***Nymphaea nouchali*** leaf extracts. The in-vitro antimicrobial activity was performed by agar well diffusion method. The ethylacetate extracts of Nymphaea nouchali leaf extract were active against all the investigated bacterial strains.

- **Nagavani, V., Raghava Rao, T., (2010).** Evaluation of Antioxidant Potential and Qualitative Analysis of major Polyphenols by RPHPLC in ***Nymphaea nouchali*** Burm.f flowers. The recent studies have investigated that the antioxidant effect of plant products is mainly attributed to phenolic compounds, such as flavonoids, phenolic acids, tannins etc. these compounds are distributed in different parts of plants such as bark, leaves, fruits and flowers etc. The flowers of *Nymphaea nouchali* Burm. f. have a wide range of applications in ayurveda and traditional medicine.

- **Bhakta, B., Raskoti., Rita Ale and Ganga, D., Bhatt., (2011)**. A new record of ***Nymphaea*** (Nymphaeaceae) for Flora of Nepal. Occurrence of *Nymphaea lotus* var. *pubescens* Willdenow (Nymphaeaceae) in Nepal is reported. Detailed description, illustration and relevant notes are provided.

- **Syed Rashel Kabir., *et al.,* (2011)**. Purification and characterization of a Ca (2+)-dependent novel lectin from ***Nymphaea nouchali*** tuber with antiproliferative activities. NNTL was a glycoprotein containing 8% neutral sugar and showed toxicity against brine shrimp nauplii with an LC(50) value of 120 ± 29 μg/ml and exerted strong agglutination activity against four pathogenic bacteria (Bacillus subtilis, Sarcina lutea, Shigella shiga and Shigella sonnei). In addition, antiproliferative activity of this lectin against EAC (Ehrlich ascites carcinoma) cells showed 56% and 76% inhibition in vivo in mice at 1.5 and 3 mg.kg(-1).day(-1) respectively.

- **Ishrat Jahan., *et al.,* (2012).** Antioxidant, Analgesic and Anti-Inflammatory activities of ***Nymphaea nouchali***. The extract MNNF at the dose of 100 and 200 mg kg^{-1}, produced a significant ($p<0.05$) increase in pain threshold in hot plate method whereas significantly ($p<0.05$) reduced the writhing caused by acetic acid and the number of licks induced by formalin in a dose dependent manner. The same ranges of doses of MNNF caused significant ($p<0.05$) inhibition of carrageenan induced paw edema after 4 h in a dose dependent manner. In DPPH, $ONOO^-$ and total ROS scavenging method, MNNF showed good antioxidant potentiality with the IC_{50} value of 10.33±1.02, 20.16±0.61 and 31.72±0.48 μg mL^{-1}, respectively.

- **Md. Al Amin Sikder., *et al.,* (2012).** Evaluation of Bioactivities of ***Nymphaea nouchali*** (Burm. f) - The National Flower of Bangladesh. The membrane stabilizing activity was assessed by hypotonic solution and heat induced methods. In the present studies, the aqueous soluble (AQSF) materials of the petals demonstrated strong membrane stabilizing activity, whereas the chloroform (CSF) and petroleum ether soluble fractions (PESF) revealed moderate membrane stabilizing activity in both methods. The total phenolic content was also determined and expressed in gallic acid equivalent.

3.2.3. Other species

- **Venu, P., (2003)** studied on the identity and sterility in ***Nymphaea rubra*** Roxb. Ex Andrews (Nymphaeaceae).

- **Awatif, A Elegami., *et al.,* (2003)** isolated three novel flavonols, myricetin-3'-O-(6"-p-coumaroyl) glucoside and two epimeric macrocyclic derivatives, as well as the known myricetin-3-O-rhamnoside and pentagalloyl glucose from the wild water lily ***Nymphaea lotus*** L.

- **Zhizhen Zhang, *et al.,* (2003)** identified two lignans, together with six known flavonol glycosides in assay-guided fractionation of the ethanol extract of ***Nymphaea odorata.***

- **Bhandakar, M, Khan., A, (2004).** The alcoholic extract (yield, 9% w/w) of the ***Nymphaea stellata*** flowers was evaluated against carbon tetrachloride-

induced hepatic damage in albino Wistar rats (8 - 10 weeks, 100 - 120 g) at 250, 500, and 750 mg/kg (orally). The oral administration of varying dosage of extract of Nymphaea stellata Willdenow, flower to rats for 10 days afforded the good hepatoprotection against carbon tetrachloride-induced elevation in serum marker enzymes, serum bilirubin, liver lipid peroxidation and reduction in liver glutathione, liver glutathione peroxidase, glycogen, superoxide dismutase and catalase activity.

- **Naghma Khan, Sarwat Sultana., (2005**) reported the prophylactic effect of ***Nymphaea alba*** against ferric nitrilotriacetate (Fe-NTA)-induced renal oxidative stress, hyperproliferative response and renal carcinogenesis in Wistar rats.

- **Naghma Khan, Sarwat Sultana., (2005**) reported the efficacy of ***Nymphaea alba*** on potassium bromate 125 mg/kg body weight, intraperitoneally) caused reduction in renal glutathione content, renal antioxidant enzymes and phase-II metabolising enzymes with enhancement in xanthine oxidase, lipid peroxidation, gamma-glutamyl transpeptidase and hydrogen peroxide (H_2O_2).

- **Dhanabal, SP., *et al.,* (2007).** The defatted ethanolic leaf extract of ***Nymphaea stellata*** Willdenow (14.26 %w/w) at a dose of 100 and 200 mg/kg was studied for hypoglycemic activity in alloxan-induced diabetic rats (Wistar, 150 – 220 g).

- **Rajagopal, K., Sasikala, K., (2008)** studied anti-hyperglycemic and anti-hyperlipidemic effects of ***Nymphaea stellata*** in alloxan-induced diabetic rats. Administration of *N. stellata* flower extract to diabetic rats reversed the changes and improved the HDL levels. The flower extract shows a significant (p-value is less than 0.001) reduction in levels of FBG, water intake, food intake, urine sugar, blood urea, TL, TC, TG, FFA, phospholipids, LDL, VLDL and AI. It also shows a significant increase in body weight, plasma insulin, protein, haemoglobin and HDL levels. These results unmistakably indicate that the flower extract could effectively manage the diabetic complications such as body weight maintenance, hyperlipidemia, cardiovascular complications in diabetes mellitus and progression of atherosclerosis.

- **Oladmeji, HO., *et al.,* (2008)** evaluated the larvicidal and anti-microbial potentials of ***Nymphaea odorata***.

- **Rakesh, SU., *et al.,* (2009).** The HPTLC method for quantitative determination of gallic acid from hydroalcoholic dried flower extract ***Nymphaea stellata*** Willdenow was reported.

- **Subash-Babu, P., Ignacimuthu, S., Agastian, P., Babu Varghese., (2009).** Partial regeneration of beta-cells in the islets of Langerhans by **Nymphayol** a sterol isolated from ***Nymphaea stellata*** (Willdenow) flowers. The present study was aimed at identifying a new agent for the control of diabetes through regeneration of pancreatic beta cells and insulin secretory potential. Nymphaea stellata flower chloroform extract (NSFCExt) showed significant plasma glucose lowering effect. Nymphayol (25, 26-dinorcholest-5-en-3beta-ol) a new crystal was reported.

- **Huang, YN., *et al.,* (2010).** Intestinal alpha-glucosidase inhibitory activity and toxicological evaluation of ***Nymphaea stellata*** flowers extract. Nymphaea stellata Willdenow flowers (NSF) are used as a traditional medicine in India and Nepal to treat diabetic disease. Different works have demonstrated that NSF extract showed antihyperglycemic effect on alloxan-induced diabetic rats. In addition, NSF extract was studied to assess its possible acute oral toxicity and genotoxicity.

- **Mohan Maruga Raja, M.K., Neeraj Kumar Sethiya and Mishra., S.H., (2010).** A comprehensive review on ***Nymphaea stellata***: A traditionally used bitter. **Nymphayol,** a steroid isolated from the flowers has been scientifically proved to be responsible for the traditionally claimed antidiabetic activity; it reverses the damaged endocrine tissue and stimulates secretion of insulin in the β-cells.

3.2.4. Common reviews

- **Gerd Bendz**, **Birgitta J~Nsson (1971).** Nymphaeceae - **Anthocyanins** in Leaves of ***Nymphaea candida***. Delphmidin-3-galactoside, cyanidin-3galactoside and a pigment tentatively identified as delphinidin-7galactoside have been isolated from the leaves of ***Nymphaea candida.***

- **Marquina, S., *et al.,* (2005)** evaluated Comparative phytochemical analysis of four Mexican Nymphaea species**.**

- **Sridhar, K.R., and Bhat, R., (2007)** described that lotus plants provide several bioactive ingredients like alkaloids, flavonoids, antioxidants, anti-steroids, antipyretic, anti-cancerous, antiviral and anti-obesity properties.

- **Agnihotri, KV., *et al.,* (2008)** isolated 20 antioxidants from the ***Nymphaea caerulea*** flowers, including two 2S, 3S, 4S-trihydroxypentanoic acid and myricetin 3-O-(3''-O-acetyl)-alpha-l-rhamnoside.

- **Kandukuri Vasu, *et al.,* (2009).** Biomolecular and phytochemical analyses of three aquatic angiosperms. Aquatic plants produce a variety of compounds of known therapeutic properties and can be utilized as food and feed. These substances are used for developing new antimicrobial drugs. The present study deals with three aquatic plants dominant in Warangal district A. P. region *Eichhornia crassipes, Ipomoea aquatica* and *Nymphaea pubescens* were selected. These three aquatic angiosperms were analysed for their biomolecules and phytochemicals.

- **Mollik MAH., *et al.,* (2009).** Medicinal plants from Sundarbans used for the prevention of cardiovascular diseases: A pragmatic randomized ethnobotanical survey in Khulna division of Bangladesh. About 33 medicinal plants being used by Khulna division as folk medicines have been identified and documented along with their curative properties for the treatment of cardiovascular diseases.

- **Patel, DK., Kumar, R., Laloo, D., Hemalatha, S., (2012).** Natural medicines from plant source used for therapy of diabetes mellitus: An overview of its pharmacological aspects. Medicinal plants play an important role in the

treatment of diabetes mellitus, especially in the developing countries due to their cost effectiveness. Currently available treatment options in modern medicine have several adverse effects.

Various plants have been found to possess significant anti-diabetic property after their preclinical and clinical evaluation. The profiles of plants with hypoglycaemic properties reported in the literature from 2009 to 2011. Use of these plants may delay the development of diabetic complications and can correct the metabolic abnormalities through variety of mechanisms.

It is an attempt to assess the available scattered literatures and compiles them under different categories in a systematic way, to provide the pharmacognostical, phytochemical and pharmacological prospective of ***Nymphaea pubescens*** **Willdenow** and ***Nymphaea nouchali*** **Burmann. F.** (**Nymphaeaceae**). It revealed that only a very few pharmacological activity and basic taxonomical characters has been reported on these plants and no phytoconstituents has been isolated and characterized except a very few antioxidants.

CHAPTER-IV

SCOPE AND PLAN OF WORK

SCOPE AND PLAN OF WORK

CHAPTER IV

SCOPE AND PLAN OF WORK

4.1. Scope of Work

Alkaloids and flavonoids are two large groups of secondary metabolites with highly diverse biological functions. An array of their biological effects is used in the production of pharmaceuticals. However, a lot of them are used only in traditional medicine. Some forgotten compounds from traditional medicine has been rediscovered, and studied for new possible medicinal effects **(Wollenweber, E., 1988, Weidenbörner, M., *et al.,* 1990, Dixon R.A., 1999).**

Alkaloids and flavonoids are members of a large group of natural compounds-the secondary metabolites. Currently, their function in plants is not clearly understood. Various kinds of secondary metabolites probably have different functions in the plant. The alkaloids are assumed to have a protective mechanism against herbivore animals and possibly some parasites **(Francois G., 1997).** Flavonoid glycosides and free aglycones are involved in the interactions of plants with microorganisms, both pathogenic and symbiotic. They also act as UV protectants in plant cells and pigment sources for flower colouring compounds, and they play an important role in interactions with insects **(Stobiecki M., 2000, Schijlen E.G.W.M, *et al.,* 2004).** These plant metabolites also affect human and animal health. Flavonoids have significance in the diet, ascribed to their antioxidant properties, estrogenic action, **(Miksicek, R.J., 1993)** and a wide spectrum of antimicrobial and pharmacological activities **(Stobiecki M., 2000, Schijlen E.G.W.M, *et al.,* 2004).** Alkaloids are used in low concentrations as efficacious remedies serving as cardiotonics, potential oncological medicines, etc.

The appearance of new and more complicated diseases in recent years, along with persistence of old ones, constrains scientists to seek new and more effective methods of treatment. The resistance of hosts is one example of the big problems of current medicaments, predominantly for antibiotics and other medicines used against infectious diseases. The growing population of the world and the migration of people increase the spread of very specific and dangerous kinds of diseases, for example, malaria. If the pathogen that causes a disease becomes resistant to the medicine used

to treat it the number of patient's increases and so does the risk that the disease will spread to other countries. Another very important concern for specialists is the problem of human cancer. This is one of WHO`s (World Health Organization) current campaigns. The following citation taken is from the WHO project outline,

In addition to prevention, it is essential to develop of more effective methods to diagnose illnesses in their early stages, when treatment can be more effective. Belated diagnosis can be a mortal danger because of the absence of effective medicines to advanced disease. Nature offers resources that include compounds which can potentially solve many of these problems. Investigation of natural products obtained from plants - the isolation of compounds and their modification, and the evaluation of their biological activities - represents an important field of biochemical and pharmaceutical research.

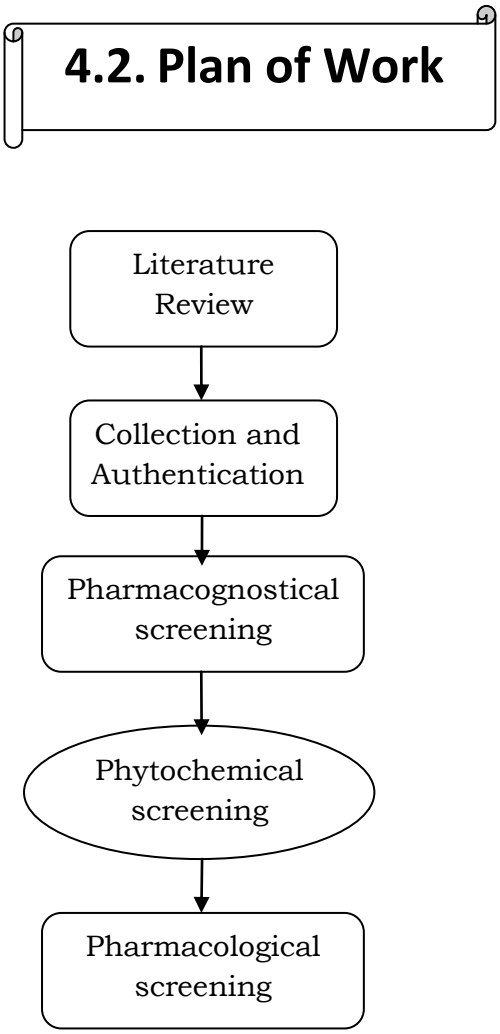
Looking at the scope of herbal drugs and increasing demand especially in cases of diseases like diabetes, liver, hypertension, cancer, renal diseases inflammation, it has been planned to studied the pharmacognostical, phytochemical and pharmacological screening of the flowers of ***Nymphaea pubescens*** Willdenow and ***Nymphaea nouchali*** Burmann. F.

Keeping in mind about the adverse effects of allopathic drugs, above said activity were studied with different parameters in order to give possible scientific validation.

Pharmacognostical and phytochemical investigation will be a useful tool for identification and authentification of the plants for research purpose.

Fig. No: 9 Plan of Work

The research work is planned systematically as follows,



I. Collection and Authentication of Flower materials

Ii. Pharmacognostical Studies of the Flower(s)

a) Macroscopy

b) Microscopy

C) Powder Analysis

d) Determination of Physico-Chemical Constants

I) Organoleptic Evaluation

Ii) Physical Evaluation

1. Moisture Content (Loss on Drying)
2. Ash values
   - i) Total Ash
   - ii) Acid Insoluble Ash
   - iii) Sulphated Ash
   - iv) Water Soluble Ash
3. Extractive Values
4. Foreign Organic Matter
5. Determination of pH Value
6. Determination of Heavy Metals
7. Solubility
8. Determination of Micro-Organism
9. Foaming Index
10. Fluorescence Analysis

II. Phytochemical studies

a) Extractive Values of Different Extracts

B) Preliminary Phytochemical Screening

C) Fluorescence Analysis of Extracts

D) Inorganic Mineral Analysis

e) Chromatography

- ♣ Thin Layer Chromatography
- ♣ HPTLC Chromatography
- ♣ Column Chromatography

Isolation of Constituents by Column Chromatography

f) Identification of Isolated Compounds by Spectral Studies

(UV, IR, NMR, MASS)

g) Screening of *In-Vitro* Anti-Oxidant Studies

a) DPPH Radical Scavenging Activity
b) FRAP Radical Scavenging Activity
c) TRAP Radical Scavenging Activity
d) ABTS Radical Scavenging Activity

h) Determination of Total Phenolic Compounds

i) Estimation of Total Flavonoid Content

III. Pharmacological and Biochemical Studies

A) Acute Oral Toxicity Studies

B) Hepatoprotective Activity

i) Biochemical Parameter Estimation

a) Serum Glutamate Pyruvate Trasaminase (SGPT)
b) Serum Glutamate Oxalocetate Transminase (SGOT)
c) Alkaline Phosphatase
d) Serum Total Protein
e) Total Bilirubin
f) Urea
g) Creatinine
h) Proteins

ii) *In-vivo* Enzymatic Antioxidant Activity

a) Catalase
b) Superoxide Dismutase (SOD)
c) Glutathione Peroxidase (GPx)

iii) *In-vivo* Non-Enzymatic Antioxidant Activity

a) Reduced Glutathione Activity (GSH)
b) Lipid Peroxidation Activity (LPX)

iv) Histopathological Studies on Liver

c) Antidiabetic activity

a) Single Dose Study
b) Oral Glucose Tolerance Test
c) Hypoglycemic Activity
d) Induction of Diabetes by Alloxan Monohydrate
e) Antioxidant ASSAYS

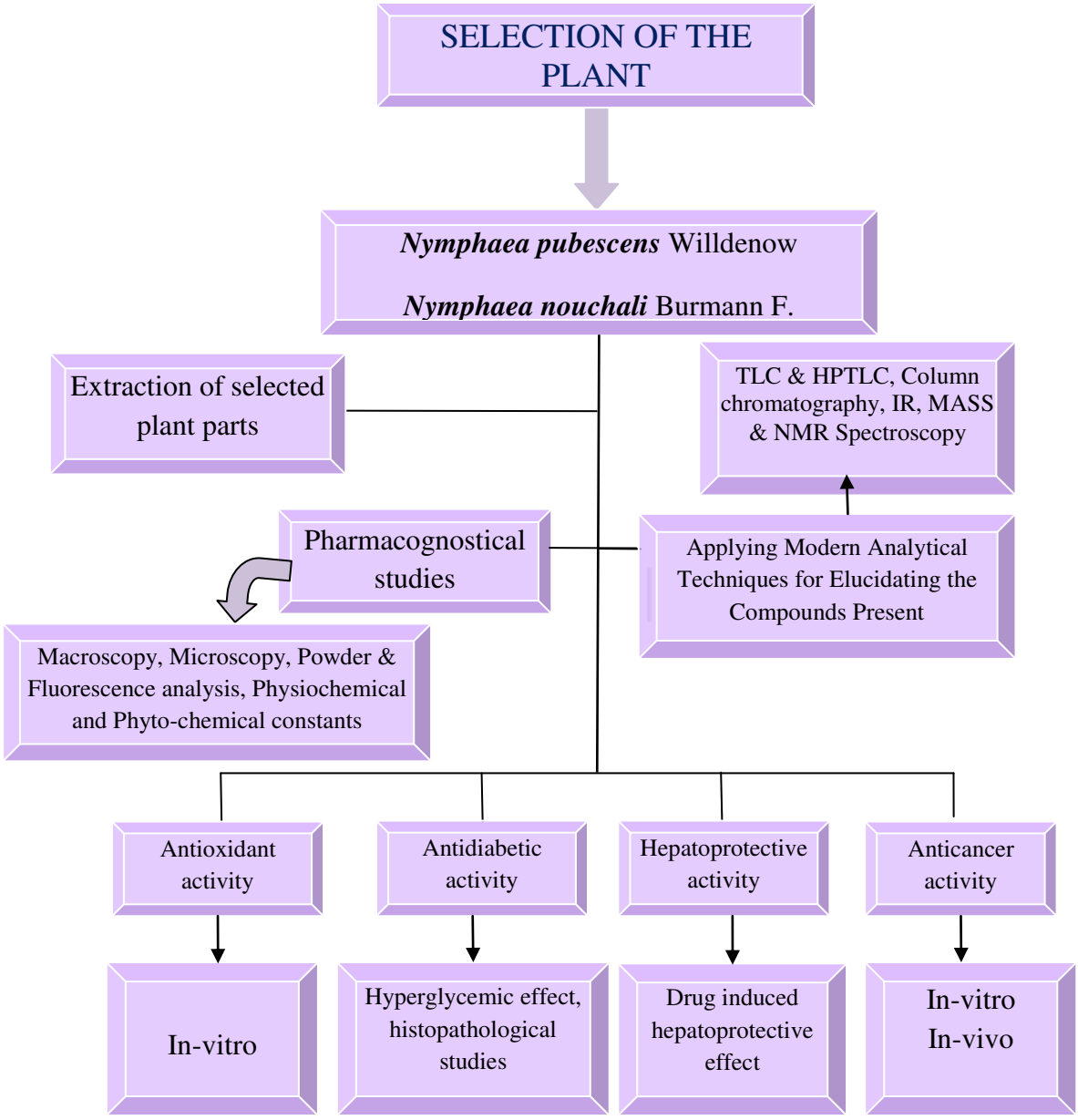
d) Anti-cancer activity

i) Tumor Cell Lines

ii).*In-vivo* Anti cancer Activity (Dalton's-Antitumor Model)

iii). Determination of Body Weight and Survival Time

Fig No: 10 Schematic Representation of Plan of Work



CHAPTER-V

MATERIALS AND METHODS

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CHAPTER V
MATERIALS AND METHODS

5.1. Chemicals, Reagents and Equipments

Solvents: Ammonia 25% AR (Batch 503542) Skylab's; Ethanol AR (Batch 3875) Associated Chemical Enterprises, RSA; Methanol Spectrophotometric grade (Batch no 68F-0898) Sigma; Methanol AR (Batch 16229) Saarchem Pvt Ltd, RSA; Toluene CP (Cat No 15, 500-4) Aldrich; Rutin, Ethyl acetate, AR (Batch no 20774) Aldrich; Formic acid (Batch 107F-0658) Sigma; Gl. Acetic acid AR (Batch 20040824P); Diethyl amine AR (Batch 43410) Microlabs; Dimethylsulphoxide AR (1.0295, 2.2500) Merck; Benzene (Batch 6317/584) Associated Chemical Enterprises, RSA; Hydrochloric acid AR (Batch 4504) Associated Chemical Enterprises, RSA; Chloroform AR (Batch 1010060) Saarchem Pvt Ltd, RSA; Petroleum ether AR (Batch 15060) Saarchem Pvt Ltd, RSA; Acetonitrile (Lot 96F 3484) Sigma, USA. Analytical grade chemicals supplied by S.D. Fine Chemicals, Sigma Chemicals Co. and Qualigens Fine Chemicals were used. All chemical solvents, enzyme kits used for this research work were of analytical reagent grade.

Chemicals: Potassium hydroxide, (Batch 19216) Saarchem; Sodium hydroxide, (Batch 1410507), Skylab's; Potassium Iodide AR (Batch 69153) Skylab's; Potassium Chloride AR (Batch 1029052) Saarchem; Sodium Chloride AR (Batch 1028306) Saarchem; Ferric chloride (Lot 37F-3478) Sigma, USA; Bismuth nitrate (No B-9383) Lot 47F- 0698, Sigma; Ninhydrin crystalline (No N-4876) Lot 97F-0081, Sigma; Vanillin (S4551918) Merck, Germany; Dinitrobenzene (S05456) Merck.

Equipments: TLC Plates (Batch 126 F-0130) T-6770, Polyester silica gel, 250µm layer thickness, 2-25µm mean particle size, 20x20cm, Sigma; Whatmann filter paper No. 1125mm (Cat No 1001125) Schleicher and Schuel; Grinding Mill, Thomas-Wiley Laboratory Mill Model 4; Rotary Evaporator, Heidolph Laborota 4000, Germany; UV-Visible Spectrophotometer, Shimadzu UV-1601, Chart no 200-91527, Japan.

5. 2. General

i). Glass ware

Clean glassware of Corning and Borosil brands were used. They were washed in acidified chromate, detergent and in running tap water. Glassware were rinsed in

distilled water and dried before use, pre-sterilized plastic containers used in this study were from Tarsons (India) and Laxbro (India).

ii). Water

Distilled water of reagent grade, from reverse osmosis unit was used for preparation of all solutions.

iii). Chemicals

Laboratory graded chemicals from Glaxo or SDS (India) were used for preparation and qualitative test. Analytical grade chemicals from Merck (India), BDH (India) and SIGMA (U.S.A) were used for preparation of reagents and solutions. Salts were weighed using a monopan balance (Sartorius, Germany).

5. 3. Purification of Solvents

1. Petroleum ether

The petroleum ether was distilled and the fraction boiling between 60°- 80°C was collected and used for extraction and chromatographic purposes.

2. Chloroform

The chloroform was shaken well with equal volume of distilled water twice to remove water soluble impurities and separated using a separating funnel. It was dried over anhydrous calcium chloride for 24 hours, filtered and dried again over anhydrous potassium carbonate for another 24 hours. This was decanted, distilled and the fraction boiling at 61-62°C was collected and stored in a dark brown bottle. As a preservative, 1mL of absolute alcohol was added per litre of chloroform.

3. Ethyl acetate

Commercial grade ethyl acetate was refluxed with 100 ml of acetic anhydride and 10 drops of concentrated sulphuric acid in one litre for 4 hours and distilled. The distilled ethyl acetate was shaken with 20-30 g of anhydrous potassium carbonate, filtered and redistilled. The fraction boiling at 77°C was collected and used.

4. Methanol

Commercial grade methanol was soaked in slaked lime overnight, distilled and used.

5. 4. Plant Materials

Fresh flowers were selected for the studies are,

1. ***Nymphaea pubescens*** **(Nymphaeaceae) Willdenow**
2. ***Nymphaea nouchali*** **(Nymphaeaceae) Burmann. F.**

5.5. Instruments

Grinding Mill	**:**	Junior Grindwell, Choudhry, J.U.C, Mumbai, India was used for powdering of plant materials.
Incinerator	**:**	Ambassador, model-2265, Matri, Pondicherry, India, was used for the determination of ash values of plant materials.
Rotary Microtome	**:**	Leica RM 2135, Leica Microsystems GmbH, Germany was used for sectioning of the paraffin embedded specimen of plants and animal tissues.
HPTLC	**:**	CAMAG Linomat IV, No-022.78.6, twin trough chamber No: 022- 5155 and TLC Scanner-3, No: 027-6480 Switzerland, were used for HPTLC studies.
Rotary evaporator	**:**	Super fit, India rotary evaporator was used for concentration of plant extracts.
ELISA reader	**:**	Bio-Rad Laboratories Inc, California, USA, model 550 was used for *In-vitro* antioxidant studies.
Centrifuge	**:**	Remi centrifuge, R-8c Laboratory centrifuge and Elvenjan homogenizer, Remi Motors Ltd, Mumbai, India were used to separate serum from blood, preparation of tissue homogenates and separation of organic layer in TBARS assay.
Autoanalyser	**:**	Merck Microlab 200 manufactured by M/S Vital Scientific N V., The Netherlands, was used to estimate various biochemical parameters viz. SGOT, SGPT, ALP (alkaline phosphatase), Total protein and albumin.

Spectrophotometer	**:**	Shimadzu 160-A UV-VIS Spectrophotometer manufactured by Shimadzu, Japan was used to estimate the absorbance range of isolated compounds, total phenol content, flavonoids content and biochemical estimations.
FTIR	**:**	Perkin Elmer, 1600 Series FTIR, Perkin Elmer (India) Pvt. Ltd., Nand Chambers, Thane, India
^{13}C NMR	**:**	DAM X- 400m/z- Bruker (India) Pvt. Ltd., Hyderabad, India, was used for ^{13}C NMR Studies
Mass Spectroscopy	**:**	MALDI, Altraflex, TOF, Bruker, Bruker Daltonics, Germany, was used for Mass spectroscopic studies

5.6. Animals

Female Albino rats-***Rattus norvegicus*** weighing 150-250g were used for the study. They were nulliparous and non-pregnant. These were acclimatized to laboratory condition for one week prior to start of dosing.

5.7. Cell Lines

In-vitro Cytotoxic Activity - DAL Cell Lines (Human Cervical Cancer (HeLa) cell lines which are obtained from National Centre for Cell Science (NCCS), Pune).

CHAPTER-VI

PHARMACOGNOSTICAL SCREENING

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PHARMACOGNOSTICAL SCREENING

6.1. Introduction

Nearly 90% of the crude drugs are obtained from the plant sources while about 10% of the drugs are derived from animal and mineral sources. These vegetable drugs are either used in dried forms or sometimes as whole fresh or their juice. The study of these crude drugs made with a view to recognize them is called Pharmacognosy. (Pharmakon=drug; Gignoso=to acquire knowledge of), meaning the knowledge or science of drugs. In Pharmacognosy a complete and systematic study is done, which comprises of,

- ♣ Origin, common names, scientific nomenclature and family
- ♣ Geographical source (and history)
- ♣ Cultivation, collection, preservation and storage
- ♣ Macroscopical, Microscopical and Sensory (organoleptic characters)
- ♣ Chemical composition wherever possible
- ♣ Identity, purity, strength and assay
- ♣ Substitutes and Adulterants etc

Such systematic study of a drug as complete as possible is claimed to be the scientific or pharmacognostical evaluation **(Anonymous, 2010).**

Crude drugs when supplied in intact form can be identified by morphological characteristics. However, in case of doubt the same can be investigated for histological characteristics to confirm the identity of the supplied drugs. Microscopical techniques provide detailed information about the crude drugs by virtue of their two main analytical uses. Firstly, their property to magnification permits the fine structures of minute objects to be visualised and thereby confirm the structural details of the plant drugs under evaluation. Secondly, determination of the optical as well as micro-chemical properties of the crude drug specimens under study.

The powdered crude drugs can be identified based on the form and the presence or absence of different cell types based on their cyto-morphological characters **e.g. parenchyma, collenchymas, fibres, stone cells, trichomes, secretory cells,**

epidermal cells etc. In the same way, the cell inclusions may also be identified such as **starch grains, aleurone grains, gums, mucilages, calcium oxalate crystals etc.**

Ash values are helpful in determining the quality and purity of the crude drugs, especially in powdered form. The objective of making ash of the vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination **(Mukherjee, 2002).** Extractive values determine the amount of active constituents in a given amount of medicinal plant material when extracted with solvents. Further, these values indicate the nature of the constituents present in the crude drug.

Physico-chemical standardization is a pre-requisite in quality control of crude drugs both single as well as compound formulation as the efficacy of the drugs mainly depends upon their chemical and physical properties.

Therefore, the objective of the present study was to evaluate various pharmacognostic standards like botanical description, microscopy, ash values, extractive values, microscopical characteristics of flower powder, heavy metals, pH, solubility and preliminary phytochemical analysis of the flowers of ***Nymphaea pubescens*** **Willdenow** and ***Nymphaea nouchali*** **Burmann. F.** for the authenticity of a drug is necessary before screening for their pharmacological activities.

6.2. Plant Collection and Authentication of the Specimen

The flowers like ***Nymphaea pubescens*** **Willdenow** and ***Nymphaea nouchali*** **Burmann. F.** was collected respectively from the months of August-October 2009 from Periyanahalli and surrounding areas of Dharmapuri District in Tamilnadu, India. It was identified and authenticated by the taxonomists of Botanical survey of India, **DR. Venu** and **DR. G.V.S. Murthy, Joint Director, Botanical Survey of India** (BSI), **Tamil Nadu Agriculture University (TNAU),** Coimbatore. A voucher specimen number PCP-205 has been kept in our research laboratory for future reference.

After authentication, the flowers were washed with tap water, air dried and then homogenized and stored in airtight bottles. The flowers were used to study for the macroscopic and microscopic characters, whereas the flowers dried under shade were milled into coarse powder and were passed through the sieve No: 40 for powder

microscopic characteristics and to determine the physicochemical parameters and phytochemical screening.

6.2.1. Botanical Description

Herbs were perennial and aquatic. **Stems** rhizomatous; rhizomes erect or repent, branched or unbranched. **Leaves** arising from rhizome, simple, alternate, floating, emersed, or submersed, long petiolate but short petiolate on submersed vernal ones; leaf blade undivided, usually with a basal sinus, often peltate.

Flowers were solitary, axillary, long pedunculate, bisexual and hypogynous to epigynous, actinomorphic, entomophilous, and mostly emergent. **Sepals** 4-7, usually green, occasionally petaloid. **Petals** were numerous (rarely absent), distinct, usually showy, often transitional to stamens. Stamens are numerous; anthers introrse, dehiscent by longitudinal slits; connective sometimes appendaged. Pistil 1, compound; carpels 5-many, partially or completely united, surrounding sometimes projecting floral axis.

Ovary is multilocular; placentation laminar; ovules numerous. Styles absent or modified into abaxially projecting carpellary appendages. Stigmas radiate on distal surface, often disclike.

Fruit are berrylike, many seeded, irregularly dehiscent. **Seeds** were mostly arillate; endosperm little, perisperm abundant; embryo small; cotyledons 2, fleshy **(Shui lian shu, 2001)**.

6.2.2. Macroscopic Evaluation

Fresh flowers were subjected to macroscopic studies which comprises of organoleptic characters of the drugs viz., color, odour, appearance, taste, smell, texture, fracture, etc in the research laboratory of Padmavathi College of Pharmacy, Dharmapuri, Tamil Nadu, India.

6.2.3. Microscopic Evaluation

The microscopic evaluation allows more detailed examination of drug and it can be used to identify the organized drugs by their known histological characters. It is mostly used for qualitative evaluation of organized crude drugs in entire and powdered forms.

Microscope, by virtue of its property to magnify, permits the minute structure under study to be enlarged and can be used to confirm the structural details of the drugs from plant origin. For the effective results, various reagents or stains can be used to distinguish cellular structure. Microscopic evaluation also covers study of the constituents by application of chemical methods to small quantities of drugs in powdered form or to histological sections of the drugs **(Kokate, C.K., 2010).**

- Transverse sections of flowers of ***Nymphaea pubescens*** **Willdenow** and ***Nymphaea nouchali*** **Burmann. F.** was taken and photomicrography was done after proper mounting and staining in **Institute of Herbal Botany,** Plant Anatomy Research Centre (PARC) West Tambaram, Chennai - 45., under the guidance of Botanist and Director **Prof. Dr. P. Jayaraman.**

The aim and objective of taxonomy is to discover the similarities and differences in the plants, including their closed relationship with their descents from common ancestry. It is a scientific way of naming, describing and arranging the plant in an orderly manner.

A. **Methodology**

The collected different flowers were fixed in Formalin Acetic Acid **(FAA)** for 24 hrs; the specimens were dehydrated with graded series of Tertiary Butyl-Alcohol **(TBA)**. Infiltrations of the specimens were carried out by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained supersaturation. The specimens were cast into paraffin blocks **(Sass, J.E. 1940).**

B. Sectioning

The paraffin embedded specimens were sectioned off with the help of **Rotary Microtome**. The thickness of the section was 10-12 μm. After Dewaxing, the sections were stained with **Toluidine blue (O'Brien, T.P, 1964).**

C. Photomicrographs

Photographs of different magnifications were taken for the study of **crystals, starch grains** and **lignified cells**; polarized light was employed **(Easu, K., 1964).**

6.2.4. Powder Microscopy

Powder microscopy of the flowers was done in Padmavathi College of Pharmacy, Dharmapuri. Powder is cleared with the clearing agent chloral hydrate, and then the powder is stained with various staining reagents like, Safronin (1 %) + Glycerin, Phloroglucinol + Con. HCl (1:1) Solution test, Sudan Red III, Iodine solution and was observed for fibres, pitted vessels, epidermal cells, starch grains, calcium oxalate crystals, stone cell etc. under microscope **(Khandelwal K.R, 2006).**

6.3. Analytical Study

a) Organoleptic evaluation

It refers to the evaluation of drugs by colour, odour, taste etc. It is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs. Organoleptic evaluation means conclusion drawn from studies resulted due to impressions on organs of senses **(Kokate, C.K., 2010).**

b) Physical evaluation

Physical standards are to be determined for drugs, wherever possible. These are rarely constant for crude drugs, but may help in evaluation, specifically with reference to moisture content, ash values, extractive values, foreign matter, pH, loss on drying, heavy metal analysis, foaming index, fluorescence analysis and solubility in different solvents **(Kokate, C.K., 2010).**

1). Moisture content (Loss on drying)

The percentage of active chemical constituents in crude drugs is mentioned on air dried basis. Hence, the moisture content of a drug should be determined, controlled and minimized in order to prevent decomposition of crude drugs either due to chemical change or microbial contamination **(Kokate, C.K., 2010).**

Method: Accurately weighed 10g of the coarsely powdered drug was taken in a dried, weighed porcelain dish. It was kept in hot air oven at 105°C for five hours after which it was taken out, cooled in a desiccator and weighed. Drug was weighed at each one hour interval and the drying was continued till constant weight was obtained. Percentage of moisture content (loss on drying) with reference to the air dried drug was calculated **(Anonymous, 2010).**

2). Ash values

Evaluation of drug basically needs its identification and can be done by morphological or microscopic characters. Many a time, even if the drug is identified, it is of substandard quality due to either faulty collection or incorrect storage. Thus to prove its acceptability as a drug, ash values and extractives should be determined. Total ash of the drug is inclusive of **"physiological ash"** as well as **"non-physiological ash"**. Physiological ash is derived from the plant tissues, while non-physiological ash consists of residues of the extraneous matter (such as sand, soil etc) adhering to the herb itself.

Many a time, the crude drugs are administered with various mineral substances like sand, soil, calcium oxalate, chalk powder or other drugs with different inorganic contents. For determining ash, the powdered drug is incinerated so as to burn out all the organic matters. Ash value is a criterion to judge the identity or purity of crude drugs. Total ash usually consists of carbonates, oxides, phosphates, silicates and silica **(Kokate, C.K., 2010).**

i). Total ash value (Anonymous, 2010, Khandelwal K.R, 2006)

Two grams of accurately weighed sample was taken and transferred to the cleaned, dried and weighed silica crucible and was subjected to ignition using electric furnace at 450°C for an hour. Silica crucible was taken out from the furnace and was allowed to cool, and weighed.

After cooling the weight of the ash obtained was calculated with reference to the air dried drug.

Calculation:

Weight of empty dish = x gm

Weight of the drug taken = y gm

Weight of dish + ash (after complete incineration) = z gm

Weight of the ash = (z-x) gm

Y gm of drug gives (z-x) gm of ash

Therefore, 100gm of crude gives $\frac{100\ (z-x)}{Y}$ gm of ash.

Total ash value of the sample = 100(z-x)/ y %

ii). Acid insoluble ash

Total ash obtained was digested with 25 ml dil hydrochloric acid for 5 min, then filtered through Whatman paper and was washed with warm water. The residue and the filter paper was taken in a crucible and heated gently until vapours cease & then more strongly until all carbon has been removed, then it was cooled, the residue was weighed and the percentage of acid insoluble ash was calculated with reference to air dried drug **(Anonymous, 2010).**

iii). Sulphated ash

About 3 gm of accurately weighed air dried powdered drug was taken in a tarred silica crucible, which was previously ignited and weighed. Then ignite gently at first until the drug was thoroughly charred. The crucible was cooled and residue was moistened with 1ml of concentrated sulphuric acid, heated gently until the white fumes were no longer evolved and ignited at $800^0C \pm 25^0C$ until all the black particles has disappeared. The crucible was allowed to cool, few drops of sulphuric acid was added and again heated. The ignition was carried out as before, allowed cooling and weighed to get a constant weight (difference not more than 0.5gm between two consecutive readings). The percentage of sulphated ash was calculated with reference to the air-dried drug.

iv). Water soluble ash

Boil the ash for 5 minutes with 25ml of water; collect the insoluble matter in an ash less filter paper; wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450°C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in the weight represents the water soluble ash. Calculate the percentage of water soluble ash with reference to the air dried drug **(Anonymous, 2010).**

3). Extractive values

The extracts obtained by exhausting crude drugs are indicative of approximate measures of their chemical constituents. Taking into consideration the diversity in chemical nature and properties of contents of drugs, various solvents are used for determination of extractive. The solvent used for extraction is in a position to dissolve appreciable quantities of substances desired **(Kokate, C.K., 2010)**.

- ♣ Extractive values of aqueous and alcohol were estimated.

25ml of the extract was transferred to a silica crucible and evaporated to dryness on a water bath and the drying was completed in an oven at 100°C for about 10-15 minutes. It was later cooled in a desiccator and weighed. The extractive value was calculated with reference to the air dried drug **(Anonymous, 2010).**

4). Foreign Organic Matter

The parts of the organ or organs other than those named in the definition and description of the drug are defined as foreign organic matter. The maximum limit for the foreign organic matter is defined in the monograph of crude drugs. If it exceeds the limits, deterioration in quality of the drug takes place. The limit for foreign organic matter is specially mentioned for natural drugs of vegetable origin in their respective monograph.

Method:

- ♣ 100 gm of crude drugs were taken and spread into thin layer
- ♣ It was examined for the presence of foreign matter like mud, leaves etc. with the help of hand lens
- ♣ The foreign matters were separated and the drugs were weighed again
- ♣ Percentage of foreign organic matter was calculated **(Kokate, C.K., 2010)**

5). Determination of pH value

The pH value of a liquid is determined by means of a glass electrode and a pH meter. Suitable glass electrode and pH meter of both potentiometer and deflection type are available. The pH meter is an electronic digital voltmeter, scaled to read pH directly, and may range from a comparatively simple hand held instrument, to more elaborate bench models, often provided with a scale expansion facility, with a resolution of 0.001 pH unit and an accuracy of + 0.001 unit.

Method:

- ♣ Standardisation of pH meter
- ♣ Take tablets/packets of different pH and dissolve one tablet/packet in 100 ml of distilled water to prepare solutions of different pH 4, 7, and 9 (buffer solutions), or as directed in the label
- ♣ Switch on the instrument
- ♣ Leave it for some time unless or on the board requirement of different pH solution appears

- ♣ Take the buffer solution in the beaker and dip the electrode in it.
- ♣ Carry out the same exercise for another buffer solution also, after washing the electrode thoroughly with distilled water.
- ♣ Take the test sample (for solid preparations 10% aqueous solution) and dip the electrode in it.
- ♣ Note the pH **(Lavekar, G.S., 2007).**

6). Determination of Heavy Metals

The analysis for heavy metals like arsenic, chromium, cobalt, lead, mercury and nickel for crude powder and ethylacetate and hydroalcoholic extracts of the flowers was done at Choksi Laboratries Limited, Vadodara.

7). Solubility

The quantitative solubility test for ethylacetate and hydroalcoholic extracts of the flowers was determined in different solvents. 50 mg of each extract was weighed for solubility test for different solvents. The extract was added in each solvent until saturated solution developed. Solubility was calculated in mg/ml.

8). Determination of Microorganisms

Total bacterial and total fungal counts as well as specific count for *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* for crude powder and ethylacetate and hydroalcoholic extracts of *both the flowers* was carried out using reported methods **(The Ayurvedic Pharmacopoeia of India, 2010).**

9). Foaming Index

Many medicinal plant materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. In order to measure the foaming ability of an aqueous decoction of plant material and their extracts, a Foaming Index is established.

Method: Weigh accurately about 1 gm of coarsely powdered flowers and are transferred to 500 mL conical flask containing 100 mL of boiling water. Maintained at moderate boiling at 80-90°C for about 30 minutes, cooled and filtered into a volumetric flask and added sufficient water through the filter to make up the volume to 100 mL (V_1). Cleaned 10-stoppered test tube of uniform dimensions were taken and marked from 1 to 10. Measured and transferred the successive portions of 1, 2, 3 mL upto 10 mL and adjusted the volume of the liquid in each tube with water to

10mL. Stoppered the tubes and shaken them in a lengthwise motion for 15 seconds uniformly and allowed to stand for 15 minutes and measure the height. If the height of the foam in every tube is less than 1cm, the Foaming Index is less than 100 (not significant).

Here, the foam was more than 1cm height after the dilution of plant material in the seventh tube. The corresponding number of the test tube was the index sought, if the height of the foam in every tube is more than 1cm, the Foaming Index is more than 1000. In this case, 10 mL of the first decoction of the plant material is measured and transferred to a 100 mL volumetric flask (V_2) and volume is made to 100 mL and followed the same procedure.

Foaming Index = 1000/a in case of V_1

Foaming Index = 1000 × 10/a in case of V_2

where, a = volume (mL) of decoction used for preparing the dilution in the tube where exactly 1 cm or more foam was observed. The Foaming Index was calculated by using this formula **(Pulok K. Mukherjee, 2008).**

10). Fluorescence Analysis (Kokoshi, J., 1958)

In the near ultraviolet region of the spectrum (150-300 nm) some of the phytoconstituents show more or less brilliant colouration when exposed to radiation. This phenomenon of emitting visible wavelengths as a result of being excited by radiation of a different wavelength is known as fluorescence. Sometimes the amount of ultra-violet light normally present with visible light is sufficient to produce the fluorescence, but more often a more powerful source of ultra-violet light is necessary, e.g a mercury vapour lamp. It is often possible to make use of this phenomenon for the qualitative examination of herbal drugs. Fluorescence analysis of the powdered flower materials were observed in daylight and UV light (254 nm & 366 nm). Also the fluorescent study was performed on treating the drug powder with different chemical reagents according to **(Chase and Pratt, 1949 & Kokoshi *et al.*, 1958).**

6.4. RESULTS AND DISCUSSION

Plants have been used as medicine for millennia; out of the estimated 2, 50, 000 to 3, 50, 000 plant species identified so far, about 35,000 are used worldwide for medicinal purposes. It has been confirmed by WHO that the herbal medicines serve the health needs of about 80 percent of the world's population; especially for millions of people in the vast rural areas of developing countries. Meanwhile, consumers in developed countries are becoming disillusioned with modern health care and are seeking alternatives.

The recent resurgence of plant medicines results from several factors;

- ♣ Effectiveness of plant medicines
- ♣ Side effect of most modern drugs and
- ♣ Development of science and technology

It has been estimated that in the mid- 1990'sover 200 companies and research organizations worldwide were screening plant and animal compounds for their medicinal properties. Actually, several important drugs used in modern medicine have come from medicinal plant studies, eg. Taxol/paclitaxel, vinblastine, vincristine, topotecan, irinotecan, etoposide, teniposide etc. As for drugs derived from orchids, some novel discoveries, both in phytochemical and pharmacological properties, were reported by some universities. However, studies on plants are very limited.

Only about one third of the million or so species of higher plants have been identified and named by scientists. Of those named, only a tiny fraction has been studied. Nowadays, the linking of the indigenous knowledge of medicinal plants to modern research activities provides a new approach, which makes the rate of discovery of drugs much more effective than with random collection **(Kong, *et al.*, 2003).** Hence, in the present study, an attempt has been made in the standardization of two aquatic plants bearing flowers with same genus but different species. The Pharmacognostical, phytochemical and pharmacological investigations were carried out.

6.4.1. PHARMACOGNOSTICAL STUDIES

Plant - A *Nymphaea pubescens* Willdenow (Nymphaeaceae)

Botanical name : *Nymphaea pubescens* Willd.

Family : *Nymphaeaceae* (Waterlily family)

Vernacular Name : **Tamil name - வெள்ளாம்பல்** Vellambel

Common Name : Pink Lotus, Pink Water Lilly

Parts Used : Flowers, Roots, Leaves, Stem

Synonym : ***Nymphaea lotus var. pubescens*** (Willdenow) *Hook.f. & Thomson* ***Nymphaea spontanea*** *K. C. Landon, nom. inval.* ***Nymphaea pubescens*** (Willdenow)

Distribution / Habitat : Throughout warmer parts of India, in tanks, ponds & ditches

6.4.1.1. Macroscopical characteristics

The Macroscopical characters of the flower of ***Nymphaea pubescens*** **Willdenow** is as follows:

Color	-	White to pink
Odour	-	Pungent
Taste	-	Tasteless
Texture	-	Smooth
Flowers	-	Emergent, (2–) 5–8(–15) cm in diameter
Calyx	-	Insertion on receptacle circular
Sepals	-	Oblong, 5–8 cm, conspicuously veined, caducous or decaying after anthesis
Petals	-	12–14(–30), white, red, or pink, oblong, 5–9 cm, transition to stamens abrupt. Filament of inner stamens only slightly wider than anther; connective apically unappendaged
Carpels	-	Completely united walls between locules of ovary single

Stigma	-	Rays 12–15(–30); carpellary appendages linear
Fruit	-	Ovoid to subglobose, 3.5–5 cm in diam
Seeds	-	Ellipsoid to globose, 1–2 mm, with longitudinal ridges
Flowering	-	Aug–Oct. 2*n* = 84
Uses: Flowers	-	Astringent & Cardiotonic **(Shui lian shu, 2001, Bhakta B. *et al.*, 2011).**

Fig. No: 11 Exomorphic View of *Nymphaea pubescens* Willdenow

Fig. No: 12 Exomorphic Features of the Flower *Nymphaea pubescens* Willdenow

A). Entire Flower Front View Petal

a

B). Sepal Inner and Outer View, and Stamen

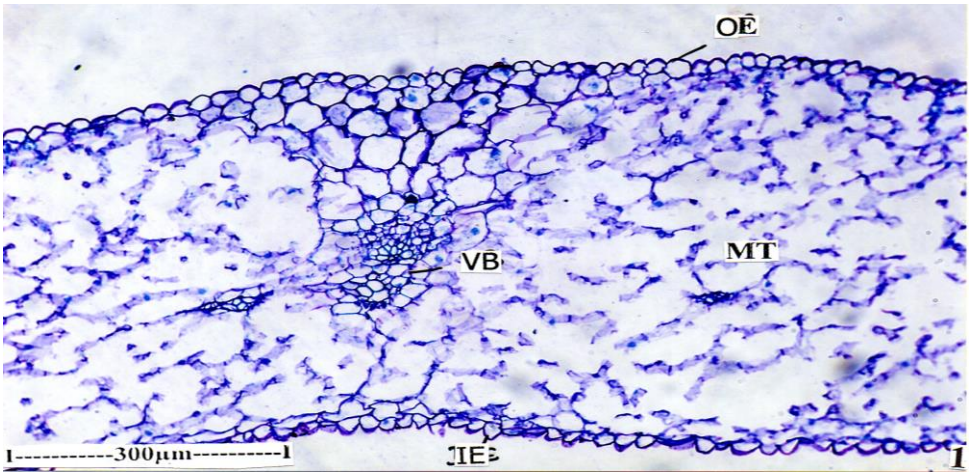
b

6.4.1.2. Microscopical characteristics

A. Anatomy of Sepal

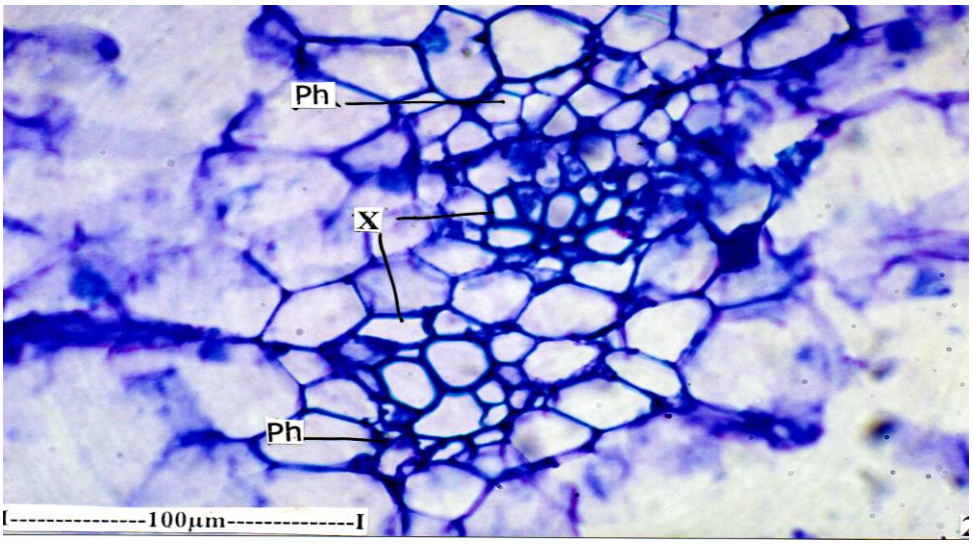
Sepal: The median part of the sepal is 550 µm thick; spindle shaped outer epidermal layers of cells, which are 20 µm thick, inner epidermal cells are papillate with thin walls; they are also 20 µm thick. The mesophyll tissue consists of 10 layers, thin walled parenchyma cells, which are not well preserved due to delicate walls **(Fig. No: 13, 14).**

Fig. No: 13 T.S. of Sepal Through Midrib Region



[**IE**-Inner Epidermis, **MT**-Mesophyll Tissue, **VB**-Vascular Bundle, **OE**-Outer Epidermis]

Fig. No: 14 T.S. of Midrib of Vascular Bundle Enlarged

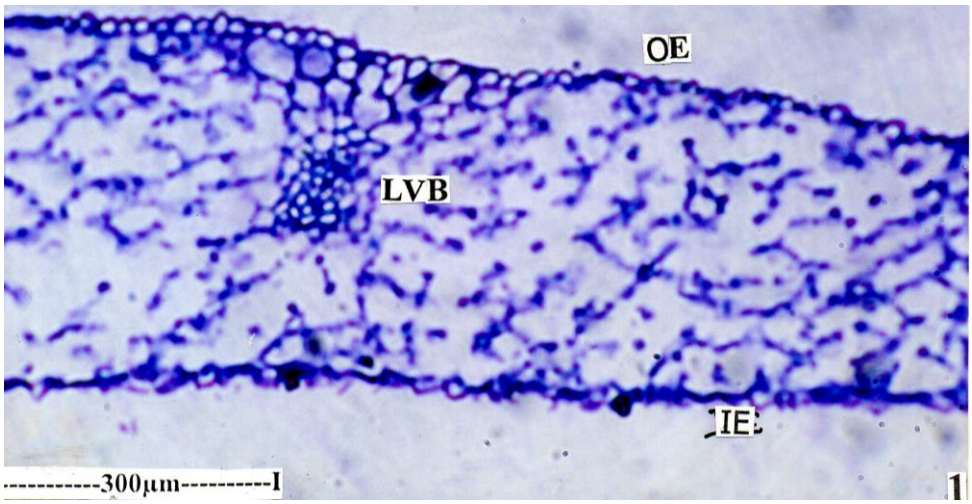


[**Ph**-Phloem, **X**-Xylem]

Anatomy of the Sepal Lateral Part and Marginal Part

A small vascular strand is seen in the middle part of the sepal; and has two clusters of xylem elements and a few phloem elements; xylem elements are wide angular, thin walled and measure 20 µm in diameter. The lateral part of the sepal is 350 µm thick; has spindle shaped outer epidermal cells and papillate inner epidermal cells; small collateral vascular strand is placed in the median position of the sepal **(Fig. No: 15).**

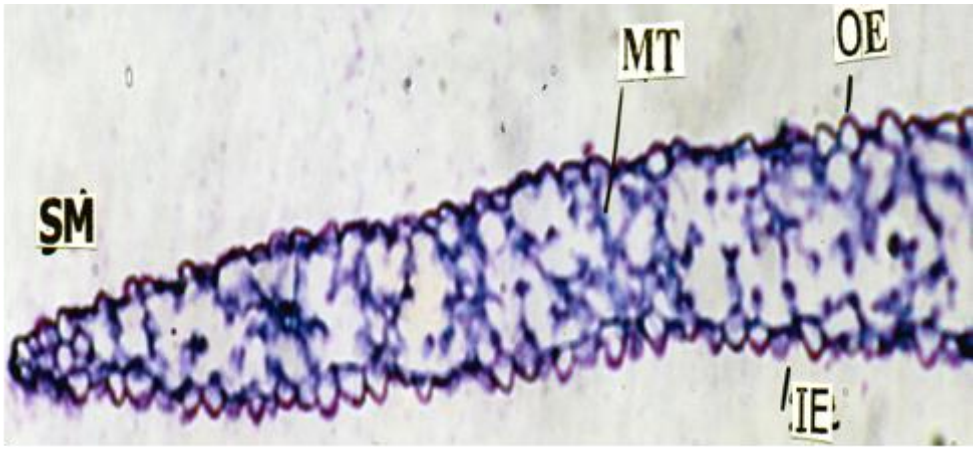
Fig. No: 15 T.S. of Sepal Lateral Part



[**LVB**-Lateral vascular Bundle, **OE**-Outer Epidermis, **IE**-Inner Epidermis]

The marginal part of the sepal is gradually narrow and conical; and has more prominent abaxial papillate epidermal cells and a few layers of mesophyll tissues; no vascular strand is seen in the marginal part **(Fig. No: 16).**

Fig. No: 16 T.S. of Sepal Marginal Part

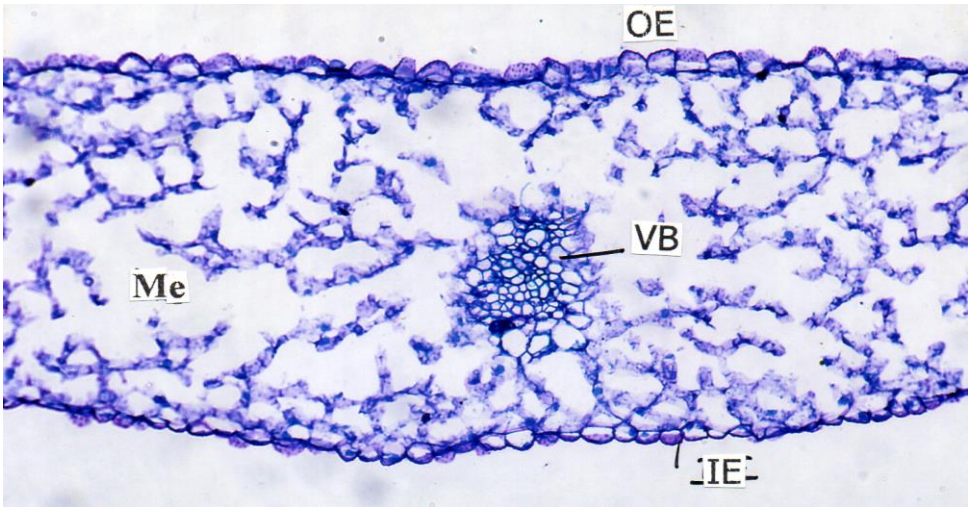


[**SM**-Sepal Margin portion, **MT**-Mesophyll Tissue, **OE**-Outer Epidermis, **IE**-Inner Epidermis]

B. Anatomy of the Petal

Petal: The midrib part of the petal is 400 µm thick; has papillate or semicircular epidermal cells on both surfaces; epidermal cells are 15 µm wide; mesophyll tissue is undifferentiated and about 8-10 layers of thin walled, fragile parenchyma cells **(Fig. No: 17.1)**.

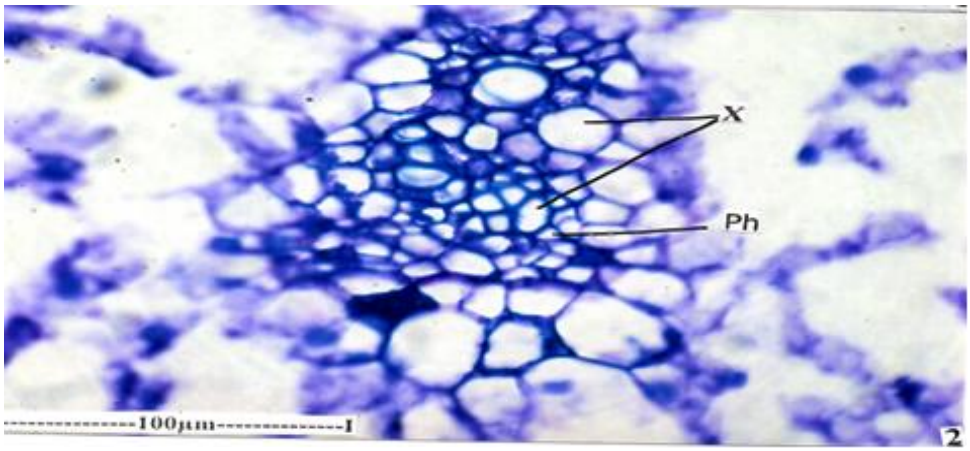
Fig. No: 17.1 T.S. of Petal through Midrib



[Me-Mesophyll tissue, **OE**-Outer Epidermis, **IE**-Inner Epidermis, **VB**-Vascular Bundle**]**

The vascular strands are placed in middle part of the ground tissue; has wide, thin walled angular xylem elements; widest xylem elements are 50-70 µm in diameter; phloem elements are seen on one side of the vascular strand **(Fig. No: 17.2).**

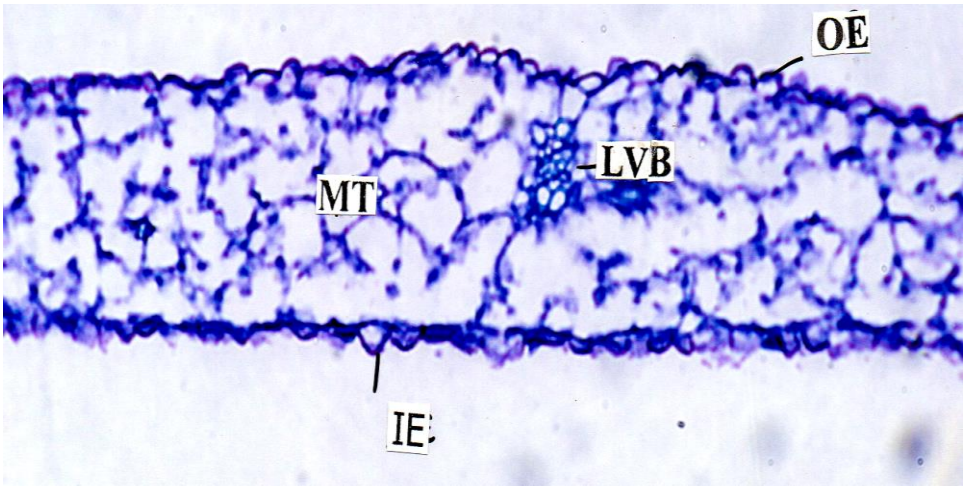
Fig. No: 17.2 T.S. of Vascular Bundle Enlarged



[Ph-Phloem, **X**-Xylem**]**

The lateral part of the petal is 200 µm thick; and has thin layer of papillate cells; palisade tissue has four layers of thin walled compact cells. The vascular bundle is small, circular and centrally placed; consists of four or five xylem elements and a few, less prominent phloem elements **(Fig. No: 18.1).**

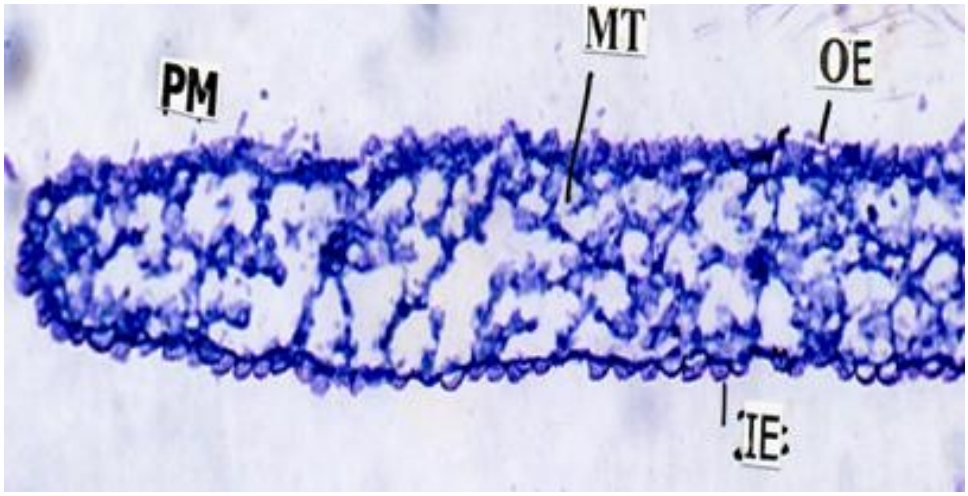
Fig. No: 18.1 T.S. of Petal through Lateral Part



[MT-Mesophyll Tissue, **OE**-Outer Epidermis, **IE**-Inner Epidermis, **LVB**-Lateral Vascular Bundle]

The petal margin has semicircular blunt end and prominent papillate epidermal cells; ground tissue is two or three layered and not well preserved, no vascular strand is evident **(Fig. No: 18.2).**

Fig. No: 18.2 T.S. of Petal through Marginal Part

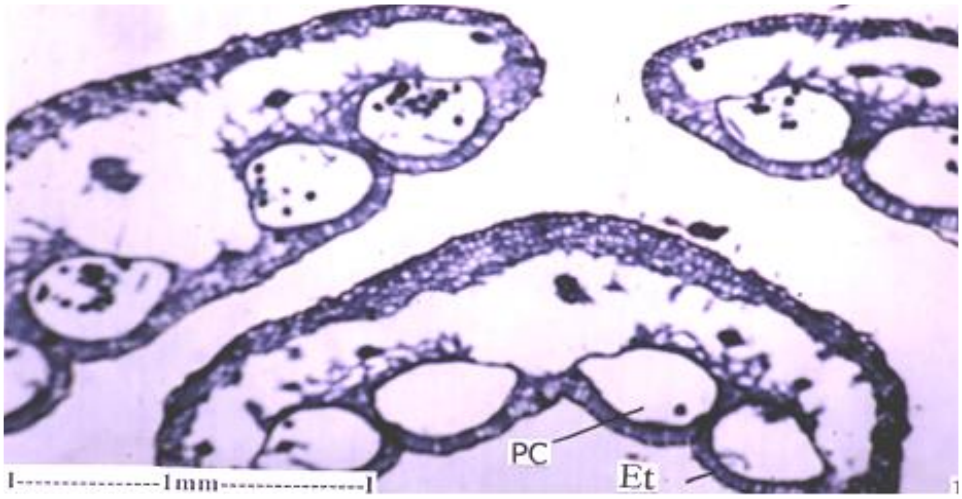


[MT-Mesophyll Tissue, **OE**-Outer Epidermis, **IE**-Inner Epidermis, **PM**-Petal Margin Portion]

C. Anatomy of the Anther (anther wall enlarged)

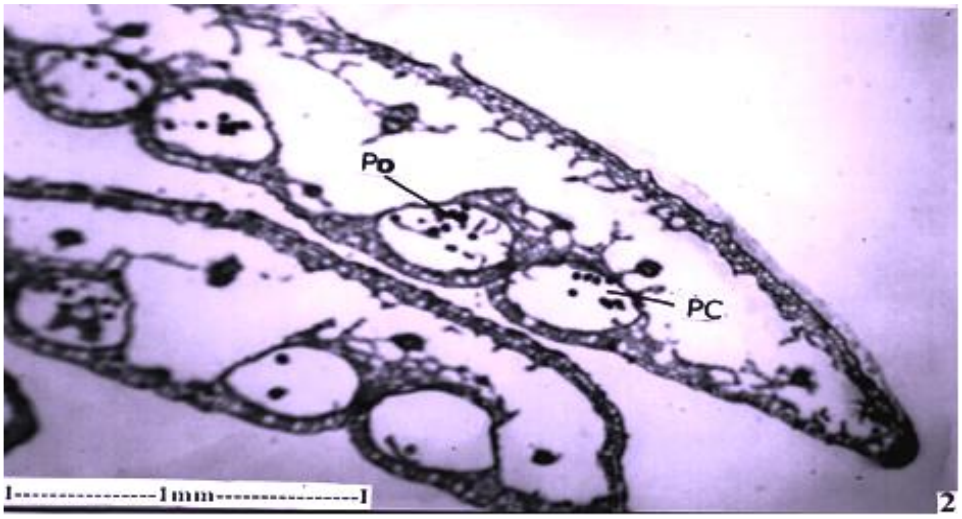
Stamens: The anther is flat, slightly curved and dorsiventral; it contains four chambers; each lobe is separated by a wide median part **(Fig. No: 19.1);** marginal portion of the anther is extending into conical wings on either side **(Fig. No: 19.2).**

Fig. No: 19.1 T.S. of Anther through Middle Portion



[**PC**-Pollen Chamber, **Et**-Endothecium]

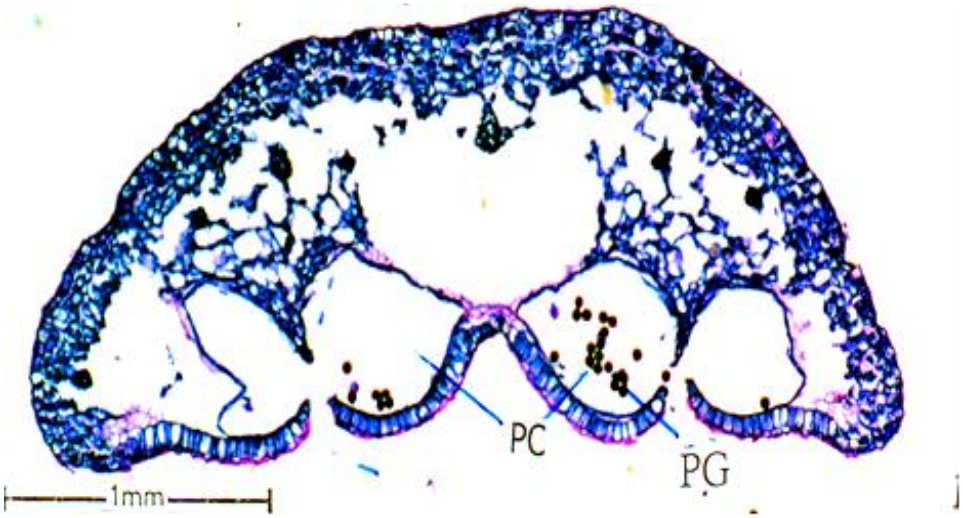
Fig. No: 19. 2 T.S. of Anther through Terminal Part



[**PC**-Pollen Chamber, **Et**-Endothecium, **Po**-Pollen]

Anther Wall enlarged: The anther wall consists of large spindle shaped epidermal cells; outer walls of the epidermal cells have echinate cuticle **(Fig. No: 20.1)**; inner wall is rectangular and wide with smooth walls.

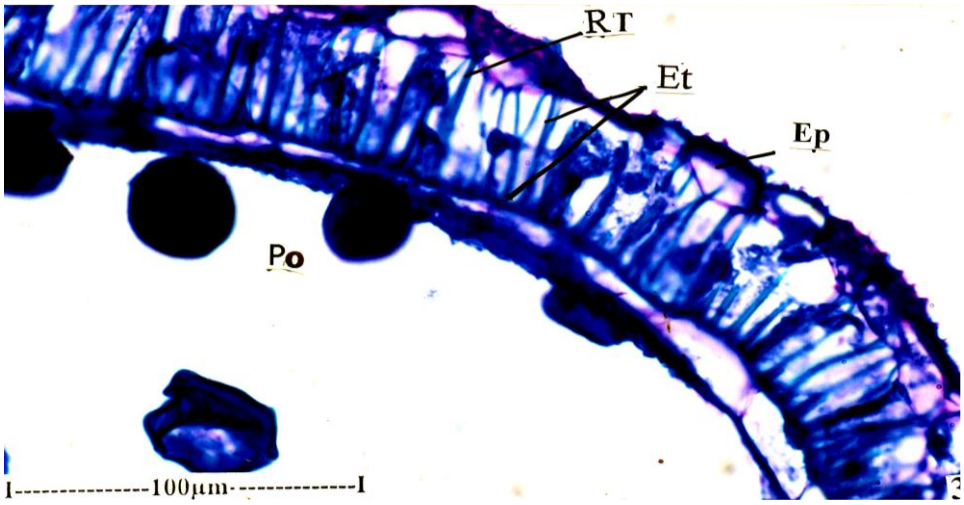
Fig. No: 20.1 T.S. of Anther



[**PC**-Pollen Chamber, **PG**-Pollen Grains]

In between the outer and inner epidermis is a wide layer of endothecium, where the cells are radially oblong and they have spirals of thickenings; anther wall is 70 µm thick; the endothecium is 40 µm thick **(Fig. No: 20.2)**.

Fig. No: 20.2 T.S. of Anther Wall Enlarged



[**RT**-Reticulate Thickening, **Et**-Endothecium, **Ep**-Epidermis, **Po**-Pollen]

D. Morphology of the Seed

Seeds: Both entire and broken seeds are seen in the powder; seeds are elliptical ovate and are 700 µm long and 450 µm wide; surface of the seed coat has vertical rows of small squarish markings. The funicle is cylindrical and is attached to the seed by a narrow short stalk. The microphlar end of the seed is shortly beaked **(Fig. No: 21.a)**.

Fruit: The fruit is multi-chambered being with thick parenchymatous pericarp. It is 80 µm thick. The pericarp has thin, less distinct epidermis and several layers of small circular compact parenchyma cells, forming the mesocarp. The inner part of the pericarp has disintegrated soft tissues.

Fig. No: 21 a). Seed Enlarged

[**Fu**-Funicle, **S**-Seed]

Fig. No: 21 b). Fruit Multi-Chambered Pericarp

Along the end of the parenchymatous mesocarp occur vascular strands. The vascular strands are two, which are juxtaposed. The outer bundle has a row of outer xylem and a thick bank of phloem on the inner side. The inner vascular bundle is reversed in having outer xylem and inner phloem **(Fig. No: 21.b)**

E. Anatomy of the Ovary

The seeds are seed free from the septa or still attached on the septal walls. They are elliptically with thick seed coat and inner embryo **(Fig. No: 22.1, 2, 3).**

Fig. No: 22.1 T.S. of Ovary a Sector

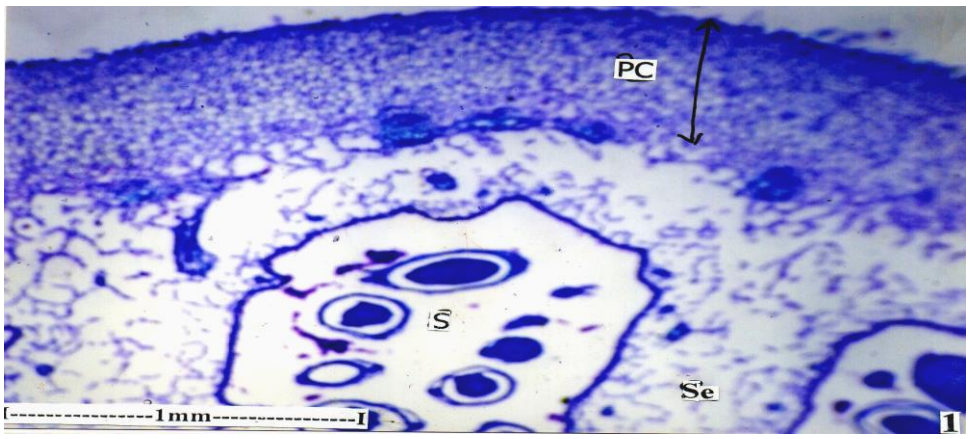


Fig. No: 22.2 T.S. of Pericarp Outer Part

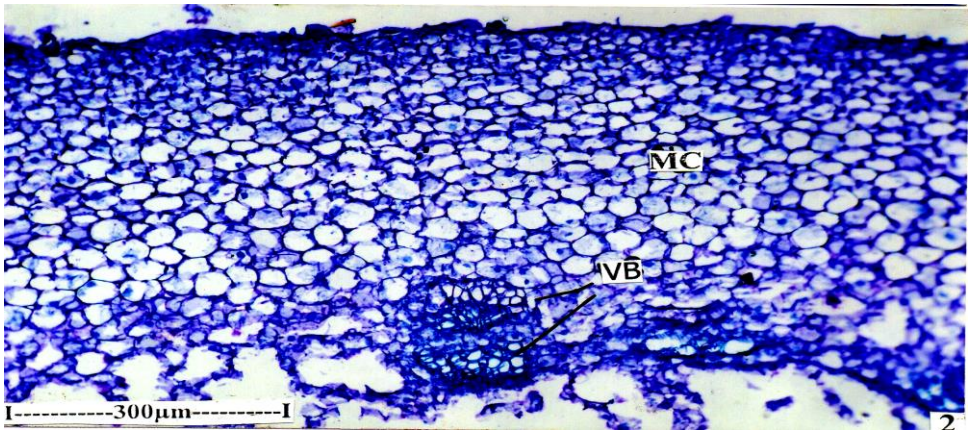
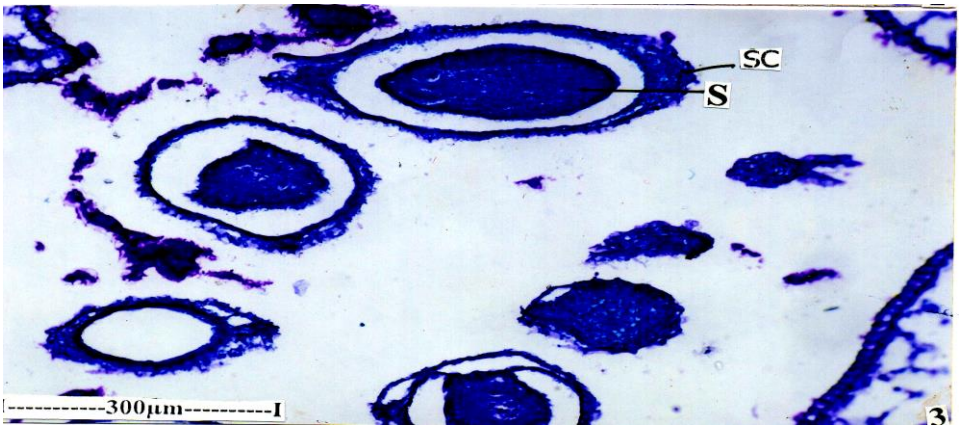


Fig. No: 22.3 T.S. of Seeds in Sectional View



[**MC**-Mesocarp, **PC**-Pericarp, **S**-Seed, **SC**-Seed Coat, **Se**-Septal]

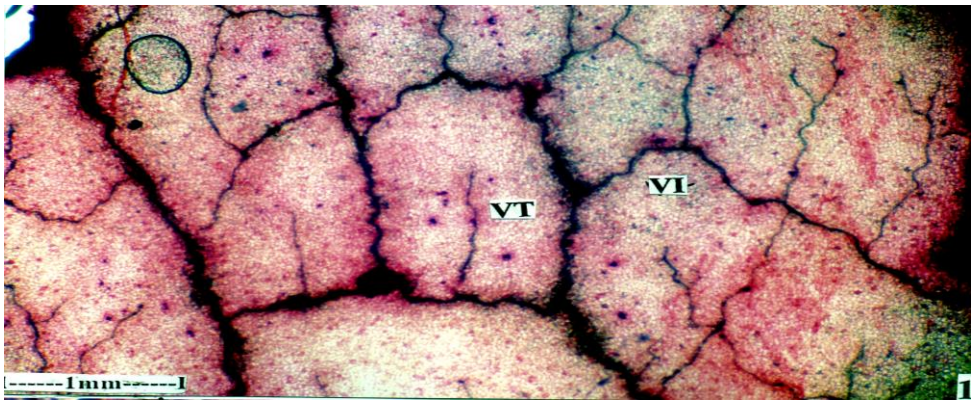
6.4.1.3. Powder characteristics

In the powder are seen fragments of sepal, petal, pollen grains, seeds and sclerenchyma cells. The following observations are made on macerated preparations of the flowers,

a. Venation of the Petal

The petal has thin lateral vein which are sinuous and wavy. They form wide rectangular vein-islets with long slender, simple or branched veins terminations **(Fig. No: 23.a).**

Fig. No: 23 a).Venation of the Petal

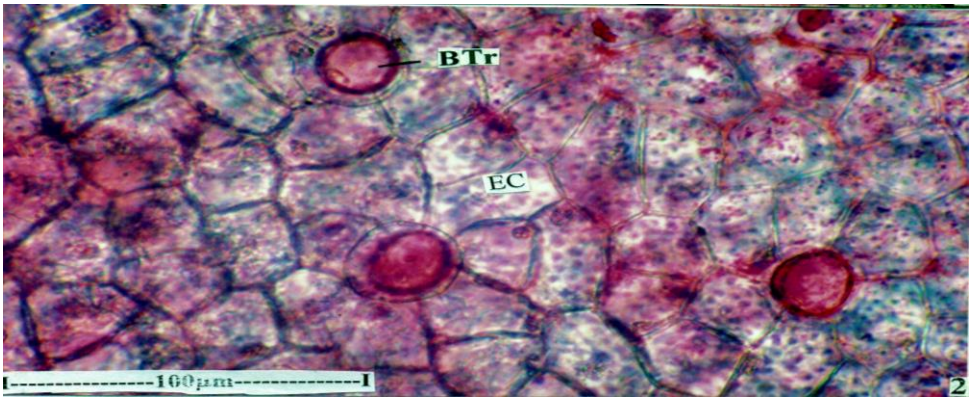


[**VI**-Vein Islets, **VT**-Vein Termination]

b. Epidermal Cells of the Petal

The lower surface of the sepal (Outer epidermis) has polygonal, thick walled cells, which are straight. These are circular darkly staining cells which are the basal cells of the epidermal trichomes**.** These circular cells are surrounded by a rosette of rectangular cells **(Fig. No: 23.b).**

Fig. No: 23 b). Epidermal Cells of the Petal

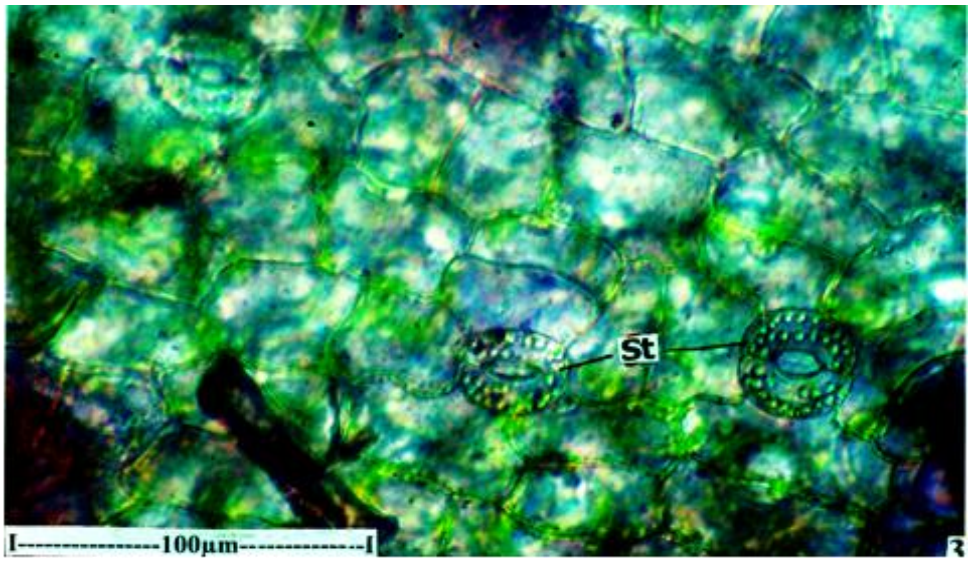


[**BTr**-Basal cell of the Trichomes, **Ec**-Epidermal cells]

c. Stomata of the Sepal

Stomata are also evident on the epidermal fragments. The guard cells are circular with wide elliptical stomatal pore. The guard cells have prominent and abundant chloroplasts in their cells **(Fig. No: 23.c)**.

