EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF ALCOHOLIC EXTRACT OF *IXORA CHENENSIS (Linn.)* LEAVES

A Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY,

CHENNAI - 600 032

In partial fulfilment of the award of the degree of

MASTER OF PHARMACY

IN

BRANCH IV: PHARMACOLOGY

Submitted by

Name: BOOPATHI.N

REG.No.261625205

Under the Guidance of

Dr. R. SHANMUGA SUNDARAM M.Pharm., Ph.D.,

HEAD OF THE DEPARTMENT

DEPARTMENT OF PHARMACOLOGY

J.K.K. NATTRAJA COLLEGE OF PHARMACY

KUMARAPALAYAM – 638183

TAMILNADU.

OCTOBER – 2018


CERTIFICATE

EVALAUTION CERTIFICATE

This is to certify that the work embodied in this dissertation entitled **"Evaluation of hepatoprotective activity of alcoholic extract of *Ixora chenensis (Linn.)* leaves",** submitted to "**The Tamil Nadu Dr.M.G.R.Medical University – Chennai**", in partial fulfilment for the award of Degree of **Master of Pharmacy in Pharmacology,** is a bonafide work carried out by **Mr. BOOPATHI.N, Reg.No. 261625205,** during the academic year 2017-2018 under my guidance and direct supervision in the Department of Pharmacology, J.K.K.Nataraja College of Pharmacy, Kumarapalayam.

Internal Examiner **External Examiner**

CERTIFICATE

This is to certify that the work embodied in this dissertation entitled **"Evaluation of hepatoprotective activity of alcoholic extract of *Ixora chenensis (Linn.)* leaves",** Submitted to "**The Tamil Nadu Dr.M.G.R.Medical University-Chennai**", in partial fulfilment and requirement of university rules and regulation for the award of Degree of **Master of Pharmacy** in **Pharmacology**, is a bonafide work carried out by **Mr. BOOPATHI.N, Reg.No. 261625205** during the academic year 2017-2018, under my guidance and supervision in the Department of Pharmacology, J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

Place: Komarapalayam
Date:

Dr.R.Shanmuga Sundaram, M.Pharm, Ph.D.
Vice Principal and Professor,
Head of the Department,
Department of Pharmacology,
J.K.K. Nattraja College of Pharmacy,
Kumarapalayam- 638 183.

CERTIFICATE

This is to certify that the work embodied in this dissertation entitled **"Evaluation of hepatoprotective activity of alcoholic extract of *Ixora chenensis (Linn.)* leaves",** submitted to "**The Tamil Nadu Dr.M.G.R.Medical University-Chennai**", in partial fulfilment and requirement of university rules and regulation for the award of Degree of **Master of Pharmacy in Pharmacology**, is a bonafide work carried out by **Mr. BOOPATHI.N, Reg.No. 261625205** during the academic year 2017-2018, under the guidance of **Dr.R.Shanmuga Sundaram, M.Pharm, Ph.D.**, Vice Principal and Head of the Department, Department of Pharmacology, J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

Place: Komarapalayam
Date:

Dr. R. Sambath Kumar, M.Pharm, Ph.D.
Principal and Professor,
Department of Pharmaceutics,
J.K.K. Nattraja College of Pharmacy.
Kumarapalayam- 638 183.

CERTIFICATE

This is to certify that the work embodied in this dissertation entitled **"Evaluation of hepatoprotective activity of alcoholic extract of *Ixora chenensis (Linn.)* leaves",** submitted to "**The Tamil Nadu Dr.M.G.R.Medical University-Chennai**", in partial fulfilment and requirement of university rules and regulation for the award of Degree of **Master of Pharmacy in Pharmacology**, is a bonafide work carried out by **Mr. BOOPATHI.N, Reg.No. 261625205** during the academic year 2017-2018, under the guidance of **Dr.R.Shanmuga Sundaram, M.Pharm, Ph.D.**, Vice Principal and Head of the Department, Department of Pharmacology, J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

Place: Komarapalayam
Date:

Dr.R.Shanmuga Sundaram, M.Pharm, Ph.D.
Vice Principal and Professor,
Head of the Department,
Department of Pharmacology,
J.K.K. Nattraja College of Pharmacy,
Kumarapalayam- 638 183.

CERTIFICATE

This is to certify that the work embodied in this dissertation entitled **"Evaluation of hepatoprotective activity of alcoholic extract of *Ixora chenensis (Linn.)* leaves",** submitted to "**The Tamil Nadu Dr.M.G.R.Medical University-Chennai**", in partial fulfilment and requirement of university rules and regulation for the award of Degree of **Master of Pharmacy** in **Pharmacology**, is a bonafide work carried out by **Mr. BOOPATHI.N, Reg.No. 261625205** during the academic year 2017-2018, under my guidance and direct supervision in Department of Pharmacology, J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

Dr. R. Sambath Kumar, M.Pharm,Ph.D.
Principal and Professor,
Department of Pharmaceutics,
J.K.K. Nattraja College of Pharmacy,
Kumarapalayam- 638 183.

Dr.R.Shanmuga Sundaram, M.Pharm, Ph.D.
Vice Principal and Professor,
Head of the Department,
Department of Pharmacology,
J.K.K. Nattraja College of Pharmacy,
Kumarapalayam- 638 183.

DECLARATION

I do hereby declared that the dissertation **"Evaluation of hepatoprotective activity of alcoholic extract of *Ixora chenensis (Linn.)* leaves"** submitted to "**The Tamil Nadu Dr.M.G.R Medical University - Chennai",** for the partial fulfilment of the degree of **Master of Pharmacy** in **Pharmacology,** is a bonafide research work has been carried out by me during the academic year 2017-2018, under the supervision of **Dr. R. Shanmuga Sundaram, M.Pharm, Ph.D.**, Vice Principal and Head of the Department, Department of Pharmacology, J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

I further declare that this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma, associate ship and fellowship or any other similar title. The information furnished in this dissertation is genuine to the best of my knowledge.

Place: Kumarapalayam

Date:

Mr. BOOPATHI.N.

Reg.no.261625205

ACKNOWLEDGEMENT

ACKNOWLEDGEMENT

It is most pleasant duty to thank my beloved guide **Dr.R.Shanmuga Sundaram, M.Pharm, Ph.D.**, Vice Principal and Head of the Department, Department of Pharmacology, J.K.K. Nattraja College of Pharmacy, Kumarapalayam for suggesting solution to problems faced by me and providing in dispensable guidance, tremendous encouragement at each and every step of this dissertation work. Without his critical advice and deep-rooted knowledge, this work would not have been a reality.

It is most pleasant duty to thank for our beloved Principal **Dr.R.Sambathkumar, M.Pharm., Ph.D.,** Professor & Head, Department of Pharmaceutics, J.K.K. Nattraja College of Pharmacy, Kumarapalayam for ensuring all the facilities were made available to me for the smooth running of this project.

My sincere thanks to **Dr.R.Shanmugasundaram, M.Pharm., Ph.D.,** Vice Principal & HOD, Department of Pharmacology, **Dr.C.Kalaiyarasi, M.Pharm., Ph.D.,** Associate professor, **Mr.V.Venkateswaran, M.Pharm.,** Assistant Professor, **Mrs.R.Elavarasi, M.Pharm.,** Lecturer, **Mrs.M.Babykala, M.Pharm.,** Lecturer, Department of Pharmacology for their valuable suggestions during my project work.

My sincere thanks to **Dr.S.Bhama, M.Pharm. Ph.D.,** Associate Professor Department of Pharmaceutics, **Mr.R.Kanagasabai, B.Pharm,M.Tech.,** Assistant Professor, **Mr.K.Jaganathan, M.Pharm.,** Assistant Professor, **Mr.C.Kannan, M.Pharm.,** Assistant Professor, and **Mr.N.Subramani, M.Pharm.,** Lecturer, Department of pharmaceutics for the in valuable help during my project.

My sincere thanks to **Dr.N.Venkateswaramurthy, M.Pharm., Ph.D.,** Professor and Head, Department of Pharmacy Practice, **Mrs.K.Krishnaveni, M.Pharm.,** Assistant Professor, **Mr.R.Kameswaran, M.Pharm,** Assistant Professor, Department of Pharmacy Practice, for their help during my project.

It is my privilege to express deepest sense of gratitude toward **Dr.M.Vijayabaskaran**, **M.Pharm., Ph.d.,** Professor & Head, Department of Pharmaceutical Chemistry,**Mrs.S.Gomathi,M.Pharm.,** Lecturer, **Mrs.B.Vasuki, M.Pharm.,** Lecturerfor their valuable suggestions and inspiration.

My sincere thanks to **Dr.V.Sekar, M.Pharm., Ph.D.,** Professor and Head, Department of Analysis, **Dr.I.Carolin Nimila, M.Pharm., Ph.D.,** Assistant Professor, **Mr.D.Kamalakannan, M.Pharm.,** Assistant Professor, Department of Pharmaceutical Analysis for their valuable suggestions.

My sincere thanks to **Mrs.Meena Prabha, M.Pharm.,** Assistant Professor, Department of Pharmacognosy for their valuable suggestions during my project work.

I greatly acknowledge the help rendered by**, Mrs.V.Gandhimathi, M.A., M.L.I.S.,** Librarian, **Mrs.S. Jayakala B.A., B.L.I.S.,** and Asst. Librarian for their co-operation. I owe my thanks to all the technical and non-technical staff members of the institute for their precious assistance and help.

I am proud to dedicate my deep sense of gratitude to the founder, (Late) Thiru **J.K.K.Nattaraja Chettiar,** providing the historical institution to study.

My sincere thanks and respectful regards to our reverent Chairperson **Smt.N.Sendamaraai, B.Com.,** and Director **Mr.S.Omm Sharravana, B.Com., LLB.,**J.K.K. Nattraja Educational Institutions, Kumarapalayam for their blessings, encouragement and support at all times.

Last, but nevertheless, I am thankful to my lovable parents, all my friends for their co-operation, encouragement and help extended to me throughout my project work.

Mr. BOOPATHI.N.

Reg.no.261625205

Dedicated to Parents,

Teachers &

My Family

CONTENTS

Chapter - I

Introduction

Chapter - II

Literature Review

Chapter - III

Plant Profile

Chapter - IV

Aim and Objectives

Chapter - V

Plan of Work

Chapter - VI

Materials & Methods

Chapter - VII

Results & Discussion

Chapter - VIII

Conclusion

Chapter - IX

References

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF ALCOHOLIC EXTRACT OF *IXORA CHENENSIS (Linn.)* LEAVES

A Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY,

CHENNAI - 600 032

In partial fulfilment of the award of the degree of

MASTER OF PHARMACY

IN

BRANCH IV: PHARMACOLOGY

Submitted by

Name: BOOPATHI.N

REG.No.261625205

Under the Guidance of

Dr. R. SHANMUGA SUNDARAM M.Pharm., Ph.D.,

HEAD OF THE DEPARTMENT

DEPARTMENT OF PHARMACOLOGY

J.K.K. NATTRAJA COLLEGE OF PHARMACY

KUMARAPALAYAM – 638183

TAMILNADU.

OCTOBER – 2018

1.0 INTRODUCTION

The liver diseases have become one of the major causes of morbidity and mortality all over world. From among, drug induced liver injury (DILI) is one of the most common causative factor that poses a major clinical and regulatory challenge. The manifestations of drug-induced hepatotoxicity are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminate hepatic failure. Paracetamol (PCM) also known as Acetaminophen, taken in overdose can cause severe hepatotoxicity and nephrotoxicity. PCM is activated and converted by cytochrome P450 enzymes to toxic metabolite NAPQI (N-acetyl-p-benzoquinoneimine) that causes oxidative stress and glutathione (GSH) depletion. In spite of tremendous advances in modem medicine, there are hardly any reliable drugs that protect the liver from damage and/or help in regeneration of hepatic cell. Many active plant extracts are frequently utilized to treat a wide variety of clinical diseases including liver disease. Therefore, searching for effective and safe drugs for liver disorders are continues to be an area of interest. Aegle mameloes, belongs to family Rutaceae is commonly known as beal. It exhibits contraceptive, hypoglycemic activity, cardio protective effects and anti-hyperlipidaemic activity. Aloe vera (family-Liliaceae) has been reported to have antifungal activity, hypoglycemic activity, anti- inflammatory, anticancer, immunomodulatory, gastro- protective and anti-hepatotoxic properties[1-5]. Eclipta alba (family-Asteraceae) commonly known as Bringraj, has been shown to posses anti- inflammatory, anti-hypertensive, anti-hepatotoxic, immunomodulatory, anthelmintic, expectorant.

Hepatic diseases are a major worldwide health problem, with frequently found in developing countries. They are mainly caused by uses of high doses of chemicals and some drugs. There is no effective drug available that stimulates liver function, offer protection to the liver from damage or help to regenerate hepatic cells. Therefore there is urgent need, for effective drugs to replace/add those in current use. Medicinal herbs are significant source of pharmaceutical drugs. Latest

trends have shown increasing demand of phytoconstituents from some medicinal herbs and those medicinal herbs have proven hepatotprotective potential.A numbers of herbal preparations are available in the market. The present review is aimed at compiling data on promising phytochemicals from medicinal plants that have been tested in hepatotoxicity models using modern scientific system. In this century clinical research has confirmed the efficacy of some herbs in the treatment of liver related disease. Hence, this review article contributes to the knowledge of reported indigenous plants, which are prevalent for prevention and treatment ofliver disorders[6-9].

The liver has more functions than any other human organ and also is a largest part of the body. The blood supply passes through the liver several times a day. The Liver has a important role in human metabolism. Liver produces and secretes bile, it also produces prothrombin and fibrinogen, both blood clotting factors, and heparin. It converts sugar into glycogen. Liver diseases have become one of the major causes of morbidity and mortality in man and animals. Hepatotoxicity due to drugs appears to be the most common factor. The most common disease that can affect the liver is 'viral hepatitis'. Hepatitis mainly caused by drugs, viruses, bacteria, parasites like amoebas or giardiasis. The use of natural remedies to treat the liver diseases has a long history. There are some medicinal plants and their derivatives which still used all over the world in one form or the other, have been tested and found to contain active principles with curative properties against a variety of diseases. Hepatoprotective plants contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthenes. Recent experience has shown that plant drugs are non-toxic, safe and even free from side effects.

There are many plants and traditional formulations available to treat the liver diseases. About 600 commercial herbal formulations with hepatoprotective activity are being sold all over the world.

Around 170 phytoconstituents isolated from 110 plants belonging to 55 families have been reported to possess hepatoprotective activity. In India, more than 93 medicinal plants are used in different combinations in the preparations of 40 patented herbal formulations. However, only a small proportion of hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated.

Table No. 1: Herbs having hepatoprotective activity

S. No.	Biological name	Common name	Extract
1.	*Andrographis lineata*nees (Acanthaceae)	Kirayat, Kalamegha	Methanol and aqueous extract
2.	*Azadirachta indica* (Meliaceae)	Neem	Methanolic extract
3.	*Careya arborea* (Myrtaceae)	Kumbhi, slow match tree	Methanolic extract
4.	*Cassia fistula*(fabaceae)	Amaltas	Methanolic extract
5.	*Cleome viscosa*linn (Capparidaceae)	Tickweed	Ethanolic extract
6.	*Eclipta alba*(Asteraceae)	Bhringaraj	Ethanol:water 1:1 extract

S. No.	Biological name	Common name	Extract
7.	*Fumaria indica*(Fumariceae)	Hauskn	Petroleum ether extract against CCl_4, methanolic extract against rifampicine, and aqueous extract against PCM.
8.	*Morinda citrifolia*Linn (Rubiaceae)	Noni	Ethanol extract
9.	*Phyllanthus amarus*(Euphorbiaceae)	Bhuiamala	Ethanolic extract
10.	*Phyllanthus emblica*(Euphorbiaceae)	Amla	Ethanolic extract
11.	*Phyllanthus polyphyllus*(Euphorbiaceae)	Dalzell	Methanolic extract
12.	*Phyllanthus reticulates*(Euphorbiaceae)	Potato bush	Ethanolic extract
13.	*Picrorhiza kurroa*(Scrophulariaceae)	Kutki	Alcoholic extract
14.	*Polygala arvensis*(Polygalaceae)	Field milkwort	Chloroform extract
15.	*Pterocarpus santalinus*(Fabaceae)	Kanak champa	Aqueous and ethanol extract

S. No.	Biological name	Common name	Extract
16.	*Ptrospermum acerifolium*(Sterculiaceae)	Maple-leaved Bayur tree	Ethanol extract
17.	*Solanum nigrum*(Solanaceae)	Makoi	Ethanol extract
18.	*Cichorium intybus*(Asteraceae)	Kasni	Ethanol extract
19.	*Swertia chirata*(Gentianaceae)	Chirayata	Ethanol extract
20.	*Wedelia calendulacea*Linn (Asteraceae)	Bhanra	Ethanolic extract
21.	*Anoectochilus formosanus*(Orchidaseae)	Jewel Orchid	Aqueous extract
23.	*Ixora coccinea*Linn (Rubiaceae)	Thetchi	Aqueous extract

1.1 Pathophysiological Mechanisms

Pathophysiological mechanisms of hepatotoxicity are still being discovered and comprise both hepatocellular/extracellular mechanisms.

