PHARMACOLOGICAL EVALUATION OF ETHANOLIC EXTRACT OF *WITHANIA COAGULANS* DUNAL (FLOWER BUDS)

A Dissertation submitted to

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

CHENNAI - 600 032.

In partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

BRANCH-IV PHARMACOLOGY

Submitted by

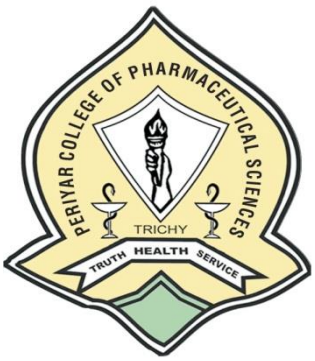
R. SARATHA

Reg. No: 261525152

Under the guidance of

MR. K. A. S. MOHAMMED SHAFEEQ, M. PHARM.,

Asst. Professor, Department of Pharmacology



PERIYAR COLLEGE OF PHARMACEUTICAL SCIENCES

TIRUCHIRAPPALLI - 620 021.

(An ISO 9001: 2008 Certified Institution)

OCTOBER – 2017

Mr. K. A. S. Mohammed Shafeeq, M. Pharm.,

Asst. Professor, Department of Pharmacology

Periyar College of Pharmaceutical Sciences

Tiruchirappalli - 620 021.

CERTIFICATE

This is to certify that the dissertation entitled **"PHARMACOLOGICAL EVALUATION OF ETHANOLIC EXTRACT OF *WITHANIA COAGULANS* DUNAL (FLOWER BUDS)"** submitted by **MS. R. SARATHA [Reg. No: 261525152]** for the award of the degree of **"MASTER OF PHARMACY"** is a bonafide research work done by her in the Department of Pharmacology, Periyar College of Pharmaceutical Sciences, Tiruchirappalli during the academic year 2016 – 2017 under my direct guidance and supervision.

Place : Tiruchirappalli

Date : **(Mr. K. A. S. Mohammed Shafeeq)**

Prof. Dr. R. Senthamarai, M. Pharm., Ph. D.,
Principal
Periyar College of Pharmaceutical Sciences
Tiruchirappalli - 620 021.

CERTIFICATE

This is to certify that the dissertation entitled **"PHARMACOLOGICAL EVALUATION OF ETHANOLIC EXTRACT OF *WITHANIA COAGULANS* DUNAL (FLOWER BUDS)"** submitted by **MS. R. SARATHA [Reg. No: 261525152]** for the award of the degree of **"MASTER OF PHARMACY"** is a bonafide research work done by her in the Department of Pharmacology, Periyar College of Pharmaceutical Sciences, Tiruchirappalli. The work was performed under the guidance and supervision of **Mr. K. A. S. Mohammed Shafeeq, M. Pharm.,** Asst. Professor, Department of Pharmacology, Periyar College of Pharmaceutical Sciences, Tiruchirappalli during the academic year 2016 – 2017.

This dissertation is submitted for acceptance as project for partial fulfillment of the degree of **"MASTER OF PHARMACY"** in Pharmacology, of The Tamilnadu Dr. M.G.R. Medical University, during November 2017.

Place : Tiruchirappalli

Date : **(Dr. R. Senthamarai)**

ACKNOWLEDGEMENT

"Success is the delivery of a product that meets expectation"

It is my pride and privilege to express my deep sense of gratitude and profound indebtedness to my inspiration and guide **Mr. K. A. S. Mohammed Shafeeq, M. Pharm.,** Asst. Professor, Department of Pharmacology, Periyar College of Pharmaceutical Sciences, Tiruchirappalli for his innovative ideas, effort, moral support and kind encouragement during the course of my work, under whose active and indescribable guidance, I am able to improve my scientific approach. Under his guidance my scientific work has been materialised and could complete this project work.

I wish to express my sincere thanks and gratitude to **Prof. Dr. R. Senthamarai, M. Pharm., Ph. D.,** Principal, Periyar College of Pharmaceutical Sciences, Tiruchirappalli for rendering all facilities and motivation to proceed with this research work.

It gives me immense pleasure to thank **Prof. Dr. A.M. Ismail, M. Pharm., Ph.D.,** Professor Emeritus and **Prof. Dr. G. Krishnamoorthy, B. Sc., M. Pharm., Ph.D.,** Vice Principal, Periyar College of Pharmaceutical Sciences, Tiruchirappalli for their moral support to complete my project work.

I extend my sincere and heartfelt deep sense of gratitude to most respected and honourable **Dr. K. Veeramani, M.A., B.L.,** Chairperson, Periyar College of Pharmaceutical Sciences, Tiruchirappalli for providing all infrastructural facilities and ample opportunity to carry out this work.