Fig. No: 23 c). Stomata of the Sepal

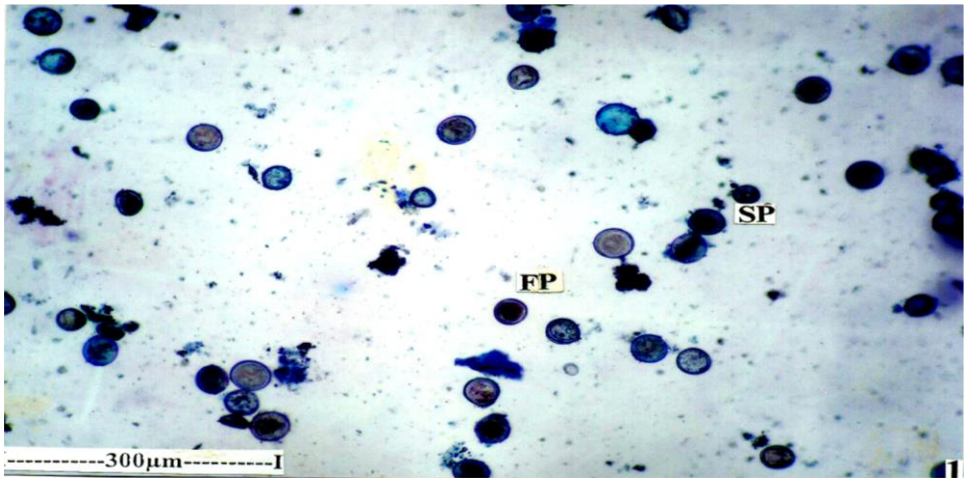


[**St**-Stomata]

d. Pollen Grains

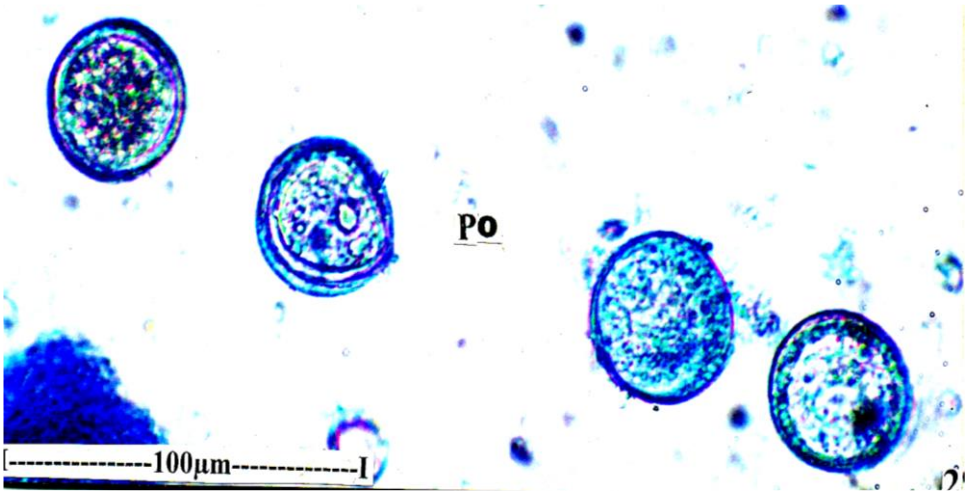
The powder contains pollen grains which include both fertile and sterile grains. The fertile larger grains measuring 40 µm in diameter the sterile grains are 20 µm in diameter **(Fig. No: 24.1)**. The pollen grains have smooth exine **(Fig. No: 24.2)**.

Fig. No: 24.1Morphology of the Pollen Grain under Low



[**SP**- Sterile Pollen, **FP**- Fertile Pollen]

Fig. No: 24. 2 Morphology of the Pollen Grain High magnification



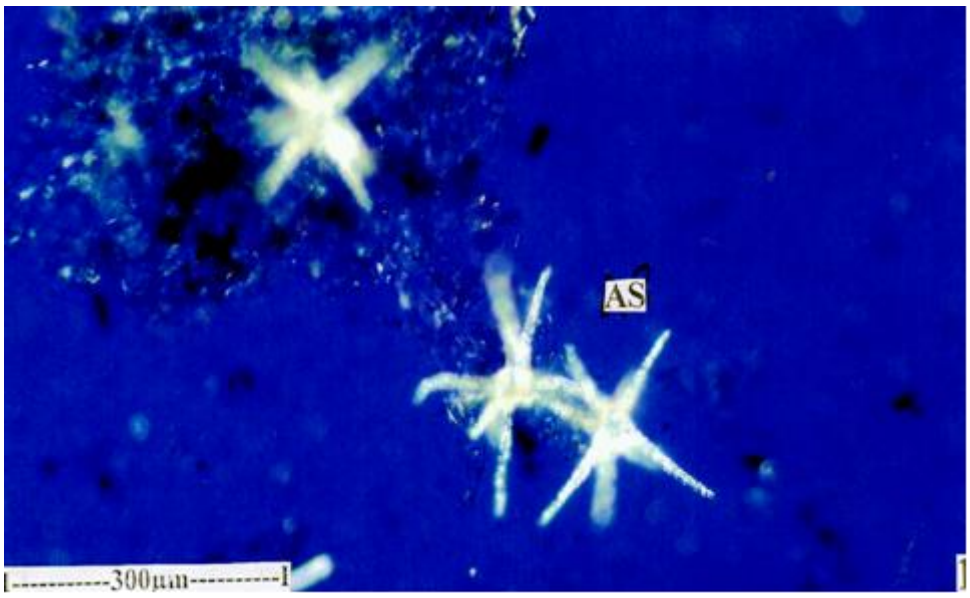
[**Po**-Pollen]

e. Sclereids

Two types of Sclereids are frequently seen in the flower powder (under polarized light microscope)-

(i) Astrosclereids: These star-shaped cells with central body and radiating arms **(Fig. No: 25.1)** they are thick walled and lignified surface has echinate or spiny out growths.

Fig. No: 25.1 Astrosclereids



[**AS**- Astrosclereids]

(ii) Acicular Sclereids: These are long, thread like cells **(Fig. No: 25.2, 3)** and also have thick lignified walls with spiny surface.

Fig. No: 25.2 Filiform Sclereids

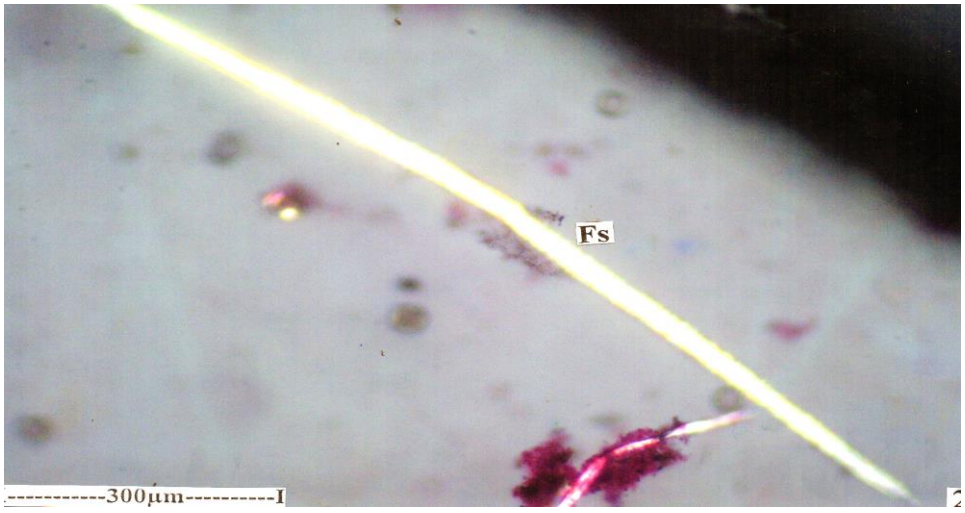
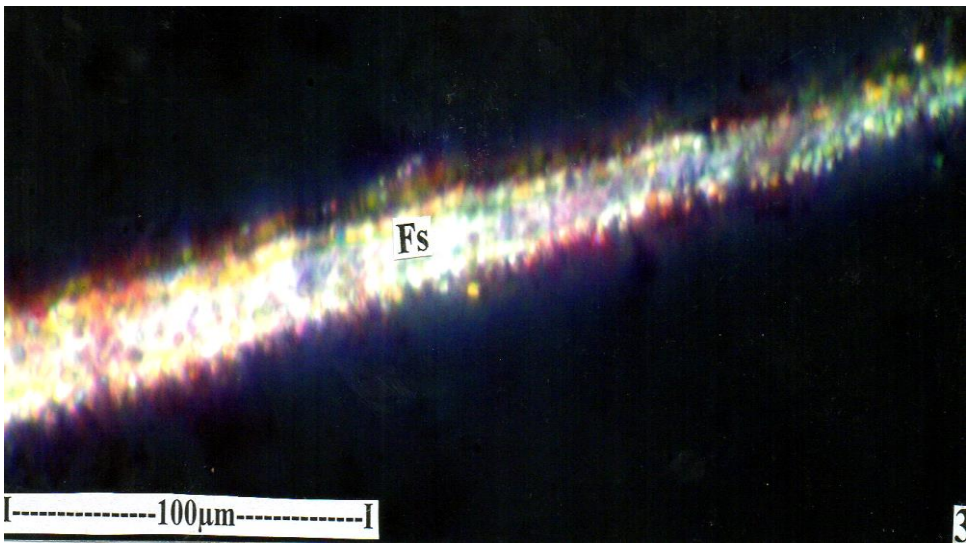


Fig. No: 25.3 Enlarged Filiform Sclereids



[**Fs**-Filiform sclereids]

6.4.1.4. Analytical study

Organoleptic evaluation

Appearance	Moderately coarse powder
Colour	Dark red to pinkish
Odour	Characteristic
Taste	Mucilagenous and bitter

Plant - B *Nymphaea nouchali* (Nymphaeaceae) Burmann. F.

Botanical name : *Nymphaea nouchali*

Family : *Nymphaeaceae* (Waterlily family)

Vernacular Name : **Tamil name - வெள்ளாம்பல்** Vellambel

Common Name : White Lotus, White Water Lilly

Parts Used : Flowers, Roots, Leaves, Stem

Synonym : ***N.lotus*** Hook., ***N.stellata*** Willdenow, ***N.rubra*** Roxb.Ex salisb **(**Vellambel, Indian Waterlily)

Distribution / Habitat : Throughout warmer parts of India, in tanks, ponds & ditches**.**

6.4.2.1. Macroscopical characteristics

Color	-	White
Odour	-	Characteristic
Taste	-	Characteristic
Flower	-	Slightly emergent, 3-15 cm in diameter
Calyx	-	Insertion on receptacle circular; sepals lanceolate to oblong- lanceolate, 2.5-8 cm, slightly veined, persistent
Petals	-	10–30, white tinged with purple, blue, or purple-red, linear-oblong to lanceolate, 4.5-5 cm, transition to stamens gradual Filament of inner stamens ± as wide as anther; connective apically appendaged
Carpels	-	Only partially united walls between locules of ovary Double
Stigma	-	Rays (8-) 10-30; carpellary appendages triangular-Tapered
Fruit	-	Globose, 1.5-4.5 cm in diameter
Seeds	-	Ellipsoid-globose, 0.5-1.3 mm, with longitudinal rows of hairs
Flowering	-	Jul–Dec. 2*n* = 28, 56, 84

Uses

Flowers - Astringent, Cardio tonic, Diabetic, Palpitation of the heart, Liver damage, Biliousness, Vomiting, Antidote in Snake Poison (Cobra bite, and in scorpion sting), Dyspepsia, Diarrhoea, Diuretic, Leprosy, Astringent, Inflammatory Diseases of Brain and Dysuria **(Shui lian shu, (2001), Bhakta, B., *et al.*, (2011).**

Fig. No: 26 a, b Habitat-Leaf and Flower of *Nymphaea nouchali* Burmann. F.

(a) (b)

Fig. No: 27 Exomorphic features of the flower *of Nymphaea nouchali* Burmann. F.

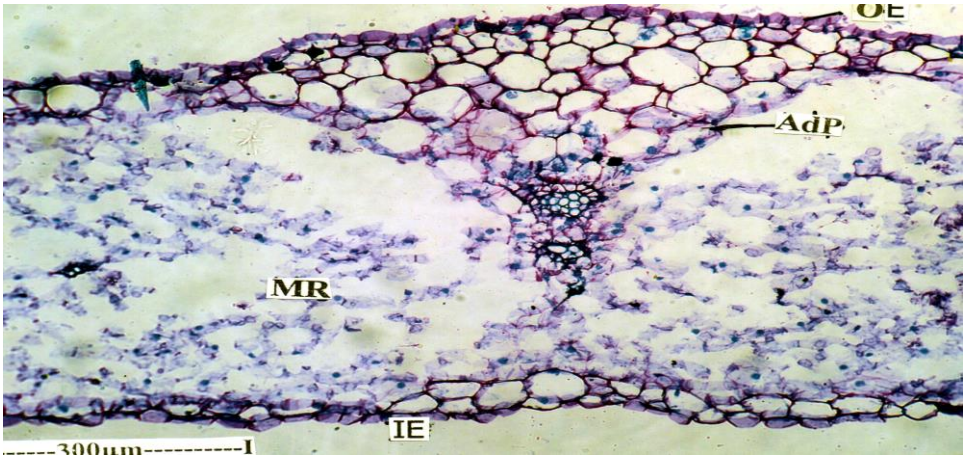
a). Entire flower front view **b). Sepal inner and outer view, petal and stamen**

6.4.2.2. Microscopic characteristics

A. Anatomy of Sepal

The sepal has smooth outer surface and slightly papillate inner surface; middle portion is 550 µm thick, the sepal becomes gradually the inner forwards the margin measuring 70 µm in thickness. It has thin epidermal layer of small spindle shaped cells with margin walls, the cells are about 15µm thick; inner epidermis has semicircular cells with slightly papillate outer tangential wall **(Fig. No: 28.1).**

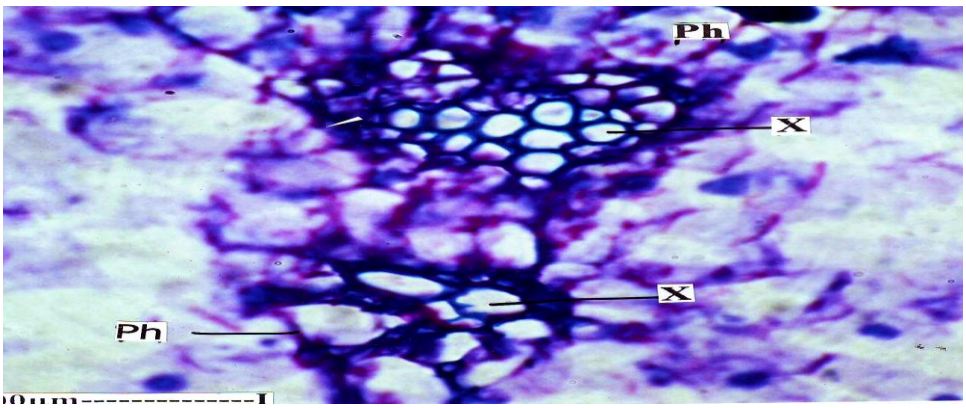
Fig. No: 28.1 T.S of Sepal along the Midrib



[**AdP**- Adaxial parenchyma cells, **IE**- Inner epidermis, **MR**- Midrib, **OE**- outer epidermis]

The vascular system of the midrib consists of a cluster of thick walled angular compact xylem elements and another small cluster of xylem elements just beneath the upper xylem mass **(Fig. No: 28.2);** xylem elements are 10 µm wide.

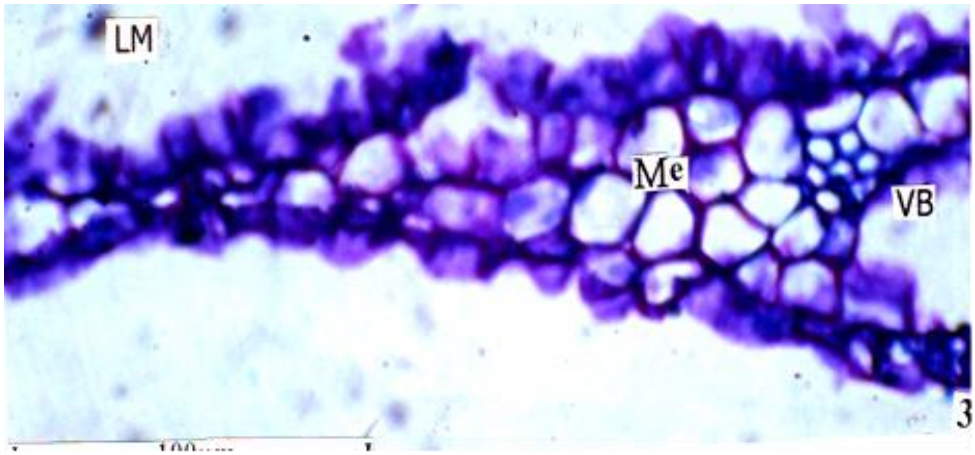
Fig. No: 28.2 Midrib of Vascular Bundle Enlarged



[**Ph**- Phloem, **X**- Xylem]

The vascular strands are supported on the upper side a conical mass of thick walled compact cells; mesophyll tissue consists of their walled delicately loosely arranged parenchyma cells. The marginal portion has larger, papillate epidermal cells and larger thick walled angular compact mesophyll cells; vascular strand in the margin is small, comprising of a circular ways of thick walled angular cells **(Fig. No: 28.3).**

Fig. No: 28.3 Marginal Portion of The Sepal Enlarged

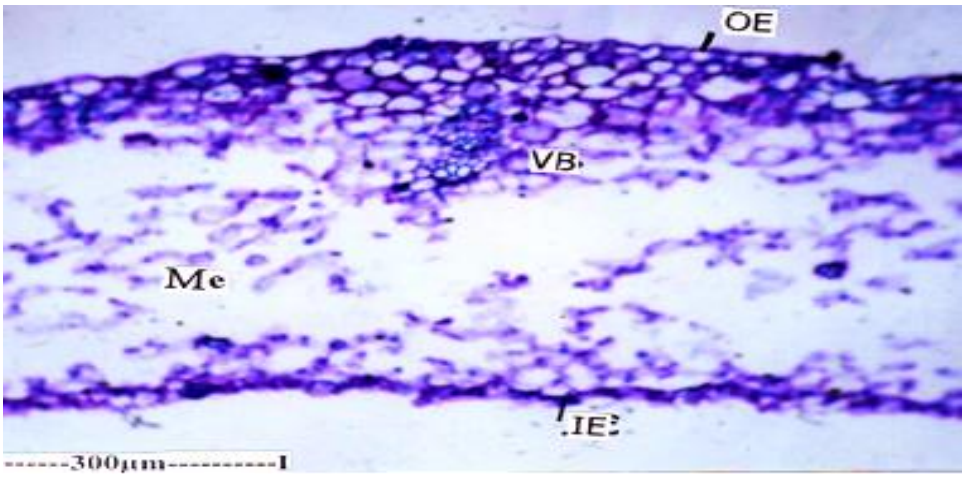


[**LM**- lateral margin, **Me**- Mesophyll tissue, **VB**- Vascular bundle]

B. Anatomy of Petal

The petal has smooth and even surface; midrib is 500 µm thick; has two segments of vascular tissues placed adaxial and abaxial position **(Fig. No: 29.1).** The vascular elements are reduced to a few narrow compact cells; the upper (adaxial) part of the midrib has about five layers of fairy thick walled compact cells offering mechanical strength to the petal.

Fig. No: 29.1 T.S of Petal through midrib

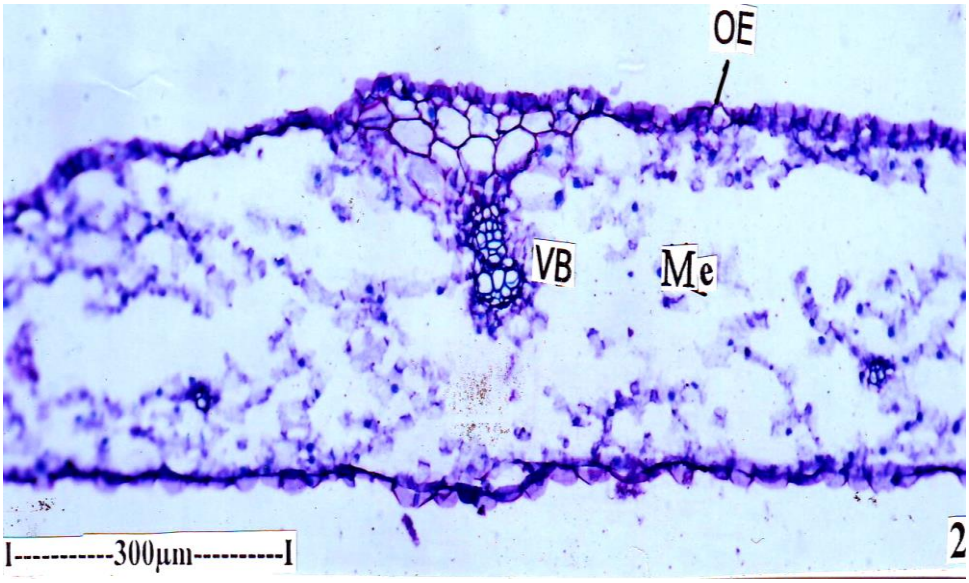


[**IE**- Inner epidermis, **Me**- Mesophyll tissue, **OE**- outer epidermis,

VB- Vascular bundle]

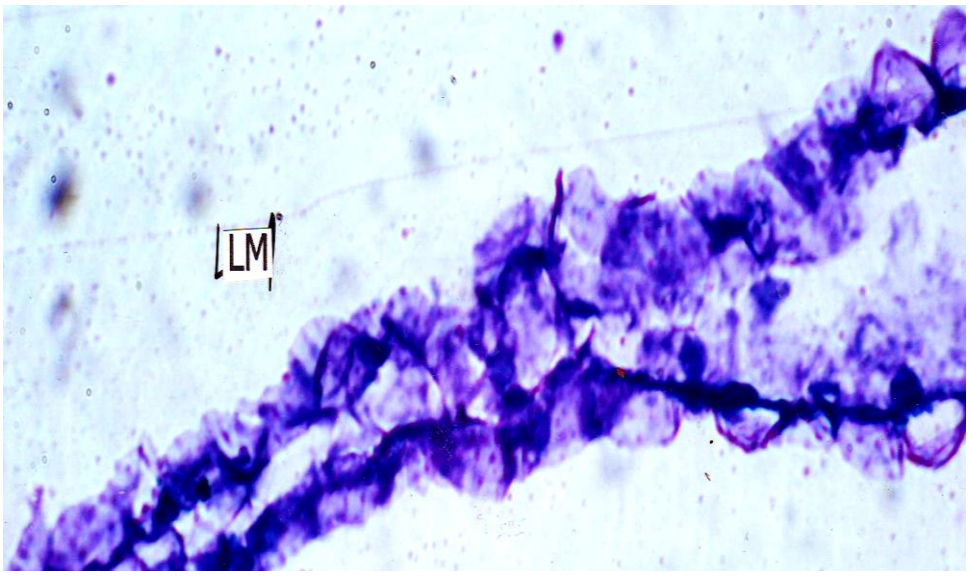
The mesophyll tissue has spongy cells with their delicate walls; lateral view has a small cluster of narrow, thick walled xylem elements and two layers of adaxial thick walked supporting cells **(Fig. No: 29.2).** The marginal portion of the petal has a central row of larger cells and hemispherical epidermal layers of cells **(Fig. No: 29.3).**

Fig. No: 29.2 T.S of Petal lateral view



[**Me**- Mesophyll tissue, **OE**- outer epidermis, **VB**- Vascular bundle]

Fig. No: 29.3 T.S of Marginal Portion of Petal

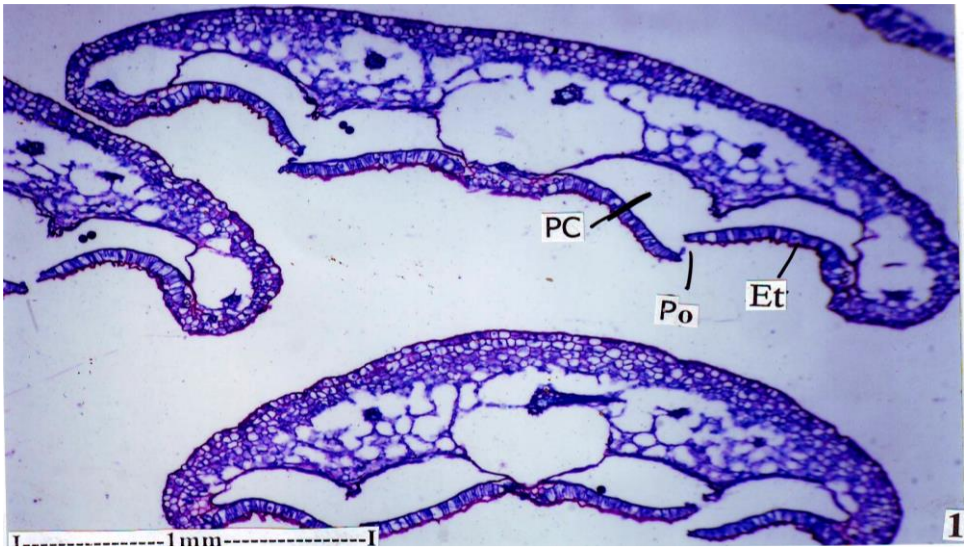


[**LM**- Lateral Margin]

C. Anatomy of Anther

The anther is flat and dorsiventral and has two pairs of longitudinal chambers on either side. The two pair of chambers is separated by a wide central gap. The pollen chambers are flat and wide **(Fig. No: 30.1).**

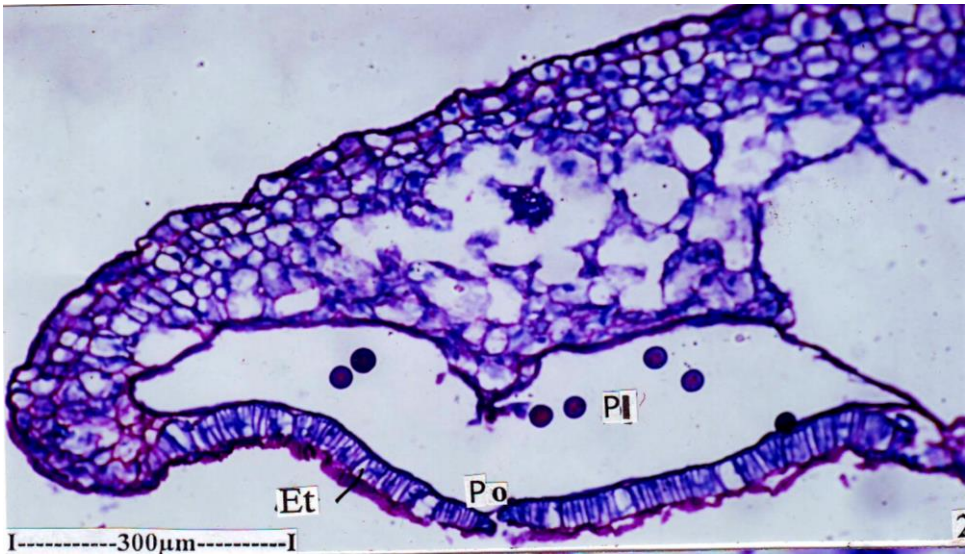
Fig. No: 30.1 T.S of Anther



[**Et** - Endothecium, **PC**- Pollen chambers, **Po**- Pollen chamber opening]

The outer part of the anther has distinct epidermis, four or five layers of small compact parenchyma cells and inner portion of aerenchyma with reticulate filaments of cells **(Fig. No: 30.2, 30.3).**The wall layer of the pollen chamber consists of an epidermal layer and thick endothecium **(Fig. No: 30.2).**

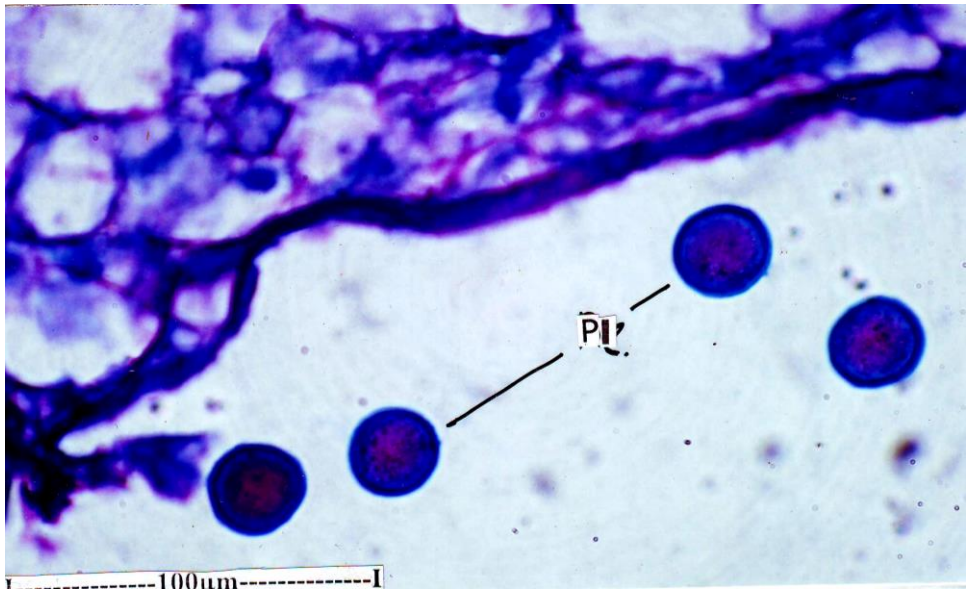
Fig. No: 30.2 T.S of Anther-A Sector Enlarged



[**Et** - Endothecium, **PI**- Pollen, **Po**- Pollen chamber opening]

The epidermal shows signs of disintegration; the endothecium is intact and the endothecial cells have annular thickenings. Circular, Smooth walled pollen grains are seen in the pollen chamber **(Fig. No: 30.3).**

Fig. No: 30.3 T.S of Anthers with Pollen Grains



[**PI**- Pollen]

D. Anatomy of Fruit

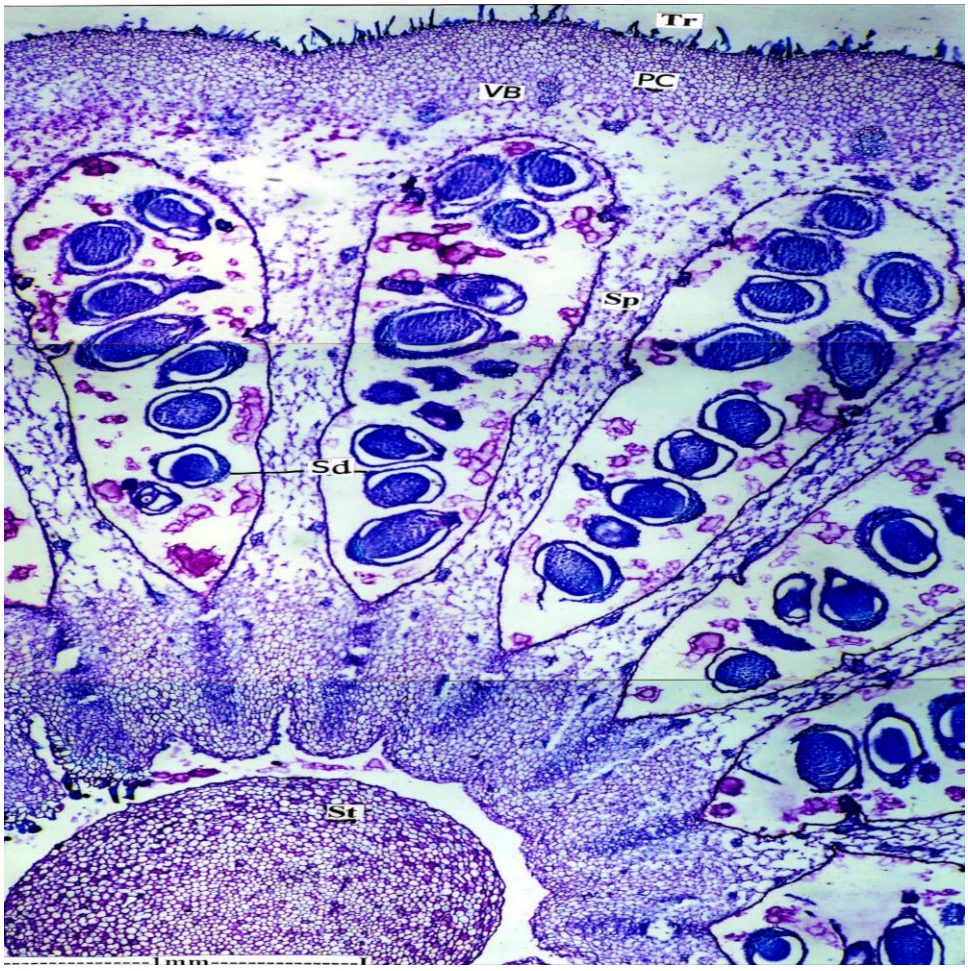
The fruit has many chambers divided by thick partitions; pericarp is thick and fleshy; is 650-800 µm thick; has dense epidermal trichomes which are two or three celled

uniseriate and unbranched; trichome strains are dark due to tannin content; mesocarp consists of small, compact thin walled parenchyma cells **(Fig. No: 32.1, 32.2).**

The vascular bundles of equal size are distributed in a circle along the inner portion of the pericarp; septa are 200µm thick, consists of soft, thin walled parenchyma cells. The septa become dilated into club shaped bodies towards the centre of the fruit and get fused with each other forming a central cavity, surrounded by ridges and furrows **(Fig. No: 31).**

The ridges have short, spindle shaped glandular trichomes **(Fig. No: 32.1, 32.2),** at the centre of the cavity is seen a wide circular parenchymatous cylinder, which is the central pillar of stigma **(Fig. No: 31).** Numerous elliptical seeds are attached on the septa **(Fig. No: 32.3).**

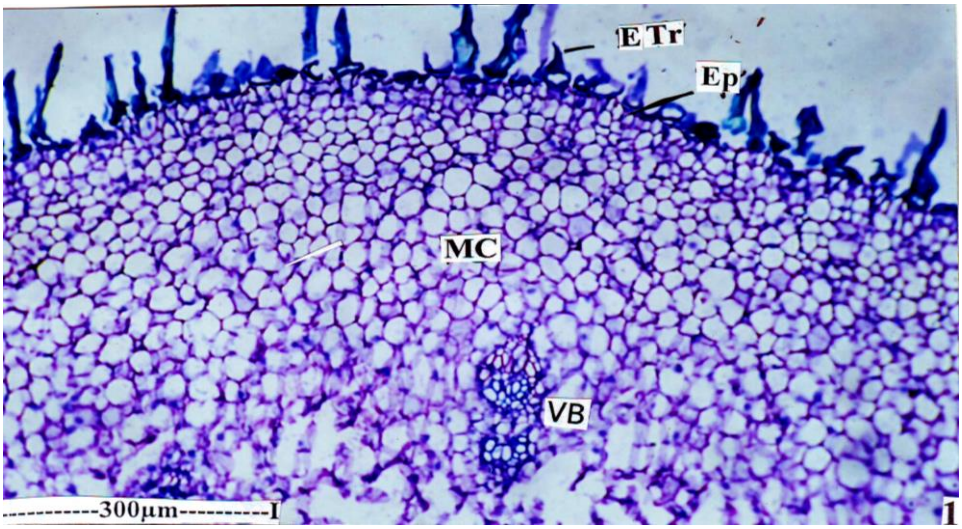
Fig. No: 31 T.S. of Fruit- A Sector Enlarged



[**PC**-Pericarp, **Sd**- Seed, **Sp**- Septae, **St**- Stigma, **Tr**- Trichome,

VB- Vascular bundle]

Fig. No: 32.1 T.S. of Pericarp Outer Sector



[**Ep**- Epidermis, **ETr**- Epidermal trichome, **MC**- Mesocarp, **VB**- Vascular bundle]

Fig. No: 32.2 T.S. of Pericarp Inner Sector Showing Ridges and Furrows

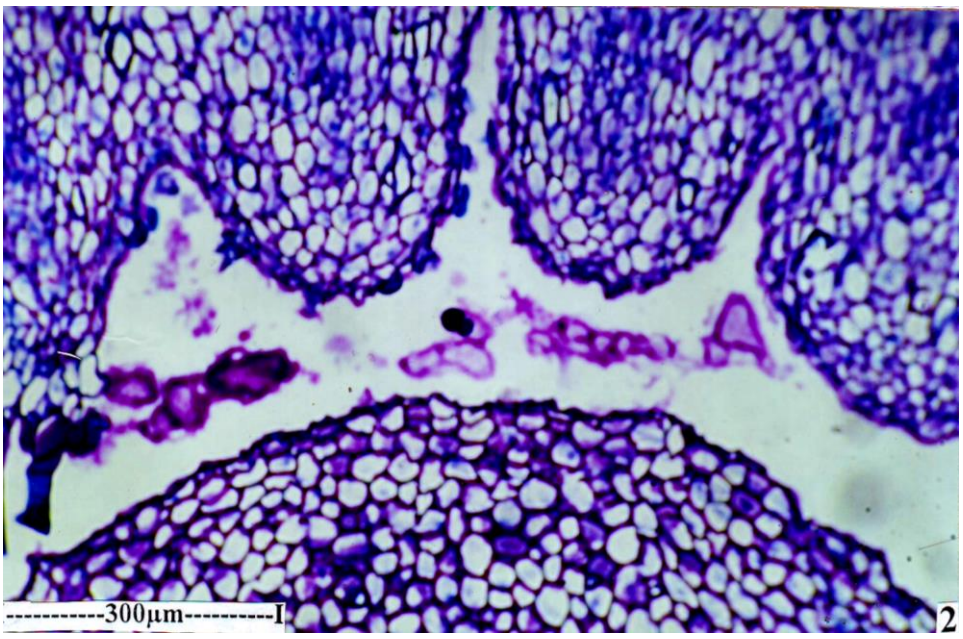
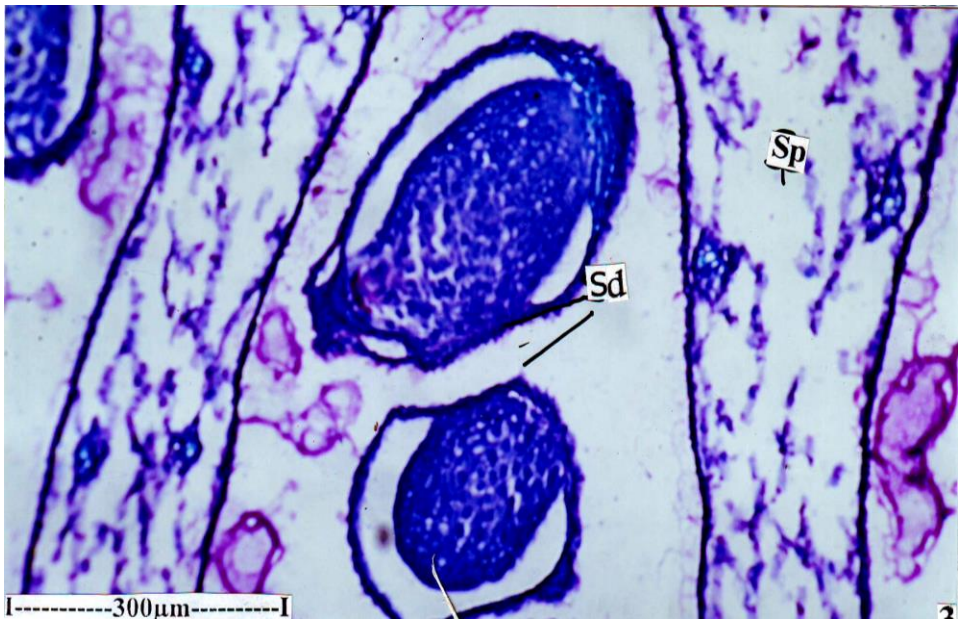


Fig. No: 32. 3. Seeds in between the Septae



[**Sd**- Seed, **Sp**- Septae]

6.4.2.3. Powder characteristics

The powder of various floral parts exhibits the following inclusion;

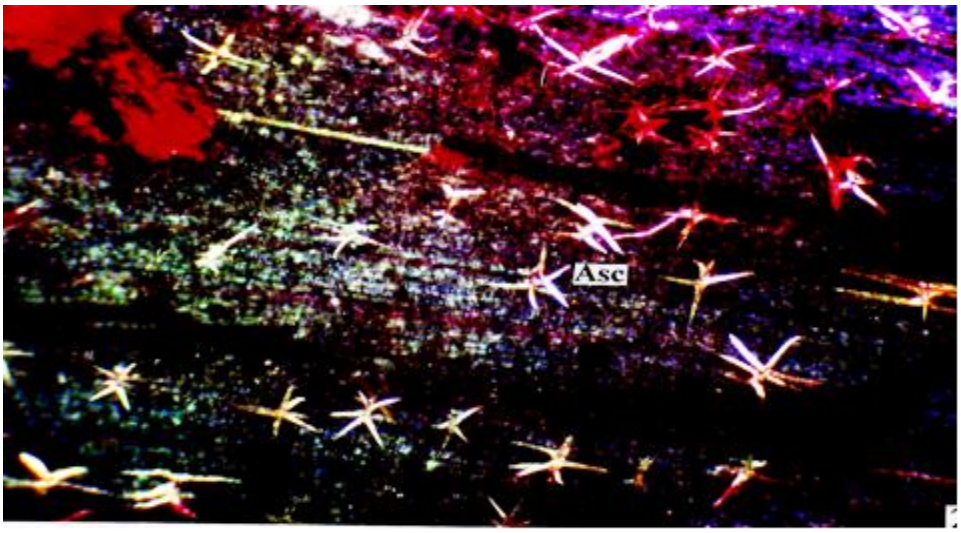
Fragments of the sepal or petal are seen in the powder. In surface view, the cells of the perianth appear vertically oblong hexagonal shape with prominent walls **(Fig. No: 33.1).** Randomly distributed on the surface are broad elliptical thick walled glandular cells with dense cell contents **(Fig. No: 33.1)**. The cells of the epidermis are 70-140 µm wide and 200-340 µm long and glandular cells are 100-140 µm wide.

Fig. No: 33. 1. Sepals Surface View

[**Ec**- Epidermal cell, **Gc**- Glandular cell]

Within the sepal and petals, especially along their basal part, are seen dense distributions of sclerenchyma cell called astrosclereids **(Fig. No: 33.2).**

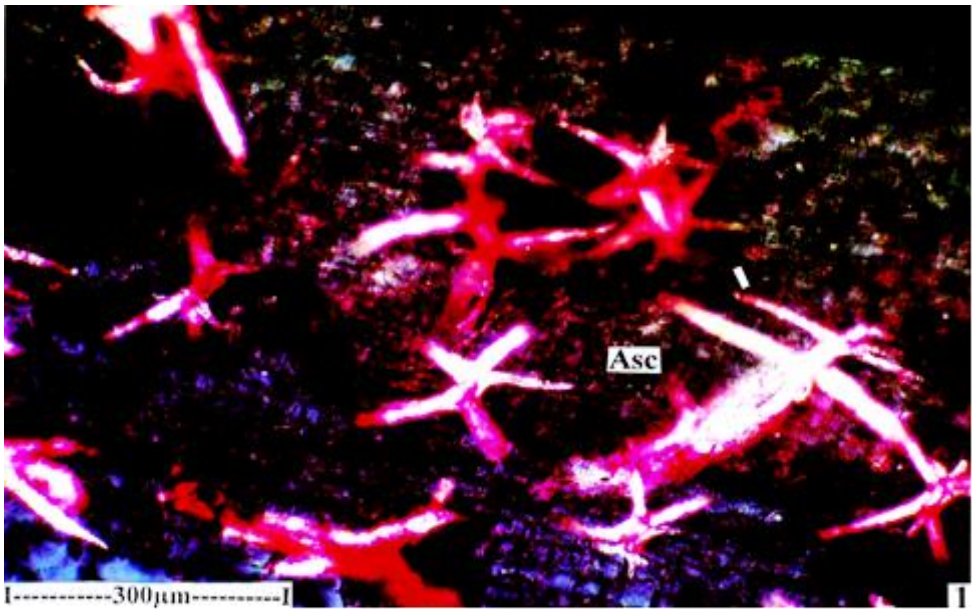
Fig. No: 33. 2. Basal Part of the Sepal Showing Distribution of Asterosclereid



[**ASc**- Astrosclereid]

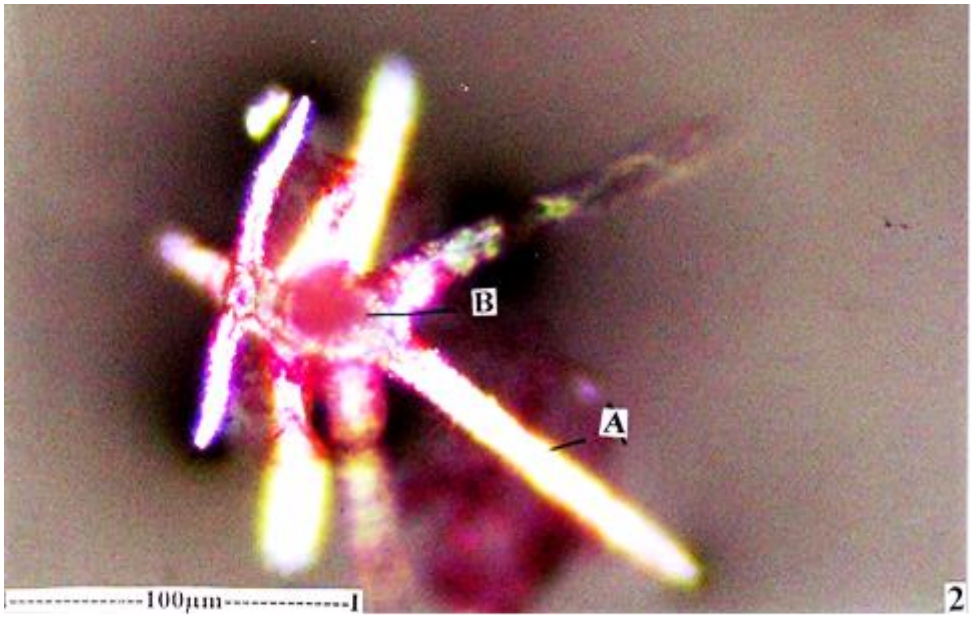
The astrosclereids have a central body and several pointed arms directed in all directions. They have thick secondary lignified walls and dense circular simple pits **(Fig. No: 34.1, 2).** The astrosclereids are random in distribution.

Fig. No: 34. 1. Astrosclereids as seen Under Polarized Light



[Asc- Astrosclereid]

Fig. No: 34.2. Astrosclereids Enlarged



[A- Arm; Asc- Astrosclereid; B- Body]

1. A second type of sclerenchyma element called acicular sclereids is seen running parallel to each other in vertical rows; they are long, narrow and pointed at the tip **(Fig. No: 35.1).** The surface has dense spiny outgrowths and circular pits **(Fig. No: 35.2).** The acicular sclereids are 1.2 mm long and 30 µm wide.

Fig. No: 35. 1. Acicular Scleried

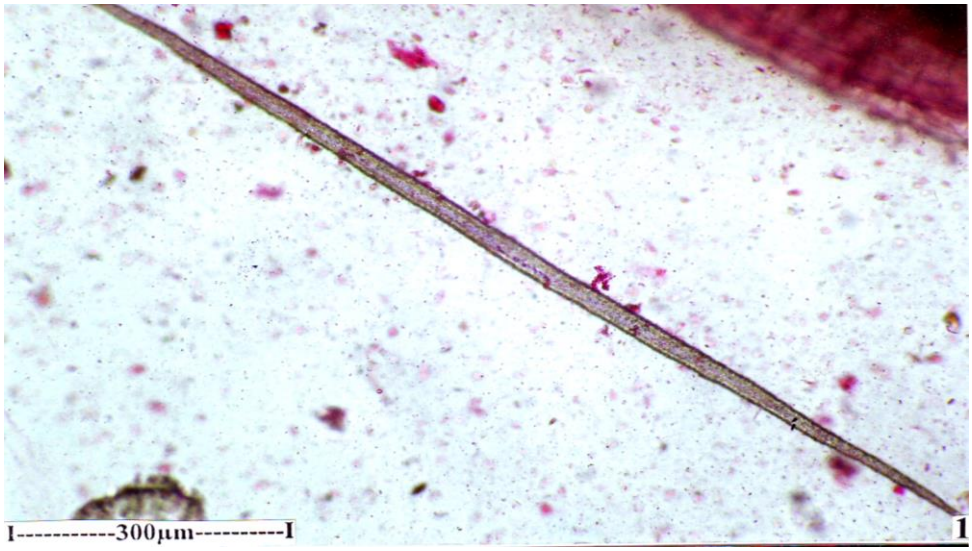
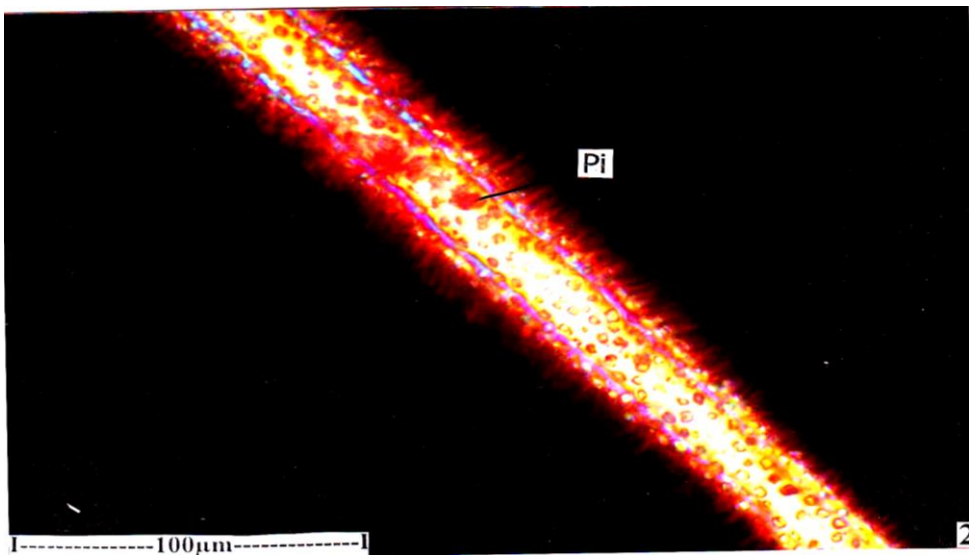


Fig. No: 35. 2. A Portion of Acicular Scleried Enlarged Under Polarized Light Microscope



[Pi- Pits]

2. Pollen **(Fig. No: 36.1, 2):** The pollen grains are circular with smooth exine and thin intine. The pollen grains are mixed with fertile and sterile grains. The fertile grains are larger and stain deeply while the sterile grains are smaller and stain lighter. The fertile pollen grains are 40 µm in diameter and the sterile ones are 25 - 30 µm wide.

Fig. No: 36. 1. Pollen Grains under Low Magnification

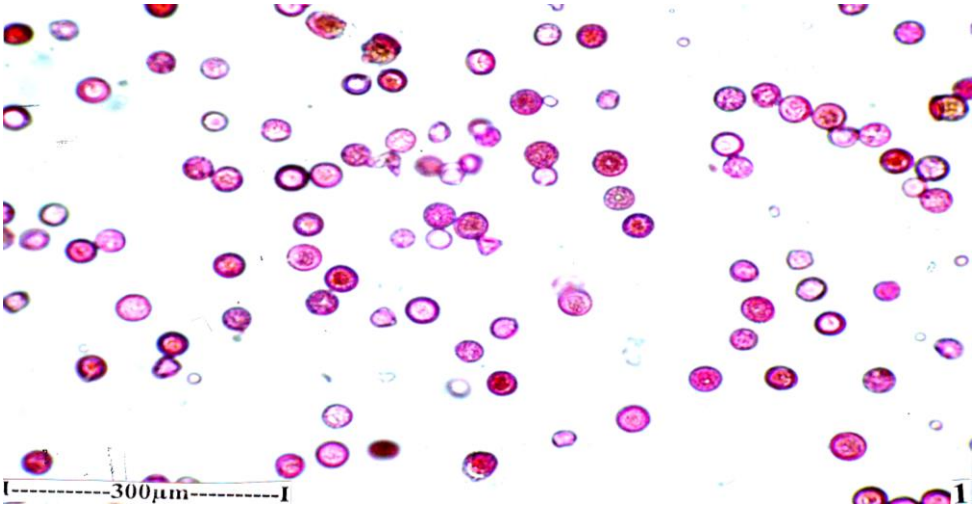
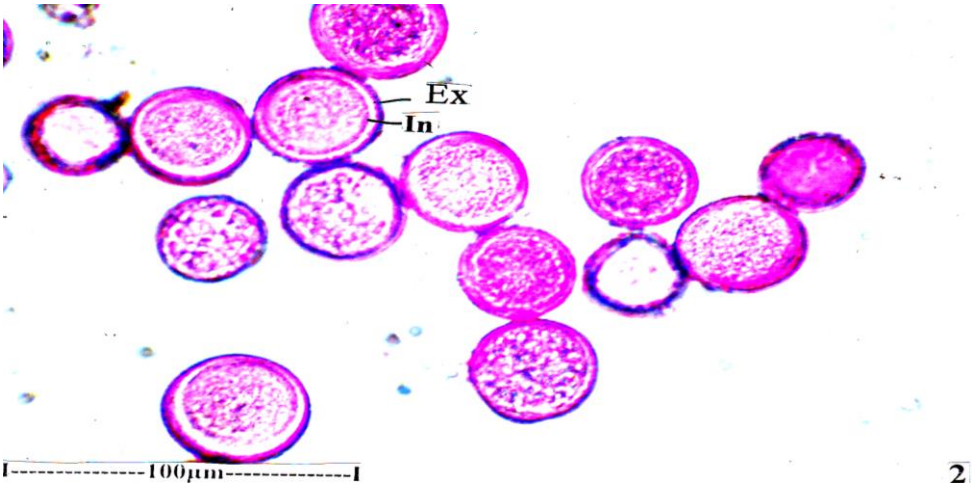


Fig. No: 36. 2. Pollen Grains under High Magnification



[Ex- Exine; In- Intine]

3. Seeds **(Fig. No: 37.1, 2):** The seeds are elliptical, dark coloured with prominent Micropyle and club shaped funicle. The surface of the seeds has reticulate. The surface of the seeds has reticulate thick endings **(Fig. No: 37.2).** The seeds are 250 µm long and 150 µm wide.

Fig. No: 37. 1. Seeds under Low Magnification

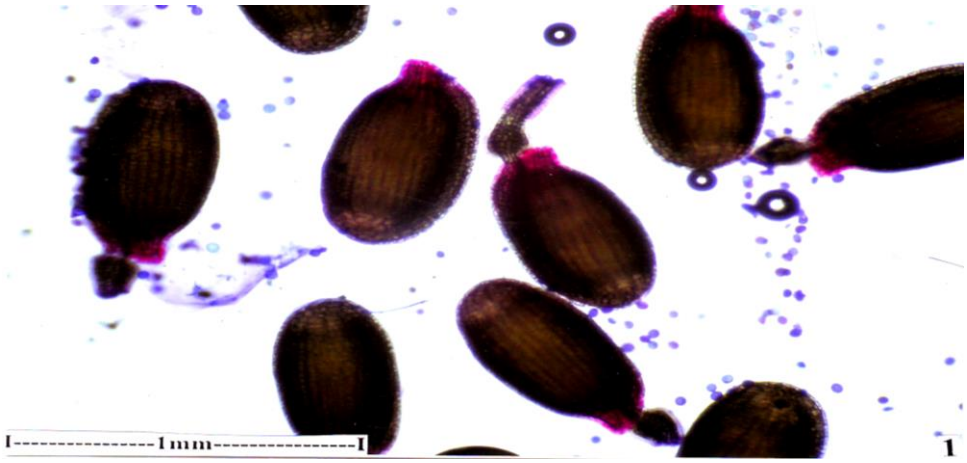
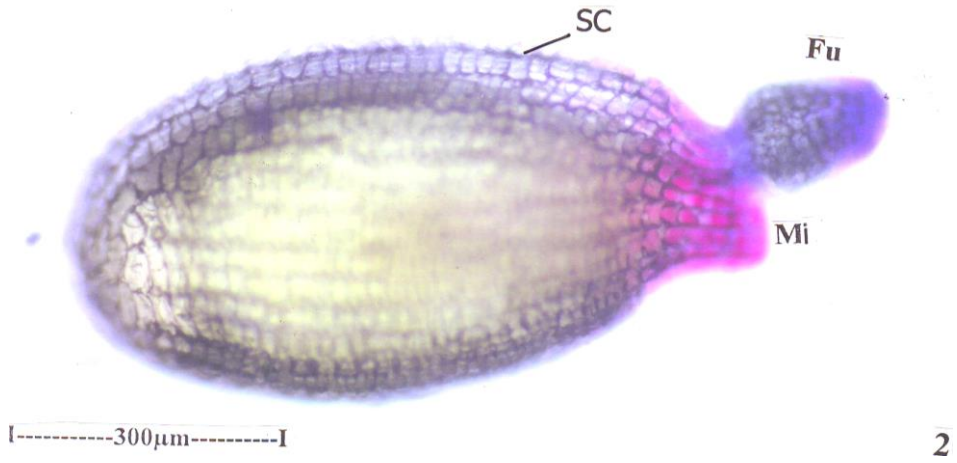


Fig. No: 37. 2. One Seed Enlarged



[Fu- Funicle; Mi- Micropyle; Sc- Seed coat]

6.4.2.4. Analytical study

Organoleptic evaluation

Appearance	Moderately coarse powder
Colour	Pale yellow to buff white
Odour	Characteristic
Taste	Mucilagenous and bitter

6.4.3. Determination of Physico-chemical constants

The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content (Loss on drying) **(7.16 and 6.99%w/w)** were shown in **Table No: 2** for both the flower powder, which is not too high, thus it could discourage bacteria, fungi or yeast growth. Equally important in the evaluation of crude drugs, is the ash value and acid insoluble ash value determination. The total ash is particularly important in the evaluation of purity

of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica **(Musa, *et al.*, 2006).** The ash values of both the flowers were shown in **Table No: 2.** The total ash and sulphated ash of both flowers were found to be highest, **10.6, 11.4, and 6.2, 5.8 %w/w** respectively. The powders of the flowers showed high water soluble extractive values. These values were found to be **12.7** and **11.9%w/w** respectively, when compared to the alcohol soluble extractive values **5.6** and **5.9% w/w** for both the flowers. Only trace amount of foreign substances are found in both the flowers. pH value of the crude flower powder of ***Nymphaea pubescens*** *and* ***Nymphaea nouchali*** was acidic **(4.4** and **4.7)** in nature. The results of Physico-chemical evaluation are given in the following **Table - 2, 3, 4**

Table No: 2 Data for Ash and extractive values of the flowers of *Nymphaea pubescens* Willdenow and *Nymphaea nouchali* Burmann. F.

S. No	Analytical parameters	*Nymphaea pubescens*	*Nymphaea nouchali*
		% w/w	% w/w
1	**Loss on drying at 105ºC**	7.16	6.99
2	**Ash values**		
	Total ash	10.6	11.4
	Acid Insoluble ash	0.62	0.43
	Sulphated ash	6.2	5.8
	Water soluble ash	4.6	5.1
3	**Extractive values**		
	Alcohol soluble extractive	5.6	5.9
	Water soluble extractive	12.7	11.9
4	**Foreign organic matter**	0.02	0.02
5	**pH**	4.4	4.7

All the values are average of three determinations.

Table No: 3 Determination of solubility of Ethylacetate and Hydroalcoholic extracts of the flowers of *Nymphaea pubescens* Willdenow and *Nymphaea nouchali* Burmann. F.

Solvent	Solubility (mg/ml)			
	Nymphaea pubescens		*Nymphaea nouchali*	
	Ethyl acetate extract	*Hydroalcoholic extract*	*Ethyl acetate extract*	*Hydroalcoholic extract*

Acetone	8	9	8	9
Chloroform	1	1	1	1
Dimethylformamide	24	25	23	24
Dimethylsulphoxide	31	33	31	32
Dioxan	3	5	3	3
Distilled water	20	22	21	20
Ethyl acetate	3	3	4	3
Hexane	-	-	-	-
Methanol	29	32	30	31
Petroleum ether	-	-	-	-
Toluene	-	-	-	-

Ethylacetate and Hydroalcoholic extract of ***Nymphaea pubescens*** was highly soluble in Dimethylsulphoxide and methanol when compared to ***Nymphaea nouchali***, while both the flowers extracts was not soluble in hexane, petroleum ether and toluene.

Contamination by toxic metals can either be accidental or intentional. Contamination by heavy metals such as mercury, lead, copper, cadmium, and arsenic in herbal remedies can be attributed to many causes, including environmental pollution, and can pose clinically relevant dangers for the health of the user and should therefore be limited **(De Smet, *et al.*, 1992; Lazarowych** and **Pekos., 1998).** Arsenic, chromium, cobalt, lead, mercury and nickel were present in trace amounts in crude flower powder and Ethylacetate and Hydroalcoholic extracts of the flowers of ***Nymphaea pubescens*** **Willdenow** and ***Nymphaea nouchali*** **Burmann. F.**

Medicinal plant materials normally carry a great number of bacteria and moulds, often originating in soil. While a large range of bacteria and fungi form the naturally occurring micro flora of herbs, aerobic spore-forming bacteria frequently predominate. Current practices of harvesting, handling and production may cause additional contamination and microbial growth. The determination of ***Escherichia coli*** and moulds may indicate the quality of production and harvesting practices **(WHO, 1998).** In the present study crude flower powder and Ethylacetate and Hydroalcoholic extracts were free from microbial contamination.

Table No: 4 Data for Foaming Index of powdered flowers of *Nymphaea pubescens* Willdenow and *Nymphaea nouchali* Burmann. F.

S. No	Test volumetric flask (10ml)	*Nymphaea pubescens*	*Nymphaea nouchali*
		Height of foam (cm)	Height of foam (cm)
1	1	0.2	0.2
2	2	0.3	0.3
3	3	0.5	0.5
4	4	0.6	0.6
5	5	0.6	0.6
6	6	0.8	0.8
7	7	0.8	0.8
8	8	0.9	0.9
9	9	0.9	1.0
10	10	1.0	1.0

Thus, the Foaming index for the powdered flowers of ***Nymphaea pubescens* Willdenow** was found to be less than 100 and for ***Nymphaea nouchali* Burmann. F.** was found to be 100.

Fluorescence Analysis

The flower powder of ***Nymphaea pubescens* Willdenow** and ***Nymphaea nouchali* Burmann F.** were treated with various reagents and visualized under ultra violet radiations, which showed several color changes indicating the presence of specific

phyto-constituents especially flavonoids. The flower powder showed dark brown color with ferric chloride indicates the presence of phenolic compounds **(tannins, flavonoids etc).** The formation of yellow color and brown color with methanol and nitric acid indicates the presence of flavonoids and proteins. Fluorescence analysis of drug powder of ***Nymphaea pubescens*** **Willdenow** and ***Nymphaea nouchali*** **Burmann F.** were furnished in **Table No: 5.**

Table No: 5 Fluorescence analysis of powdered flowers of *Nymphaea pubescens* Willdenow and ***Nymphaea nouchali*** **Burmann F.**

S.No:	Chemical Treatment with powder	Day light	*Nymphaea pubescens* Willdenow		Day light	*Nymphaea nouchali* Burmann	
			UV Light (nm)			UV Light (nm)	
			254	366		254	366
1.	Powder as such	Greenish brown	Dark greenish black	Light greenish black	Dark Green	Dark Greenish black	Light Greenish Black
2.	Water	Pale yellowish green	Dark green	Light green	Pale Yellowish Green	Dark Brown	Light Brown
3.	1 N HCl	Red	Dark greenish black	Light greenish black	Dark Greenish black	Dark Green	Light Green
4.	5% NaOH	Orange	Dark greenish black	Light greenish black	Dark Reddish Brown	Dark Greenish Black	Light Greenish Black
5.	1 N NaOH in methanol	Pale yellow	Dark green	Light green	Pale Yellow	Dark Greenish	Light Greenish
6.	50% HNO_3	Light orange	Dark green	Light green	Pale Yellow	Dark Green	Light Green
7.	50% H_2SO_4	Red	Dark green	Light green	Yellow	Dark Green	Light Green
8.	Methanol	Yellow	Dark yellow	Light brown	Pale Yellow	Dark yellow	Dark brown
9.	Picric acid	Yellowish green	Dark yellowish green	Light yellowish green	Yellowish Green	Dark Yellowish Green	Light Yellowish Green
10.	Acetic acid	Light red	Dark black	Light black	Green	Light brown	Light brown
11.	5% $FeCl_3$	Dark greenish black	Dark black	Light brown	Dark Greenish Black	Light brown	Dark brown
12.	HNO_3+ NH_3	Brown	Dark black	Light brown	Yellowish	Dark Greenish	Light brown

CHAPTER-VII

PHYTOCHEMICAL SCREENING

PHYTOCHEMICAL SCREENING

7.11. RESULTS AND DISCUSSION

7.11.1. Quantitative Phytochemical studies

Many of the plant extracts have given good results for most of the assays. Some of these names were pronounced as best results at each assay. Therefore, it would be wise to first discuss the findings according to each assay and later examine each flower individually.

The continuous interest in laboratory screening of medicinal plants is not only to determine the scientific rationale for their usage, but also to discover new active principles. Flavonoids have been well investigated for many pharmacological properties including anti-diabetic, cytotoxic, anti-hepatotoxicity, anti-oxidant properties but there are also few reports about their antimicrobial properties **(Kiran Kumar, A., *et al.,* 2008).** In a study in 2010, **Nagavani, V.,** have revealed the presence of phenolic compounds in different parts of the flowers of ***Nymphaea nouchali*** and have a wide range of applications in ayurveda and traditional medicine.

i). Plant Extraction

Percentage yield of successive solvent extractive values of the flower parts of ***Nymphaea pubescens*** **Willdenow** and ***Nymphaea nouchali*** **Burmann. F.** was given in **Table No.7.**

Table No: 7 Extractive values of different extracts of the flower of *Nymphaea pubescens* Willdenow and *Nymphaea nouchali* Burmann. F.

Plants	Extracts	Yield in gms	%W/W	Color and Appearance of the Extract
Plant-A ***Nymphaea pubescens*** **Willdenow (Nymphaeaceae)**	Petroleum ether	246	12.30	Brownish green, waxy
	Chloroform	225	11.25	Brown, waxy
	Ethyl acetate	254	12.70	Yellowish green, waxy
	Hydro-alcoholic	286	14.30	Deep brown, oily
Plant – B ***Nymphaea nouchali*** **Burmann. F. (Nymphaeaceae)**	Petroleum ether	253	12.65	Brownish green, waxy
	Chloroform	227	11.35	Brown, waxy
	Ethyl acetate	244	12.20	Yellowish green, waxy
	Hydro-alcoholic	281	14.10	Deep brown, oily

ii). Fluorescence Analysis

Fluorescence analysis of different extracts of the flower parts of ***Nymphaea pubescens*** **Willdenow** and ***Nymphaea nouchali*** **Burmann. F.** was observed under ultra violet light and the findings are furnished in **Table No.8.**

Table No: 8 Fluorescence analysis of various extracts of the flowers of *Nymphaea pubescens* Willdenow and *Nymphaea nouchali* Burmann. F.

Plants	Name of the extract	Day light	UV Light (nm)	
			254	366
Plant-A *Nymphaea pubescens* Willdenow (Nymphaeaceae)	Petroleum ether	Brownish green	Dark greenish brown	Light greenish black
	Chloroform	Pale yellow	Dark yellow	Light yellow
	Eth ylacetate	Yellowish green	Dark yellowish green	Light yellowish green
	Hydroalcoholic	Deep brown	Dark brown	Light brown
Plant – B *Nymphaea nouchali* Burmann. F. (Nymphaeaceae)	Petroleum ether	Brownish green	Dark greenish brown	Light greenish black
	Chloroform	Pale yellow	Dark yellow	Light yellow
	Ethylacetate	Yellowish green	Dark yellowish green	Light yellowish green
	Hydroalcoholic	Deep brown	Dark brown	Light brown

The formation of yellow color and brown color indicates the presence of flavonoids and proteins respectively. Dark brown color indicates the presence of phenolic compounds.

iii). Qualitative Phytochemical Analysis

Qualitative Phytochemical analysis of various extracts of the flower parts of ***Nymphaea pubescens*** **Willdenow** and ***Nymphaea nouchali*** **Burmann. F.** with various reagents was observed and the findings are furnished in **Table No.9.**

Table No: 9 Qualitative Phytochemical analysis of various extracts of *Nymphaea pubescens* Willdenow and *Nymphaea nouchali* Burmann. F.

Phytoconstituents	Pet. Ether		Chloroform		Ethylacetate		Hydroalcoholic	
	N.p	*N.n*	*N.p*	*N.n*	*N.p*	*N.n*	*N.p*	*N.n*
Alkaloids								
Mayer's Reagent	+	+	+	+	+	+	+	+
Wagner's Reagent	-	-	+	+	++	++	++	++
Hager's Reagent	-	-	+	+	++	++	++	++
Dragendroff's Reagent	-	-	+	+	++	++	++	++
Phenolic compounds and Tannins								
Ferric chloride test	+	+	++	+	++	++	+	+
Gelatin test	-	-	++	++	++	++	+	+
Lead acetate test	-	-	++	++	+	+	+	+
Alkaline reagent test	-	-	+	+	++	++	+	+
Magnesium & HCl reduction	+	+	++	++	+	+	+	+
Carbohydrates								
Molisch's Reagent	+	+	++	++	++	++	++	++
Fehling's Reagent	+	+	++	++	++	++	++	++
Barfoed's Reagent	+	+	++	++	++	++	++	++
Benedict's Reagent	+	+	++	++	++	++	++	++
Proteins & Amino acids								
Biuret's test	-	-	+	+	+	+	+	+
Millon's test	+	+	+	+	+	+	+	+
Xanthoproteic test	-	-	+	+	+	+	+	+
Ninhydrin test	-	-	+	+	+	+	+	+
Steroids and Sterols								
Libermann's Burchard's test	-	-	++	++	++	++	+	+
Salkowaski's test	-	-	++	++	++	++	+	+
Gums and Mucilage								
Alcohol 95% test	-	-	-	-	-	-	-	-
Ruthenium red test	-	-	-	-	-	-	-	-
Glycosides								
Baljet's Test	-	-	+	+	+	+	-	-
Borntrager's Test	-	-	+	+	+	+	-	-
Modified Borntrager's test	-	-	-	-	-	-	-	-

Schoentetens reaction (Borax test)	-	-	-	-	-	-	-	-
Legal's Test	-	-	+	+	+	+	-	-
Killer-Killiani Test	-	-	+	+	+	+	-	-
Flavones and Flavanoids								
Extract with ammonia	++	++	+++	+++	+++	+++	++	++
Extract with NaOH	+	++	+++	+++	+++	+++	++	++
Extract with Conc. H_2So_4	++	++	+++	+++	+++	+++	++	++
Extract with Mg/HCl(Shinoda's Test)	+	++	++	++	+++	+++	++	++
Fixed oils and Fats								
Spot test	-	-	-	-	-	-	-	-
Saponification test	-	-	-	-	-	-	-	-
Triterpenoids								
Noller's test	-	-	++	++	++	++	+	+
Modified Libermann's Burchard's test	-	-	++	++	++	++	+	+
Saponins								
Foam test	-	-	-	-	-	-	-	-
Lignins								
Phloroglucinol staining method	-	-	-	-	-	-	-	-
Volatile oil								
Steam distillation	***Nymphaea pubescens***				***Nymphaea nouchali***			
	++				++			

+++ ↱ more intense ++ ↱ moderate intense

+ ↱ less intense - ↱ Absence

N.p: Nymphaea pubescens Willdenow***, N.n: Nymphaea nouchali*** Burmann. F.

The results of preliminary phytochemical analysis of ***Nymphaea pubescens*** **Willdenow** and ***Nymphaea nouchali*** **Burmann. F.** extracts is shown in **Table No: 9.** All the extracts showed the presence of various phytochemical constituents like alkaloids, phenolic compounds and tannins, carbohydrates, proteins and amino acids, flavanoids, steroids, terpenoids, sterols and only few glycosides. However the extracts showed absence for gum and mucilages, fixed oils, lignins and saponins **(Wealth of India, 2003).** In 2010 **Nagavani, V.,** evaluated the presence of phenolic compounds, such as flavonoids, phenolic acids, tannins etc. these compounds are distributed in different parts of plants such as bark, leaves, fruits and flowers etc. High levels of enzymatic antioxidants were found in the fresh flower extracts **(Nagavani, V., 2010).**

7.11.2. Inorganic Mineral Analysis

In nutrition, minerals are those elements for which the body's requirements are at least 100 mg/day and trace minerals are those elements that are needed in smaller amounts. The minerals include calcium, chloride, magnesium, phosphorus, potassium, sodium and sulphur. There are fourteen essential trace minerals. They are chromium, cobalt, copper, fluorine, iodine, iron, manganese, molybdenum, nickel, selenium, silicon, tin, vanadium and zinc. However, evidence for the requirement of nickel, silicon, tin and vanadium are still incomplete **(Gennare, 2004).** The amount of Sodium and Potassium estimated by flame photometry and copper, zinc, lead, etc., estimated by atomic absorption spectroscopy present in 100 g of dried flower material were presented in **Table No.10.**

Table No: 10 Amount of minerals present in wet and dried materials of flowers of *Nymphaea pubescens* and *Nymphaea nouchali*

S. No	Parameter	Unit	*Nymphaea pubescens*	*Nymphaea nouchali*
1.	Copper (DB)	mg/Kg	118	112
2.	Zinc (DB)	mg/Kg	63	56
3.	Lead (DB)	mg/Kg	11	10.5
4.	Nickel(DB)	mg/Kg	100	102
5.	Cadmium (DB)	mg/Kg	< 0.0008	< 0.0009
6.	Cyanide (DB)	mg/Kg	< 0.005	< 0.004
7.	Arsenic (WB)	mg/Kg	< 0.001	< 0.001
8.	Manganese (DB)	mg/Kg	72	71
9.	Total Chromium (DB)	mg/Kg	< 0.01	< 0.01
10.	Cobalt (DB)	mg/Kg	92	91
11.	Sodium	mg/Kg	0.01	0.01
12.	Potassium	mg/Kg	0.01	0.01

< ⇗ Indicates less than minimum detection limit

DB ⇗ Dry basis, WB ⇗ Wet basis

7.11.3. Thin Layer Chromatography

Petroleum ether, Chloroform, Ethyl acetate and Hydroalcoholic extracts of ***Nymphaea pubescens*** **Willdenow** and ***Nymphaea nouchali*** **Burmann. F.** was subjected to thin layer chromatography for alkaloids, flavanoids, glycosides and anthocyanins with the respective solvent system. Their R_f values were determined and furnished in **Table Nos: 11, 12, 13** and **14.**

Table No: 11 TLC for Alkaloids

Plants	Extracts	Solvent System	Detecting agent	No of spots	R_f
Nymphaea pubescens **Willdenow**	Pet-ether	Chloroform: Methanol (80:20)	**Dragendroff's reagent, UV-254, 366nm**	**3**	**0.23 0.34 0.46**
	Chloroform	Ethylacetate: Methanol: Water (100:13.5:10)		**5**	**0.09 0.11 0.22 0.46 0.63**
	Ethyl acetate	Chloroform: Methanol (85:15)		**4**	**0.21 0.25 0.39 0.57**
	Hydroalcoholic	Toluene: Ethylacetate: Diethylamine (7:2:1)		**4**	**0.43 0.46 0.54 0.57**
Nymphaea nouchali **Burmann. F.**	Pet-ether	Chloroform: Methanol (80:20)		**3**	**0.24 0.35 0.53**
	Chloroform	Ethylacetate: Methanol: Water (100:13.5:10)		**6**	**0.09 0.12 0.27 0.45 0.54 0.64**
	Ethyl acetate	Chloroform: Methanol (85:15)		**3**	**0.22 0.29 0.72**
	Hydroalcoholic	Toluene: Ethylacetate: Diethylamine (7:2:1)		**5**	**0.24 0.36 0.42 0.53 0.60**

Table No: 12 TLC for Flavanoids

Plants	Extracts	Solvent System	Detecting agent	No of spots	R_f
***Nymphaea pubescens* Willdenow**	Pet-ether	Toluene: Ethylacetate: Formic acid (5:4:1)	**UV - 254 and 366nm**	**4**	**0.34 0.43 0.52 0.68**
	Chloroform	Chloroform: Acetone: Formic acid (76: 16.5: 8.5)		**6**	**0.27 0.30 0.34 0.37 0.42 0.47**
	Ethyl acetate	Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:27)		**6**	**0.16 0.23 0.34 0.47 0.73 0.82**
	Hydroalcoholic	n- Butanol: Acetic acid: Water (40:10:20)		**6**	**0.24 0.31 0.33 0.41 0.56 0.72**
***Nymphaea nouchali* Burmann. F.**	Pet-ether	Toluene: Ethylacetate: Formic acid (5:4:1)		**4**	**0.34 0.42 0.53 0.69**
	Chloroform	Chloroform: Acetone: Formic acid (76: 16.5: 8.5)		**5**	**0.25 0.33 0.38 0.45 0.55**
	Ethyl acetate	Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:27)		**6**	**0.22 0.38 0.46 0.54 0.73 0.81**
	Hydroalcoholic	n-Butanol: Acetic acid: Water (40:10:20)		**6**	**0.24 0.30 0.35 0.41 0.57 0.73**

Table No: 13 TLC for Glycosides

Plants	Extracts	Solvent System	Detecting agent	No of spots	R_f
***Nymphaea pubescens* Willdenow**	Pet-ether	Ethyl acetate: Methanol: water (75:15:10)	**UV - 254 and 366nm**	**2**	**0.24 0.45**
	Chloroform	Ethyl acetate: Ethanol: water (70:20:10)		**3**	**0.41 0.56 0.72**
	Ethyl acetate	Ethylacetate: Ethylmethyl ketone: Formic acid: Water (5:3:1:1)		**3**	**0.35 0.41 0.57**
	Hydroalcoholic	Benzene: Ethanol: Formic acid: (9:7:4)		**4**	**0.27 0.45 0.54 0.64**
***Nymphaea nouchali* Burmann. F.**	Pet-ether	Ethyl acetate: Methanol: water (75:15:10)		**3**	**0.47 0.67 0.73**
	Chloroform	Ethyl acetate: Ethanol: water (70:20:10)		**3**	**0.42 0.54 0.73**
	Ethyl acetate	Ethylacetate: Ethylmethyl ketone: Formic acid: Water (5:3:1:1)		**3**	**0.43 0.56 0.71**
	Hydroalcoholic	Benzene; Ethanol: Formic acid: (9:7:4)		**4**	**0.29 0.44 0.55 0.63**

Table No: 14 TLC for Anthocyanins

Plants	Extracts	Solvent System	Detecting agent	No of spots	R_f
***Nymphaea pubescens* Willdenow**	Pet-ether	n-Butanol: Acetic acid: Water (4:1:5)	**Anisaldehyde sulphuric acid reagent**	**3**	**0.09 0.27 0.54**
	Chloroform			**4**	**0.11 0.22 0.46 0.63**
	Ethyl acetate			**5**	**0.11 0.31 0.43 0.56 0.71**
	Hydroalcoholic			**4**	**0.24 0.33 0.41 0.72**
***Nymphaea nouchali* Burmann. F.**	Pet-ether	n-Butanol: Acetic acid: Water (4:1:5)		**3**	**0.42 0.54 0.73**
	Chloroform			**3**	**0.41 0.56 0.72**
	Ethyl acetate			**5**	**0.27 0.45 0.54 0.64 0.70**
	Hydroalcoholic			**4**	**0.23 0.35 0.42 0.70**

In the TLC studies for each of the extract, several solvent systems were tried to identify suitable solvent system exhibiting more number of spots and good separation indicated by the difference of R_f values. The petroleum ether, chloroform, ethyl acetate and hydroalcoholic extract of the flowers ***Nymphaea pubescens* Willdenow** and ***Nymphaea nouchali* Burmann. F.** showed more spots when separated using Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:27) as a solvent system for flavonoids. Similarly n-Butanol: Acetic acid: Water (40:10:20) was found to be the better solvent system for both flavonoids and anthocyanins. **Anthocyanins** are present in the flowers of ***Nymphaea nouchali* Burmann. F.** for the first time and it is also confirmed by TLC.