1.2 Disruption of hepatocyte

Medications can bound to intracellular proteins by covalent tying which bring about a lessening in ATP levels prompting actin interruption. Part of actin fibrils at the surface of the hepatocyte causes blebs and burst of the layer.

1.3 Disruption of transport protein

Bile stream may be interrupted by medications that influence transport proteins at canalicular film. Loss of villous procedures and intrusion of transport pumps, for example, multidrug resistance- related protein 3 forestall discharge of bilirubin bringing about cholestasis

1.4 Cytolytic T-cell activation

Co-valent tying of medication to Cytochrom P-450 enzyme goes about as an immunogen activating T-cells and cytokines and animating multifaceted immune reactions.

1.5 Apoptosis of hepatocytes

Enhancement of apoptotic pathways by tumor necrosis factor-alpha receptor of Fas may trigger the course of intercellular caspases, which bring about customized cell death.

1.6 Mitochondrial disruption:

A few medications restrain mitochondrial capacity by double impact on both beta- oxidation energy productions by hindering the synthesis of nicotinamide adenine dinucleotide and flavin adenine dinucleotide, bringing about diminished ATP generation.

1.7 Bile duct injury

Dangerous metabolites disposed of in bile may bring about harm to bile conduit epithelium[10].

1.8 Drug Induced Hepatotoxicity

Medication/drug prompted liver damage is a well-being issue, and is relied upon to increment as the quantity of medications being devoured increments, both prescription and non-prescription, and because of the present pattern of utilization of pharmacologically active substances in complementary and alternative medicine. Medication/drug prompted hepatotoxicity is the most well-known reason referred for withdrawal of officially approved medications from the business. It additionally represents more than 50 percent of instances of intense liver failure in the United States. The definite frequency of medication/drug prompted liver damage is hard to gauge, and all in all, studies going to measuring its occurrence experience the ill effects of disadvantages, for example, under-reporting and that information by large originate from review studies. Regularly, there is likewise an absence of data about self-solution and utilization of herbal product that may interact with medicine and non-physician endorsed medications[11-13].

Notwithstanding the recurrence of medication instigated liver damage being low, information from the Centers for Disease Control and Prevention in the U.S. report more or less 1600 new intense instances of liver failure yearly, of which Paracetamol hepatotoxicity represents give or take 41%. At the point when taking a gender at hospitalized patients, the rate of antagonistic medication responses is evaluated to be 6.7%, and lethal unfriendly medication responses add up to 0.32%, as controlled by a meta-analysis of around 40 prospective studies. During the period 1995 to 2005, the reports of unfavorable medication responses and additionally deaths identified with these, have dramatically multiplied. Numerous instances of medication triggered liver damage are idiosyncratic, i.e. the response is capricious

taking into account the known pharmacological properties of the drug, and henceforth is barely noticeable during preclinical phases of improvement. There are however studies to show that these responses may be subject to an expanded affectability of the patient to the medication being referred to, contingent upon such components as other accompanying infections or other corresponding medications. Certain hereditary variables, for example, HLA-type, can now and again add to the affectability of a person to antagonistic medication responses. Ordinarily, clinically clear unfriendly medication responses happen when some time of idleness, anyplace in the compass going from one to 12 months (most generally inside of 90 days), and about dependably vanish after evacuation of the medication. Medication investigated liver harm may give a few distinctive clinical components; hepatitis /hepatocellular, cholestatic or mixed.

Despite their etiology, medication/drug induced hepatotoxicity remains a noteworthy issue during medication development in the pharmaceutical industry, both concerning expanded danger for patients experiencing clinical trials, furthermore patient-risk after the introduction of new medication to the treatment. Additionally, due to the expanded expenses that take after failure of a medication to-be at a late stage in medication development or after its launch.

1.9 Drug Toxicity Mechanisms

Typical division of medication reactions is of not less than 2 noteworthy gatherings which include:

- ✓ Drugs which straightforwardly influence liver.
- ✓ Drugs which intercede an immune reaction.

1.10 Intrinsic / predictable drug reactions

Drugs that belongs into this classification cause reproducible wounds in animals and harm is identified with dose. Harm can be because of medication itself or to its metabolite. Acetaminophen is the most appropriate illustration of a known natural or unsurprising hepatotoxin at super therapeutic dosages. Another illustration is carbon tetrachloride.

1.11 Idiosyncratic/unpredictable drug reactions

These drug responses can be segmented into those that are classified as hypersensitivity or immunoallergic and those that are metabolic-idiosyncratic. It happens without obvious.

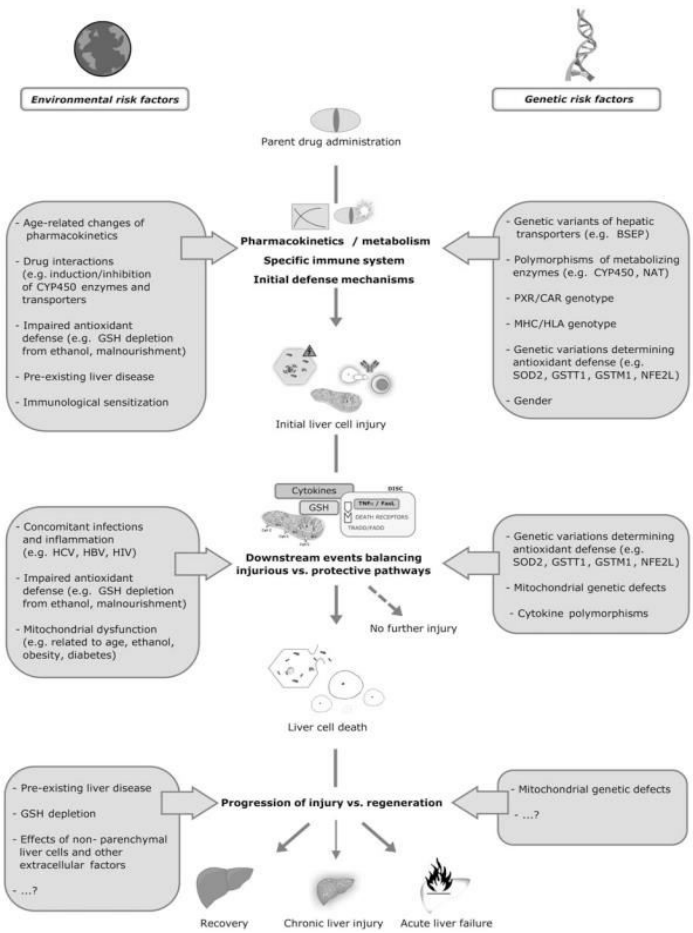

Figure No. 1: Risk factors for hepatotoxicity

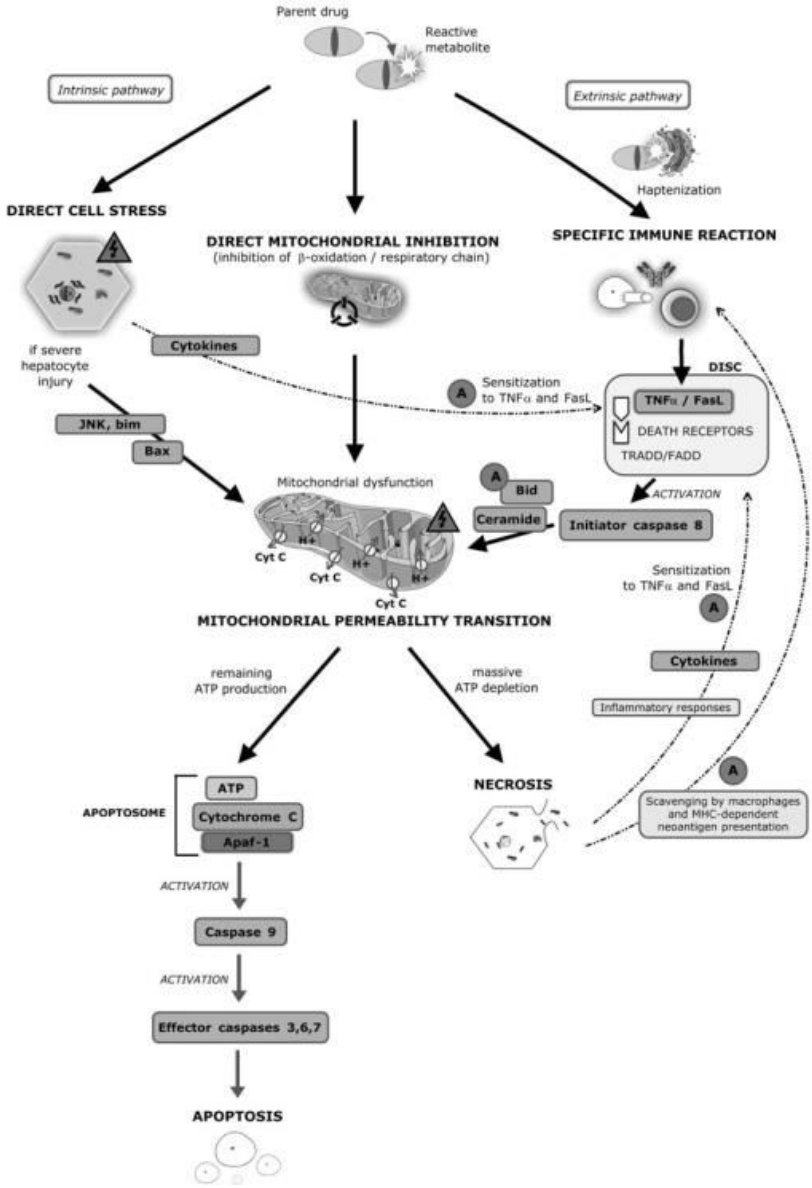

Figure No. 2: Three-step mechanistic working model of hepatotoxicity

Hepatotoxicity largely indicates the chemical compelled liver destruction. Some medications when consumed in overdose and occasionally even when taken within recommend dose may damage many internal organs. Few compound/substances comprising those that are used in laboratories (Example: CCl_4 and Paracetamol) and industries (Lead, and arsenic) and natural compounds (microcystine and aflatoxins) and herbal therapies (cascara sagrada, ephedra) can root hepatotoxicity. Chemicals/Compounds that cause liver damage are branded as hepatotoxins[14-21].

- ✓ NSAIDS (Acetaminophen, Aspirin, Ibuprofen)
- ✓ Glucocorticoids.
- ✓ Anti-Tubercular drug (Isoniazid).
- ✓ Industrial toxins (arsenic, carbon tetrachloride, vinyl chloride).
- ✓ Herbal remedies (Ackee fruit, camphor, cycasin, kava leaves, valerian, comfrey).

1.12 Alcohol Hepatotoxicity

Alcohol is one of the fundamental inducer of end-stage liver damage around the world. In the United States, alcoholic liver disease is the second most regular purpose behind liver transplantation. The Dionysos Study, a cohort investigation of the predominance of ceaseless liver disease in an Italian populace, demonstrated that 21% of the populace considered was at danger for creating liver damage. Of these, just 5.5% of the people at danger hinted at real liver damage. Around 50 years prior it was accepted that alcohol in itself was not harmful, rather that the dietary inadequacies frequently going hand in hand with it were the real reasons for liver harm. In any case, it was indicated by Lieber and De Carli that in rats, alcoholic liver damage created in spite of adequate sustenance. The lethality of alcohol was later on demonstrated to be identified with its digestion system by alcohol dehydrogenases (ADHs) furthermore to the digestion system by CYP2E1. There is additionally a part of digestion system by catalase. The fundamental pathway for ethanol (EtOH) oxidation in the liver is by means of ADH to acetaldehyde, which is connected with the reduction of NAD to NADH. NADH thus builds xanthine oxidase action, which rises generation of superoxide. Metabolic system of EtOH by alcohol dehydrogenase impacts the redox status of the liver likewise in different ways. Elevated acetaldehyde creation after EtOH metabolism diminishes hepatic glutathione (GSH)

content. The diminishing in GSH is both because of an expanded misfortune, and also a lower rate of synthesis

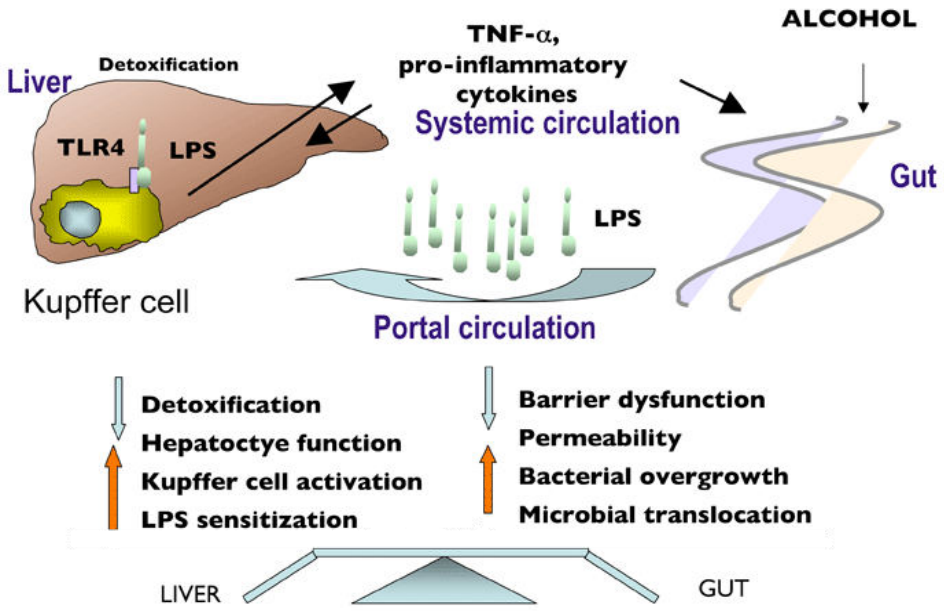

Figure No. 3: Alcohol Hepatotoxicity – Mechanism

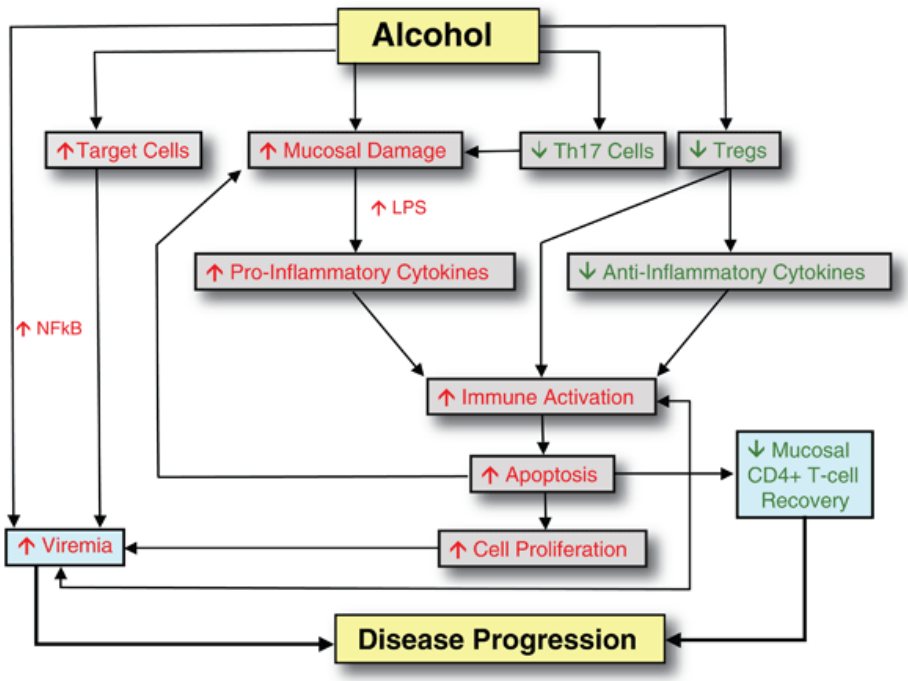

Figure No. 4: Alcohol Hepatotoxicity – Disease Progression

Ethanol induces number of deleterious metabolic changes in liver. Intake of ethanol for long time leads to development of steatosis, alcoholic hepatitis and cirrhosis resulting in weight and volume changes. About 80% of heavy drinkers had been reported to develop steatosis, 10-35% alcoholic hepatitis and approximately 10% liver cirrhosis.

1.13 Mechanism underlying Ethanol induced hepatotoxicity

Alcohol consumption results in increase in release of endotoxin from gut bacteria and membrane permeability of gut to endotoxin or both. Females are more often sensitive to these changes. Blood endotoxin is elevated and enters liver where it is engulfed by Kupffer cells that become activated releasing TNF- alpha, PGE2 and free radical. Prostaglandins increase oxygen uptake and are responsible for hypermetabolic state in liver. Increase in oxygen demand leads to hypoxia of liver and on reperfusion alpha - hydroxyethyl free radicals are formed that leads to tissue damage in oxygen poor pericentral regions of liver lobule. Blocking of these events can be done by sterilization of gut using antibiotics or destruction of Kupffer cells with Gdcl3 and thus prevents liver injury.

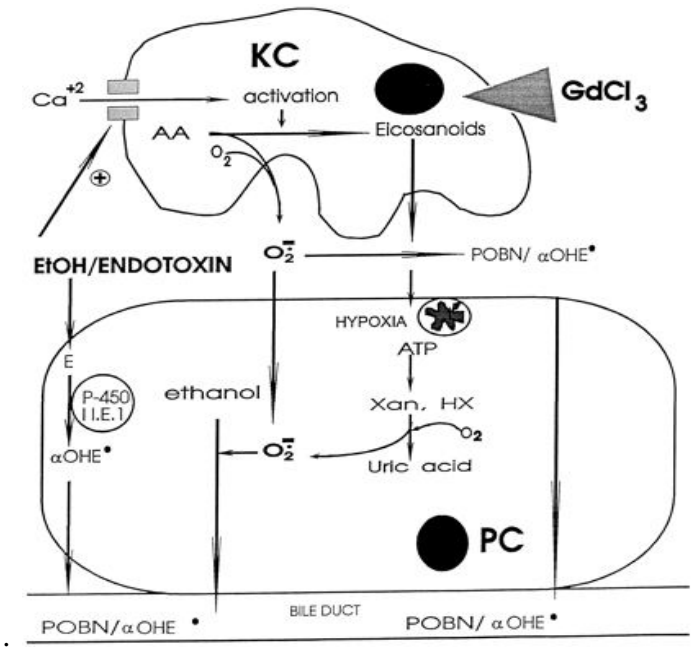

Figure No. 5: Mechanism of ethanol induced hepatotoxicity

1.14 Symptoms of Hepatotoxicity

List of signs and symptoms depicted in various causes for Hepatotoxicity include 15 symptoms as listed below:

- ✓ Nausea
- ✓ Vomiting
- ✓ Abdominal pain
- ✓ Loss of appetite
- ✓ Diarrhea
- ✓ Tiredness
- ✓ Weakness
- ✓ Jaundice
- ✓ Yellow eyes
- ✓ Yellow skin
- ✓ Hepatomegally
- ✓ Abnormal liver function test results
- ✓ Swelling in feet
- ✓ Weight gain due to water retention
- ✓ Prolonged bleeding time.

1.15 Treatment for Hepatotoxicity

The list of treatments mentioned in various sources for hepatotoxicity includes the following. Always follow professional medical advice about any treatment or change in treatment plans. Treatment of hepatotoxicity is depends upon causative agent, degree of liver dysfunction and age and general health of patient. Treatments for hepatotoxicity include:

- ✓ Withdrawal of causative medication or removal from exposure to causative agent.
- ✓ Regular monitoring of patient and review of liver function – where liver dysfunction is mild to moderate and liver function is improving.
- ✓ Complete avoidance of alcohol and medication that may contribute to further liver damage.
- ✓ N-Acetylcysteine is used for paracetamol toxicity.
- ✓ Management of symptoms of liver damage.
    - Nutrition – with vitamin supplementation as required
    - Regular exercise in order to maintain muscle mass.
    - Ursodeoxycholic acid.
- ✓ Management of pruritus
    - Cholestyramine
    - Antihistamines.
- ✓ Management of ascites
    - Low sodium diet.

- Diuretics – furosemide, spironolactone.
- Removal of fluid via a needle in the abdomen – Paracentesis.
- Portosystemic shunting.

✓ Management of portal hypertension

- Beta – blockers
- Oesophageal variceal banding
- Portocaval shunt

✓ Management of acute liver failure due to hepatotoxicity

- Supportive care always in intensive care unit – airway protection, fluid and electrolyte management.
- Management of complications such as bleeding problems and hepatic encephalopathy.

✓ Liver transplantation – for acute fulminant liver failure or end stage cirrhosis.

1.16 Modern Medicines for Treatment of Liver Diseases

Liver diseases can be treated using allopathic as well as by using herbal drugs.

1.17 Hepatoprotective Allopathic Treatment

Few modern medicines are available for treating liver diseases that includes:

- Ursodeoxycholic acid (Ursodiol): Ursodiol decreases intestinal absorption and suppresses hepatic synthesis and storage of cholesterol. It is mainly used in management of chronic hepatic diseases in humans.

- Penicillamine: Penicillamine chelates several metals like copper, iron, lead and mercury forming stable water soluble complexes which are renally excreted[22-28].

Other drugs:

Antiviral medication such as alpha interferon, ribavirin, steroids, antibiotics etc. are also used in liver diseases. Drugs like tricholinecitrate, trithioparamethoxy phenyl propane, essential phospholipids, combination of drugs such as L-ornithine, L-aspartate and pancreatin, silymarin and Ursodeoxycholic acid are usually prescribed for hepatitis, cirrhosis and other liver diseases. N-acetylcysteine is used in early phases of acetaminophen toxicity. L-carnitine is potentially valuable during valproate toxicity. Cholestyramine can be used to alleviate pruritus.

1.18 Disadvantages of allopathic drugs

Side effects of many modern medicines are mostly alarming. Interactions, contra-interactions, side effects and toxicity of synthetic medicine vary from mild to severe that includes insomnia, vomiting, fatigue, dry mouth, diarrhea, constipation, dizziness, suicidal thought, depression, seizures, anemia, hair loss, high blood sugar, swelling, impotency, confusion, fainting and finally death. Antibiotics usually cause stomach upset or allergic reactions. Interferon shows side effects as flu-like illness with fever and body aches[29].

1.19 Herbal Hepatoprotective Drug Treatment

A number of polyherbal preparations have been used in treating various liver disorders since ages. Some herbal formulations include:

a) Liv-52: It is a non-toxic hepatoprotective drug from Himalaya Drug Co. Liv-52 can improve clinical parameters in patients having liver damage mainly in alcoholic liver cirrhosis.

b) LIMARIN®: It has potent hepatoprotective and free radical scavenging (antioxidant) activity. It is derived from active extract of fruit of silybum marianum.

Some of the polyherbal formulations have been evidenced for hepatoprotective activity against chemical driven liver damage in experimental animals which include Liv52, Liv42, Jigrine, Koflet, Cirrhitin, Livex and Hepatomed[10] etc.

1.20 Limitations of herbal preparations

Herbal- based preparations for treating liver disorders has been used in India for long time and has been popularized worldwide by leading pharmaceuticals. Despite of popularity of herbal medicines for liver diseases in particular, are still unacceptable treatment modalities for liver diseases. Limiting factors include:

- ✓ Lack of standardization procedures of herbal preparations.
- ✓ Lack of identification of active components and principles.
- ✓ Lack of randomized controlled clinical trials (RCTs).
- ✓ Lack of toxicological evaluation[67].
- ✓ Poor solubility.
- ✓ Poor bioavailability.
- ✓ Poor hepatic cell regeneration[30,31].

2.0 LITERATURE REVIEW

Marklund S, et al., (2011)[32] evaluated the hepatoprotective activity of Andrographis paniculata in ethanol induced hepatotoxicity in albino rats. In the study the aqueous extract of A. paniculata (50mg/kg, 100mg/kg, 200mg/kg body weight) was found to protect the lever from hepatotoxic action of ethanol by significant reduction in the transaminase level.

Martin P, et al., (2008) [33]aimed to find out the hepatoprotective activity of Orthosiphon stamineus on liver damage caused by paracetamol in rats. The methanolic extract of leaves in the dose of 100mg and 200mg/kg body weight was given to the rats affected by paracetamol and investigated and found that the extract showed siginificant recovery and suggested that O. stamineus methanol leaf extract possessed hepatoprotective activity.

Muriel P, et al., (2011)[34] studied the hepatoprotective active of Mimosa pudica Linn. In carbon tetrachloride indued hepatotoxicity in rats. Mimosa pudica Linn. Is commonly used herbs against many disease. The hydroalcoholic extract of dried powder of whole plant was investigated in CCl4 hepatotoxicity rats modle at the dose of 200mg/kg, 400mg/kg and 800mg/kg body weight and found recovery in the liver function biochemical parameters. Histology of liver sections of animals treated with extract showed the hepatic cell regeneration. The results support the hepatoprotective effect of Mimosa pudica Linn.

Nkosi CZ, et al., (2005)[35] investigated hepatoprotective activity of aqueous extract of Lawsonia lnermis against paracetamol induced rats. The aqueous extract of Lawsonia lnermis was administered orally to the animals with hepatotoxicity induced by paracetamol. Silymarin was was given as reference standard. The plant aqueous extract was found to be effective in protecting the liver against the injury induced by paracetamol in rats. The result proved the hepatoprotective activity of Lawsonia lnermis agsinst paracetamol induced rats.

Okokon JE, et al., (2007)[37] studied the hepatoprotective activity of Dregea volubilis fruit against paracetamol induced liver damage in rats. The liver of the rats was affected with paracetamol and the damaged liver were treated with the extract in dose of 100mg/kg and 200mg/kg body weight and sylimerin 25mg/kg body weight was also given as reference standard and compaired the different liver function test and found safe recovery of the treated group. The results concluded that the Dregea volubilis fruit has hepatoprotective activity.

Plaa GL, et al., (2006)[38] studied on preparation of quercetin solid lipid nanoparticles and oral absorption in mice model. In this study nanoparticles were prepared by emulsion evaporation at a high temperature and solidification method at low temperature. Result shows QT-SLN morphology was sphere like and smooth in nature. Mean diameter was 217.3nm. Entrapment efficiency was 48.50%. The mice oral absorption of QT-SLN was much better than quercetin solution.

Prakash J, et al., (2001)[39] discussed the various techniques for the fabrication of nano drug delivery system (NDDS) like nanoparticles, nanocrystals, nanosuspension, and nanoemulsions by various techniques such as bottom-up (Microprecipitation, microemulsion and melt emulsification) top –down method (High pressure homogenization and milling method).

Reitman S, et al., (2012)[40]produced small anionic polymeric (Eudragit) nanoparticles containing quercetin in a non-crystalline form. They proved that after mouth stomach and small intestine stages the amount of quercetin released was around 43%,16% and 7% for free quercetin dispersed in water and around 5%, 3%, 2% of quercetin encapsulated within polymeric nanoparticles. These results suggested that nanoparticles protected quercetin from dissolution in mouth and stomach but promoted its release in small intestine.

Setzer WN, et al., (2007)[41] prepared quercetin-loaded nanoparticles with gelation of chitosan with tripolyphosphate anions poly-D, L- lactide (PLA) nanoparticles by

solvent evaporation method inorder to improve aqueous solubility and stability of quercetin. Nano-encapsulation of quercetin into PLA nanoparticles significantly improves therapeutic efficacy and bioavailability of this molecule. The *in vitro* release studies showed that 40-45% quercetin was released within 0-0.5h showing rapid burst release.

Shahank T, et al., (2011)[42] Carried out the physicochemical characterization of nanoparticles using different methods like transmission electron microscopy (TEM), differential scanning calorimetry (DSC), powder X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR) and dissolution study. Particle size determination, encapsulation efficiency, drug release and SEM studies have also been used for characterization of nanoparticles.

Streeter A.J, et al., (2008)[43] developed quercetin loaded- nanoparticles (QUEN) by nanoprecipitation technique with Eudragit E (EE) and polyvinyalcohol (PVA) as carriers and to evaluate antioxidant effects of quercetin (QU) and its nanoparticles. Results from powder X-ray diffraction (XRD) and differential scanning calorimetry (DSC) of QUEN showed that crystal of drug might be converted to an amorphous state. Release of drug from QUEN was 74- fold higher compared with pure drug. In addition antioxidant activity of QUEN was more effective than pure QU.

Hussain MA, et al., (2014)[44] performed Intracellular ROS protection efficiency and free radical-scavenging activity of quercetin and quercetin-encapsulated liposomes.this study indicated that liposomal quercetin can significantly improve the solubility and bioavailability of quercetin and can be used as an effective antioxidant for ROS protection within the polar cytoplasm, and the nano-sized quercetin encapsulated by liposomes enhanced the cellular uptake (cancer cell human MCF_7). Quercetin has many pharmaceutical applications, many of which arise from its potent antioxidant properties.

Zakaria ZA, et al., (2014)[45] developed Quercetin-containing self-nanoemulsifying drug delivery system for improving oral bioavailability Q-SNEDDS formed a nanoemulsion having a droplet size of 208.8 ± 4.5 nm and zeta potential of -26.3 ± 1.2 mV. The presence of Tween® 80 and PEG 400 increased quercetin solubility and maintained supersaturated quercetin concentrations (5 mg/mL) for >1 month. The optimized Q-SNEDDS significantly improved quercetin transport across a human colon carcinoma (Caco-2) cell monolayer. Fluorescence imaging demonstrated rapid absorption of the Q-SNEDDS within 40 min of oral ingestion. Following oral administration of Q-SNEDDS in rats (15 mg/kg), the area under the concentration curve and maximum concentration of plasma quercetin after 24 h increased by approximately twofold and threefold compared with the quercetin control suspension. These data suggest that this Q-SNEDDS formulation can enhance the solubility and oral bioavailability of quercetin for appropriate clinical application.

Mukherjee PK, et al., (2013)[46]formulated and optimized the topical delivery of quercetin from solid lipid based nanosystems. In this research work, a solvent-free solid lipid based nanosystem has been developed and evaluated for topical delivery of quercetin. Systematic screening of the formulation and process parameters led to the development of a solid lipid (glyceryl dibehenate) based nanosystem using a probe ultrasonication method. The selected variant demonstrated good physical stability for up to 8 weeks at 2-8 °C. Transmission electron microscopy (TEM) images showed spherical particles in the nanometer range. In vitro release studies showed biphasic release of quercetin from the SLN formulation, with an initial burst release followed by prolonged release for up to 24h. In vitro permeation studies using full thickness human skin showed higher amounts of quercetin to be localized within the skin compared to a control formulation with particles in the micrometer range. Such accumulation of quercetin in the skin is highly desirable since the efficacy of quercetin in delaying ultra-violet radiation mediated cell damage and eventual necrosis mainly occurs in the epidermis.