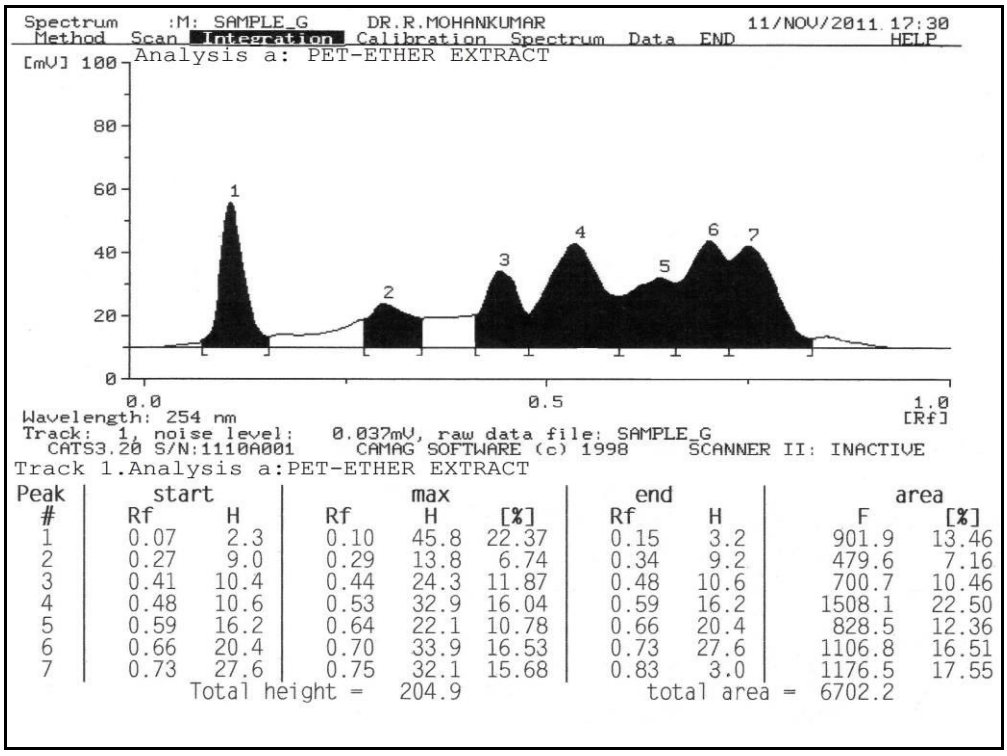
7.11.4. HPTLC Finger Prints - *Nymphaea pubescens* Willdenow

In the HPTLC studies, the same solvent system used in TLC were used for the separation. The number of compounds separated, their R_f values and their percentage were noted. The chloroform and ethylacetate extract of ***Nymphaea pubescens*** flowers showed the presence of 8 spots and 6 of them with R_f values **0.11, 0.29, 0.45, 0.62, 0.72** and **0.80**, were found to be the major ones with about **23** and **18%, 30** and **19%, 55** and **11%, 58** and **11%** peak areas for pet ether, chloroform, ethylacetate and hydroalcoholic extracts respectively and the results were furnished in **Table No: 15** and **Fig No: 44 - 48.**

Table No: 15 R_f values of various extracts of *Nymphaea pubescens* obtained by HPTLC

Name of the extract	Total number of spots	R_f value	Total Height	Total Area
Pet-ether	**7**	**0.10 0.29 0.44 0.53 0.64 0.70 0.75**	**204.9**	**6702.2**
Chloroform	**8**	**0.11 0.29 0.38 0.45 0.55 0.62 0.72 0.80**	**111.2**	**3030.2**
Ethylacetate	**8**	**0.11 0.25 0.29 0.45 0.56 0.62 0.72 0.80**	**141.3**	**4886.8**
Hydroalcoholic	**7**	**0.11 0.28 0.46 0.56 0.62 0.72 0.83**	**59.7**	**1775.1**

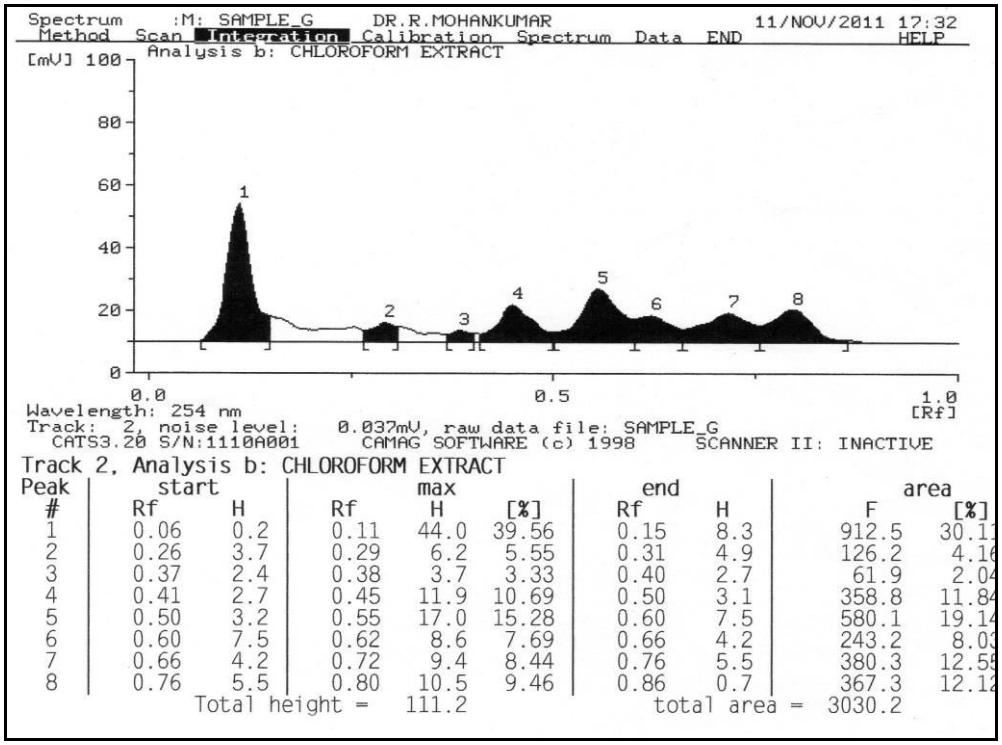
Fig. No: 44 HPTLC FINGER PRINT OF PET-ETHER EXTRACT AT 366 nm



Peak	start		max			end		area	
#	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.07	2.3	0.10	45.8	22.37	0.15	3.2	901.9	13.46
2	0.27	9.0	0.29	13.8	6.74	0.34	9.2	479.6	7.16
3	0.41	10.4	0.44	24.3	11.87	0.48	10.6	700.7	10.46
4	0.48	10.6	0.53	32.9	16.04	0.59	16.2	1508.1	22.50
5	0.59	16.2	0.64	22.1	10.78	0.66	20.4	828.5	12.36
6	0.66	20.4	0.70	33.9	16.53	0.73	27.6	1106.8	16.51
7	0.73	27.6	0.75	32.1	15.68	0.83	3.0	1176.5	17.55

Total height = 204.9 total area = 6702.2

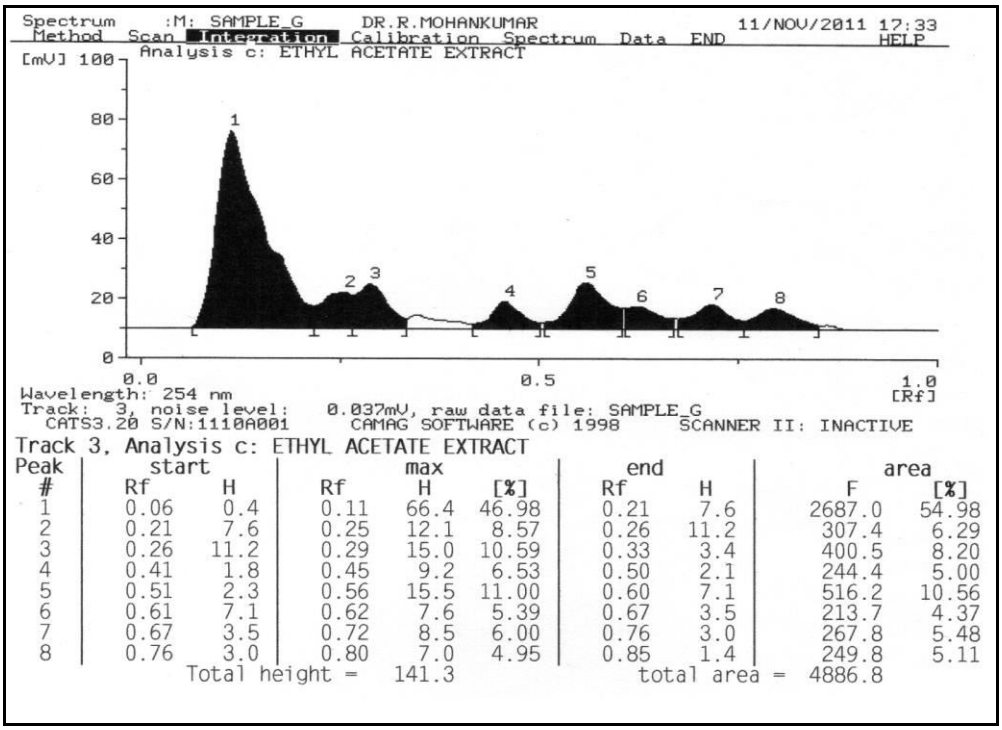
Fig. No: 45 HPTLC FINGER PRINT OF CHLOROFORM EXTRACT AT 366 nm



Peak	start		max			end		area	
#	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.06	0.2	0.11	44.0	39.56	0.15	8.3	912.5	30.1
2	0.26	3.7	0.29	6.2	5.55	0.31	4.9	126.2	4.16
3	0.37	2.4	0.38	3.7	3.33	0.40	2.7	61.9	2.04
4	0.41	2.7	0.45	11.9	10.69	0.50	3.1	358.8	11.84
5	0.50	3.2	0.55	17.0	15.28	0.60	7.5	580.1	19.14
6	0.60	7.5	0.62	8.6	7.69	0.66	4.2	243.2	8.03
7	0.66	4.2	0.72	9.4	8.44	0.76	5.5	380.3	12.55
8	0.76	5.5	0.80	10.5	9.46	0.86	0.7	367.3	12.12

Total height = 111.2 total area = 3030.2

Fig. No: 46 HPTLC FINGER PRINT OF ETHYLACETATE EXTRACT AT 366 nm

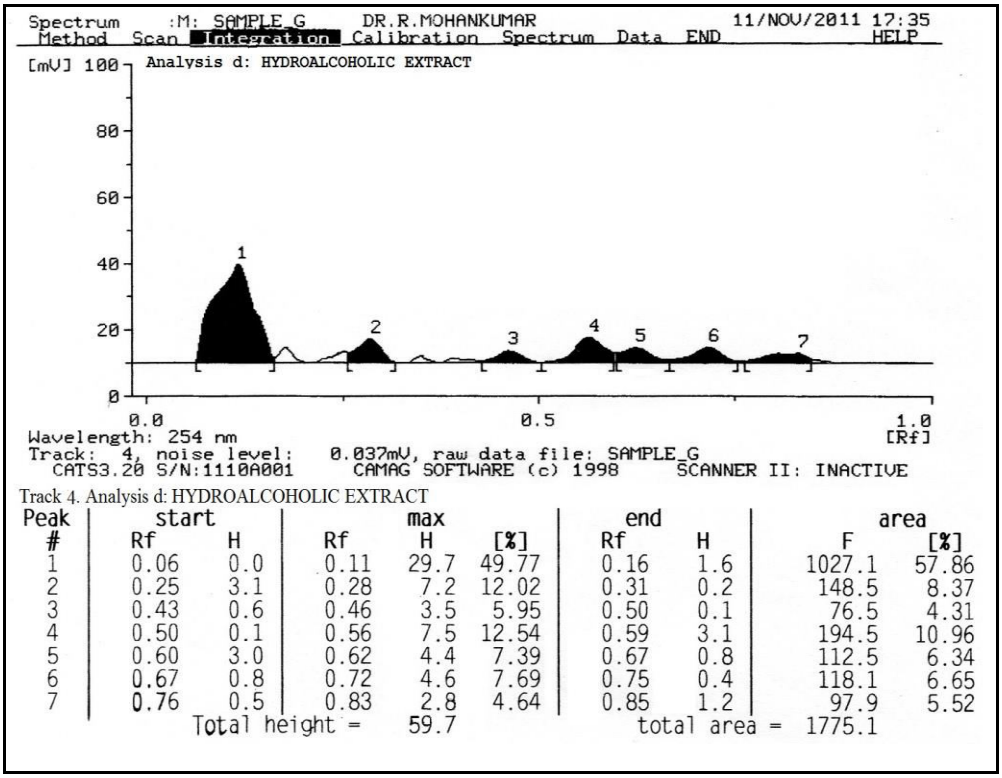


Track 3, Analysis c: ETHYL ACETATE EXTRACT

Peak	start		max			end		area	
#	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.06	0.4	0.11	66.4	46.98	0.21	7.6	2687.0	54.98
2	0.21	7.6	0.25	12.1	8.57	0.26	11.2	307.4	6.29
3	0.26	11.2	0.29	15.0	10.59	0.33	3.4	400.5	8.20
4	0.41	1.8	0.45	9.2	6.53	0.50	2.1	244.4	5.00
5	0.51	2.3	0.56	15.5	11.00	0.60	7.1	516.2	10.56
6	0.61	7.1	0.62	7.6	5.39	0.67	3.5	213.7	4.37
7	0.67	3.5	0.72	8.5	6.00	0.76	3.0	267.8	5.48
8	0.76	3.0	0.80	7.0	4.95	0.85	1.4	249.8	5.11

Total height = 141.3 total area = 4886.8

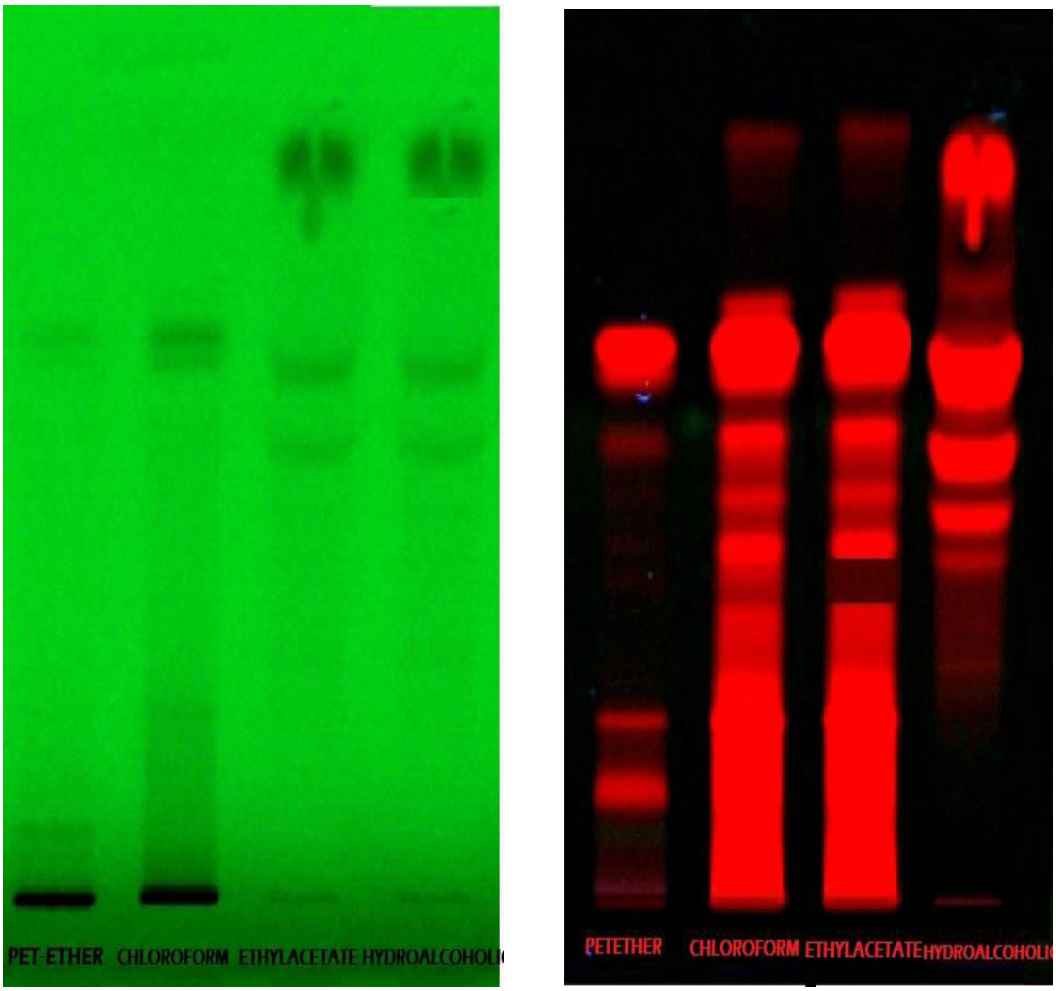
Fig. No: 47 HPTLC FINGER PRINT OF HYDROALCOHOLIC EXTRACT AT 366 nm



Track 4. Analysis d: HYDROALCOHOLIC EXTRACT

Peak	start		max			end		area	
#	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.06	0.0	0.11	29.7	49.77	0.16	1.6	1027.1	57.86
2	0.25	3.1	0.28	7.2	12.02	0.31	0.2	148.5	8.37
3	0.43	0.6	0.46	3.5	5.95	0.50	0.1	76.5	4.31
4	0.50	0.1	0.56	7.5	12.54	0.59	3.1	194.5	10.96
5	0.60	3.0	0.62	4.4	7.39	0.67	0.8	112.5	6.34
6	0.67	0.8	0.72	4.6	7.69	0.75	0.4	118.1	6.65
7	0.76	0.5	0.83	2.8	4.64	0.85	1.2	97.9	5.52

Total height = 59.7 total area = 1775.1



254 nm **366nm**

Fig. No: 48 HPTLC for *Nymphaea pubescens* at 254nm and 366nm

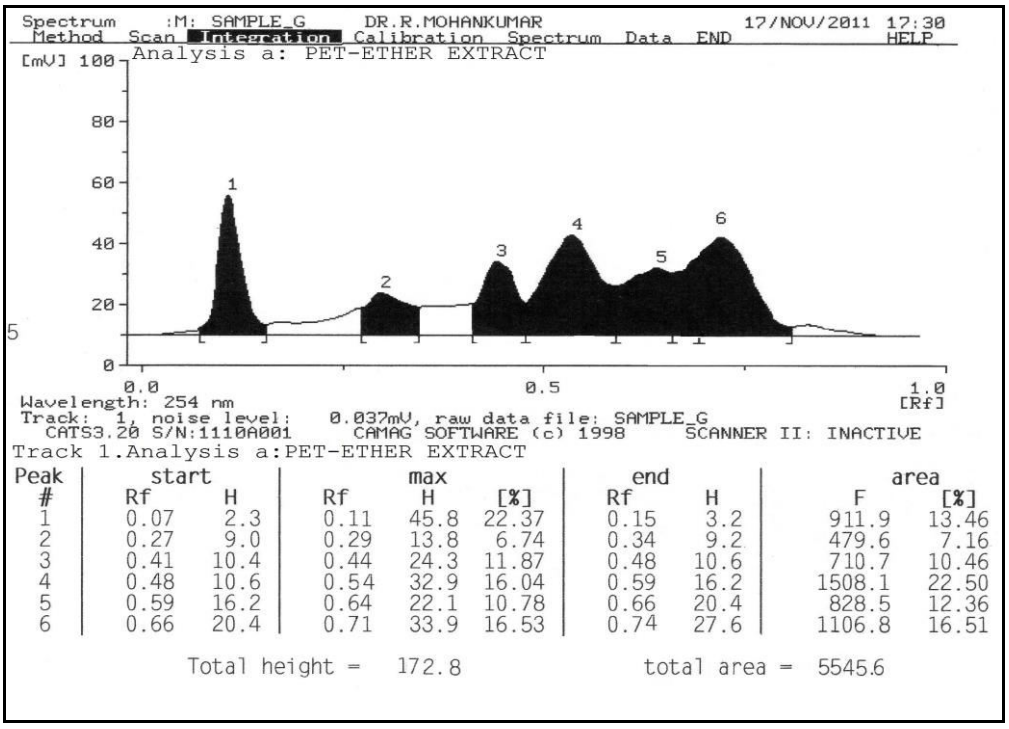
7.11.5. HPTLC Finger Prints - *Nymphaea nouchali* Burmann. F.

The chloroform and ethylacetate extract of ***Nymphaea nouchali*** flowers showed the presence of 8 spots and 4 of them with R_f values **0.29, 0.45, 0.72** and **0.80**, were found to be the major ones with about **23** and **17%, 30** and **19%, 55** and **19%, 58** and **11%** peak areas for pet ether, chloroform, ethylacetate and hydroalcoholic extracts respectively and the results were furnished in **Table No: 16 and Fig No: 49 - 53.**

Table No: 16 R_f values of various extracts of *Nymphaea nouchali* obtained by HPTLC

Name of the extract	Total number of spots	Rf value	Total Height	Total Area
Pet-ether	**6**	**0.11 0.29 0.44 0.54 0.64 0.71**	**172.8**	**5545.6**
Chloroform	**7**	**0.10 0.29 0.38 0.45 0.55 0.60 0.72**	**105.5**	**2674.9**
Ethylacetate	**7**	**0.11 0.29 0.38 0.45 0.52 0.62 0.72**	**109.8**	**2650.9**
Hydroalcoholic	**7**	**0.11 0.28 0.46 0.56 0.62 0.72 0.83**	**59.7**	**1775.1**

Fig. No: 49 HPTLC FINGER PRINT OF PET-ETHER EXTRACT AT 366 nm

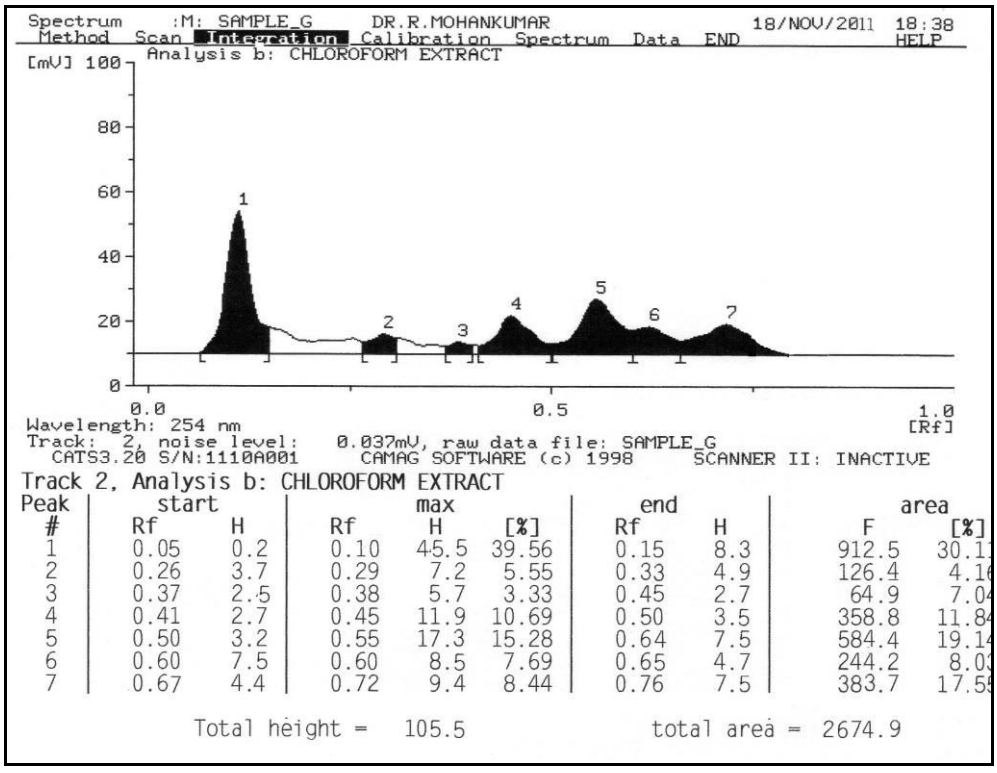


Track 1.Analysis a:PET-ETHER EXTRACT

Peak	start		max			end		area	
#	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.07	2.3	0.11	45.8	22.37	0.15	3.2	911.9	13.46
2	0.27	9.0	0.29	13.8	6.74	0.34	9.2	479.6	7.16
3	0.41	10.4	0.44	24.3	11.87	0.48	10.6	710.7	10.46
4	0.48	10.6	0.54	32.9	16.04	0.59	16.2	1508.1	22.50
5	0.59	16.2	0.64	22.1	10.78	0.66	20.4	828.5	12.36
6	0.66	20.4	0.71	33.9	16.53	0.74	27.6	1106.8	16.51

Total height = 172.8 total area = 5545.6

Fig. No: 50 HPTLC FINGER PRINT OF CHLOROFORM EXTRACT AT 366 nm

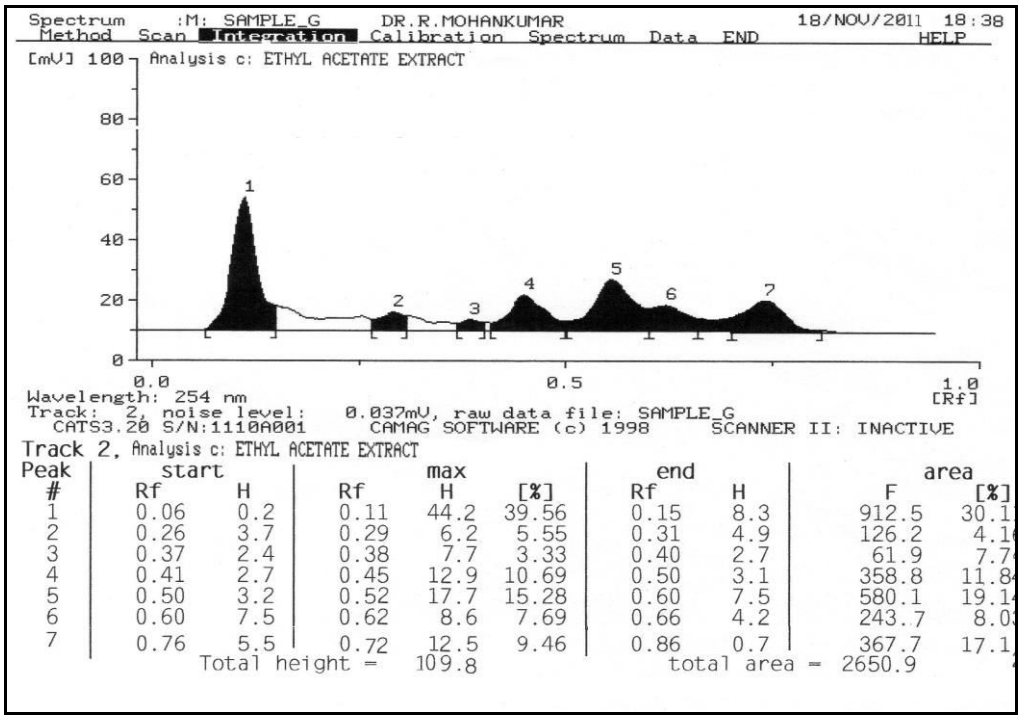


Track 2, Analysis b: CHLOROFORM EXTRACT

Peak	start		max			end		area	
#	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.05	0.2	0.10	45.5	39.56	0.15	8.3	912.5	30.1
2	0.26	3.7	0.29	7.2	5.55	0.33	4.9	126.4	4.1
3	0.37	2.5	0.38	5.7	3.33	0.45	2.7	64.9	7.0
4	0.41	2.7	0.45	11.9	10.69	0.50	3.5	358.8	11.8
5	0.50	3.2	0.55	17.3	15.28	0.64	7.5	584.4	19.1
6	0.60	7.5	0.60	8.5	7.69	0.65	4.7	244.2	8.0
7	0.67	4.4	0.72	9.4	8.44	0.76	7.5	383.7	17.5

Total height = 105.5 total area = 2674.9

Fig. No: 51 HPTLC FINGER PRINT OF ETHYLACETATE EXTRACT AT 366 nm

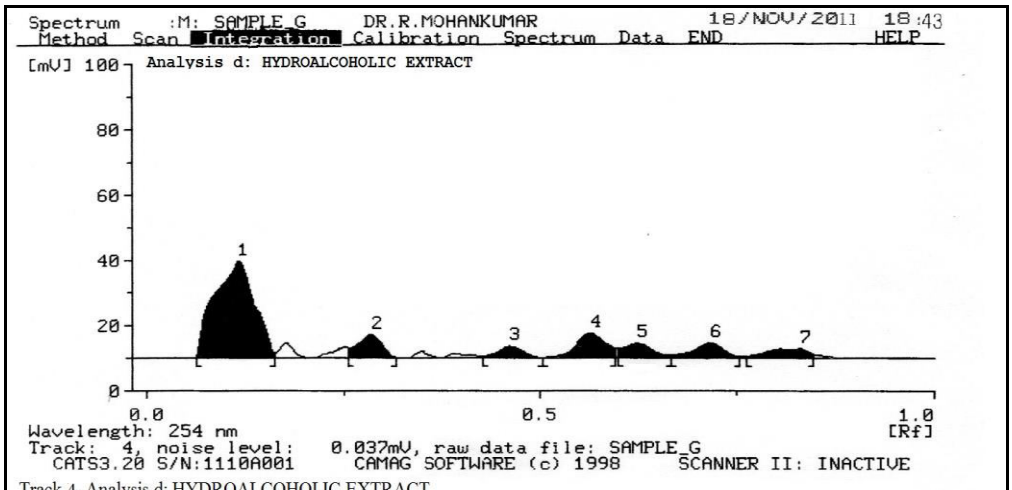


Track 2, Analysis c: ETHYL ACETATE EXTRACT

Peak	start		max			end		area	
#	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.06	0.2	0.11	44.2	39.56	0.15	8.3	912.5	30.1
2	0.26	3.7	0.29	6.2	5.55	0.31	4.9	126.2	4.1
3	0.37	2.4	0.38	7.7	3.33	0.40	2.7	61.9	7.7
4	0.41	2.7	0.45	12.9	10.69	0.50	3.1	358.8	11.8
5	0.50	3.2	0.52	17.7	15.28	0.60	7.5	580.1	19.1
6	0.60	7.5	0.62	8.6	7.69	0.66	4.2	243.7	8.0
7	0.76	5.5	0.72	12.5	9.46	0.86	0.7	367.7	17.1

Total height = 109.8 total area = 2650.9

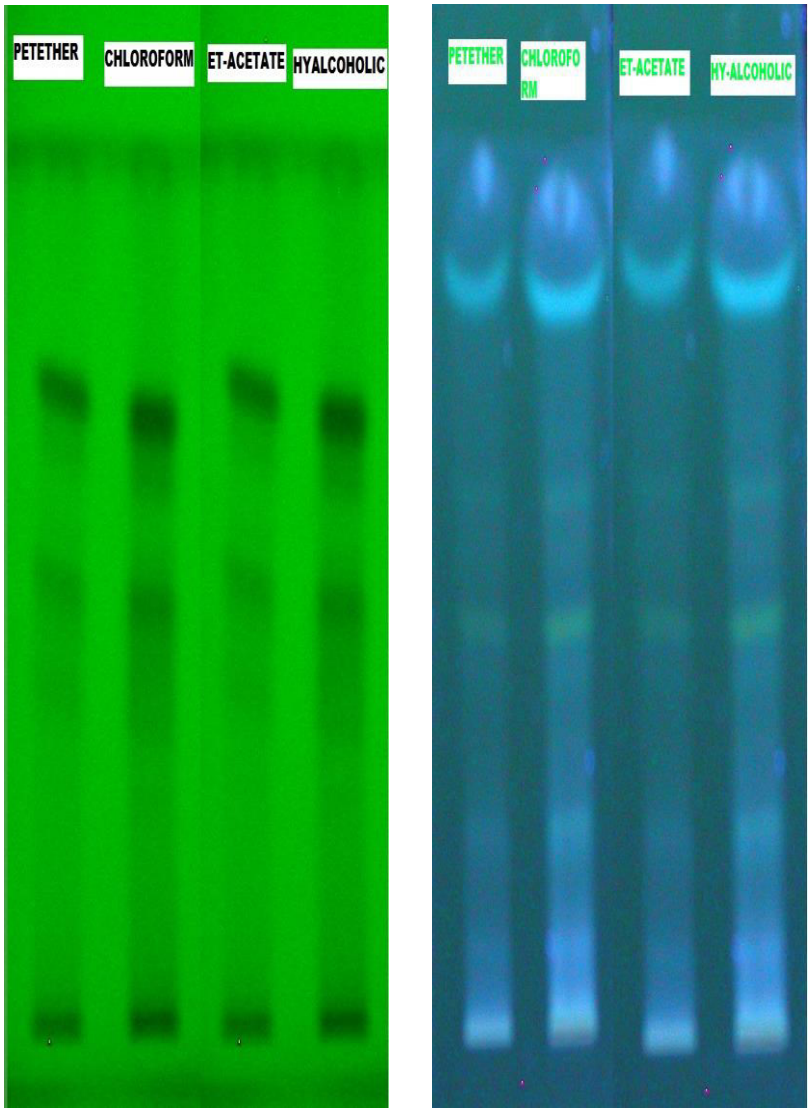
Fig. No: 52 HPTLC FINGER PRINT OF HYDROALCOHOLIC EXTRACT AT 366 nm



Track 4. Analysis d: HYDROALCOHOLIC EXTRACT

Peak	start		max			end		area	
#	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.06	0.0	0.11	29.7	49.77	0.16	1.6	1027.1	57.86
2	0.25	3.1	0.28	7.2	12.02	0.31	0.2	148.5	8.37
3	0.43	0.6	0.46	3.5	5.95	0.50	0.1	76.5	4.31
4	0.50	0.1	0.56	7.5	12.54	0.59	3.1	194.5	10.96
5	0.60	3.0	0.62	4.4	7.39	0.67	0.8	112.5	6.34
6	0.67	0.8	0.72	4.6	7.69	0.75	0.4	118.1	6.65
7	0.76	0.5	0.83	2.8	4.64	0.85	1.2	97.9	5.52

Total height = 59.7 total area = 1775.1



254 nm **366nm**

Fig. No: 53 HPTLC for *Nymphaea nouchali* at 254nm and 366nm

7.11.6. Characterization of Isolated Compounds

The chemical investigation of ethylacetate extracts of the flowers of ***Nymphaea pubescens*** and ***Nymphaea nouchali*** had isolated 2 compounds each (EANP-1, 2 and EANN-1, 2). The characterization the compounds were given below.

The isolated compounds were characterized using physical properties, chemical tests, m.p and spectral data especially IR, MASS and NMR.

i). Analytical Data of Isolated Compound-I

Physical and chemical properties of **Compound I - (EANP-1)**

Physical state	:	Cream coloured amorphous powder
Melting point	:	241-242°C
Molecular weight	:	456
Chemical test	:	It gave positive test for $FeCl_3$, lead acetate, test for Schinoda of Flavonoids.
Eluent	:	50% Ethyl acetate in Hexane

This indicates the presence of tri flavonoids.

Molecular Formula : $C_{27}H_{30}O_{16}$

ii). Spectral Data

UV Spectrum : The ethylacetate extract of the sample is 10µg/mL, should be maximum absorbance at 214.0 nm **(Fig. No: 55).**

IR Spectrum : Indicated bands at 3132.79 (Phenolic-OH stretch), 2924.52 (Aromatic C-H stretching) 1732.73 (Aromatic C=O stretching) 1400.07 (Aromatic C=C stretching). **(Fig. No: 56).**

^{1}H & ^{13}C NMR Data : ^{1}HNMR (400MHz, MeOD, δ ppm) has given signals at δ 7.6 (1H, doublet, J = 2 Hz), δ 7.5(1H, doublet, J = 2.4 Hz) and at δ6.7 (1H, doublet, J = 8.4 Hz) and at δ 6.3(1H, doublet, J = 2 Hz) and at δ 6.1(1H, doublet, J = 2 Hz) and at δ 3.15 -3.8 (11 H, sugar protons) which are characteristic for Rutin. **(Fig. No: 57, 58).**

Mass Spectrum : In the mass spectrum $[MH]^-$ ion peak was observed at m/z value 610.4 **(Fig. No: 59).**

The above spectral data (MASS, NMR) is tallied well with that of Rutin.

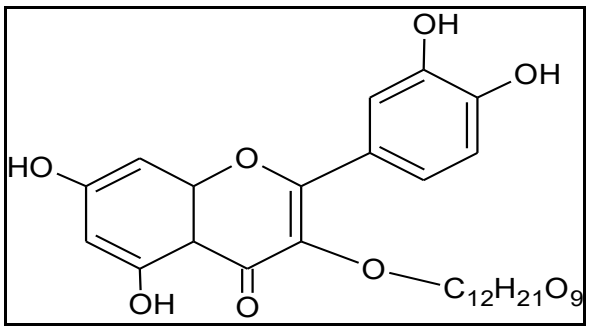
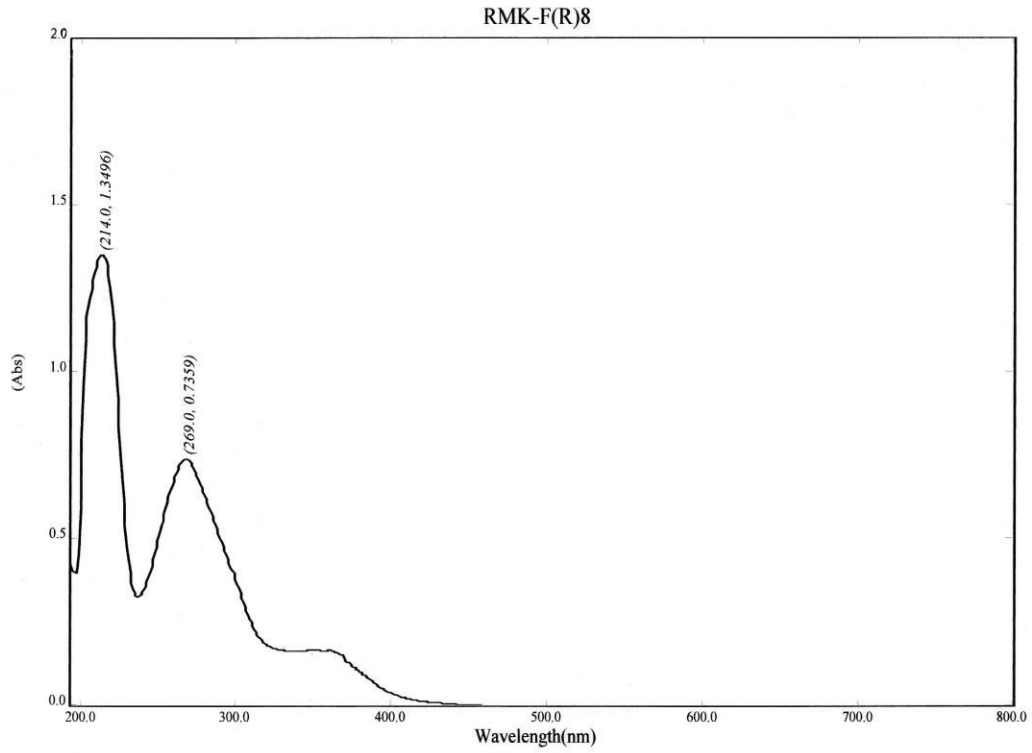


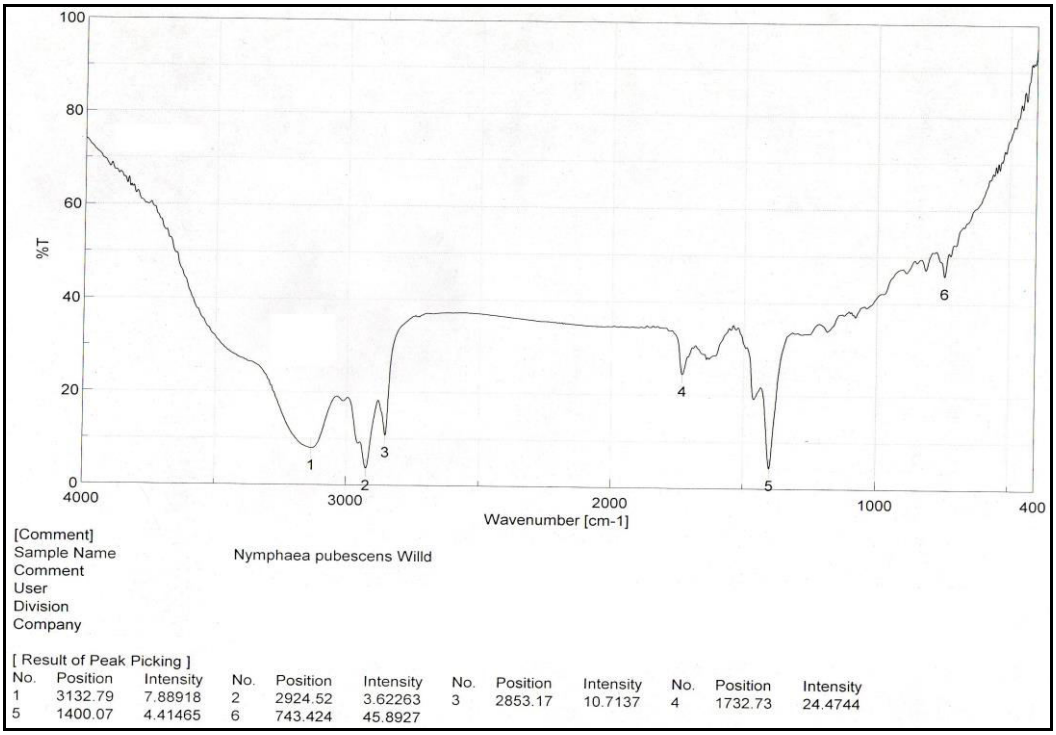
Fig. No: 54 Rutin (EANP-1)

Fig. No: 55 UV Spectrum of Rutin (EANP-1)



Scan Range:180.0-1100.0nm
Scan Step: 1.0nm

Fig. No: 56 IR Spectrum of Rutin (EANP-1)



[Comment]
Sample Name Nymphaea pubescens Willd
Comment
User
Division
Company

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3132.79	7.88918	2	2924.52	3.62263	3	2853.17	10.7137	4	1732.73	24.4744
5	1400.07	4.41465	6	743.424	45.8927						

Fig. No: 57 ^{1}H NMR Spectrum of Rutin (EANP-1)

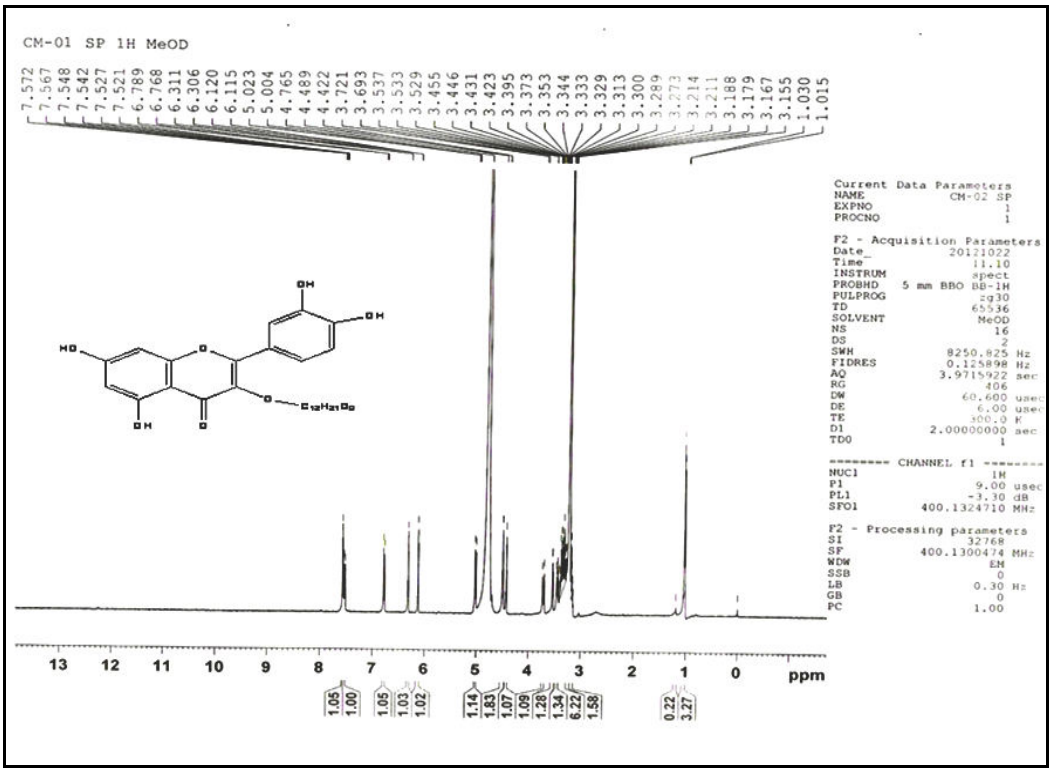


Fig. No: 58 ^{13}C NMR Spectrum of Rutin (EANP-1)

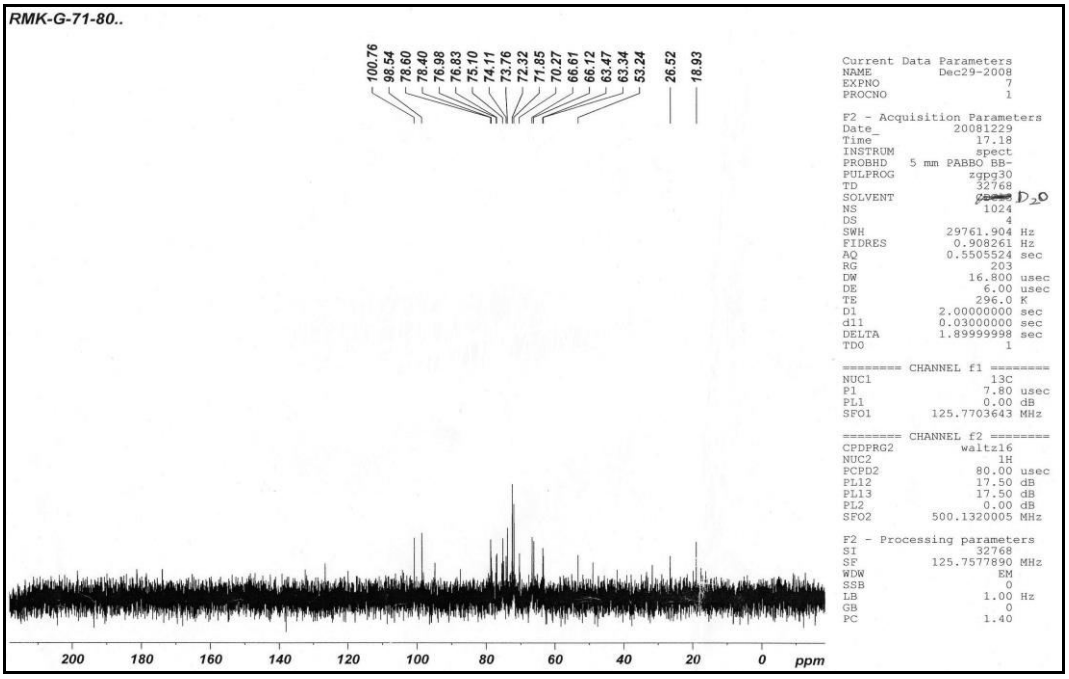
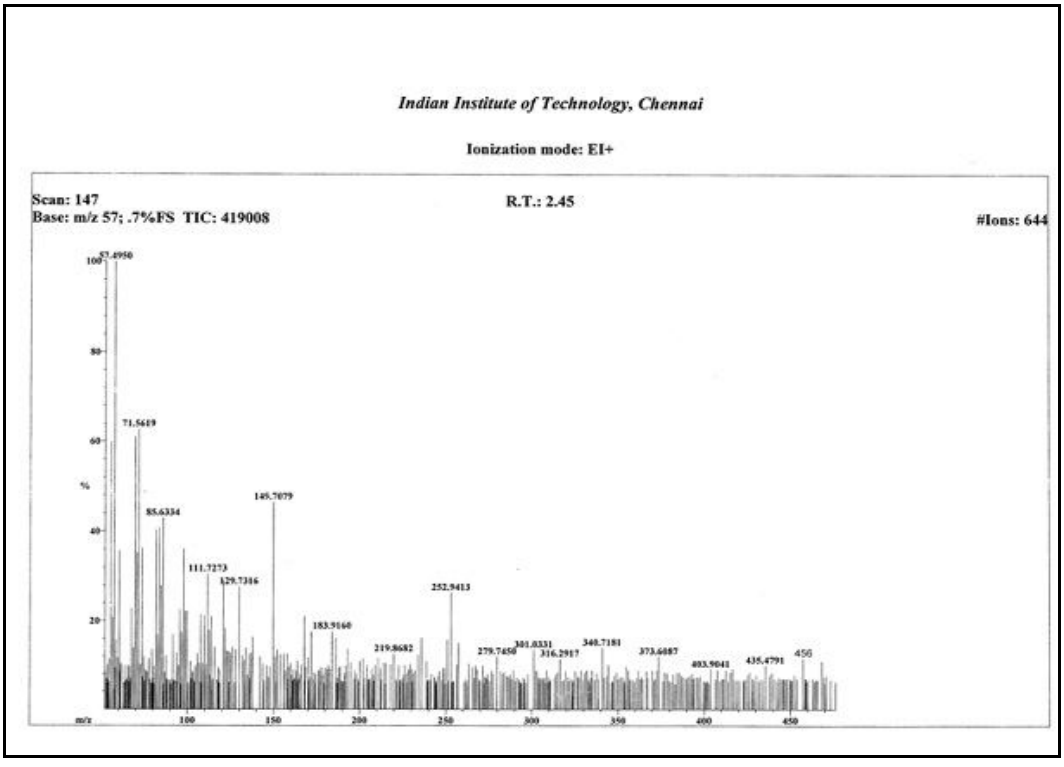


Fig. No: 59 MASS Spectrum of Rutin (EANP-1)



ii). Analytical Data of Isolated Compound-II

Physical and chemical properties of **compound II - (EANP-2)**

Physical state	:	Yellowish green needle shaped crystals
Melting point	:	284-286°C
Molecular weight	:	456
Chemical test	:	It gave positive test for Borntragers (Anthraquinone) test
Eluent	:	75% Ethyl acetate in Hexane

This indicates the presence of tri terpenoids.

Molecular Formula : $C_{14}H_8O_6$

ii). Spectral Data

UV Spectrum : The ethylacetate extract of the sample is 10µg/mL, should be maximum absorbance at 214.0 nm **(Fig. No: 61).**

IR Spectrum : Indicated bands at 3426.61 (Phenolic -OH), 2925 (Aromatic C-H Stretch), 1721 (C=O Stretch) **(Fig. No: 62).**

^{1}H & ^{13}CNMR Data : ^{1}HNMR (400MHz, MeOD, δ ppm) has given signals at δ 7.4 (1H, Characteristic for α type of proton in Anthraquinone) and at δ 7.9 (1H, Characteristic for β type of protons in Anthraquinone) **(Fig. No: 63, 64).**

Mass Spectrum : In the mass spectrum $[MH]^-$ ion peak was observed at m/z value 271.2**(Fig. No: 65).**

The above spectral data (IR, MASS, NMR) is tallied well with that of 1,3,6,8 tetra hydroxy Anthraquinone.

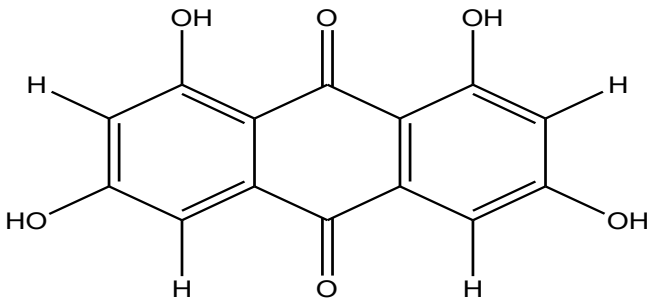


Fig. No: 60-1, 3, 6, 8 Tetra Hydroxy Anthraquinone (EANP-2)

Fig. No: 61 UV Spectrum of 1, 3, 6, 8 Tetra Hydroxy Anthraquinone (EANP-2)

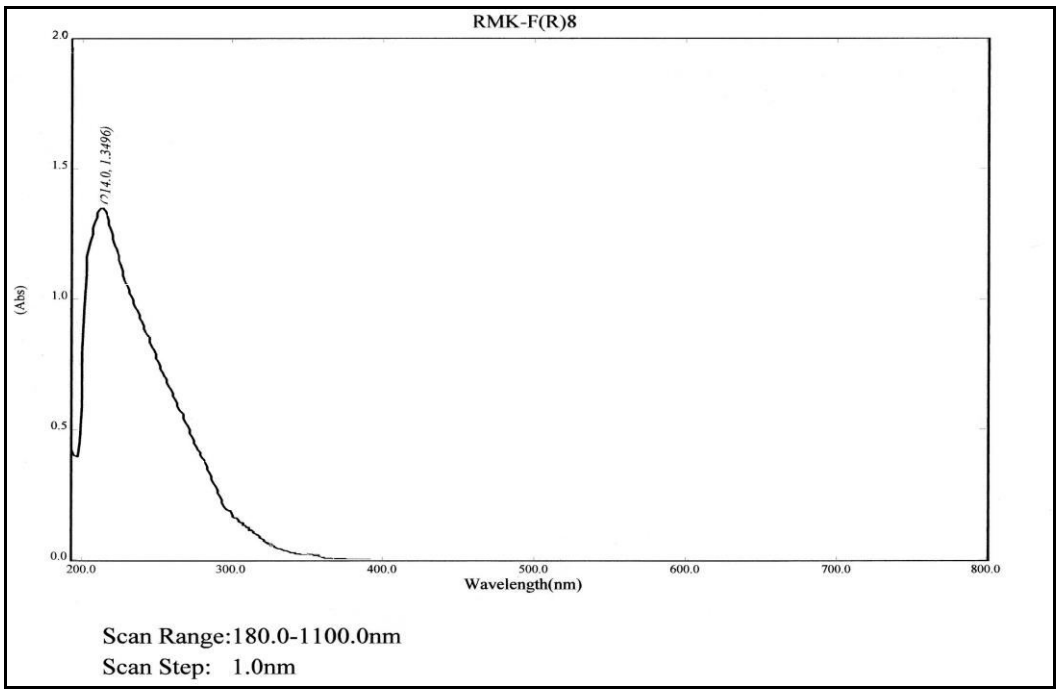


Fig. No: 62 IR Spectrum of 1, 3, 6, 8 Tetra Hydroxy Anthraquinone (EANP-2)

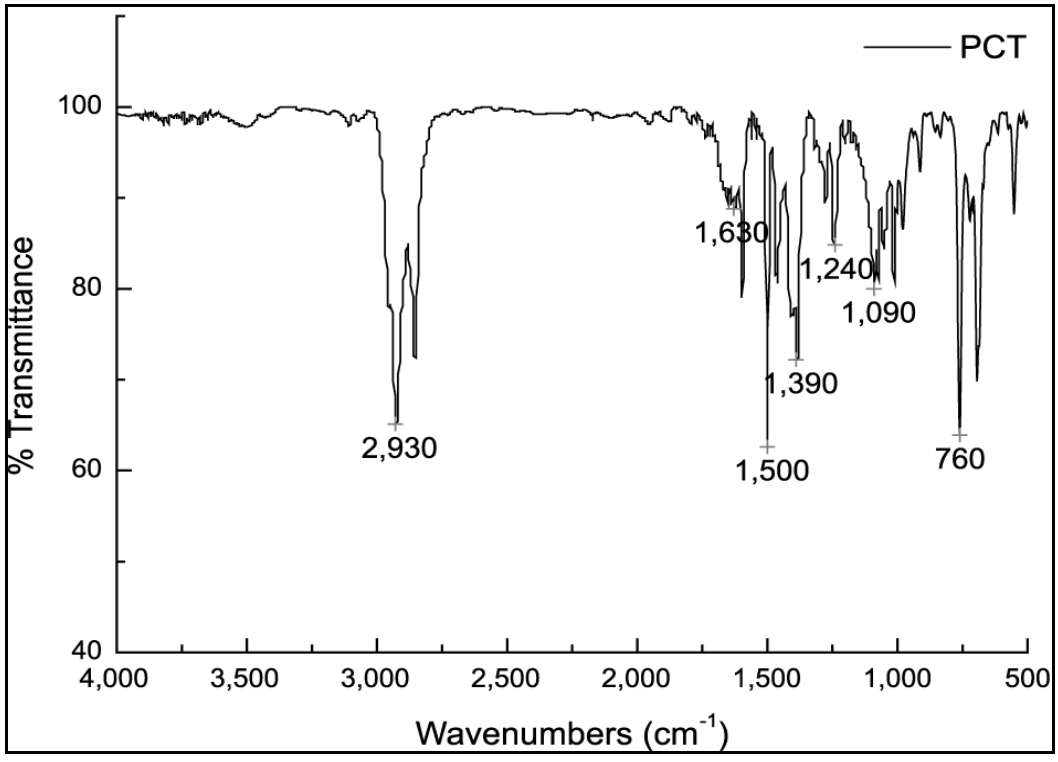


Fig. No: 63 ^{1}H NMR Spectrum of 1, 3, 6, 8 Tetra Hydroxy Anthraquinone (EANP-2)

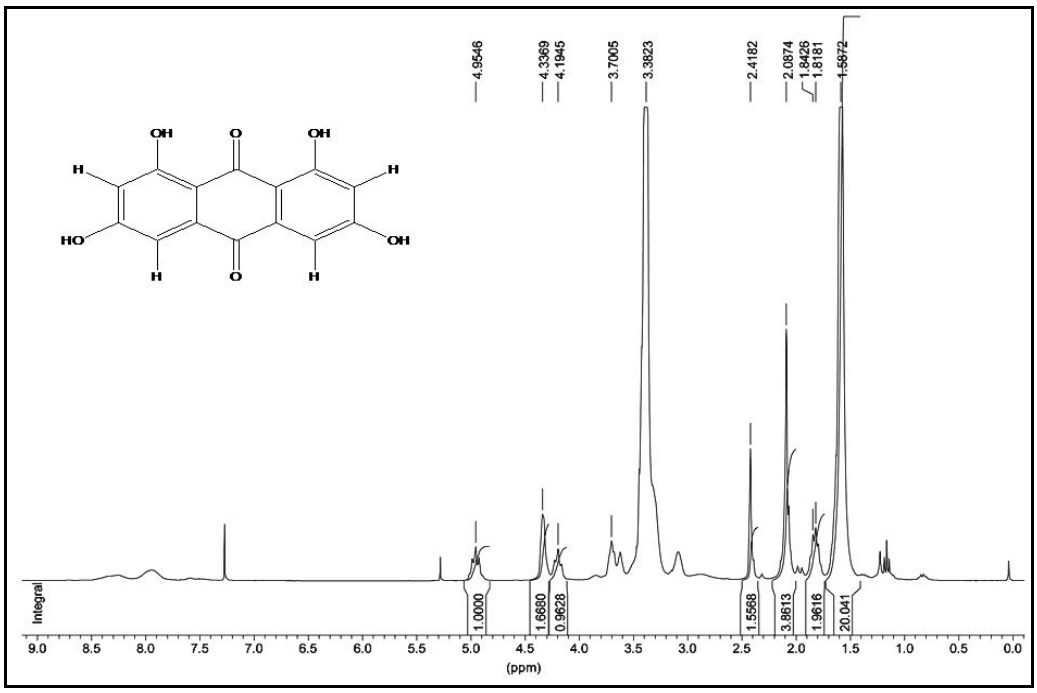


Fig. No: 64 ^{13}C NMR Spectrum of 1, 3, 6, 8 Tetra Hydroxy Anthraquinone (EANP-2)

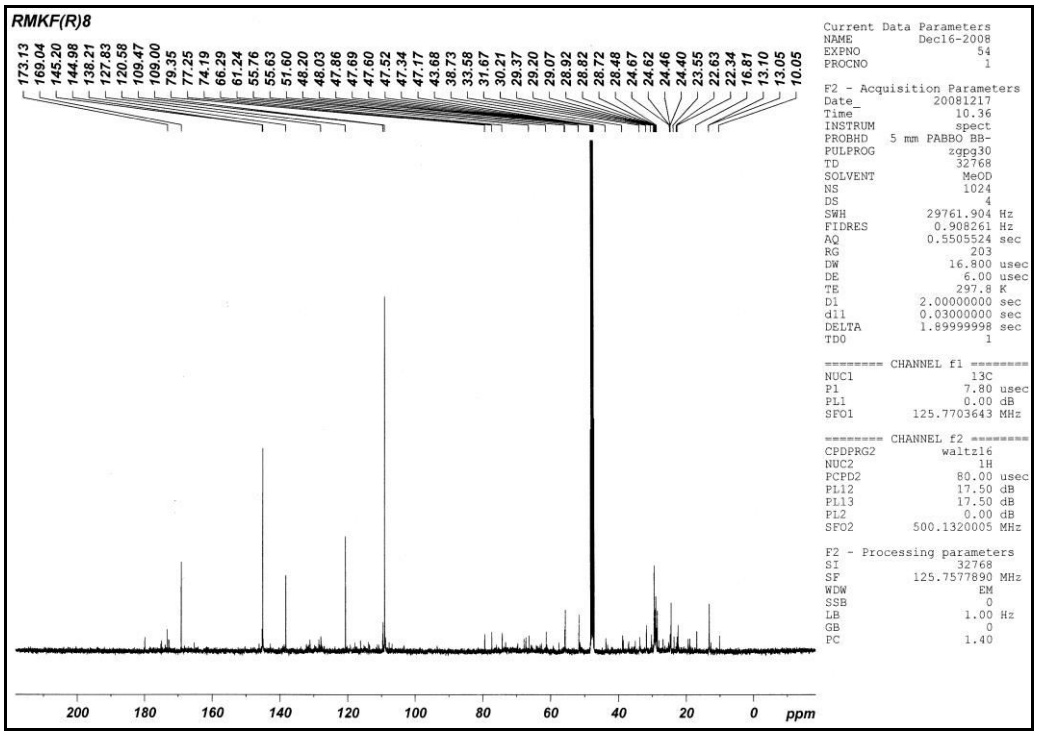
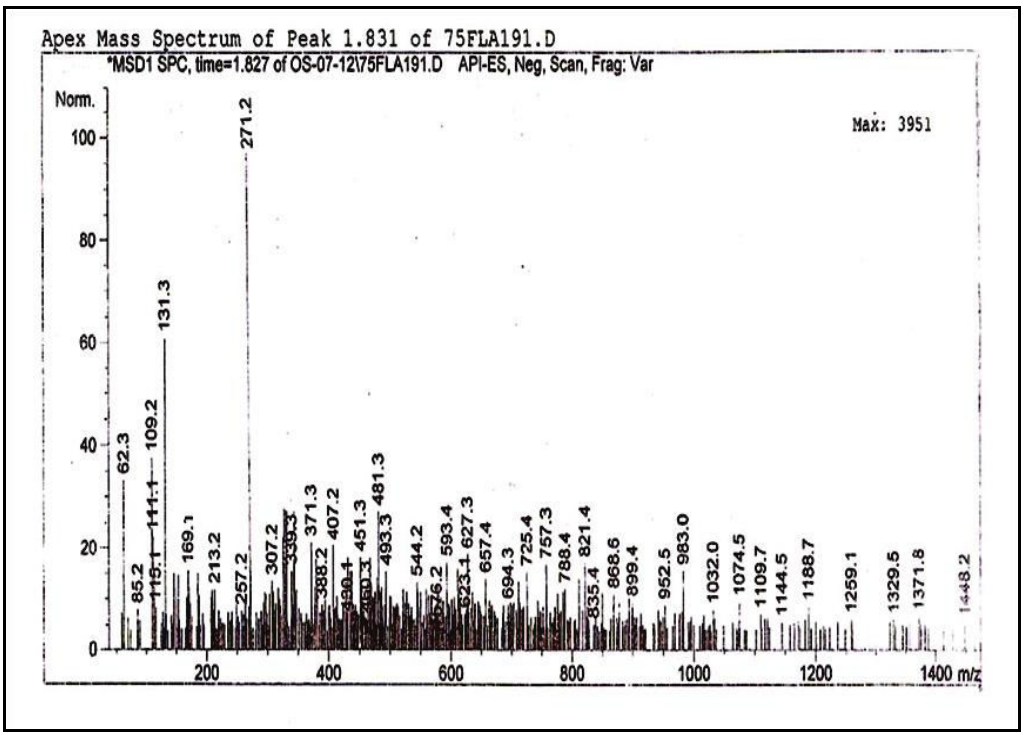


Fig. No: 65 MASS Spectrum of 1, 3, 6, 8 Tetra Hydroxy Anthraquinone (EANP-2)



iii). Analytical Data of Isolated Compound-I

Physical and chemical properties of **Compound I - (EANN-1)**

The isolated **Compound I - (EANN-1)** is similar to that of **compound-I** of **EANP-I,** having similar nature of isolate and the R_f value is also compared and found to be same.

iv). Analytical Data of Isolated Compound-II

Physical and chemical properties of **Compound II - (EANN-2)**

Physical state	:	Fluorescent yellow powder
Melting point	:	235-236°C
Molecular weight	:	432
Chemical test	:	It gave positive test for Schinoda of Flavonoids.
Eluent	:	50% Ethyl acetate in Hexane

This indicates the presence of flavone.

Molecular Formula : $C_{21}H_{20}O_{10}$

ii). Spectral Data

UV Spectrum : The ethylacetate extract of the sample is 10µg/mL, should be maximum absorbance at 220.0 nm **(Fig. No: 67).**

IR Spectrum : The IR spectrum showed bands at 3127 cm^{-1}, for the presence of hydroxyl and 1642 cm^{-1}, (carbonyl group), 2924 cm^{-1} (C-O Strech), Bands in 1727 cm^{-1} range are typical of a flavone skeleton. **(Fig. No: 68).**

^{1}H NMR Data : The H^1 NMR spectrum (400MHz, MeOD, δ ppm) showed the presence of a downfield methylene proton (δ 6.609, 1H, s) characteristic of H-3 of flavonoids (δ 6.410, 1H, s) assigned to H-6 of ring A of 5,7-dihydroxy flavonoids. The ^{1}H NMR spectrum also showed the presence of a signal at δ 8.014 (d, 2H, J = 11.6Hz), δ 6.819 (d, 2H, J = 10.6 Hz) characteristic of 1, 4 – disubstituted phenyl unit. The ^{1}H NMR spectrum also showed the presence of a signal at δ 5.300 (1H, d, J= 4.1Hz) characteristic H of anomeric carbon , δ 3.898 (m) characteristic of H-6 , δ 3.524 to 3.797 (m) characteristic of H-3,45 of sugar moiety **(Fig. No: 69).**

Mass Spectrum : The Mass spectrum showed ESI-MS $(M+H)^+$ molecular ion peak at m/z 433.1, The molecular formula $C_{21}H_{19}O_{10}$ was deduced from its positive ion MS $(M+H)^+$ 433. The structure of **EANN-2** was established as 5, 7-dihydroxy-2-(4-hydroxyphenyl)-4*H*-chromen-4-one -8-glucoside. **(Fig. No: 70).**

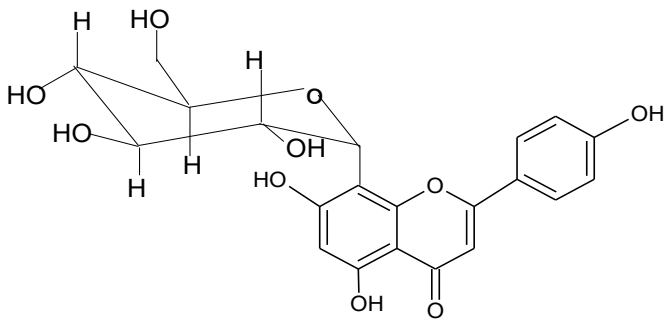


Fig. No: 66 Apigenin -8-C Glycoside (EANN-2)

Fig. No: 67 UV Spectrum of Apigenin -8-C Glycoside (EANN-2)

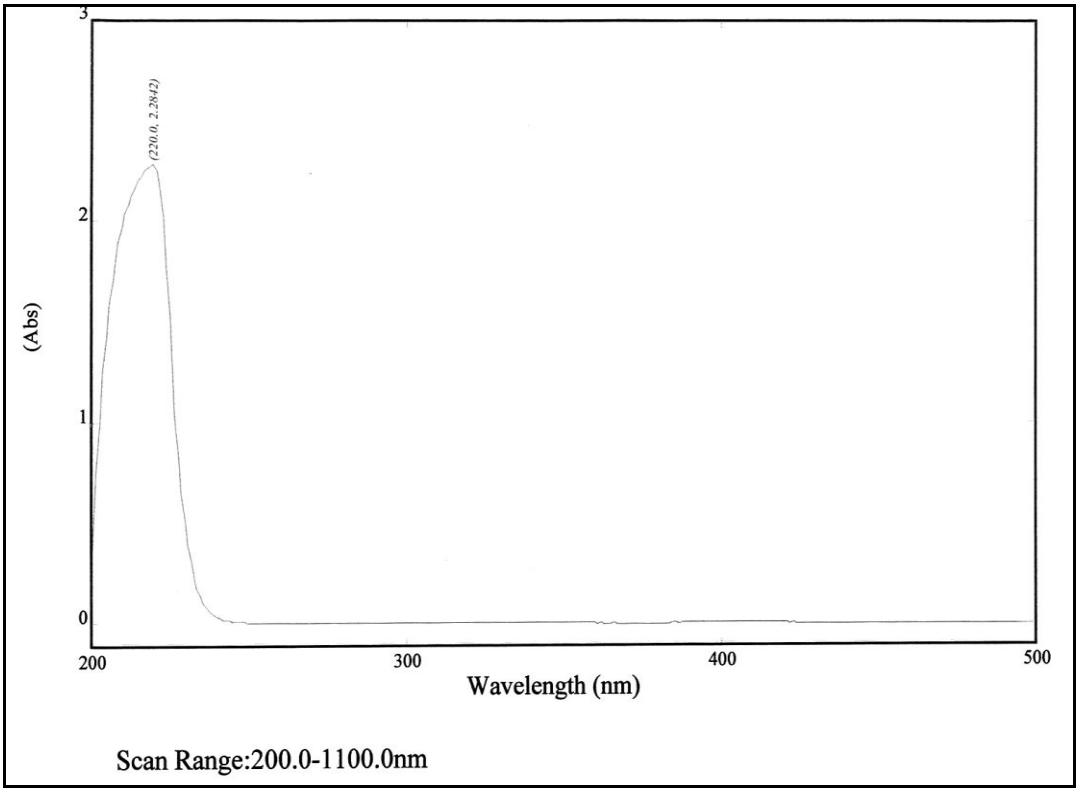


Fig. No: 68 IR Spectrum of Apigenin -8-C Glycoside (EANN-2)

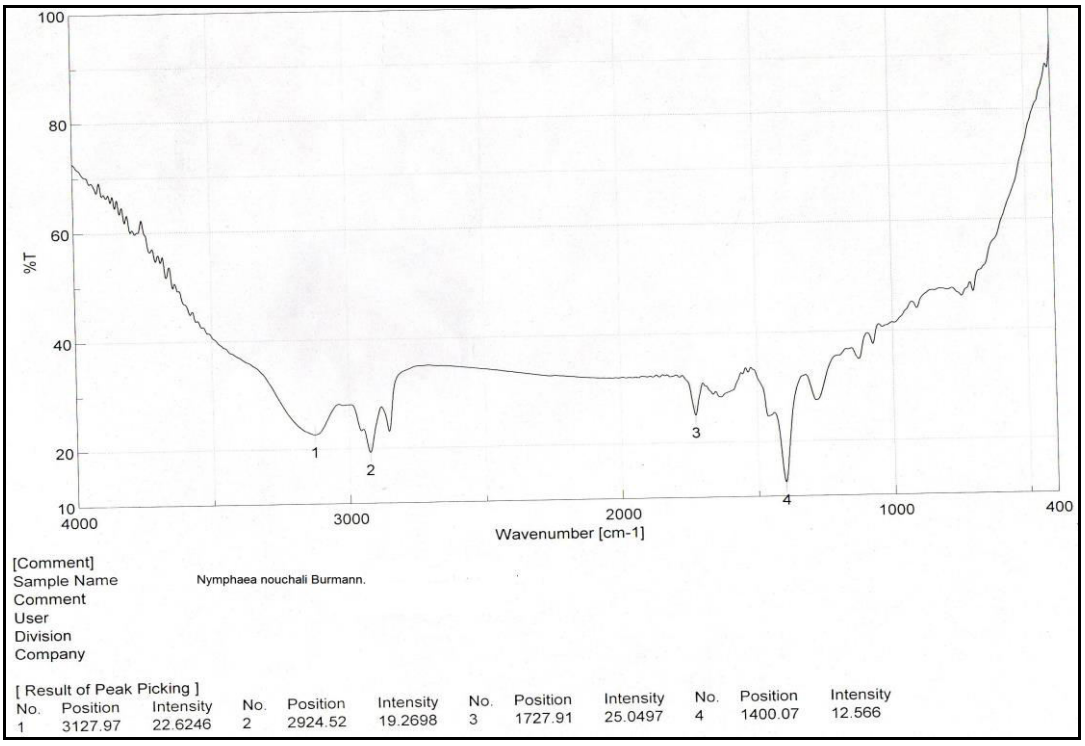


Fig. No: 69 ^{1}H NMR Spectrum of Apigenin -8-C Glycoside (EANN-2)

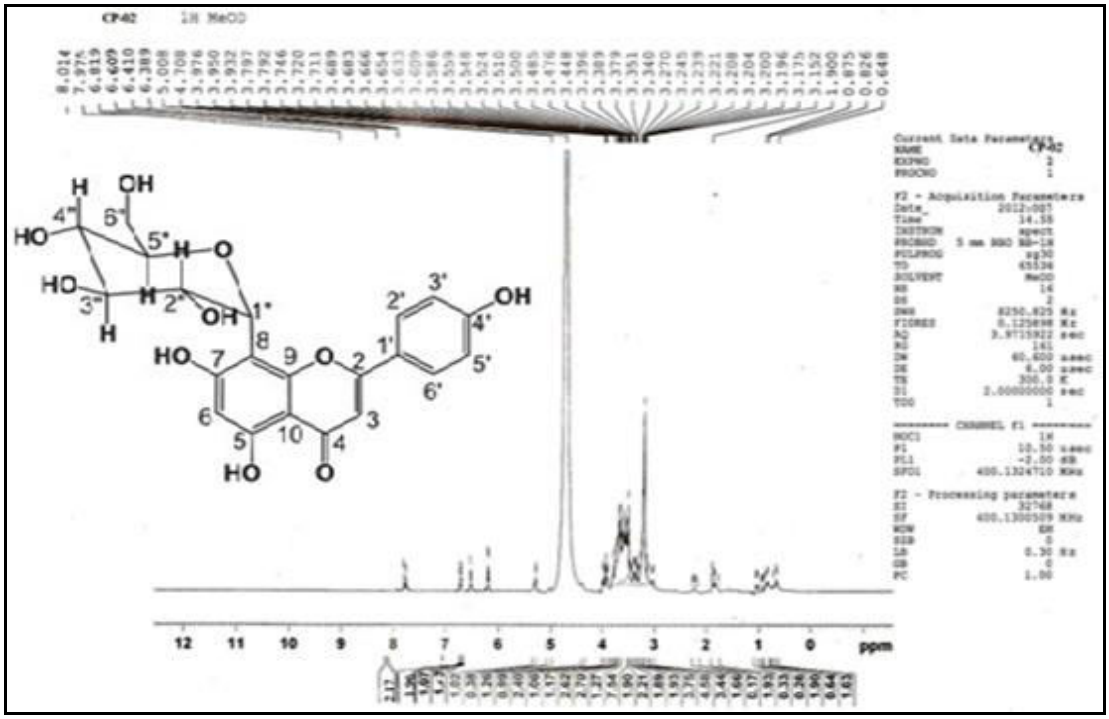
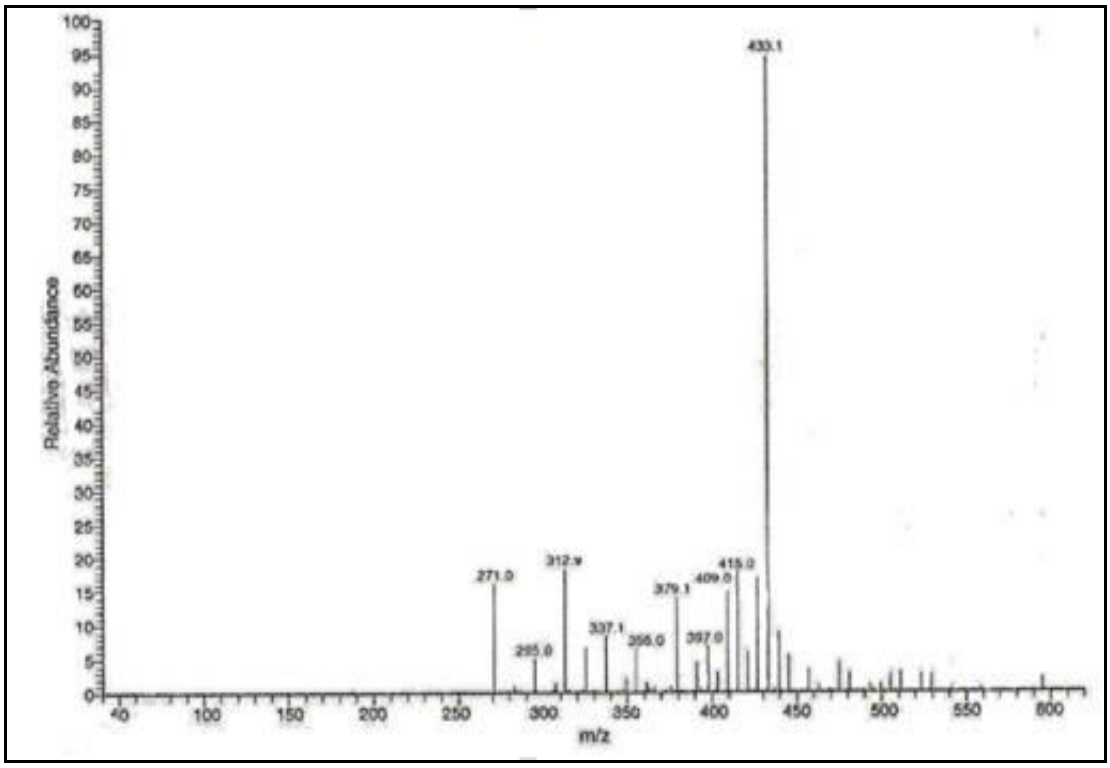


Fig. No: 70 MASS Spectrum of Apigenin -8-C Glycoside (EANN-2)



7.11.7. *In-vitro* Antioxidant Activity

The extracts of the flowers was studied for antioxidant potential. Four different *In-vitro* methods namely DPPH, FRAP, TRAP and ABTS scavenging activity methods were employed. Ascorbic acid was used as a standard.

7.11.7.1. DPPH Free Radical Scavenging Activity

In the DPPH Free radical scavenging activity was evaluated for the ethylacetate and hydroalcoholic extracts of the flowers of ***Nymphaea pubescens*** and ***Nymphaea nouchali*** and its free radical scavenging activity is compared with ascorbic acid as standard compound. The scavenging effect increased with the increasing concentrations of test compounds. It exhibited a significant antioxidant activity in dose dependent manner. From the results of DPPH, it showed that the Hydro-alcoholic (70%v/v) extract of ***Nymphaea pubescens*** and Ethylacetate extract of ***Nymphaea nouchali*** compounds are effective antioxidant when compared to ascorbic acid. The IC_{50} was calculated for each extract with different doses as well as ascorbic acid as standard and summarized in **Table No: 17-20** and graphically represented in **Fig. No: 71-74.**

i). DPPH Radical Scavenging Activity of *Nymphaea pubescens*

Table No: 17 DPPH Radical Scavenging Activity of EAENP

Groups	Absorbance (Mean ± SEM)	% Decrease
Control	1.282 ± 0.003	---
Ascorbic acid 10 µg	0.108 ± 0.002***	91.57%
EAENP 10 µg	0.932 ± 0.003***	27.30%
EAENP 30 µg	0.705 ± 0.002***	45.00%
EAENP 50 µg	0.584 ± 0.002***	54.44%
EAENP 70 µg	0.409 ± 0.002***	68.09%
EAENP 100 µg	0.271 ± 0.003***	78.86%

Values are the mean ± S.E.M., n=3, ***Significant at **p<0.001** compared to control.
Standard: Ascorbic acid, **EAENP**: Ethylacetate extract of *Nymphaea pubescens*.

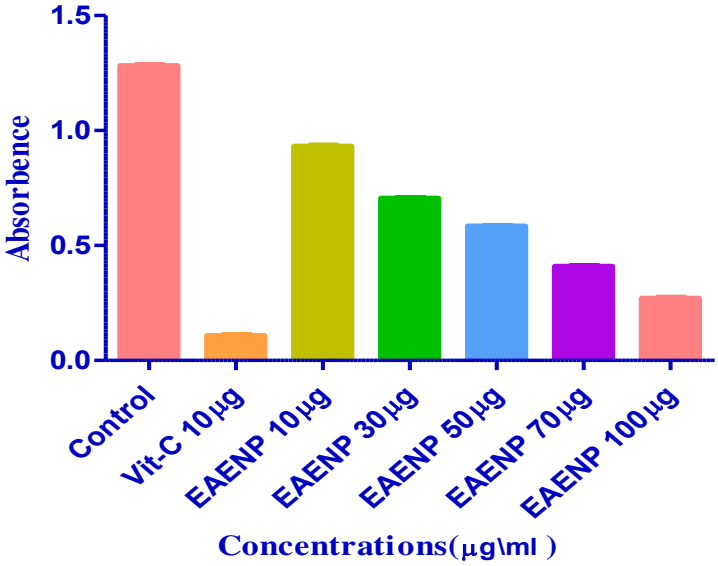


Fig. No: 71 DPPH Radical Scavenging Activity of EAENP

Table No: 18 DPPH Radical Scavenging Activity of HAENP

Groups	Absorbance (Mean ± SEM)	% Decrease
Control	1.282 ± 0.003	---
Ascorbic acid 10 µg	0.108 ± 0.002***	91.57%
HAENP 10 µg	1.109 ± 0.003***	13.49%
HAENP 30 µg	0.919 ± 0.002***	28.31%
HAENP 50 µg	0.689 ± 0.003***	46.25%
HAENP 70 µg	0.477 ± 0.003***	62.79%
HAENP 100 µg	0.309 ± 0.002***	75.89%

Values are the mean ± S.E.M., n=3, ***Significant at **p<0.001** compared to control. Standard: Ascorbic acid, **HAENP**: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

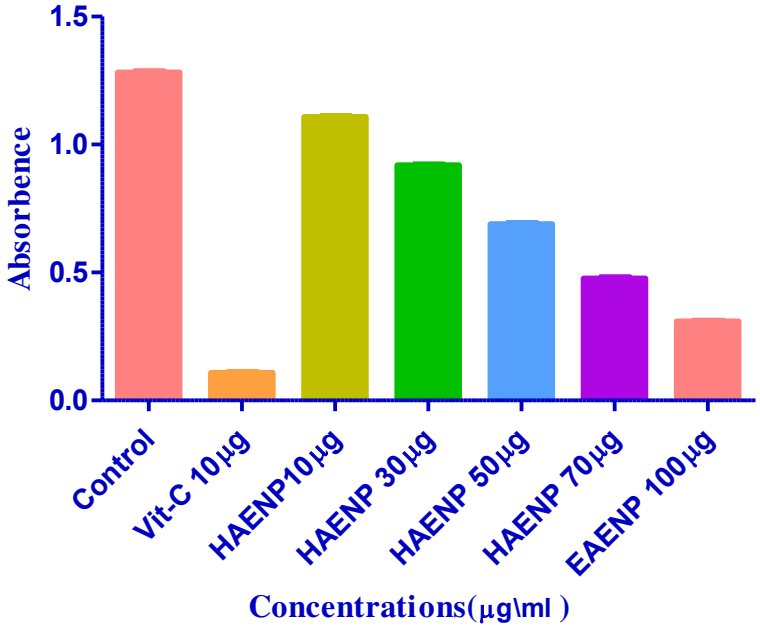


Fig. No: 72 DPPH Radical Scavenging Activity of HAENP

ii). DPPH Radical scavenging activity of *Nymphaea nouchali*

Table No: 19 DPPH Radical Scavenging Activity of EAENN

Groups	Absorbance (Mean ± SEM)	% Decrease
Control	1.282 ± 0.003	---
Ascorbic acid 10 µg	0.108 ± 0.002***	91.57%
EAENN 10 µg	1.022 ± 0.003***	20.28%
EAENN 30 µg	0.874 ± 0.002***	31.82%
EAENN 50 µg	0.619 ± 0.003***	51.71%
EAENN 70 µg	0.395 ± 0.003***	69.18%
EAENN 100 µg	0.248 ± 0.002***	80.65%

Values are the mean ± S.E.M., n=3, ***Significant at **p<0.001** compared to control.

Standard: Ascorbic acid, **EAENN**: Ethylacetate extract of *Nymphaea nouchali*.