Panda S, et al., (2012)[47] studied Anticancer effect and mechanism of polymer micelle-encapsulated quercetin on ovarian cancer. In this work, encapsulated QU into biodegradable monomethoxy poly(ethylene glycol)-poly(ε-caprolactone) (MPEG-PCL) micelles and tried to provide proof-of-principle for treating ovarian cancer with this nano-formulation of quercetin. These QU loaded MPEG-PCL (QU/MPEG-PCL) micelles with drug loading of 6.9% had a mean particle size of 36 nm, rendering the complete dispersion of quercetin in water. QU inhibited the growth of A2780S ovarian cancer cells on a dose dependent manner in vitro. Intravenous administration of QU/MPEG-PCL micelles significantly suppressed the growth of established xenograft A2780S ovarian tumors through causing cancer cell apoptosis and inhibiting angiogenesis in vivo. Furthermore, the anticancer activity of quercetin on ovarian cancer cells was studied in vitro. Quercetin treatment induced the apoptosis of A2780S cells associated with activating caspase-3 and caspase-9. MCL-1 downregulation, Bcl-2 downregulation, Bax upregulation and mitochondrial transmembrane potential change were observed, suggesting that quercetin may induce apoptosis of A2780S cells through the mitochondrial apoptotic pathway. Otherwise, quercetin treatment decreased phosphorylated p44/42 mitogen-activated protein kinase and phosphorylated Akt, contributing to inhibition of A2780S cell proliferation. This data suggested that QU/MPEG-PCL micelles were a novel nano-formulation of quercetin with a potential clinical application in ovarian cancer therapy.

Jatwa R, et al., (2013) [48]developed A transdermal delivery system to enhance quercetin nanoparticle permeability . A quercetin nanoemulsion was prepared using poly(lactic-co-glycolic acid) (PLGA), hyaluronic acid (HA) and emulsifier (Tween-20) through a solvent evaporation technique. The efficiency of the nanoemulsion was evaluated with and without chemical permeation enhancer (CPE). The FT-IR result shows no interaction between quercetin and polymer that proves excellent compatibility. The transdermal delivery ability was evaluated using in vitro release and ex vivo permeation analysis. The transdermal drug-release mechanism was

studied by the mathematical model and was found to obey a zero-order, diffusion-controlled mechanism. In vitro toxicity and cell behavior, including cell adhesion, proliferation and cell death of quercetin-nanoemulsion-treated L929 cells.The produced nanoemulsion showed a high encapsulation efficiency, less toxicity, controlled delivery with enhanced transdermal drug permeation and effective scavenging of free radicals.

Murugan M, et al., (2013)[49] performed a Long-term stability of quercetin nanocrystals prepared by different methods. Quercetin nanocrystals were prepared by high pressure homogenization, bead milling and cavi-precipitation. The nanocrystals produced by these methods were compared for particle size, saturation solubility and dissolution of the drug particles, and were subjected to stability testing. Key finding of this study the X-ray diffraction study and microscopic pictures taken under polarized light indicated the crystalline nature of the nanocrystals produced by the three methods. As the crystalline state is relatively more stable than the amorphous state, a good physical stability was expected from the quercetin nanocrystals prepared. The high-pressure homogenized and bead-milled quercetin nanocrystals showed excellent physical stability when stored under refrigeration (4±2°C) and at room temperature (25±2°C) for 180 days. The dissolution properties were not significantly affected on storage at room temperature. However, increase in the storage temperature to 40±2°C led to physical instability. On the other hand, the cavi-precipitated quercetin nanocrystals exhibited a lower stability than the bead-milled and homogenized formulations and did not show the optimum zeta potential values as well. In the case of cavi-precipitated nanocrystals, recrystallization and agglomeration were responsible for the increasing particle size besides the Ostwald ripening phenomenon. The solvents used during cavi-precipitation might have competed with the surfactant for hydration leading to a partial dehydration of the surfactant, which subsequently affected the stability of the quercetin nanocrystals.This study concluded that High-pressure homogenized and bead-milled quercetin nanocrystals showed better physical stability than the cavi-

precipitated ones. Freeze drying immediately after nanocrystal production can help to prevent their agglomeration and thus improve physical stability.

Boonphong S, et al., (2010)[50] Design and characterization of protein-quercetin bioactive nanoparticles. Induced by dimethyl sulfoxide (DMSO), BSA, Lys, and Mb formed spherical nanocarriers with sizes less than 70 nm. After loading Q, the size was further reduced by 30%. The adsorption of Q on protein is mainly hydrophobic, and is related to the synergy of Trp residues with the molecular environment of the proteins. Seven Q molecules could be entrapped by one Lys molecule, 9 by one Mb, and 11 by one BSA. The controlled releasing measurements indicate that these bioactive nanoparticles have long-term antioxidant protection effects on the activity of Q in both acidic and neutral conditions. The antioxidant activity evaluation indicates that the activity of Q is not hindered by the formation of protein nanoparticles. Other flavonoids, such as kaempferol and rutin, were also investigated. These studies conclude the BSA exhibits the most remarkable abilities of loading, controlled release, and antioxidant protection of active drugs, indicating that such type of bionanoparticles is very promising in the field of bionanotechnology.

Lakshmi BVS, et al., (2009)[51] performed the Development of a quercetin-loaded nanostructured lipid carrier formulation for topical delivery. QT-NLCs were prepared by the method of emulsion evaporation-solidification at low temperature. The average entrapment efficiency and drug loading of the optimized QT-NLCs were 89.95 ± 0.16% and 3.05 ± 0.01%, respectively. Under the transmission electron microscope, the nanoparticles were spherically shaped. The average particle size was 215.2 nm, the zeta potential was -20.10 ± 1.22 mV and pH value of QT-NLCs system was 4.65. Topical delivery of QT in the form of NLCs was investigated in vitro and in vivo. The results showed that QT-NLCs could promote the permeation of QT, increase the amount of QT retention in epidermis and dermis, and enhance the effect of anti-oxidation and anti-inflammation exerted by QT. Then the

mechanism of NLCs for facilitating drug penetration was further investigated through histological sections. In conclusion, NLCs could be a promising vehicle for topical delivery of QT.

Anand KV, et al., (2010)[52] formulated oral active nanomicellar of quercetin in the treatment of lung cancer. Quercetin-loaded nanomicelles were prepared by using the film casting method, and were evaluated in terms of drug incorporation efficiency, micelle size, interaction with Caco-2 cells, and anticancer activity in the A549 lung cancer cell line and murine xenograft model. In this study result shows that the incorporation efficiency into the nanomicelles was ≥88.9% when the content of quercetin was up to 4% w/w, with sizes of 15.4-18.5 nm and polydispersity indices of <0.250. Solubilization of quercetin by the nanomicelles increased its aqueous concentration by 110-fold. The quercetin nanomicelles were stable when tested in simulated gastric (pH 1.2) and intestinal (pH 7.4) fluids, and were non-toxic to the Caco-2 cells as reflected by reversible reduction in transepithelial electrical resistance and ≤25% lactose dehydrogenase release. The anticancer activity of quercetin could be significantly improved over the free drug through the nanomicellar formulation when tested using the A549 cancer cell line and murine xenograft model. The nanomicellar quercetin formulation was well tolerated by the tumor-bearing animals, with no significant weight loss observed at the end of the 10-week study period. Concluded that a stable PEG-PE nanomicellar formulation of quercetin was developed with enhanced per oral anticancer activity and no apparent toxicity to the intestinal epithelium.

Annegowda HV, et al., (2012)[53] Development of biodegradable nanoparticles for delivery of quercetin. The antioxidant molecule quercetin has been encapsulated on poly-D,L-lactide (PLA) nanoparticles by solvent evaporation method for the improvement of its poor aqueous solubility and stability. The result shows that surface morphology and average size of PLA and quercetin loaded PLA nanoparticles are 170+/-25 and 130+/-30 nm respectively. The antioxidant activities

of the PLA encapsulated quercetin nanomedicine are identical to free quercetin. The nanoencapsulation efficiency of quercetin evaluated by HPLC and antioxidant assay is 96.7%. The in vitro release kinetics under physiological condition show initial burst release followed by slow and sustained release. The complete release and maximum retention of quercetin is 72 and 96h respectively. The less fluorescence quenching efficiency of quercetin-PLA nanoparticles than free quercetin on BSA confirms the controlled release of quercetin from PLA nanoparticles. These properties of PLA encapsulated quercetin molecule pave way for encapsulating various therapeutically less useful highly active antioxidant molecules towards the development of better therapeutic compounds.

Nagi BS, et al ., (2008)[54] prepared Dual agents loaded PLGA nanoparticles: systematic study of particle size and drug entrapment efficiency.PLGA nanoparticles simultaneously loaded with vincristine sulfate (VCR) and quercetin (QC) were prepared via O/W emulsion solvent evaporation. Six independent processing parameters and PLGA characteristics were assessed systematically to enhance the incorporation of the dual agents with different properties (VCR and QC, hydrophilic and hydrophobic molecule, respectively) into PLGA nanoparticles and control particle size. Approaches investigated for the enhancement of drug entrapment efficiencies and the controlling of particle size included the influence of the molecular weight (MW) of PLGA and the lactide-to-glycolide (L:G) ratio of PLGA, PLGA concentration, PVA concentration, initial QC content, acetone-to-dichloromethane (A/D) volume ratio, aqueous phase pH and aqueous to organic phase (W/O) volume ratio. The nanoparticles produced by optimal formulation were submicron size (139.5+/-4.3 nm, n=3) with low polydispersity index (0.095+/-0.031, n=3). Nanoparticles observed by transmission electron microscopy (TEM) showed extremely spherical shape. The entrapment efficiencies determined by high performance liquid chromatography (HPLC) by ultracentrifuge method were 92.84+/-3.37% for VCR and 32.66+/-2.92% for QC (n=3). The drug loadings were 0.0037+/-0.0001% for VCR and 1.36+/-0.12% for QC (n=3).

Fang EF, et al., (2009)[55] developed quercetin by solid lipid nanoparticles that Enhance gastrointestinal absorption. The aim of the present study is to design and characterize quercetin-loaded solid lipid nanoparticles (QT-SLNs), clarify the absorption mechanism of QT-SLNs and to evaluate the potential of using solid lipid nanoparticles (SLNs) as an oral delivery carrier for poorly water soluble drugs. QT-SLNs were prepared by an emulsification and low-temperature solidification method. The QT-SLNs presented as spherically shaped under transmission electron microscopy, with an average diameter of 155.3 nm. The average drug entrapment efficiency, drug loading and zeta potential were 91.1%, 13.2% and -32.2 mV, respectively. Drug release from QT-SLNs was fitted to a double phase kinetics model and the equation was as follows: 100-Q=98.87e(-0.1042t)+42.45e(-0.0258t). The absorption of QT-SLNs in the gastrointestinal (GI) tract was studied using an in situ perfusion method in rats. It was found that the absorption percent in the stomach for 2 h was only 6.20%, the absorption process of intestine was first-process with passive diffusion mechanism, and the main absorptive segments were ileum and colon. A pharmacokinetic study was conducted in rats after oral administration of quercetin at 50 mg/kg in the form of either QT-SLNs or suspension. The plasma concentration-time curves were both fitted to a one-compartment model. The relative bioavailability of QT-SLNs to quercetin suspension was 571.4%. The T(max) and MRT for quercetin in plasma were both delayed. Our studies provide evidence that SLNs are valuable as an oral delivery carrier to enhance the absorption of a poorly water soluble drug, quercetin.

Naravana KR, et al., (2013)[56] developed the Co-encapsulation of tamoxifen and quercetin in polymeric nanoparticles: implications on oral bioavailability, antitumor efficacy, and drug-induced toxicity. The developed formulation was found to have particle size 185.3 ± 1.20 nm, PDI 0.184 ± 0.004, entrapment efficiency 67.16 ± 1.24% Tmx, 68.60 ± 1.58% QT at a Tmx/QT ratio of 1:2 w/w. The stability of the freeze-dried formulation was established in simulated gastrointestinal fluids for 8 h and at accelerated stability condition for 3 months. DPPH free radical scavenging

assay confirmed that the functional architecture of QT was retained in freeze-dried NPs. Higher cellular uptake, cytotoxicity, and nuclear co-localization of Tmx-QT-NPs in MCF-7 cells revealed higher efficiency of the formulation. At the same time, higher Caco-2 cell uptake revealed its potential for oral delivery, which was well corroborated with in vivo pharmacokinetics, which suggested ~ 5-fold and ~ 3-fold increase in oral bioavailability as compared to the free Tmx citrate and free QT, respectively. Concomitantly, significantly higher tumor suppression was observed in the case of the developed formulation in contrast to respective free drug(s) and their combination when tested against a DMBA-induced breast cancer model in female SD rats. Multiple oral administrations of Tmx-QT-NPs efficiently controlled the tumor angiogenesis as revealed by normalized levels of respective markers (MMP-2 and MMP-9). The safety profile of Tmx-QT-NPs was also established, and no measurable hepatotoxicity or oxidative stress was observed when measured as a function of respective biochemical markers in contrast to free drug(s) and their combinations. In a nutshell, the co-encapsulation strategy with PLGA-NPs could be a promising approach in improving oral delivery of Tmx and QT for cancer therapy.

3.0. PLANT PROFILE

KINGDOM : Plantae

CLADE : Angiosperms

ORDER : Gentianales

FAMILY : Rubiaceae

GENUS : Ixora

TAMIL NAME : Vetchi

Fig. No. 6: Leaves of *Ixora chenensis*

Ixora is a large genus of about 400 species of popular evergreen shrubs and small trees, many of which are native to southern china, India and Srilanka and belong to the taxonomic family rubiaceae. A few of these species are grown in gardens for their vibrantly coloured flowers produced in large clusters at the tips of branches. Various species of *Ixora* are otherwise called as West Indian Jasmine , Jungle Flame , Flame of the World , Jungle Geranium etc., but are very popular by the genus name, *Ixora.*

CHARACTERISTICS:

Ixora is a compact, multibranched evergreen shrubs or small tree, commonly 4 to 6 feet in height, but can reach up to 12 feet height. Leaves are glossy, leathery, oblong, about 4 to 6 inches long and are arranged in opposite pairs.

PHYTOCHEMICAL CONSTITUENTS :

***** D- Mannitol

* Stearic acid

* 1,5-Cyclooctadiene

* Beta-Sitosterol

* Azelaic acid

* Dihydromasticadienolic acid

* Ixoraliphatic acid

MEDICINAL USES :

A decoction of the root is used after child birth. A decoction is used against bronchial disorders. An infusion of the fresh flowers is said to be a remedy against incipient tuberculosis and haemorrhage. A decoction is prescribed in the treatment of amenorrhoea and hypertension. An infusion of leaves or flowers is used against headache.

4.0 AIM & OBJECTIVE

Herbal drugs play a vital role in the management of various liver disorder, most of them speed up the natural healing process of liver. Numerous medicinal plants and their formulations are used in liver disorders in ethno medicinal practices as well as traditional system of medicine in India. According to WHO about 18,000 people die every year due to hepatic disease. Liver diseases remain one of the serious health problems is the absence of variable liver protective drugs. The common ailments of liver are cirrhosis, cholestasis, hepatitis, portal hypertension, hepatic encephalopathy, hepatic failure and certain tomours like hepatoma.

The aim of the research is to find out new hepatoprotective drugs from indigenous plants which are potent and nontoxic agents.. Present study deals with the hepatoprotective activity leaves of *Ixora chenensis*.

5.0 PLAN OF THE WORK

- To investigate the organoleptic and morphological characteristics of *Ixora chenensis* leaves to establish the pharmacognostical standards of the plants.
- To carry out preliminary phytochemical analysis of extracts of leaves of *Ixora chenensis* to establish the different classes of compounds present their leaves.
- To evaluate the extracts of *Ixora chenensis* leaves for antioxidant activity by different radical scavenging methods and methods based on reducing ability of extracts.
- To evaluate the extracts of *Ixora chenensis* for acute toxicity potential and establish their margin of therapeutic safety.
- To evaluate the hepatoprotective activity of extracts of *Ixora chenensis* leaves against carbon tetrachloride induced liver damage in rats.

6.0 MATERIALS AND METHODS

6.1 Plant collection and authentification

The leaves of *Ixora chenensis* were collected from Kolli forest area, Tamilnadu and which was authenticated.

6.2 Preparation of coarse powder and Extraction technique

The leaves were shade dried at room temperature for 10 days. Then these were milled into powder by mechanical grinder. This powder was sequentially extracted to their increasing polarity with Petroleum ether, Ethyl acetate, Ethanol respectively. About 500gm of powdered leaves was uniformly packed into a thimble in a soxhlet apparatus and extracted with 1000ml Petroleum ether, Ethyl acetate and Ethanol, respectively. Constant heat was provided by Mantox heater for recycling of the solvent. The process of extraction continues for 1-2 hours for each solvent. The excess solvent was evaporated and the dried extracts were kept in refrigerator at 4ºC for their future use in phytochemical analysis and pharmacological screenings.