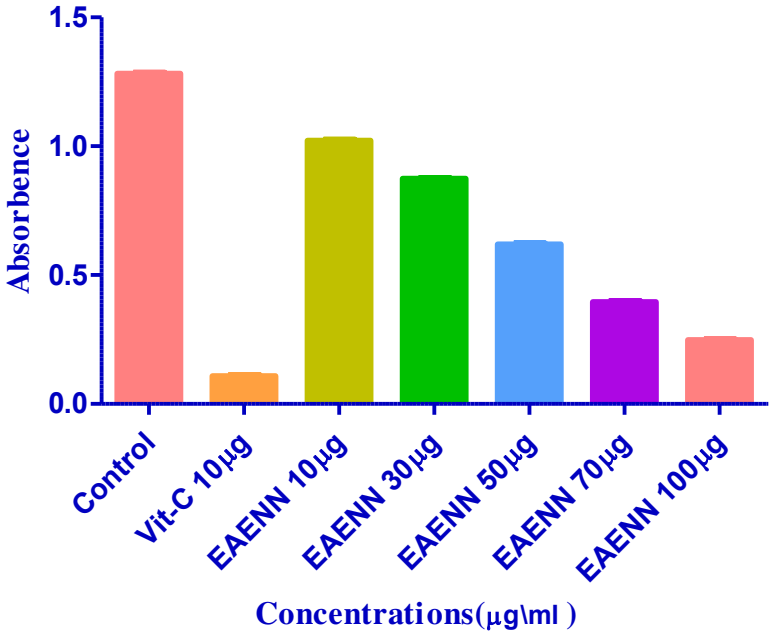


Fig. No: 73 DPPH Radical Scavenging Activity of EAENN

Table No: 20 DPPH Radical Scavenging Activity of HAENN

Groups	Absorbance (Mean ± SEM)	% Decrease
Control	1.282 ± 0.003	---
Ascorbic acid 10 µg	0.108 ± 0.002***	91.57%
HAENN 10 µg	0.979 ± 0.002***	23.63%
HAENN 30 µg	0.768 ± 0.003***	40.09%
HAENN 50 µg	0.585 ± 0.002***	54.36%
HAENN 70 µg	0.488 ± 0.003***	61.93%
HAENN 100 µg	0.357 ± 0.001***	72.15%

Values are the mean ± S.E.M., n=3, ***Significant at **p<0.001** compared to control. Standard: Ascorbic acid, **HAENN**: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

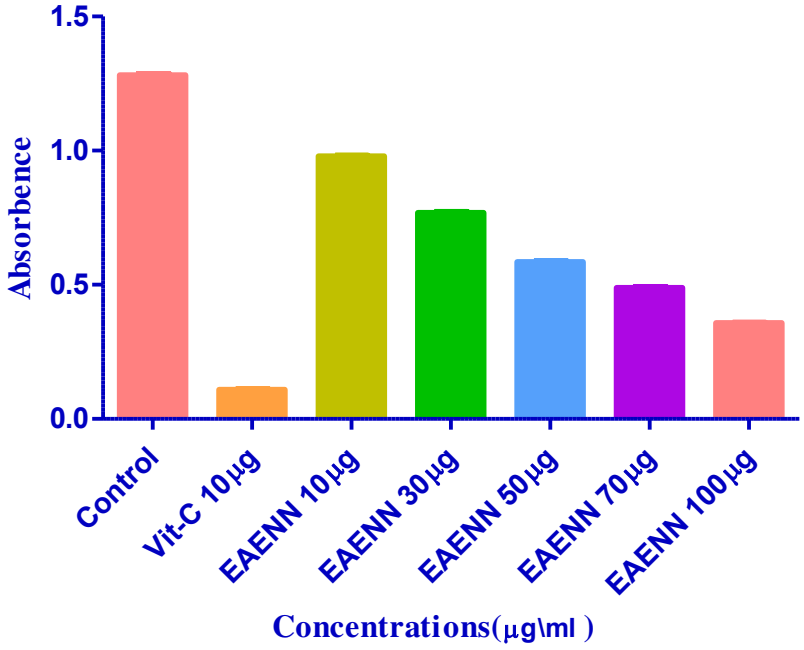


Fig. No: 74 DPPH Radical Scavenging Activity of HAENN

7.11.7.2. Ferric Reducing Ability of Plasma (FRAP) Radical Scavenging Activity

The Ferric Reducing Ability of Plasma (FRAP) radical scavenging activity of the flower extracts are concentration dependent and the IC_{50} value results are shown in **Table No: 21, 22** and **Fig. No: 75-78.**

Table No: 21 FRAP Free Radical Scavenging Activity of EAENP and HAENP

Test	Sample	% Inhibition for concentrations (Mean±SEM)				
		10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50 µg/mL
FRAP	EAENP	0.295±0.001	0.373±0.001	0.716±0.002	0.976±0.002	1.205±0.002
	HAENP	0.201±0.002	0.293±0.002	0.405±0.002	0.668±0.001	0.834±0.001

Values are the mean ± S.E.M., n=3, **EAENP**: Ethylacetate extract of *Nymphaea pubescens*, **HAENP**: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

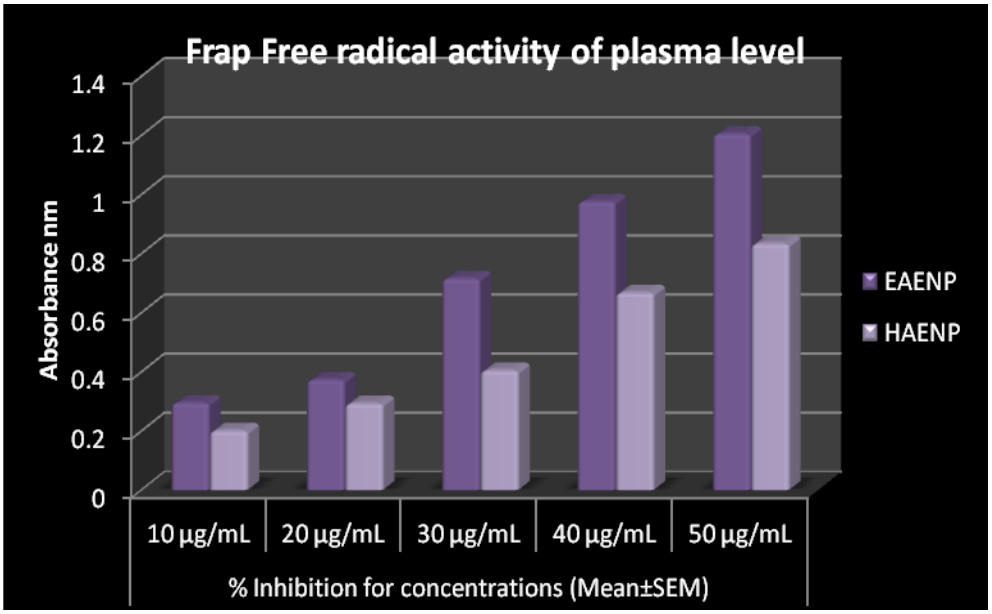


Fig. No: 75 FRAP Free Radical Scavenging Activity of EAENP and HAENP

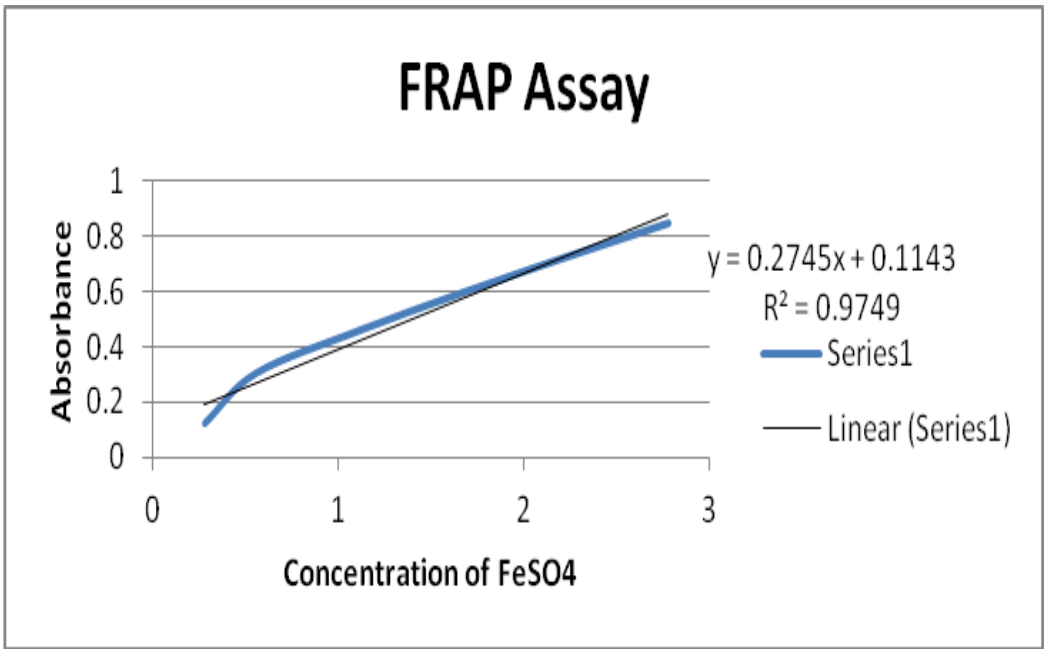


Fig. No: 76 Linearity of FRAP Assay Method *Nymphaea pubescens*

ii). Ferric Reducing Ability of Plasma (FRAP) Radical Scavenging Activity *Nymphaea nouchali*

Table No: 22 FRAP Free Radical Scavenging Activity of EAENN and HAENN

Test	Sample	% Inhibition for concentrations (Mean±SEM)				
		10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50 µg/mL
FRAP	EAENN	0.245±0.002	0.398±0.001	0.590±0.002	0.852±0.011	1.103±0.002
	HAENN	0.189±0.002	0.363±0.002	0.487±0.001	0.788±0.001	1.024±0.002

Values are the mean ± S.E.M., n=3, **EAENN**: Ethylacetate extract of *Nymphaea nouchali,* **HAENN**: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

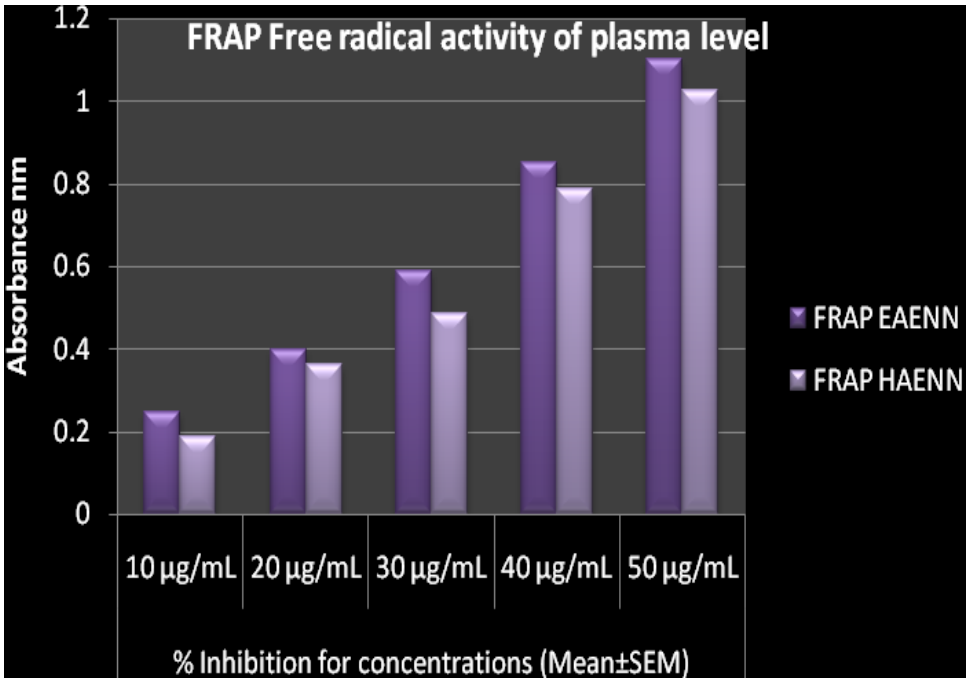


Fig. No: 77 FRAP Free Radical Scavenging Activity of EAENN and HAENN

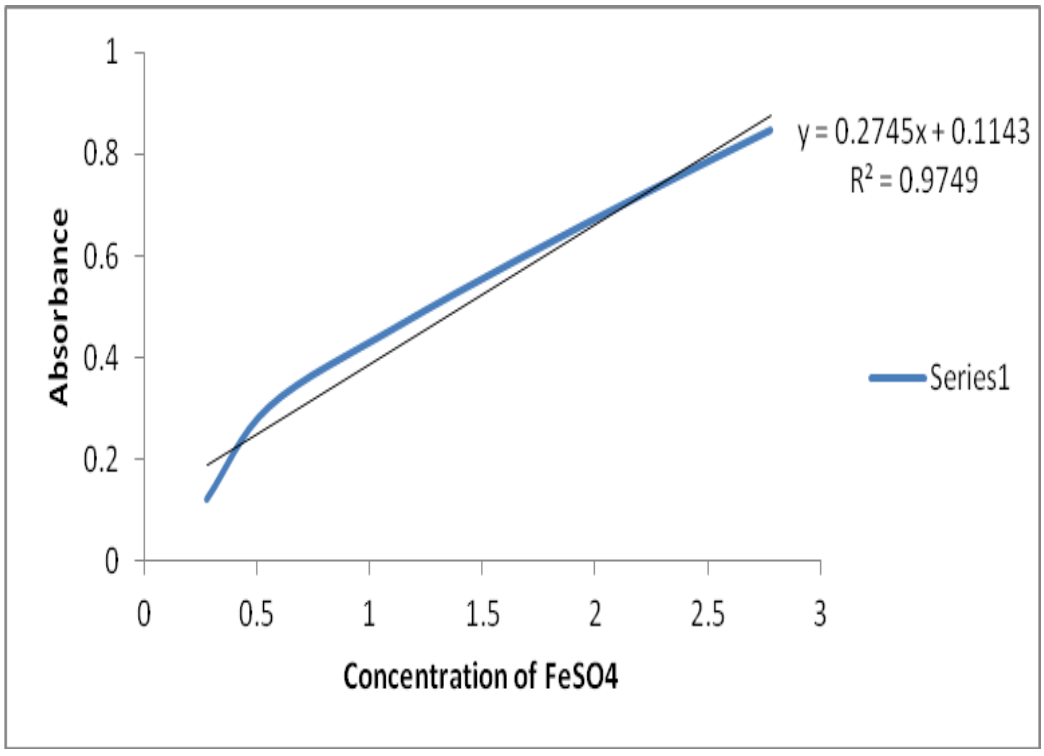


Fig. No: 78 Linearity of FRAP Assay Method *Nymphaea nouchali*

7.11.7.3. Dichlorofluorescin-Diacetate (DCF/AAPH assay) (TRAP)

The Ethylacetate and Hydroalcoholic extracts of ***Nymphaea pubescens*** and ***Nymphaea nouchali*** of **TRAP** radical scavenging activity with corresponding increase in its concentration and have good IC_{50} values which are furnished in **Table No: 23, 24** and **Fig. No: 79-82.**

Table No.: 23 DCF/AAPH Assay (TRAP) Radical Scavenging Activity of EAENP and HAENP

Test	Sample	% Inhibition for concentrations (Mean±SEM)					IC_{50} µg/mL
		10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50 µg/mL	
DCF/ AAPH Assay	EAENP	28.86±0.023	34.13±0.035	42.71±0.035	50.31±0.015	58.82±0.036	39.24µg/mL
	HAENP	22.52±0.097	29.50±0.031	36.84±0.034	45.73±0.017	51.90±0.017	46.93µg/mL

Values are the mean ± S.E.M., n=3, **EAENP**: Ethylacetate extract of *Nymphaea pubescens*, **HAENP**: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

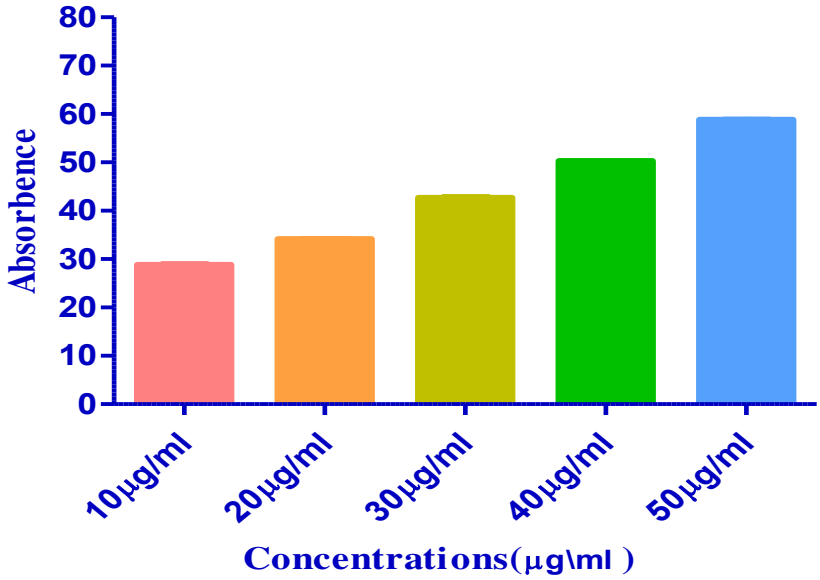


Fig. No: 79 DCF/AAPH Assay (TRAP) Free Radical Scavenging Activity of EAENP

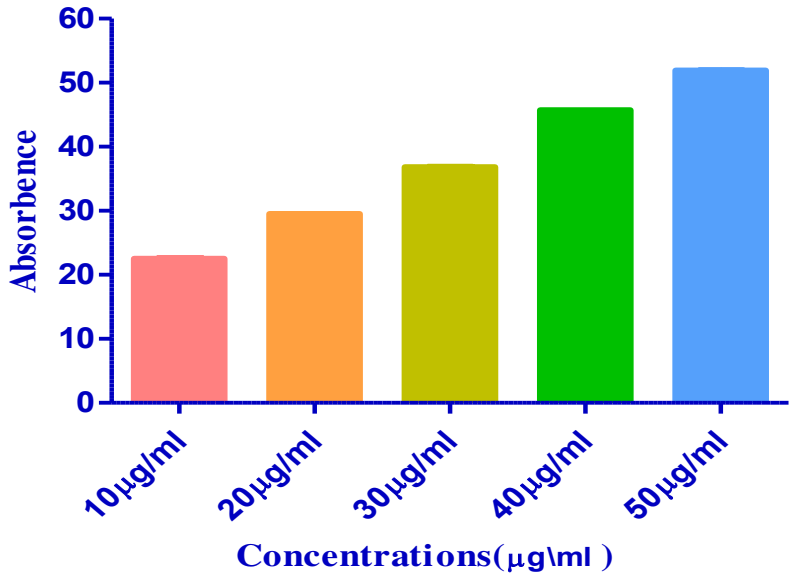


Fig. No: 80 DCF/AAPH Assay (TRAP) Free Radical Scavenging Activity of HAENP

ii). Dichlorofluorescin-diacetate (DCF/AAPH assay) (TRAP) Free Radical Scavenging Activity

Table No.: 24 DCF/AAPH Assay (TRAP) Radical Scavenging Activity of EAENN and HAENN

Test	Sample	% Inhibition for concentrations (Mean±SEM)					IC_{50} µg/mL
		10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50 µg/mL	
DCF/ AAPH Assay	EAENN	31.58±0.026	40.29±0.031	48.91±0.024	55.24±0.029	62.81±0.029	32.88µg/mL
	HAENN	24.47±0.044	33.59±0.028	39.72±0.030	47.32±0.011	56.22±0.023	42.60µg/mL

Values are the mean ± S.E.M., n=3, **EAENN**: Ethylacetate extract of *Nymphaea nouchali,* **HAENN**: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

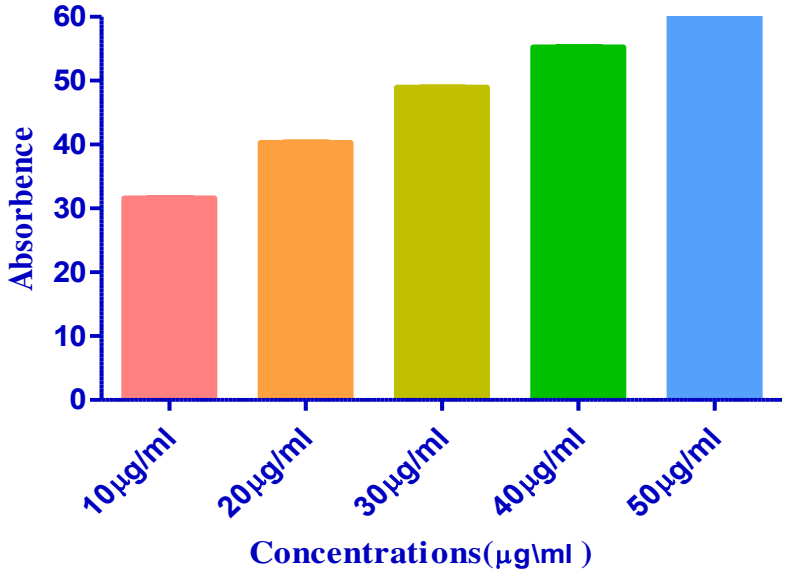


Fig. No: 81 DCF/AAPH Assay (TRAP) Free Radical Scavenging Activity of EAENN

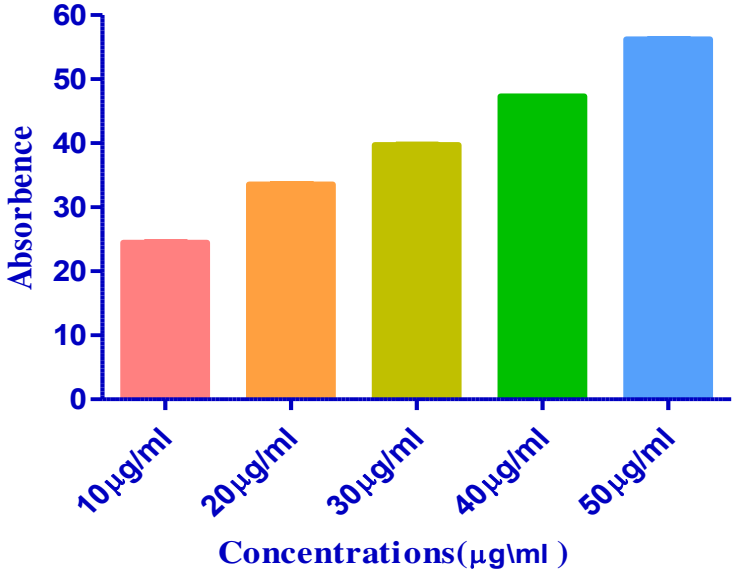


Fig. No: 82 DCF/AAPH Assay (TRAP) Free Radical Scavenging Activity of HAENN

7.11.7.4. ABTS radical scavenging activity

The Ethylacetate and Hydroalcoholic extracts of ***Nymphaea pubescens*** and ***Nymphaea nouchali*** showed ABTS scavenging activity with corresponding increase in its concentration and the results are showed in **Table No: 25, 26** and **Fig. No: 83-86.**

Table No.: 25 ABTS Free Radical Scavenging Activity of EAENP and HAENP

Test	Sample	% Inhibition for concentrations (mean±SEM)				
		10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50 µg/mL
ABTS	TROLOX	44.62±0.026	49.29±0.032	58.82±0.041	66.84±0.034	80.25±0.049
	EAENP	40.78±0.063	46.50±0.031	52.88±0.012	63.15±0.031	76.46±0.060
	HAENP	35.81±0.040	41.30±0.033	48.60±0.031	54.26±0.039	63.40±0.028

Values are the mean ± S.E.M., n=3, **EAENP**: Ethylacetate extract of *Nymphaea pubescens*, **HAENP**: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

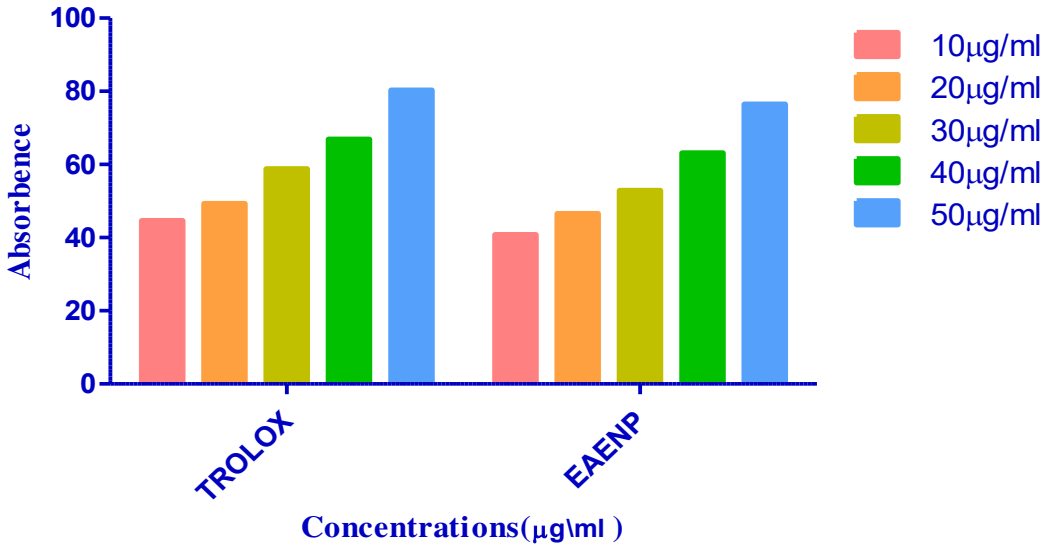


Fig. No: 83 ABTS Free Radical Scavenging Activity of EAENP

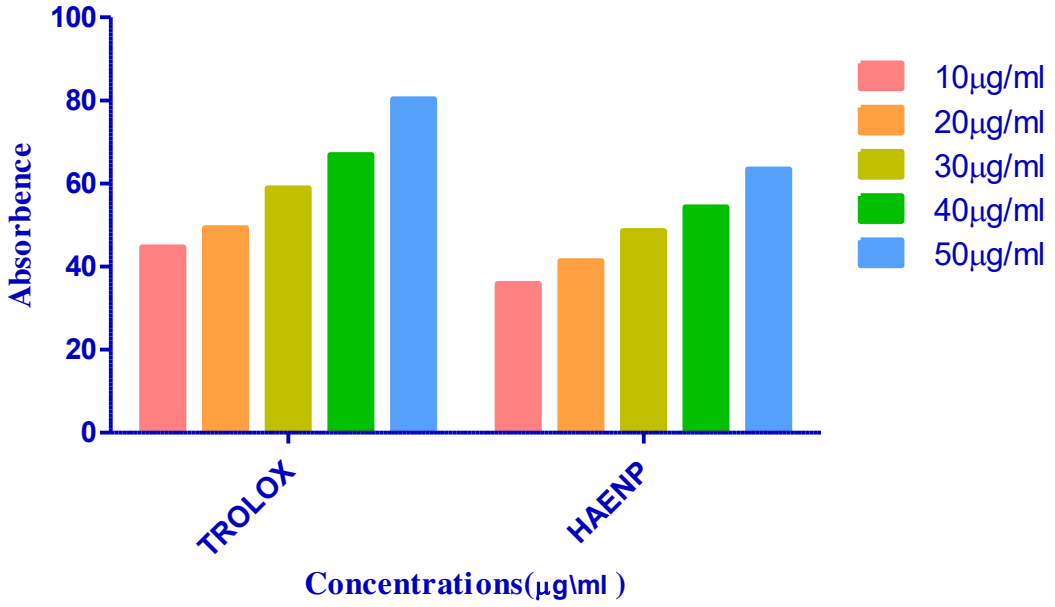


Fig. No: 84 ABTS Free Radical Scavenging Activity of HAENP

Table No.: 26 ABTS Free Radical Scavenging Activity of EAENN and HAENN

Test	Sample	% Inhibition for concentrations (Mean±SEM)				
		10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50 µg/mL
ABTS	TROLOX	44.62±0.026	49.29±0.032	58.82±0.041	66.84±0.034	80.25±0.049
	EAENN	42.24±0.031	50.50±0.037	57.61±0.017	66.15±0.025	72.38±0.043
	HAENN	38.39±0.017	45.28±0.034	51.92±0.025	58.41±0.018	67.17±0.026

Values are the mean ± S.E.M., n=3, **EAENN**: Ethylacetate extract of *Nymphaea nouchali,* **HAENN**: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

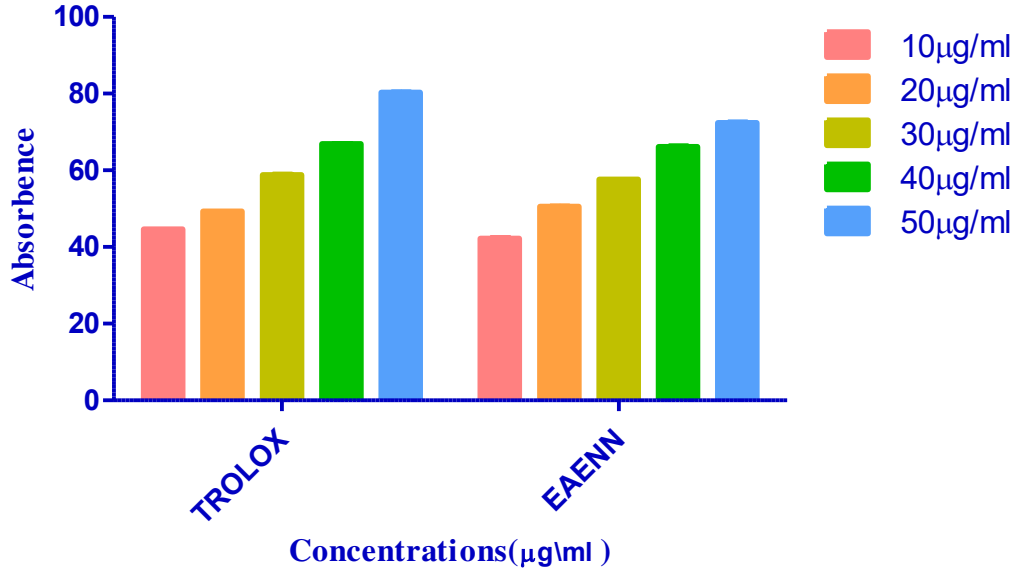


Fig. No: 85 ABTS Free Radical Scavenging Activity of EAENN

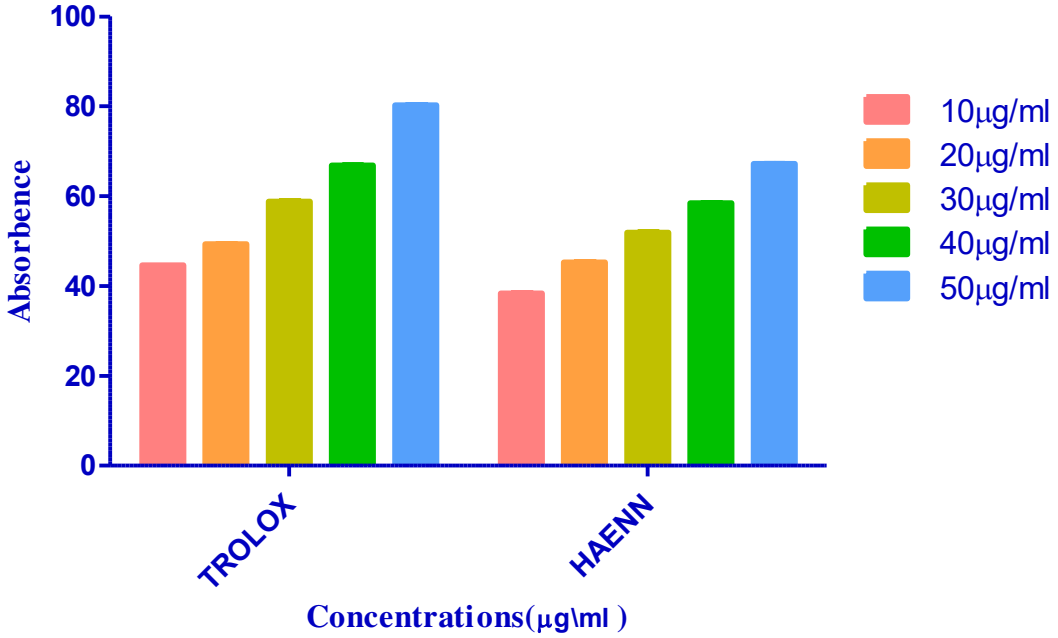


Fig. No: 86 ABTS Free Radical Scavenging Activity of HAENN

7.11.8. Estimation of Total Phenolic content

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. The total phenolic content of ethyl acetate and hydroalcoholic extracts of ***Nymphaea pubescens*** **Willdenow** flowers are found to be **101.71±0.09µg** and **101.40±0.04µg** GAE/g of extracts and that of ethyl acetate and hydroalcoholic extracts of ***Nymphaea nouchali*** **Burmann. F.** flowers are found to be **100.70±0.70µg** and **100.36±0.43µg** GAE/g of extracts with reference to the standard Gallic acid equivalent respectively.

7.11.9. Estimation of Total flavonoid content

The total flavonoid content of ethyl acetate and hydroalcoholic extracts of *Nymphaea pubescens* Willdenow flowers are found to be **104.71±0.09µg** and **103.40±0.04µg** QE/mg of extract and that of ethyl acetate and hydroalcoholic extracts of *Nymphaea nouchali* Burmann. F. flowers are found to be **103.80±0.70µg** and **103.36±0.43µg** QE/mg of extracts with reference to the standard Quercetin equivalent respectively.

The total flavonoid content was found to be high **(101.71±0.09µg GAE/g)** in the ethyl acetate extract of *Nymphaea pubescens* Willdenow when compared to all extracts and the total flavonoid content was found to be high **(104.71±0.09µg QE/mg)** in the ethyl acetate extract of *Nymphaea pubescens* Willdenow when compared to all extracts.

CHAPTER-VIII

PHARMACOLOGICAL SCREENING

PHARMACOLOGICAL SCREENING

CHAPTER – VIII

PHARMACOLOGICAL SCREENING

8.1. Introduction

Pharmacology is the branch of medicine and biology concerned with the study of drug action, **(Vallance, P., 2006)** where a drug can be broadly defined as any man-made, natural, or endogenous (within the body) molecule which exerts a biochemical and/or physiological effect on the cell, tissue, organ, or organism. More specifically, it is the study of the interactions that occur between a living organism and chemicals that affect normal or abnormal biochemical function. If substances have medicinal properties, they are considered pharmaceuticals.

The field encompasses drug composition and properties, synthesis and drug design, molecular and cellular mechanisms, organ/systems mechanisms, signal transduction/cellular communication, molecular diagnostics, interactions, toxicology, chemical biology, therapy, and medical applications and antipathogenic capabilities. The two main areas of pharmacology are pharmacodynamics and pharmacokinetics. The former studies the effects of the drug on biological systems, and the latter the effects of biological systems on the drug. In broad terms, pharmacodynamics discusses the chemicals with biological receptors, and pharmacokinetics discusses the absorption, distribution, metabolism, and excretion **(ADME)** of chemicals from the biological systems.

Early pharmacologists focused on natural substances, mainly plant extracts. Pharmacology developed in the 19^{th} century as a biomedical science that applied the principles of scientific experimentation to therapeutic contexts **(Rang, H.P., 2007).** Today Pharmacologists harness the power of genetics, molecular biology, chemistry, and other advanced tools to transform information about molecular mechanisms and targets into therapies directed against disease, defects or pathogens, and create methods for preventative care, diagnostics, and ultimately personalized me to study the toxicity and other pharmacological studies.

Toxicology basically is defined traditionally as "**the science of poisons".** The word toxicology is derived from **toxicon** - a poisonous substance into which arrow heads were dipped and **toxikos** - a bow **(Robyn, 1996).** A poison is any substance has a harmful effect on a living system **(EHSC, 2004).** A more descriptive definition of toxicology is **"the study of the adverse effects of chemicals or physical agents on biological material with special emphasis on the harmful effects"**. After gaining relevant information on the harmful effects of a compound the levels for its safe usage or the degree of its safeness is established, this is known as its **"Bio-safety level".**

Toxicology is a relatively young biological science that involves a complex interrelationship among dose, absorption, distribution, metabolism and elimination **(Robyn, 1996).** A toxic substance is a material which has toxic properties. Knowledge of how toxic agents damage the body has progressed along with medical knowledge. Toxicity depends not only on the dose of the substance but also on the toxic properties of the substance. The relationship between these two factors is important in the assessment of therapeutic dosage in pharmacology and herbalism **(Klaassen, *et al.,* 1995; Hayes, 2001).**

8.1.1. Toxicity Effects

In traditional toxicology the organism tends to be regarded as the primary unit for the expression of toxic effects. The adverse effects caused by a toxic substance may occur in many forms, ranging from immediate death to subtle changes not realized until months or years later. Consequently, the onset and duration of effects, as well as the effects themselves (e.g., changes in behaviour and alterations in blood flow, renal functions, and metabolic parameters), become signs of impaired homeostasis and are inevitably bound to the intact organism. All chemicals are toxic under some condition of exposure. Therefore, it is necessary to define these conditions as well as the quantity involved in the exposure in order to compare the toxicity characteristics of chemicals. Toxic effects are classified as either acute, sub chronic or chronic. The dose response concept is the basis for all toxicity assessments. It is used differently to evaluate acute effects, sub chronic effects and chronic effects.

8.1.2. Acute Toxicity

Acute toxicity studies are the most common of the toxicity or safety evaluations. Acute toxicity is usually defined as the adverse change(s) occurring immediately or after short time following a single dose or short period of exposure to a substance or substances within 24 h. An adverse effect is any effect that results in functional impairment and/or biochemical lesions that may affect the performance of the whole organism or that reduce the organ's ability to respond to an additional challenge. Consequently, a chemical that enters the organism via the oral route during a restricted time and produces any adverse effect with little delay is acutely toxic. However, the term acute oral toxicity is most often used in connection to lethality and LD_{50} determinations.

8.1.3. Objective

In the assessment and evaluation of the toxic characteristics of a substance, determination of acute oral toxicity is usually an initial step. Acute studies are generally performed first to generate data to improve the design and conduct of longer-term studies including sub chronic and chronic toxicity tests and others devoted to reproductivity, carcinogenicity and neurotoxicity testing. If designed appropriately, these studies may also provide initial information on the biological activity, toxico-kinetics of a chemical and useful to gain insight into its mechanism of action **(Fan and Chang, 1996; Walum, 1998).**

The short-term hazard from a chemical is determined by identifying the acute toxicity by the most likely routes of exposure. Acute toxicity effects are also investigated when exposed to a chemical taken orally, absorbed through the skin or by inhalation. A study of acute toxicity determines the dose-dependent adverse effect and from the comprehensive acute toxicity profile of a substance various appropriate data may be collected. This may include the incidence of lethality. If the dose-dependent lethality incidence is determined in a precise manner, it is usually expressed as an LD_{50} **(Gad and Chengelis, 1988).**

8.1.4. Median lethal dose test (LD_{50})

The term LD_{50} (lethal dose 50 or median lethal dose) is defined as statistically 50 derived dose of a substance that, when administered in an acute toxicity test, is expected to cause death in 50 % of the treated animals exposed for a specified time **(Oliver, 1986).** For a classical LD_{50} study, laboratory mice and rats species are typically selected. Materials can be classified as hazardous waste on the basis of LD_{50} of the material. A number of protocols exist for conducting LD_{50} studies **(Litchfield and Wilcoxon, 1949; Bruce, 1985).**

Determination of lethality (LD_{50}) is one of the many measures of toxicity evaluated in acute toxicity studies. The focus of acute toxicity is to identify lethal effects and target organs, with emphasis on behavioural, gross anatomical, haematological biochemical and histopathological changes. Dose selection in acute toxicity tests should aim to produce a dose-response curve that will enable an acceptable estimation of the median lethal dose (LD_{50}) which usually occurs within 24 h after initiation of exposure **(Fan and Chang, 1996).**

The LD_{50} value (precise or approximate) is currently the basis for toxicological classification of chemicals. Thus, LD_{50} values based on animal experiments are used to estimate the lethal dose of substances for humans **(Martin, *et al.,* 1989).**

8.1.5. Application

Acute toxicity studies in animals are usually necessary for any pharmaceutical intended for human use. The information obtained from these studies is useful in choosing doses for repeat-dose studies, providing preliminary identification of target organs of toxicity, and, occasionally, revealing delayed toxicity. Acute toxicity studies may also aid in the selection of starting doses for Phase I human studies, and provide information relevant to acute overdosing in humans **(Guidance for Industry, 1996).**

8.1.6. Botanicals: Toxicological evaluation

Plants are an important part of culture and traditions all over the globe. Most of the people today are reliant on herbal medicines for their health care needs. Herbal prescriptions and natural remedies are commonly employed in developing countries for

the treatment of various diseases, which is an alternative way to compensate for some deficiencies in orthodox pharmacotherapy **(Sofowora, 1989; Zhu, *et al.,* 2002).** The fact that herbal medicines contain ingredients to maintain health and to cure ailments is well known. However, the fact that they may contain toxic substances which are harmful or even dangerous to health is least known. Moreover there is limited scientific evidence regarding safety and efficacy to back up the continued therapeutic application of these remedies.

Plants commonly used in traditional medicine are assumed to be safe. This safety is based on their long usage in the treatment of diseases according to knowledge accumulated over centuries. Virtually all our knowledge is derived from human exposures to herbs leading to acute toxicities and little information is available from prior animal experimentation **(Sheehan, 1998).**

It has been reported by researchers that many plants used as food or in traditional medicine are potentially toxic, mutagenic and carcinogenic **(Schimmer, *et al.,* 1988, 1994; Higashimoto, *et al.,* 1993; Kassie, *et al.,* 1996; De Sa Ferrira and Ferrao Vargas, 1999).** Herbs may be contaminated with undeclared toxic botanicals or heavy metals **(De Smet, 1992).** Several plants used in traditional medicine can cause damage to the genetic material and therefore have the potential to cause long-term damage in patients when administered as medical preparations. Thus, the rationale for their utilization has rested largely on the treatment of various ailments with caution and rigorous long-term toxicological and clinical studies **(Zhu, *et al.,* 2002; Santamaria et al., 2007).** Now, with the upsurge in the use of herbal medicines, a thorough scientific investigation of these plants will go a long way in validating their folkloric usage **(Sofowora, 1989).**

It is essential to evaluate the quality and safety of herbs before being prescribed for any medicinal use. Considering the aforesaid, the flower powder extracts of ***Nymphaea pubescens*** **Willdenow** and ***Nymphaea nouchali*** **Burmann. F.** was evaluated for the acute toxicity studies.

8.2. Acute Toxicity Studies (LD_{50}) (OECD Guidelines, 2008)

Acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance, or multiple doses given within 24 hours.

Animals

Female Albino rats-***Rattus norvegicus*** weighing 150-250g were used for the study. They were nulliparous and non-pregnant. These were acclimatized to laboratory condition for one week prior to start of dosing.

Preparation of Dose

EAENP**,** HANP and EAENN, HANN was dissolved in distilled water, to prepare a dose of 2000mg/kg p.o. The dose was selected according to the OECD guideline no. 425.

Procedure

The procedure was divided into two phases. **Phase I** (observation made on day one) and **Phase II** (observed the animals for next 14 days of drug administration). Two sets of healthy female rats (each set of 3 rats) were used for this experiment. First set of animals were divided into three groups, each of one in a group. Animals were fasted overnight with water ***ad libitum***. Animals received a single dose of 2000 mg/kg; p.o. was selected for the test, as the test item was a source from herb. After administration of extract, food was withheld for 3-4 hrs. If the animal dies, conduct the main test to determine the LD_{50}. If the animal survives, dose four additional animals sequentially so that a total of five animals are tested. However, if three animals die, the limit test is terminated and the main test is performed. The LD_{50} is greater than 2000 mg/kg p.o, if three or more animals survive. If an animal unexpectedly dies late in the study, and there are other survivors, it is appropriate to stop dosing and observe all animals to see if other animals will also die during a similar observation period. Late deaths should be counted the same as other deaths. The same procedure was repeated with another set of animals to nullify the errors.

8.3. Screening of *In-vivo* Antioxidant Activity

i). Antioxidants

Anti-Oxidants are our first line of defence against free radical damage, and are critical for maintaining optimum health and well-being. The need for anti-oxidants becomes even more critical with increased exposure to free radicals.

Plant derived substances collectively termed **"Phytonutrients"**, or **"Phytochemical"**, are becoming increasingly known for their anti-oxidant activity.

ii). Animals

Healthy adult female ***Albino Wister*** **rats** between 2-3 months of aged and weighing 180-200 gm were procured from the animal house used for the study. The animals were housed in standard environmental conditions at 25±20°C, relative humidity 50±15% and normal photo period (12 hr dark and 12 hr light) for the experiment. The animals were fed with standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and provided water ***ad libitum*****.** The experimental protocol has been approved by the Institutional Animal Ethics Committee **(CPCSEA/PCP/IAEC/Ph.D/128/2010)** prior to the beginning of the thesis work.

8.3.1. Screening of Paracetamol Induced Hepatoprotective Activity

Introduction

Liver, the largest organ in vertebrate body, plays a major role in metabolic activities like detoxification and excretion of many exogenous and endogenous compounds. Liver injury caused by toxic chemicals and certain drugs, has been recognized as a toxicological problem. In the absence of reliable liver protective drugs in modern system of allopathic medical practice, herbal drugs are playing important roles in health care programmes worldwide and there is a resurgence of interest in herbal treatment of various hepatic ailments **(Shivkumar, *et al.*, 2006).**

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. And it functions as a centre of metabolism of nutrients such as

carbohydrates, proteins and lipids and excretion of waste metabolites. The bile secreted by the liver has, among other things, plays an important role in digestion. Therefore, maintenance of a healthy liver is essential for the overall well being of an individual **(Smuckler, EA., 1975).** Liver cell injury caused by various toxicants such as certain chemotherapeutic agents, carbon tetrachloride, thio-acetamide, chronic alcohol consumption and microbes are common. Enhanced lipid per oxidation during metabolism of ethanol may result in development of hepatitis leading to cirrhosis **(Agarwal, SS., 2001)**.

Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. The Indian Traditional Medicine like Ayurveda, Siddha and Unani are predominantly based on the use of plant materials. The association of medical plants with other plants in their habitat also influences their medicinal values in some cases. One of the important and well documented uses of plant products is their use as hepatoprotective agents.

Hence, there is an ever increasing need for safe hepatoprotective agent **(Ward, FM., 1999)**. In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help regeneration of hepatic cell. Many formulations containing herbal extracts are sold in the Indian market for liver disorders **(Achuthan, CR., *et al.,* 2003)**.

But management of liver disorders by a simple and precise herbal drug is still an intriguing problem. Several Indian medicinal plants have been extensively used in the Indian traditional system of medicine for the management of liver disorder. Some of these plants have already been reported to posse's strong antioxidant activity **(Aniya, Y., *et al.,* 2002, Gupta, AK., 2006)**.

8.3.2. Liver Diseases and Medicinal Plants

Liver has a pivotal role in regulation of physiological processes. It is involved in several vital functions such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs and xenobiotics occurs in liver. The bile secreted by the liver has, among other things, an important role in digestion. Liver diseases are among the most

serious ailment **(Samir, S., 2001)**. They may be classified as acute or **chronic hepatitis** (inflammatory liver diseases), **hepatosis** (non inflammatory diseases) and **cirrhosis** (degenerative disorder resulting in fibrosis of the liver). Liver diseases are mainly caused by toxic chemicals (certain antibiotics, paracetamol, chemotherapeutics, peroxidised oil, aflatoxin, carbon-tetrachloride, chlorinated hydrocarbons, etc.), excess consumption of alcohol, infections and autoimmune/disorder **(Malhotra S, 2001)**. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver. Enhanced lipid peroxidation produced during the liver microsomal metabolism of ethanol may result in hepatitis and cirrhosis **(Ross and Wilson 2005, Tortora and Grabowski, 2003)**.

Medicinal herbs are significant source of pharmaceutical drugs. Latest trends have shown increasing demand of phytodrugs and some medicinal herbs have proven hepatoprotective potential. **Silymarin**, a flavonol lignan mixture extracted from the milk thistle (*Silybum marianum*) is a popular remedy for hepatic diseases **(PDR for herbal medicines, 1998)**. Today every herbal company is marketing formulations for liver disorders but the actual scene is that only selected medicinal herbs have been tested for hepatoprotective activity. Some herbal formulations claiming to be hepatoprotective may actually contain chemical constituents having hepatotoxic potential. Andrographolide (*Andrographis paniculata*), Glycyrrhizin (*Glychyrrhiza glabra*), Picrrorihzin (*Picrorrhiza kurroa*) and Hypo-phyllanthin (*Phyllanthus niruri*) are potential candidates with hepatoprotective activity.

8.3.3. Experimental Design

The method of **Chattopadhyay, R R., (2003)** was used in the study. Animals were divided into 15 groups of 6 animals each. Group I received saline 1 mL/kg for one week (control). Group II received saline 1 mL/kg for one week (positive control). Remaining all the groups III-XV received silymarin (100mg/kg p.o.) (Sigma chem. Mumbai) and 100, 200 and 400 mg/kg of ethylacetate and hydroalcoholic extracts of the flowers of *Nymphaea pubescens* Willdenow and *Nymphaea nouchali* Burmann. F. respectively once daily for seven days. On the fifth day, 30 min after administration of the respective treatments, all the animals of groups III to XV were administered with paracetamol 2

g/kg orally. On the seventh day after 2 hr of respective treatments the blood samples were collected from the retro orbital sinus for the estimation of serum biochemical marker enzymes. Then animals were given ether as anaesthesia and were sacrificed. The livers from all the animals were collected, washed and used for the estimation of tissue GSH and lipid peroxide levels **(Ramachandra Setty, S., 2007).**

Treatment Protocol and method

The animals (albino mice weighing 20-25g) were divided in to fifteen groups of six animals each and the doses were calculated to the b.w, p.o.

Group-I	**S**erved as Negative control group animal received saline 0.5 ml/Kg p.o.
Group-II	Served as Positive control and received paracetamol 2g/ kg p.o.
Group-III	Served as Standard received paracetamol 2g/ kg p.o. + Silymarin 100mg/kg p.o.
Group-IV	Served as test group received paracetamol 2g/ kg p.o + EAENP 100mg/kg p.o
Group-V	Served as test group received paracetamol 2g/ kg p.o + EAENP 200mg/kg p.o
Group-VI	Served as test group received paracetamol 2g/ kg p.o + EAENP 400mg/kg p.o
Group-VII	Served as test group received paracetamol 2g/ kg p.o + HAENP 100mg/kg p.o
Group-VIII	Served as test group received paracetamol 2g/ kg p.o + HAENP 200mg/kg p.o
Group-IX	Served as test group received paracetamol 2g/ kg p.o + HAENP 400mg/kg p.o
Group-X	Served as test group received paracetamol 2g/ kg p.o + EAENN 100mg/kg p.o
Group-XI	Served as test group received paracetamol 2g/ kg p.o + EAENN 200mg/kg p.o
Group-XII	Served as test group received paracetamol 2g/ kg p.o + EAENN 400mg/kg p.o
Group-XIII	Served as test group received paracetamol 2g/ kg p.o + HAENN 100mg/kg p.o
Group-XIV	Served as test group received paracetamol 2g/ kg p.o + HAENN 200mg/kg p.o
Group-XV	Served as test group received paracetamol 2g/ kg p.o + HAENN 400mg/kg p.o

8.3.4. Estimation of Serum Biochemical Parameters

The serum collected blood samples were used for the analysis of biochemical markers SGPT, SGOT, ALP, Bilirubin, Urea, Creatinine and Protein levels.

8.3.5. Estimation of Serum Glutamate Pyruvate Transaminase (SGPT)

Addition of pyridoxal-5-phosphate (P-5-P) stabilizes the transaminases and avoids falsely low values in samples containing insufficient endogenous P-5-P, eg. from patients with myocardial infarction liver diseases and intensive care patients.

Method: Kinetic UV test, according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) **(Bradley, DW., *et al.,* 1972).**

8.3.6. Estimation of Serum Glutamate Oxalo-Acetate Transaminase (SGOT)

Addition of pyridoxal-5 phosphate (P-5-P) stabilizes the transaminases and avoids falsely low values in samples containing insufficient endogenous P-5-P, eg. from patients with myocardial infarction, liver diseases and intensive care patients.

Method: Optimized UV- test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) **(Rej, R, 1973).**

8.3.7. Estimation of Alkaline Phosphatase

Method: Kinetic photometric test, according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) **(Mac Comb, RB, 1972).**

8.3.8 Estimation of Bilirubin

Principle: Bilirubin is coupled with diazotized sulfanilic acid in the presence of caffeine to give an azo dye. No caffeine is added when direct bilirubin is determined by **Jendrassik method (Pearlman, PC, 1974).**

8.3.9 Estimation of Urea

Urea is the nitrogen-containing end product of protein catabolism. States associated with elevated levels of urea in blood are referred to as hyperuremia or azotemia. Estimation of urea was done by Urease-GLDH: enzymatic UV test **(Lipika Patnaik., 2010).**

Procedure

- ♣ Take 1000 µl of reagent 1 and 250 µl of reagent 2 in 5mL test tube
- ♣ To this add 10 µl of serum
- ♣ Mix well and immediately read the test sample at 340 nm, Hg 334 nm, Hg 365 nm, optical path 1 cm against reagent blank (2-point kinetic)
- ♣ And note down the value

8.3.10 Estimation of Creatinine

Estimation of Creatinine by **Jaffe Method** (modified)

Principle: Creatinine forms a colored complex with picrate in alkaline medium. The rate of formation of the complex is measured **(Lipika Patnaik., 2010).**

Procedure

- ♣ Take 500 µl of reagent -2 and 500 µl of reagent -3 in a 5mL test tube
- ♣ To this add 100 µl of serum
- ♣ Mix well and immediately read the test sample at Hg 492 nm 1cm light path and note down the values

8.3.11 Estimation of Proteins

Principle: Procedure described by **Lowery, OH., (1951)** was used for protein estimation. The method is based on the Biuret reaction, formation of a protein-copper complex and reduction of phosphomolybdo tungstate reagent (Folin-ciocalteu phenol reagent) by tyrosine and tryptophan residues of protein to form a colored product **(Lowery, OH., 1951).**

8.3.12 Estimation of *In-vivo* Enzymatic Antioxidant Activity

Liver slices were collected from the above groups of animals were subjected to the enzymatic anti oxidant determination of CAT, SOD, GPx and non-enzymatic level GSH and LPx. The tissue GSH was evaluated according to **(Ayake, *et al.,* 1985).**

a. Estimation of Catalase Activity

Catalase activity was assayed calorimetrically at 620 nm and expressed as µmoles of H_2O_2 consumed/min/mg protein as described by **Sinha, (1972).** The reaction mixture may (1.5 mL, vol) contain 1.0 mL of 0.01 M pH 7.0 phosphate buffer, 0.1 mL of tissue homogenate (supernatant) and 0.4 mL of 2 M H_2O_2. The reaction is to be stopped by the addition of 2.0 mL of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio) **(Sinha, AK., 1972).**

b. Estimation of Super Oxide Dismutase (SOD) Activity

The Super Oxide Dismutase activity in 0.5 mL of tissue homogenate was assayed by the method of **Kakkar, *et al*., (1972).** 0.5 mL of tissue homogenate was diluted with 1 mL of

water. In this mixture, 2.5 mL of ethanol and 1.5 mL of chloroform (all reagents chilled) are to be added and shaken for 1 min at 4°C then centrifuged. The enzyme activity in the supernatant may be determined. The assay mixture contains 1.2 mL of sodium pyrophosphate buffer (0.025 M, pH 8.3), 0.1 mL of 186 µM PMS, 0.3 mL of 30 µM NBT, 0.2 mL of 780 µM NADH, appropriately diluted enzyme preparation and water in a total volume of 3 mL. Reaction was started by the addition of NADH. After incubation at 30°C for 90 seconds the reaction was stopped by the addition of 1mL glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4mL of n-butanol. The intensity of the chromogen in the butanol layer may be measured at 560 nm against butanol blank. A system devoid of enzyme serves as control. One unit of the enzyme activity was defined as the enzyme reaction, which will give 50% inhibition of NBT reduction in one minute under the assay conditions **(Kakkar, *et al.,* 1972).**

c. Estimation of Glutathione Peroxidase (GPx) Activity

Glutathione Peroxidase activity was measured by the method described by **Rotruck, *et al*., (1973)**. Briefly, reaction mixture contains 0.2 mL of 0.4 M Tris-HCl buffer pH 7.0, 0.1 mL of 10 Mm sodium azide, 0.2 mL of tissue homogenate (homogenized in 0.4 M, Tris-HCl buffer, pH 7.0), 0.2 mL glutathione, and 0.1 mL of 0.2 mM hydrogen peroxide. The contents are incubated at 37°C for 10 min. The reaction was arrested by 0.4 mL of 10% TCA, and centrifuged. Supernatant was assayed for glutathione content by using Ellman's reagent (19.8 mg of 5, 5'-dithiobis nitro benzoic acid (DTNB) in 100 mL of 0.1% sodium nitrate) **(Rotruck, J.T., 1973).**

8.3.13 Estimation of *In-vivo* Non-Enzymatic Antioxidant Activity

a. Estimation of Reduced Glutathione (GSH) activity

Reduced Glutathione activity was determined by the method of **Ellman (1959).** A known weight of tissue homogenized in phosphate buffer from this 0.5 mL was pipetted out and precipitated with 2 mL of 5% TCA.1 mL of the supernatant is taken after centrifugation at 3200 x g for 20 min and added to it 0.5 mL of Ellman's reagent and 3 mL of phosphate buffer (pH 8.0). Then the absorbance may be read at 412 nm. A series of standard was

treated in a similar manner along with a blank containing 3.5 mL of buffer. The values are expressed as mg/100 g tissue **(Moron, M.S., 1979).**

b. Assay of Lipid Peroxidation (LPX) Activity

Lipid peroxidation in liver was estimated colorimetrically by thiobarbituric acid reactive substances TBARS and hydro peroxides by the method of **Niehius and Samuelsson and Jiang, *et al.,*** respectively. In brief, 0.1 mL of tissue homogenate (Tris -HCl buffer, pH 7.5) was treated with 2 mL of (1:1:1 ratio) TBA-TCA-HCl reagent (thiobarbituric acid 0.37 %, 0.25 N HCl and 15 % TCA) and placed in water bath for 15 min, cooled. The absorbance of clear supernatant was measured against reference blank at 535 nm **(Ohkawa, H., 1979).**

8.3.14. Histopathological Studies

Paraffin sections (7 μm thick) of buffered formalin-fixed liver samples were stained with haematoxylin-eosin was used to study the histological structure of control and treated rats liver.

Statistical Analysis

The results are expressed as mean ± SEM, (N=6) statistical significance was determined by one way analysis of variance (ANOVA) Tukey followed by comparison of all groups. $P < 0.05$ considered statistically significant.

8.4. Screening of Anti Diabetic activity

8.4.1. Introduction

The term ***diabetes*** **(Greek: *διαβήτης*)** was coined by **Aretaeus of Cappadocia.** It is derived from the **Greek *διαβαίνειν*, *diabaínein*** that literally means **"passing through",** or **"siphon",** a reference to one of diabetes major symptoms - excessive urine production. In **1675 Thomas Willis** added ***mellitus*** from the Latin word for honey **(*mel*** in the sense of **"honey sweet")** when he noted that the blood and urine of a diabetic has a sweet taste. This had been noticed long before in ancient times by the Greeks, Chinese, Egyptians, and Indians **(Patlak, M., 1853).**

Diabetes mellitus **(DM)** is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves **(Nagappa, AN., 2003).** Medicinal plants continue to provide valuable therapeutic agents, in both modern medicine and in traditional system **(Demerdash, FM., 2005).**

DM is not a single disease entity; it is a group of metabolic disorders with a common feature of *hyperglycemia.* Diabetes is often called **"the silent killer"**, because it causes serious complications without serious symptoms, and can affect many of the major organs in the body. Hyperglycemia in diabetes affects multiple organ systems in the body especially kidneys, eyes, nerves, and blood vessels. It is an endocrine disorder of pancreas.

8.4.2. Classification of Diabetes

DM is the most common endocrine disorder reported among the 10% to 15% population in the world. DM is known to Indians from Vedic periods which were termed as ***Asrava*** or ***Prameha***. The word ***prameha*** derived from the root of ***mihsechane*** which means dilution of everything in the body. According to ayurveda, DM **(*prameha*)** is again divided into 4 major types and 21 sub types **(Krishna Kumar, CS., 2011)**. In Ayurveda

it is also termed as Madhumeha. The ancient physicians called this diabetes as a ***maharoga*** (major disease). It affects the most of organs in the body.

"Mudhura Yachch Mehesu Proyo Madhvint Maihati"

"Sarve Api Madhumehachya Madhuyachchi Manorath"

It means Madhumeha is a disease in which a patient passes sweet urine and exhibits sweetness all over the body i.e. in sweat, mucus, breath, blood etc. **(Subbulakshmi, G., 2010).**

DM causes disturbances in carbohydrate, protein and lipid metabolism. It damages multi organ systems, especially the kidneys, eyes, nerves, and blood vessels which lead to complications such as diabetic nephropathy, retinopathy, neuropathy and microangiopathy.

Mainly diabetes is classified into following types,

- Type 1 Diabetes Mellitus
- Type 2 Diabetes Mellitus
- Gestational Diabetes
- Miscellaneous

Type 1 DM is an auto immune disorder; the main cause of this is beta cell loss by T-cell mediated autoimmune attack. It is also characterized by loss of the insulin-producing beta cells of the Islets of Langerhans in the pancreas, leading to a deficiency of insulin.

Type 2 DM characterized by insulin resistance or reduced insulin sensitivity, combined with reduced insulin secretion. Variants in 11 genes significantly associated with the risk of Type 2 diabetes of these 8 genes are responsible for impaired beta-cell function.

Gestational diabetes is defined as, diabetes that begins during pregnancy where there is a change in the glucose metabolism and resistance to insulin or glucose tolerance test. It is more common in obese patients **(www.cdc.gov/diabetes/pubs/factsheet11.htm).**

Diabetes is currently the sixth leading cause of death, and the end stage complications like kidney failure, **(diabetic nephropathy),** blindness **(diabetic retinopathy),** and amputations **(diabetic neuropathy).** It also increases the chance of heart attacks by 2-4

times, and 60-70% diabetic patients reporting with nerve damage. There are many factors that contribute to it, like stress, an inactive lifestyle, poor nutrition and genetic predisposition. It is the most prevalent disease in the world affecting 25% of population and afflicts 150 million people and is set to rise to 300 million by 2025. Today, India has become the diabetic capital of the world with over 32 million diabetics and this number is set to increase to 80 million by 2030.

8.4.3. Prevalence of Diabetes

Data on diabetes prevalence by age and sex from a limited number of countries were extrapolated to all 191 World Health Organization member states and applied to United Nations population estimates for 2000 and 2030. Urban and Rural populations were considered separately for developing countries. The prevalence of diabetes is higher in men than women, but in some countries there are more women with diabetes than men. The urban population in developing countries is projected to double between the year 2000 and 2030. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people above 65 years of age **(www.diabetesinformationhub.com/).**

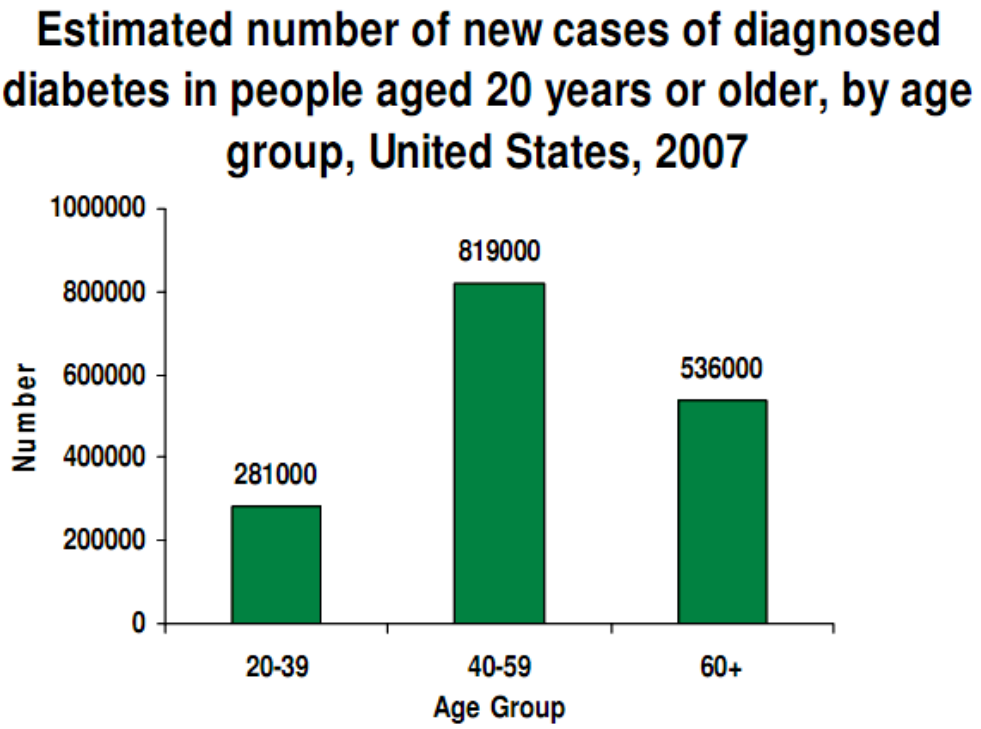
It is estimated that there are about 143 million people are suffering from diabetes, but it is almost five times more than the estimation ten years ago. According to the survey in 2003, there were about 16 million people in United States affected by this disease. According to the recent survey of U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, there are 25.8 million people i.e., almost 8.3% of the U.S. population. Among U.S. residents; aged 65 years and older, 10.9 million, or 26.9%, had diabetes in 2010. About 215,000 people younger than 20 years had diabetes (Type 1or Type 2) in the United States in 2010. About 1.9 million people aged 20 years or older were newly diagnosed with diabetes in 2010 in the United States **(www.cdc.gov/ diabetes/pubs/factsheet07.htm., (Sarah Wild, *et al*., 2004).**

Diabetes is a leading cause of end stage renal disease, adult-onset blindness, and non-traumatic. As a major non-communicable disease, diabetes mellitus claims on average around 8% of total health budgets in developed countries. Type 2 diabetes is the more common form and accounts for 90-95% of all diabetes cases worldwide.

8.4.4. Global Prevalence of Diabetes

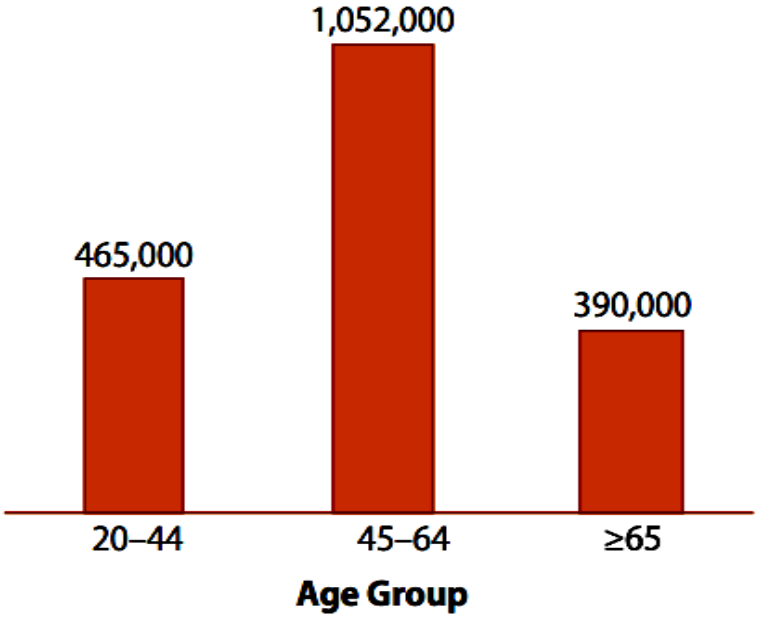
The global prevalence of diabetes, an estimation study shows that 2.8% in 2000 and 4.4% in 2030 will suffer from this disease. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. The top three countries India, China and U.S. will be same for 2030 also. In the below list the Russian and Italy is replaced with Philippines and Egypt because there will be an anticipated changes in the population growth during these years **(Baily, CJ., 1989).**

Fig. No: 87 Prevalence of Diabetes in US during the period of 2007 and 2010



Source: 2004–2006 National Health Interview Survey estimates projected to year 2007.

About 1.9 million people aged 20 years or older were newly diagnosed with diabetes in 2010.



Source: 2007–2009 National Health Interview Survey estimates projected to the year 2010

The global prevalence of diabetes is estimated to increase, from 4% in 1995 to 5.4% by the year 2025. WHO has predicted that the major burden will occur in developing countries. Studies conducted in India in the last decade have highlighted that not only is the prevalence of diabetes high but also that it is increasing rapidly in the urban population. It is estimated that there are approximately 33 million adults with diabetes in India. This number is likely to increase to 57.2 million by the year 2025.

Although, herbal medicines have long been used effectively in treating disease in Asian communities and also throughout the world, yet the mechanism of most of the herbals used has not been defined **(Elder, C., 2004).** It has been attributed that the anti-hyperglycaemic effect of these plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or facilitation of metabolites in insulin-dependent process. Hence, treatment with herbal drugs has an effect on protecting cells and smoothing out fluctuation in glucose levels **(Jia, W., 2003, Loew, D., 2002).**

Table No: 27 Prevalence of Expected Data during the Period of 2000 and 2030 in Some Countries

Ranking	Country	People with diabetes (millions) 2000	Country	People with diabetes (millions) 2030
1	India	31.7	India	79.4
2	China	20.8	China	42.3
3	U.S.	17.7	U.S.	30.3
4	Indonesia	8.4	Indonesia	21.3
5	Pakistan	6.8	Pakistan	13.9
6	Brazil	5.2	Brazil	11.3
7	Bangladesh	4.6	Bangladesh	11.1
8	Japan	4.6	Japan	8.9
9	Italy	4.3	Philippines	7.8
10	Russian Federation	3.2	Egypt	6.7

Most of the plants have been found to contain substance like glycosides, alkaloids, terpenoids, flavonoids etc, that are frequently implicated as having a specific mode of action of these plant drug or herbal formulation used for treating diabetes. The ethanobotanical information reports states that about 800 plants may possess antidiabetic potential. Based on the WHO recommendations hypoglycemic agents of plants origin used in traditional medicine are important. 12 Plant drugs and herbal formulation are frequently considered to be less toxic and freer from side effects than the synthetic ones **(Aguliara, FJA., 1998), (WHO Expert, 1980).**

8.4.5. International Status on Diabetes

According to the International Diabetes Federation (**IDF**), it is revealed that the country with the largest numbers of people with diabetes is India (40.9 million), followed by China (39.8 million), the United States (19.2 million) In Canada, 2.4 million people live with diabetes. About 90% of diabetic people die of diabetic cardio vascular diseases. Russia (9.6 million) and Germany (7.4 million) by the year 2025, there will be as many as seven million new diabetic cases in the world **(Diabetes Statistics, 2010).**

8.4.6. National Status on Diabetes

The Indian Diabetic population is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken. The prevalence of type 2 diabetes in different parts of the nation are as follows Kashmir 6.1% (2000), New Delhi 10.3% (2001), Rajasthan 8.6% (2003), Guwahati 8.3% (1999), Kolkatta 11.7% (2001), Mumbai 9.3% (2001), Hyderabad 16.6% (2001), Chennai 14.3% (2006), Bengaluru 12.4% (2001), Ernakulam 19.5% (2006) **(Rajiv Gupta., 2011, Usha Menon, V., 2008).**

Diabetes becomes a real problem of public health in developing countries, where its prevalence is increasing steadily**.** In such countries, adequate treatment is often expensive or unavailable. The pathogenesis of Diabetes mellitus and the possibility of its management by existing therapeutic agents without any side effects have stimulated great interest in recent years. Alternative strategies to the current modern pharmacotherapy of diabetes mellitus are urgently needed **(WHO 1999),** because of the inability of existing modern therapies to control all the pathological aspects of the disorders, as well as the enormous cost and poor availability of the modern therapies for many rural populations in developing countries.

Although oral hypoglycemic agents / insulin or the mainstay of treatment of diabetes and are effective in controlling hyperglycemic, but they have prominent side effects and fail to significantly after the course of diabetic complication. Due to the higher side effects there is a need to look for more efficacious agents with a lesser side effect.

Plants have been used in the treatment of various diseases because; they are one of the rich and important sources of medicine, science human civilization. Many of herbal products are presently used in modern days. The latest trend in advanced countries indicates **"PREFERENCE FOR NATURAL DRUGS OVER SYNTHETIC DRUGS".** Herbal medicines are preferred from the ancient time but they are not having a significant scientific data.

8.4.7. Classification of Herbal Antidiabetics

The wide range of structures of the plants constituents, which appear to be the active hypoglycemic principles, suggests different sites of action within the body **(Goyal, RK., 2006).** Based on the possible mechanism of action, plants antidiabetics may be classified as follows,

- Drugs acting like insulin e.g. ***Momordica charantia, Panax ginseng***
- Drugs increasing insulin secretion from beta cells of pancreas e.g. ***Azadirachta indica, Syzigium cumini***
- Drugs acting by regeneration of ß-cells of Islets of Langerhans e.g. ***Pterocarpus marsupium, Tinospora crispa, Gymnema sylvestre***
- Drugs inhibiting glucagon secretion from alpha cells in pancreas
- Drugs reducing absorption of glucose from GIT e.g. ***Ocimum sanctum, Cyamposis tetragonoloba***
- Drug inhibiting aldose reductase activity e.g. ***Glycyrrhiza glabra, Cinnamomum cortex***
- Drugs increasing glucose utilization e.g. ***Zingiber officinale, Nelumbo nucifera*** (lily flower)
- Drugs reducing lactic dehydrogenase and γ-glutamyl transpeptidase e.g. ***Lythrum salicaria***
- Drug inhibiting glycogen-metabolizing enzyme e.g. ***Bryonia alba***
- Drug acting on Liver glycogen e.g. ***Allium sativum, Coccinia indica***
- Drug increasing glyoxalase 1 activity in liver e.g. ***Trigonella foenumgraecum***
- Drug increasing glucose uptake in lipocytes e.g. ***Swertia japonica***
- Drug inhibiting glucose-6-phosphate system e.g. ***Bauhinia megalandra*** *enz*yme
- Drug increasing the creatine kinase levels in tissues e.g. ***Trigonella foenumgraecum***
- Drug having oxygen radical scavenging activity e.g. ***Momordica charantia, Pterocarpus marsupium***

From ancient period, people are using medicinal plants for the treatment of diabetes and WHO estimates that 80% of the populations presently use herbal medicine for primary

health care. Antidiabetic plants have the ability to restore the function of damaged pancreatic tissue by increasing the insulin or inhibiting the intestinal absorption of glucose. Administration of appropriate antioxidants from plant source could prevent or retard the diabetic complications to some extent.

In India around 25,000 plant-based formulations are used as traditional and folk medicine. In the present scenario, the demand for herbal products is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on plant materials for their potential medicinal value. Although the therapeutic effects of many herbs has not been scientifically proven, research continues to identify the active ingredients that may form the basis of drugs to fight diseases like cancer, AIDS, diabetes mellitus, asthma, physiological disorders and many more chronic diseases in future **(Madan, MP., 2008).**

8.4.8. Discovery of Oral Agents in the Treatment of Diabetes

In 1918 C.K.Watanabe discovered that certain compounds of Guanidine showed hypoglycemic effect. Initially it shows a hyperglycemia followed by hypoglycemia. By finding this **E. Frankl** and his team modified guanidine compound and synthesized a different derivatives of mono and biguanidine. But the bi-guanidine derivative has shown better hypoglycemic effect. Whereas, the first commercial available, guanidine was introduced in the year 1928 and marketed as Synthalin (Decamethyl-diguanide).

In 1957, Phenylethyl-bi-guanidine was discovered in US, it is available for the clinical use from 1959 under the name of Phenformin. Later these drugs were removed from the usage due to its toxicity to liver, kidney and an increased in frequency of lactic acidosis.

Another bi-guanide, Metformin has been used in Europe without significant adverse effects and was approved in US from 1995 **(Laurence Brunton, 2006)**. Whereas in the year 1942 a French professor of pharmacology at Montpellier University **M.J.Janbon** discovered that some of the sulfonylurea agents tested for the treatment of typhoid fever. These drugs are having side effects which include confusion, cramps, and coma to hypoglycemia. Later researchers worked a lot and found out the mechanism of action of

these drugs and they found these are ineffective when they are administered to the pancreatectomized animals.

In 1956 Tolbutamide became the first commercially available sulfonylurea compound in US. About 20 different agents of this class have been in use since that time. In 1997, repaglinide, the first member of a new class of oral insulin secretagogues called meglitinides was approved for clinical uses; these agents are used as therapy for postprandial hyperglycemia. Where, Thiazolidinediones were introduced as a second major class of insulin sensitizer. In this Troglitazone (2000) is the first drug use in US where it is removed from usage due to hepatic toxicity. Other two agents of this class, Rosiglitazones and Pioglitazones are having a very less side effects and are used world widely **(Sanders, L J., 2002).**

Since, DM is a clinical syndrome characterized by inappropriate hyperglycemia caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level. Plant materials which are being used as traditional medicine for the treatment of diabetes are considered as one of the good sources for a lead to make of a new drug. Plant extracts or different folk plant preparations are being prescribed by the traditional practioners also accepted by the users for diabetes like other diseases in many countries.

8.4.9. Oral Glucose Tolerance Test in Normal Rats (Bonner-Weir S, 1988)

The oral glucose tolerance test **(OGTT) (Chattopadhyay RR, 1993)** was performed in overnight fasted (18 hours) normal rats but allowed free access to water. Rats were divided into six groups, each consisting of six rats was administered 0.9% (w/v) saline, metformin 250 mg/kg (p.o), and 200 mg/kg, of ethylacetate and hydroalcoholic extracts of the flowers of ***Nymphaea pubescens*** **Willdenow** and ***Nymphaea nouchali*** **Burmann. F.** p.o. respectively. Glucose (2 g/kg) was fed 30 min after the administration of extract. Blood was withdrawn from the retro orbital sinus under ether inhalation at 0, 30, 60, and 120 min of glucose administration and blood glucose level was estimated by glucose oxidase–peroxidase method, by **Trinder, P., (1969), (Trinder, P., 1969).**

8.4.10. Hypoglycemic Activity

Fasted rats were divided into six groups of six animals each. Group I animals received only vehicle (0.9% w/v saline) orally in a volume of 0.5 mL/kg b.w. and served as a control. Group II received metformin (250 mg/kg, b.w. p.o) as a reference drug suspended in vehicle. The ethylacetate and hydroalcoholic extracts of the flowers of ***Nymphaea pubescens*** **Willdenow** and ***Nymphaea nouchali*** **Burmann. F.** p.o. extract, suspended in vehicle, was administered at dose of 100, 200, 400 mg/kg (b.w. p.o.) to the animals of groups III, IV, V, VI, VII and VIII respectively. Blood samples were collected from the retro orbital sinus under ether inhalation at 0, 30, 60, and 120 min for glucose estimation after dosing **(Santosh KS, *et al.,* 2007).**

8.4.11. Induction of Diabetes by Alloxan Monohydrate

Diabetes was induced in *Albino Wistar* rats by single intraperitoneal injection of freshly prepared solution of alloxan monohydrate [150 mg/kg b.w. (p.o.)] by **(Nagappa *et al*., 2003)** in sterile normal saline to overnight fasted animals. The fasting blood glucose level was determined after 72 hours of alloxan injection. The rats having fasting blood glucose (FBG) levels above 250 mg/dl were selected for the study. The diabetic animals were allowed free access to tap water and pellet diet, and were maintained at room temperature in plastic cages.

8.4.12. Experimental Design

Overnight fasted diabetic rats were divided into 15 groups of six animals in each group. Group 1 animals served as normal control, which received 0.9% w/v saline, Group 2 served as diabetic control-Alloxan monohydrate (150 mg/kg b.w., p.o.) in sterile normal saline (0.9%). Group 3 was treated with alloxan and Metformin (Glenmark) (250mg/kg/day b.w., p.o.), Group 4-15 received 100, 200 and 400 mg/kg (b.w., p.o.) of ethylacetate and hydro-alcoholic extracts of the flowers of ***Nymphaea pubescens*** **Willdenow** and ***Nymphaea nouchali*** **Burmann. F.** respectively. All the treatments were carried out for a period of 21 days. Body weight of the animals was recorded every week. The fasting blood samples were collected on days 0, 7, 14, and 21 to determine the glucose level by glucose oxidase method, by **Trinder, P., (1969).**

8.4.13. Sample Collection

At the end of the treatments, the blood samples were collected by retro orbital puncture and serum was separated by centrifugation at 4000 rpm for 15 min. The separated serum was used for biochemical estimation. Serum triglycerides, serum total cholesterol, total protein, albumin, creatinine, and HDL- cholesterol were analyzed from the serum by auto analyzer using Ecoline kits (Merck, Mumbai, India). Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol were calculated as per Friedewald's equation **(Friedewald WT, 1972)** i.e., **VLDL-Cholesterol=Serum triglyceride/5**; and **LDL-cholesterol = Serum total-cholesterol-(VLDL cholesterol+HDL cholesterol).** Results were expressed in mg/dl.

8.4.14. Antioxidant Assays

The Serum glutamate pyruvate transaminase (SGPT) and Serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by utilizing the method of Reitman and Frankel. Serum Alkaline Phosphatase (ALP) was measured by the method of King and Armstrong. Catalase (CAT), Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) Lipid Peroxidation (LPO), Reduced Glutathione (GSH) were analyzed in the normal, diabetic induced and drug treated rats **(Shanmugasundaram R., 2011).**

Statistical Comparison

One way ANOVA Tukey, followed by comparison of all pairs of columns test was performed. Alloxan only (**Group 2**) was compared with normal control (**Group1**), STD (**Group 3**) Metformin was compared with various doses 100, 200 and 400mg/kg b.w. of the extracts of the flowers of *Nymphaea pubescens* Willdenow and *Nymphaea nouchali* Burmann. F. respectively.

8.5. *In-Vitro* Screening of Anti-Cancer Activity

8.5.1. Introduction

Cancer is the uncontrolled growth of abnormal cells anywhere in a body. The abnormal cells are termed cancer cells, malignant cells, or tumor cells. Many cancers and the abnormal cells that compose the cancer tissue are further identified by the name of the tissue that the abnormal cells originated from (for example, breast, cancer, lung, colon cancer). Cancer is not confined to humans; animals and other living organisms can get cancer. Below is a schematic that shows normal cell division and how when a cell is damaged or altered without repair to its system, the cell usually dies. Also shown is what can occur when such damaged or unrepaired cells do not die and become cancer cells and proliferate with uncontrolled growth; a mass of cancer cells develop. Frequently, cancer cells can break away from this original mass of cells, travel through the blood and lymph systems, and lodge in other organs where they can again repeat the uncontrolled growth cycle. This process of cancer cells leaving an area and growing in another body area is termed metastatic spread or metastatic disease. For example, if breast cancer cells spread to a bone (or anywhere else), it means that the individual has metastatic breast cancer. **(www.trigon.com)**

Cancer is one of the most life-threatening diseases and serious public health problems in both developed and developing countries. It is a group of diseases characterized by the disregulate proliferation of abnormal cells that invade and disrupt surrounding tissues **(Gennari, C.D., 2007)**. Due to the toxic and adverse side effects of synthetic drugs as well as conventional treatments are being failed to fulfil their objectives (tumor control), for these consequence herbal medicine has made a comeback to improve the fulfilment of our present and future health needs **(Harun-ur-Rashid, M., 2002).**

Today one of the most serious problems of the public health is the evaluation of the efficient methods to stoppage of the cancer in different cell events **(Vimala, S., 1999).** A recent survey list out approximately 1400 genera's of herbal plants is used in the cancer treatment **(Anajwala Chetan C., 2010).** The phytochemical and non nutritive plant substances that have health beneficial effect so that wise most of the scientific research people and common mans are showing good interest in the naturopathy **(Yogeshwar**

Shukla, 2007). Lack of proper standards of medicinal plants may result in the usage of improper drugs which in turn will cause damage not only to the individual using it, but also to the respect gained by the well known ancient system of medicine. Therefore scientific method must be resorted to identify and maintain quality of plant drugs to be used in the traditional system of medicine **(Jia-Ming Chang, 2010)**.

Furthermore, cancer is an abnormal, malignant growth of body tissue or cell. A cancerous growth is called a malignant tumor or malignancy. A non cancerous growth is called benign tumor. The process of cancer metastasis is consisting of series of sequential interrelated steps, each of which is rate limiting. Plants loaded with chemical and chemo-protective activities of some of them are undergoing clinical trial. Inhibition of angiogenesis is a novel process of cancer therapy. The selected plant is used in anti-angiogenic therapy and also in cancer management **(www.diadetesinformationhub. com).**

8.5.2. Definition

A disease in which there is an uncontrolled multiplication and spread of abnormal forms of bodies own cells within the body. In simple words some cells get out of control during cell division and developed into a lump, which is called a tumor/neoplasm. These tumors destruct the vital structures of the body and it leads to disruptions of bodily functions.

Cancer is a general term applied of series of malignant diseases that may affect different parts of body. These diseases are characterized by a rapid and uncontrolled formation of abnormal cells, which may mass together to form a growth or tumor, or proliferate throughout the body, initiating abnormal growth at other sites. If the process is not arrested, it may progress until it causes the death of the organism. The main forms of treatment for cancer in humans are surgery, radiation and drugs (cancer chemotherapeutic agents). Cancer chemotherapeutic agents can often provide temporary relief of symptoms, prolongation of life, and occasionally cures. In recent years, a lot of effort has been applied to the synthesis of potential anticancer drugs. Many hundreds of chemical variants of known class of cancer chemotherapeutic agents have been synthesized but have a more side effects.

8.5.3. Brief History

According to celsus **"carinos"** means cancer (**crab in Latin**). According to Hippocrates **"Oncos"** means benign tumors. **"Carcinos"** means malignant tumors. The suffix-**oma** means swelling. Hippocrates described drawings of outwardly visible tumors on the skin, nose and breasts. Treatment was based on the humor theory of four body fluids (black and yellow bile, blood and phlegm).

In 1020 Annicena conducted first surgical treatment for cancer. In the 16^{th} and 17^{th} century Doctors are acceptable to dissect bodies to discover cause of death. In 18^{th} century Nicholas Tulip described Metastasis by using Microscope. In 19^{th} century removal of tumors was primary treatment for cancer. In the end of the 19^{th} century radiation was introduced as non surgical treatment by Madam Marie Curie and Pierrie Curie. After World War II Japanese Medical Community discovered Bone marrow transplantation for Leukaemia. In 1943 Nitrogen Mustard is used to cure Haemotologic Neoplasms. In 1946 Sidney Farber observed Folic acid had proliferative effect on Leukemic cell growth **(www.mpdfoundation.org).**

A successful anticancer drug should kill or incapacitate cancer cells without causing excessive damage to normal cells. This ideal is difficult, or perhaps impossible, to attain and is why cancer patients frequently suffer unpleasant side effects when under-going treatment. Synthesis of modifications of known drug continues as an important aspect of research. However, a waste amount of synthetic work has given relatively small improvements over the prototype drugs. There is a continued need for new prototype-new templates to use in the design of potential chemotherapeutic agents: natural products are providing such templates. Recent studies of tumor-inhibiting compounds of plant origin have yielded an impressive array of novel structures. Herbal medicines have a vital role in the prevention and treatment of cancer and medicinal herbs are commonly available and comparatively economical.