6.3 Phytochemical Analysis

6.3.1 Test for carbohydrates

- Molisch's test: Dissolved small quantity of 300mg alcoholic and dried leaves extract powder of Pimenta dioica separately in 4ml distilled water and filtered. The filtrate was subjected to Molisch's test.

- Fehling's test: Dissolve a small portion of extract in water and treat with Fehling's solution.

- Phenols test: The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapours.

6.3.2 Test for flavanoids

- Shinoda test: To 2 to 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added .
- .Lead acetate test: To 5ml of extract 1ml of lead acetate solution was added.

6.3.3 Test for tannins

- Braemer's test: To a 2 to 3ml of extract, 10% alcoholic ferric chloride solution was added.

6.3.4 Test for steroid/terpenoid

- Liebermann-Burchardt test: To 1ml of extract, 1ml of chloroform, 2 to3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added.

6.3.5 Test for alkaloids

- Draggendorf's test: A drop of extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Draggendorf's reagent.
- Hager's test: The extract was treated with few ml of Hager's reagen.
- Wagner's test: The extract was treated with few ml of Wagner's reagent.

6.3.6 Tests for Glycosides

- Legal's test: Dissolved the extract [0.1g] in pyridine [2ml], added sodium nitroprusside solution [2ml] and made alkaline with Sodium hydroxide solution.

6.3.7 Test for Saponins

- Foam test: 1ml of extract was dilute with 20ml of distilled water and shaken with a graduated cylinder for 15 minutes.

6.3.8 Test for Anthraquinones

- Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml of concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia.

6.3.9 Test for Amino acids

- Ninhydrin test: Dissolved a small quantity of the extract in few ml of water and added 1ml of ninhydrin reagent.

6.3.10 Test for fixed oils and fats

Press small quantity of the petroleum ether extract between two filter paper.

Note: the results for the above experiments can be noted as follows.

- If the response to the test is high it can be noted as +++which indicates that the particular group is present as the major class.
- If the response is average then note it as ++ indicates the presence in moderate quantity.
- If the response is very small then note it as + indicating the presence of only in traces.
- If no response is then negative.

6.4 Pharmacological screenings

6.4.0 *Invitro* Antioxidant activity

6.4.1 Reducing power by Ferric Chloride(FRAP)

- ✓ The standard stock solution was prepared by dissolving ascorbic acid (Standard Sample) in suitable solvent (methanol) with a final concentration of 1000 µg/ml and different concentration of 100, 200, 300, 400, 500 µg/ml were prepared by distilled water.

- ✓ About 100 mg of *Ixora chenensis extract* were dissolved in 100 ml of methanol to obtain a solution of 1000µg/ml. From this stocking solutions, various working conc. were produced to get concentration of 100, 200, 300, 400, 500 µg/ml with distilled water.

- ✓ About 1.5 milliter of 10 percentage tri-chloro aceticacid was then dropped to the mixture and the contents were centrifuged at 3000 rpm for 10 minutes and about 1.5 ml of supernatant liquid was collected and mixed with 1 ml of distilled water and 0.5 ml of 1 % $FeCl_3$.

- ✓ The absorbance noted at seven hundred nanometer using UV-Visible Spectrophotometer.Difference in the absorbance between test and control was calculated and expressed as reducing power in percentage.[95-104]

- ✓ Ability of reducing power of test and control in percentage was calculated by following formula:

$$IC_{50}\% = 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of Control}} X\,100$$

- ✓ All experimentsdone three times.

6.4.2 DPPH assay

- ✓ 0.3mM DPPH solution was prepared by dissolving DPPH (5.91mg) in 50 ml of methanol.This stock solution was prepared freshly and kept in the dark at ambient temperature when not in used.

- ✓ The standard stock solution was prepared by dissolving ascorbic acid (Standard Sample) in suitable solvent (methanol) with a final concentration of 1000 µg/ml and different concentration of 100, 200, 300, 400, 500 µg/ml were prepared by distilled water.

- ✓ About 100 mg of *Ixora chenensisextract* were dissolved in 100 ml of methanol to obtain a solution of 1000µg/ml. From this stocking solutions, various working conc. were produced to get concentration of 100, 200, 300, 400, 500 µg/ml with distilled water.

- ✓ The standard stock solution was prepared by dissolving ascorbic acid (Standard Sample) in suitable solvent (methanol) with a final concentration of 1000 µg/ml and different concentration of 100, 200, 300, 400, 500 µg/ml were prepared by distilled water.

- ✓ 0.3 mM solution of free radical standard in CH_3OH produced and 1 ml of this solution was admixed to three milliliter of test solution in water at various different conc and kept for 30 minutes, the absorbance was taken at 517nm. Difference between the absorbance value of sample and control of (DPPH) was obtained and given as percent scavenging of free radical.[95-104]

$$IC_{50}\% = 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of Control}} \times 100$$

- ✓ All experimentswere performed in triplicate.

6.4.3 $ABTS^+$ Radical Scavenging Assay

- ✓ The standard stock solution was prepared by dissolving ascorbic acid (Standard Sample) in suitable solvent (methanol) with a final concentration of 1000 µg/ml and different concentration of 100, 200, 300, 400, 500 µg/ml were prepared by distilled water.

- ✓ About 100 mg of *Ixora chenensisextract* were dissolved in 100 ml of methanol to obtain a solution of 1000µg/ml. From this stocking solutions, various working conc. were produced to get concentration of 100, 200, 300, 400, 500 µg/ml with distilled water.

- ✓ The standard stock solution was prepared by dissolving ascorbic acid (Standard Sample) in suitable solvent (methanol) with a final concentration of 1000 µg/ml and different concentration of 100, 200, 300, 400, 500 µg/ml were prepared by distilled water.

- ✓ $ABTS^+$ radical was freshly prepared by adding 5 ml of 4.9 nM ammonium persulfate solution to 5ml of 14 mM ABTS solution and kept for 16 hrs in the dark ambience. Resultant solution was dispersed with ethyl alcohol (99.5 percentages) to gained value of 0.700 ± 0.020 at 734 nanometer and the similar was utilized for assay purpose.

- ✓ About 950µl of ABTS radical solution has been added with 50 microliter of sample or referrals solution and the reaction mixture was vortexed for 10 sec and kept for 6 minutes.

- ✓ The absorbance was recorded at 734 nm compared with the manage ABTS. %age suppression was calculated from following formula. [95-104]

$$IC_{50}\% = 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of Control}} \times 100$$

- ✓ All experimentswere performed in triplicate.

6.4.4 Nitric Oxide Scavenging Method

- ✓ The standard stock solution was prepared by dissolving ascorbic acid (Standard Sample) in suitable solvent (methanol) with a final concentration of 1000 µg/ml and different concentration of 100, 200, 300, 400, 500 µg/ml were prepared by distilled water.

- ✓ About 100 mg of *Ixora chenensisextract* were dissolved in 100 ml of methanol to obtain a solution of 1000µg/ml. From this stocking solutions, various working conc. were produced to get concentration of 100, 200, 300, 400, 500 µg/ml with distilled water.

- ✓ The standard stock solution was prepared by dissolving ascorbic acid (Standard Sample) in suitable solvent (methanol) with a final concentration of 1000 µg/ml and different concentration of 100, 200, 300, 400, 500 µg/ml were prepared by distilled water.

- ✓ Sodium nitroprusside 5mM in phosphate buffer at pH 7.4 saline was added with a range of concentrations of the test sample or standard and incubated at 25^{o}C for 150 minutes. At regular intervals, 1.5 ml of samples (incubated test sample) were taken off and a poured with 1.5 ml Griess reagent (1% Sulphanilamide, phosphoric acid (2 percent), and 0.1 percent NEDA 2.HCL.

- ✓ The absorbance was read at 540 nm.The difference in the absorbance between test and control on nitric oxide was determined and depicted as percent scavenging of NO radical. [95-104]

✓ Capability to scavenge the NO radical was designed by using formulas.

$$IC_{50}\% = 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of Control}} \times 100$$

✓ All experiments were performed in triplicate.

6.5 Acute Oral Toxicity Study

Acute toxicity study was carried using albino rats by up and down/staircase method as per OECD guidelines. The methanolic extract of *Ixora chenensis* Linn. (MEIC) was orally administered to different groups of rats at the doses of 50, 300, 1000, 2000 and 3000 mg/kg body weight, respectively. The animals were observed for 48 hr to study the general behavior and for any sign of discomfort to the animals. There was no mortality found upto dose 3000 mg/kg.

6.6 *In-vivo* Hepatoprotective Activity of *Ixora chenensis* Leaves Extract in CCL_4 Induced Liver Damage in Wistar Rats

Wistar rats (150-200grams) of Male sex were used in the study and were obtained from the Animal house, JKKNCP, Kumarapalayam. The studies were performed with the approved by the institutional animal ethical committee, and these animals were used to evaluate Hepatoprotective activity of *Ixora chenensis* leaves extract. Prior to the experiment the rats were housed in a clean poly propylenecages (6rats/cages) for a period of 7 days under standard temperature (25-30^{o} c), relative humidity (45–55%), dark/lightcycle (12/12hrs). The animals were put in overnight fasting were deprived of food for 16 hrs but allowed free access of water.

6.6.1 Chemicals Required

Carbon Tetra Chloride, Standard Liver Tonic (silymerin), Leaves extract of *Ixora chenensis.*

6.6.2 Animals Required

Species/common name	:	wistar rats
Age/weight/size	:	3 months/180- 200 gms
Gender	:	male
Number of animals to be used:		6 nos

Number of days each animal will be housed in 60 days

6.6.3 Experimental Design

The animals will divided into 4 groups of 6 rats in each.

Group-I- Positive control

Group-II- CCl4 (Carbon tetra chloride) will given intra peritonially (1ml/ kg) with 1:1 dilution of coconut oil on the 5^{th}day.

Group-III- Administered with liver tonic marketed product (5ml/kg) daily for 7 days and on 5^{th} day the CCl4 is induced through intra peritonially (1 ml / kg).

Group-IV- Was treated with alocoholic extract of *Ixora chenensis* high dose for 7 days, CCl4 is administered on day 5. On the 8th day, all rats will be sacrificed and the blood collected, centrifuged and the collected serum samples will studied for Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT) and bilirubin (through kits) tests for the study of the toxic

effect of CCl4 and also the therapeutic effect of the plant extracts. The livers will fixed in the fixative (Bouin's fluid) for the histological study.

6.6.4 Collection of serum

Fasting blood samples were drawn from tail vein of rats at weekly intervals till the end of the study 5, 7, and 8 days.

6.6.5 Estimation of biochemical parameters Serum

On5, 7, and8 days fasting blood samples were collected and analyzed the serum levels.

6.6.6 Statistical analysis

Statistical analysis was done by using GRAPH PAD PRISM 5.0. All the values of Biochemical parameters and body weight were expressed as Mean ± Standard Error Mean (SEM). The values were analyzed for statistical significance using one- way analysis of variance (ANOVA), comparison was done by using Dunnett'st test. P values < 0.05 were considered as significant, P values < 0.01were considered as very significant, P values < 0.001 were considered as highly significant and P > 0.05 were considered as not significant.

7.0 RESULTS & DISCUSSION

7.1 Appearance and percentage yield of Extract

AEIC were a semisolid brownish color extract and the percentage yield was found to be 19.35%

7.2. Phyochemical Analysis

Below two observations indicated absence of Saponins because not Formed stable foam confirmed the test &formation of a soluble emulsion confirmed the test. The formation of blue colour in acetic acid layer confirmed the test. Formed red color was formed in test tube in the presence of glycosides.

The phytochemical screening results revealed that the after which it was observed whether the alkaloids were present by the indication of turbidity and/or precipitate formation. The colour changed from violet to blue or green in some samples indicated the presence of steroids. An interface with a reddish brown coloration was formed in the presence of terpenoids, as positive result. Red coloration identifies the presence of flavonoids (Shinado's test). A colour change was observed in the test tube, which indicated in the presence of tannins. A brown ring formation at the junction and the turning of the upper layer to dark green color confirmed the test for the absence of phytosterols. Some o the test indicated no characteristic changes on test tube by the absence of fixed oil, liginin and gum mucilage.

Table No. 2: Phytochemical screening of the leaf *Ixora chinensis*

S.No	Constituents	Methanol
1	Alkaloids	+
2	Carbohydrates	+
3	Glycosides	+
4	Phytosterol	-
5	Fixed oil	-
6	Saponins	-
7	Tannins	+
8	Proteins & amino acids	+
9	Gum & mucilage	-
10	Flavonoids	+
11	Lignin	-

***Invitro* antioxidant**

The extracts was showed similar increasing trend in activity with increase in extract concentration. There was a strong correlation FRAP values supporting the fact will that phenolics and flavonoids are highly potent antioxidants.

Table No. 3: *Invitro* antioxidant activity byferric Chloride method (FRAP)

S.No.	Concentration (µg/ml)	Leaf extract	Standard (Ascorbic acid)
1.	100	27.08± 0.42	40.96 ± 0.24
2.	200	33.05± 0.18	52.50 ± 0.27
3.	300	40.05 ± 0.35	65.51 ± 0.34
4.	400	51.03 ± 0.96	77.14 ± 0.65
5.	500	67.28± 0.67	88.26 ± 0.48

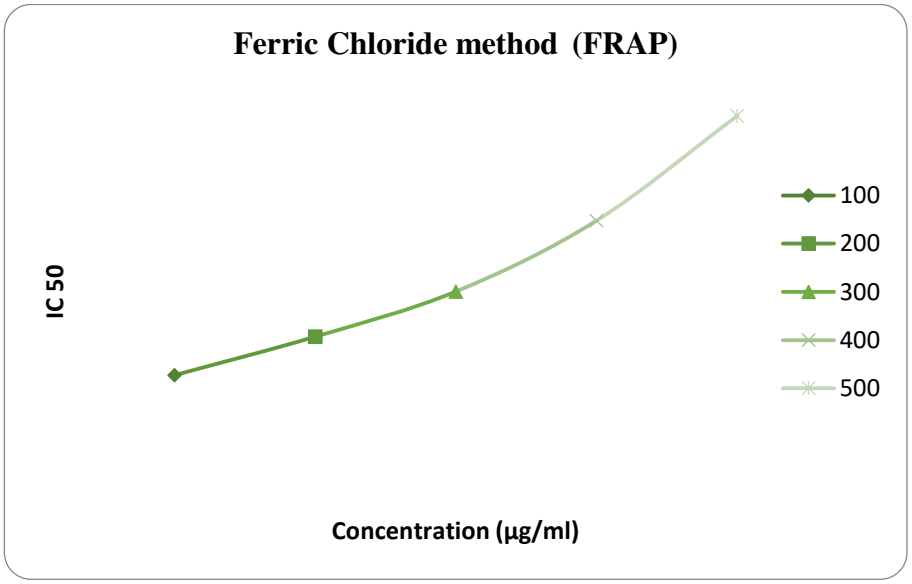

Figure No. 7: Ferric chloride Method- Leaf extract

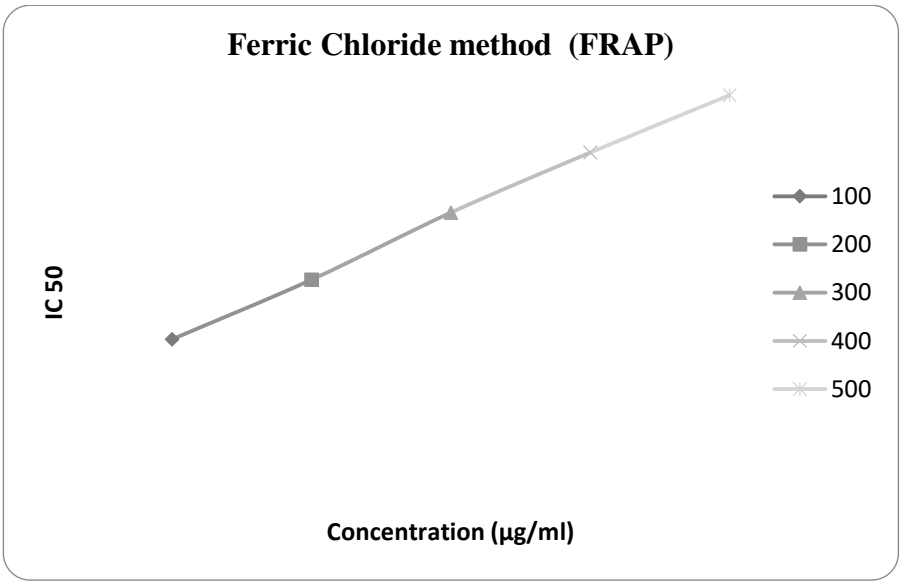

Figure No. 8: Ferric chloride Method- Standard (Ascorbic acid)

***Invitro* antioxidant (DPPH Assay)**

Extract was demonstrate similar rising trend in activity with augment in extract concentration. There was a strong correlation DPPH values supporting the fact will that phenolics and flavonoids are highly potent antioxidants.