A great deal of pharmaceutical research done in technologically advanced countries like USA, Germany, France, Japan and China has considerably improved quality of the herbal medicines used in the treatment of cancer. Some herbs protect the body from cancer by

enhancing detoxification functions of the body. Certain biological response modifiers derived from herbs are known to inhibit growth of cancer by modulating the activity reduce toxic side effects. The mechanism of cancer therapy involves inhibiting cancer cell proliferation directly by stimulating macrophage phagocytosis, enhancing natural killer cell activity, Promoting apoptosis of cancer cells by increasing production of interferon, interleukin-2 immunoglobulin and complement in blood serum, Enforcing the necrosis of tumor and inhibiting its translocation and spread by blocking the blood source of tumor tissue, Enhancing the number of leukocytes and platelets by stimulating the hemopoietic function, Promoting the reverse transformation from tumor cells into normal cells, Promoting metabolism and preventing carcinogenesis of normal cells and Stimulating appetite, improving quality of sleep, relieving pain, thus benefiting patients health **(Sakarkar, D.M., 2011).**

8.5.4. Prevalence of Cancer

Cancer remains the main cause of death in man and women worldwide. It is becoming an increasingly important factor in the global burden of disease. There are 24.6 million people living with cancer at the moment by 2020 there will be an estimated 30 million and it could kill over 10 million people / year. Almost 7 million people now die each year from cancer. Some cancers are more common in developed countries. Lung, prostate, breast and colon cancer, liver, stomach and cervical cancer are more common in developing countries. The world cancer reports tells us that cancer rates are set increased at an alarming rate globally.

Origin of Cancer: All cancers begin in cells, the body's basic unit of life. The body is made up of many types of cells. These cells grow and divide in a controlled way to produce more cells as they are needed to keep the body healthy. When cells become old or damaged, they die and are replaced with new cells. However, sometimes this orderly process goes wrong. The genetic material (DNA) of a cell can become damaged or changed, producing mutations that affect normal cell growth and division. When this happens, cells do not die when they should and new cells form when the body does not need them. The extra cells may form a mass of tissue called a tumor.

Fig. No: 88 Percentage of Cancer Death due to Various Factors

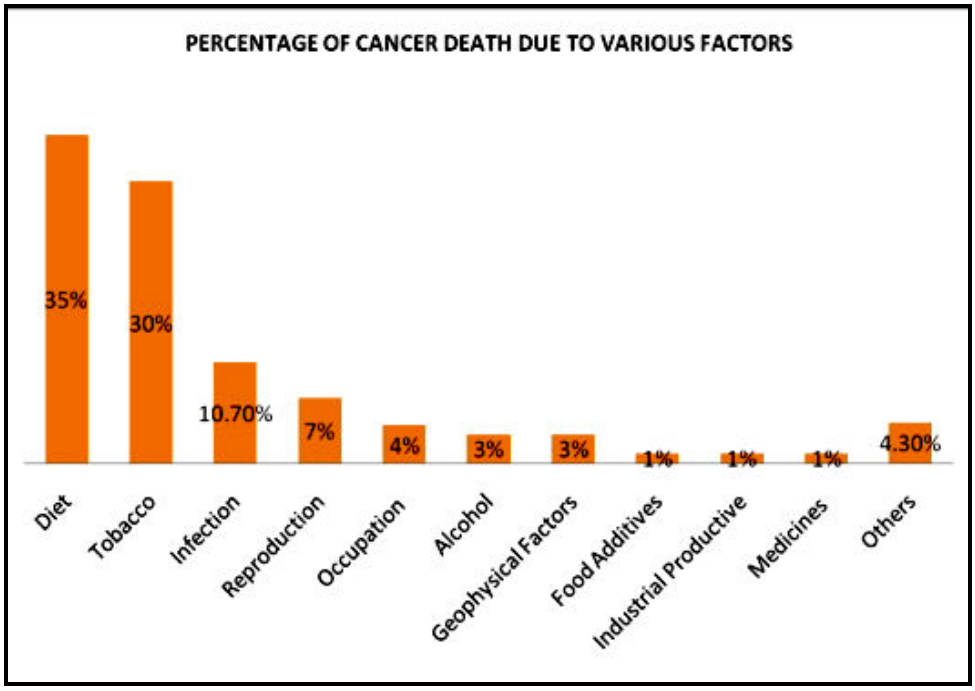
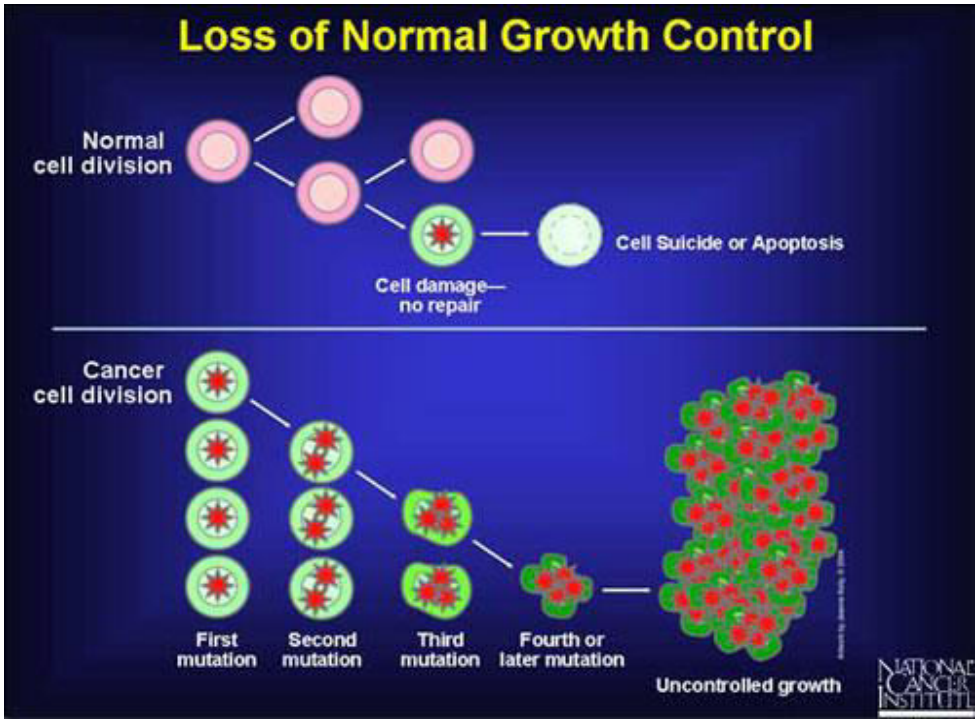


Fig. No: 89 Development of Cancer



8.5.5. Incidence of Cancer

Cancer may affect people at all ages, even foetus. Risk of more common varieties of cancer tends to increase with age. Cancer cells are formed continuously in the organism. Those which have the potential to divide and form cancer are effectively destroyed by the various mechanisms available to immune system. Cancer causes about 13% of all deaths **(www.asterpnc.com).**

8.5.6. Plants as Anti-Cancer Agents

Cancer is a major public health burden in both developed and developing countries. Plants have long been used in the treatment of cancer. The National cancer institute collected about 35,000 plant samples from 20 countries and has screened around 114,000 extracts for anti-cancer activity. Of the 92 anti-cancer drugs commercially available prior to 1983 in the US; among worldwide approved anti-cancer drugs between 1983 and 1994 **(Shoep, M., 2006).** Hartwell, in his review of plants used against cancer, lists more than 3000 plant species **(Graham, J.G., 2000).**

In many instances, however, the **"cancer"** is undefined, or reference is made to conditions such as **"hard swelling"**, abscesses, calluses, corns, warts, polyps or tumors to name a few. These symptoms would generally applied to skin, "tangible" or visible conditions, and may indeed sometimes correspond to a cancerous condition.

Cancer, as a specific disease entity, is likely to the poorly defined in terms of folklore and traditional medicine. This is in contrast to other traditional medicine for the treatment of malaria and pain, which are more easily defined. It is significant that over 60% of currently used anti-cancer agents are derived in one way or another from natural sources, including plants, marine organisms and micro-organisms. Indeed, these sources have played, and continue to play, a dominant role in the discovery of leads for the treatment of most human diseases.

The following table gives the estimated numbers of new cases and deaths for each common cancer type:

Table: 28 Estimated Numbers of New Cases and Deaths for Each Common Cancer Type

Cancer Type	Estimated New Cases	Estimated Deaths
Bladder	70,980	14,330
Breast (Female - Male)	192,370 - 1,910	40,170 - 440
Colon & Rectal (Combined)	146,970	49,920
Endometrial	42,160	7,780
Kidney(Renal Cell) Cancer	49,096	11,033
Leukemia (ALL)	44,790	21,870
Lung (Including Bronchus)	219,440	159,390
Melanoma	68,720	8,650
Non-Hodgkin Lymphoma	65,980	19,500
Pancreatic	42,470	35,240
Prostate	192,280	27,360
Skin (Non-melanoma)	>1,000,000	<1,000
Thyroid	37,200	1,630

The search for anticancer agents from plant sources started in 1950's with the discovery and development of vinca alkaloids, vincristine vinblastine and the isolation of cytotoxic podophyllotoxin. These discoveries in turn lead to discovery of taxanes and camptothecins but their development into clinically active agent spanned period of 30 years from 1960's to 1990's.

8.5.7. Tumor Cell Line

The Cell viability was assessed by **Wang *et al.,* (2001**) for the flowers of ***Nymphaea pubescens* Willdenow** and ***Nymphaea nouchali* Burmann. F.** in human cervical cancer (HeLa) cell lines which are obtained from National Centre for Cell Science (NCCS), Pune. Briefly cells were seeded concentrations of hydroalcoholic extracts of flowers (10, 25, 50, 100 and 200µg/mL) were added separately. Since 1% ethanol was added to drug treatment group. Positive control (5-flurouracil), control group was added with 1% ethanol. Each concentration was repeated three times. These cells were incubated in a humidified atmosphere with 5% CO_2 for three days. At concentration of $1x10^6$ cells in a 0.1 mL in a 96 well plates. After overnight incubation, serial 20µl MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) was added to each well and incubated at 37° C for 4 hrs. The medium was removed and formazan was dissolved in DMSO and the absorbance is taken at 570nm.

The growth inhibition was determined using growth inhibition = (Control O.D. - Sample O.D/Control O.D) and further IC_{50} value were determined. IC_{50} was defined as the concentration of the extract that produced a 50% decrease in cell viability relative to the negative control which was wells exposed to the solvent without any extract **(Mosaddegh M., 2006), (Mosaddegh M., 2010).**

Non-linear regression graph was plotted between % Cell inhibition and concentration and IC_{50} was determined using GraphPad Prism software.

8.5.8. *In-vivo* screening of Anti Cancer Activity - Dalton's-Induced Ascitic Antitumor Model

Method: 0.5mL of 0.4% Tryphan blue, 0.3mL of PBS and 0.2mL of cell suspension were mixed and kept aside for 5min and not more than 15min. From this one drop of solution was taken on a neubar chamber and a cover slip is placed. This is placed on Haemocytometer and the viable and non-viable cells were counted fewer than 10X power. Viable cells doesn't take colour and these cells appear in white colour on blue background Non-viable cells(dead cells) take blue colour and give dark blue shading to the cells, cell count was calculated using formula**.**

Cell count = No. of cells × Dilution factor × Volume factor

The anti-tumor activity of the extract was determined by injecting DAL cell suspension (1×10^6 cells per mouse) in to the peritoneal cavity of the animals and treatment was started after 24 hours of the tumor inoculation continued once daily for 14 days. The antitumor efficacy of extract was compared with that of 5-Fu (20mg/kg, i.p) and DAL control by **Talwar, (Talwar GP., 1974).**

8.5.9. Experimental Protocol

This method in designed to evaluate anticancer activity by **mean survival time, body weight analysis**. The animals were divided into 15 groups. Each group contains 6 animals. Induction of DAL cell suspension (1×10^6 cells per mouse) into the peritoneal cavity of the animals and treatment was started after 24 hours of the tumor inoculation continued once daily for 14 days. Group 1 as normal control, group 2 as DAL cell induced lymphoma, group 3 DAL+5FU 20 mg/kg, group IV-XV received 100, 200 and 400 µg/kg of the flower extracts of EAENP and HAENP, EAENN and HAENN respectively.

8.5.10. Determination of Body Weight and Survival Time

a. Body Weight Analysis

All the mice were weighed for every five days, after tumor inoculation. Average gain in body weight was determined and % decrease in body weight was calculated by the formula.

% Decrease in body weight = (Decrease in body weight/initial body weight) ×100

b. Mean Survival Time (MST*)*

After induction every day checks all the groups for mortality & record how many days the mouse is survived the mean survival time (MST) and percentage increase in life span (ILS %) was calculated by using the formula,

Mean survival time = [1st Death + Last Death] / 2

ILS (%) = [(Mean survival of treated group/ Mean survival of control group)-1] x 100

8.6. RESULTS AND DISCUSSION

8.6.1. Acute Toxicity Studies (LD_{50})

It is the normal practice to determine the LD_{50} value, it is worthwhile to study an acute toxicity studies by employing several doses including reasonably high doses. Acute toxicity studies were conducted using a dose of 2000 mg/kg, p.o. with two different extracts of ***Nymphaea pubescens*** **Willdenow** and ***Nymphaea nouchali*** **Burmann. F.** in female albino rats according to the OECD guidelines. Even at this high dose EAENP, HAENP, EAENN and HAENN did not exhibit any sign or symptoms of toxicity and mortality. Hence low dose (100 mg/kg, p.o.) medium dose (200 mg/kg, p.o.) and high dose of (400 mg/kg, p.o.) of Ethylacetate and Hydroalcoholic extracts of the flowers of ***Nymphaea pubescens*** **Willdenow** and ***Nymphaea nouchali*** **Burmann. F.** was selected for further studies in animals.

8.6.2. PARACETAMOL INDUCED HEPATOPROTECTIVE ACTIVYTY

8.6.2.1. Effect of EAENP and HAENP on Paracetamol Induced Hepatotoxicity

i). Physical Parameters

Paracetamol treatment in rats resulted in enlargement of liver which was evident by increase in the wet liver weight and volume. The groups were treated with Silymarin and EAENP and HAENP showed significant restoration of wet liver weight and wet liver volume nearer to normal. These values are tabulated in the **Table No. 29** and graphically represented in **Fig. No: 90, 91.**

Table No: 29 Effect of EAENP and HAENP on Wet Liver Weight & Wet Liver Volumes in Paracetamol Induced Hepatotoxic Rats

Groups	Treatment	Wet Liver weight (gm/100gm) (Mean ± SEM)	Wet Liver volumes (ml/100gm) (Mean ±SEM)
Group I	Negative Control (0.5ml saline)	4.15 ± 0.14	4.31 ± 0.19
Group II	Positive Control Paracetamol (2 g/kg p.o.)	5.72 ± 0.16	5.91 ± 0.14
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o. + 100 mg/kg p.o.)	4.13 ± 0.30 ***	4.27 ± 0.028***
Group IV	Paracetamol + EAENP (2 g/kg p.o. + 100 mg/kg p.o.)	4.83 ± 0.18^{ns}	4.99 ± 0.19*
Group V	Paracetamol + EAENP (2 g/kg p.o. + 200 mg/kg p.o.)	4.32 ± 0.15**	4.52 ± 0.16***
Group VI	Paracetamol + EAENP (2 g/kg p.o. + 400 mg/kg p.o.)	4.24 ± 0.23***	4.35 ± 0.22***
Group VII	Paracetamol + HAENP (2 g/kg p.o. + 100 mg/kg p.o.)	4.89 ± 0.21^{ns}	4.96 ± 0.20*
Group VIII	Paracetamol + HAENP (2 g/kg p.o. + 200 mg/kg p.o.)	4.59 ± 0.13*	4.69 ± 0.14**
Group IX	Paracetamol + HAENP (2 g/kg p.o. + 400 mg/kg p.o.)	4.25 ± 0.26***	4.52 ± 0.18***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** **P<0.001**, ** **P<0.01**, * **P<0.05** and ns represents Not significant. All the values are compared to Paracetamol treated group.

EAENP: Ethylacetate extract of *Nymphaea pubescens*

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

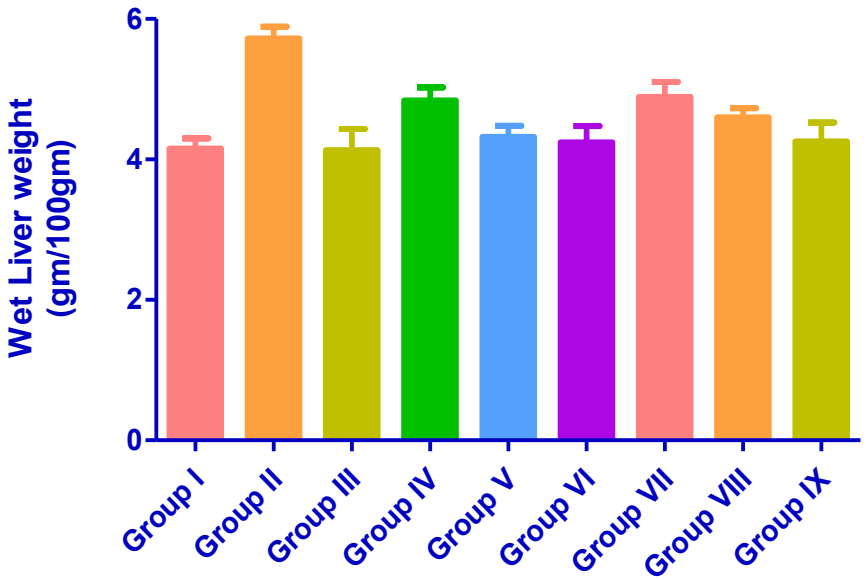


Fig. No: 90 Effect of EAENP and HAENP on Wet Liver Weight in Paracetamol Induced Hepatotoxic Rats

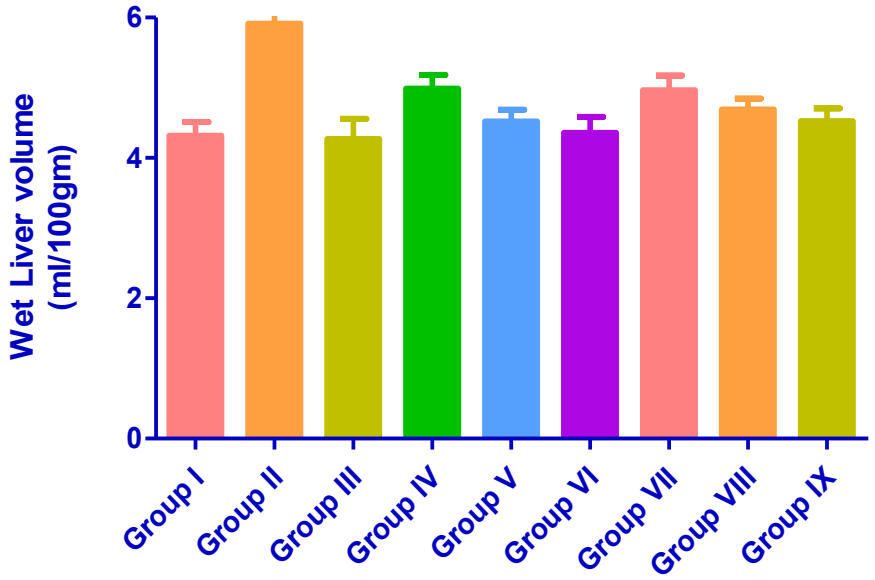


Fig. No: 91 Effect of EAENP and HAENP on Wet Liver Volumes in Paracetamol Induced Hepatotoxic Rats

8.6.2.1.1. Biochemical Parameters

i). Effect on Serum Marker Enzyme

There is a marked increase in SGPT levels observed in Paracetamol treated group. However the SGPT levels were decreased by EAENP and HAENP dose dependently. In addition the standard silymarin has restored the SGPT levels significantly.

Serum SGOT levels have been also elevated in the Paracetamol treated groups. Treatment with standard silymarin has brought back the SGOT levels to the near normal levels. However treatment with the EAENP and HAENP has decreases the SGOT levels in a dose dependent manner, which statistically significant.

Rise in ALP serum levels observed in Paracetamol treated group, and was remarkable decreased significantly by the EAENP and HAENP by dose dependent manner and standard silymarin treatment.

The results are summarized in **Table No: 30** and graphically depicted in **Fig. No: 92**

Table No.: 30 Effect of EAENP and HAENP on SGPT, SGOT and ALP Levels in Paracetamol Induced Hepatotoxic Rats

Groups	Treatment	SGPT Levels (U/L) (Mean ± SEM)	SGOT Levels (U/L) (Mean ± SEM)	ALP (U/L) (Mean ± SEM)
Group I	Negative Control (0.5ml saline)	51.09 ± 3.10	88.04 ± 2.51	126.5 ± 3.52
Group II	Positive Control Paracetamol (2 g/kg p.o.)	289.1 ± 8.50	406.2 ± 6.97	246.6 ± 3.61
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o. + 100 mg/kg p.o.)	62.27 ± 4.74***	121.2 ± 4.68***	90.52 ± 4.20***
Group IV	Paracetamol + EAENP (2 g/kg p.o. + 100 mg/kg p.o.)	126.4 ± 4.90***	232.5 ± 4.71***	151.3 ± 5.01***
Group V	Paracetamol + EAENP (2 g/kg p.o. + 200 mg/kg p.o.)	100.5 ± 4.71***	173.2 ± 6.32***	123.2 ± 5.15***
Group VI	Paracetamol + EAENP (2 g/kg p.o. + 400 mg/kg p.o.)	71.87 ± 4.38***	139.4 ± 3.96***	102.1 ± 5.63***
Group VII	Paracetamol + HAENP (2 g/kg p.o. + 100 mg/kg p.o.)	134.9 ± 2.47***	244.6 ± 4.22***	165.2 ± 5.06***
GroupVIII	Paracetamol + HAENP (2 g/kg p.o. + 200 mg/kg p.o.)	107.3 ± 6.29***	177.1 ± 6.67***	128.4 ± 3.66***
Group IX	Paracetamol + HAENP (2 g/kg p.o. + 400 mg/kg p.o.)	78.88 ± 4.35***	142.2 ± 4.38***	106.2 ±5.71***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** **P<0.001,** ** **P<0.01,** * **P<0.05** and ns represents Not significant. All the values are compared to Paracetamol treated group.

EAENP: Ethylacetate extract of *Nymphaea pubescens,*

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

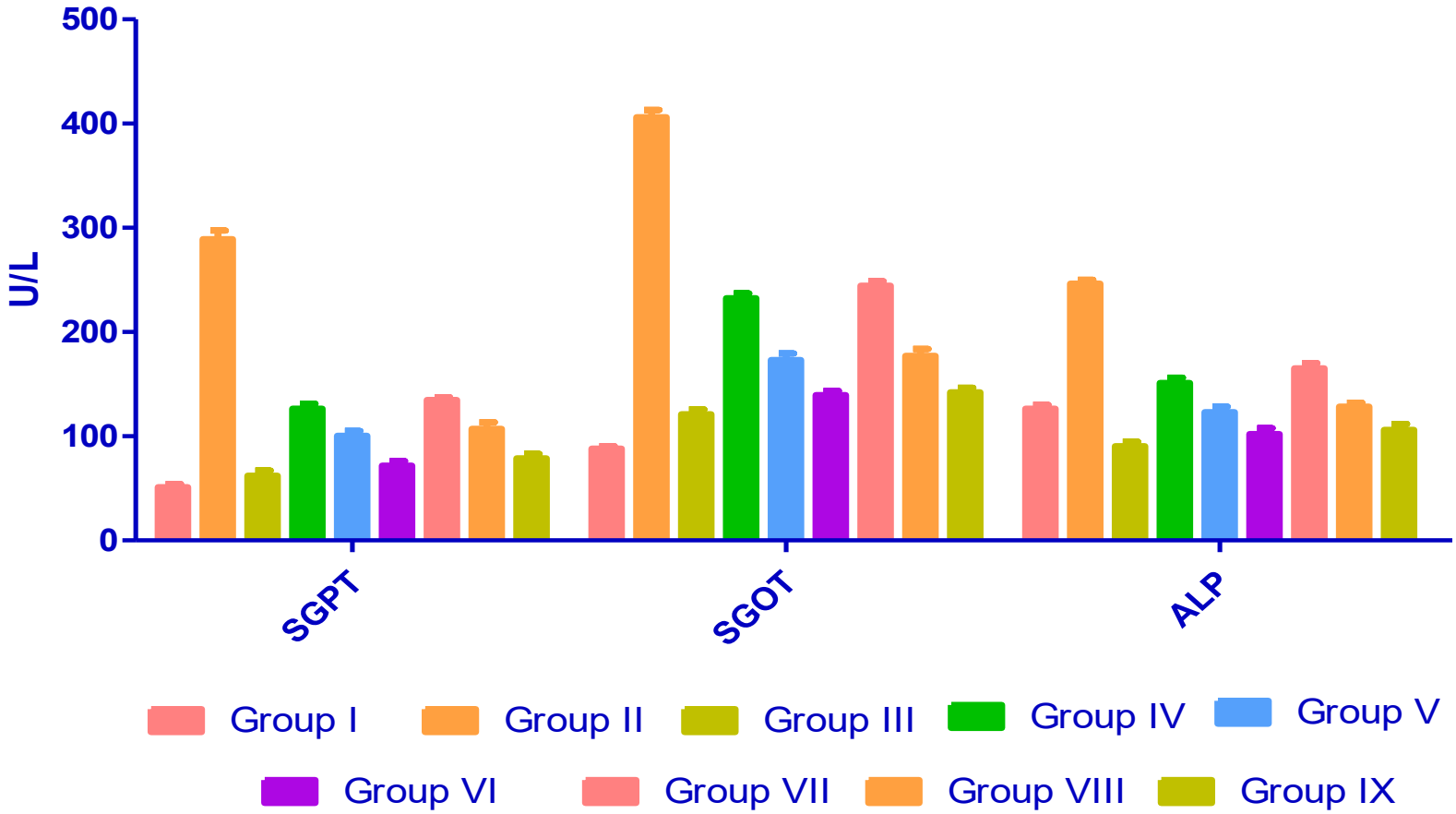


Fig. No: 92 Effect of EAENP and HAENP on SGPT, SGOT and ALP levels in Paracetamol Induced Hepatotoxic Rats

ii). Effect of EAENP and HAENP on Direct Bilirubin, Total Bilirubin Levels

In case of Direct and Total Bilirubin there is a noticeable rise in Serum Levels on Paracetamol treatment observed. Treatment with EAENP and HAENP has reversed the total and a direct bilirubin serum level by dose dependent manner, which is statistically significant when compared with Paracetamol treated group. By reversal treatment with standard Silymarin which was also significant. The results are summarized in **Table No: 31** and graphically depicted in **Fig. No: 93.**

Table No: 31 Effect of EAENP and HAENP on Direct Bilirubin, Total Bilirubin levels in Paracetamol Induced Hepatotoxic Rats

Groups	Treatment	Direct Bilirubin (Mean ± SEM)	Total Bilirubin (Mean ± SEM)
Group I	Negative Control (0.5ml saline)	0.213 ± 0.015	0.986 ± 0.039
Group II	Positive Control Paracetamol (2 g/kg p.o.)	1.727 ± 0.109	4.563 ± 0.237
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o. + 100 mg/kg p.o.)	0.371 ± 0.028***	1.315 ± 0.176***
Group IV	Paracetamol + EAENP (2 g/kg p.o. + 100 mg/kg p.o.)	0.825 ± 0.037***	2.620 ± 0.294***
Group V	Paracetamol + EAENP (2 g/kg p.o. + 200 mg/kg p.o.)	0.646 ± 0.029***	1.773 ± 0.172***
Group VI	Paracetamol + EAENP (2 g/kg p.o. + 400 mg/kg p.o.)	0.463 ± 0.036***	1.420 ± 0.142***
Group VII	Paracetamol + HAENP (2 g/kg p.o. + 100 mg/kg p.o.)	0.845 ± 0.034***	2.890 ± 0.322***
Group VIII	Paracetamol + HAENP (2 g/kg p.o. + 200 mg/kg p.o.)	0.695 ± 0.028***	1.898 ± 0.204***
Group IX	Paracetamol + HAENP (2 g/kg p.o. + 400 mg/kg p.o.)	0.486 ± 0.028***	1.498 ± 0.108***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, ***** P<0.001, ** P<0.01, * P<0.05** and ns represents Not significant. All the values are compared to Paracetamol treated group.

EAENP: Ethylacetate extract of *Nymphaea pubescens*

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

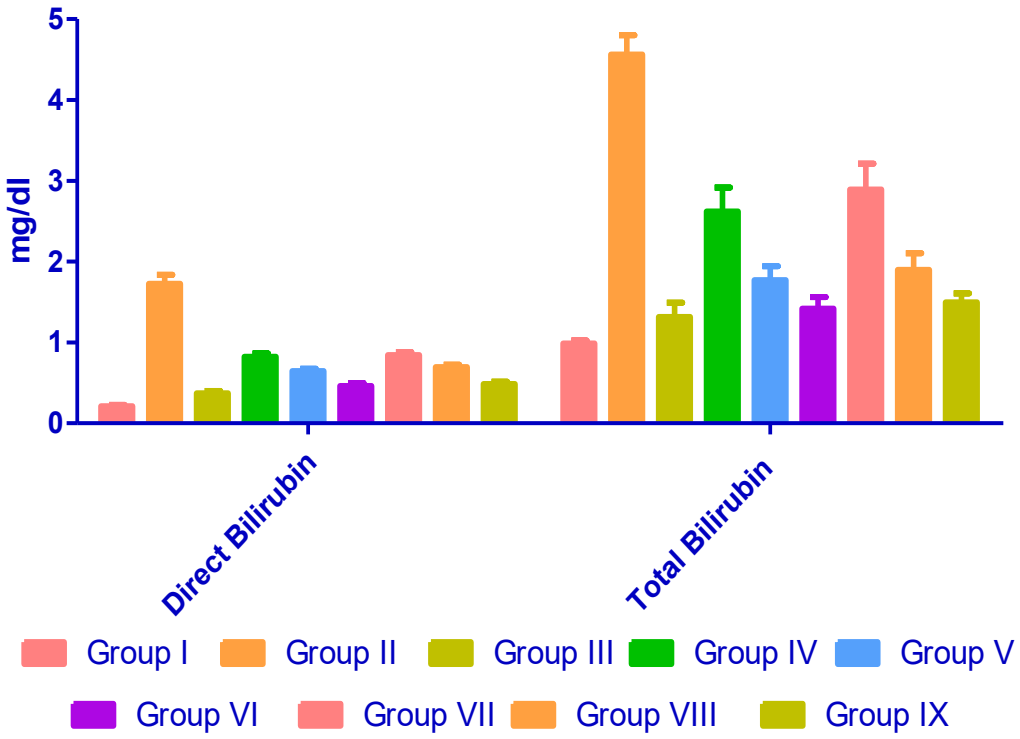


Fig. No: 93 Effect of EAENP and HAENP on Direct Bilirubin, Total Bilirubin levels in Paracetamol Induced Hepatotoxic Rats

iii). Serum Urea, Creatinine

Paracetamol treatment rats showed an increased in the Levels of Serum Urea and Creatinine. Treatment of these rats with the EAENP and HAENP and Silymarin showed a decrease in the serum urea and creatinine levels when compared with the normal animals. Serum Urea and Creatinine Levels of treated and normal rats are expressed in the **Table No: 32** and shown in **Fig. No: 94, 95.**

Table No: 32 Effect of EAENP and HAENP on Urea, Creatinine Levels in Paracetamol Induced Hepatotoxic Rats

Groups	Treatment	Urea (Mean ± SEM)	Creatinine (Mean ± SEM)
Group I	Negative Control (0.5ml saline)	32.57 ± 2.346	0.812 ± 0.034
Group II	Positive Control Paracetamol (2 g/kg p.o.)	64.16 ± 5.053	2.292 ± 0.159
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o. + 100 mg/kg p.o.)	35.31 ± 2.532***	0.853 ± 0.027***
Group IV	Paracetamol + EAENP (2 g/kg p.o. + 100 mg/kg p.o.)	47.60 ± 1.427**	1.337 ± 0.179***
Group V	Paracetamol + EAENP (2 g/kg p.o. + 200 mg/kg p.o.)	43.41 ± 2.966***	0.986 ± 0.088***
Group VI	Paracetamol + EAENP (2 g/kg p.o. + 400 mg/kg p.o.)	37.34 ± 2.629***	0.883 ± 0.047***
Group VII	Paracetamol + HAENP (2 g/kg p.o. + 100 mg/kg p.o.)	46.98 ± 3.494**	1.521 ± 0.174***
Group VIII	Paracetamol + HAENP (2 g/kg p.o. + 200 mg/kg p.o.)	41.73 ± 2.101***	1.033 ± 0.069***
Group IX	Paracetamol + HAENP (2 g/kg p.o. + 400 mg/kg p.o.)	39.09 ± 2.697***	0.905 ± 0.062***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** **P<0.001,** ** **P<0.01,** * **P<0.05** and ns represents Not significant. All the values are compared to Paracetamol treated group.

EAENP: Ethylacetate extract of *Nymphaea pubescens*

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

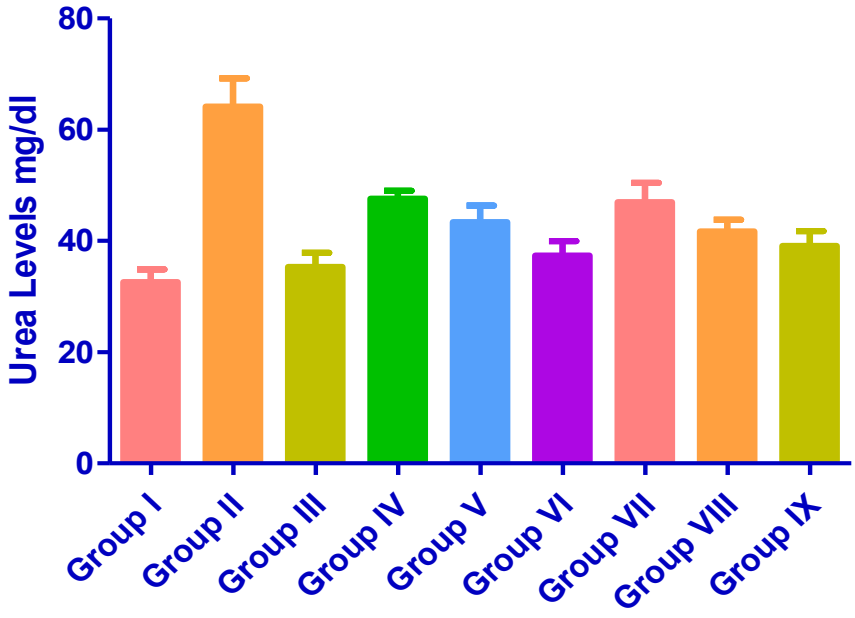


Fig. No: 94 Effect of EAENP and HAENP on Urea levels in Paracetamol Induced Hepatotoxic Rats

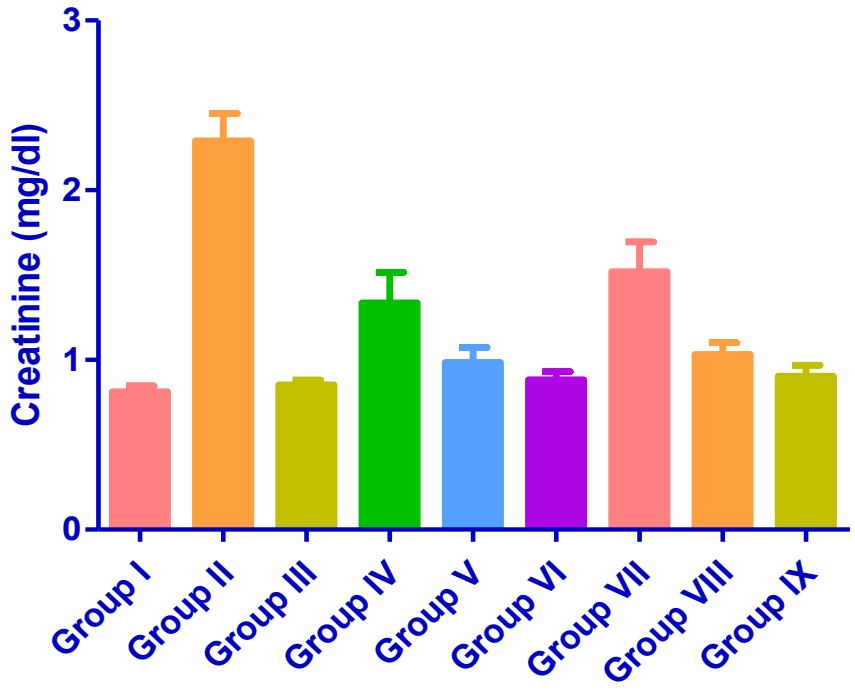


Fig. No: 95 Effect of EAENP and HAENP on Creatinine levels in Paracetamol Induced Hepatotoxic Rats

iv). Serum Total Protein

Paracetamol treatment considerably reduced Serum Total Protein levels. Pretreatment with Silymarin and EAENP and HAENP showed a significant increase in Total Protein levels as compared with toxicant control group.

The results are summarized in **Table No: 33** and graphically depicted in **Fig. No: 96**

Table No: 33 Effect of EAENP and HAENP on Serum Total Protein Levels in Paracetamol Induced Hepatotoxic Rats

Groups	Treatment	Total Protein levels (gm/dl) (Mean ± SEM)
Group I	Negative Control (0.5ml saline)	8.218 ± 0.258
Group II	Positive Control Paracetamol (2 g/kg p.o.)	3.810 ± 0.179
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o. + 100 mg/kg p.o.)	8.068 ± 0.104***
Group IV	Paracetamol + EAENP (2 g/kg p.o. + 100 mg/kg p.o.)	6.085 ± 0.227***
Group V	Paracetamol + EAENP (2 g/kg p.o. + 200 mg/kg p.o.)	7.580 ± 0.449***
Group VI	Paracetamol + EAENP (2 g/kg p.o. + 400 mg/kg p.o.)	7.968 ± 0.416***
Group VII	Paracetamol + HAENP (2 g/kg p.o. + 100 mg/kg p.o.)	5.840 ± 0.096***
Group VIII	Paracetamol + HAENP (2 g/kg p.o. + 200 mg/kg p.o.)	7.193 ± 0.315***
Group IX	Paracetamol + HAENP (2 g/kg p.o. + 400 mg/kg p.o.)	7.865 ±0.307***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, ***** P<0.001, ** P<0.01, * P<0.05** and ns represents Not significant. All the values are compared to Paracetamol treated group.

EAENP: Ethylacetate extract of *Nymphaea pubescens*

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

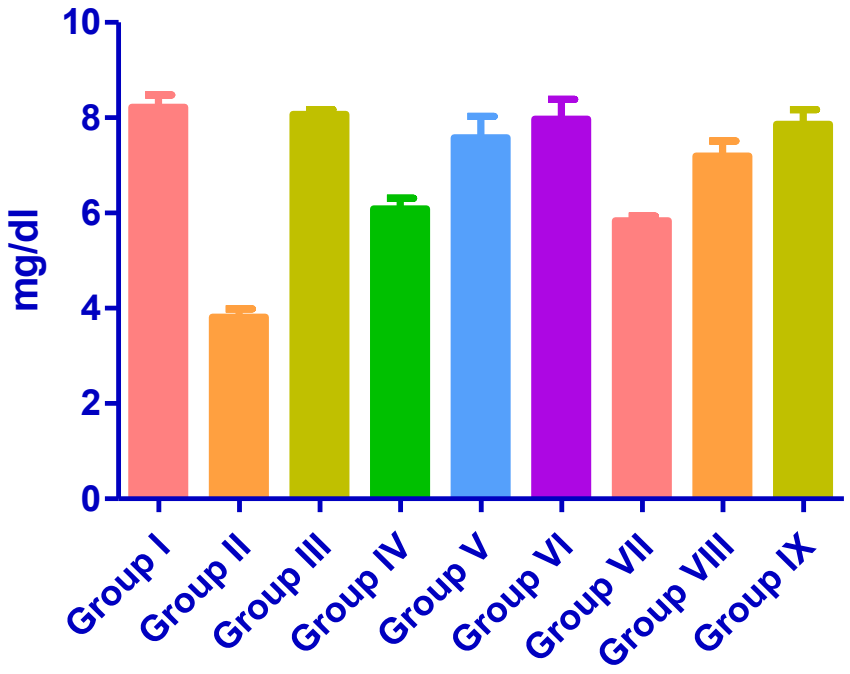


Fig. No: 96 Effect of EAENP and HAENP on Serum Total Protein Levels in Paracetamol Induced Hepatotoxic Rats

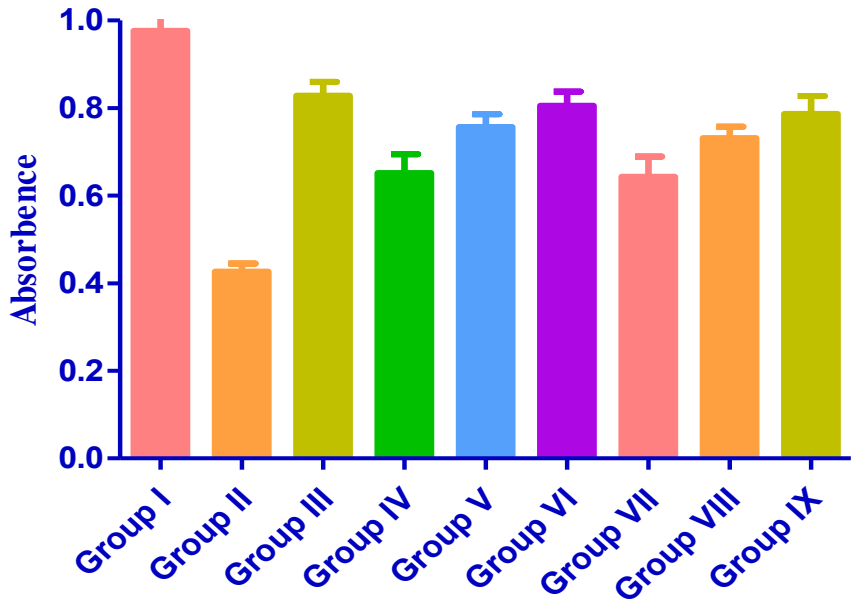


Fig. No: 97 Effect of EAENP and HAENP on Tissue GSH Levels in Paracetamol Induced Hepatotoxic Rats

8.6.2.1.2. *In-vivo* Non-Enzymatic Antioxidant Activity

i). Effect of EAENP and HAENP on GSH in Paracetamol Induced Hepatotoxicity

There is a marked depletion of GSH levels in paracetamol treated group. 100 mg/kg Silymarin has increased it by 94.36%, EAENP and HAENP has shown a dose dependent increase in the levels of GSH.

The results are summarized in **Table No: 34** and graphically depicted in **Fig. No: 97**

Table No: 34 Effect of EAENP and HAENP on Tissue GSH Levels in Paracetamol Induced Hepatotoxic Rats

Groups	Treatment	Absorbance Mean ± SEM	% Increase
Group I	Negative Control (0.5ml saline)	0.976 ± 0.058	--
Group II	Positive Control Paracetamol (2 g/kg p.o.)	0.426 ± 0.018	--
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o. + 100 mg/kg p.o.)	0.828 ± 0.031***	94.36%
Group IV	Paracetamol + EAENP (2 g/kg p.o. + 100 mg/kg p.o.)	0.651 ± 0.042**	52.81%
Group V	Paracetamol + EAENP (2 g/kg p.o. + 200 mg/kg p.o.)	0.756 ± 0.029***	77.46%
Group VI	Paracetamol + EAENP (2 g/kg p.o. + 400 mg/kg p.o.)	0.805 ± 0.032***	88.96%
Group VII	Paracetamol + HAENP (2 g/kg p.o. + 100 mg/kg p.o.)	0.643 ± 0.045**	50.93%
Group VIII	Paracetamol + HAENP (2 g/kg p.o. + 200 mg/kg p.o.)	0.731 ± 0.025***	71.59%
Group IX	Paracetamol + HAENP (2 g/kg p.o. + 400 mg/kg p.o.)	0.786 ± 0.041***	84.50%

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, ***** P<0.001, ** P<0.01, * P<0.05** and ns represents Not significant. All the values are compared to Paracetamol treated group.

EAENP: Ethylacetate extract of *Nymphaea pubescens*

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

ii). Effect of EAENP and HAENP on *In-vivo* Lipid Peroxidation in Paracetamol Induced Hepatotoxicity

There is a dose dependent inhibition of *In-vivo* Lipid Peroxidation by EAENP and HAENP. 100 mg/kg silymarin has **64.49%** inhibition whereas 400 mg/kg of EAENP and HAENP has **63.18%** and **61.35%** inhibition of Lipid Peroxidation.

The results are summarized in **Table No: 35** and graphically depicted in **Fig. No: 98**

Table No: 35 Effect of EAENP and HAENP on *In-vivo* Lipid Peroxidation in Paracetamol Induced Hepatotoxic Rats

Groups	Treatment	Absorbance Mean ± SEM	% Decrease
Group I	Negative Control (0.5ml saline)	0.251 ± 0.017	--
Group II	Positive Control Paracetamol (2 g/kg p.o.)	0.383 ± 0.019	--
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o. + 100 mg/kg p.o.)	0.136 ± 0.016***	64.49%
Group IV	Paracetamol + EAENP (2 g/kg p.o. + 100 mg/kg p.o.)	0.208 ± 0.020***	45.69%
Group V	Paracetamol + EAENP (2 g/kg p.o. + 200 mg/kg p.o.)	0.163 ± 0.016***	57.44%
Group VI	Paracetamol + EAENP (2 g/kg p.o. + 400 mg/kg p.o.)	0.141 ± 0.017***	63.18%
Group VII	Paracetamol + HAENP (2 g/kg p.o. + 100 mg/kg p.o.)	0.218 ± 0.016***	43.08%
Group VIII	Paracetamol + HAENP (2 g/kg p.o. + 200 mg/kg p.o.)	0.171 ± 0.014***	55.35%
Group IX	Paracetamol + HAENP (2 g/kg p.o. + 400 mg/kg p.o.)	0.148 ± 0.017***	61.35%

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, ***** P<0.001, ** P<0.01, * P<0.05** and ns represents Not significant. All the values are compared to Paracetamol treated group.

EAENP: Ethylacetate extract of *Nymphaea pubescens*

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

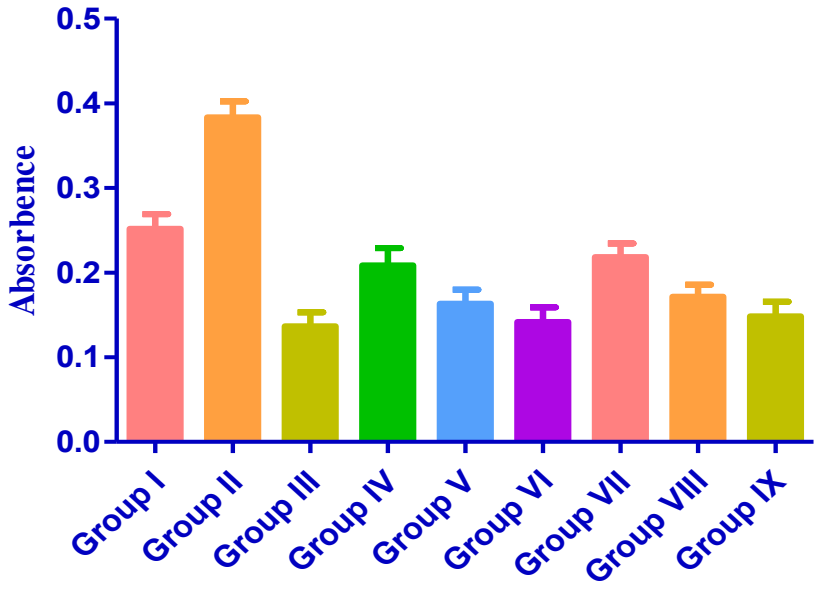


Fig. No: 98 Effect of EAENP and HAENP on *In-vivo* Lipid Peroxidation in Paracetamol Induced Hepatotoxic Rats

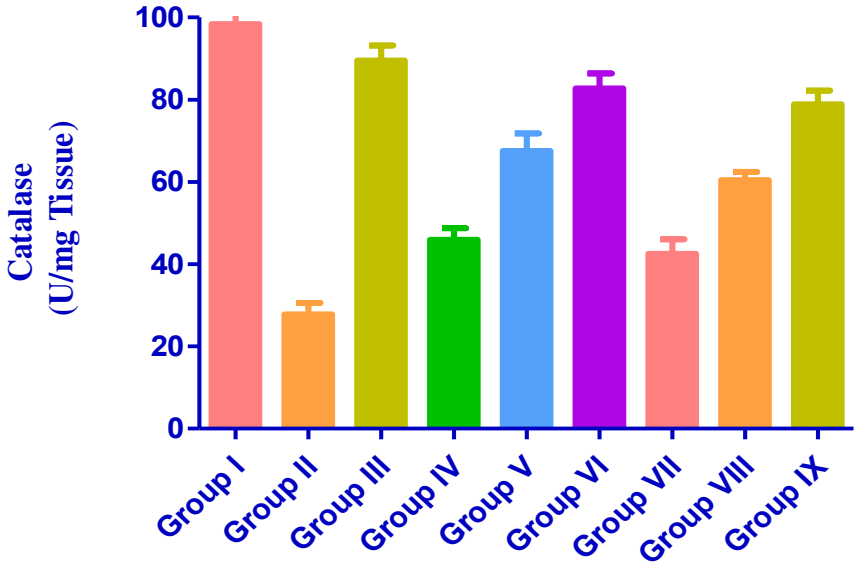


Fig. No: 99 Effect of EAENP and HAENP on Catalase in Paracetamol Induced Hepatotoxic Rats

8.6.2.1.3. *In-vivo* Enzymatic Antioxidant Activity

i). Effect of EAENP and HAENP on Catalase in Paracetamol Induced Hepatotoxicity

Paracetamol Induced Hepatotoxic rats exhibited significant lower Catalase **(27.84 ± 2.747)** as compared to those of negative control rats **(98.44 ± 5.137)** treatment with the plant extract significantly elevated the reduced catalase levels. The 100mg/kg, 200mg/kg, 400mg/kg p.o dose of EAENP, HAENP and Silymarin showed a marked increase in the catalase levels **(P<0.001)** compared to the positive control.

The results are summarized in **Table No: 36** and graphically depicted in **Fig. No: 99.**

Table No: 36 Effect of EAENP and HAENP on Catalase in Paracetamol Induced Hepatotoxic Rats

Groups	Treatment	Catalase (U/mg protein)
Group I	Negative Control (0.5ml saline)	98.44 ± 5.137
Group II	Positive Control Paracetamol (2 g/kg p.o.)	27.84 ± 2.747
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o. + 100 mg/kg p.o.)	89.51 ± 3.697***
Group IV	Paracetamol + EAENP (2 g/kg p.o. + 100 mg/kg p.o.)	45.97 ± 2.771**
Group V	Paracetamol + EAENP (2 g/kg p.o. + 200 mg/kg p.o.)	67.54 ± 4.258***
Group VI	Paracetamol + EAENP (2 g/kg p.o. + 400 mg/kg p.o.)	82.76 ± 3.629***
Group VII	Paracetamol + HAENP (2 g/kg p.o. + 100 mg/kg p.o.)	42.54 ± 3.474*
Group VIII	Paracetamol + HAENP (2 g/kg p.o. + 200 mg/kg p.o.)	60.48 ± 2.006***
Group IX	Paracetamol + HAENP (2 g/kg p.o. + 400 mg/kg p.o.)	78.91 ± 3.277***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, ***** P<0.001, ** P<0.01, * P<0.05** and ns represents Not significant. All the values are compared to Paracetamol treated group.

EAENP: Ethylacetate extract of *Nymphaea pubescens*

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

ii). Effect of EAENP and HAENP on SOD in Paracetamol Induced Hepatotoxicity

Paracetamol induced hepatotoxic rats exhibited significant lower SOD **(5.07 ± 0.488)** as compared to those of negative control rats **(15.39 ± 1.269)** treatment with the plant extract significantly elevated the reduced SOD levels. EAENP, HAENP and Silymarin showed a marked increase in the SOD levels **(P<0.001)** compared to the positive control.

These values are tabulated in the **Table No: 37** and graphically represented in **Fig. No: 100**

Table No: 37 Effect of EAENP and HAENP on SOD in Paracetamol Induced Hepatotoxic Rats

Groups	Treatment	SOD (U/mg protein)
Group I	Negative Control (0.5ml saline)	15.39 ± 1.269
Group II	Positive Control Paracetamol (2 g/kg p.o.)	5.07 ± 0.488
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o. + 100 mg/kg p.o.)	13.51 ± 0.609***
Group IV	Paracetamol + EAENP (2 g/kg p.o. + 100 mg/kg p.o.)	8.76 ± 0.243**
Group V	Paracetamol + EAENP (2 g/kg p.o. + 200 mg/kg p.o.)	11.08 ± 0.688***
Group VI	Paracetamol + EAENP (2 g/kg p.o. + 400 mg/kg p.o.)	12.40 ± 0.533***
Group VII	Paracetamol + HAENP (2 g/kg p.o. + 100 mg/kg p.o.)	8.573 ± 0.614**
Group VIII	Paracetamol + HAENP (2 g/kg p.o. + 200 mg/kg p.o.)	10.68 ± 0.437***
Group IX	Paracetamol + HAENP (2 g/kg p.o. + 400 mg/kg p.o.)	12.16 ± 0.251***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, ***** P<0.001, ** P<0.01, * P<0.05** and ns represents Not significant. All the values are compared to Paracetamol treated group.

EAENP: Ethylacetate extract of *Nymphaea pubescens*

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

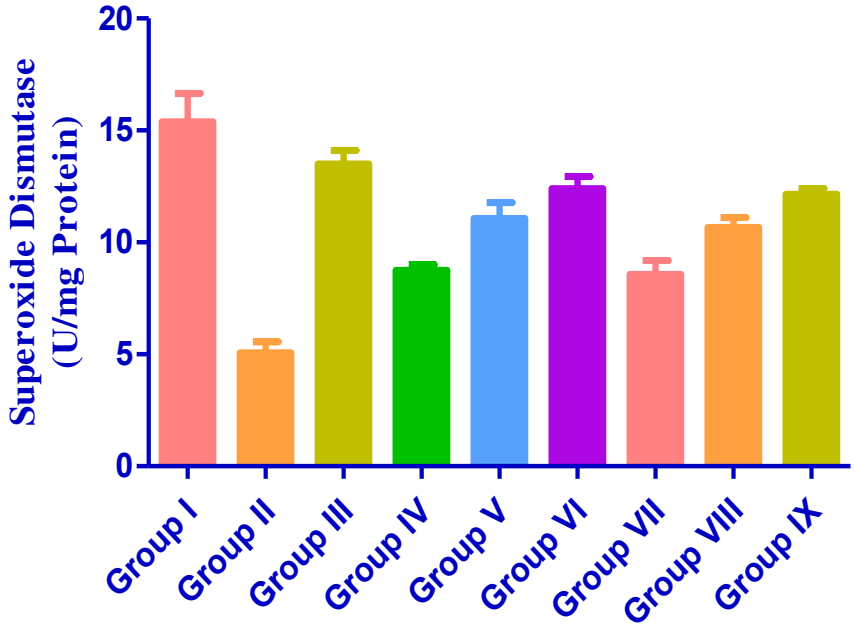


Fig. No: 100 Effect of EAENP and HAENP on SOD in Paracetamol Induced Hepatotoxic Rats

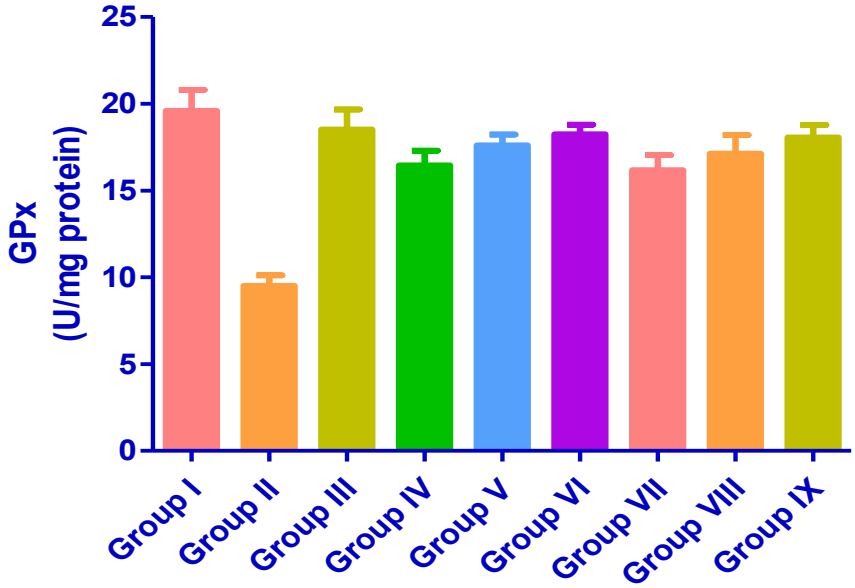


Fig. No: 101 Effect of EAENP and HAENP on GPx in Paracetamol Induced Hepatotoxic Rats

iii). Effect of EAENP and HAENP on GPx in Paracetamol Induced Hepatotoxicity

A significant decrease in antioxidant enzyme GPx activity was observed in the liver of paracetamol administered rats when compared to normal rats that had received vehicle alone. Treatment with EAENP and HAENP shows increase GPx levels in paracetamol treated rats. These values are having a significant higher **(P<0.001)** when compared to GPx levels in Positive control rats.

The values are tabulated in the **Table No: 38** and graphically represented in **Fig. No: 101**

Table No: 38 Effect of EAENP and HAENP on GPx in Paracetamol Induced Hepatotoxic Rats

Groups	Treatment	GPx (U/mg protein)
Group I	Negative Control (0.5ml saline)	19.60 ± 1.211
Group II	Positive Control Paracetamol (2 g/kg p.o.)	9.51 ± 0.594
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o. + 100 mg/kg p.o.)	18.52 ± 1.143***
Group IV	Paracetamol + EAENP (2 g/kg p.o. + 100 mg/kg p.o.)	16.46 ± 0.836***
Group V	Paracetamol + EAENP (2 g/kg p.o. + 200 mg/kg p.o.)	17.60 ± 0.636***
Group VI	Paracetamol + EAENP (2 g/kg p.o. + 400 mg/kg p.o.)	18.25 ± 0.537***
Group VII	Paracetamol + HAENP (2 g/kg p.o. + 100 mg/kg p.o.)	16.19 ± 0.847***
Group VIII	Paracetamol + HAENP (2 g/kg p.o. + 200 mg/kg p.o.)	17.13± 1.062***
Group IX	Paracetamol + HAENP (2 g/kg p.o. + 400 mg/kg p.o.)	18.06 ± 0.707***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, ***** P<0.001, ** P<0.01, * P<0.05** and ns represents Not significant. All the values are compared to Paracetamol treated group.

EAENP: Ethylacetate extract of *Nymphaea pubescens*

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

8.6.2.1.4. Histopathological Studies of the Liver in Paracetamol Induced Hepatotoxicity

Group I: Section studied shows liver parenchyma with intact architecture. Most of the perivenular hepatocytes, periportal hepatocytes and midzonal hepatocytes appear normal. Within the hepatic parenchyma, the sinusoids appear normal.

Group II: Section studied shows liver parenchyma with effaced architecture. Most of the hepatocytes show macrosteatosis, while some show degenerative changes. There are seen focal aggregates of mononuclear inflammatory cells within the parenchyma. Scattered, mononuclear inflammatory infiltration present within the parenchyma.

Group III: Section studied shows liver parenchyma with partially effaced architecture. Some of the sinusoids appear congested. Most of the hepatocytes show macrosteatosis, while few show microsteatosis. There are seen scattered mononuclear inflammatory infiltrations within the parenchyma.

Group IV: Section studied shows liver parenchyma with effaced architecture. Most of the hepatocytes show microsteatosis, while some show macrosteatosis. Some of the hepatocytes show degenerative changes. Intervening the hepatocytes are seen aggregates of mononuclear inflammatory cells.

Group V: Section studied shows liver parenchyma with intact architecture. Most of the hepatocytes, central veins and sinusoids appear normal. Also seen are scattered regenerative hepatocytes. There are seen focal aggregates of mononuclear inflammatory cells within the parenchyma.

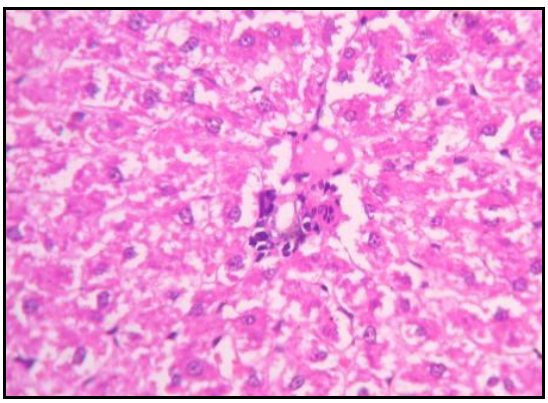
Group VI: Section studied shows liver parenchyma with intact architecture. The sinusoids and central veins appear congested. Also seen are few scattered hepatocytes with macrosteatosis. Intervening the hepatocytes are seen focal aggregates of mononuclear inflammatory cells within the parenchyma.

Group VII: Section studied shows liver parenchyma with effaced architecture. Most of the hepatocytes show degenerative changes, while some show regenerative changes. The central veins and sinusoids appear dilates. There are seen periportal and perivenular aggregates of mononuclear inflammatory cells.

Group VIII: Section studied shows liver parenchyma with intact architecture. Few of the central veins and sinusoids appear congested. Intervening the hepatocytes are seen scattered mononuclear inflammatory cells within the parenchyma.

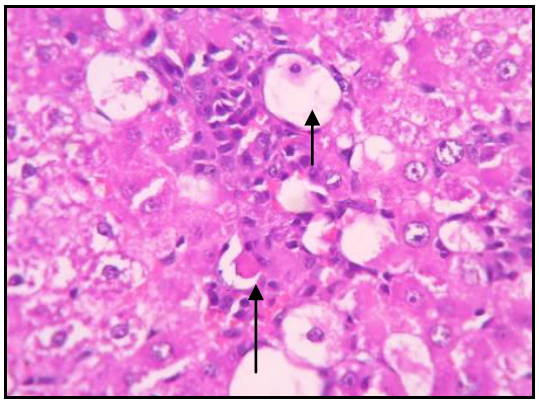
Group IX: Section studied shows liver parenchyma with partially effaced architecture. Some of the central veins and sinusoids appear congested. Also seen are few epitheloid granulomas within the parenchyma**.**

Fig. No: 102 Histopathological Studies of the Rat Liver in Paracetamol Induced Hepatotoxicity



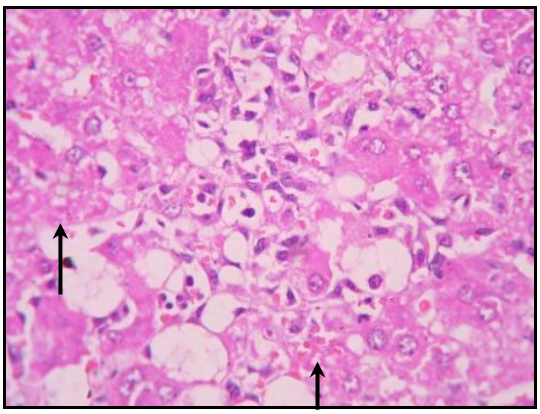
Group I

Negative Control - Saline



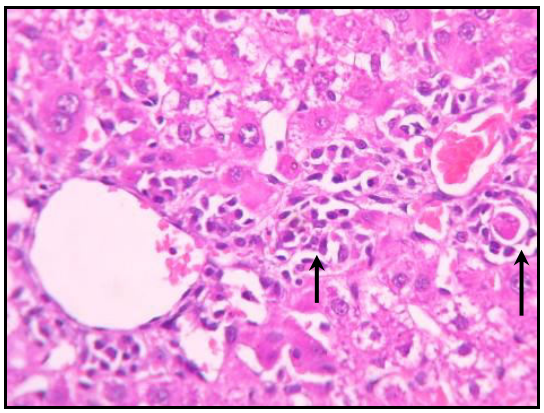
Group II

Positive Control Paracetamol [2 g/kg p.o.]



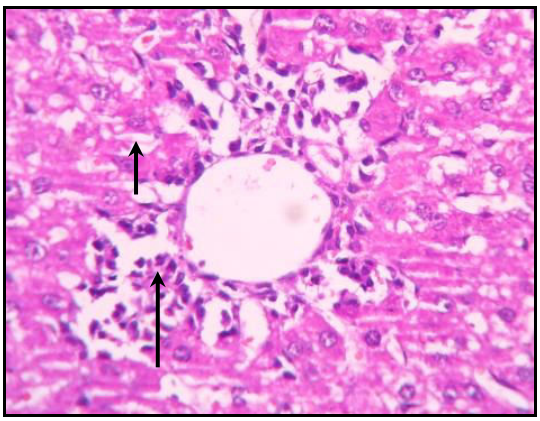
Group III

Paracetamol [2 g/kg p.o.] + Silymarin [100mg/kg]



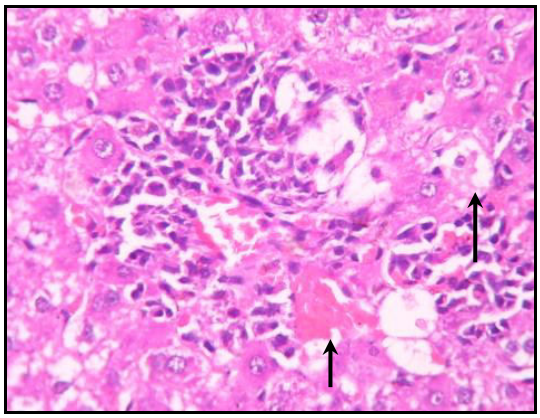
Group IV

Paracetamol [2 g/kg p.o.] + EAENP [100mg/kg]



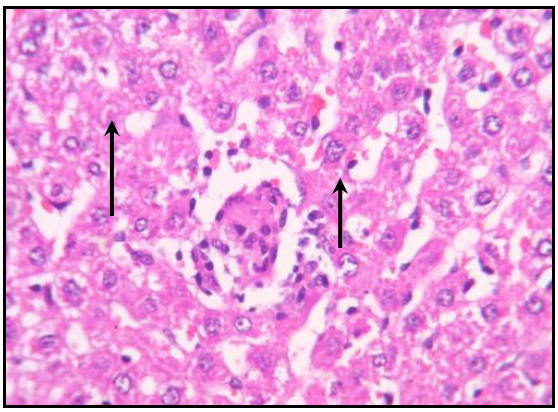
Group V

Paracetamol [2 g/kg p.o.] + EAENP [200mg/kg]



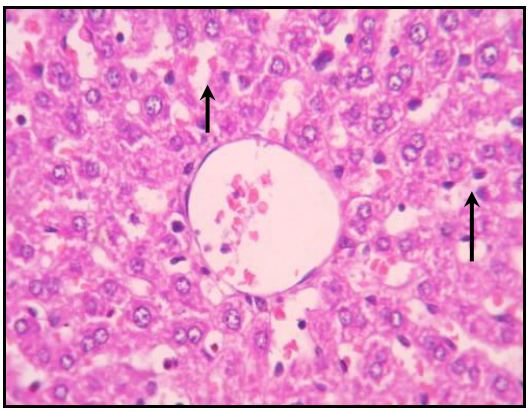
Group VI

Paracetamol [2 g/kg p.o.] + EAENP [400mg/kg]



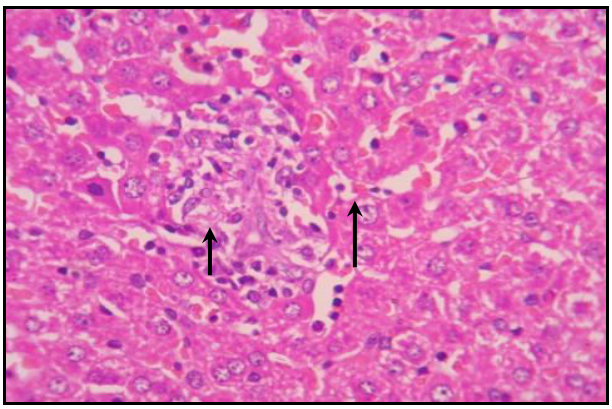
Group VII

Paracetamol [2 g/kg p.o.] + HAENP [100mg/kg]



Group VIII

Paracetamol [2 g/kg p.o.] + HAENP [200mg/kg]



Group IX

Paracetamol [2 g/kg p.o.] + HAENP [400mg/kg]

Fig. No: 102 Histopathological Studies of the Rat Liver in Paracetamol Induced Hepatotoxicity

8.6.2.2. Effect of EAENN and HAENN on Paracetamol Induced Hepatotoxicity

i). Physical Parameters

Paracetamol treatment in rats resulted in enlargement of Liver which was evident by increase in the Wet Liver Weight and Volume. The groups were treated with Silymarin and EAENN and HAENN showed significant restoration of wet liver weight and wet liver volume nearer to normal. These values are tabulated in the **Table No: 39** and graphically represented in **Fig No: 103, 104.**

Table No: 39 Effect of EAENN and HAENN on Wet Liver Weight & Wet Liver Volumes in Paracetamol Induced Hepatotoxic Rats

Groups	Treatment	Wet Liver weight (gm/100gm) (Mean ± SEM)	Wet Liver volumes (ml/100gm) (Mean ±SEM)
Group I	Negative Control (0.5ml saline)	4.15 ± 0.14	4.31 ± 0.19
Group II	Positive Control Paracetamol (2 g/kg p.o.)	5.72 ± 0.16	5.91 ± 0.14
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o. + 100 mg/kg p.o.)	4.13 ± 0.30 ***	4.27 ± 0.028***
Group IV	Paracetamol + EAENN (2 g/kg p.o. + 100 mg/kg p.o.)	4.66 ± 0.12*	4.74 ± 0.15*
Group V	Paracetamol + EAENN (2 g/kg p.o. + 200 mg/kg p.o.)	4.39 ± 0.15**	4.45 ± 0.16***
Group VI	Paracetamol + EAENN (2 g/kg p.o. + 400 mg/kg p.o.)	4.18 ± 0.09***	4.22 ± 0.05***
Group VII	Paracetamol + HAENN (2 g/kg p.o. + 100 mg/kg p.o.)	5.01 ± 0.28^{ns}	5.07 ± 0.29^{ns}
Group VIII	Paracetamol + HAENN (2 g/kg p.o. + 200 mg/kg p.o.)	4.46 ± 0.27**	4.52 ± 0.27**
Group IX	Paracetamol + HAENN (2 g/kg p.o. + 400 mg/kg p.o.)	4.23 ± 0.25***	4.26 ± 0.26***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, ***** P<0.001, ** P<0.01, * P<0.05** and ns represents Not significant. All the values are compared to Paracetamol treated group.

EAENN: Ethylacetate extract of *Nymphaea nouchali*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

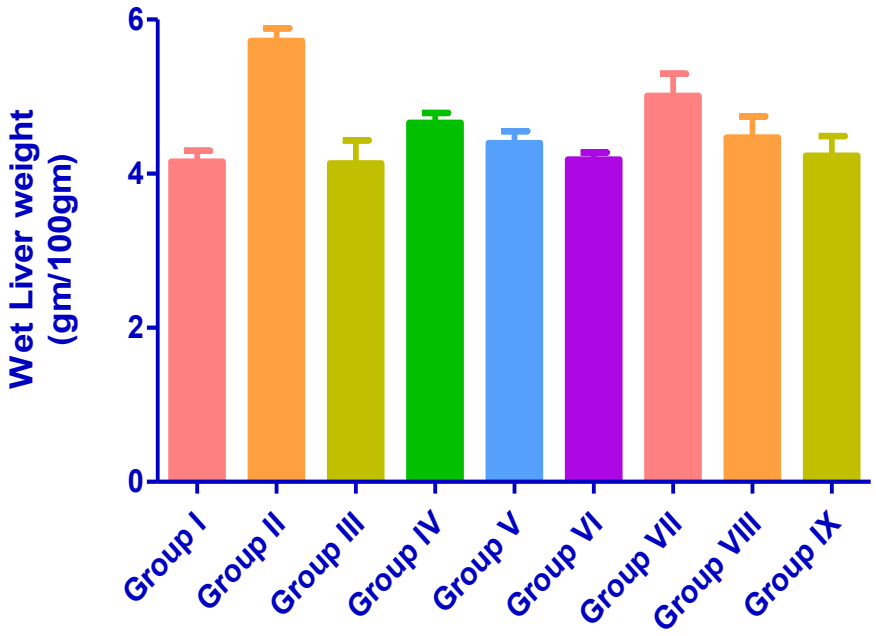


Fig. No: 103 Effect of EAENN and HAENN on Wet Liver Weight in Paracetamol Induced Hepatotoxic Rats

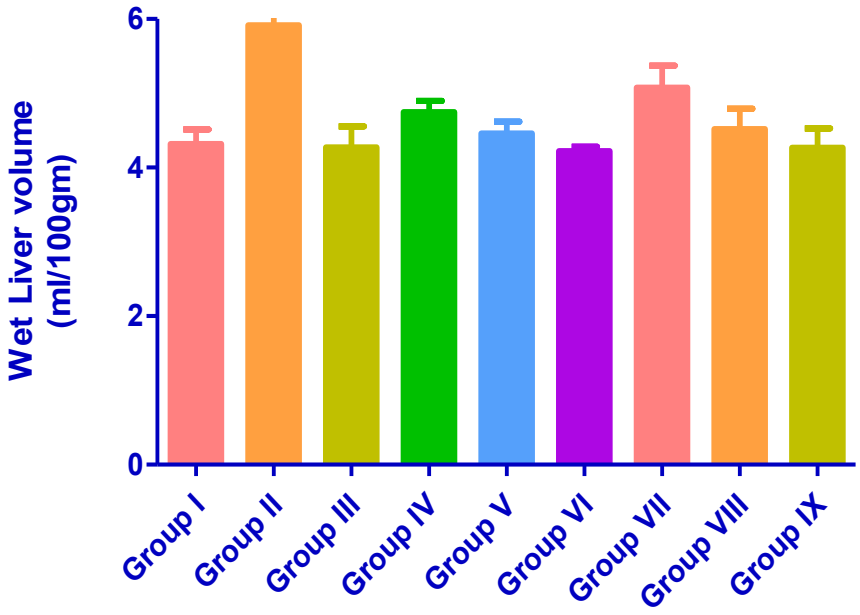


Fig. No: 104 Effect of EAENN and HAENN on Wet Liver Volumes in Paracetamol Induced Hepatotoxic Rats

8.6.2.2.1. Biochemical Parameters

i). Effect on Serum Marker Enzymes

There is a marked increase in SGPT Levels observed in Paracetamol treated group. However the SGPT levels were decreased by EAENN and HAENN dose dependently. In addition the standard Silymarin has restored the SGPT levels significantly.

Serum SGOT Levels have been also elevated in the Paracetamol treated groups. Treatment with standard Silymarin has brought back the SGOT Levels to the near normal levels. However treatment with the EAENN and HAENN has decreases the SGOT levels in a dose dependent manner, which statistically significant.

Rise in ALP Serum Levels observed in Paracetamol treated group, and was remarkable decreased significantly by the EAENN and HAENN by dose dependent manner and standard Silymarin treatment.

The results are summarized in **Table No: 40** and graphically depicted in **Fig. No: 105.**

Table No.: 40 Effect of EAENN and HAENN on SGPT, SGOT and ALP Levels in Paracetamol Induced Hepatotoxic Rats

Groups	Treatment	SGPT Levels (U/L) (Mean ± SEM)	SGOT Levels (U/L) (Mean ± SEM)	ALP (U/L) (Mean ± SEM)
Group I	Negative Control (0.5ml saline)	51.09 ± 3.10	88.04 ± 2.51	126.5 ± 3.52
Group II	Positive Control Paracetamol (2 g/kg p.o.)	289.1 ± 8.50	406.2 ± 6.97	246.6 ± 3.61
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o. + 100 mg/kg p.o.)	62.27 ± 4.74***	121.2 ± 4.68***	90.52 ± 4.20***
Group IV	Paracetamol + EAENN (2 g/kg p.o. + 100 mg/kg p.o.)	152.6 ± 3.45***	217.1 ± 9.38***	158.4 ± 3.36***
Group V	Paracetamol + EAENN (2 g/kg p.o. + 200 mg/kg p.o.)	104.8 ± 3.81***	167.5 ± 4.84***	126.2 ± 3.02***
Group VI	Paracetamol + EAENN (2 g/kg p.o. + 400 mg/kg p.o.)	82.59 ± 2.85***	132.4 ± 6.58***	98.55 ± 3.84***
Group VII	Paracetamol + HAENN (2 g/kg p.o. + 100 mg/kg p.o.)	162.6 ± 2.64***	229.8 ± 8.29***	162.3 ± 5.64***
Group VIII	Paracetamol + HAENN (2 g/kg p.o. + 200 mg/kg p.o.)	113.4 ± 1.89***	171.2 ± 3.52***	124.2 ± 3.01***
Group IX	Paracetamol + HAENN (2 g/kg p.o. + 400 mg/kg p.o.)	89.75 ± 2.09***	137.8 ± 4.60***	103.2 ± 4.99***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared to Paracetamol treated group.

EAENN: Ethylacetate extract of *Nymphaea nouchali*, **HAENN**: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

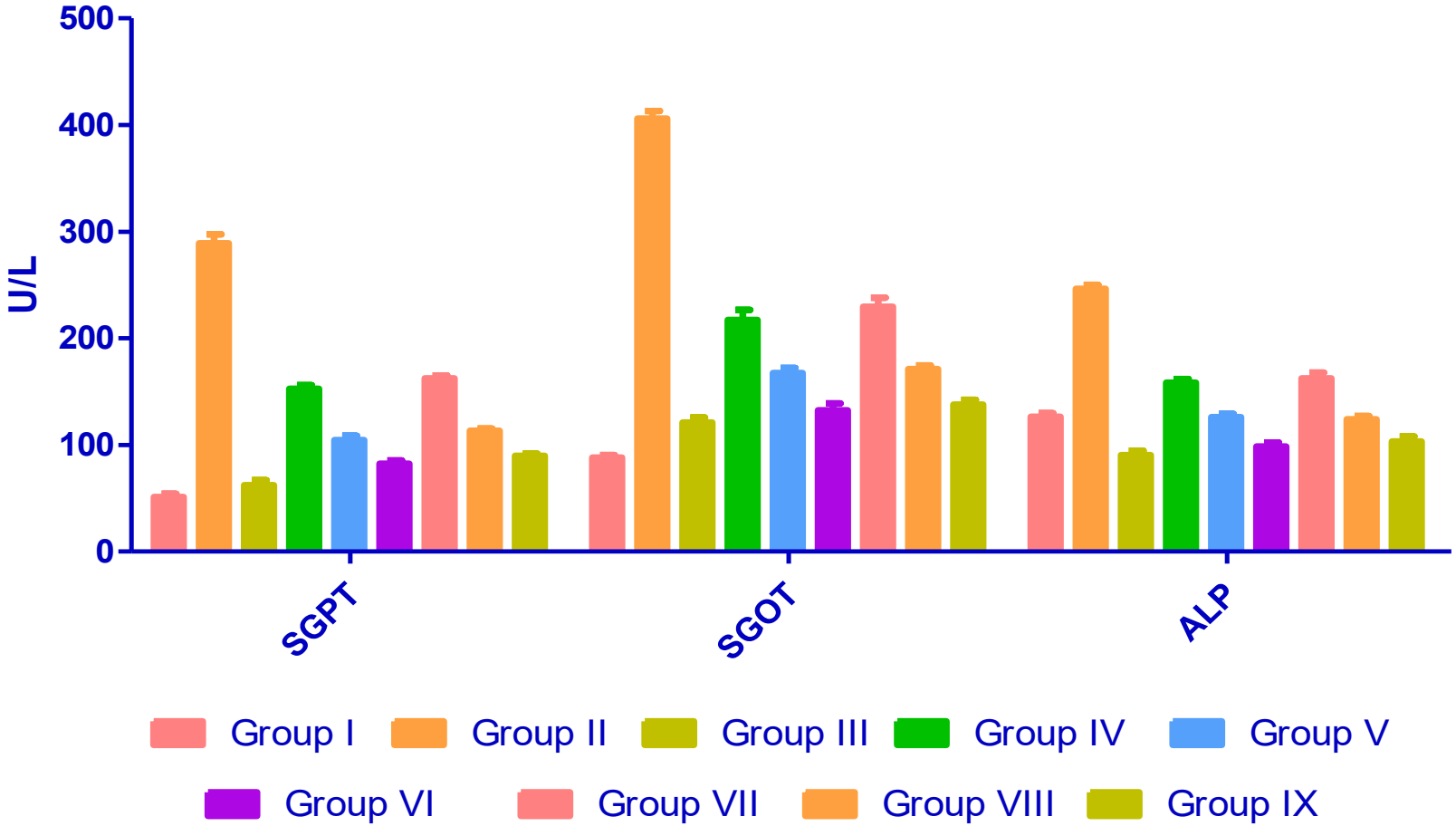


Fig. No: 105 Effect of EAENN and HAENN on SGPT, SGOT and ALP Levels in Paracetamol Induced Hepatotoxic Rats

ii). Direct Bilirubin, Total Bilirubin

In case of total and direct bilirubin there is a noticeable rise in serum levels on Paracetamol treatment observed. Treatment with EAENN and HAENN has reversed the total and a direct bilirubin serum level by dose dependent manner, which is statistically significant when compared with Paracetamol treated group. By reversal treatment with standard silymarin which was also significant and are expressed in the **Table No: 41** and shown in **Fig. No: 106.**

.**Table No.: 41 Effect of EAENN and HAENN on Direct Bilirubin, Total Bilirubin levels in Paracetamol Induced Hepatotoxic Rats**

Groups	Treatment	Direct Bilirubin, (Mean ± SEM)	Total Bilirubin, (Mean ± SEM)
Group I	Negative Control (0.5ml saline)	0.213 ± 0.015	0.986 ± 0.039
Group II	Positive Control Paracetamol (2 g/kg p.o.)	1.727 ± 0.109	4.563 ± 0.237
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o. + 100 mg/kg p.o.)	0.371 ± 0.028***	1.315 ± 0.176***
Group IV	Paracetamol + EAENN (2 g/kg p.o. + 100 mg/kg p.o.)	0.896 ± 0.026***	2.510 ± 0.145***
Group V	Paracetamol + EAENN (2 g/kg p.o. + 200 mg/kg p.o.)	0.661 ± 0.027***	1.815 ± 0.143***
Group VI	Paracetamol + EAENN (2 g/kg p.o. + 400 mg/kg p.o.)	0.491 ± 0.032***	1.492 ± 0.160***
Group VII	Paracetamol + HAENN (2 g/kg p.o. + 100 mg/kg p.o.)	0.915 ± 0.048***	2.612 ± 0.171***
Group VIII	Paracetamol + HAENN (2 g/kg p.o. + 200 mg/kg p.o.)	0.718 ± 0.033***	2.010 ± 0.173***
Group IX	Paracetamol + HAENN (2 g/kg p.o. + 400 mg/kg p.o.)	0.520 ± 0.024***	1.518 ± 0.120***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** **P<0.001, ** P<0.01, * P<0.05** and ns represents Not significant. All the values are compared to Paracetamol treated group.

EAENN: Ethylacetate extract of *Nymphaea nouchali*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

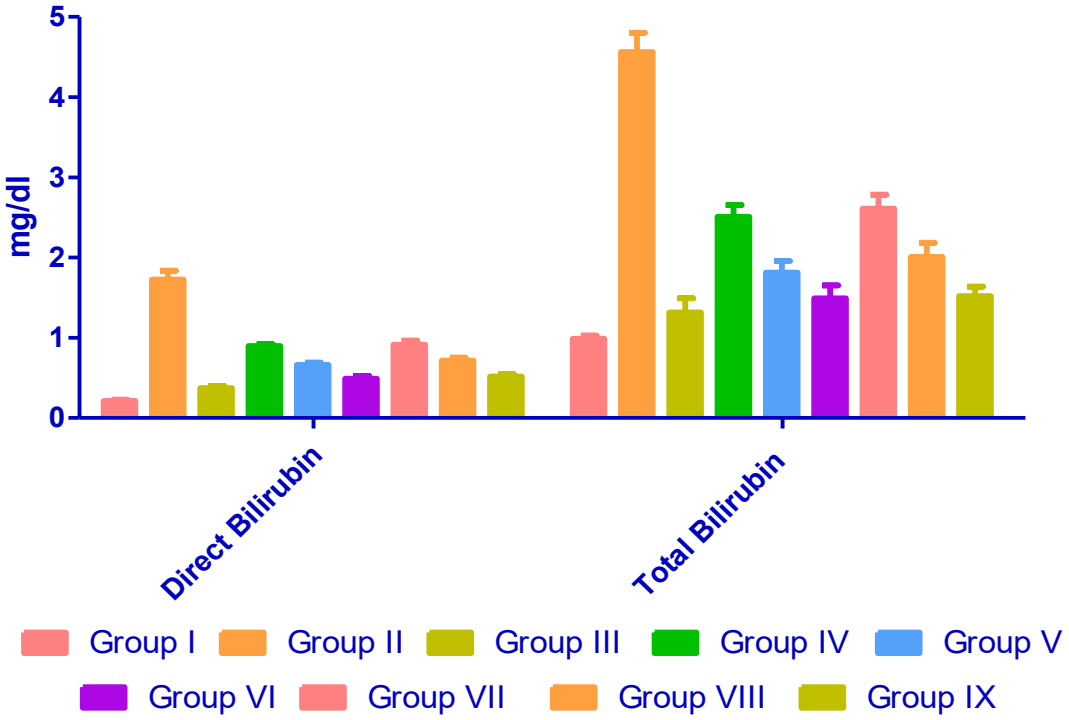


Fig. No: 106 Effect of EAENN and HAENN on Direct Bilirubin, Total Bilirubin levels in Paracetamol Induced Hepatotoxic Rats

iii). Serum Urea, Creatinine

Paracetamol treatment rats showed an increased in the Levels of Serum Urea and Creatinine. Treatment of these rats with the EAENN and HAENN and Silymarin showed a decrease in the serum urea and creatinine levels when compared with the normal animals. Serum urea and creatinine levels of treated and normal rats are expressed in the **Table No: 42** and shown in **Fig. No: 107, 108.**

Table No.: 42 Effect of EAENN and HAENN on Urea, Creatinine levels in Paracetamol Induced Hepatotoxic Rats

Groups	Treatment	Urea (Mean ± SEM)	Creatinine (Mean ± SEM)
Group I	Negative Control (0.5ml saline)	32.57 ± 2.346	0.812 ± 0.034
Group II	Positive Control Paracetamol (2 g/kg p.o.)	64.16 ± 5.053	2.292 ± 0.159
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o. + 100 mg/kg p.o.)	35.31 ± 2.532***	0.853 ± 0.027***
Group IV	Paracetamol + EAENN (2 g/kg p.o. + 100 mg/kg p.o.)	45.40 ± 3.203**	1.406 ± 0.172***
Group V	Paracetamol + EAENN (2 g/kg p.o. + 200 mg/kg p.o.)	42.46 ± 2.599***	0.997 ± 0.073***
Group VI	Paracetamol + EAENN (2 g/kg p.o. + 400 mg/kg p.o.)	39.63 ± 1.811***	0.903 ± 0.074***
Group VII	Paracetamol + HAENN (2 g/kg p.o. + 100 mg/kg p.o.)	48.16 ± 2.527*	1.389 ± 0.266***
Group VIII	Paracetamol + HAENN (2 g/kg p.o. + 200 mg/kg p.o.)	43.40 ± 2.900***	1.108 ± 0.087***
Group IX	Paracetamol + HAENN (2 g/kg p.o. + 400 mg/kg p.o.)	40.88 ± 3.053***	0.893 ± 0.019***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *****P<0.001, **P<0.01, *P<0.05** and ns represents Not significant. All the values are compared to Paracetamol treated group.

EAENN: Ethylacetate extract of *Nymphaea nouchali*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

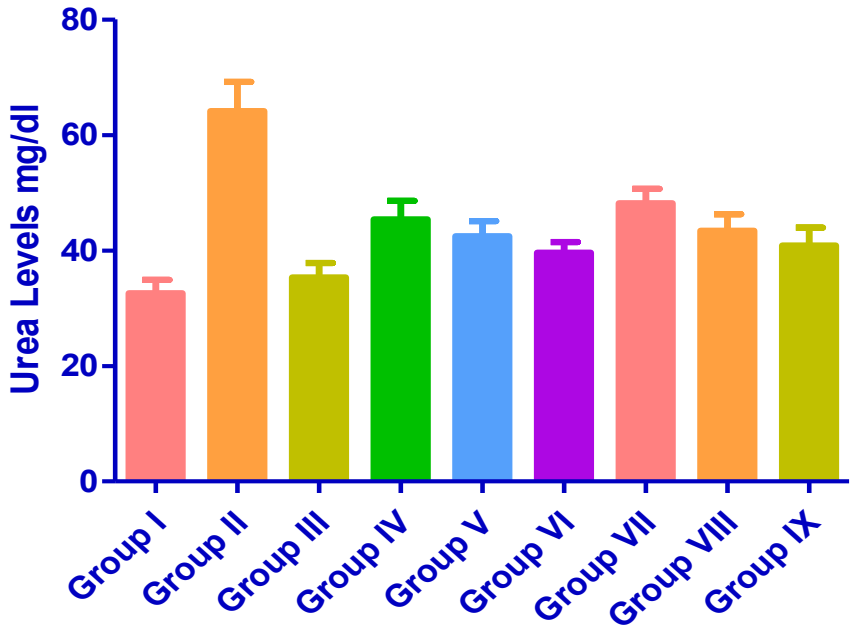


Fig. No: 107 Effect of EAENN and HAENN on Urea levels in Paracetamol Induced Hepatotoxic Rats

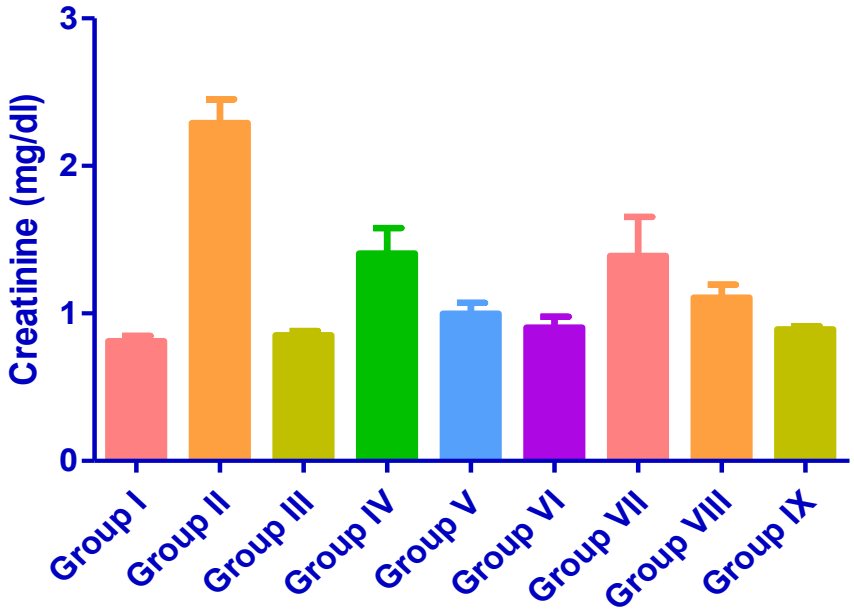


Fig. No: 108 Effect of EAENN and HAENN on Creatinine levels in Paracetamol Induced Hepatotoxic Rats

iv). Serum Total Protein

Paracetamol treatment considerably reduced Serum Total Protein Levels. Pretreatment with Silymarin and EAENN and HAENN showed a significant increase in Total Protein Levels as compared with toxicant control group.

The results are summarized in **Table No: 43** and graphically depicted in **Fig. No: 109.**

Table No.: 43 Effect of EAENN and HAENN on Serum Total Protein Levels in Paracetamol Induced Hepatotoxic Rats

Groups	Treatment	Total Protein levels (gm/dl) (Mean ± SEM)
Group I	Negative Control (0.5ml saline)	8.218 ± 0.258
Group II	Positive Control Paracetamol (2 g/kg p.o.)	3.810 ± 0.179
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o. + 100 mg/kg p.o.)	8.068 ± 0.104***
Group IV	Paracetamol + EAENN (2 g/kg p.o. + 100 mg/kg p.o.)	5.855 ± 0.152***
Group V	Paracetamol + EAENN (2 g/kg p.o. + 200 mg/kg p.o.)	7.468 ± 0.382***
Group VI	Paracetamol + EAENN (2 g/kg p.o. + 400 mg/kg p.o.)	7.892 ± 0.308***
Group VII	Paracetamol + HAENN (2 g/kg p.o. + 100 mg/kg p.o.)	5.748 ± 0.199***
Group VIII	Paracetamol + HAENN (2 g/kg p.o. + 200 mg/kg p.o.)	7.250 ± 0.225***
Group IX	Paracetamol + HAENN (2 g/kg p.o. + 400 mg/kg p.o.)	7.745 ± 0.160***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *****P<0.001, **P<0.01, *P<0.05** and ns represents Not significant. All the values are compared to Paracetamol treated group.

EAENN: Ethylacetate extract of *Nymphaea nouchali*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

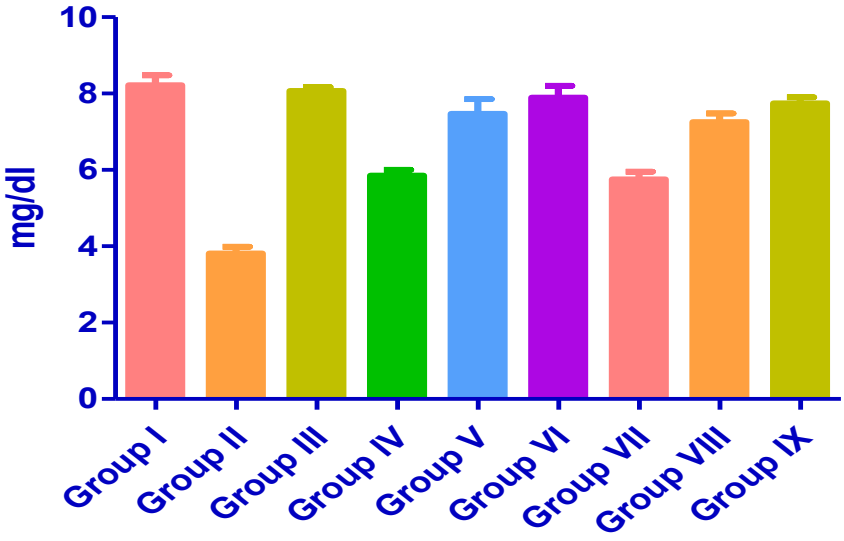


Fig. No: 109 Effect of EAENN and HAENN on Serum Total Protein Levels in Paracetamol Induced Hepatotoxic Rats

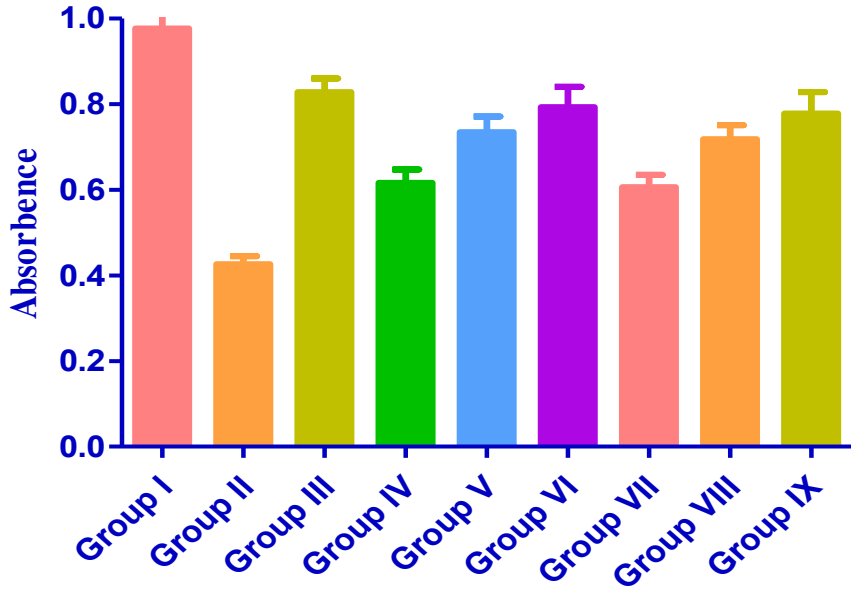


Figure No: 110 Effect of EAENN and HAENN on Tissue GSH Levels in Paracetamol Induced Hepatotoxic Rats

8.6.2.2.2. *In-vivo* Non-enzymatic Antioxidant Activity

i). Effect of EAENN and HAENN on GSH in Paracetamol Induced Hepatotoxicity

There is a marked depletion of GSH Levels in Paracetamol treated group. 100 mg/kg Silymarin has increased it by **94.36%,** EAENN and HAENN has shown a dose dependent increase in the levels of GSH.

The results are summarized in **Table No: 44** and graphically depicted in **Fig. No: 110.**

Table No.: 44 Effect of EAENN and HAENN on Tissue GSH Levels in Paracetamol Induced Hepatotoxic Rats

Groups	Treatment	Absorbance Mean ± SEM	% Increase
Group I	Negative Control (0.5ml saline)	0.976 ± 0.058	--
Group II	Positive Control Paracetamol (2 g/kg p.o.)	0.426 ± 0.018	--
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o. + 100 mg/kg p.o.)	0.828 ± 0.031***	94.36%
Group IV	Paracetamol + EAENN (2 g/kg p.o. + 100 mg/kg p.o.)	0.616 ± 0.030*	44.60%
Group V	Paracetamol + EAENN (2 g/kg p.o. + 200 mg/kg p.o.)	0.735 ± 0.036***	72.53%
Group VI	Paracetamol + EAENN (2 g/kg p.o. + 400 mg/kg p.o.)	0.793 ± 0.047***	86.15%
Group VII	Paracetamol + HAENN (2 g/kg p.o. + 100 mg/kg p.o.)	0.606 ± 0.028*	42.25%
Group VIII	Paracetamol + HAENN (2 g/kg p.o. + 200 mg/kg p.o.)	0.718 ± 0.032***	68.54%
Group IX	Paracetamol + HAENN (2 g/kg p.o. + 400 mg/kg p.o.)	0.778 ± 0.049***	82.62%

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, ***P<0.001, **P<0.01, *P<0.05 and ns represents Not significant. All the values are compared to Paracetamol treated group.

EAENN: Ethyl acetate extract of *Nymphaea nouchali*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

ii). Effect of EAENN and HAENN on *In-vivo* Lipid Peroxidation in Paracetamol Induced Hepatotoxicity

There is a dose dependent inhibition of *In-vivo* Lipid Peroxidation by EAENN and HAENN. 100 mg/kg silymarin has **64.49%** inhibition whereas 400 mg/kg of EAENN and HAENN has **61.87%** and **60.57%** inhibition of Lipid Peroxidation.

The results are summarized in **Table No: 45** and graphically depicted in **Fig. No: 111.**

Table No.: 45 Effect of EAENN and HAENN on *In-vivo* Lipid Peroxidation in Paracetamol Induced Hepatotoxic Rats

Groups	Treatment	Absorbance Mean ± SEM	% Decrease
Group I	Negative Control (0.5ml saline)	0.251 ± 0.017	--
Group II	Positive Control Paracetamol (2 g/kg p.o.)	0.383 ± 0.019	--
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o. + 100 mg/kg p.o.)	0.136 ± 0.016***	64.49%
Group IV	Paracetamol + EAENN (2 g/kg p.o. + 100 mg/kg p.o.)	0.215 ± 0.014***	43.86%
Group V	Paracetamol + EAENN (2 g/kg p.o. + 200 mg/kg p.o.)	0.158 ± 0.014***	58.74%
Group VI	Paracetamol + EAENN (2 g/kg p.o. + 400 mg/kg p.o.)	0.146 ± 0.013***	61.87%
Group VII	Paracetamol + HAENN (2 g/kg p.o. + 100 mg/kg p.o.)	0.203 ± 0.014***	46.99%
Group VIII	Paracetamol + HAENN (2 g/kg p.o. + 200 mg/kg p.o.)	0.166 ± 0.016***	56.65%
Group IX	Paracetamol + HAENN (2 g/kg p.o. + 400 mg/kg p.o.)	0.151 ± 0.013***	60.57%

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *****P<0.001, **P<0.01, *P<0.05** and ns represents Not significant. All the values are compared to Paracetamol treated group.

EAENN: Ethylacetate extract of *Nymphaea nouchali*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

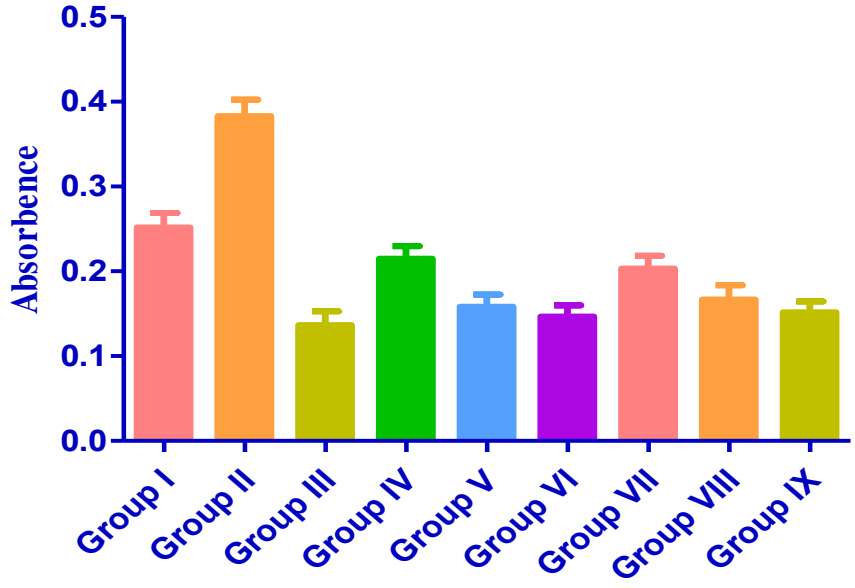


Fig. No: 111 Effect of EAENN and HAENN on *In-vivo* Lipid Peroxidation in Paracetamol Induced Hepatotoxic Rats

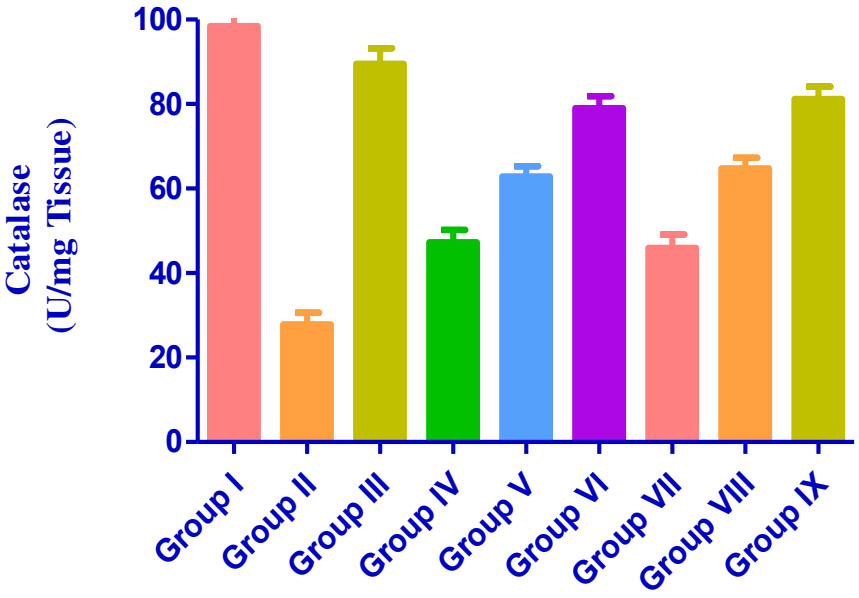


Fig. No: 112 Effect of EAENN and HAENN on Catalase in Paracetamol Induced Hepatotoxic Rats

8.6.2.2.3. *In-vivo* Enzymatic Antioxidant Activity

i). Effect of EAENN and HAENN on Catalase in Paracetamol Induced Hepatotoxicity

Paracetamol induced hepatotoxic rats exhibited significant lower Catalase (27.84±2.747) as compared to those of negative control rats (98.44±5.137) treatment with the plant extract significantly elevated the reduced catalase levels. The 100mg/kg, 200mg/kg, 400mg/kg p.o dose of EAENN, HAENN and Silymarin showed a marked increase in the catalase levels (P<0.001) compared to the positive control.

The results are summarized in **Table No: 46** and graphically depicted in **Fig. No: 112.**

Table No: 46 Effect of EAENN and HAENN on Catalase in Paracetamol Induced Hepatotoxic Rats

Groups	Treatment	Catalase (U/mg protein)
Group I	Negative Control (0.5ml saline)	98.44 ± 5.137
Group II	Positive Control Paracetamol (2 g/kg p.o.)	27.84 ± 2.747
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o.+ 100 mg/kg p.o.)	89.51 ± 3.697***
Group IV	Paracetamol + EAENN (2 g/kg p.o.+ 100 mg/kg p.o.)	47.27 ± 2.924**
Group V	Paracetamol + EAENN (2 g/kg p.o.+ 200 mg/kg p.o.)	62.88 ± 2.386***
Group VI	Paracetamol + EAENN (2 g/kg p.o.+ 400 mg/kg p.o.)	79.05 ± 2.833***
Group VII	Paracetamol + HAENN (2 g/kg p.o.+ 100 mg/kg p.o.)	45.92 ± 3.166**
Group VIII	Paracetamol + HAENN (2 g/kg p.o.+ 200 mg/kg p.o.)	64.81 ± 2.485***
Group IX	Paracetamol + HAENN (2 g/kg p.o.+ 400 mg/kg p.o.)	81.22 ± 2.922***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *****P<0.001, **P<0.01, *P<0.05** and ns represents Not significant. All the values are compared to Paracetamol treated group.

EAENN: Ethylacetate extract of *Nymphaea nouchali*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

ii). Effect of EAENN and HAENN on SOD in Paracetamol Induced Hepatotoxicity

Paracetamol induced hepatotoxic rats exhibited significant lower SOD **(5.07 ± 0.488)** as compared to those of negative control rats **(15.39 ± 1.269)** treatment with the plant extract significantly elevated the reduced SOD levels. EAENN, HAENN and Silymarin showed a marked increase in the SOD levels **(P<0.001)** compared to the positive control.

These values are tabulated in the **Table No: 47** and graphically represented in **Fig No: 113.**

Table No: 47 Effect of EAENN and HAENN on SOD in Paracetamol Induced Hepatotoxic Rats

Groups	Treatment	SOD (U/mg protein)
Group I	Negative Control (0.5ml saline)	15.39 ± 1.269
Group II	Positive Control Paracetamol (2 g/kg p.o.)	5.07 ± 0.488
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o. + 100 mg/kg p.o.)	13.51 ± 0.609***
Group IV	Paracetamol + EAENN (2 g/kg p.o. + 100 mg/kg p.o.)	9.14 ± 0.418**
Group V	Paracetamol + EAENN (2 g/kg p.o. + 200 mg/kg p.o.)	11.46 ± 0.533***
Group VI	Paracetamol + EAENN (2 g/kg p.o. + 400 mg/kg p.o.)	12.78 ± 0.718***
Group VII	Paracetamol + HAENN (2 g/kg p.o. + 100 mg/kg p.o.)	8.908 ± 0.392*
Group VIII	Paracetamol + HAENN (2 g/kg p.o. + 200 mg/kg p.o.)	11.23 ± 0.600***
Group IX	Paracetamol + HAENN (2 g/kg p.o. + 400 mg/kg p.o.)	12.27 ± 0.868***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *****P<0.001, **P<0.01, *P<0.05** and ns represents Not significant. All the values are compared to Paracetamol treated group.

EAENN: Ethylacetate extract of *Nymphaea nouchali*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

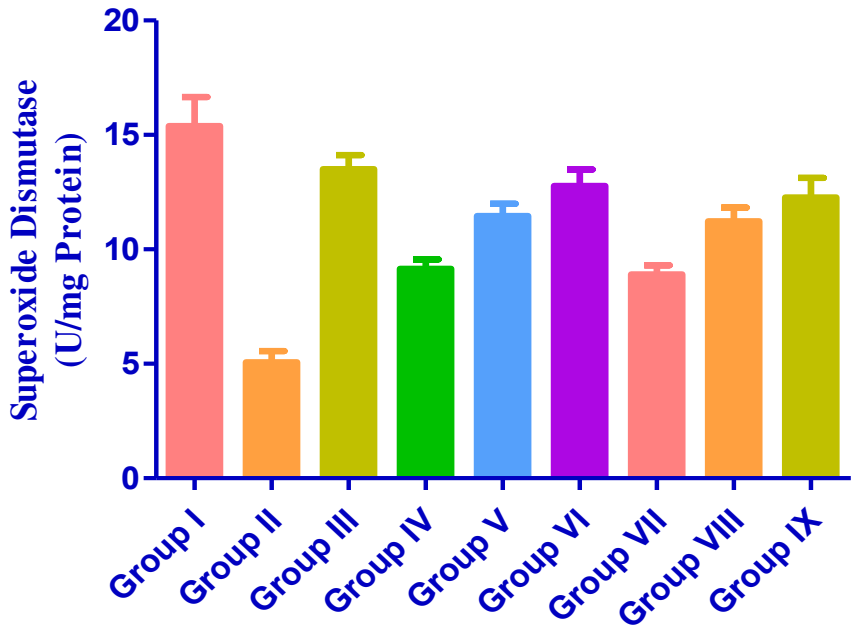


Fig. No: 113 Effect of EAENN and HAENN on SOD in Paracetamol Induced Hepatotoxic Rats

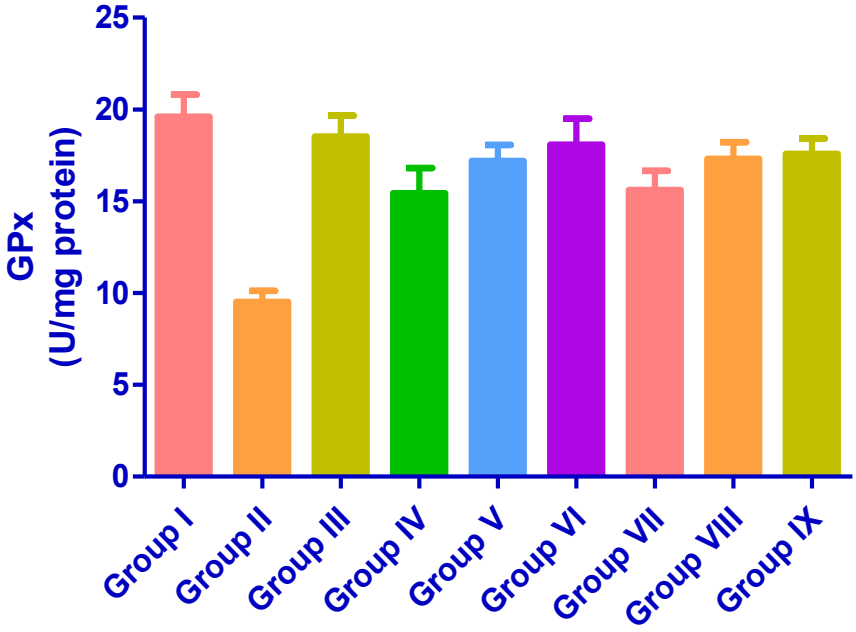


Fig. No: 114 Effect of EAENN and HAENN on GPx in Paracetamol Induced Hepatotoxic Rats

iii). Effect of EAENN and HAENN on GPx in Paracetamol Induced Hepatotoxicity

A significant decrease in antioxidant enzyme GPx activity was observed in the liver of paracetamol administered rats when compared to normal rats that had received vehicle alone. Treatment with EAENN and HAENN shows increase GPx levels in paracetamol treated rats. These values are having a significant higher **(P<0.001)** when compared to GPx levels in Positive control rats.

The values are tabulated in the **Table No: 48** and graphically represented in **Fig No: 114.**

Table No: 48 Effect of EAENN and HAENN on GPx in Paracetamol Induced Hepatotoxic Rats

Groups	Treatment	GPx (U/mg protein)
Group I	Negative Control (0.5ml saline)	19.60 ± 1.211
Group II	Positive Control Paracetamol (2 g/kg p.o.)	9.51 ± 0.594
Group III	Paracetamol + Standard (Silymarin) (2 g/kg p.o. + 100 mg/kg p.o.)	18.52 ± 1.143***
Group IV	Paracetamol + EAENN (2 g/kg p.o. + 100 mg/kg p.o.)	15.45 ± 1.362**
Group V	Paracetamol + EAENN (2 g/kg p.o. + 200 mg/kg p.o.)	17.20 ± 0.859***
Group VI	Paracetamol + EAENN (2 g/kg p.o. + 400 mg/kg p.o.)	18.09 ± 1.413***
Group VII	Paracetamol + HAENN (2 g/kg p.o. + 100 mg/kg p.o.)	15.62 ± 1.030**
Group VIII	Paracetamol + HAENN (2 g/kg p.o. + 200 mg/kg p.o.)	17.31 ± 0.923***
Group IX	Paracetamol + HAENN (2 g/kg p.o. + 400 mg/kg p.o.)	17.58 ± 0.841***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *****P<0.001, **P<0.01, *P<0.05** and ns represents Not significant. All the values are compared to Paracetamol treated group.

EAENN: Ethylacetate extract of *Nymphaea nouchali*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

8.6.2.2.4. Histopathological Studies of the Liver in Paracetamol Induced Hepatotoxicity

Group I: Section studied shows liver parenchyma with intact architecture. Most of the perivenular hepatocytes, periportal hepatocytes and midzonal hepatocytes appear normal. Within the hepatic parenchyma, the sinusoids appear normal.

Group II: Section studied shows liver parenchyma with effaced architecture. Most of the hepatocytes show macrosteatosis, while some show degenerative changes. There are seen focal aggregates of mononuclear inflammatory cells within the parenchyma. Scattered, mononuclear inflammatory infiltration present within the parenchyma.

Group III: Section studied shows liver parenchyma with partially effaced architecture. Some of the sinusoids appear congested. Most of the hepatocytes show macrosteatosis, while few show microsteatosis. There are seen scattered mononuclear inflammatory infiltrations within the parenchyma.

Group IV: Section studied shows liver parenchyma with effaced architecture. Most of the hepatocytes show microsteatosis while few show macrosteatosis. Also seen are scattered degenerative hepatocytes. Intervening the hepatocytes are seen scattered mononuclear inflammatory cells.

Group V: Section studied shows liver parenchyma with partially effaced architecture. Most of the hepatocytes show microsteatosis. Also seen are scattered degenerative and regenerative hepatocytes. Intervening the hepatocytes are seen focal aggregates of mononuclear inflammatory cells.

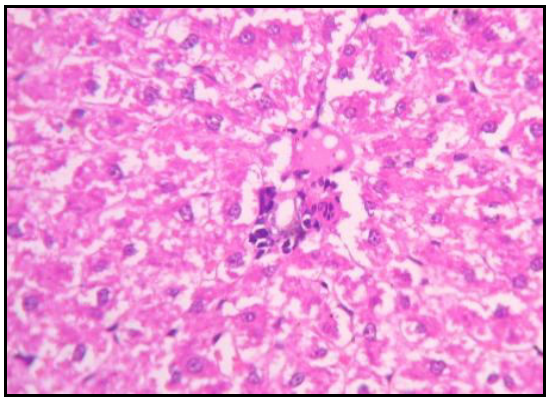
Group VI: Section studied shows liver parenchyma with intact architecture. Few of the sinusoids and central veins show congestion. Also seen are regenerative hepatocytes. Intervening the hepatocytes are seen focal aggregates of mononuclear inflammatory cells within the parenchyma.

Group VII: Section studied shows liver parenchyma with effaced architecture. Most of the hepatocytes show microsteatosis and macrosteatosis (Long-Arrow, Fig.1). Also seen are scattered degenerative hepatocytes (Arrow, Fig.2). Intervening the hepatocytes are seen scattered mononuclear inflammatory cells.

Group VIII: Section studied shows liver parenchyma with partially effaced architecture. Few of the hepatocytes show microsteatosis. Also seen are scattered degenerative and regenerative hepatocytes. Intervening the hepatocytes are seen scattered mononuclear inflammatory cells.

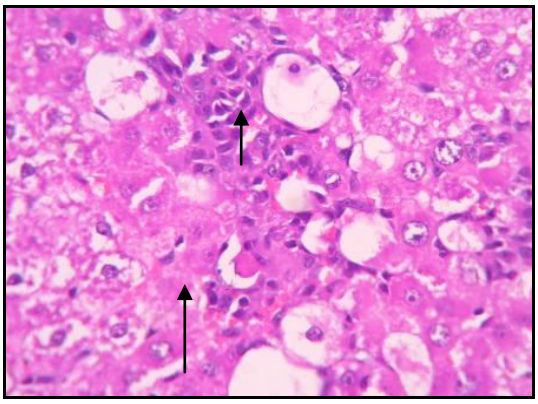
Group IX: Section studied shows liver parenchyma with intact architecture. Few of the sinusoids and central veins show congestion. Also seen are regenerative hepatocytes. Intervening the hepatocytes and periportal region, are seen focal aggregates of mononuclear inflammatory cells within the parenchyma.

Fig. No: 115 Histopathological Studies of the Rat Liver in Paracetamol Induced Hepatotoxicity



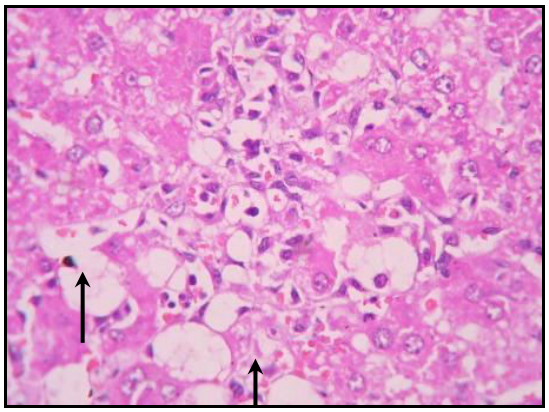
Group I

Negative Control - Saline



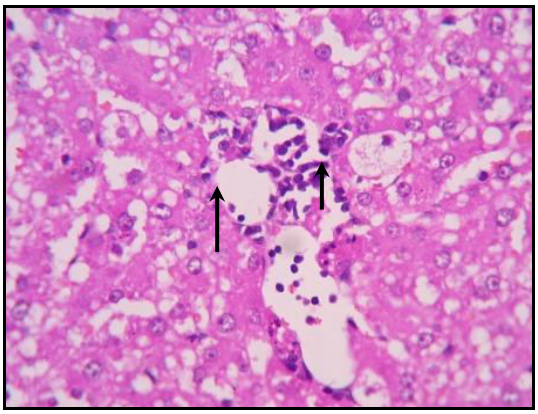
Group II

Positive Control Paracetamol [2 g/kg p.o.]



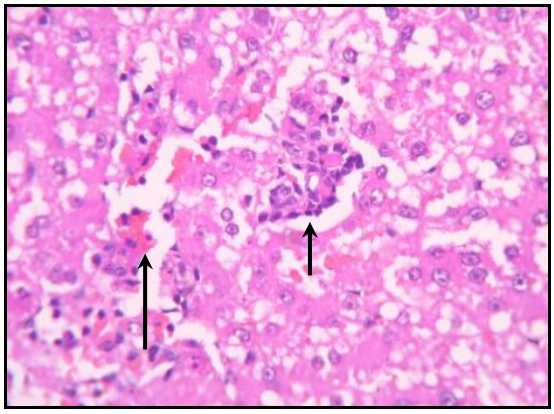
Group III

Paracetamol [2 g/kg p.o.] + Silymarin [100mg/kg]

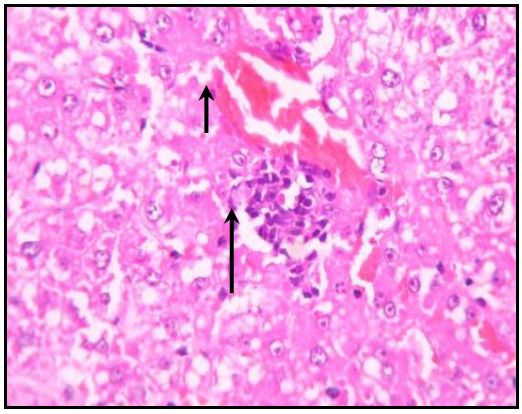


Group IV

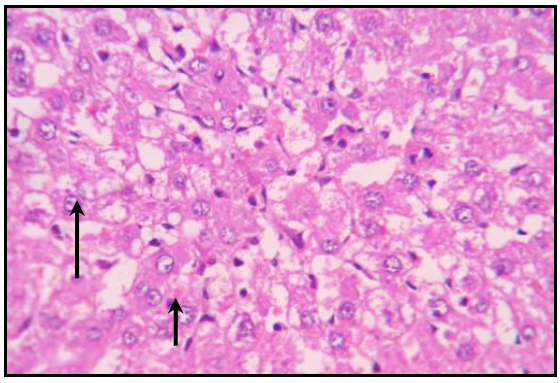
Paracetamol [2 g/kg p.o.] + EAENN [100mg/kg]



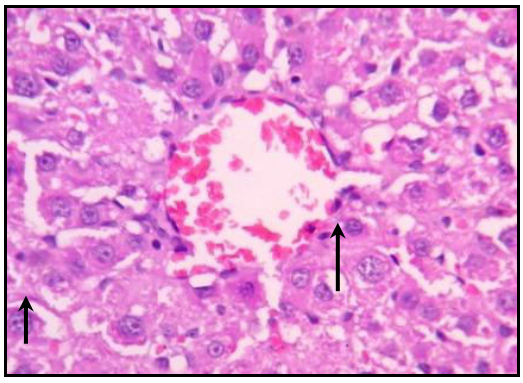
Group V
Paracetamol [2 g/kg p.o.] + EAENN [200mg/kg]



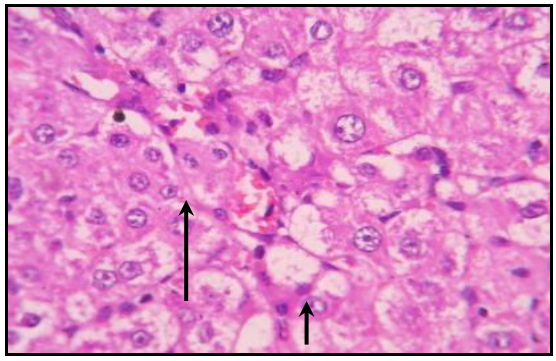
Group VI
Paracetamol [2 g/kg p.o.] + EAENN [400mg/kg]



Group VII
Paracetamol [2 g/kg p.o.] + HAENN [100mg/kg]



Group VIII
Paracetamol [2 g/kg p.o.] + HAENN [200mg/kg]



Group IX
Paracetamol [2 g/kg p.o.] + HAENN [400mg/kg]

Fig. No: 115 Histopathological Studies of the Rat Liver in Paracetamol Induced Hepatotoxicity

8.6.2.3. Discussion

Paracetamol is a commonly band widely used analgesic and antipyretic agent. Hepatotoxic doses of paracetamol deplete the normal levels of hepatic glutathione, when NAPQI covalently binds to cysteine groups on proteins to form 3-(cysteine-S-yl) acetaminophen adducts **(Tirmenstein, MA., (1989).** The glutathione protects hepatocytes by combining with the reactive metabolite of paracetamol thus preventing their covalent binding to liver proteins **(Vermeulen, NPE., 1992).**

In case of toxic liver, Wet liver weight and wet liver volumes are increased. Toxicants induced hepatotoxicity produce fatty changes and also it is observed that there is a fall in serum lipids in another series of experiments. In this case water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass and volume **(Childs, JFL., *et al.,* 1946).** It is reported that liver mass and volume are important parameters in ascertaining the hepatoprotective effect of the drugs. Treatment with of EAENP, HAENP, EAENN and HAENN (100, 200 and 400 mg/kg, p.o.) and Silymarin significantly reduced the wet liver weight and wet liver volumes of animals and hence it possesses statistically significant hepatoprotective activity.

Estimating the activities of **serum marker enzymes**, like SGOT, SGPT and ALP can make assessment of liver function. When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol, are released into the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage **(Mitra, SK., *et al.*, 1998).** The study of different enzyme activities such as SGOT, SGPT, SALP, total bilirubin and total protein have been found to be of great value in the assessment of clinical and experimental liver damage **(Vaishwanar, I., 1976).** In the present investigation it was observed that the animals treated with paracetamol resulted in significant hepatic damage as shown by the elevated levels of serum markers. These changes in the marker levels will reflect in hepatic structural integrity. The rise in the SGOT is usually accompanied by an elevation in the levels of SGPT, which play a vital role in the conversion of amino acids to keto acids **(Sallie, R., 1999).** The pre-treatment with EAENP, HAENP, EAENN and HAENN (100, 200 and 400 mg/kg, p.o.) and Silymarin significantly attenuated the elevated levels of the serum markers. The normalization of serum markers by EAENP, HAENP, EAENN and

HAENN suggests that they are able to condition the hepatocytes so as to protect the membrane integrity against paracetamol induced leakage of marker enzymes into the circulation. The above changes can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells. Serum ALP and bilirubin levels, on the other hand are related to hepatic cell damage. Increase in serum level of ALP is due to increased synthesis in presence of increasing biliary pressure **(Moss, DW., 1974).** Effective control of bilirubin level and alkaline phosphatase activity points towards an early improvement in the secretory mechanism of the hepatic cell.

Several metabolic disorders including urea and creatinine derangements are possible in the presence of paracetamol over dosage **(Kale, R H., 2012).** Increased concentration of serum urea and creatinine are considered for investigating drug induced nephrotoxicity in animals and man **Bennit, W M., *et al.,* 1982).** Paracetamol treatment obviously interfered with kidney filtration functions as seen by its elevated values in rats. Urea, a waste product of protein catabolism can rise when the kidney is defective. In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of its clearance (**Mayne, P D., 1994).** The pre-treatment with EAENP, HAENP, EAENN and HAENN (100, 200 and 400 mg/kg, p.o.) and Silymarin had a dose dependent reversal of the effects on this parameter. The present study showed the hepatoprotective effects of **the *Nymphaea pubescens*** and ***Nymphaea nouchali*** in paracetamol -induced toxicity.

There are reports that paracetamol induced hepatotoxicity is due to activation of PCM to a toxic electrophile N-acetyl p-benzoquinine amine (NAPQI) by a number of iso-enzyme of CYP-450 namely CYP $2E_1$, CYP$1A_2$, CYP$2A_6$, CYP$3A_4$, CYP$2D_6$. Normally PCM is eliminated from the body as sulphate and glucuronide to the extents of 95% before oxidation. However, 5% of PCM is undergoing bio-activation by above mentioned iso-enzymes of CYP to a highly reactive NAPQI **(Janbaz, H. Khalid., *et al.,* 2002).** After the over dosage of paracetamol, routs of sulphation and glucuronidation saturates. As a consequence oxidation of PCM, CYP-450 iso enzymes are increased leading to the increased concentration of NAPQI. This NAPQI further loses one electron resulting into the toxic radical. This radical interact covalently with membrane macromolecules and damage the membrane. However this reaction is countered by inbuilt tissue antioxidants systems like GSH. Excessive concentration of NAPQI radical over

powers the inbuilt protecting mechanisms thereby damages the cell membrane. This results into the leakage of biochemical markers into the serum. It is apparent from the results that treatment with Treatment with of EAENP, HAENP, EAENN and HAENN reversed the elevated levels of all the biochemical markers to the near normal levels in this model also. The histopathological parameters of PCM induced hepatotoxicity were normalized by the treatment of EAENP, HAENP, EAENN and HAENN. These observations indicate that the EAENP, HAENP, EAENN and HAENN possess hepatoprotective activity against PCM induced hepatotoxicity. Prevents the formation of one electron reduced metabolite of NAPQI (which mediates cytotoxic effects of NAPQI) due to its antioxidant property i.e. hydroxyl and superoxide anion scavenging activities. Further, this may be helpful in retaining the membrane GSH contents, reduced lipid peroxidation and prevents the tissue damage **(Yun-Hee Shon, 2004), (Premila Abraham, 2005).**

The **non-enzymic antioxidant**, glutathione is one of the most abundant tripeptides present in the liver. Its functions are mainly concerned with the removal of free radical species such as hydrogen peroxide, superoxide radicals, alkoxy radicals, and maintenance of membrane proteinthiols and as a substrate for glutathione peroxidase and GST **(Prakash J, *et al.,* 2001).** In our present study the decreased level of GSH has been associated with an enhanced lipid peroxidation in paracetamol treated rats. Administration of EAENP, HAENP, EAENN and HAENN (100, 200 and 400 mg/kg, p.o.) and Silymarin significantly increased the level of glutathione in a dose-dependent manner.

Lipid peroxidation has been postulated to the destructive process of liver injury due to acetaminophen administration. In the present study the elevations in the levels of end products of lipid peroxidation in the liver of rat treated with paracetamol were observed. The increase in malondialdehyde (MDA) levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Treatment with EAENP, HAENP, EAENN and HAENN (100, 200 and 400 mg/kg, p.o.) and Silymarin has reversed the paracetamol induced elevated lipid peroxidation was decreased. Hence it may be possible that the mechanism of hepatoprotection by EAENP, HAENP, EAENN and HAENN is due to its antioxidant effect. The **enzymic antioxidant** defence system is the nature protector against lipid peroxidation. SOD, CAT and GPx enzymes

are important scavengers of superoxide ion and hydrogen peroxide. These enzymes prevent generation of hydroxyl radical and protect the cellular constituents from oxidative damage **(Scott, MD., *et al.,* 1991).** In the present study, it was observed that the EAENP, HAENP, EAENN and HAENN significantly increased the hepatic SOD activity in paracetamol induced liver damage in rats. This show EAENP, HAENP, EAENN and HAENN (100, 200 and 400 mg/kg, p.o.) and Silymarin can reduce reactive free radicals that might lessen oxidative damage to the tissues and improve the activities of the hepatic antioxidant enzyme.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity is found in the red cells and in the liver. CAT decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals **(Chance, B, *et al.,* 1952).** Therefore the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. Administration of EAENP, HAENP, EAENN and HAENN (100, 200 and 400 mg/kg, p.o.) and Silymarin increased the activities of CAT in paracetamol-induced liver damage in rats to prevent the accumulation of excessive free radicals and protected the liver from paracetamol intoxication.

The **histological** evidence authenticated the injury caused by paracetamol and the protection offered by the flowers of EAENP, HAENP, EAENN and HAENN to hepatocytes. Microscopical examination revealed loss of architecture and cell necrosis with inflammatory collections in the central zone in paracetamol - induced rats. Prior oral administration with EAENP, HAENP, EAENN and HAENN extracts and Silymarin prevented completely the histopathological changes in liver induced by paracetamol. Thus the histopathological studies serve as a direct evidence of efficacy of drug as protectant. The results of histopathological study also support the result of biochemical. These observations indicate that of ***Nymphaea pubescens*** and ***Nymphaea nouchali*** possess hepatoprotective activity against PCM induced hepatotoxicity.

It seems that the protective activities of the flower may be, by strengthening the inbuilt antioxidant systems of the antioxidant principles that are present in the flower. However, further studies are needed to completely establish the mechanism of hepatoprotective effect of the flower in this model.

8.6.3. ANTI-DIABETIC ACTIVITY

8.6.3.1. Single Dose Study in Normal Rats

Table No.: 49 Effects of EAENP and HAENP on Blood Glucose Levels on Single Dose Study in Normal Rats

Groups	Treatment	Blood Glucose Levels (mg/dl)			
		0	30	60	120
Group-I	Normal Control Saline	93.50 ± 3.80	98.17 ± 3.85	92.50 ± 3.16	88.67 ± 2.30
Group-II	Metformin (250 mg/kg b.w. p.o)	90.17 ± 3.43	71.50 ± 2.34***	58.00 ± 3.06***	51.67 ± 1.97***
Group-III	EAENP (100 mg/kg b.w. p.o)	85.67 ± 2.97	84.33 ± 3.25^{ns}	78.50 ± 3.32^{ns}	74.67 ± 3.57*
Group-IV	EAENP (200 mg/kg b.w. p.o)	89.83 ± 3.93	81.17 ± 2.83*	74.67 ± 3.40**	67.83 ± 3.26***
Group-V	EAENP (400 mg/kg b.w. p.o)	84.50 ± 4.83	76.50 ± 3.99**	67.67 ± 2.40***	59.17 ± 2.54***
Group-VI	HAENP (100 mg/kg b.w. p.o)	88.50 ± 6.08	83.50 ± 5.79^{ns}	79.00 ± 5.13^{ns}	73.33 ± 4.14*
Group-VII	HAENP (200 mg/kg b.w. p.o)	87.67 ± 4.68	82.33 ± 2.51^{ns}	75.50 ± 2.07*	69.50 ± 1.17***
Group-VIII	HAENP (400 mg/kg b.w. p.o)	87.33 ± 4.55	77.50 ± 3.28**	69.50 ± 2.89***	61.17 ± 3.29***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Kramer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant.

EAENP: Ethylacetate extract of *Nymphaea pubescens*,

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

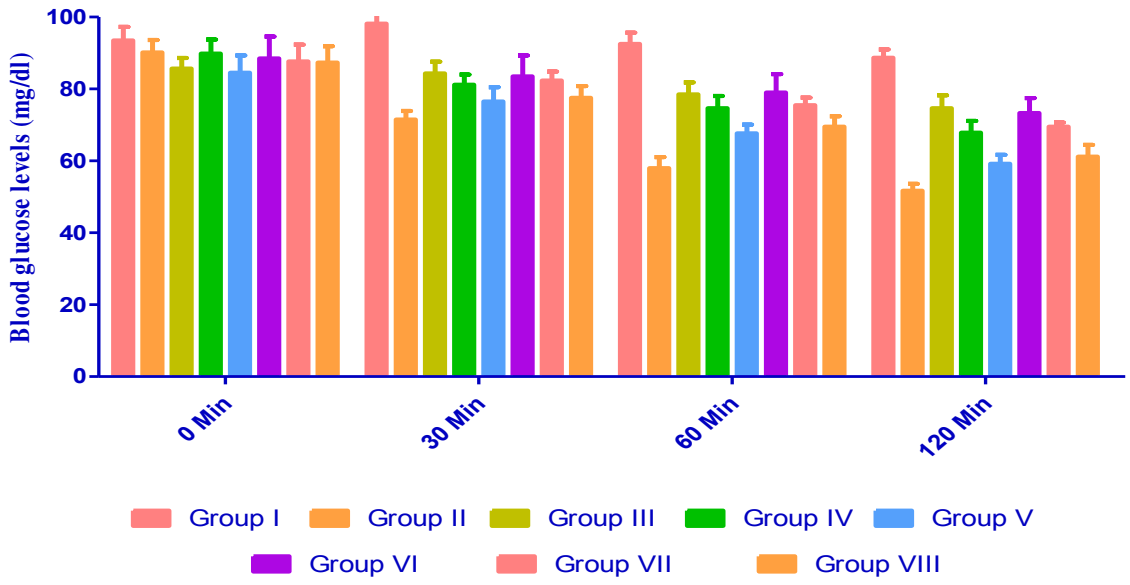


Fig. No: 116 Effects of EAENP and HAENP on Blood Glucose Levels in Normal Rats

Table No.: 50 Effects of EAENN and HAENN on Blood Glucose Levels on Single Dose Study in Normal Rats

Groups	Treatment	Blood Glucose Levels (mg/dl)			
		0	30	60	120
Group-I	Normal Control Saline	93.50 ± 3.80	98.17 ± 3.85	92.50 ± 3.16	88.67 ± 2.30
Group-II	Metformin (250 mg/kg, b.w. p.o)	90.17 ± 3.43	71.50 ± 2.34***	58.00 ± 3.06***	51.67 ± 1.97***
Group-III	EAENN (100 mg/kg b.w. p.o)	97.83 ± 3.59	93.00 ± 4.37^{ns}	82.67 ± 3.35^{ns}	74.00 ± 2.46**
Group-IV	EAENN (200 mg/kg b.w. p.o)	88.33 ± 1.76	85.00 ± 1.862^{ns}	74.67 ± 2.02**	66.50 ± 2.46***
Group-V	EAENN (400 mg/kg b.w. p.o)	89.67 ± 6.11	78.33 ± 5.51*	69.67 ± 4.49***	61.00 ± 4.10***
Group VI	HAENN (100 mg/kg b.w. p.o)	94.17 ± 4.69	91.50 ± 3.62^{ns}	87.00 ± 3.42^{ns}	78.50 ± 3.26^{ns}
Group VII	HAENN (200 mg/kg b.w. p.o)	93.00 ± 4.62	83.17 ± 3.17^{ns}	74.83 ± 2.65**	68.67 ± 2.52***
Group VIII	HAENN (400 mg/kg b.w. p.o)	91.00 ± 4.43	80.50 ± 3.61*	71.17 ± 2.60***	63.67 ± 1.89***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant.

EAENN: Ethylacetate extract of *Nymphaea nouchali,* **HAENN**: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

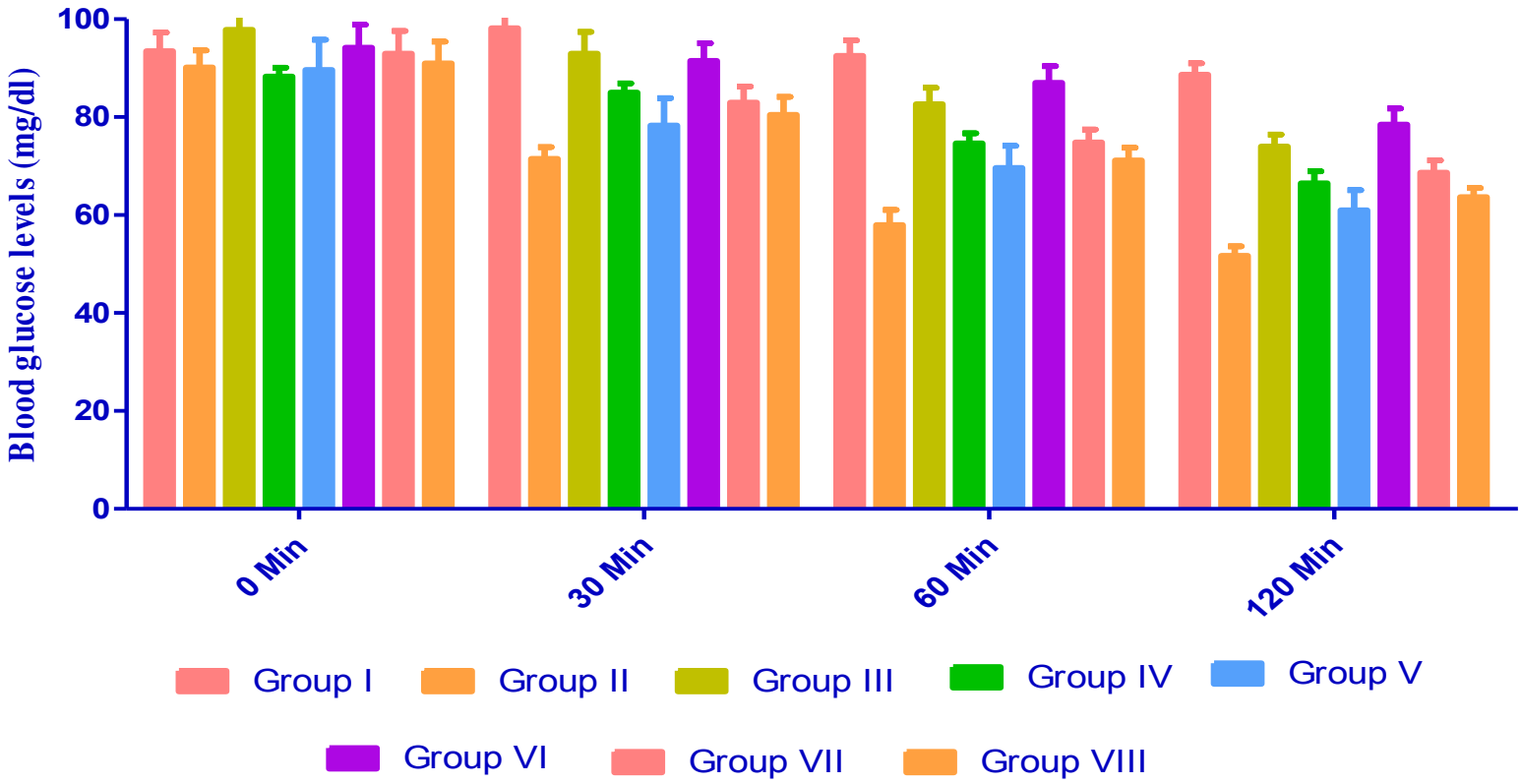


Fig. No: 117 Effects of EAENN and HAENN on Blood Glucose Levels in Normal Rats

8.6.3.2. Oral Glucose Tolerance Test in Normal Rats

Table No: 51 Effect of EAENP and HAENP on Blood Glucose Levels on Oral Glucose Tolerance Test in Normal Rats

Groups	Treatment	Blood Glucose Levels (mg/dl) and Time in min			
		0	30	60	120
Group-I	Saline + Glucose 2g/kg	92.50 ± 4.13	141.0 ± 4.22	137.7 ± 4.04	119.3 ± 3.29
Group-II	Metformin (250mg/kg) + Glucose 2g/kg	90.17 ± 3.02	103.0 ± 3.38***	84.67 ± 3.91***	73.83 ± 3.32***
Group-III	EAENP (100 mg/kg b.w. p.o) + Glucose 2g/kg	87.33 ± 4.69	120.3 ± 6.00^{ns}	111.8 ± 6.45**	96.67 ± 4.08**
Group-IV	EAENP (200 mg/kg b.w. p.o) + Glucose 2g/kg	86.17 ± 2.56	116.8 ± 3.29*	103.7 ± 3.47***	90.50 ± 3.24***
Group-V	EAENP (400 mg/kg b.w. p.o) + Glucose 2g/kg	84.83 ± 3.71	109.2 ± 4.16**	96.50 ± 4.65***	82.33 ± 4.50***
Group VI	EAENP (100 mg/kg b.w. p.o) + Glucose 2g/kg	88.33 ± 5.42	122.5 ± 7.88^{ns}	113.3 ± 5.81*	99.17 ± 5.33*
Group VII	EAENP (200 mg/kg b.w. p.o) + Glucose 2g/kg	85.67 ± 2.78	118.5 ± 6.14^{ns}	106.2 ± 5.97***	92.33 ± 5.64***
Group VIII	EAENP (400 mg/kg b.w. p.o) + Glucose 2g/kg	89.50 ± 4.66	110.7 ± 3.26**	98.17 ± 2.45***	84.17 ± 2.73***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant.

EAENP: Ethylacetate extract of *Nymphaea pubescens*, **HAENP**: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

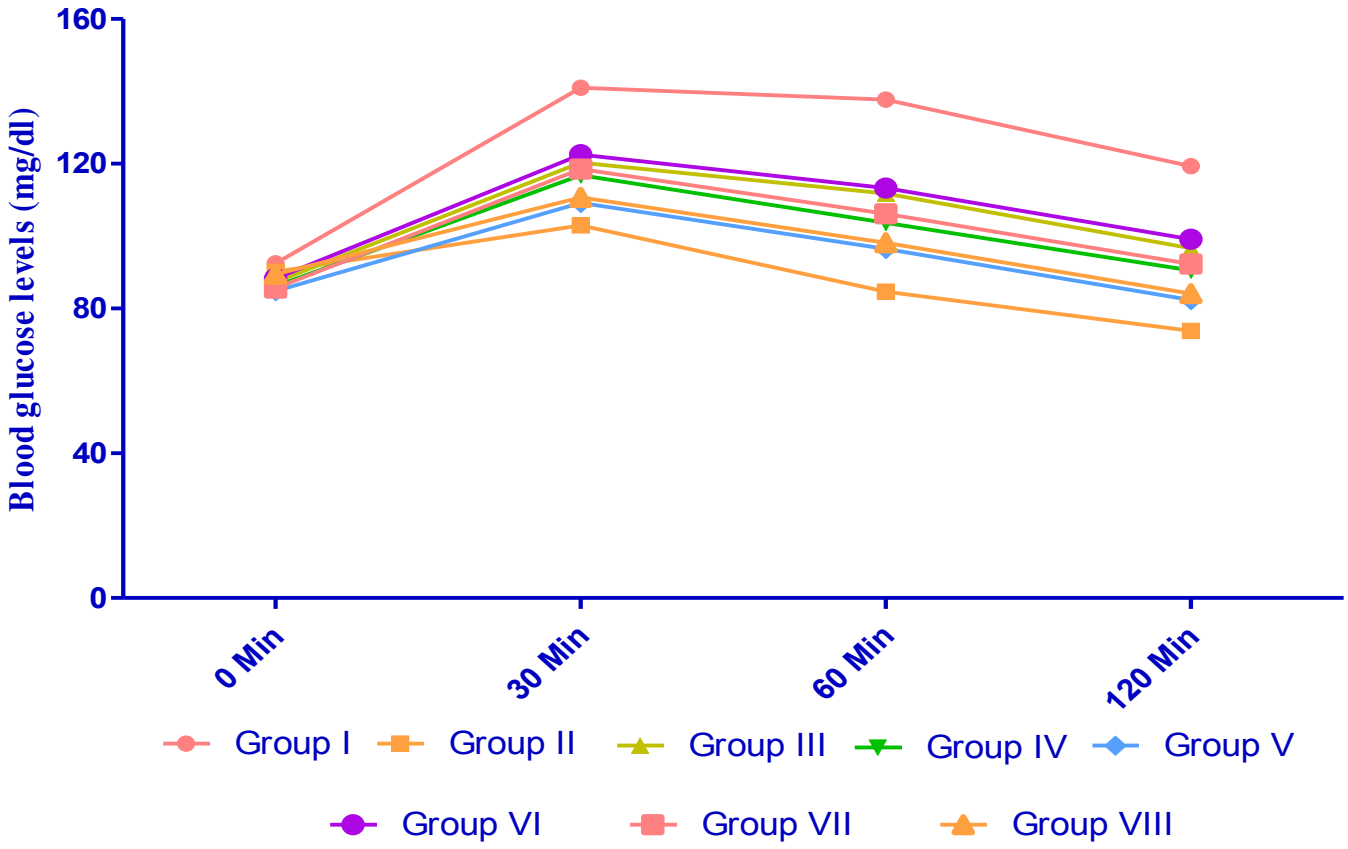


Fig. No: 117, a Effect of EAENP and HAENP on Blood Glucose Levels on Oral Glucose Tolerance Test in Normal Rats

Table No: 52 Effect of EAENN and HAENN on Blood Glucose Levels on Oral Glucose Tolerance Test in Normal Rats

Groups	Treatment	Blood Glucose Levels (mg/dl) and Time in min			
		0	30	60	120
Group-I	Saline + Glucose 2g/kg	92.50 ± 4.13	141.0 ± 4.22	137.7 ± 4.04	119.3 ± 3.29
Group-II	Metformin (250mg/kg) + Glucose 2g/kg	90.17 ± 3.02	103.0 ± 3.38***	84.67 ± 3.91***	73.83 ± 3.32***
Group-III	EAENN (100 mg/kg b.w. p.o) + Glucose 2g/kg	89.67 ± 4.30	118.3 ± 5.56*	109.8 ± 4.63***	93.50 ± 4.48***
Group-IV	EAENN (200 mg/kg b.w. p.o) + Glucose 2g/kg	91.83 ± 3.47	114.2 4.24**	102.2 ± 3.56***	88.50 ± 3.72***
Group-V	EAENN (400 mg/kg b.w. p.o) + Glucose 2g/kg	88.67 ± 3.52	106.7 ± 3.095***	93.67 ± 2.56***	79.67 ± 3.45***
Group VI	EAENN (100 mg/kg b.w. p.o) + Glucose 2g/kg	90.83 ± 5.31	120.3 ± 4.34*	108.7 ± 3.89***	97.17 ± 4.23**
Group VII	EAENN (200 mg/kg b.w. p.o) + Glucose 2g/kg	89.33 ± 3.57	115.2 ± 4.71**	104.0 ± 3.77***	89.17± 3.49***
Group VIII	EAENN (400 mg/kg b.w. p.o) + Glucose 2g/kg	87.67 ± 5.308	105.5 ± 4.73***	96.33 ± 4.38***	81.33 ± 4.51***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant.

EAENN: Ethylacetate extract of *Nymphaea nouchali,* **HAENN**: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

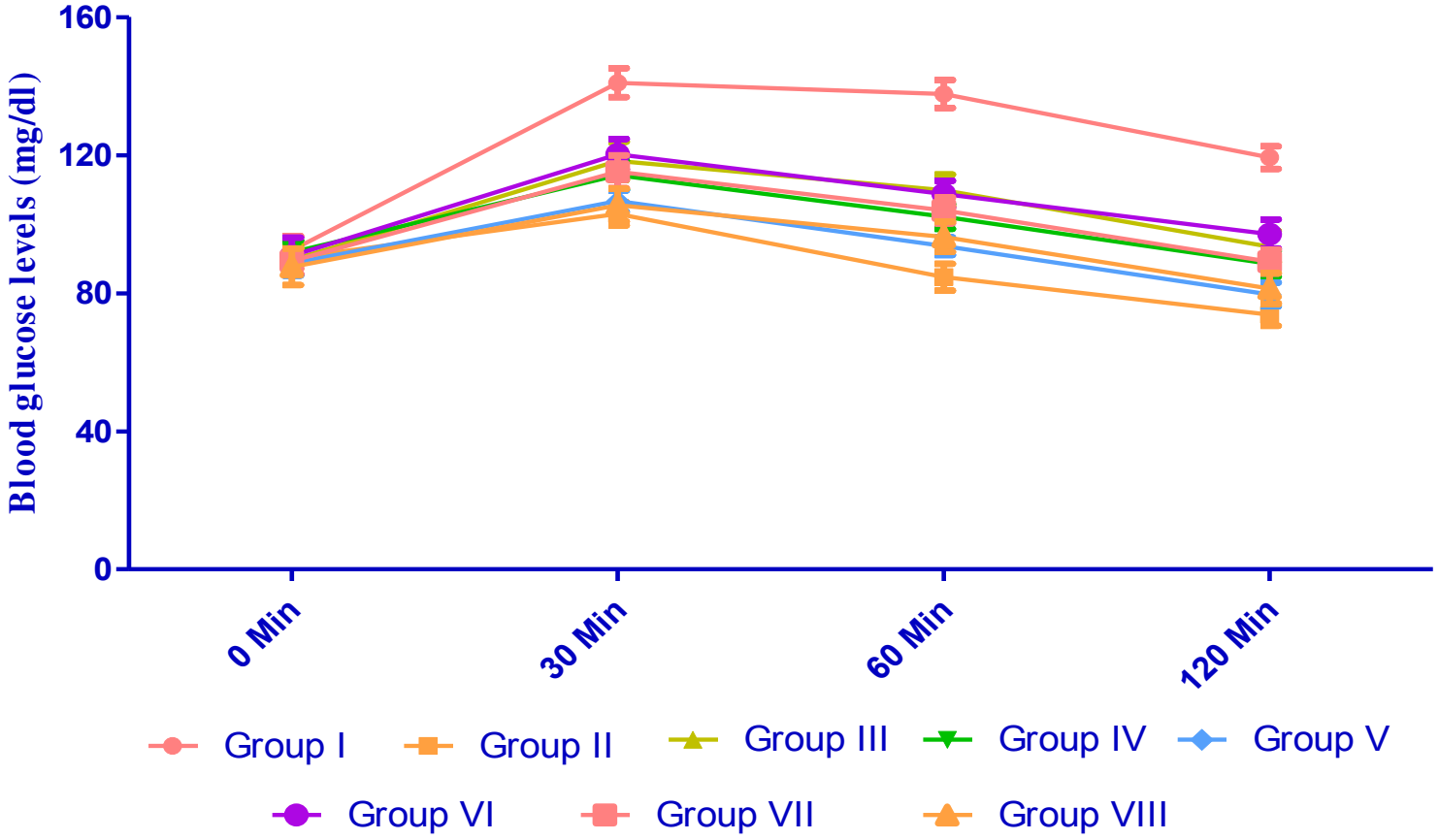


Fig. No: 118 Effects of EAENN and HAENN on Blood Glucose Levels on Oral Glucose Tolerance Test in Normal Rats

8.6.3.3. Effect of Extracts in Alloxan Induced Diabetic Rats

Table No: 53 Effect of EAENP and HAENP on Blood Glucose Levels in ALX Induced Diabetic Rats

Groups	Treatment	Blood Glucose Levels (mg/dl)			
		0 day	7 day	14 day	21 day
Group-I	Saline	95.50 ± 5.10	97.67 ± 4.34	92.17 ± 4.57	104.5 ± 4.395
Group-II	ALX (150mg/kg) + Saline	390.8 ± 18.41	423.3 ± 21.62	388.2 ± 17.00	348.7 ± 14.01
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	398.2 ± 25.97	314.8 ± 15.31***	207.2 ± 12.32***	110.2 ± 7.952***
Group-IV	ALX(150mg/kg) + EAENP (100 mg/kg b.w. p.o)	392.2 ± 25.15	360.3 ± 16.59^{ns}	301.0 ± 17.57**	226.3 ± 18.15***
Group-V	ALX(150mg/kg) + EAENP (200 mg/kg b.w. p.o)	387.3 ± 12.42	354.5 ± 9.670^{ns}	278.5 ± 11.08***	189.7± 19.28***
Group-VI	ALX(150mg/kg) + EAENP (400 mg/kg b.w. p.o)	379.7 ± 17.51	335.2 ± 13.64**	233.0 ± 13.30***	128.5 ± 4.16***
Group-VII	ALX(150mg/kg) + HAENP (100 mg/kg b.w. p.o)	373.5 ± 17.31	362.5 ± 15.89^{ns}	317.7 ± 13.26*	238.8 ± 12.10***
Group-VIII	ALX(150mg/kg) + HAENP (200 mg/kg b.w. p.o)	380.3 ± 18.28	351.7 ± 14.38*	288.3 ± 12.90***	195.3 ± 11.85***
Group-IX	ALX(150mg/kg) + HAENP (400 mg/kg b.w. p.o)	396.3 ± 19.29	341.0 ± 16.97**	257.2 ± 20.70***	137.5 ± 17.68***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group. **EAENP**: Ethylacetate extract of *Nymphaea pubescens*, **HAENP**: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

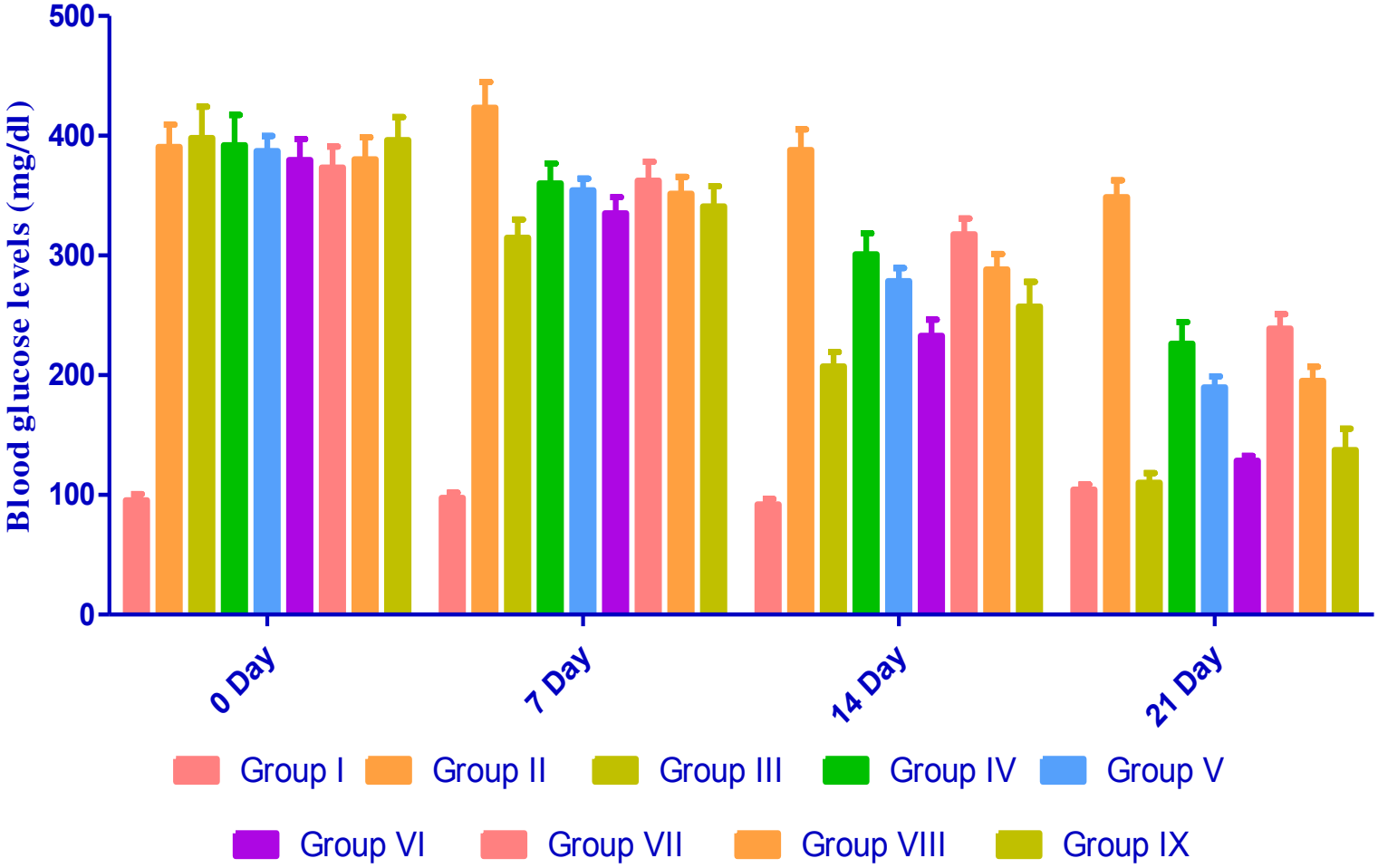


Fig. No: 119 Effect of EAENP and HAENP on Blood Glucose Levels in ALX Induced Diabetic Rats

Table No: 54 Effect of EAENN and HAENN on Blood Glucose Levels in ALX Induced Diabetic Rats

Groups	Treatment	Blood Glucose Levels (mg/dl)			
		0 day	7 day	14 day	21 day
Group-I	Saline	95.50 ± 5.10	97.67 ± 4.34	92.17 ± 4.57	104.5 ± 4.395
Group-II	ALX (150mg/kg) + Saline	390.8 ± 18.41	423.3 ± 21.62	388.2 ± 17.00	348.7 ± 14.01
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	398.2 ± 25.97	314.8 ± 15.31***	207.2 ± 12.32***	110.2 ± 7.952***
Group-IV	ALX(150mg/kg) + EAENN (100 mg/kg b.w. p.o)	401.8 ± 17.77	353.2 ± 13.91^{ns}	297.0 ± 16.45**	227.0 ± 12.76***
Group-V	ALX(150mg/kg) + EAENN (200 mg/kg b.w. p.o)	389.7 ± 30.15	339.3 ± 18.33*	278.8 ± 12.71***	179.3 ± 11.19***
Group-VI	ALX(150mg/kg) + EAENN (400 mg/kg b.w. p.o)	385.5 ± 15.14	329.8 ± 12.46**	235.3 ± 13.14***	122.8 ± 10.16***
Group-VII	ALX(150mg/kg) + HAENN (100 mg/kg b.w. p.o)	396.8 ± 24.89	357.7 ± 23.19^{ns}	301.8 ± 18.63**	231.7 ± 18.66***
Group-VIII	ALX(150mg/kg) + HAENN (200 mg/kg b.w. p.o)	395.2 ± 24.57	346.5 ± 21.12*	271.3 ± 22.85***	182.3 ± 19.52***
Group-IX	ALX(150mg/kg) + HAENN (400 mg/kg b.w. p.o)	392.0 ± 10.47	332.5 ± 8.358**	243.3 ± 12.27***	126.5 ± 12.56***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENN: Ethylacetate extract of *Nymphaea nouchali,* **HAENN**: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

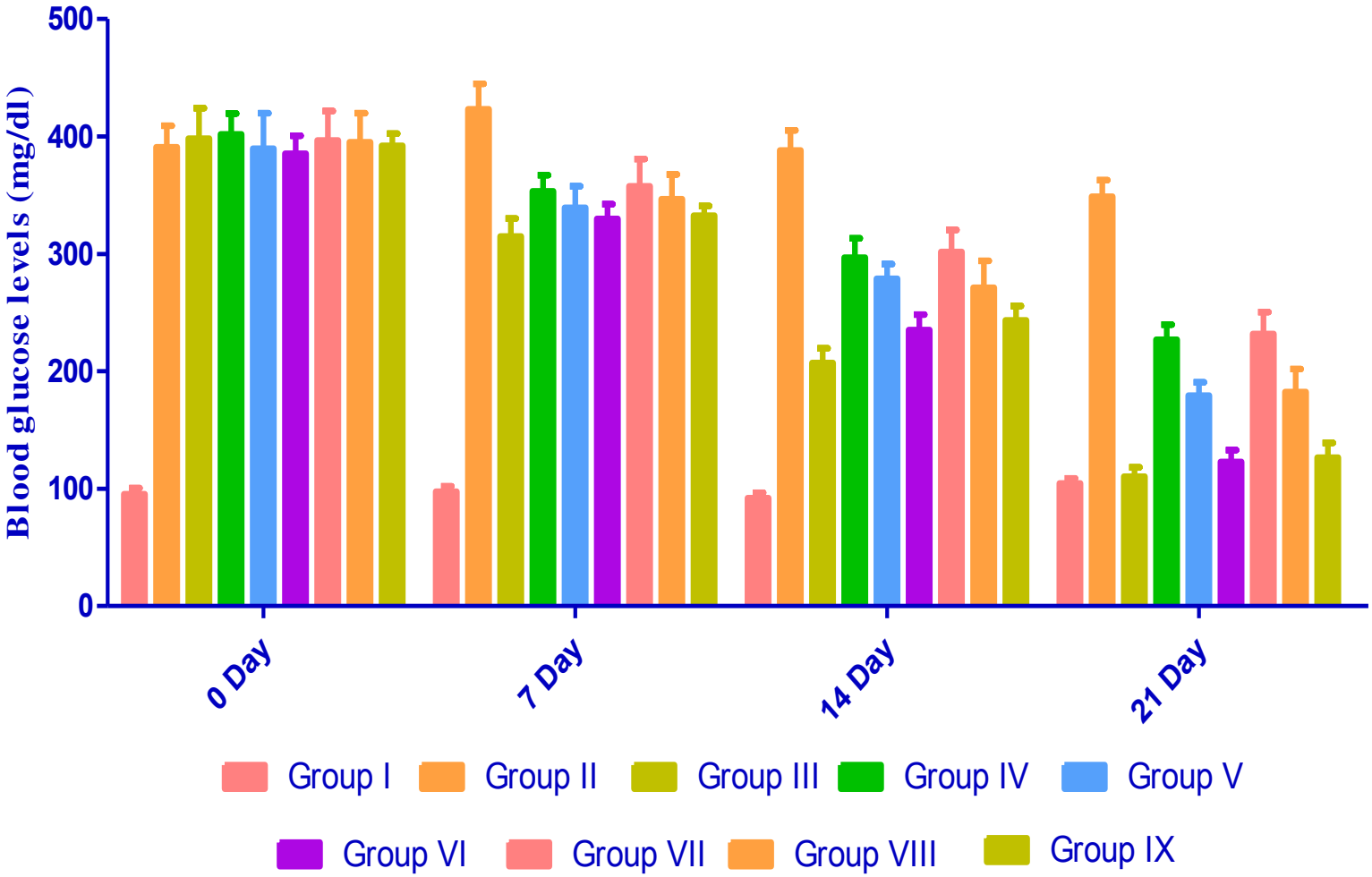


Fig. No: 120 Effect of EAENN and HAENPN on Blood Glucose Levels in ALX Induced Diabetic Rats

8.6.3.4. Effect of extract on Physical Parameters of ALX Induced Diabetic Rats: Body Weight

Table No: 55 Effect of EAENP and HAENP on Body Weight in ALX Induced Diabetic Rats

Groups	Treatment	Body Weight (gms)			
		0 Day	7 Day	14 Day	21 Day
Group-I	Saline	185.2 ± 5.72	193.8 ± 6.45	201.5 ± 6.52	207.8 ± 5.74
Group-II	ALX (150mg/kg) + Saline	181.7 ± 3.81	143.7 ± 2.69	139.7 ± 33.23	144.2 ± 2.24
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	184.7 ± 6.03	179.7 ± 3.93***	186.5 ± 3.73***	193.5 ± 3.27***
Group-IV	ALX(150mg/kg) + EAENP (100 mg/kg b.w. p.o)	191.0 ± 5.78	161.0 ± 3.19^{ns}	168.5 ± 2.43***	172.2 ± 2.83***
Group-V	ALX(150mg/kg) + EAENP (200 mg/kg b.w. p.o)	189.7 ± 5.10	165.0 ± 3.75*	172.7 ± 3.89***	179.5 ± 3.06***
Group-VI	ALX(150mg/kg) + EAENP (400 mg/kg b.w. p.o)	180.8 ± 3.21	170.0 ± 3.31**	177.0 ± 3.34***	187.0 ± 3.19***
Group-VII	ALX(150mg/kg) + HAENP (100 mg/kg b.w. p.o)	193.7 ± 6.11	159.8 ± 4.79^{ns}	163.8 ± 3.62**	170.7 ± 3.10***
Group-VIII	ALX(150mg/kg) + HAENP (200 mg/kg b.w. p.o)	187.7 ± 3.85	162.2 ± 6.15^{ns}	167.2 ± 4.96***	176.2 ± 5.20***
Group-IX	ALX(150mg/kg) + HAENP (400 mg/kg b.w. p.o)	179.2 ± 3.29	168.2 ± 3.76**	174.3 ± 3.41***	183.7 ± 2.83***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENP: Ethylacetate extract of *Nymphaea pubescens*, **HAENP**: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

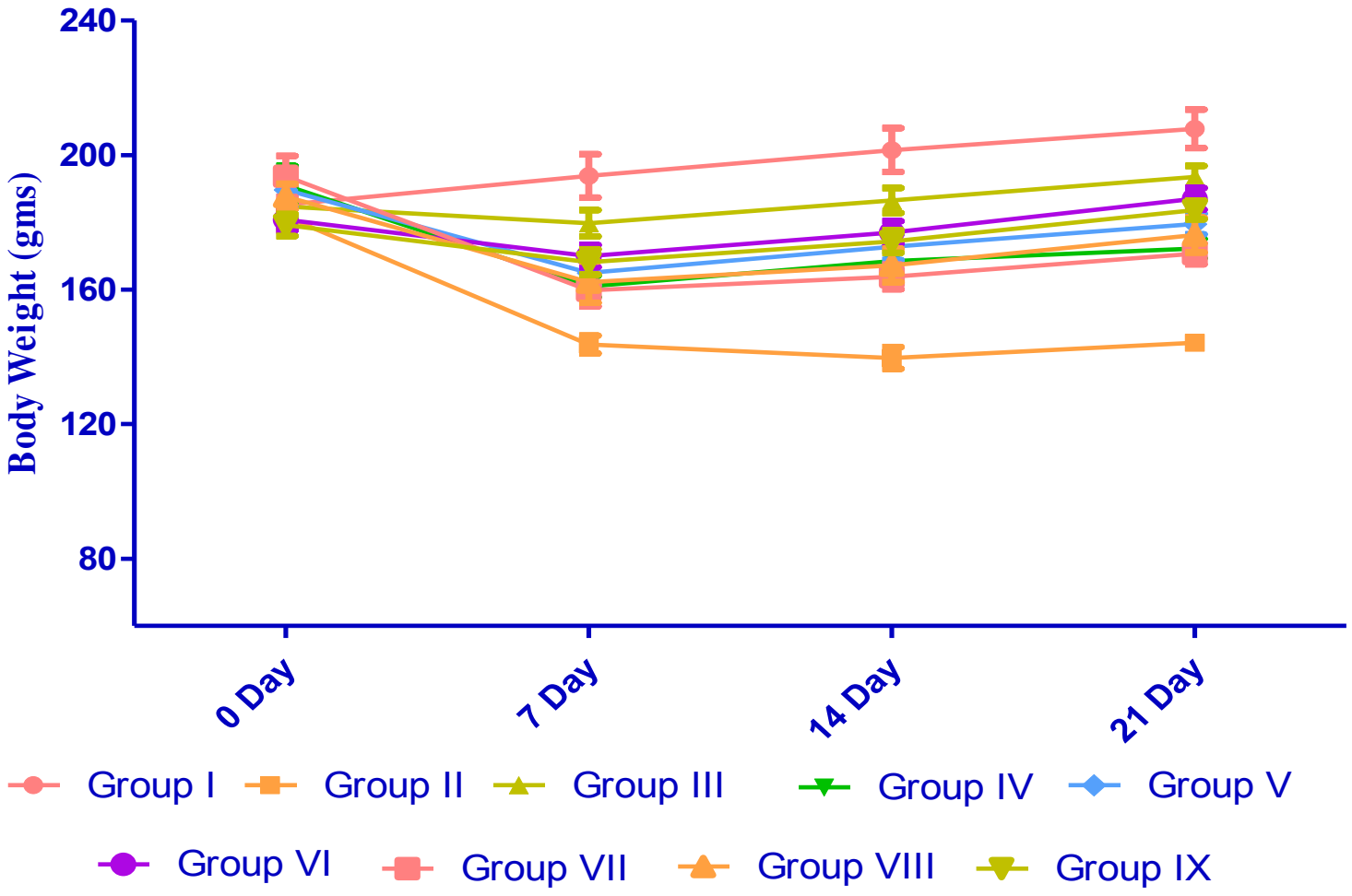


Fig. No: 121 Effect of EAENP and HAENP on Body Weight in ALX Induced Diabetic Rats