Table No. 4: *Invitro* antioxidant activity byDPPH Assay

S.No.	Concentration (µg/ml)	Leaf extract	Standard (Ascorbic acid)
1.	100	58.26 ± 0.39	63.60 ± 0.68
2.	200	59.18 ± 0.29	67.10 ± 0.52
3.	300	60.30 ± 0.61	73.23 ± 0.46
4.	400	61.23 ± 0.23	77.00 ± 0.56
5.	500	71.52± 0.28	84.30 ± 0.36

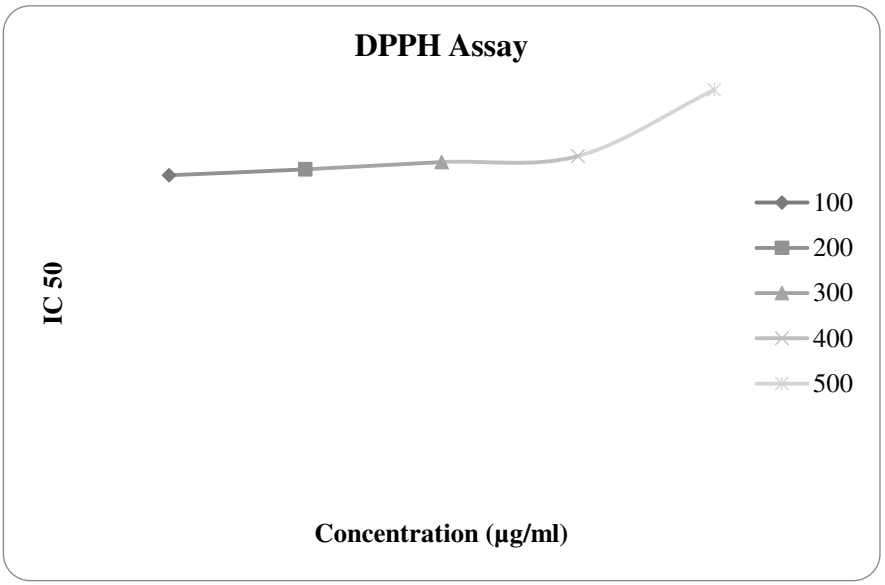

Figure No. 9: DPPH- Leaf extract

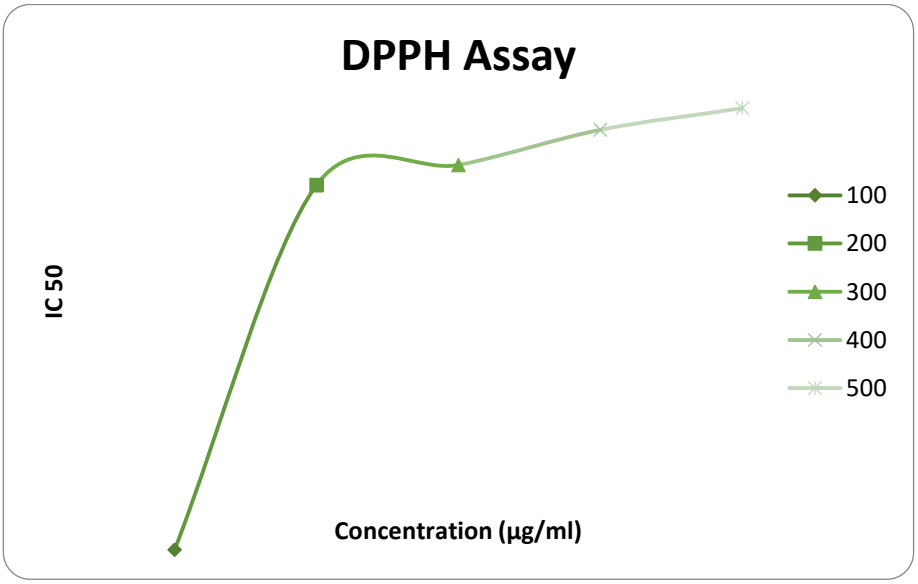

Figure No. 10: DPPH- Leaf extract

***In vitro* Antioxidant ($ABTS^+$)**

Extract was representative alike rising tendency in activity with enhance in extract concentration. There was a strong correlation $ABTS^+$ values supporting the fact will that phenolics and flavonoids are highly potent antioxidants.

Table No. 5: *In vitro* Antioxidant activity by $ABTS^+$

S.No.	Concentration (µg/ml)	Leaf extract	Standard (Ascorbic acid)
1.	100	35.19± 0.48	79.59 ± 0.26
2.	200	45.32 ± 0.38	84.02 ± 0.52
3.	300	56.56 ± 0.28	86.79 ± 0.48
4.	400	61.56 ± 0.26	91.56 ± 0.58
5.	500	71.28± 0.26	95.44 ± 0.45

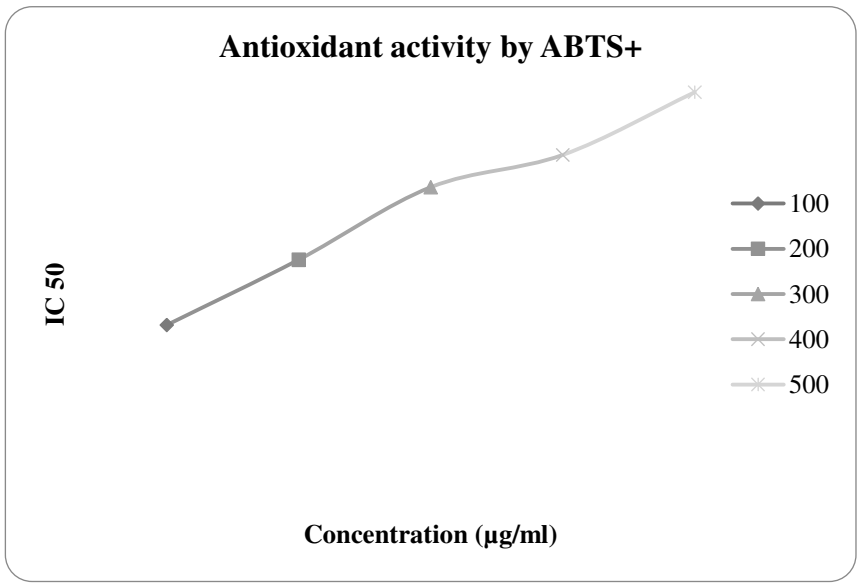

Figure No. 11: DPPH- Leaf extract

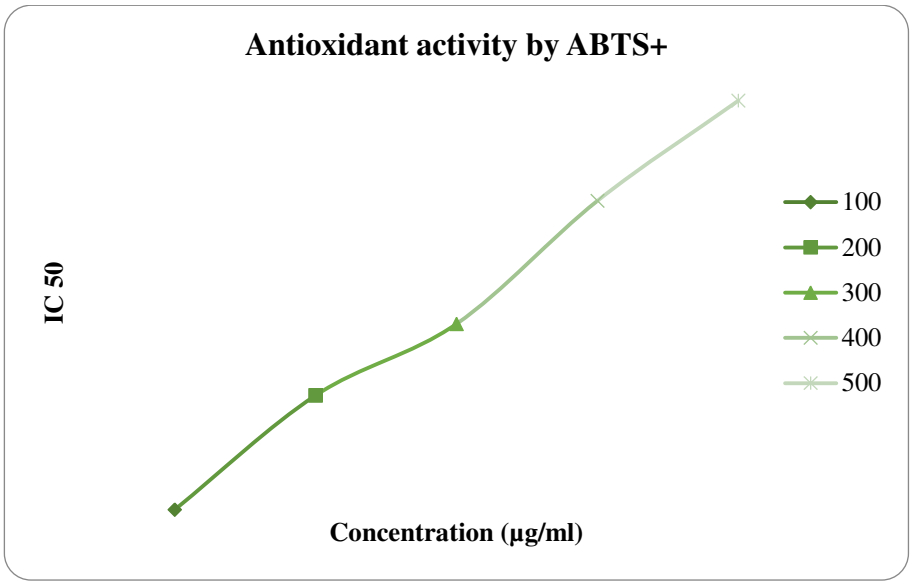

Figure No. 12: DPPH- Standard (Ascorbic acid)

In vitro Antioxidant (Nitric Oxide)

The extracts was showed similar increasing trend in activity with increase in extract concentration. There was a strong correlation Nitric Oxide values supporting the fact will that phenolics and flavonoids are highly potent antioxidants.

Table No. 6: *In vitro* Antioxidant activity by Nitric Oxide Scavenging Method

S.No.	Concentration (µg/ml)	Leaf extract	Standard (Ascorbic acid)
1.	100	27.08± 0.42	40.96 ± 0.24
2.	200	33.05± 0.18	52.50 ± 0.27
3.	300	40.05 ± 0.35	65.51 ± 0.34
4.	400	51.03 ± 0.96	77.14 ± 0.65
5.	500	67.28± 0.67	88.26 ± 0.48

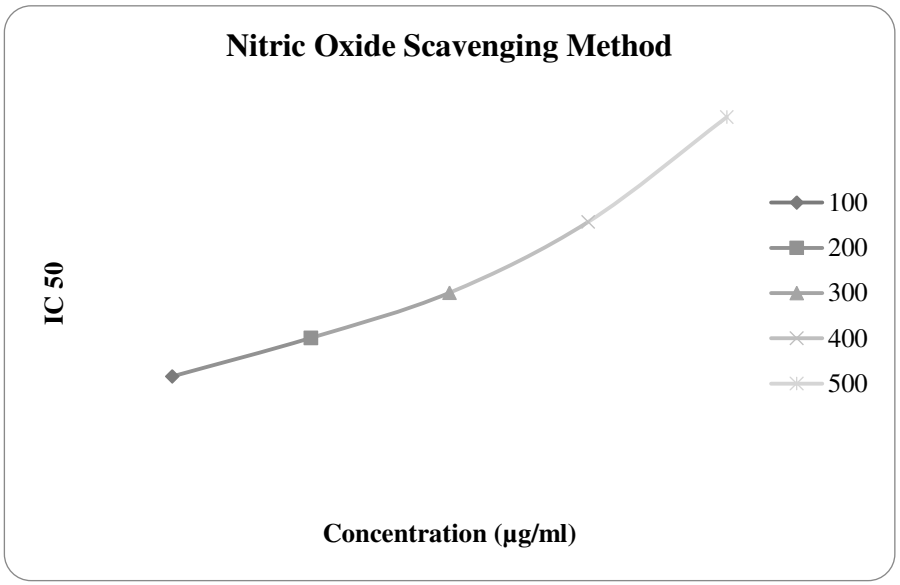

Figure No. 13: Nitric oxide Scavenging- Leaf extract

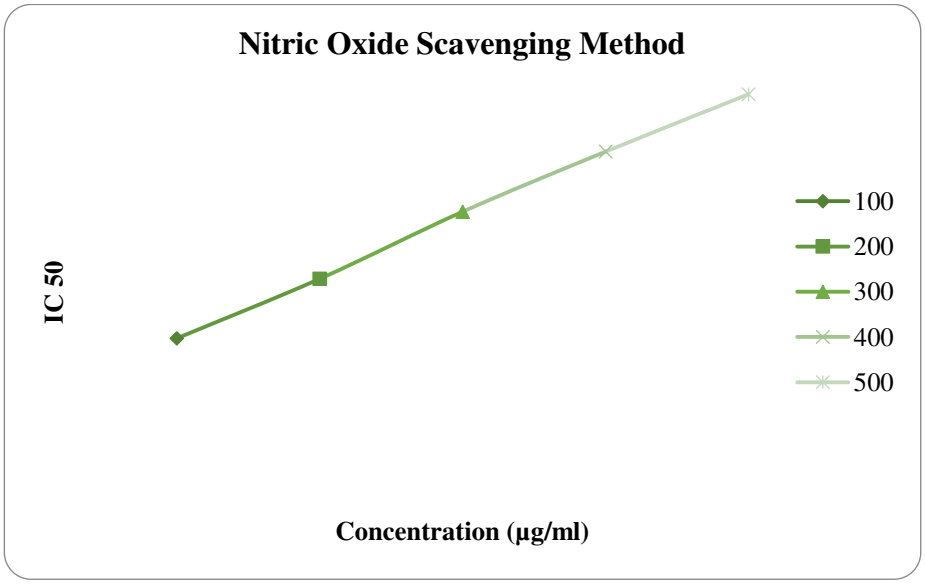

Figure No. 14: Nitric oxide Scavenging- Standard (Ascorbic acid)

Invivo Hepatoprotective Studies

Carbon Tetra Chloride Induced Hepatoprotective Activity

CCl4 is one of the most powerful Hepatotoxins which is able to induce liver damage through the formation of reactive Hepatoprotective activity of Otostegia persica in rats. Free radicals such as Trichloromethyl (CCl3) or Trichloroperoxyl radicals (CCl3 O°3). It can cause severe damages to the liver such as fatty changes Centrilobular steatosis, inflammation, Apoptosis, and Cell necrosis. Therefore, the main intercellular structures which are affected by CCl4 are Plasma membrane, Endoplasmic reticulum, Mitochondria, and Golgi apparatus. As a result of damaging the cell membrane of Hepatocytes, enzymes release in circulation. In the CCl4-treated group, the levels of SGOT, SGPT, AP, Bilurubin, HDL reduced and the levels of Triglyceride, LDL and Total cholesterol increased compared to the normal and control group, indicating severe Hepatocellular damage. After the drug and extract treated group of animalsSGOT, SGPT, AP, Bilurubin, HDL increased and the levels of Triglyceride, LDL and Total cholesterol lowered compared to the normal and control group by the illustration has expressed reduction of Hepatic damage.