Table No: 56 Effect of EAENN and HAENN on Body Weight in ALX Induced Diabetic Rats

Groups	Treatment	Body Weight (gms)			
		0 day	7 day	14 day	21 day
Group-I	Saline	185.2 ± 5.72	193.8 ± 6.45	201.5 ± 6.52	207.8 ± 5.74
Group-II	ALX (150mg/kg) + Saline	181.7 ± 3.81	143.7 ± 2.69	139.7 ± 33.23	144.2 ± 2.24
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	184.7 ± 6.03	179.7 ± 3.93***	186.5 ± 3.73***	193.5 ± 3.27***
Group-IV	ALX(150mg/kg) + EAENN (100 mg/kg b.w. p.o)	177.8 ± 3.49	159.3 ± 1.22^{ns}	165.8 ± 2.41***	172.8 ± 2.05***
Group-V	ALX(150mg/kg) + EAENN (200 mg/kg b.w. p.o)	182.3 ± 4.63	164.7 ± 3.27**	170.7 ± 3.10***	180.2 ± 2.58***
Group-VI	ALX(150mg/kg) + EAENN (400 mg/kg b.w. p.o)	178.7 ± 4.01	168.8 ± 3.48***	179.0 ± 3.27***	187.2 ± 2.81***
Group-VII	ALX(150mg/kg) + HAENN (100 mg/kg b.w. p.o)	180.2 ± 3.60	158.7 ± 3.49^{ns}	166.3 ± 3.23**	174.2 ± 2.98***
Group-VIII	ALX(150mg/kg) + HAENN (200 mg/kg b.w. p.o)	179.5 ± 2.91	163.5 ± 1.96**	172.2 ± 2.58***	181.2 ± 1.88***
Group-IX	ALX(150mg/kg) + HAENN (400 mg/kg b.w. p.o)	176.5 ± 3.12	167.3 ± 3.11***	177.2 ± 3.59***	185.3 ± 2.98***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENN: Ethylacetate extract of *Nymphaea nouchali,*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

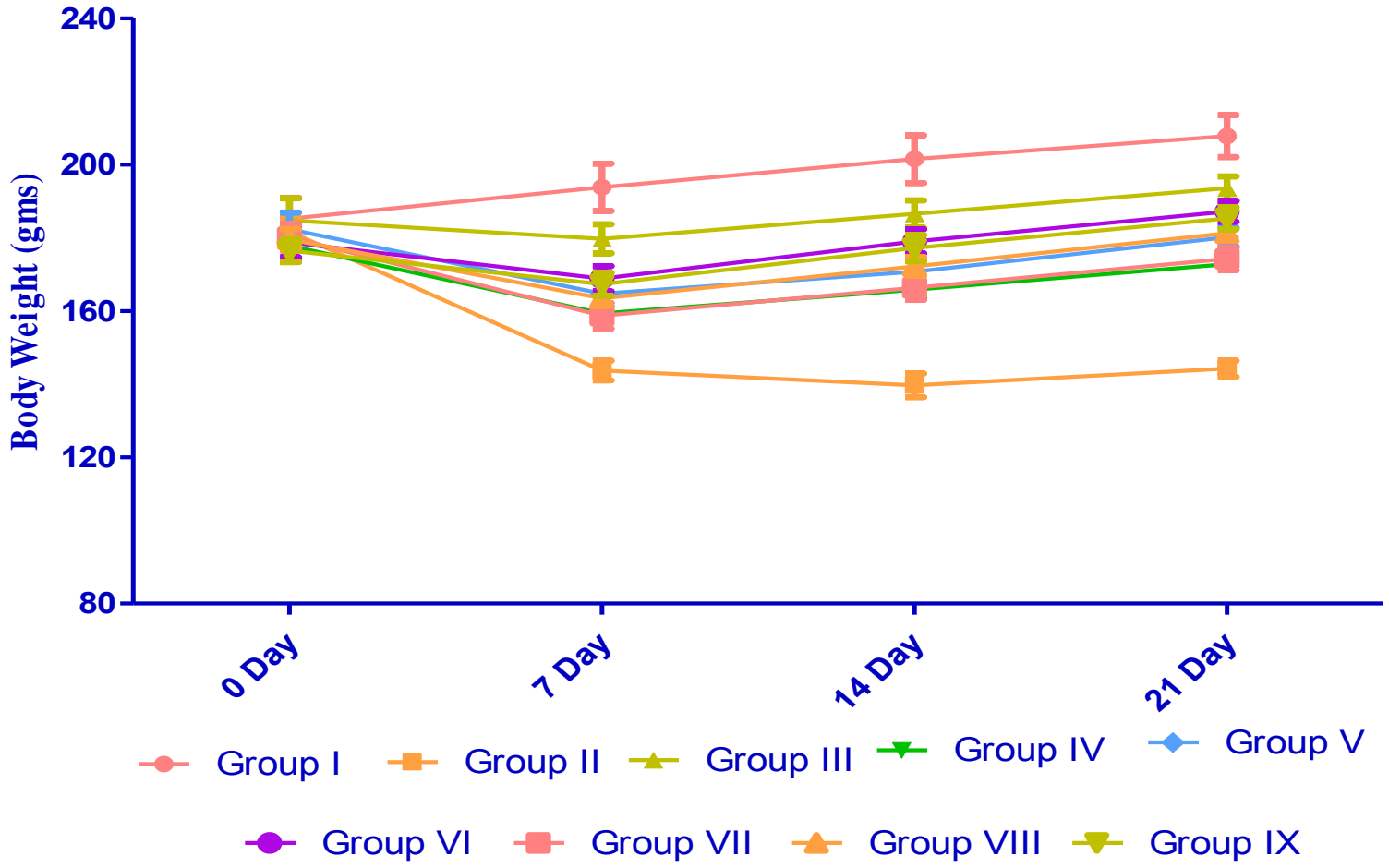


Fig. No: 122 Effect of EAENN and HAENN on Body Weight in ALX Induced Diabetic Rats

8.6.3.5. Effect of extract on Biochemical Parameters of ALX Induced Diabetic Rats

i. Serum Albumin Levels

Table No: 57 Effect of EAENP and HAENP on Serum Albumin Levels in ALX Induced Diabetic Rats

Groups	Treatment	Serum Albumin Levels (g/dl)
Group-I	Saline	4.48 ± 0.118
Group-II	ALX (150mg/kg) + Saline	2.24 ± 0.132
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	4.32 ± 0.112***
Group-IV	ALX(150mg/kg) + EAENP (100 mg/kg b.w. p.o)	3.18 ± 0.136**
Group-V	ALX(150mg/kg) + EAENP (200 mg/kg b.w. p.o)	3.52 ± 0.201***
Group-VI	ALX(150mg/kg) + EAENP (400 mg/kg b.w. p.o)	4.13 ± 0.172***
Group-VII	ALX(150mg/kg) + HAENP (100 mg/kg b.w. p.o)	3.26 ± 0.166**
Group-VIII	ALX(150mg/kg) + HAENP (200 mg/kg b.w. p.o)	3.46 ± 0.199***
Group-IX	ALX(150mg/kg) + HAENP (400 mg/kg b.w. p.o)	4.03 ± 0.224***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENP: Ethylacetate extract of *Nymphaea pubescens*,

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

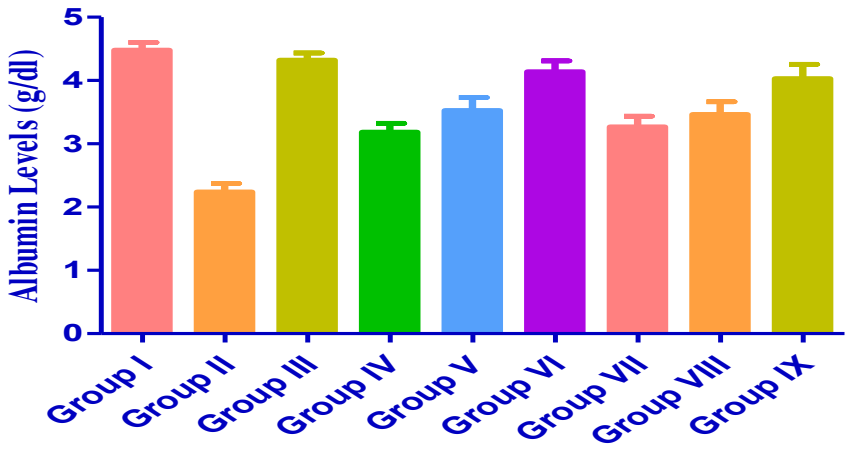


Fig. No: 123 Effect of EAENP and HAENP on Serum Albumin Levels in ALX Induced Diabetic Rats

Table No: 58 Effect of EAENN and HAENN on Serum Albumin Levels in ALX Induced Diabetic Rats

Groups	Treatment	Serum Albumin Levels (g/dl)
Group-I	Saline	4.48 ± 0.118
Group-II	ALX (150mg/kg) + Saline	2.24 ± 0.132
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	4.32 ± 0.112***
Group-IV	ALX(150mg/kg) + EAENN (100 mg/kg b.w. p.o)	3.46 ± 0.138***
Group-V	ALX(150mg/kg) + EAENN (200 mg/kg b.w. p.o)	3.73 ± 0.269***
Group-VI	ALX(150mg/kg) + EAENN (400 mg/kg b.w. p.o)	4.20 ± 0.131***
Group-VII	ALX(150mg/kg) + HAENN (100 mg/kg b.w. p.o)	3.15 ± 0.226**
Group-VIII	ALX(150mg/kg) + HAENN (200 mg/kg b.w. p.o)	3.57 ± 0.184***
Group-IX	ALX(150mg/kg) + HAENN (400 mg/kg b.w. p.o)	4.11 ± 0.110***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENN: Ethylacetate extract of *Nymphaea nouchali,*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

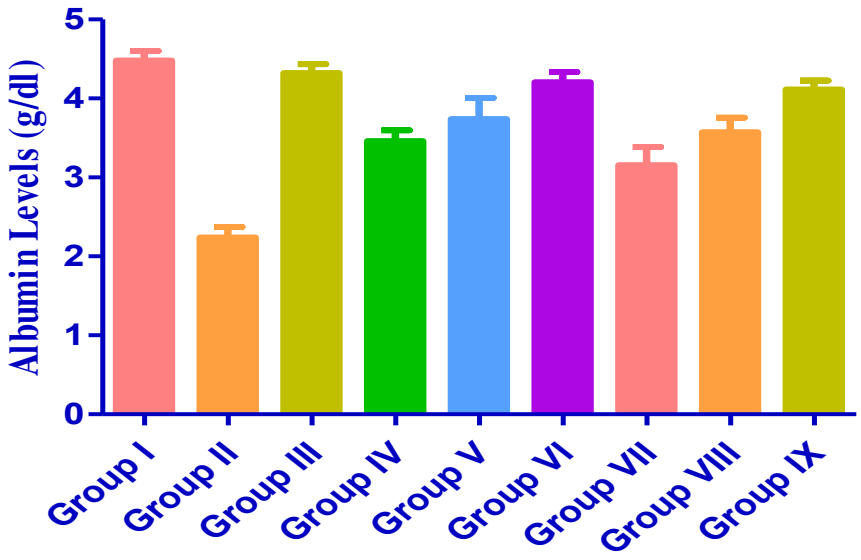


Fig. No: 124 Effects of EAENN and HAENN on Serum Albumin Levels in ALX Induced Diabetic Rats

ii. Serum Total Protein

Table No: 59 Effect of EAENP and HAENP on Serum Total Protein levels in ALX Induced Diabetic Rats

Groups	Treatment	Serum Total Protein Levels (mg/dl)
Group-I	Saline	8.98 ± 0.269
Group-II	ALX (150mg/kg) + Saline	5.15 ± 0.192
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	8.46 ± 0.270***
Group-IV	ALX(150mg/kg) + EAENP (100 mg/kg b.w. p.o)	7.01 ± 0.181***
Group-V	ALX(150mg/kg) + EAENP (200 mg/kg b.w. p.o)	7.54 ± 0.228***
Group-VI	ALX(150mg/kg) + EAENP (400 mg/kg b.w. p.o)	8.25 ± 0.116***
Group-VII	ALX(150mg/kg) + HAENP (100 mg/kg b.w. p.o)	6.99 ± 0.278***
Group-VIII	ALX(150mg/kg) + HAENP (200 mg/kg b.w. p.o)	7.64 ± 0.329***
Group-IX	ALX(150mg/kg) + HAENP (400 mg/kg b.w. p.o)	8.12 ± 0.281***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENP: Ethylacetate extract of *Nymphaea pubescens*,

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

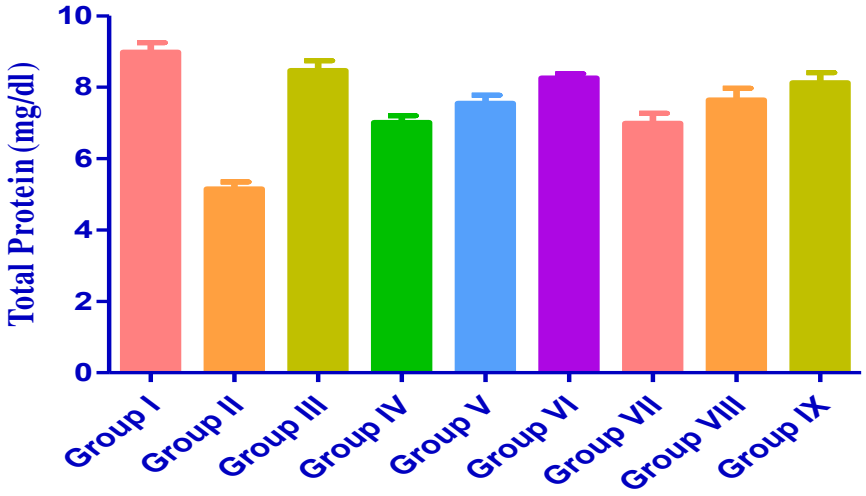


Fig. No: 125 Effect of EAENP and HAENP on Serum Total Protein levels in ALX Induced Diabetic Rats

Table No.: 60 Effect of EAENN and HAENN on Serum Total Protein levels in ALX Induced Diabetic Rats

Groups	Treatment	Serum Total Protein Levels (mg/dl)
Group-I	Saline	8.98 ± 0.269
Group-II	ALX (150mg/kg) + Saline	5.15 ± 0.192
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	8.46 ± 0.270***
Group-IV	ALX(150mg/kg) + EAENN (100 mg/kg b.w. p.o)	6.89 ± 0.212*
Group-V	ALX(150mg/kg) + EAENN (200 mg/kg b.w. p.o)	7.34 ± 0.326**
Group-VI	ALX(150mg/kg) + EAENN (400 mg/kg b.w. p.o)	8.16 ± 0.364***
Group-VII	ALX(150mg/kg) + HAENN (100 mg/kg b.w. p.o)	7.14 ± 0.502**
Group-VIII	ALX(150mg/kg) + HAENN (200 mg/kg b.w. p.o)	7.47 ± 0.312***
Group-IX	ALX(150mg/kg) + HAENN (400 mg/kg b.w. p.o)	8.18 ± 0.443***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENN: Ethylacetate extract of *Nymphaea nouchali,*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

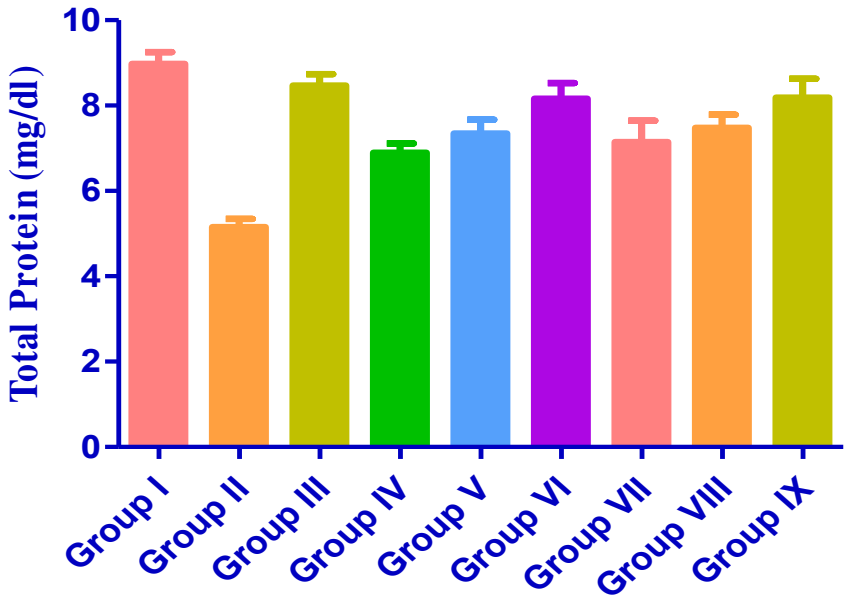


Fig. No: 126 Effect of EAENN and HAENN on Serum Total Protein levels in ALX Induced Diabetic Rats

iii). Serum Creatinine

Table No: 61 Effect of EAENP and HAENP on Serum Creatinine Levels in ALX Induced Diabetic Rats

Groups	Treatment	Serum Creatinine Levels (mg/dl)
Group-I	Saline	0.675 ± 0.020
Group-II	ALX (150mg/kg) + Saline	1.205 ± 0.065
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	0.684 ± 0.029***
Group-IV	ALX(150mg/kg) + EAENP (100 mg/kg b.w. p.o)	0.969 ± 0.048*
Group-V	ALX(150mg/kg) + EAENP (200 mg/kg b.w. p.o)	0.750 ± 0.031***
Group-VI	ALX(150mg/kg) + EAENP (400 mg/kg b.w. p.o)	0.709 ± 0.042***
Group-VII	ALX(150mg/kg) + HAENP (100 mg/kg b.w. p.o)	0.940 ± 0.053*
Group-VIII	ALX(150mg/kg) + HAENP (200 mg/kg b.w. p.o)	0.819 ± 0.080***
Group-IX	ALX(150mg/kg) + HAENP (400 mg/kg b.w. p.o)	0.742 ± 0.032***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENP: Ethylacetate extract of *Nymphaea pubescens*,

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

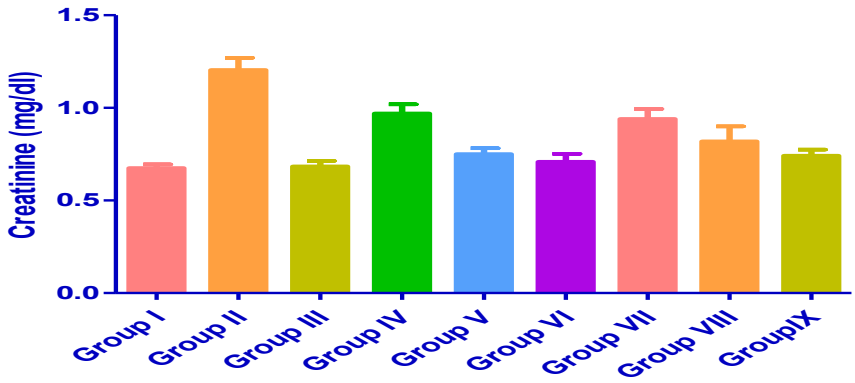


Fig. No: 127 Effect of EAENP and HAENP on Serum Creatinine levels in ALX Induced Diabetic Rats

Table No: 62 Effect of EAENN and HAENN on Serum Creatinine levels in ALX Induced Diabetic Rats

Groups	Treatment	Serum Creatinine Levels (mg/dl)
Group-I	Saline	0.675 ± 0.020
Group-II	ALX (150mg/kg) + Saline	1.205 ± 0.065
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	0.684 ± 0.029***
Group-IV	ALX(150mg/kg) + EAENN (100 mg/kg b.w. p.o)	0.948 ± 0.054**
Group-V	ALX(150mg/kg) + EAENN (200 mg/kg b.w. p.o)	0.778 ± 0.032***
Group-VI	ALX(150mg/kg) + EAENN (400 mg/kg b.w. p.o)	0.696 ± 0.040***
Group-VII	ALX(150mg/kg) + HAENN (100 mg/kg b.w. p.o)	0.913 ± 0.074**
Group-VIII	ALX(150mg/kg) + HAENN (200 mg/kg b.w. p.o)	0.760 ± 0.042***
Group-IX	ALX(150mg/kg) + HAENN (400 mg/kg b.w. p.o)	0.716 ± 0.033***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENN: Ethylacetate extract of *Nymphaea nouchali,*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

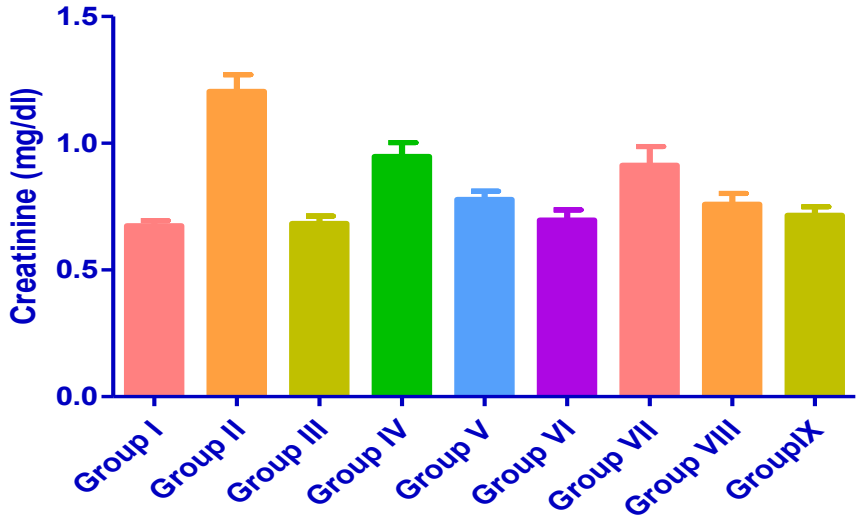


Fig. No: 128 Effect of EAENN and HAENN on Serum Creatinine levels in ALX Induced Diabetic Rats

iv). Serum Lipid Profile

Table No.: 63 Effect of EAENP and HAENP on Serum Lipid Profile of ALX Induced Diabetic Rats

Groups	Treatment	Serum Lipid Profile mg/dl				
		TC	TG	HDL-C	LDL-C	VLDL-C
Group-I	Saline	67.92 ± 2.59	77.55 ± 4.41	24.48 ± 0.70	27.93 ± 2.20	15.51 ± 0.88
Group-II	ALX (150mg/kg) + Saline	133.8 ± 3.96	141.9 ± 8.99	15.52 ± 0.69	89.84 ± 4.76	28.39 ± 1.79
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	76.69 ± 3.54***	81.96 ±4.13***	23.07 ± 0.81***	37.23 ± 4.20***	16.39 ± 0.82***
Group-IV	ALX(150mg/kg) + EAENP (100 mg/kg b.w. p.o)	104.4 ± 3.85***	109.6 ± 4.89**	19.37 ± 0.870*	63.15 ± 4.19**	21.92 ± 0.97**
Group-V	ALX(150mg/kg) + EAENP (200 mg/kg b.w. p.o)	91.57 ± 3.80***	94.11 ±4.21***	20.36 ± 0.90**	52.40 ± 4.20***	18.82 ± 0.84***
Group-VI	ALX(150mg/kg) + EAENP (400 mg/kg b.w. p.o)	84.06 ± 4.73***	86.81 ±5.40***	22.14 ± 0.61***	44.56 ± 3.89***	17.36 ± 1.08***
Group-VII	ALX(150mg/kg) + HAENP (100 mg/kg b.w. p.o)	108.2 ± 6.38**	111.5 ± 4.91**	18.80 ± 0.94^{ns}	67.12 ± 7.55*	22.31 ± 0.98**
GroupVIII	ALX(150mg/kg) + HAENP (200 mg/kg b.w. p.o)	95.01 ± 3.07***	104.6 ±5.39***	20.21 ± 0.75**	53.88 ± 3.91***	20.92 ± 1.07***
Group-IX	ALX(150mg/kg) + HAENP (400 mg/kg b.w. p.o)	86.20 ± 4.67***	88.57 ±2.62***	21.87 ± 0.99***	46.62 ± 5.59***	17.71 ± 0.52***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENP: Ethylacetate extract of *Nymphaea pubescens*, **HAENP**: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

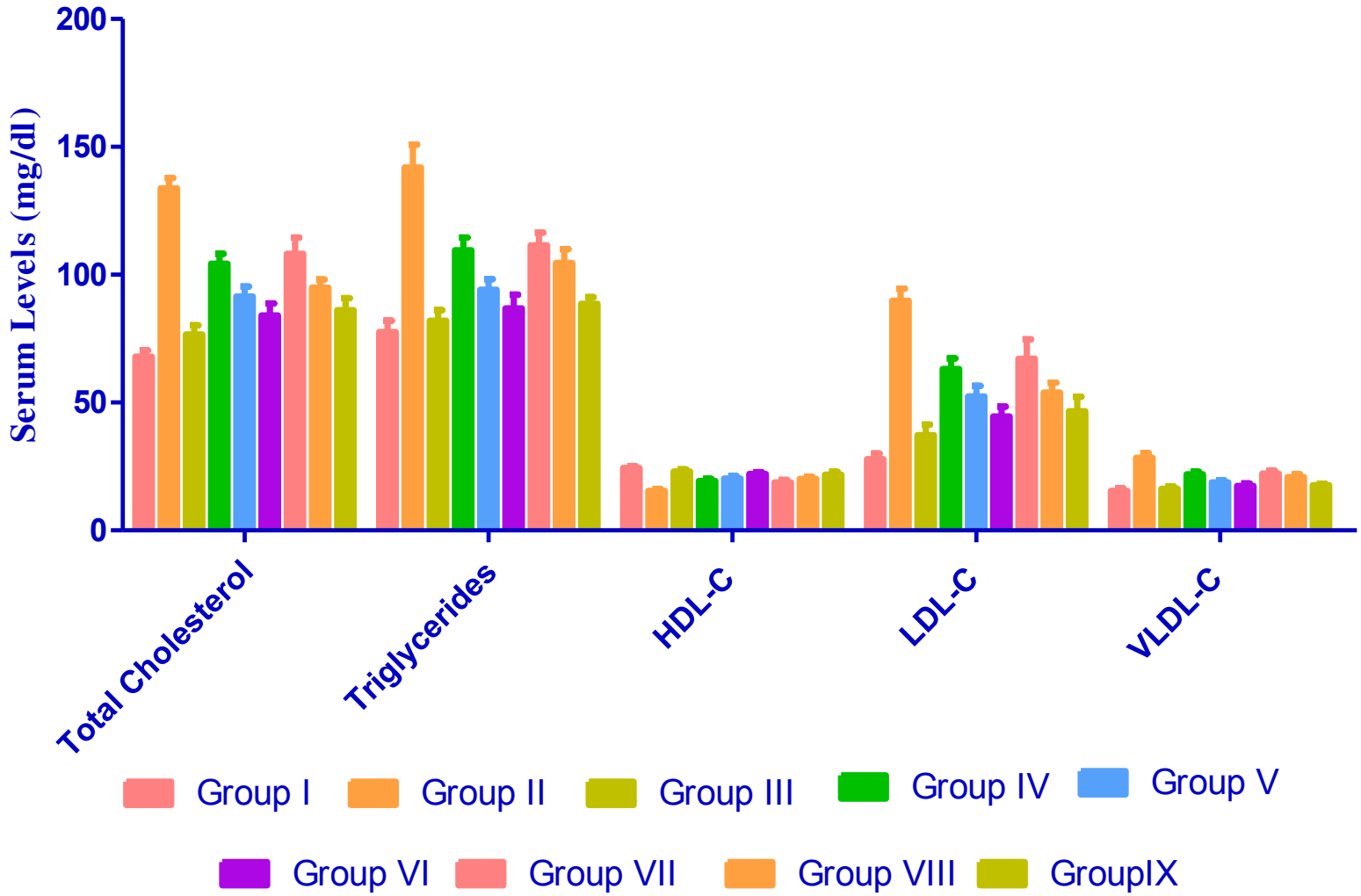


Fig. No: 129 Effect of EAENP and HAENP on Serum Lipid Profile of ALX Induced Diabetic Rats

Table No: 64 Effect of EAENN and HAENN on Serum Lipid Profile of ALX Induced Diabetic Rats

Groups	Treatment	Serum Lipid Profile mg/dl				
		TC	TG	HDL-C	LDL-C	VLDL-C
Group-I	Saline	67.92 ± 2.59	77.55 ± 4.41	24.48 ± 0.70	27.93 ± 2.20	15.51 ± 0.88
Group-II	ALX (150mg/kg) + Saline	133.8 ± 3.96	141.9 ± 8.99	15.52 ± 0.69	89.84 ± 4.76	28.39 ± 1.79
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	76.69 ± 3.54***	81.96 ± 4.13***	23.07 ± 0.81***	37.23 ± 4.20***	16.39 ± 0.82***
Group-IV	ALX(150mg/kg) + EAENN (100 mg/kg b.w. p.o)	101.2 ± 5.04***	113.3 ± 6.68*	18.90 ± 0.39*	59.58 ± 4.85***	22.67 ± 1.33*
Group-V	ALX(150mg/kg) + EAENN (200 mg/kg b.w. p.o)	90.25 ± 4.25***	98.52 ± 5.25***	20.79 ± 0.59***	49.76 ± 4.21***	19.70 ± 1.05***
Group-VI	ALX(150mg/kg) + EAENN (400 mg/kg b.w. p.o)	81.55 ± 4.60***	89.62 ± 4.76***	22.26 ± 0.39***	41.37 ± 4.62***	17.92 ± 0.95***
Group-VII	ALX(150mg/kg) + HAENN (100 mg/kg b.w. p.o)	98.28 ± 3.93***	115.7 ± 7.39^{ns}	18.63 ± 0.34*	56.52 ± 5.01***	23.13 ± 1.48^{ns}
Group-VIII	ALX(150mg/kg) + HAENN (200 mg/kg b.w. p.o)	87.01 ± 4.98***	102.7 ± 5.55***	19.43 ± 0.55**	47.04 ± 5.29***	20.54 ± 1.11***
Group-IX	ALX(150mg/kg) + HAENN (400 mg/kg b.w. p.o)	79.81 ± 4.14***	91.62 ± 4.79***	22.07 ± 0.89***	39.41 ± 3.41***	18.32 ± 0.95***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENN: Ethylacetate extract of *Nymphaea nouchali,* **HAENN**: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

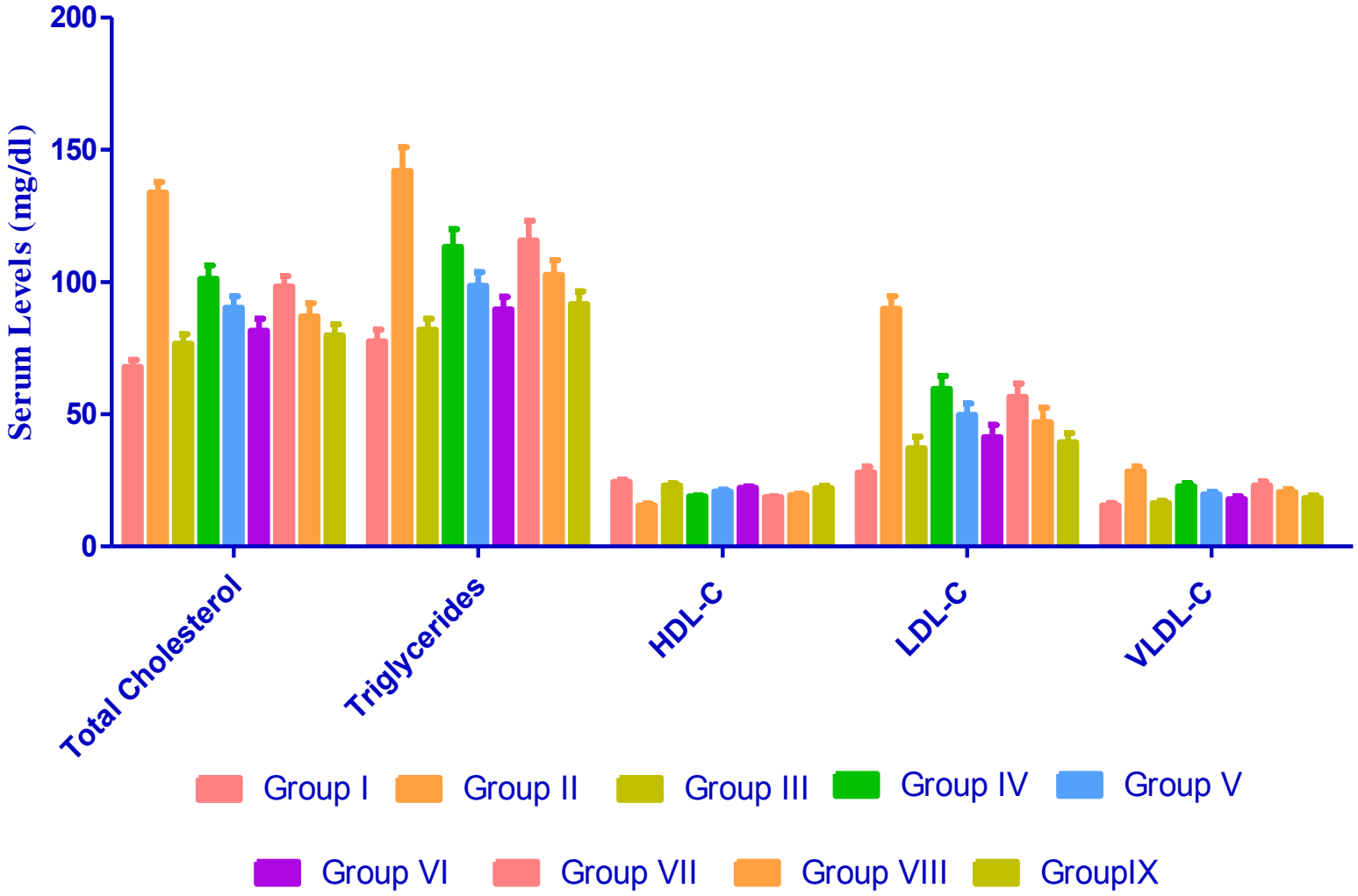


Fig. No: 130 Effect of EAENN and HAENN on Serum Lipid Profile of ALX Induced Diabetic Rats

8.6.3.6. Effect of Extract on Antioxidant Levels

i. Superoxide Dismutase Levels

Table No: 65 Effect of EAENP and HAENP on SOD Levels in ALX Induced Diabetic Rats

Groups	Treatment	SOD (U/mg protein)
Group-I	Saline	14.36 ± 0.36
Group-II	ALX (150mg/kg) + Saline	7.81 ± 0.491
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	13.23 ± 0.46***
Group-IV	ALX(150mg/kg) + EAENP (100 mg/kg b.w. p.o)	10.57 ± 0.42***
Group-V	ALX(150mg/kg) + EAENP (200 mg/kg b.w. p.o)	11.87 ± 0.46***
Group-VI	ALX(150mg/kg) + EAENP (400 mg/kg b.w. p.o)	12.68± 0.32***
Group-VII	ALX(150mg/kg) + HAENP (100 mg/kg b.w. p.o)	9.97 ± 0.38**
Group-VIII	ALX(150mg/kg) + HAENP (200 mg/kg b.w. p.o)	11.56 ± 0.35***
Group-IX	ALX(150mg/kg) + HAENP (400 mg/kg b.w. p.o)	12.42 ± 0.32***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENP: Ethylacetate extract of *Nymphaea pubescens*,

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

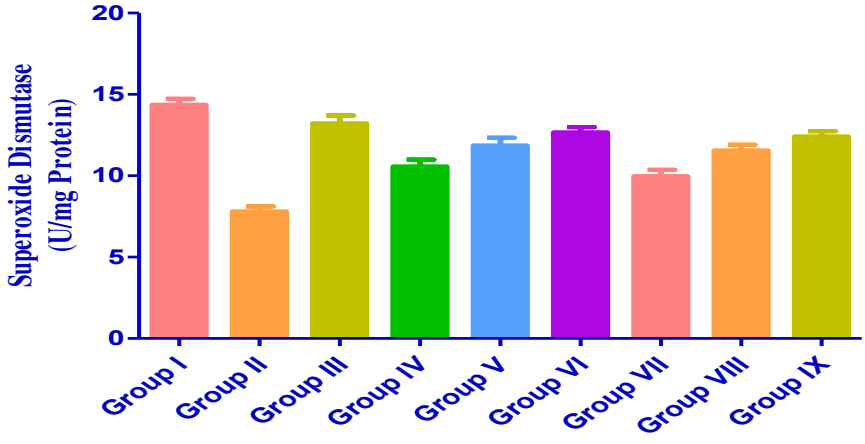


Fig. No: 131 Effect of EAENP and HAENP on SOD Levels in ALX Induced Diabetic Rats

Table No: 66 Effect of EAENN and HAENN on SOD Levels in ALX Induced Diabetic Rats

Groups	Treatment	SOD (U/mg protein)
Group-I	Saline	14.36 ± 0.36
Group-II	ALX (150mg/kg) + Saline	7.81 ± 0.491
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	13.23 ± 0.46***
Group-IV	ALX(150mg/kg) + EAENN (100 mg/kg b.w. p.o)	10.52 ± 0.47***
Group-V	ALX(150mg/kg) + EAENN (200 mg/kg b.w. p.o)	12.09 ± 0.42***
Group-VI	ALX(150mg/kg) + EAENN (400 mg/kg b.w. p.o)	13.01 ± 0.32***
Group-VII	ALX(150mg/kg) + HAENN (100 mg/kg b.w. p.o)	10.39 ± 0.33***
Group-VIII	ALX(150mg/kg) + HAENN (200 mg/kg b.w. p.o)	11.94 ± 0.37***
Group-IX	ALX(150mg/kg) + HAENN (400 mg/kg b.w. p.o)	12.84 ± 0.27***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENN: Ethylacetate extract of *Nymphaea nouchali,*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

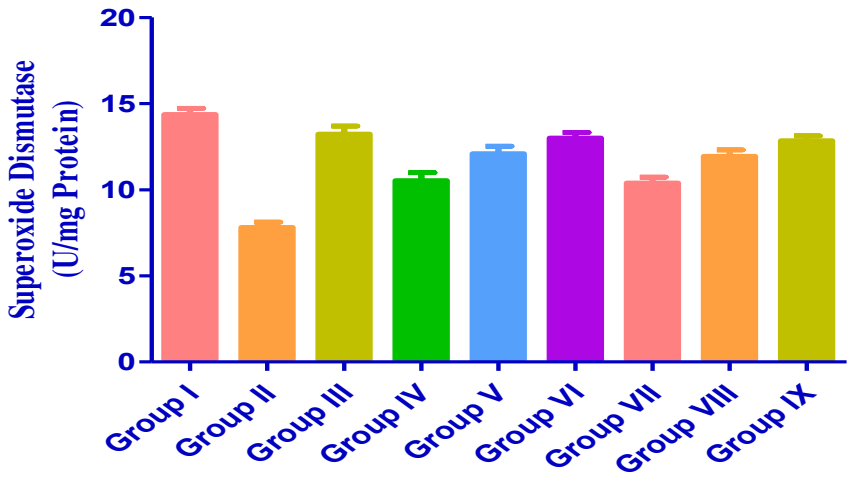


Fig. No: 132 Effect of EAENN and HAENN on SOD Levels in ALX Induced Diabetic Rats

ii). Glutathione Estimation

Table No: 67 Effect of EAENP and HAENP on GSH Levels in ALX Induced Diabetic Rats

Groups	Treatment	GSH (mM/ 100 mg of tissue)
Group-I	Saline	44.21 ± 1.21***
Group-II	ALX (150mg/kg) + Saline	28.13 ± 1.38
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	42.06 ± 1.33***
Group-IV	ALX(150mg/kg) + EAENP (100 mg/kg b.w. p.o)	35.91 ± 0.875**
Group-V	ALX(150mg/kg) + EAENP (200 mg/kg b.w. p.o)	37.60 ± 2.16***
Group-VI	ALX(150mg/kg) + EAENP (400 mg/kg b.w. p.o)	41.11 ± 1.09***
Group-VII	ALX(150mg/kg) + HAENP (100 mg/kg b.w. p.o)	36.92 ± 1.85***
Group-VIII	ALX(150mg/kg) + HAENP (200 mg/kg b.w. p.o)	38.81 ± 0.72***
Group-IX	ALX(150mg/kg) + HAENP (400 mg/kg b.w. p.o)	40.53 ± 0.61***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENP: Ethylacetate extract of *Nymphaea pubescens*,

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

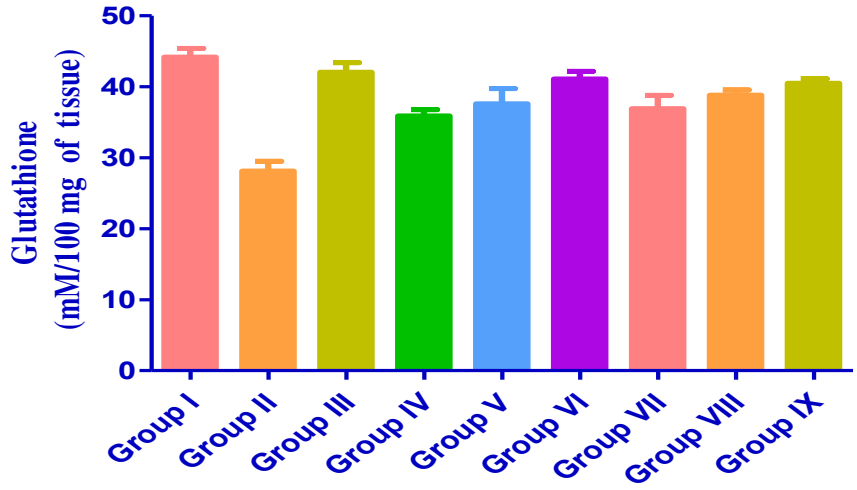


Fig. No: 133 Effect of EAENP and HAENP on GSH Levels in ALX Induced Diabetic Rats

Table No: 68 Effect of EAENN and HAENN on GSH Levels in ALX Induced Diabetic Rats

Groups	Treatment	GSH (mM/ 100 mg of tissue)
Group-I	Saline	44.21 ± 1.21***
Group-II	ALX (150mg/kg) + Saline	28.13 ± 1.38
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	42.06 ± 1.33***
Group-IV	ALX(150mg/kg) + EAENN (100 mg/kg b.w. p.o)	36.67 ± 0.95***
Group-V	ALX(150mg/kg) + EAENN (200 mg/kg b.w. p.o)	38.27 ± 0.75***
Group-VI	ALX(150mg/kg) + EAENN (400 mg/kg b.w. p.o)	39.72 ± 0.51***
Group-VII	ALX(150mg/kg) + HAENN (100 mg/kg b.w. p.o)	37.02 ± 0.86***
Group-VIII	ALX(150mg/kg) + HAENN (200 mg/kg b.w. p.o)	39.05 ± 0.84***
Group-IX	ALX(150mg/kg) + HAENN (400 mg/kg b.w. p.o)	40.65 ± 0.62***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENN: Ethylacetate extract of *Nymphaea nouchali,*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

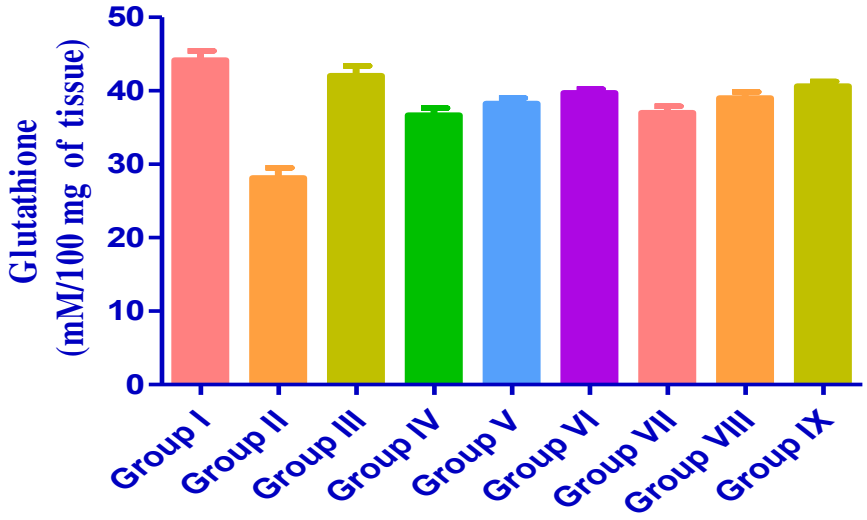


Fig. No: 134 Effect of EAENN and HAENN on GSH Levels in ALX Induced Diabetic Rats

iii) Catalase Levels

Table No: 69 Effect of EAENP and HAENP on Catalase Levels in ALX Induced Diabetic Rats

Groups	Treatment	CAT (U/mg tissue)
Group-I	Saline	11.23 ± 0.53
Group-II	ALX (150mg/kg) + Saline	4.69 ± 0.48
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	10.79 ± 0.39***
Group-IV	ALX(150mg/kg) + EAENP (100 mg/kg b.w. p.o)	7.03 ± 0.42**
Group-V	ALX(150mg/kg) + EAENP (200 mg/kg b.w. p.o)	8.95 ± 0.44***
Group-VI	ALX(150mg/kg) + EAENP (400 mg/kg b.w. p.o)	9.82 ± 0.26***
Group-VII	ALX(150mg/kg) + HAENP (100 mg/kg b.w. p.o)	7.14 ± 0.39**
Group-VIII	ALX(150mg/kg) + HAENP (200 mg/kg b.w. p.o)	8.64 ± 0.38***
Group-IX	ALX(150mg/kg) + HAENP (400 mg/kg b.w. p.o)	9.98 ± 0.39***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENP: Ethylacetate extract of *Nymphaea pubescens*,

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

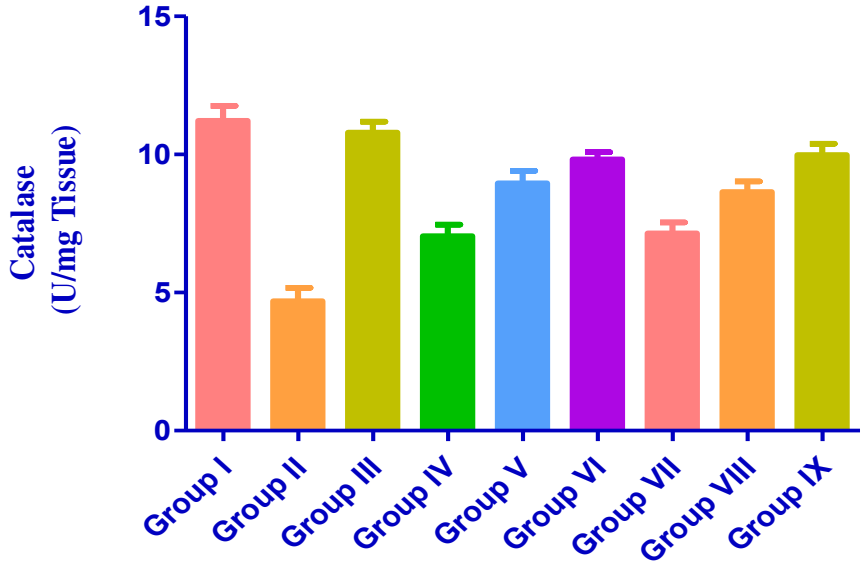


Fig. No: 135 Effect of EAENP and HAENP on Catalase Levels in ALX Induced Diabetic Rats

Table No: 70 Effect of EAENN and HAENN on Catalase Levels in ALX Induced Diabetic Rats

Groups	Treatment	CAT (U/mg tissue)
Group-I	Saline	11.23 ± 0.53
Group-II	ALX (150mg/kg) + Saline	4.69 ± 0.48
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	10.79 ± 0.39***
Group-IV	ALX(150mg/kg) + EAENN (100 mg/kg b.w. p.o)	7.36 ± 0.27**
Group-V	ALX(150mg/kg) + EAENN (200 mg/kg b.w. p.o)	7.96 ± 0.28***
Group-VI	ALX(150mg/kg) + EAENN (400 mg/kg b.w. p.o)	9.70 ± 0.26***
Group-VII	ALX(150mg/kg) + HAENN (100 mg/kg b.w. p.o)	7.53 ± 0.45***
Group-VIII	ALX(150mg/kg) + HAENN (200 mg/kg b.w. p.o)	8.03 ± 0.44***
Group-IX	ALX(150mg/kg) + HAENN (400 mg/kg b.w. p.o)	9.64 ± 0.28***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENN: Ethylacetate extract of *Nymphaea nouchali,*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

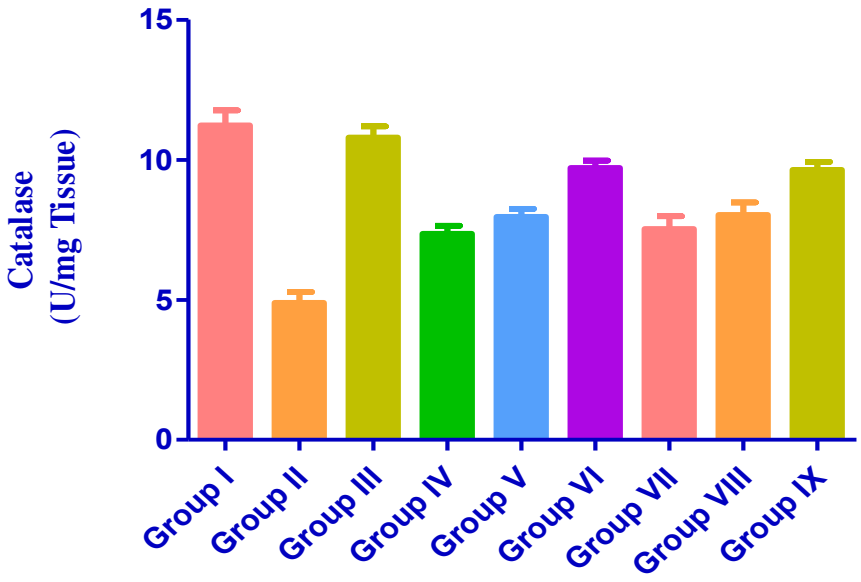


Fig. No: 136 Effect of EAENN and HAENN on Catalase Levels in ALX Induced Diabetic Rats

iv). Lipid Peroxidation Levels

Table No: 71 Effect of EAENP and HAENP on Lipid Peroxidation Levels in ALX Induced Diabetic Rats

Groups	Treatment	LPO (mM/ 100 mg of tissue)
Group-I	Saline	11.23 ± 0.53
Group-II	ALX (150mg/kg) + Saline	4.69 ± 0.48
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	10.79 ± 0.39***
Group-IV	ALX(150mg/kg) + EAENP (100 mg/kg b.w. p.o)	7.03 ± 0.42**
Group-V	ALX(150mg/kg) + EAENP (200 mg/kg b.w. p.o)	8.95 ± 0.44***
Group-VI	ALX(150mg/kg) + EAENP (400 mg/kg b.w. p.o)	9.82 ± 0.26***
Group-VII	ALX(150mg/kg) + HAENP (100 mg/kg b.w. p.o)	7.14 ± 0.39**
Group-VIII	ALX(150mg/kg) + HAENP (200 mg/kg b.w. p.o)	8.64 ± 0.38***
Group-IX	ALX(150mg/kg) + HAENP (400 mg/kg b.w. p.o)	9.98 ± 0.39***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENP: Ethylacetate extract of *Nymphaea pubescens*

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

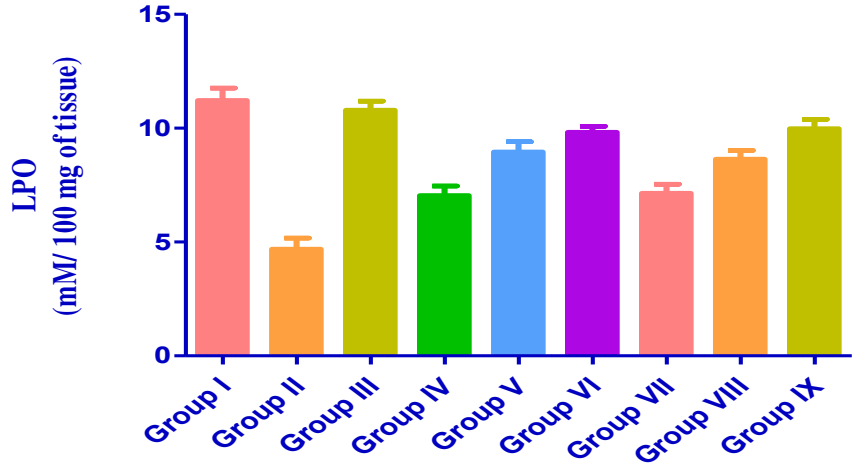


Fig. No: 137 Effect of EAENP and HAENP on Lipid Peroxidation Levels in ALX Induced Diabetic Rats

Table No: 72 Effect of EAENN and HAENN on Lipid Peroxidation Levels in ALX Induced Diabetic Rats

Groups	Treatment	LPO (mM/ 100 mg of tissue)
Group-I	Saline	11.23 ± 0.53
Group-II	ALX (150mg/kg) + Saline	4.69 ± 0.48
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	10.79 ± 0.39***
Group-IV	ALX(150mg/kg) + EAENN (100 mg/kg b.w. p.o)	7.36 ± 0.27**
Group-V	ALX(150mg/kg) + EAENN (200 mg/kg b.w. p.o)	7.96 ± 0.28***
Group-VI	ALX(150mg/kg) + EAENN (400 mg/kg b.w. p.o)	9.70 ± 0.26***
Group-VII	ALX(150mg/kg) + HAENN (100 mg/kg b.w. p.o)	7.53 ± 0.45***
Group-VIII	ALX(150mg/kg) + HAENN (200 mg/kg b.w. p.o)	8.03 ± 0.44***
Group-IX	ALX(150mg/kg) + HAENN (400 mg/kg b.w. p.o)	9.64 ± 0.28***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENN: Ethylacetate extract of *Nymphaea nouchali*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

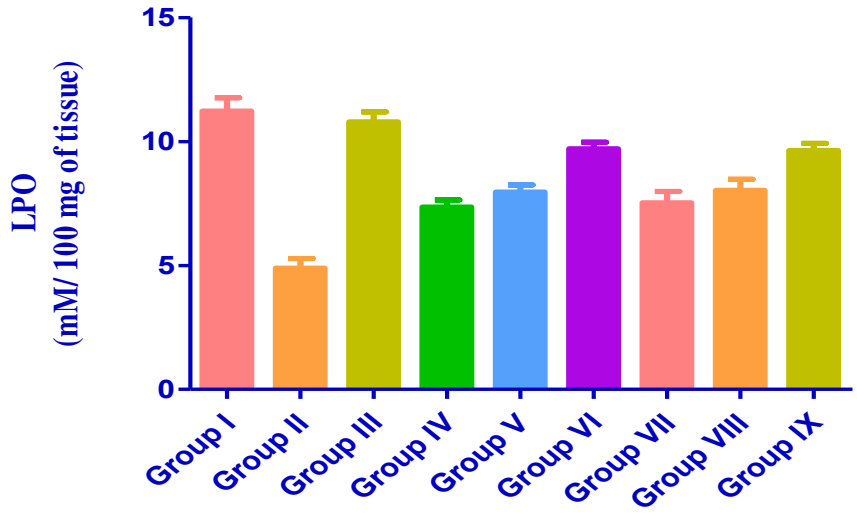


Fig. No: 138 Effect of EAENN and HAENN on Lipid Peroxidation Levels in ALX Induced Diabetic Rats

v). Glutathione Peroxidase (GPx) Levels

Table No: 73 Effect of EAENP and HAENP on Glutathione Peroxidase (GPx) Levels in ALX Induced Diabetic Rats

Groups	Treatment	GPx (U/mg protein)
Group-I	Saline	7.95 ± 0.36
Group-II	ALX (150mg/kg) + Saline	3.12 ± 0.29
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	7.03 ± 0.36***
Group-IV	ALX(150mg/kg) + EAENP (100 mg/kg b.w. p.o)	5.08 ± 0.41**
Group-V	ALX(150mg/kg) + EAENP (200 mg/kg b.w. p.o)	5.71 ± 0.31***
Group-VI	ALX(150mg/kg) + EAENP (400 mg/kg b.w. p.o)	6.56 ± 0.27***
Group-VII	ALX(150mg/kg) + HAENP (100 mg/kg b.w. p.o)	5.12 ± 0.28**
Group-VIII	ALX(150mg/kg) + HAENP (200 mg/kg b.w. p.o)	5.42 ± 0.40***
Group-IX	ALX(150mg/kg) + HAENP (400 mg/kg b.w. p.o)	6.34 ± 0.36***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENP: Ethylacetate extract of *Nymphaea pubescens*

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens*

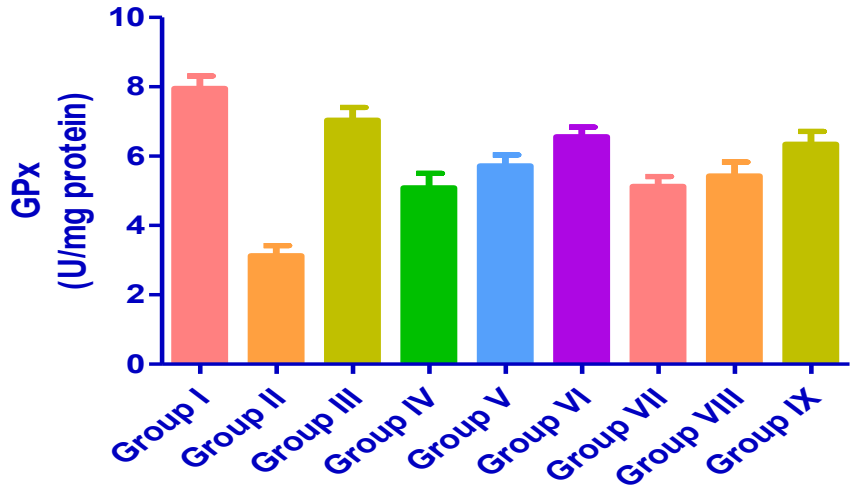


Fig. No: 139 Effect of EAENP and HAENP on Glutathione Peroxidase (GPx) Levels in ALX Induced Diabetic Rats

Table No: 74 Effect of EAENN and HAENN on Glutathione Peroxidase (GPx) Levels in ALX Induced Diabetic Rats

Groups	Treatment	GPx (U/mg protein)
Group-I	Saline	7.95 ± 0.36
Group-II	ALX (150mg/kg) + Saline	3.12 ± 0.29
Group-III	ALX (150mg/kg) + Metformin (250mg/kg)	7.03 ± 0.36***
Group-IV	ALX(150mg/kg) + EAENN (100 mg/kg b.w. p.o)	5.18 ± 0.25**
Group-V	ALX(150mg/kg) + EAENN (200 mg/kg b.w. p.o)	5.87 ± 0.29***
Group-VI	ALX(150mg/kg) + EAENN (400 mg/kg b.w. p.o)	6.76 ± 0.31***
Group-VII	ALX(150mg/kg) + HAENN (100 mg/kg b.w. p.o)	4.87 ± 0.27*
Group-VIII	ALX(150mg/kg) + HAENN (200 mg/kg b.w. p.o)	5.63 ± 0.36***
Group-IX	ALX(150mg/kg) + HAENN (400 mg/kg b.w. p.o)	6.23 ± 0.45***

Values are Mean ± SEM (n=6) one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with the diabetic control group.

EAENN: Ethylacetate extract of *Nymphaea nouchali*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

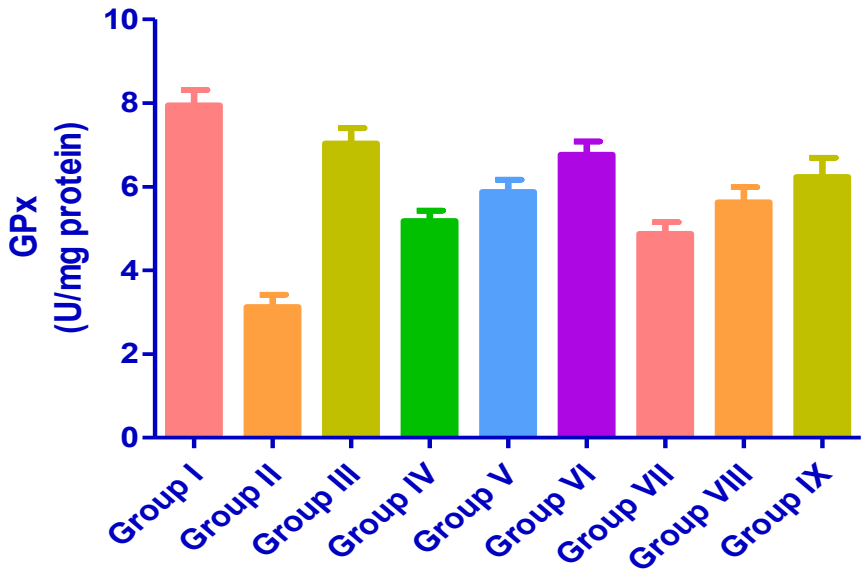


Fig. No: 140 Effect of EAENN and HAENN on Glutathione Peroxidase (GPx) Levels in ALX Induced Diabetic Rats

8.6.3.7. Histopathological Study of Pancreas of *Nymphaea pubescens*

Group –I (Normal Control + Saline)

Section studied shows pancreatic lobules separated by connective tissue septa. The pancreatic lobules consist largely of the exocrine acini and their intralobular ducts. Most of the lobules show small, round, light-staining islets of langerhans. The centre of islet cells consist of aggregates of small Beta-cells (70%, Short-arrow), while the periphery comprises of large Alpha-cells (25%, Long-arrow). Intervening these cells are seen thin walled capillaries.

Group –II (Diabetic Control + Alloxan [150mg/kg)

Section studied shows pancreatic lobules separated by connective tissue septa. The number of islets appears reduced in number. The center of islet cells consist of quantitative decrease in Beta-cells (30%, Long-arrow) having basophilic granules, while the periphery comprises of large Alpha-cells (65%, Short-arrow) having eosinophilic granules. Also seen are some degenerated beta cells and lymphocytic infiltration amidst these islet cells.

Group –III (Alloxan [150mg/kg] + Metformin [250mg/kg])

Section studied shows pancreatic lobules separated by connective tissue septa. Most of the lobules show areas of light-staining islets of langerhans. The center of islet cells consist of quantitative increase in Beta-cells [compared to Diabetes control] (75%, Long-arrow), while the periphery comprises of Alpha-cells (20%, Short-arrow). Also seen are few congested vascular spaces amidst these cells.

Group – IV Alloxan [150mg/kg] + EAENP [100mg/kg])

Section studied shows pancreatic lobules separated by connective tissue septa. Most of the lobules show small, round, light-staining islets of langerhans. The centre of islet cells consist of aggregates of small Beta-cells (50%, Short-arrow), while the periphery comprises of large Alpha-cells (45%, Long-arrow). Intervening these cells are seen thin walled capillaries.

Group – V Alloxan [150mg/kg] + EAENP [200mg/kg])

Section studied shows pancreatic lobules separated by connective tissue septa. The number of islets appears reduced in number. The center of islet cells consist of quantitative decrease in Beta-cells (65%, short-arrow), while the periphery comprises of large Alpha-cells (30%, long-arrow).

Group – V Alloxan [150mg/kg] + EAENP [400mg/kg])

Section studied shows pancreatic lobules separated by connective tissue septa. Most of the lobules show large areas of light-staining islets of langerhans. The center of islet cells consist of small Beta-cells (70%, short-arrow), while the periphery comprises of large Alpha-cells (25%, long-arrow). Also seen are congested vascular spaces amidst these cells.

Group – VII (Alloxan [150mg/kg] + HAENP [100mg/kg])

Section studied shows pancreatic lobules separated by thin connective tissue septa. The center of islet cells consists of quantitative decrease in Beta-cells (50%, Long-arrow), while the periphery comprises of Alpha-cells (45%, Short-arrow).

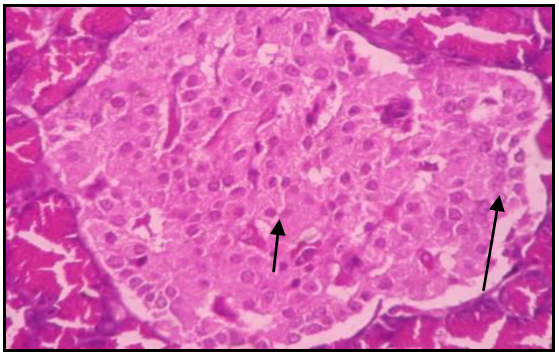
Group – V III (Alloxan [150mg/kg] + HAENP [200mg/kg])

Section studied shows pancreatic lobules separated by thin connective tissue septa. The center of islet cells consists of quantitative decrease in Beta-cells (55%, Long-arrow) having basophilic granules, while the periphery comprises of Alpha-cells (40%, Short-arrow)) having eosinophilic granules. There are seen degenerated beta cells amidst these islets.

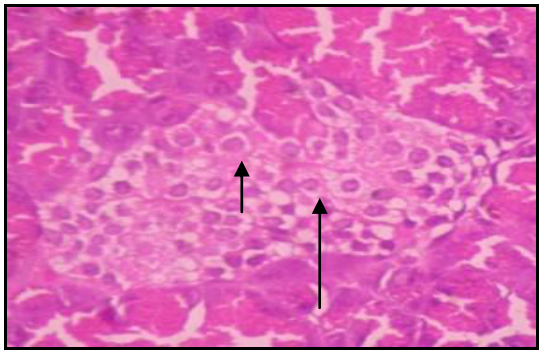
Group – IX (Alloxan [150mg/kg] + HAENP [400mg/kg])

Section studied shows pancreatic lobules separated by thin fibro vascular septa. The center of islet cells consist of quantitative increase in Beta-cells [compared to positive control] (65%, Short-arrow), while the periphery comprises of Alpha-cells (30%, Long-arrow). Also seen are few degenerated beta cells.

Fig. No: 141 Histopathology of Pancreas



Group I



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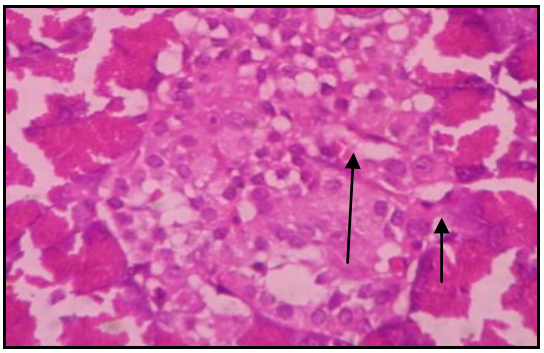

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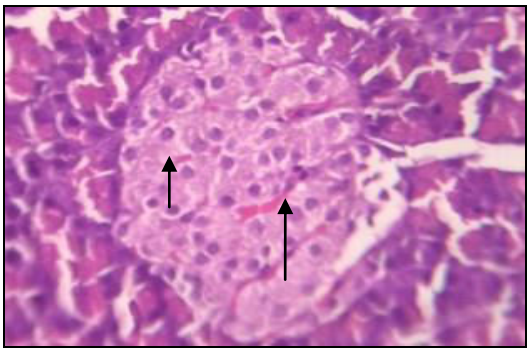

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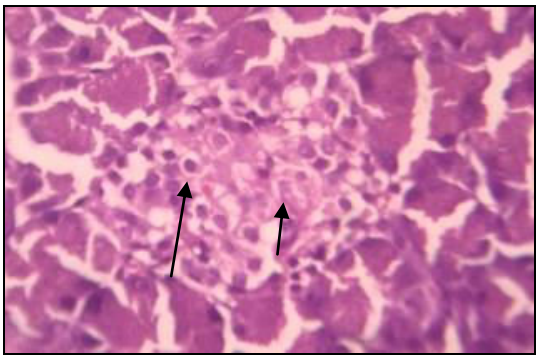

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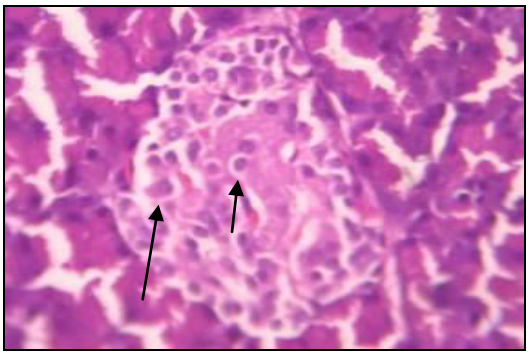
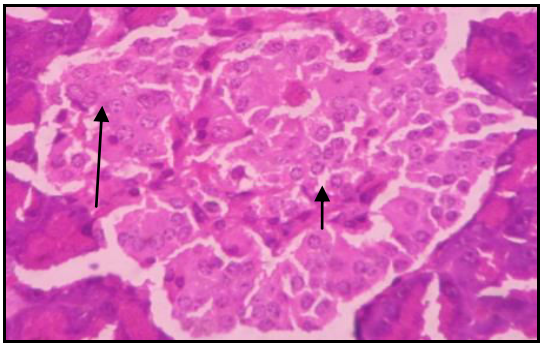
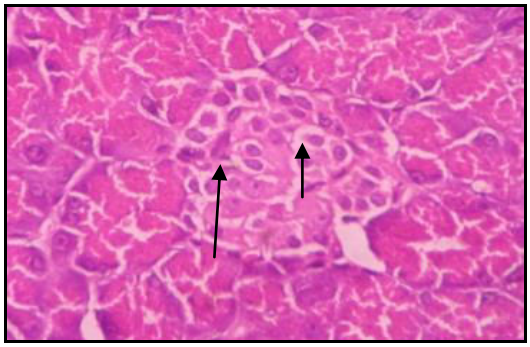


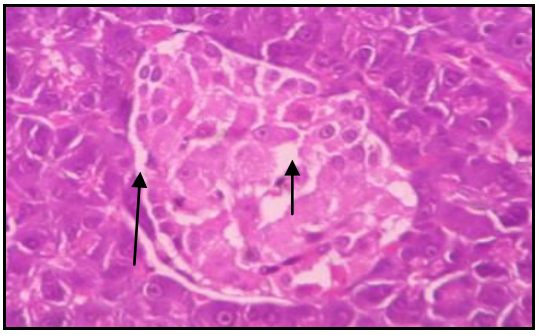
Group VI



Group VII



Group VIII



Group IX

Fig. No: 141 Histopathology of Pancreas

Group –I **(Normal control- Saline)**

Group –II **(Diabetic control- Alloxan [150mg/kg)**

Group –III **(Alloxan [150mg/kg] + Metformin [250mg/kg])**

Group – IV **(Alloxan [150mg/kg] + EAENP [100mg/kg])**

Group – V **(Alloxan [150mg/kg] + EAENP [200mg/kg])**

Group – VI **(Alloxan [150mg/kg] + EAENP [400mg/kg])**

Group – VII **(Alloxan [150mg/kg] + HAENP [100mg/kg])**

Group – VIII **(Alloxan [150mg/kg] + HAENP [200mg/kg])**

Group – IX **(Alloxan [150mg/kg] + HAENP [400mg/kg])**

8.6.3.8. Histopathological Study of Pancreas of *Nymphaea nouchali*

Group –I (Normal Control + Saline)

Section studied shows pancreatic lobules separated by connective tissue septa. The pancreatic lobules consist largely of the exocrine acini and their intralobular ducts. Most of the lobules show small, round, light-staining islets of langerhans. The center of islet cells consist of aggregates of small Beta-cells (70%, Short-arrow), while the periphery comprises of large Alpha-cells (25%, Long-arrow). Intervening these cells are seen thin walled capillaries.

Group –II (Diabetic Control + Alloxan [150mg/kg)

Section studied shows pancreatic lobules separated by connective tissue septa. The number of islets appears reduced in number. The center of islet cells consist of quantitative decrease in Beta-cells (30%, Long-arrow) having basophilic granules, while the periphery comprises of large Alpha-cells (65%, Short-arrow) having eosinophilic granules. Also seen are some degenerated beta cells and lymphocytic infiltration amidst these islet cells.

Group –III (Alloxan [150mg/kg] + Metformin [250mg/kg])

Section studied shows pancreatic lobules separated by connective tissue septa. Most of the lobules show areas of light-staining islets of langerhans. The center of islet cells consist of quantitative increase in Beta-cells [compared to Diabetes control] (75%, Long-arrow), while the periphery comprises of Alpha-cells (20%, Short-arrow). Also seen are few congested vascular spaces amidst these cells.

Group – IV Alloxan [150mg/kg] + EAENN [100mg/kg])

Section studied shows pancreatic lobules separated by connective tissue septa. The pancreatic lobules consist largely of the exocrine acini and their intralobular ducts. Most of the lobules show small, round, light-staining islets of langerhans. The center of islet cells consist of aggregates of small Beta-cells (60%, Short-arrow), while the periphery comprises of large Alpha-cells (35%, Long-arrow). Intervening these cells are seen thin walled capillaries.

Group – V Alloxan [150mg/kg] + EAENN [200mg/kg])

Section studied shows pancreatic lobules separated by connective tissue septa. The number of islets appears reduced in number. The center of islet cells consist of quantitative decrease in Beta-cells (65%, long-arrow), while the periphery comprises of large Alpha-cells (30%, short-arrow). Also seen are some degenerated beta cells.

Group – VI Alloxan [150mg/kg] + EAENN [400mg/kg])

Section studied shows pancreatic lobules separated by connective tissue septa. Most of the lobules show large areas of light-staining islets of langerhans. The centre of islet cells consist of quantitative increase in Beta-cells (70%, short-arrow), while the periphery comprises of Alpha-cells (25%, long-arrow). Also seen are congested vascular spaces amidst these cells.

Group – VII (Alloxan [150mg/kg] + HAENN [100mg/kg])

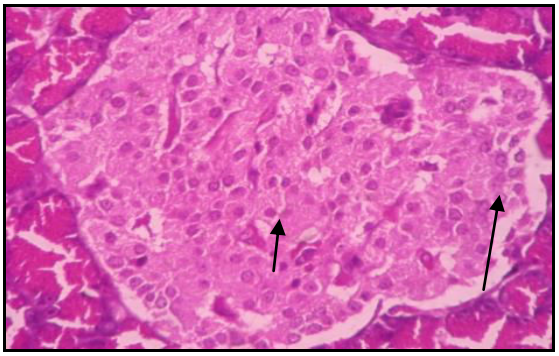
Section studied shows pancreatic lobules separated by connective tissue septa. Some of the lobules show small, round, light-staining islets of langerhans. The center of islet cells consist of small Beta-cells (50%, Short-arrow), while the periphery comprises of large Alpha-cells (45%, Long-arrow). Intervening these cells are seen degenerated beta cells.

Group – VIII Alloxan [150mg/kg] + HAENN [200mg/kg])

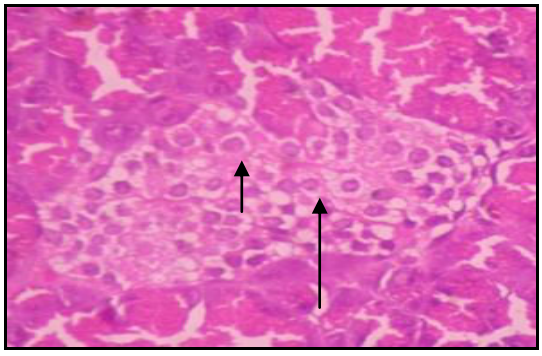
Section studied shows pancreatic lobules separated by connective tissue septa. Some of the lobules show small, round, light-staining islets of langerhans. The center of islet cells consist of small Beta-cells (60%, Short-arrow), while the periphery comprises of large Alpha-cells (35%, Long-arrow). Intervening these cells are seen degenerated beta cells.

Group – IX Alloxan [150mg/kg] + HAENN [400mg/kg])

Section studied shows pancreatic lobules separated by connective tissue septa. Most of the lobules show large areas of light-staining islets of langerhans. The center of islet cells consist of quantitative increase in Beta-cells (70 %, long-arrow), while the periphery comprises of Alpha-cells (25%, long-arrow). Also seen are congested vascular spaces amidst these cells.



Group I



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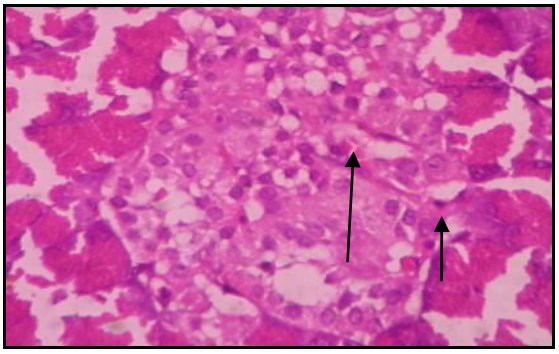
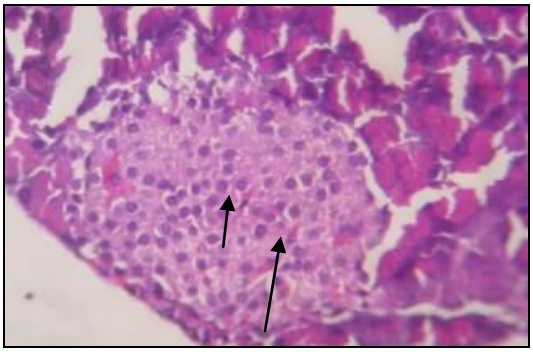


Group III



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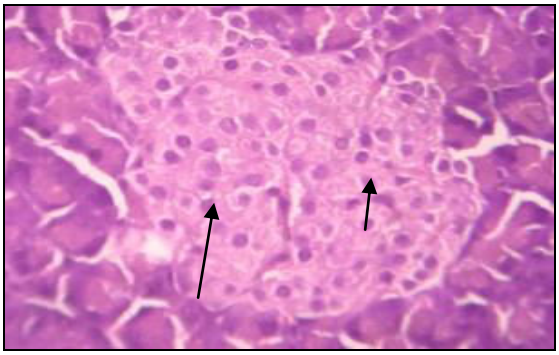
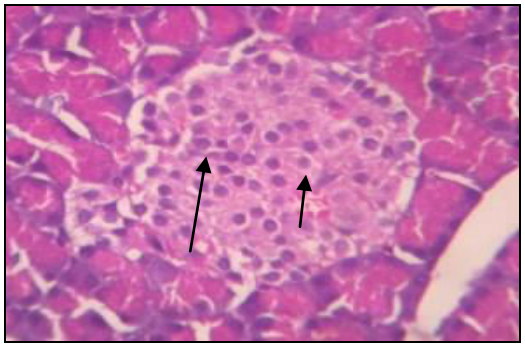
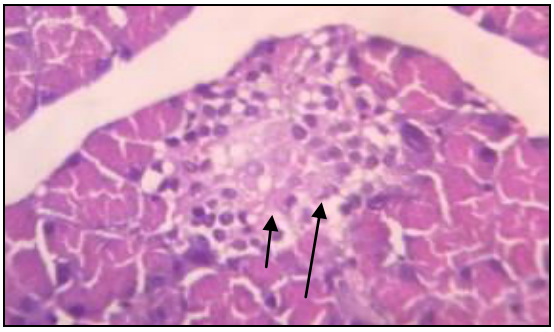


Group V

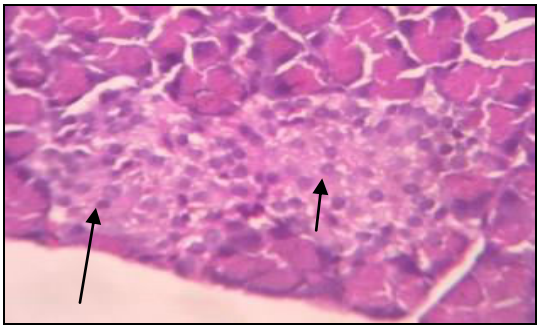


Group VI

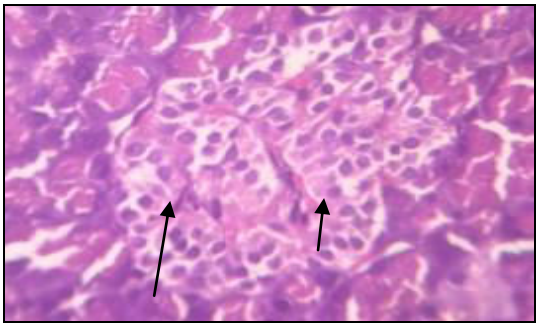
Fig. No: 142 Histopathology of Pancreas



Group VII



Group VIII



Group IX

Fig. No: 142 Histopathology of Pancreas

Group –I	**(Normal control- Saline)**
Group –II	**(Diabetic control- Alloxan [150mg/kg)**
Group –III	**(Alloxan [150mg/kg] + Metformin [250mg/kg])**
Group – IV	**(Alloxan [150mg/kg] + EAENN [100mg/kg])**
Group – V	**(Alloxan [150mg/kg] + EAENN [200mg/kg])**
Group – VI	**(Alloxan [150mg/kg] + EAENN [400mg/kg])**
Group – VII	**(Alloxan [150mg/kg] + HAENN [100mg/kg])**
Group – VIII	**(Alloxan [150mg/kg] + HAENN [200mg/kg])**
Group – IX	**(Alloxan [150mg/kg] + HAENN [400mg/kg])**

8.6.3.9. DISCUSSION

It is the normal practice to determine the LD_{50} value, it is worthwhile to study an acute toxicity studies by employing several doses including reasonably high doses. Acute toxicity studies were conducted using a dose of 2000 mg/kg, p.o. with two different extracts of ***Nymphaea pubescens*** and ***Nymphaea nouchali*** in female albino rats according to the OECD guidelines. Even at this high dose EAENP HAENP, EAENN and HAENN did not exhibit any sign or symptoms of toxicity and mortality. Hence low dose (100 mg/kg, p.o.) medium dose (200 mg/kg, p.o.) and high dose of HAEF (400 mg/kg, p.o.) were selected for further studies in animals.

Diabetes mellitus is a complex and diverse group of disorders characterized by hyperglycaemia that has reached epidemic proportions in the present century. Several synthetic drugs being utilized, but those have several side effects to avoid such effects researchers come up with new class of compounds which are having essential targets to overcome these problems. Globally, there is a positive trend in favour of traditional and integrative medicine in both research and practice. So, traditional antidiabetic plants might provide new oral hypoglycaemic compounds, which can encounter the high cost and poor specificity of the current medicines. A wide range of chemical compounds are present in the herbal plant, which are having a similar action like synthetic drugs (**Piyush M Patel, 2006).**

Single Dose Study for 120 min was carried out in normo-glycemic rats. High dose of EAENP HAENP, EAENN and HAENN (400 mg/kg, p.o.) showed maximum decrease in blood glucose levels at 120 min compared to normal group. High dose of EAENP HAENP, EAENN and HAENN also showed a significant decrease from the 60 min of the drug administration. Medium dose of EAENP HAENP, EAENN and HAENN showed a significant decrease in blood glucose level at 120 min compared to normal levels. It also showed its activity at 120 min after the drug administration when compared with the normal control. Low dose shown less significant compared with medium and high dose of EAENP HAENP, EAENN and HAENN. It may produce hypoglycemia in normal animals by stimulating the pancreatic beta-cells to produce more Insulin and by increasing the glycogen deposition in the liver **(Jimam NS, 2010).**

Metformin (250 mg/kg, b.w. p.o) showed a maximum decrease of blood glucose levels in normoglycemic rats at 120 min of our study.