Table No. 7: Serum Biochemical extracts

Groups	Category	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Total (g/dl)	Total Bilirubin (mg/dl)	SGOT (IU/L)	SGPT (IU/L)	Alkaline Phosphatase (kA units)
I	Normal Control	150.26±0.05	37.90 ±1.20	15.35±0.81	8.12±0.16	0.26±0.54	37.22±0.22	35.65±0.56	7.43±0.41
II	CCl_4	270.56±0.56	28.67 ±0.56	57.33±1.74	4.22±0.68	1.04±0.21	110.3±0.43	83.76±0.51	25.67±0.92
III	Standard liver tonic + CCl_4	166.67±0.56 ***	32.81±0.79 ***	38.50±0.92 ***	4.01±0.68 ***	1.00±0.31 ***	61.56±0.57 ***	39.05±0.67 ***	12.54±0.38 ***
IV	Extract (400 mg/kg) + CCl_4	190.23±0.58 **	26.13±0.57 **	23.09±0.42 ***	6.04±0.73 **	1.03±0.61 **	62.45±0.58 ***	56.22±0.46 **	13.56±0.88 ***

The Serum levels were analysed by using kits and all values are expressed as mean ±SEM (n=6) group 2 was compared with group 3 were compared with group 4; *p<0.05,**p<0.01,***p<0.001 evaluated by one way ANOVA followed by Dunnet 't' test

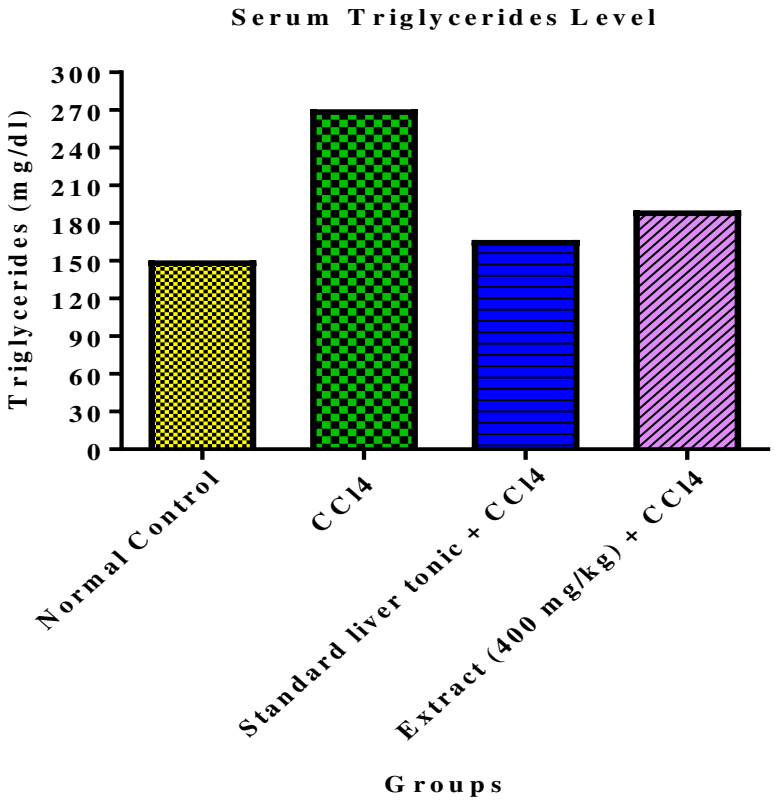

Figure No. 15: Serum Triglyceride Level

In Present Study, CCL4 Induced Hepatoprotective Activity in Wistar Rat significantly ($p<0.001$) increased TG Level. Where as Standard Livertonic group(III) significantly reduced ($p<0.001$) TG Levels. Similarly, the *Ixora Chenensis* Extract dose level of 400mg ($p<0.001$) significantly reduced when compared to Group (II).

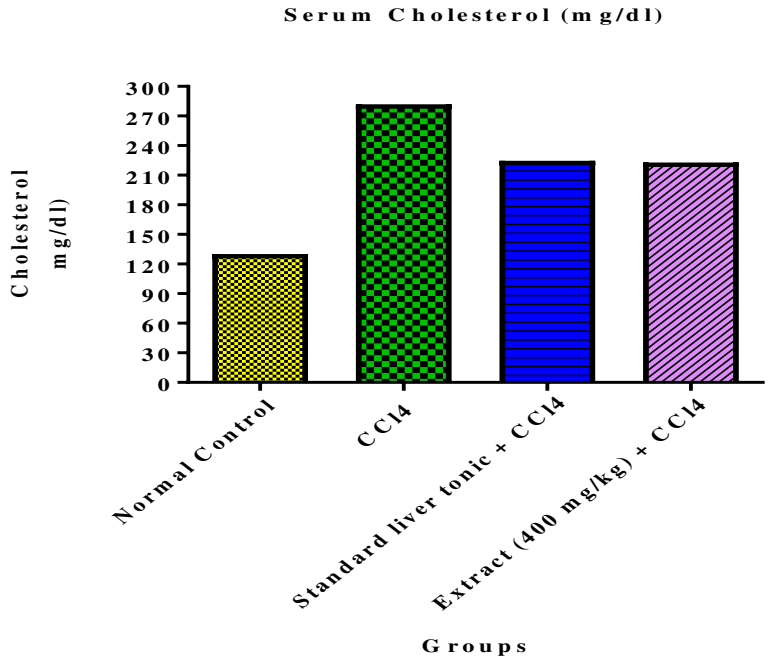

Figure No. 16: Serum Cholesterol Level

In Present Study, CCL4 Induced Hepatoprotective Activity in Wistar Rat significantly ($p<0.001$) increased SC Level. Where as Standard Livertonic group(III) significantly reduced ($p<0.001$) SCLevels. Similarly, the *Ixora Chenensis* Extract dose level of 400mg ($p<0.001$) significantly reduced when compared to Group(II).

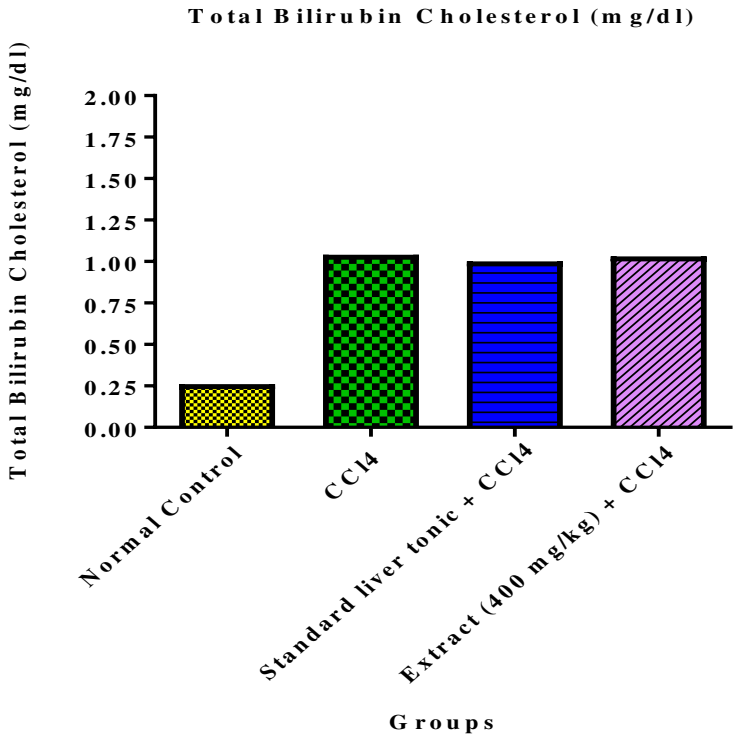

Figure No. 17: Total Bilirubin Cholesterol Level

In Present Study, CCL4 Induced Hepatoprotective Activity in Wistar Rat significantly ($p<0.001$) increased TBC Level When compared to Normal Control. Where as Standard Livertonic group(III) significantly reduced ($p<0.001$) TBC Levels compared to Group(II). The *Ixora Chenensis* Extract dose level of 400mg ($p<0.001$) significantly Increased when compared to Group(III).

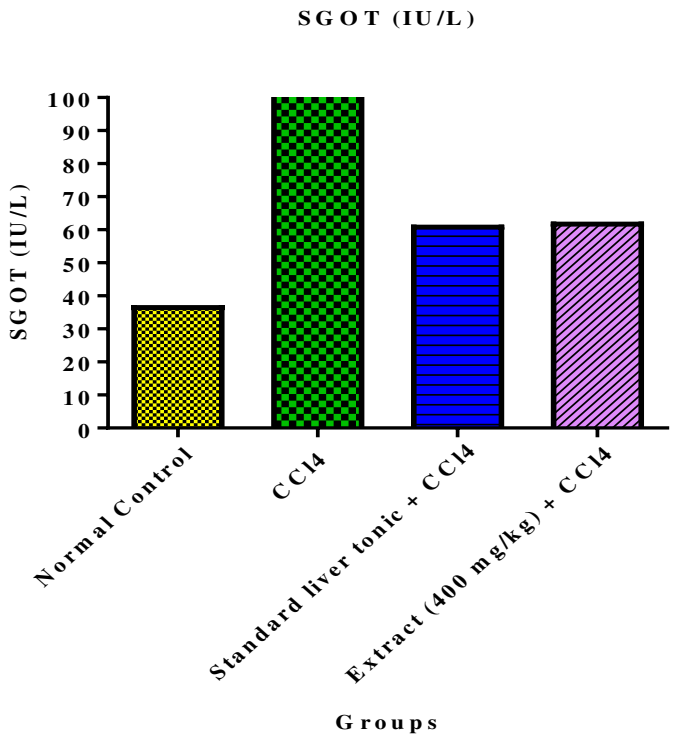

Figure No. 18: Serum Glutamic Oxaloacetic Transaminase Level

In Present Study, CCL4 Induced Hepatoprotective Activity in Wistar Rat significantly ($p<0.001$) increased SGOT Level When compared to Normal Control. Where as Standard Livertonic group(III) significantly reduced ($p<0.001$) SGOT Levels compared to Group(II). The *Ixora Chenensis* Extract dose level of 400mg ($p<0.001$) significantly Increased when compared to Group(III).

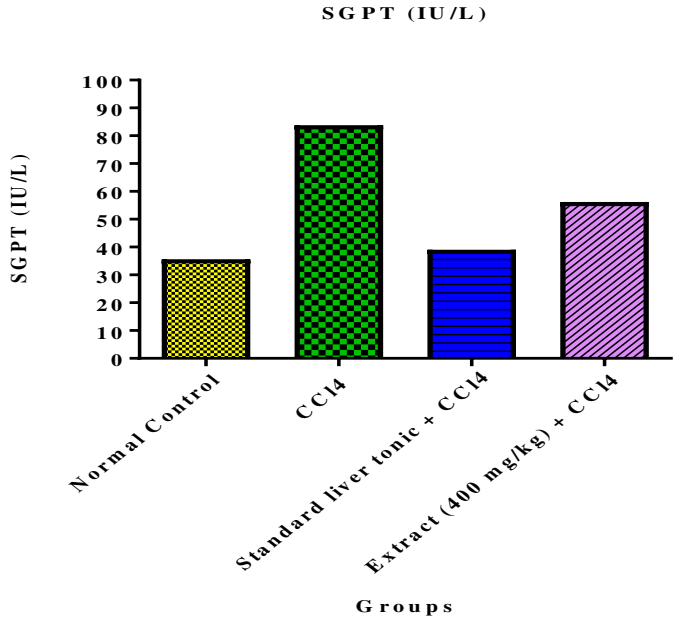

Figure No. 19: Serum Glutamate-Pyruvate Transaminase

In Present Study, CCL4 Induced Hepatoprotective Activity in Wistar Rat significantly ($p<0.001$) increased SGPT Level When compared to Normal Control. Where as Standard Livertonic group(III) significantly reduced ($p<0.001$) SGPT Levels compared to Group(II). The *Ixora Chenensis* Extract dose level of 400mg ($p<0.001$) significantly Increased when compared to Group(III).

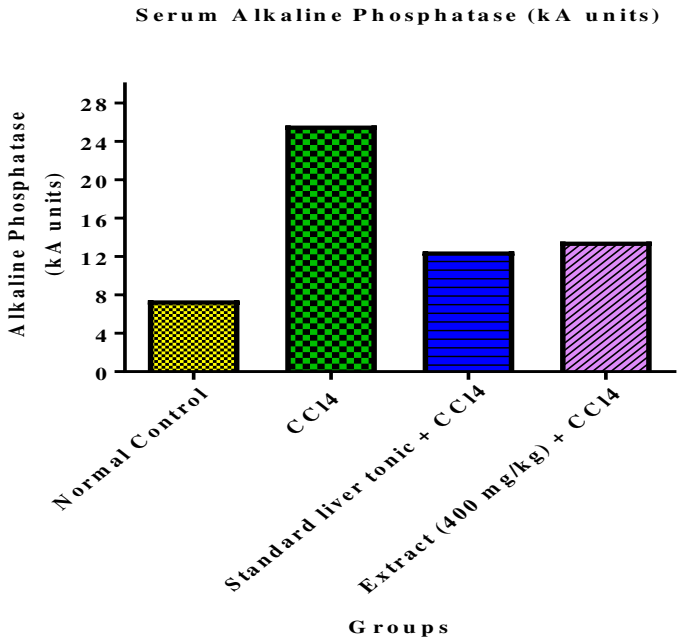

Figure No. 20: Serum Alkaline Phosphatase

In Present Study, CCL4 Induced Hepatoprotective Activity in Wistar Rat significantly ($p<0.001$) increased SKP Level When compared to Normal Control. Where as Standard Livertonic group(III) significantly reduced ($p<0.001$) SKP Levels compared to Group(II). The *Ixora Chenensis* Extract dose level of 400mg ($p<0.001$) significantly Increased when compared to Group(III).

DISCUSSION

For screening the hepatoprotective drugs, liver damage is usually induced by CCL_4. As a result there is a rise in different liver enzyme levels in serum that is SGOT, SGPT, TB, AP and also for cholesterol which have been recognized as a sign to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages.

8.0 CONCLUSION

In conclusion, from the overall result of the biochemical and histopathological examinations, it will be inferred that *Ixora chenensis* which may be have highest hepatoprotective activity among the four tested plant extracts. This work will be useful for extended for the isolation and structure determination of the hepatoprotective principles.

9.0 REFERENCES

1. Sethuraman MG, Lalitha KG, Kapoor BR (2003). Hepatoprotective activity of Sarcostemma brevistigma against carbon tetrachlorideinduced hepatic damage in rats. Curr. Sci., 84(9): 1186-1187.

2. Shahjahan M, Sabitha KE, Mallika J, and Shyamala-Devi CS (2004). Effect of Solanum trilobatum against carbon tetrachloride-induced hepatic damage in albino rats. Indian J. Med. Res., 120: 194-198.

3. Shanani S (1999). Evaluation of hepatoprotective efficacy of APCL-A polyherbal formulation in vivo in rats. Indian Drugs. 36: 628-631.

4. Shim SB, Kim NJ, Kim DH (2000). β-Glucuronidase inhibitory activity and hepatoprotective effect of 18β-glycyrrhetinic acid from the rhizomes of Glycyrrhiza uralensis. Planta Med., 66(1): 40-43.

5. Singanan V, Singanan M, Begum H (2007). The Hepatoprotective Effect of Bael Leaves (Aegle marmalos) in alcohol induced liver injury in albino rats. Int. J. Sci. Technol., 2(2): 83-92.

6. Singh A, Handa SS (1995). Hepatoprotective activity of Apium graveolens and Hygrophila auriculata against paracetamol and thioacetamide intoxication in rats. J. Ethnopharmacol., 49(3): 119- 126.

7. Singh B, Saxena AK, Chandan BK, Agarwal SG, Bhatia MS, Anand KK (1993). Hepatoprotective effect of ethanolic extract of Eclipta alba on experimental liver damage in rats and mice. Phytother. Res., 7(2): 154-158.

8. Singh B, Saxena AK, Chandan BK, Bhardwaj V, Gupta VN, Suri OP, Handa SS (2001). Hepatoprotective activity of indigtone: A bioactive fraction from Indigofera tinctoria Linn. Phytother. Res., 15(4): 294- 297.

9. Song EK, Kim JH, Kim JS, Cho H, Nan JX, Soku DH, Ko GJ , Oh H, Ki YC (2003). Hepatoprotective phenolic constituents of Rhodiola sachalinensis on tacrine-induced cytotoxicity in Hep G2 cells. Phytother. Res., 17(5): 563-565.

10. Subramoniam A, Pushpangadan P (1999). Development of phytomedicine for liver diseases. Indian J. Pharmacol., 31: 166-175.

11. Suja SR, Latha PG, Pushpangadan P, Rajasekharan S (2003). Evaluation of hepatoprotective effects of Rhinacanthus nasuta root extracts. J. Trop. Med. Plants 4(2): 151-157.

12. Suja SR, Latha PG, Pushpangadan P, Rajasekharan S (2004). Evaluation of hepatoprotective effects of Helminthostachys zeylanica (L.) Hook against carbon tetrachloride-induced liver damage in Wistar rats. J. Ethnopharmacol., 92: 61-66.

13. Dams RP. Identification of Essential oils by Gas Chromatography Quadrupole Mass Spectrometry.Carol Stream, USA: Allured Publishing Corporation; 2001.

14. Adeneye AA, Olagunju JA, Benebo AS, Elias SO, Adisa AO, Idowu BO, Oyedeji MO, Isioye EO, Braimoh OB, Oladejo OO, Alana EO. Nephroprotective effects of the aqueous root extract of Harungana madagascariensis [L] in acute and repeated dose acetaminophen renal injured rats. Int J Appl Res Nat Prod. 2008;1:6–14.

15. Aldridge WN. Mechanism of toxicity: New concepts are required in toxicology. Trends in Pharmacol Sci. 1981;2:228–231.

16. Amazu LU, Okokon JE, Nwidu LL. Antiulcer activity of stem extract and fractions of Homalium letestui. Int J Pharmacog. 2015;2:242–247.

17. Antia BS, Okokon JE, Nwidu LL. Antidiarrhoeal activity of stem extract of Homalium letestui. Int J Phytother. 2015;5:86–89.

18. Cooper RA, Durocher JR, Leslie MH. Decreased fluidity of red cell membrane lipids in a beta lipoproteinemia. J Clin Investig. 1977;60:115–121.

19. Dahlin D, Miwa G, Lee A. N-acetyl-pbenzoquinonamine: A cytochrome P450 dependent oxidation product of acetaminophen. Proc Natl Acad Sci. 1984;81:327–331.

20. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82:70–77.

21. Gilani AH, Jabeen Q, Ghayur MN, Janbaz KH, Akhtar MS. Studies on the antihypertensive, antispasmodic, bronchodilator and hepatoprotective activities of the Carum copticum seed extract. J Ethnopharmacol. 2005;98:127–135.

22. Halliwell B, Gutteridge JM. Oxygen toxicity, oxygen radicals, transition metals and disease.Biochem J. 1984;219:1–14.

23. Hutchinson T, Daziel JM. Flora of West Tropical Africa. Vol 2. London: Crown Agents for overseas government; 1963.

24. Ita B, Ngochindo R. Fatty Acid Composition, antioxidant and antimicrobial activity of Homalium letestui Stem. Austral J Basic Appl Sci. 2014;8:416–422.

25. Kamat JP, Ghosh A, Devasagayam TP. Vanillin as an antioxidant in rat liver mitochondria: inhibition of protein oxidation and lipid peroxidation induced by photosensitization. Mol Cell Biochem. 2000;209:47–53.

26. Keay RWJ. Trees of Nigeria A revised version of Nigerian trees. In: Keay RWJ, Onoche CFA, Stanfield DP, editors. 1989. (Vol 1 and 2) Clarendon Press; Oxford.

27. Kumar S, Priyadarsini K, Sainis K. Free radical scavenging activity of vanillin and o-vanillin using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. Redox Rep. 2002;7:35–40.

28. Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium- deficient rat liver.BiochemBiophys Res Commun. 1976;71:952–958.

29. Lin CC, Shieh DE, Yen MH. Hepatoprotective effect of the fractions of Ban-zhi-lian on experimental liver injuries in rats. J Ethnopharmacol. 1997;56:193–200.

30. Kramb JP, Suthiphongchai T, Surarit R, Srisomsap C, Dannhardt G, Svasti J. Vanillin suppresses metastatic potential of human cancer cells through PI3K inhibition and decreases angiogenesis in vivo. J Agr Food Chem. 2009;57:3055–3063.

31. Manokaran S, Jaswanth A, Sengottuvelu S, Nandhakumar J, Duraisamy R, Karthikeyan D, Mallegaswari R. Hepatoprotective activity of Aerva lanata Linn against paracetamol induced hepatotoxicity in rats. Res J Pharm Technol. 2008;1:398–400.

32. Marklund S, Marklund G. Involvement of superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 2011;47:469–474.

33. Martin P, Friedman LS. Assessment of liver function and diagnostic studies. In: Friedman LS, Keeffe EB, editors. Hand Book of Liver Disease. Philadelphia: Churchill Livingstone; 2008. pp. 1–14.

34. Muriel P, Garcipiana T. Silymarin protects against paracetamol-induced lipid peroxidation and liver damage. J ApplToxicol. 2011,12:439–442.

35. Nkosi CZ, Opoku AR, Terblanche SE. Effect of pumpkin seed (Cucurbita pepo) protein isolate on the activity levels of certain plasma enzymes in CCl4-induced liver injury in low protein fed rats.Phytother Res. 2005;19:341–345.

36. Okokon JE, Davies K. Psychopharmacological studies of Homalium letestui stem extract. J Pharmaceut Biol. 2014;4:158–164.

37. Okokon JE, Antia BS, Ita BN. Antidiabetic effects of Homalium letestui (Flacourtiaceae) in streptozotocin induced diabetic rats. Res J Med Plants. 2007;1:134–138.

38. Plaa GL, Hewitt WR. Detection and evaluation of chemically induced liver injury. In: Wallace Hayes, A., editor. Principles and Methods of Toxicology. New York: Raven Press; 2006. pp. 399–628.

39. Prakash J, Gupta SK, Kochupillai V, Singh N, Gupta YK, Joshi S. Chemopreventive activity of Withania somnifera in experimentally induced fibrosarcoma tumours in Swiss albino mice. Phytother Res. 2001;15:240–244.

40. Reitman S, Frankel S. Determination of Glutamate-Pyruvate transaminase (ALT) and Aspartate Aminotransfrase (AST) J ClinPathol. 2012;28:56.

41. Setzer WN, Stokes SL, Penton AF, Takaku S, Haber WA, Hansell E, Caffrey CR, McKerrow JH. Cruzain Inhibitory activity of leaf essential oils of neotropicallauraceae and essential oil components.Nat Prod Comm. (2007);2:1203–1210.

42. Shashank T, EllandalaR , Yasmeen N, Sujatha K, Vishal Reddy k. Evaluation of hepatoprotective activity of stem bark of Homalium zeylanicum in rats. Int J Pharm Tech Res. 2011;3:1630–1634.

43. Streeter AJ, Dahlin DC, Nelson SD, Baillie TA. The covalent binding of acetaminophen to protein. Evidence for cysteine residues as major sites of arylation in vitro. Chem Biol Interact. 2008;48:349–66.

44. Hussain MA, Khan MQ, Hussain N, Habib T. Antibacterial and antifungal potential of leaves and twigs of *Viscum album* L. *Journal of Medicinal Plant Research*. 2014;5(23):5545–5549.

45. Zakaria ZA, Wen LY, Abdul Rahman NI, Abdul Ayub AH, Sulaiman MR, Gopalan HK. Antinociceptive, anti-inflammatory and antipyretic properties of the aqueous extract of *Bauhinia purpurea* leaves in experimental animals. *Medical Principles and Practice*. 2014;16(6):443–449.

46. Mukherjee PK, Gopal TK, Subburaju T, et al. Studies on the anti-diarrheal profiles of *Bauhinia purpurea*linn leaves extract. *Natural Product Sciences*. 2013;4(4):234–237.

47. Panda S, Kar A. *Withania somnifera* and *Bauhinia purpurea* in the regulation of circulating thyroid hormone concentrations in female mice. *Journal of Ethnopharmacology*. 2012;67(2):233–239.

48. Jatwa R, Kar A. Amelioration of metformin-induced hypothyroidism by *Withania somnifera* and *Bauhinia purpurea* extracts in type 2 diabetic mice. *Phytotherapy Research*. 2013;23(8):1140–1145.

49. Murugan M, Mohan VR. Evaluation of phytochemical analysis and antibacterial activity of *Bauhinia purpurea* L. and *Hiptage benghalensis* L. Kurz. *Journal of Applied Pharmaceutical Science*. 2013;1:157–160.

50. Boonphong S, Puangsombat P, Baramee A, Mahidol C, Ruchirawat S, Kittakoop P. Bioactive compounds from *Bauhinia purpurea* possessing antimalarial, antimycobacterial, antifungal, anti-inflammatory, and cytotoxic activities. *Journal of Natural Products*. 2010;70(5):795–801.

51. Lakshmi BVS, Neelima N, Kasthuri N, Umarani V, Sudhakar M. Protective effect of *Bauhinia purpurea* on gentamicin-induced nephrotoxicity in rats. *Indian Journal of Pharmaceutical Sciences*. 2009;71(5):551–554.

52. Ananth KV, Asad M, Kumar NP, Asdaq SMB, Rao GS. Evaluation of wound healing potential of *Bauhinia purpurea* leaf extracts in rats. *Indian Journal of Pharmaceutical Sciences*. 2010;72(1):122–127.

53. Annegowda HV, Mordi MN, Ramanathan S, Hamdan MR, Mansor SM. Effect of extraction techniques on phenolic content, antioxidant and antimicrobial activity of *Bauhinia purpurea*: HPTLC determination of antioxidants. *Food Analytical Methods*. 2012;5(2):226–233.

54. Negi BS, Dave BP, Agarwal YK. Evaluation of antimicrobial activity of *Bauhinia purpurea* leaves under *in vitro* conditions. *Indian Journal of Microbiology*. 2012;52(3):360–365.

55. Fang EF, Bah CSF, Wong JH, et al. A potential human hepatocellular carcinoma inhibitor from *Bauhinia purpurea* L. seeds: from purification to mechanism exploration. *Archives of Toxicology*. 2012;86(2):293–304.

56. Narayana KR, Reddy MS, Chaluvadi MR, Krishna DR. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. *Indian Journal of Pharmacology*. 2001;33(1):2–16.

57. Zabidi Z, Wan Zainulddin WN, Mamat SS, et al. Antiulcer activity of methanol extract of *Melastoma malabathricum* leaves in rats. *Medical Principles and Practice*. 2012;21(5):501–503.

58. Singleton VL, Rossi JA., Jr. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*. 1965;16:144–158.

59. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature*. 1958;181(4617):1199–1200.

60. Mujeeb M, Aeri V, Bagri P, Khan S. Hepatoprotective activity of the methanolic extract of *Tylophora indica* (Burm. f.) Merill. leaves. *International Journal of Green Pharmacy*. 2009;3(2):125–127.

61. El-Beshbishy HA, Mohamadin AM, Nagy AA, Abdel-Naim AB. Amelioration of tamoxifen-induced liver injury in rats by grape seed extract, black seed extract and curcumin. *Indian Journal of Experimental Biology*. 2010;48(3):280–288.

62. Shaker E, Mahmoud H, Mnaa S. Silymarin, the antioxidant component and *Silybum marianum* extracts prevent liver damage. *Food and Chemical Toxicology*. 2010;48(3):803–806.

63. Zakaria ZA, Rofiee MS, Somchit MN, et al. Hepatoprotective activity of dried- and fermented-processed virgin coconut oil. *Evidence-Based Complementary and Alternative Medicine*. 2011;2011:8 pages.142739

64. Hazai E, Vereczkey L, Monostory K. Reduction of toxic metabolite formation of acetaminophen. *Biochemical and Biophysical Research Communications*. 2002;291(4):1089–1094.

65. Rajkapoor B, Venugopal Y, Anbu J, Harikrishnan N, Gobinath M, Ravichandran V. Protective effect of *Phyllanthus polyphyllus* on acetaminophen induced hepatotoxicity in rats. *Pakistan Journal of Pharmaceutical Sciences*. 2008;21(1):57–62.

66. Katzung BG. *Basic and Clinical Pharmacology*. 9th edition. New York, NY, USA: McGrawHill; 2004.

67. Ha H, Lee N, Seo C, et al. Hepatoprotective and antioxidative activities of cornus officinalis against acetaminophen-induced hepatotoxicity in mice. *Evidence-Based Complementary and Alternative Medicine*. 2012;2012:8 pages.804924

68. Farghaly HS, Hussein MA. Protective effect of curcumin against paracetamol-induced liver damage. *Australian Journal of Basic and Applied Sciences*. 2010;4(9):4266–4274.

69. Kerksick C, Willoughby D. The antioxidant role of glutathione and N-acetyl-cysteine supplements and exercise-induced oxidative stress. *Journal of the International Society of Sports Nutrition*. 2005;2(2):38–44.

70. Yanpallewar SU, Sen S, Tapas S, Kumar M, Raju SS, Acharya SB. Effect of *Azadirachta indica* on paracetamol-induced hepatic damage in Albino rats. *Phytomedicine*. 2003;10(5):391–396.

71. Shnoy AK, Somayaji SN, Bairy KL. Hepatoprotective activity of ethanolic extract of *Ginkgo biloba*against carbon tetrachloride induced hepatic injury in rats. *Indian Journal Pharmacology*. 2002;46:167–174.

72. Amacher DE. Serum transaminase elevations as indicators of hepatic injury following the administration of drugs. *Regulatory Toxicology and Pharmacology*. 1998;27(2):119–130.

73. Uma N, Fakurazi S, Hairuszah I. *Moringa oleifera* enhances liver antioxidant status via elevation of antioxidant enzymes activity and counteracts paracetamol-induced hepatotoxicity. *Malaysian Journal of Nutrition*. 2010;16(2):293–307.

74. Somchit MN, Zuraini A, Bustamam AA, Somchit N, Sulaiman MR, Noratunlina R. Protective activity of turmeric (*Curcuma longa*) in paracetamol-induced hepatotoxicity in rats. *International Journal of Pharmacology*. 2005;1:252–256.

75. Kumar G, Banu GS, Pappa PV, Sundararajan M, Pandian MR. Hepatoprotective activity of *Trianthema portulacastrum* L. against paracetamol and thioacetamide intoxication in albino rats. *Journal of Ethnopharmacology*. 2004;92(1):37–40.

76. Gupta AK, Chitme H, Dass SK, Misra N. Hepatoprotective activity of *Rauwolfia serpentina* rhizome in paracetamol intoxicated rats. *Journal of Pharmacological and Toxicological*. 2006;1:82–88.

77. Luster MI, Simeonova PP, Gallucci RM, Matheson JM, Yucesoy B. Immunotoxicology: role of inflammation in chemical-induced hepatotoxicity. *International Journal of Immunopharmacology*. 2000; 22(12): 1143–1147.

78. Yadav S, Bhadoria BK. Two dimeric flavonoids from *Bauhinia purpurea* . *Indian Journal of Chemistry B*. 2005;44(12):2604–2607.

79. Ferreira JFS, Luthria DL, Sasaki T, Heyerick A. Flavonoids from *Artemisia annua* L. As antioxidants and their potential synergism with artemisinin against malaria and cancer. *Molecules*. 2010;15(5):3135–3170.

80. Tapas AR, Sakarkar DM, Kakde RB. Flavonoids as nutraceuticals: a review. *Tropical Journal of Pharmaceutical Research*. 2008;7(3):1089–1099.

81. Sandhar HK, Kumar B, Prasher S, Tiwari P, Salhan M, Sharma P. A review of phytochemistry and pharmacology of flavonoids. *Internationale Pharmaceutica Sciencia*. 2011;1:24–41.

82. Pithayanukul P, Nithitanakool S, Bavovada R. Hepatoprotective potential of extracts from seeds of *Areca catechu* and nutgalls of *Quercus infectoria* . *Molecules*. 2009;14(12):4987–5000.

83. Elekofehinti OO, Adanlawo IG, Komolafe K, Ejelonu OC. Saponins from *Solanum anguivi* fruits exhibit antioxidant potential in Wistar rats. *Annals of Biological Research*. 2012;3(7):3212–3217.

84. Akkol EK, Tatli II, Akdemir ZS. Antinociceptive and anti-inflammatory effects of saponin and iridoid glycosides from *Verbascum pterocalycinum* var. mutense Hub.-Mor. *Zeitschrift fur Naturforschung C*. 2007;62(11-12):813–820.

85. Porchezhian E, Ansari SH. Hepatoprotective activity of *Abutilon indicum* on experimental liver damage in rats. *Phytomedicine*. 2005;12(1-2):62–64.

86. Mehendale HM. PPAR-α: a key to the mechanism of hepatoprotection by clofibrate. *Toxicological Sciences*. 2000;57(2):187–190.

87. Manautou JE, Hart SGE, Khairallah EA, Cohen SD. Protection against acetaminophen hepatotoxicity by a single dose of clofibrate: effects on selective protein arylation and glutathione depletion. *Fundamental and Applied Toxicology*. 1996;29(2):229–237.

88. Liang Y, Tsai S, Tsai D, Lin-Shiau S, Lin J. Suppression of inducible cyclooxygenase and nitric oxide synthase through activation of peroxisome proliferator-activated receptor-γ by flavonoids in mouse macrophages. *FEBS Letters*. 2001;496(1):12–18.

89. Han KL, Jung MH, Sohn JH, Hwang J. Ginsenoside 20(S)-protopanaxatriol (PPT) activates peroxisome proliferator-activated receptor γ (PPARγ) in 3T3-L1 adipocytes. *Biological and Pharmaceutical Bulletin*. 2006;29(1):110–113.