Oral Glucose Tolerance Test was studied on the normal rats. The lowering of glucose can be seen well in assay of glucose tolerance **(Versphol EJ., 2002).** The fasting blood glucose levels decreases in Metformin, along with EAENP HAENP, EAENN and HAENN high dose and medium dose treated rats. Low dose shows reduced activity at 120 min. Such a phenomenon was already seen in the indigenous plants and reported. The lowering of glucose levels is may be due to the inhibition of intestinal absorption, or it may act by potentiating the secretion of Insulin and by increase in the utilization of glucose levels in muscles **(Venkatesh S, 2012).**

Diabetes mellitus is a chronic metabolic disorder for which extensive researches being carried out. Various animals models had been standardized and being used for the screening of the hypoglycemic agents **(Szkudelski T., 2001).** The discovery of alloxan was a major breakthrough in the screening of drugs for the treatment of diabetes mellitus. It has been reported that, this diabetogenic agent acts by forming free reactive oxygen species which selectively destroys the pancreatic β-cells **(Srinivasan K, 2007).** Though this pancreatic β-cell cytotoxic agent has narrow dose for inducing diabetes mellitus, a variety of doses had been studied for the induction of diabetes mellitus in rats. Its dose ranges from 40-200 mg/kg body weight, and the dose depends on the route of administration like i.v., s.c., i.p. **(Szkudelski T., 2001).** In this study we have chosen i.p., route which is the easiest way for rapid administration of the highly unstable alloxan solution. The very most commonly used doses in the case of intraperitoneal administration of alloxan were 120 mg/kg. Variety of vehicles has been used for administration of this diabetogenic agent. Most of the previous works used saline (0.9 % NaCl w/v) as a vehicle to administer alloxan.

A dose of 100, 200 and 400 mg/kg, p.o. Body Weight of EAENP HAENP, EAENN and HAENN is administered for a period of 21 days in ALX treated rats. EAENP, HAENP, EAENN and HAENN showed a significant reduction in the blood glucose levels than the control group rats. This hypoglycemic activity may be due to the stimulation of surviving β-cells to release more Insulin. ***Nymphaea pubescens*** and ***Nymphaea nouchali*** may act by

inhibiting hepatic gluconeogenesis or inhibiting α-glucosidase enzyme in the intestine, which is the enzyme helpful for breakdown of disaccharides to form glucose **(Okwuosa CN, 2011).**

Induction of diabetes with Alloxan (ALX) is associated with a characteristic decrease in body weight than the normal rats, this may be due to the wasting and loss of tissue protein. Whereas, diabetic rats treated with 100, 200 and 400mg/kg, p.o. of EAENP HAENP, EAENN and HAENN showed an improved result when compared with normal diabetic control. Which may be due to the protective effect in controlling muscle wasting i.e., reversal of gluconeogenesis and may also be due to the improvement of glycaemia control **(Salahuddin M, 2006).**

A marked reduction in the levels of Total Protein and Albumin Levels was observed in the diabetic rats. The decrease in the albumin levels may be due to the increased protein catabolism. Present study showed that the treatment of diabetic rats with the 200 and 400mg/kg p.o dose of EAENP HAENP, EAENN and HAENN and Metformin showed increased albumin levels and protein levels significantly.

An enhanced increase in Plasma Serum Creatinine Levels was found in the diabetic rats when compared with the respective control group rats. While after the treatment with EAENP, HAENP, EAENN and HAENN levels were significantly decreased.

In the present study, the groups of normal rats have shown gain in the body weight while fasting serum glucose was maintained in the normal range throughout the study period. The serum cholesterol and serum triglyceride levels of the normal rats were found to be increasing within the normal range during the four weeks of study period.

Under normal condition, Insulin activates enzyme lipoprotein lipase and hydrolyses triglycerides. Insulin deficiency results in failure to activate the enzymes there by causing hypertriglyceridemia **(Eidi A, 2006).** This altered lipid metabolism leads to diabetic complications. Practically it has been observed that there is an altered in levels of serum cholesterol and triglycerides levels in ALX treated rats, causes hypercholesterolemia and hyper-triglyceridemia **(Okwuosa CN, 2011).** Diabetic rats treated with the medium, high dose of (200 and 400mg/kg, p.o.) EAENP, HAENP, EAENN and HAENN and Metformin has shown a significant decrease in the levels of TG, TC, LDL-C and VLDL-C, where as it

increases the levels of HDL-C when compared to the normal diabetic control rats. In low dose of EAENP, HAENP, EAENN and HAENN treated rats HDL-C levels is less significant.

Diabetic rats treated with Metformin (250mg/kg p.o.) showed significant protection from the body weight loss and progressive reduction of fasting serum glucose levels after a daily dose for 21 days. The metformin treatment also showed the reduced elevated serum cholesterol, albumin, creatinine and total protein levels produced significant reduction in elevated serum triglycerides during the period of study when compared with the diabetic group of rats. In agreement with the present results, several studies have shown protection in body weight loss **(Paul Desire Dzeufiet Djomeni, 2006),** anti-diabetic activity, reduction in serum cholesterol and serum triglyceride **(Tao Xia, 2006).**

Superoxide Dismutase is an enzymatic antioxidant which reduces superoxide radical to hydrogen peroxide and oxygen. A decrease in the antioxidant activity in liver results in the accumulation of free radicals (hydroxyl radical) in diabetic rats. Administration of the high dose, medium dose, low dose of EAENP, HAENP, EAENN and HAENN (100, 200 and 400 mg/kg, p.o.) and Metformin increased the activity of SOD levels to a significant level of $P<0.001$. While the SOD levels of untreated diabetic control rats having lowered levels. The *Nymphaea pubescens* and *Nymphaea nouchali* may act by either directly scavenging the reactive oxygen metabolites or by increasing the anti-oxidant molecules.

Antioxidants scavenge a number of highly reactive oxygen free radicals rapidly. They have a great potential role in inhibiting the fibrotic process which is induced during the cell damage. Medicine plants are used as source of antioxidants in traditional medicine and their therapeutic properties may be due to their capacity for scavenging oxygen free radicals. It is well known that CAT, SOD, and Glutathione (GPx) play an important role as protective enzymes against free radical formation in tissues **(Maeuthuoandian A, 2011).** Significant increased levels of CAT and GPx were shown in the diabetic rats treated with the high dose, medium dose, low dose of EAENP, HAENP, EAENN and HAENN (100, 200 and 400 mg/kg, p.o.) and Metformin.

Glutathione which is a tripeptide normally present at high concentrations intracellularly. Glutathione is helpful for reducing the toxic effects of lipid peroxidation. Decreased level of GSH in liver during diabetes represents its increased utilization due to oxidative stress. Significant increased levels of GSH were shown in the diabetic rats treated with the high dose, medium dose, low dose of EAENP, HAENP, EAENN and HAENN (100, 200 and 400 mg/kg, p.o.) and Metformin.

In Diabetes, lipid peroxidation is one of the characteristic features of chronic diabetes. The increased free radicals react with the polyunsaturated fatty acids in cell membrane leading to lipid peroxidation. This in turn results in the development of free radicals. Low levels of lipoxygenase peroxides stimulate the release of Insulin. But, if the concentration of this peroxidase increases it results in uncontrolled release of lipid peroxidation. The most commonly used indicator of lipid peroxidation is TBARS. Administration of high dose, medium dose, low dose of EAENP HAENP, EAENN and HAENN (100, 200 and 400 mg/kg, p.o.) and Metformin significantly increase the lipid peroxidation levels. The effect shown is may be due to prevention of potential glycation of anti-oxidant enzymes.

Metformin is the only drug of the biguanide class that is used clinically. AMP-Activated Protein Kinase (AMPK) is an enzyme that works as a fuel gauge which becomes activated in situations of energy consumption. AMPK is involved in the mechanism of action of metformin and thiozolidinediones, and the adipocytokines leptin and adiponectin. These data, along with evidence that pharmacological activation of AMPK *In-vivo* improves blood glucose homeostasis **(Schimmack G, 2006).** Reduce hepatic glucose production (gluconeogenesis), which is markedly, increased in type 2 diabetes. Increase glucose uptake and utilisation in skeletal muscle (i.e. they reduce Insulin resistance) reduce carbohydrate absorption. increase fatty acid oxidation. Reduce circulating low-density and very-low-density lipoprotein (LDL and VLDL, respectively). Reduced hepatic gluconeogenesis is especially important. The mechanism involves activation in hepatocytes of AMP-activated protein kinase (AMPK), an important enzyme in metabolic control. Activation of AMPK increases expression of a nuclear receptor that inhibits expression of genes that are important for gluconeogenesis in the liver

The histological evidence showed in the authenticated injury caused by ALX and the protection offered by EAENP, HAENP, EAENN and HAENN (100, 200 and 400 mg/kg, p.o.) and Metformin in pancreatic cells were shown. Microscopically examination revealed loss of architecture and cell necrosis with inflammatory collections in the central zone in ALX induced rats. Histopathological study showed that ***Nymphaea pubescens*** and ***Nymphaea nouchali*** has the capacity to increase Islet cell mass. However, the expansion was better with medium, high dose of EAENP, HAENP, EAENN and HAENN dose.

8.6.4. Anti-Cancer Activity

8.6.4.1. Anti-Cancer Activity in Cancer cell Lines

Table No: 75 The Cytotoxic Effect of EAENP and HAENP Extracts on Cancer Cell Lines

Groups	Extract	Cancer Cell Line	IC_{50}
Group-I	EAENP	HeLa	35.12
		HepG2	28.01
Group-II	HAENP	HeLa	18.37
		HepG2	21.47

8.6.4.2. Anti-Cancer Activity Viable and Non-Viable Tumor Cell Count

Table No: 76 Effect of EAENP and HAENP on DAL Cell Line against by Viable and Non-Viable Tumor Cell Count

Groups	Treatment	Viable tumor cell count×10^6 cells/ml	Non-viable tumor cell count×10^6 cells/ml
Group-I	Normal Mice	-	-
Group-II	DAL + Solvent 20ml/kg	10.57 ± 0.632	0.89 ± 0.085
Group-III	DAL+5-FU (20 mg/kg, b.w. p.o)	2.98 ± 0.140***	5.68 ± 0.248***
Group-IV	DAL + EAENP (100mg/kg, b.w. p.o)	7.35 ± 0.416***	4.01 ± 0.326***
Group-V	DAL + EAENP (200mg/kg, b.w. p.o)	5.14 ± 0.348***	4.87 ± 0.145***
Group-VI	DAL + EAENP (400mg/kg, b.w. p.o)	4.38 ± 0.219***	5.33 ± 0.298***
Group-VII	DAL + HAENP (100mg/kg, b.w. p.o)	7.28 ± 0.376***	3.75 ± 0.374***
Group-VIII	DAL + HAENP (200mg/kg, b.w. p.o)	6.11 ± 0.425***	4.51 ± 0.215***
Group-IX	DAL + HAENP (400mg/kg, b.w. p.o)	4.79 ± 0.260***	5.26 ± 0.230***

Values are mean ± S.E.M. (n=6). One way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with DAL control + solvent.
EAENP: Ethylacetate extract of *Nymphaea pubescens*,
HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens.*

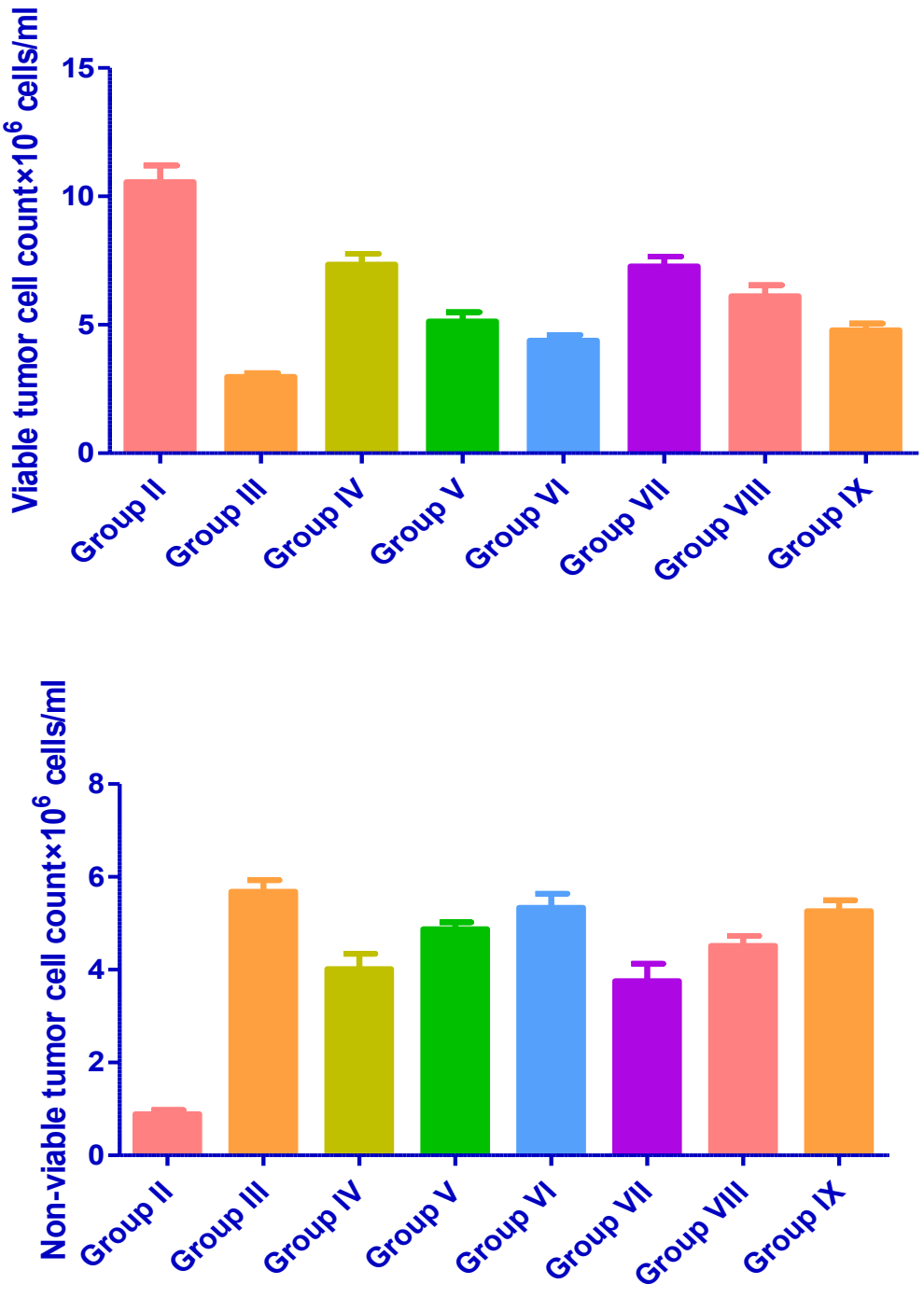


Fig. No: 143 Effects of EAENP and HAENP on DAL Cell Line against by Viable and Non-Viable Tumor Cell Count

8.6.4.3. *In-vivo* screening of Anti Cancer Activity of the Flower Extracts EAENP and HAENP on Mean Survival Time

Table No: 77 Effect of EAENP and HAENP on Mean Survival Time in DAL Tumour Bearing Mice

Groups	Treatment	Mean Survival Time	ILS %
Group I	Normal Mice	-	-
Group II	DAL + Solvent 20ml/kg	18.54 ± 1.369	-
Group III	DAL+5-FU (20 mg/kg, b.w. p.o)	35.64 ± 2.067***	92.23%
Group IV	DAL + EAENP (100mg/kg, b.w. p.o)	22.23 ± 1.162^{ns}	19.90%
Group V	DAL + EAENP (200mg/kg, b.w. p.o)	27.98 ± 1.328***	47.68%
Group VI	DAL + EAENP (400mg/kg, b.w. p.o)	31.63 ± 1.125***	70.60%
Group VII	DAL + HAENP (100mg/kg, b.w. p.o)	20.95 ± 1.815^{ns}	12.99%
Group VIII	DAL + HAENP (200mg/kg, b.w. p.o)	26.63 ± 0.963**	43.63%
Group IX	DAL + HAENP (400mg/kg, b.w. p.o)	30.76 ± 1.418***	65.91%

Values are mean ± S.E.M. (n=6). One way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with DAL control + solvent.

EAENP: Ethylacetate extract of *Nymphaea pubescens*

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens.*

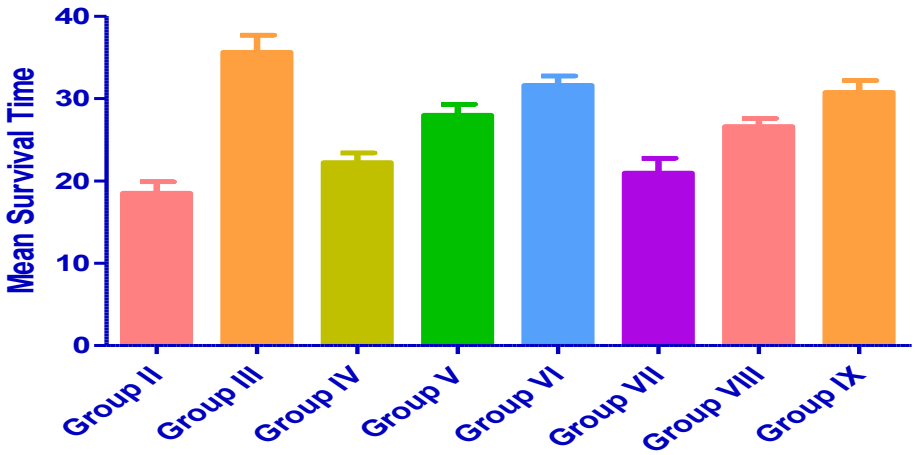


Fig. No: 144 Effects of EAENP and HAENP on Mean Survival Time in DAL Tumour Bearing Mice

8.6.4.4. Determination of Body Weight and Survival Time

Table No: 78 Effect of EAENP and HAENP on Body Weight Analysis and Tumor Growth Response against DAL Induced Animals

Groups	Treatment	Increase in Body Weight (g)	Tumour Volume (ml)	Packed Cell Volume(ml)	% Decrease In Body Weight
Group I	Normal Mice	0.97 ± 0.101	-	-	-
Group II	DAL + Solvent 20ml/kg	8.26 ± 0.433	8.13 ± 0.267	4.39 ± 0.252	-
Group III	DAL+5-FU (20 mg/kg, b.w. p.o)	1.87 ± 0.142***	1.66 ± 0.164***	0.72 ± 0.079***	77.36%
Group IV	DAL + EAENP (100mg/kg, b.w. p.o)	6.28 ± 0.278***	6.16 ± 0.251***	2.96 ± 0.231***	23.97%
Group V	DAL + EAENP (200mg/kg, b.w. p.o)	4.94 ± 0.209***	4.95 ± 0.198***	2.39 ± 0.130***	40.19%
Group VI	DAL + EAENP (400mg/kg, b.w. p.o)	3.85 ± 0.130***	3.87 ± 0.205***	1.87 ± 0.091***	53.38%
Group VII	DAL + HAENP (100mg/kg, b.w. p.o)	6.55 ± 0.149***	5.96 ± 0.350***	2.72 ± 0.085***	20.70%
Group VIII	DAL + HAENP (200mg/kg, b.w. p.o)	5.18 ± 0.217***	5.03 ± 0.338***	2.23 ± 0.136***	37.28%
Group IX	DAL + HAENP (400mg/kg, b.w. p.o)	4.01 ± 0.167***	4.05 ± 0.219***	2.06 ± 0.139***	51.45%

Values are mean ± S.E.M. (n=6). One way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with DAL control + solvent.

EAENP: Ethylacetate extract of *Nymphaea pubescens*

HAENP: Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens.*

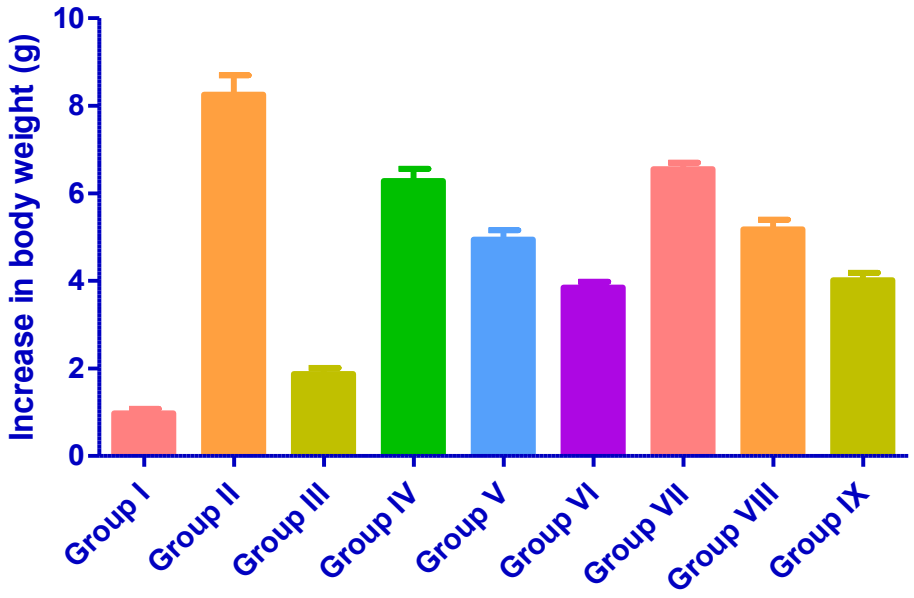


Fig. No: 145 Effect of EAENP and HAENP on Body Weight Response against DAL Induced Animals

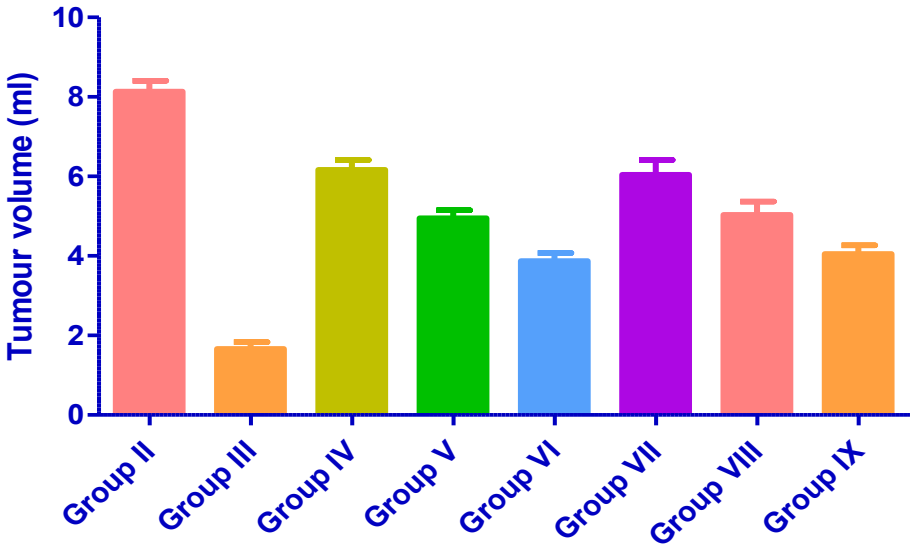


Fig. No: 146 Effect of EAENP and HAENP on Tumor Volume against DAL Induced Animals

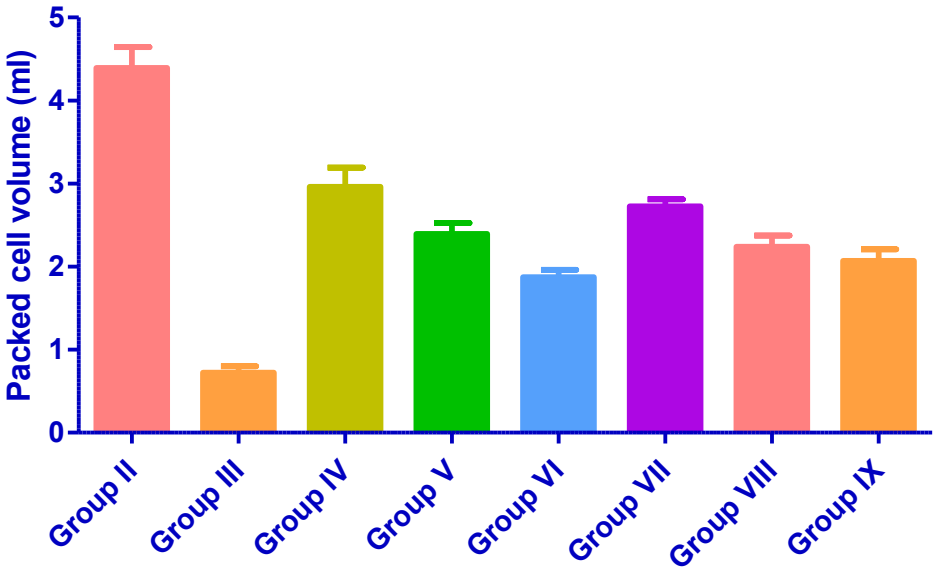


Fig. No: 147 Effect of EAENP and HAENP on Packed Cell Volume against DAL Induced Animals

8.6.4.5. Haematological Parameters on 14th day in Normal and DAL Tumour Bearing Mice

Table No: 79 Effect of EAENP and HAENP on Haematological Parameters on 14th day in Normal and DAL Tumour Bearing Mice

Groups	Treatment	Hb (%)	RBC ($x10^6$ cell/mm^3)	WBC ($x10^4$ cell/mm^3)
Group I	Normal Mice	13.61±0.289	6.58 ± 0.341	8.67 ± 0.211
Group II	DAL + Solvent 20ml/kg	6.71±0.177	3.11 ± 0.302	22.64 ± 1.218
Group III	DAL+5-FU (20 mg/kg, b.w. p.o)	12.78±0.208***	6.06 ± 0.205***	10.51 ± 0.62***
Group IV	DAL + EAENP (100mg/kg, b.w. p.o)	8.49 ± 0.177**	4.45 ± 0.144**	17.88 ± 0.584***
Group V	DAL + EAENP (200mg/kg, b.w. p.o)	9.80 ± 0.377***	5.06 ± 0.137***	16.56 ± 0.676***
Group VI	DAL + EAENP (400mg/kg, b.w. p.o)	12.21±0.355***	5.76 ± 0.170***	13.62 ± 0.549***
Group VII	DAL + HAENP (100mg/kg, b.w. p.o)	8.31±0.309*	4.10 ± 0.157^{ns}	17.56 ± 0.654***
Group VIII	DAL + HAENP (200mg/kg, b.w. p.o)	9.41±0.374***	4.96 ± 0.149***	17.06 ± 0.727***
Group IX	DAL + HAENP (400mg/kg, b.w. p.o)	11.31 ± 0.327***	5.52 ± 0.250***	14.89 ± 0.68***

Values are mean ± S.E.M. (n=6). one way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with DAL control + solvent.

EAENP: Ethylacetate extract of *Nymphaea pubescens,* **HAENP:** Hydro-alcoholic (70%v/v) extract of *Nymphaea pubescens.*

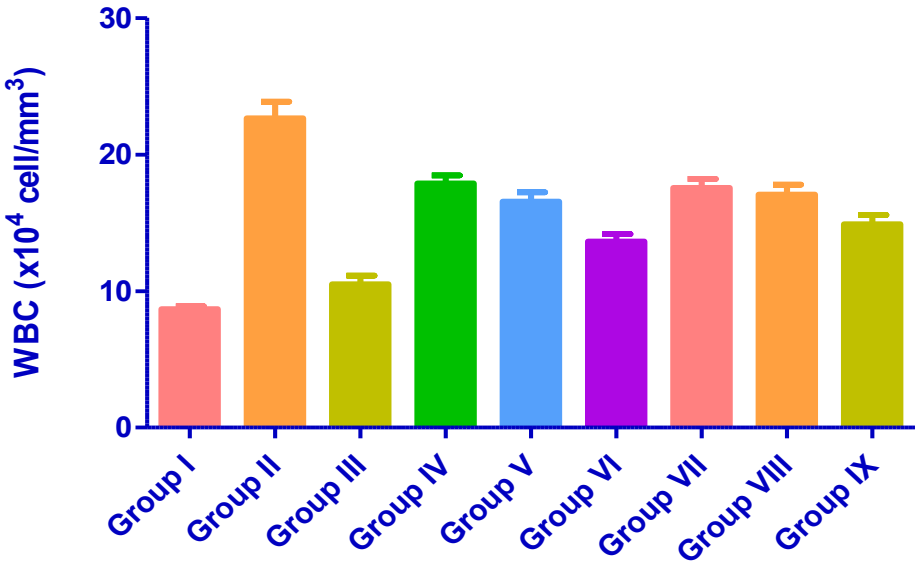


Fig. No: 148 Effect of EAENP and HAENP on WBC Count on 14th Day in Normal and DAL Tumour Bearing Mice

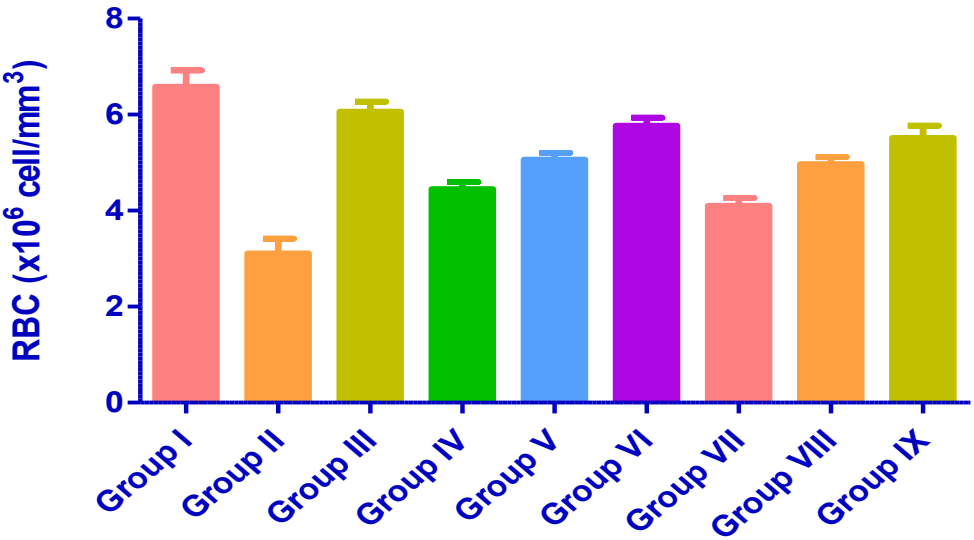


Fig. No: 149 Effect of EAENP and HAENP on RBC Count on 14th Day in Normal and DAL Tumour Bearing Mice

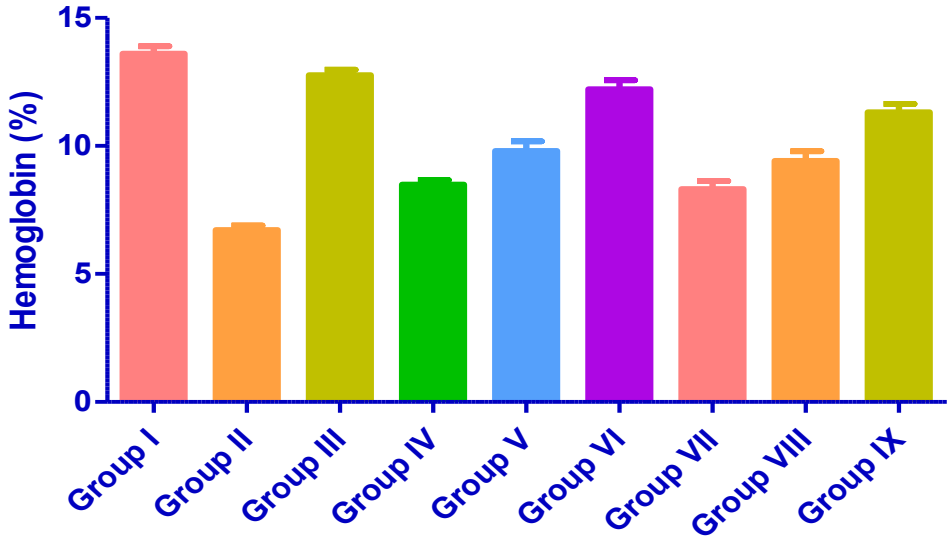


Fig. No: 150 Effects of EAENP and HAENP on Haemoglobin on 14th Day in Normal and DAL Tumour Bearing Mice

8.6.4.6. Anti-Cancer Activity in Cancer cell Lines

Table No: 80 The Cytotoxic Effect of EAENN and HAENN Extracts on Cancer Cell Lines

Groups	Extract	Cancer Cell Line	IC_{50}
Group-I	EAENP	HeLa	38.20
		HepG2	21.16
Group-II	HAENP	HeLa	17.06
		HepG2	23.16

8.6.4.7. Anti-Cancer Activity Viable and Non-Viable Tumor Cell Count

Table No: 81 Effect of EAENN and HAENN on DAL Cell Line against By Viable and Non-Viable Tumor Cell Count

Groups	Treatment	Viable Tumor Cell Count×10^6 cells/ml	Non-Viable Tumor Cell Count×10^6 cells/ml
Group-I	Normal Mice	-	-
Group-II	DAL + Solvent 20ml/kg	10.57 ± 0.632	0.89 ± 0.085
Group-III	DAL+5-FU (20 mg/kg, b.w. p.o)	2.98 ± 0.140***	5.68 ± 0.248***
Group-IV	DAL + EAENN (100mg/kg, b.w. p.o)	6.54 ± 0.423***	3.54 ± 0.315***
Group-V	DAL + EAENN (200mg/kg, b.w. p.o)	4.97 ± 0.205***	4.25 ± 0.235***
Group-VI	DAL + EAENN (400mg/kg, b.w. p.o)	3.96 ± 0.200***	5.07 ± 0.114***
Group-VII	DAL + HAENN (100mg/kg, b.w. p.o)	7.49 ± 0.345***	3.25 ± 0.282***
Group-VIII	DAL + HAENN (200mg/kg, b.w. p.o)	5.56 ± 0.249***	4.69 ± 0.271***
Group-IX	DAL + HAENN (400mg/kg, b.w. p.o)	4.47 ± 0.265***	4.94 ± 0.240***

Values are mean ± S.E.M. (n=6). One way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with DAL control + solvent.

EAENN: Ethylacetate extract of *Nymphaea nouchali,*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

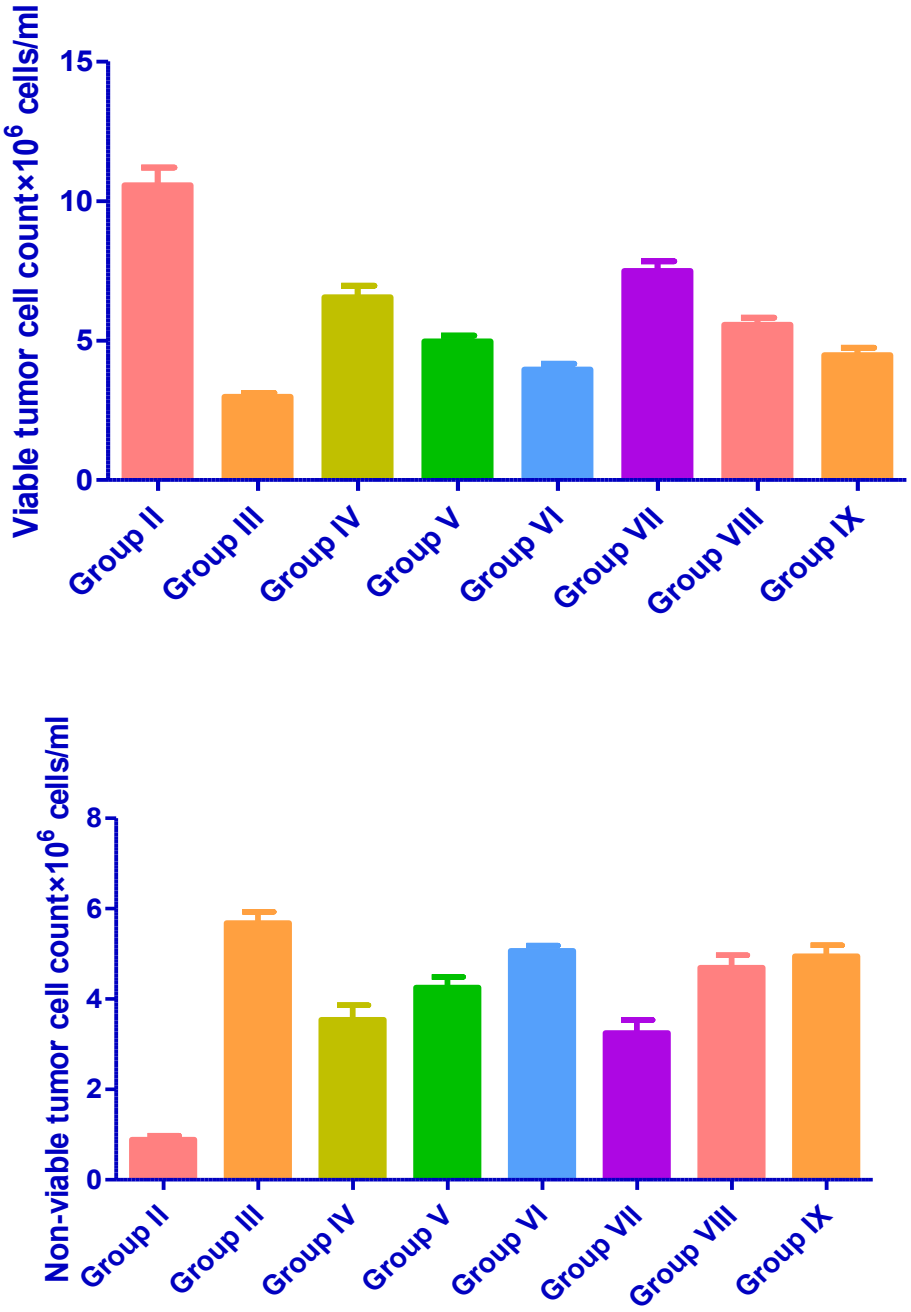


Fig. No: 151 Effect of EAENN and HAENN on DAL Cell Line against By Viable and Non-Viable Tumor Cell Count

8.6.4.8. *In-vivo* screening of Anti Cancer Activity of the Flower Extracts EAENN and HAENN on Mean Survival Time

Table No: 82 Effect of EAENN and HAENN on Mean Survival Time in DAL Tumour Bearing Mice

Groups	Treatment	Mean Survival Time	ILS %
Group I	Normal Mice	-	-
Group II	DAL + Solvent 20ml/kg	18.54 ± 1.369	-
Group III	DAL+5-FU (20 mg/kg, b.w. p.o)	35.64 ± 2.067***	92.23%
Group IV	DAL + EAENN (100mg/kg, b.w. p.o)	23.18 ± 1.131^{ns}	25.02%
Group V	DAL + EAENN (200mg/kg, b.w. p.o)	28.61± 1.052***	54.31%
Group VI	DAL + EAENN (400mg/kg, b.w. p.o)	32.10 ± 1.477***	73.13%
Group VII	DAL + HAENN (100mg/kg, b.w. p.o)	21.36 ± 1.119^{ns}	15.21%
Group VIII	DAL + HAENN (200mg/kg, b.w. p.o)	27.56 ± 1.295***	48.65%
Group IX	DAL + HAENN (400mg/kg, b.w. p.o)	31.89 ± 1.101***	72.00%

Values are mean ± S.E.M. (n=6). One way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and **ns** represents Not significant. All the values are compared with DAL control + solvent.

EAENN: Ethylacetate extract of *Nymphaea nouchali,*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

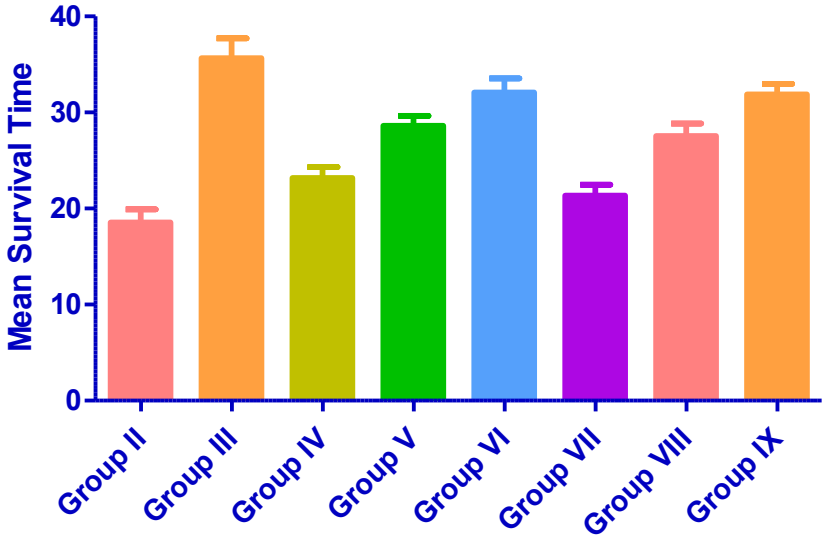


Fig. No: 152 Effect of EAENN and HAENN on Mean Survival Time in DAL Tumour Bearing Mice

8.6.4.9. Determination of Body Weight and Survival Time

Table No. : 83 Effect of EAENN and HAENN on Body Weight Analysis and Tumor Growth Response against DAL Induced Animals

Groups	Treatment	Increase In Body Weight (G)	Tumour Volume (Ml)	Packed Cell Volume (Ml)	% Decrease In Body Weight
Group I	Normal Mice	0.97 ± 0.101	-	-	-
Group II	DAL + Solvent 20ml/kg	8.26 ± 0.433	8.13 ± 0.267	4.39 ± 0.252	-
Group III	DAL+5-FU (20 mg/kg, b.w. p.o)	1.87 ± 0.142***	1.66 ± 0.164***	0.72 ± 0.079***	77.36%
Group IV	DAL + EAENN (100mg/kg, b.w. p.o)	6.48 ± 0.353**	5.94 ± 0.232***	2.76 ± 0.154***	21.54%
Group V	DAL + EAENN (200mg/kg, b.w. p.o)	5.30 ± 0.316***	5.13 ± 0.347***	2.64 ± 0.130***	35.83%
Group VI	DAL + EAENN (400mg/kg, b.w. p.o)	3.93 ± 0.303***	4.08 ± 0.136***	1.98 ± 0.220***	52.42%
Group VII	DAL + HAENN (100mg/kg, b.w. p.o)	6.75 ± 0.328*	6.08 ± 0.277***	3.04 ± 0.157***	18.28%
Group VIII	DAL + HAENN (200mg/kg, b.w. p.o)	5.52 ± 0.219***	5.32 ± 0.267***	2.53 ± 0.135***	33.17%
Group IX	DAL + HAENN (400mg/kg, b.w. p.o)	4.17 ± 0.145***	4.21 ± 0.191***	2.11 ± 0.148***	49.51%

Values are mean ± S.E.M. (n=6). One way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with DAL control + solvent.

EAENN: Ethylacetate extract of *Nymphaea nouchali,* **HAENN:** Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

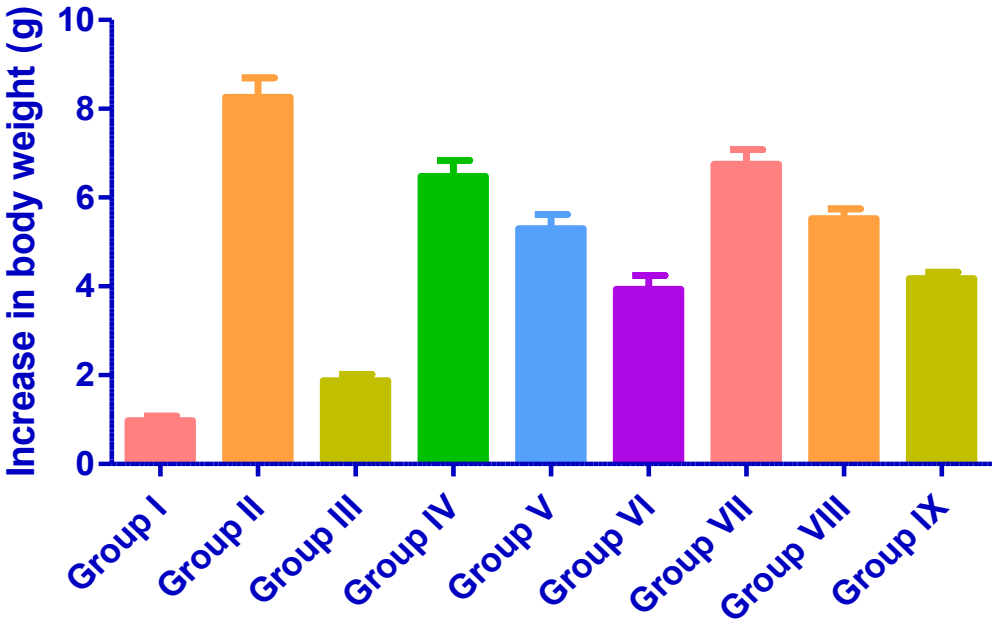


Fig. No: 153 Effect of EAENN and HAENN on Body Weight Response against DAL Induced Animals

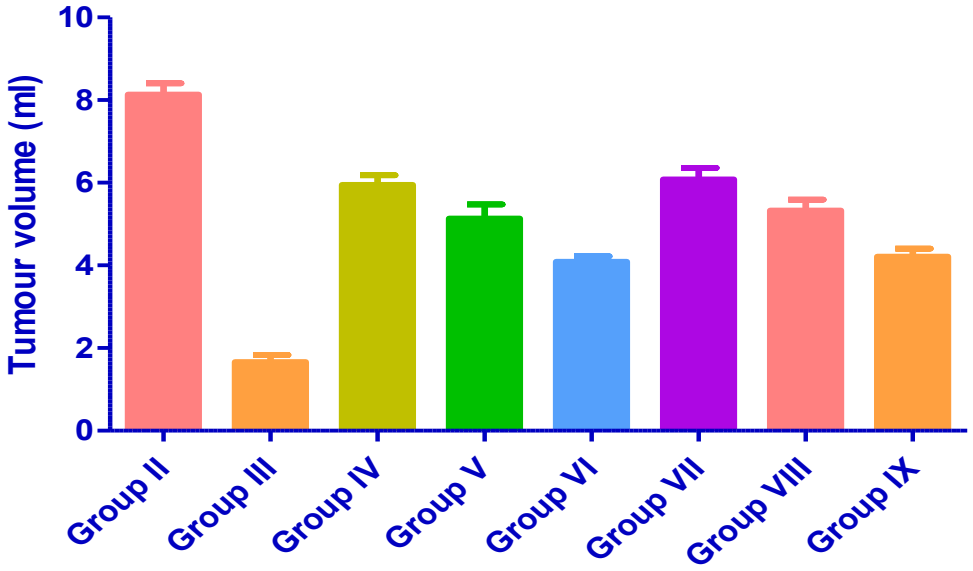


Fig. No: 154 Effect of EAENN and HAENN on Tumor Volume against DAL Induced Animals

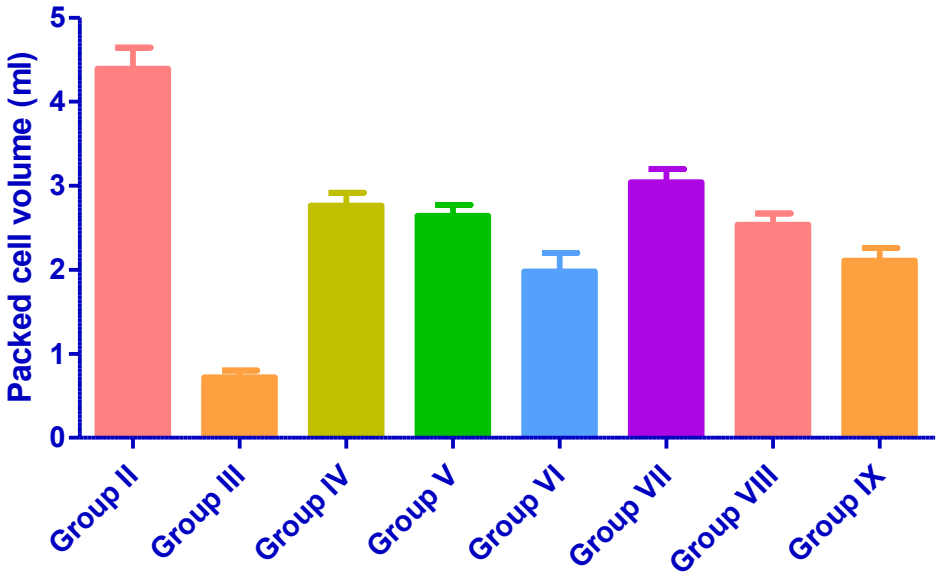


Fig. No: 155 Effect of EAENN and HAENN on Packed Cell Volume against DAL Induced Animals

8.6.4.10. Haematological Parameters on 14th day in Normal and DAL Tumour Bearing Mice

Table No: 84 Effects of EAENN and HAENN on Haematological Parameters on 14th Day in Normal and DAL Tumour Bearing Mice

Groups	Treatment	Hb (%)	RBC ($x10^6$ cell/mm^3)	WBC ($x10^4$ cell/mm^3)
Group I	Normal Mice	13.61±0.289	6.58 ± 0.341	8.67 ± 0.211
Group II	DAL + Solvent 20ml/kg	6.71±0.177	3.11 ± 0.302	22.64 ± 1.218
Group III	DAL+5-FU (20 mg/kg, b.w. p.o)	12.78±0.208***	6.06 ± 0.205***	10.51 ± 0.62***
Group IV	DAL + EAENN (100mg/kg, b.w. p.o)	8.61 ± 0.238***	4.13 ± 0.216*	16.89 ± 0.732***
Group V	DAL + EAENN (200mg/kg, b.w. p.o)	10.32 ± 0.344***	4.93 ± 0.113***	15.76 ± 0.803***
Group VI	DAL + EAENN (400mg/kg, b.w. p.o)	12.08 ± 0.378***	5.56 ± 0.210***	13.28 ± 0.617***
Group VII	DAL + HAENN (100mg/kg, b.w. p.o)	8.85± 0.242***	3.90 ± 0.097^{ns}	17.37 ± 0.684***
Group VIII	DAL + HAENN (200mg/kg, b.w. p.o)	9.87 ± 0.302***	4.73 ± 0.119***	15.34 ± 0.799***
Group IX	DAL + HAENN (400mg/kg, b.w. p.o)	11.69 ± 0.359***	5.28 ± 0.217***	14.16 ± 0.887***

Values are mean ± S.E.M. (n=6). One way ANOVA followed by Tukey-Karmer's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All the values are compared with DAL control + solvent.

EAENN: Ethylacetate extract of *Nymphaea nouchali,*

HAENN: Hydro-alcoholic (70%v/v) extract of *Nymphaea nouchali*

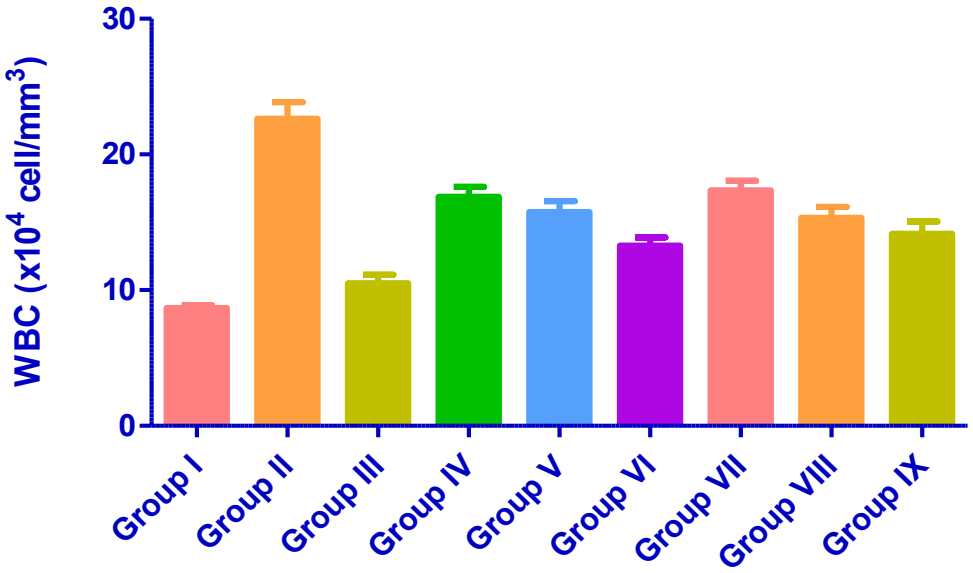


Fig. No: 156 Effect of EAENN and HAENN on WBC Count on 14th Day in Normal and DAL Tumour Bearing Mice

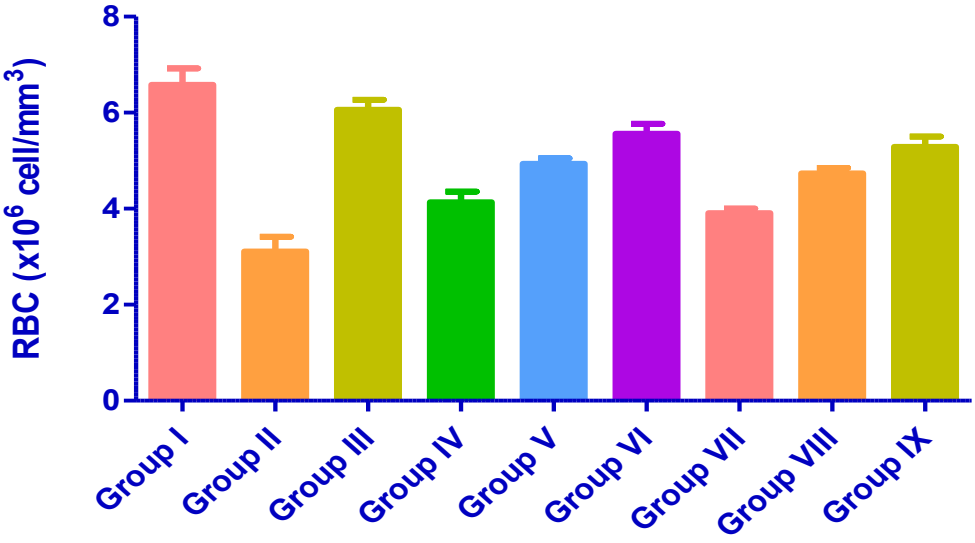


Fig. No: 157 Effect of EAENN and HAENN on RBC Count on 14th Day in Normal and DAL Tumour Bearing Mice

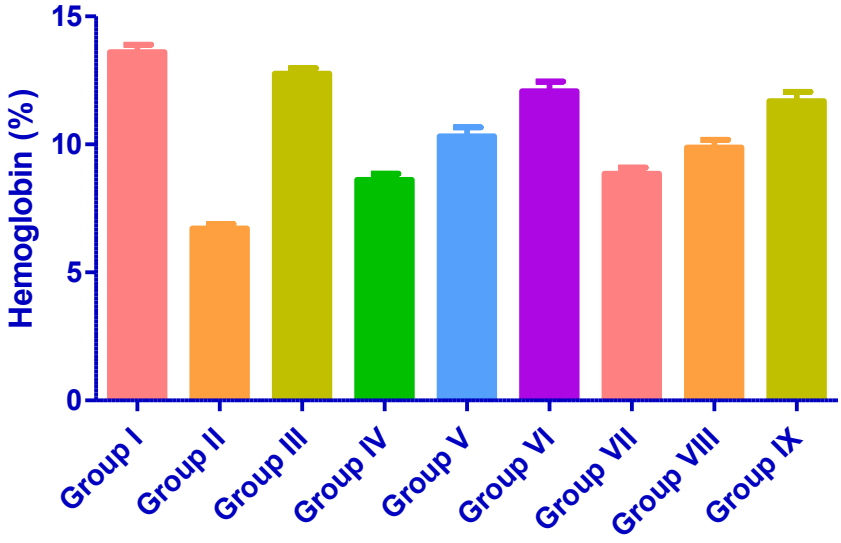


Fig. No: 158 Effects of EAENN and HAENN on Haemoglobin on 14th Day in Normal and DAL Tumour Bearing Mice

8.6.4.11. DISCUSSION

The use of Chemo-Therapeutic Drugs in cancer therapy involves the risk of life threatening host toxicity. The search therefore continues, to develop drugs which selectively act on tumor cells **(Durairaj AK., 2009).** The search for new anti-cancer agents from herbal plants has been extensively studied.

Traditional Indian system of medicine has many plants with versatile medicinal properties, which require detailed investigation for effective drug development. The natural products are economical and of low toxicity and this is perhaps the advantage over synthetic agents, which exhibit normal tissue toxicity. With the desire to find new and different types of compounds with anticancer and radio sensitizing activity, various plants have been used in cancer therapy as direct anticancer agents, chemotherapeutic agents, and radiosensitive or immunity enhancers.

While most of the research has been with experimental animals and helps to validate the applicability on the human system; *In-vitro* studies will facilitate a better understanding of the mode action responsible for the chemo preventive, radio protective and chemo preparations effects. The mechanism of action of polyherbal drugs and their extracts preparations differs in many respects from that of synthetic drugs or single substances. Antioxidant principles showed cytotoxicity towards tumor cells and antitumor activity in experimental animals **(Jain S, 2003; Shahedur Rahman, 2011; Krishnaveni, 2012).**

Cell deaths in mammalian cells are divided into two morphologically and biochemically distinct modes namely apoptosis and necrosis. Apoptosis is an organized, pre-programmed response of cell to shifting of environmental conditions. Characteristics of apoptotic cell include cell shrinkage, nuclear and DNA fragmentation and breaking up of the cell into membrane-bounded vesicles, termed **'apoptotic bodies'**, which are subsequently ingested by macrophages **(Doyle A, 1988).** Apoptosis plays a vital role in regulating growth, development and immune response, and also clearing abnormal cells **(Fan T J, *et al.,* 2005).** This apoptosis program becomes important in medical study in order to cure the cancerous cell without give the inflammatory effect. Aberrant cell death processes may underlie many human diseases including cancers, autoimmune,

neurodegenerative and immunodeficiency disorders **(Baehrecke EH, 2002; Graham-Evans B, 2003).**

Cytotoxic has been defined as the cell killing property of a chemical compound independent from the mechanism of death. Cytotoxicity assay is an appropriate method for screening new substances within a short time in order to determine cytotoxicity on cancer cell **(Obeling C, 1954).**

The anti-cancer potential of the extracts was assessed by change in survival time, body weight, total ascites fluid volume, packed cell volume, tumor cell count and haematological parameters. The ascites fluid is the direct nutritional source for tumor cells, and the faster increase in ascites fluid with tumor growth could possibly be a means to meet the nutritional requirements of tumor cells. The reliable criteria for judging the value of any anticancer drug are the prolongation of lifespan of the animal and control of WBC count in blood **(Durairaj AK., 2009).**

The ethylacetate and hydro-alcoholic extracts of ***Nymphaea pubescens*** and ***Nymphaea nouchali*** treated animals at doses of 100, 200 and 400 mg/kg b.w. p.o., inhibited the body weight, tumor volume, packed cell volume, tumor cell count, and also reverted the haematological parameters to approximately normal levels when compared DAL tumor bearing mice showing varied protection. In DAL-bearing hosts, regular rapid increase in ascites tumor volume was observed.

For judging the value of any anticancer drug is the prolongation of life span of animal and decrease in the WBC cells **(Prasad SB, 1994).** Ethylacetate and hydro-alcoholic extracts of *Nymphaea pubescens* and *Nymphaea nouchali* decreased the ascites fluid volume and thereby increased the Percentage of lifespan. It may be said that all three drugs, by decreasing the nutritional fluid volume and arresting the tumor growth, thereby increased the lifespan of treated DAL-bearing mice. The beneficial effect of EAENP, HAENP, EAENN and HAENN on mean survival time and % ILS against EAC induced mice. In the DAL control group, the median survival time was 14 days and which increased significantly to 22 days with EAENP, HAENP, EAENN and HAENN 400mg/kg b.w. p.o when compared to DAL group, which showed maximum increase in

the lifespan when compared with other doses of EAENP, HAENP, EAENN and HAENN and in comparison with DAL control.

In Ascites model following the inoculation of DAL tumor cell lines a marked decrease in life span and increase in body weight of mice were observed. Ascites fluid is the direct nutritional source to tumor cells and the faster increase in ascites fluid with tumor growth could possibly be a means to meet more nutritional requirement of tumor cells. A regular rapid increase in ascites tumor volume was noted in tumor bearing mice (increase in body weight) **(Hogland HC, 1982).** The percentage decrease in the body weight after treatment with EAENP, HAENP, EAENN and HAENN for 14 days, was reported. EAENP, HAENP, EAENN and HAENN400 mg/kg b.w. p.o., showed maximum percentage decrease in body weight when compared with vehicle treated cancerous animals. Treatment with EAENP, HAENP, EAENN and HAENN 400 mg/kg b.w. p.o., significantly ($p < 0.001$) decreased the tumor volume and packed cell volume when compared to that of DAL control group.

Usually in cancer chemotherapy, the major problems encountered are of myelo-suppression and anaemia **(Hogland HC, 1982).** The anaemia encountered in tumor-bearing mice is mainly due to reduction in RBC or haemoglobin percentage, and this may occur either due to iron deficiency or due to haemolytic or myelopathic conditions. Treatment with ethylacetate and hydro-alcoholic extracts of *Nymphaea pubescens* and *Nymphaea nouchali* restored the haemoglobin content, RBC and WBC cell count approximately to normal levels. This indicates that all three drugs possess protective action on the hemopoietic system but in varied protection.

The effect EAENP, HAENP, EAENN and HAENN100, 200 and 400 mg/kg b.w. p.o dose of haematological parameters against DAL induced animals estimated on 14^{th} day of treatment. EAENP, HAENP, EAENN and HAENN 400mg/kg b.w. p.o., showed better improvement in the haematological parameters than the rest of the doses among the compared groups. The total WBC count found significantly ($p < 0.001$) increased in the DAL control group. All the test drugs when administered to the EAC bearing mice showed the significant ($p < 0.001$) decrease in the WBC count when compared with the

DAL control group, with EAENP, HAENP, EAENN and HAENN 400 mg/kg b.w. p.o., showing the significant decrease in the WBC when compared to the DAL control ($p < 0.001$) groups. RBC count and Hb content in the DAL groups were significantly ($p < 0.001$) decreased as compared to the normal group. Treatment with EAENP, HAENP, EAENN and HAENN 400mg/kg b.w. p.o., significantly ($p < 0.001$) increased the RBC and Hb content when compared with the DAL control. All the test drugs have showed the significant increase but EAENP, HAENP, EAENN and HAENN 400mg/kg b.w. p.o. showed the better activity compared to rest of the drugs and doses.

Furthermore, it may be that the increase of lifespan of tumor-bearing mice by treatment is a positive result and supports the anti-cancer effect of *Nymphaea pubescens* and *Nymphaea nouchali.* The results of the present study are encouraging, as all the extracts have shown significant prolongation of lifespan, reduction in tumor volume, improvement in the haematological parameters of the hosts. The above parameters are responsible for the anti-cancer activity *Nymphaea pubescens* and *Nymphaea nouchali.*

CHAPTER – IX

SUMMARY AND CONCLUSION

The herbal medicines are the major remedy in traditional medicinal system and are being used in medicinal practices for thousands of years. They have made a great contribution in maintaining human health. The practice continues even today because of its biomedicinal benefits in many parts of the world. There is a phenomenal increase in the demand for the herbal medicines especially for those, which have been scientifically validated. These drugs are invariably single plant extracts or fractions thereof, which have been carefully standardized and their efficacy and safety for a suggested application, well demonstrated.

In the present study an attempt has been made to standardize two flowers of same families traditionally used for medicine plants by carrying out their pharmacognostical, phytochemical and pharmacological studies as per the standard procedures. The evaluation of potent flower extracts for their phytochemical, ***In-vitro*** and ***In-vivo*** **antioxidant, antidiabetic** and **anticancer properties** were carried out. Isolation of the phyto-constituents from the potent flower extracts using column chromatography and their characterization was also carried out.

The plant materials were collected from in and around Dharmapuri and Krishnagiri districts lakes, Tamilnadu, India and authenticated. The fresh flower materials were collected and used for morphological identification and microscopical studies. The dried flower materials were coarsely powdered and used for extraction with pet-ether, chloroform, ethylacetate and hydroalcoholic extracts using hot continuous soxhlet apparatus individually. All the extracts were concentrated to dryness under reduced pressure and controlled temperature (40-50ºC).

Pharmacognostical evaluation was carried out in order to establish the identity and to standardize the flowers. Morphological and microscopical characters, determination of various Physico-chemical parameters like loss on drying, ash values like total ash, acid insoluble ash, sulphated ash and water soluble ash, extractive values like water soluble and alcohol soluble extractive values, foreign organic matter, pH, heavy metals, solubility, micro-organisms, foaming index and fluorescence analysis were determined.

In fluorescence analysis, the flower powders and their extracts were treated with several acids and reagents. The color changes were observed under UV and visible lights. In the micro-chemical analysis, the flower powders were treated with several acids and reagents and the color changes were observed under microscope. These results gave clues regarding the presence of some particular phytoconstituents in the respective flower powders and extracts.

The phytochemical studies of both the flower extracts showed the major presence of **carbohydrates, flavonoids, proteins and amino acids, triterpenoids, phenolic compounds**. Alkaloids and steroids were found to be present in all except pet-ether extracts.

All the extracts were subjected to TLC and HPTLC analysis using several solvent systems. The solvent system **Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:27)** was found to be the better solvent system for the separation of all extracts especially for flavonoids. All the extracts were subjected to HPTLC standardization using the same solvent systems used in TLC. The R_f values of the separated components and their quantities in the extracts were found out.

The total phenolic contents of the extracts were estimated using Folin-Ciocalteu method. The total phenolic content was found to be high **(101.71±0.09µg GAE/g)** in the ethyl acetate extract of *Nymphaea pubescens* Willdenow when compared to all extracts and the total flavonoid content was found to be high **(104.71±0.09µg QE/mg)** in the ethyl acetate extract of ***Nymphaea pubescens* Willdenow** when compared to all extracts. So the ethyl acetate extracts *Nymphaea pubescens* was found to be the highest among all the other extracts estimated. From this it concludes that the flowers could serve as a source of **Natural Antioxidants**.

In the HPTLC studies, the same solvent system used in TLC were used for the separation. The number of compounds separated, their R_f values and their percentage were noted. The chloroform and ethylacetate extract of ***Nymphaea pubescens*** flowers showed the presence of 8 spots and 6 of them with R_f values **0.11, 0.29, 0.45, 0.62, 0.72** and **0.80**, were found to be the major ones with about **23** and **18%, 30** and **19%, 55** and **11%, 58** and **11%** peak areas for pet ether, chloroform, ethylacetate and hydroalcoholic extracts respectively.

The chloroform and ethylacetate extract of ***Nymphaea nouchali*** flowers showed the presence of 8 spots and 4 of them with R_f values **0.29, 0.45, 0.72** and **0.80**, were found to be the major ones

with about **23** and **17%, 30** and **19%, 55** and **19%, 58** and **11%** peak areas for pet ether, chloroform, ethylacetate and hydroalcoholic extracts respectively.

The HPTLC studies reveal the presence of major active components particularly flavonoids, the extracts of ***Nymphaea pubescens* Willdenow** and ***Nymphaea nouchali*** Burmann. F. flowers showed the presence of flavonoids. Two compounds were isolated from the ethylacetate extracts of *Nymphaea pubescens* and *Nymphaea nouchali* flowers individually. The isolated compounds were subjected to Physico-chemical and Spectral studies. The isolated and characterized compounds include rutin, 1, 3, 6, 8 Tetra Hydroxy Anthraquinone and Apigenin -8-C Glycoside which are found to be the major flavonoids present in the flowers was identified for the first time in this research. The isolated compounds were subjected to spectral studies to give the possible structure for the compounds.

Among the two flower extracts tested for ***In-vitro* antioxidant activity**, the ethylacetate extract of *Nymphaea pubescens* exhibit potent antioxidant activity with low IC_{50} **DPPH, FRAP, TRAP** and **ABTS** radical scavenging methods. The IC_{50} values were found to be **78.86%, 1.205±0.002µg/mL, 39.24µg/mL** and **76.46±0.060µg/mL** for DPPH, FRAP, TRAP and ABTS methods respectively. However, these IC_{50} values were found to be higher or comparable with those obtained for the standards used. In the other extracts of the flowers showed IC_{50} values indicating moderate to low antioxidant activity in other methods.

Based on the results of ***In-vitro*** studies, the flower extracts was selected for ***In-vivo*** antioxidant and Hepatoprotective studies.

In the present investigation, it was observed that the animals treated with paracetamol resulted in significant hepatic damage as shown by the elevated levels of serum markers. These changes in the marker levels will reflect in hepatic structural integrity. The rise in the SGOT is usually accompanied by an elevation in the levels of SGPT, which play a vital role in the conversion of amino acids to keto acids**.** The pre-treatment with EAENP, HANP, EAENN and HANN (100, 200 and 400 mg/kg, p.o.) and Silymarin significantly attenuated the elevated levels of the serum markers. The normalization of serum markers by EAENP, HANP, EAENN and HANN suggests that they are able to condition the hepatocytes so as to protect the membrane integrity against paracetamol induced leakage of marker enzymes into the circulation. Serum ALP and bilirubin

levels, on the other hand are related to hepatic cell damage. Increase in serum level of ALP is due to increased synthesis in presence of increasing biliary pressure. Effective control of bilirubin level and alkaline phosphatase activity points towards an early improvement in the secretory mechanism of the hepatic cell.

The Non-Enzymic Antioxidant, glutathione is one of the most abundant tripeptides present in the liver. Its functions are mainly concerned with the removal of free radical species such as hydrogen peroxide, superoxide radicals, alkoxy radicals, and maintenance of membrane proteinthiols and as a substrate for glutathione peroxidase and GST.

Lipid peroxidation has been postulated to the destructive process of liver injury due to acetaminophen administration. The increase in malondialdehyde (MDA) levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms to prevent formation of excessive free radicals. Treatment with EAENP, HANP, EAENN and HANN (100, 200 and 400 mg/kg, p.o.) and Silymarin has reversed the paracetamol induced elevated lipid peroxidation was decreased. Hence it may be possible that the mechanism of hepatoprotection by EAENP, HANP, EAENN and HANN is due to its antioxidant effect.

The **Enzymic Antioxidant** defence system is the nature protector against lipid peroxidation. SOD, CAT and GPx enzymes are important scavengers of superoxide ion and hydrogen peroxide. These enzymes prevent generation of hydroxyl radical and protect the cellular constituents from oxidative damage. In the present study, it was observed that the EAENP, HANP, EAENN and HANN significantly increased the hepatic SOD activity in paracetamol induced liver damage in rats. This show EAENP, HANP, EAENN and HANN (100, 200 and 400 mg/kg, p.o.) and Silymarin can reduce reactive free radicals that might lessen oxidative damage to the tissues and improve the activities of the hepatic antioxidant enzyme.

The **histological evidence** authenticated the injury caused by paracetamol and the protection offered by the flowers of EAENP, HANP, EAENN and HANN to hepatocytes. Microscopical examination revealed loss of architecture and cell necrosis with inflammatory collections in the central zone in paracetamol - induced rats. Prior oral administration with EAENP, HANP, EAENN and HANN extracts and Silymarin prevented completely the histopathological changes in liver induced by paracetamol. Thus the histopathological studies serve as a direct evidence of

efficacy of drug as protectant. The results of histopathological study also support the result of biochemical. These observations indicate that of ***Nymphaea pubescens*** and ***Nymphaea nouchali*** possess hepatoprotective activity against PCM induced hepatotoxicity.

Diabetes mellitus is a complex and diverse group of disorders characterized by hyperglycaemia that has reached epidemic proportions in the present century. Several synthetic drugs being utilized, but those have several side effects to avoid such effects researchers come up with new class of compounds which are having essential targets to overcome these problems. Globally, there is a positive trend in favour of traditional and integrative medicine in both research and practice. So, traditional antidiabetic plants might provide new oral hypoglycaemic compounds, which can encounter the high cost and poor specificity of the current medicines. A wide range of chemical compounds are present in the herbal plant, which are having a similar action like synthetic drugs (**Piyush M Patel, 2006).**

Single Dose Study for 120 min was carried out in normo-glycemic rats. High dose of EAENP HAENP, EAENN and HAENN (400 mg/kg, p.o.) showed maximum decrease in blood glucose levels at 120 min compared to normal group. It may produce hypoglycemia in normal animals by **stimulating the pancreatic beta-cells** to produce more Insulin and by increasing the glycogen deposition in the liver.

Oral Glucose Tolerance Test was studied on the normal rats. The lowering of glucose can be seen well in assay of glucose tolerance **(Versphol EJ., 2002).** The fasting blood glucose levels decreases in Metformin, along with EAENP HAENP, EAENN and HAENN high dose and medium dose treated rats. Low dose shows reduced activity at 120 min. Such a phenomenon was already seen in the indigenous plants and reported.

Induction of Diabetes with Alloxan (ALX) is associated with a characteristic decrease in body weight than the normal rats, this may be due to the wasting and loss of tissue protein. Whereas, diabetic rats treated with 100, 200 and 400mg/kg, p.o. of EAENP HAENP, EAENN and HAENN showed an improved result when compared with normal diabetic control. Which may be due to the protective effect in controlling muscle wasting i.e., reversal of gluconeogenesis and may also be due to the improvement of glycaemia control.

Diabetic rats treated with the medium, high dose of (200 and 400mg/kg, p.o.) EAENP, HAENP, EAENN and HAENN and Metformin has shown a significant decrease in the levels of TG, TC, LDL-C and VLDL-C, where as it increases the levels of HDL-C when compared to the normal diabetic control rats. In low dose of EAENP, HAENP, EAENN and HAENN treated rats HDL-C levels is less significant.

The histological evidence showed in the authenticated injury caused by ALX and the protection offered by EAENP, HAENP, EAENN and HAENN (100, 200 and 400 mg/kg, p.o.) and Metformin in pancreatic cells were shown. Microscopically examination revealed loss of architecture and cell necrosis with inflammatory collections in the central zone in ALX induced rats. Histopathological study showed that ***Nymphaea pubescens*** and ***Nymphaea nouchali*** has the capacity to increase Islet cell mass.

The **Anti-Cancer** potential of the extracts was assessed by change in survival time, body weight, total ascites fluid volume, packed cell volume, tumor cell count and haematological parameters. The ascites fluid is the direct nutritional source for tumor cells, and the faster increase in ascites fluid with tumor growth could possibly be a means to meet the nutritional requirements of tumor cells.

The ethylacetate and hydro-alcoholic extracts of ***Nymphaea pubescens*** and ***Nymphaea nouchali*** treated animals at doses of 100, 200 and 400 mg/kg b.w. p.o., inhibited the body weight, tumor volume, packed cell volume, tumor cell count, and also reverted the haematological parameters to approximately normal levels when compared DAL tumor bearing mice showing varied protection. In DAL-bearing hosts, regular rapid increase in ascites tumor volume was observed.

In Ascites model following the inoculation of DAL tumor cell lines a marked decrease in life span and increase in body weight of mice were observed. Ascites fluid is the direct nutritional source to tumor cells and the faster increase in ascites fluid with tumor growth could possibly be a means to meet more nutritional requirement of tumor cells. A regular rapid increase in ascites tumor volume was noted in tumor bearing mice (increase in body weight)**.** The percentage decrease in the body weight after treatment with EAENP, HAENP, EAENN and HAENN for 14 days, was reported. EAENP, HAENP, EAENN and HAENN400 mg/kg b.w. p.o., showed maximum percentage decrease in body weight when compared with vehicle treated cancerous

animals. Treatment with EAENP, HAENP, EAENN and HAENN 400 mg/kg b.w. p.o., significantly ($p < 0.001$) decreased the tumor volume and packed cell volume when compared to that of DAL control group.

The effect EAENP, HAENP, EAENN and HAENN 100, 200 and 400 mg/kg b.w. p.o dose of haematological parameters against DAL induced animals estimated on 14^{th} day of treatment. EAENP, HAENP, EAENN and HAENN 400mg/kg b.w. p.o., showed better improvement in the haematological parameters than the rest of the doses among the compared groups. The total WBC count found significantly ($p <0.001$) increased in the DAL control group. All the test drugs when administered to the EAC bearing mice showed the significant ($p <0.001$) decrease in the WBC count when compared with the DAL control group, with EAENP, HAENP, EAENN and HAENN 400 mg/kg b.w. p.o., showing the significant decrease in the WBC when compared to the DAL control ($p <0.001$) groups. RBC count and Hb content in the DAL groups were significantly ($p < 0.001$) decreased as compared to the normal group. Treatment with EAENP, HAENP, EAENN and HAENN 400mg/kg b.w. p.o., significantly ($p <0.001$) increased the RBC and Hb content when compared with the DAL control. All the test drugs have showed the significant increase but EAENP, HAENP, EAENN and HAENN 400mg/kg b.w. p.o. showed the better activity compared to rest of the drugs and doses. The above parameters are responsible for the anti-cancer activity *Nymphaea pubescens* and *Nymphaea nouchali.*

The present research work provides the pharmacognostical and phytochemical evaluation profiles to identity the two flowers drugs. HPTLC fingerprint of the flowers are useful in identifying the chemical entities and their quantities present therein. The ethylacetate extract of the flowers of *Nymphaea pubescens* shows potent antioxidant properties when compared to all other extracts of the flowers.

In *In-vivo* Antioxidant Studies, the ethylacetate extracts of both the flowers, EAENP, HAENP, EAENN and HAENN (100, 200 and 400 mg/kg, p.o.) and Silymarin on treatment shows significant reduction in the wet liver weight and wet liver volumes of animals and hence it possesses statistically significant hepatoprotective activity.

Alloxan Induced Diabetic rats is associated with a characteristic decrease in body weight than the normal rats, this may be due to the wasting and loss of tissue protein. Whereas, diabetic rats

treated with 100, 200 and 400mg/kg, p.o. of EAENP HAENP, EAENN and HAENN showed an improved result when compared with normal diabetic control. Which may be due to the protective effect in controlling muscle wasting i.e., reversal of gluconeogenesis and may also be due to the improvement of glycaemia control.

Anti-Cancer potential of the extracts was assessed by change in survival time, body weight, total ascites fluid volume, packed cell volume, tumor cell count and haematological parameters. The ethylacetate and hydro-alcoholic extracts of ***Nymphaea pubescens*** and ***Nymphaea nouchali*** treated animals at doses of 100, 200 and 400 mg/kg b.w. p.o., inhibited the body weight, tumor volume, packed cell volume, tumor cell count, and also reverted the haematological parameters to approximately normal levels when compared DAL tumor bearing mice showing varied protection. In DAL-bearing hosts, regular rapid increase in ascites tumor volume was observed.

Based on the above results obtained and observations we can infer that the flowers under study, ***Nymphaea pubescens*** and ***Nymphaea nouchali*** could be used for the supportive treatment of hepatotoxicity, diabetes mellitus and cancer as the flowers also offers effective protection against the attack of free radicals that forms the basis for the development of diabetic complications, hepatic damage and for certain case of viral treatment.

Such antioxidant and antidiabetic herbal drugs developed through standardization and validation studies will certainly contribute to combat this deadly disease.

Further in depth molecular level isolation and screened for clinical as well as toxicological studies can result in an eco-friendly human compatible antioxidant, hepatoprotective and antidiabetic herbal drug or herbal molecule from these selected traditional as well as tribal flower drug sources.

CHAPTER X

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ANNEXURE

NATIONAL INSTITUTE OF HERBAL SCIENCE

Plant Anatomy Research Centre : PARC

Prof.P. Jayaraman, Ph.D
Director
(Retd,Professor,Presidency College Chennai-5).

 # 4, 2nd Street, Sakthi Nagar,
West Tambaram, Chennai-600 045.
Ph:044-22263236, 9444385098
E.mail:herbalparc@yahoo.com

AUTHENTICATION CERTIFICATE

Based upon the organoleptic /macroscopic / microscopic examination of fresh / market sample, it is certified that the specimen given by Mrs. I. Karthiyayini, M.Pharm, Dept. of Pharmacognosy, Padmavathi College of Pharmacy, Dharmapuri -01 is identified as below :

Binomial: Nymphaea nouchali Burm.f. (Flowers)

Family: Nymphaeaceae

Synonym(s): Nymphaea stellata Willd.

Regional names: Hindi - Nilkamal ; Sanskrit - Nilotpala ; Tamil - Alli ; Telugu - Nitikulava

Reg.No of the certificate: PARC/2008/240

References : Nair, N.C & Henry, A.N. Flora of Tamilnadu, India 1: 9 .1983.

Henry, A.N. *et al.* Ibid. II: — .1987.

Ibid. III: — .1989.

Date:

(Prof.P.JAYARAMAN)

Director, Prof. P. JAYARAMAN, Ph.D
PLANT ANATOMY RESEARCH CENTRE
PHARMACOGNOSY INSTITUTE
No. 4-II Street, Sakthi Nagar, W. Tambaram, Chennai-45.
PH.: 22263236, CELL: 9444385098

PARC **PLANT ANATOMY RESEARCH CENTRE**
PHARMACOGNOSY INSTITUTE
Medicinal Plant Research Unit

Dr. P. Jayaraman, Ph.D.
Director, PARC,
Retd. Professor, Presidency College

4, II Street, Sakthi Nagar, Mudichoor Road,
West Tambaram, Chennai - 600 045.
Ph : 044-222 63 236, 9444385098, 9841273236

AUTHENTIFICATION CERTIFICATE

Based upon the organoleptic / macroscopic / microscopic examination of fresh / market sample, it is certified that the specimen given by T. Karthiyayini, M. Pharm., Dept. of Pharmacognosy, Padmavathi College of Pharmacy, Dharmapuri is identified as below:

Binomial : Nymphaea pubescens Willd.

Family : Nymphaeaceae

Synonym (s) : Nymphaea lotus L.

Regional names : Hindi - Kanval; Tamil - Vellambal; Telugu - Tella-kalava

References : Nair N.C. et al., 1983, Vol-I, Flora of Tamil Nadu, BSI, Coimbatore-03.

Nature of the specimen : Fresh Flower

Voucher no. of the specimen : PARC/2008/193

Date : 03.10.08

(Prof. P. JAYARAMAN)

Director, Prof. P. JAYARAMAN, Ph.D.,
PLANT ANATOMY RESEARCH CENTRE
PHARMACOGNOSY INSTITUTE
No. 4-II Street, Sakthi Nagar, W. Tambaram, Chennai-
PH.: 22263236, CELL: 9444385098, 984127323

Fax : 04348 - 247020

Ph : 04348 - 247020, 111, 326
04342 - 260343, 262810

PADMAVATHI COLLEGE OF PHARMACY

(Approved by the AICTE, New Delhi and Pharmacy Council of india, New Delhi)
(Affiliated to the T.N.Dr. M.G.R. Medical University, Chennai)
(AN ISO 9001 - 2008 Certified Institution)
Krishnagiri Main Road, PERIYANAHALLI - 635 205. DHARMAPURI (Dt), TAMIL NADU
Website : www.sapthagirigroups.Com
E-mail :padpharm1994@gmail.com

Dr. **K.L. Senthilkumar,** M.Pharm., Ph.D., M.B.A.,
Principal

Thiru. **M.G. Sekhar,** BA., BL.,
Chairman

Ref: / / Pharm / 20

Date :

CERTIFICATE

This is to certify that the investigation in the Thesis entitled **"PHARMACOGNOSTICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL SCREENING OF NYMPHAEA SPECIES LINN. (NYMPHAEACEAE)"** submitted to the Tamilnadu Dr.M.G.R.Medical University, Chennai, for the partial fulfillment of the award of the Degree of **DOCTOR OF PHILOSOPHY** in Pharmacy, was carried out by **Tmt. T. Karthiyayini, M.Pharm., Rc.No.ACAD-I(2)/33745/2008** at Padmavathi College of Pharmacy and Research Institute, Dharmapuri - 635205, under the guidance and supervision of **Dr. Nagesh R Sandu, M.Pharm., Ph.D.,** Professor, Department of Pharmaceutics, Padmavathi College of Pharmacy and Research Institute, Dharmapuri - 635 205.

This work is original and has not been submitted in part or full to any other Degree or Diploma of this or any other University.

Place: Dharmapuri

Date:

Dr. K.L.SENTHIL KUMAR, M.Pharm, Ph.D.,
PRINCIPAL

PADMAVATHI COLLEGE OF PHARMACY & RESEARCH INSTITUTE, PERIYANAHALLI, DHARMAPURI-635 205.

1. Title :

"Pharmacognostical, Phytochemical Screening and Pharmacological Screening of *Nymphaea* Species Linn., (Nymphaeaceae)"

2. Authors	:	**DR. Nagesh R. Sandu**
3. Proposal Number	:	**CPCSEA/PCP/IAEC/Ph.D/128/10**
4. Date of First Received	:	31.09.2009
5. Date of Received After Modification (If any)	:	Nil
6. Date of Received Second Modification (If any)	:	Nil
7. Approval Date	:	18.09.2010
8. Expiry Date	:	19.09.2012
9. Name of IAEC/CPCSEA Chair Person	:	**DR.K.L.Senthil Kumar**

Date : 18.09.2010
Place : Periyanahalli

CHAIRMAN
Institutional Animal Ethical Committee
Padmavathi College of Pharmacy & Research Institute
Dharmapuri.

PADMAVATHI COLLEGE OF PHARMACY & RESEARCH INSTITUTE, PERIYANAHALLI, DHARMAPURI-635 205.

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Dharmapuri.