I vouch my sincere thanks and gratitude to respected **Thiru. Gnana Sebastian**, Correspondent, Periyar College of Pharmaceutical Sciences, Tiruchirappalli for his constant support and encouragement to carry out this work

I express my sincere thanks and gratitude to **Dr. S. Karpagam Kumara Sundari, M. Pharm., Ph.D.,** Head, Department of Pharmacology, Periyar College of Pharmaceutical Sciences, Tiruchirappalli for her great support and timely interventions to do my work.

I convey my thankfulness to **Dr. T. Shri Vijaya Kirubha, M. Pharm., Ph.D.,** Head, Department of Pharmacognosgy, Periyar College of Pharmaceutical Sciences, Tiruchirappalli for providing workplace and guidance to do the extraction and Phytochemical screening of the dissertation.

I express my earnest thanks to **Dr. V. Nandagopalan, M.Sc., M.Phil., Ph.D., SLST.,** Dean of sciences, Associate Professor, National College, Tiruchirappalli for his valuable help in authentication of sample.

I express our gratitude to **Prof. G. Uma Devi**, Head, Department of Medical Laboratory Technology, Periyar College of Pharmaceutical Sciences, Tiruchirappalli for her earnest support and guidance on antimicrobial screening work.

I extend my thanks to all the **Teaching Faculties** of Periyar College of Pharmaceutical Sciences, Tiruchirappalli for their valuable support. I extend thankfulness to the **Non Teaching Faculty** for their care and help.

I thank sincerely the **Librarian and Assistant Librarian** for the reference to the resource of knowledge and wisdom.

This acknowledgement would be incomplete without thanking my **Family members** who has always been very supportive in all endeavours of my life. It gives me utmost satisfaction to end this acknowledgement by thanking them for their care and encouragement for the successful completion of my post graduate studies.

R. SARATHA

PERIYAR COLLEGE OF PHARMACEUTICAL SCIENCES

DEPARTMENT OF PHARMACOLOGY

INSTITUTIONAL ANIMAL ETHICAL COMMITTEE (IAEC)

CENTRAL ANIMAL HOUSE REGISTRATION NUMBER: 265/2000/CPCSEA

Title of the project	: Pharmacological Evaluation of Ethanolic Extract of *Withania Coagulans* Dunal (Flower Buds)
Authors	: R. Saratha & Mr. K. A. S. Mohammed Shafeeq
Proposal number	: PCP/IAEC/002/2017
Date first received	: 03.11.2016
Date received after modification (if any)	: 15.03.2017
Date received after Second modification (if any)	: Nil
Approval date	: 15.05.2017
Expiry date	: 15.05.2018
Name of IAEC/ CPCSEA Chairperson	: The HoD Department of Pharmacology Periyar College of Pharmaceutical Sciences Trichy- 21

Date: 15. 05. 2017

CHAIRMAN

INSTITUTIONAL ANIMAL ETHICS COMMITTEE

PERIYAR COLLEGE OF PHARMACEUTICAL SCIENCES

CONTENTS

S. No.	Chapters	Page No.
1	Introduction	01
2	Review of Literature	49
3	Objective of the work	77
4	Plan of the work	78
5	Plant profile	79
6	Methodology	85
7	Results and Discussion	103
8	Conclusion	126
9	Bibliography	128

LIST OF TABLES

Table No.	Particulars	Page No.
1.	Statins and dose recommended	25
2.	Doses of commonly used statins in Asia Population	28
3.	Choice of lipid lowering drugs	32
4.	Taxonomical classification of *Withania coagulans* Dunal	82
5.	Common names of *Withania coagulans* Dunal	82
6.	Specification of FTIR Spectrophotometer	89
7.	Composition of Nutrient Agar Medium	101
8.	Composition of Sabouraud Dextrose Agar Medium	102
9.	Preliminary Phytochemical Studies	103
10.	IR Spectral Data of the Ethanolic extract of *Withania coagulans*	104
11.	Chromatogram Data of 10µL of test solution (Alkaloids)	105
12.	Chromatogram Data of 20µL of test solution (Alkaloids)	105
13.	Chromatogram Data of 10µL of test solution (Flavanoids)	106
14.	Chromatogram Data of 20µL of test solution (Flavanoids)	106
15.	Chromatogram Data of 10µL of test solution (Glycosides)	107
16.	Chromatogram Data of 20µL of test solution(Glycosides)	107
17.	Behavioral Changes in Acute Oral Toxicity in Albino rats	108
18.	Effect of Test compound on Body Weight in Acute oral toxicity in Albino rats	110
19.	Effect of Test compound on Biochemical parameters in Acute oral toxicity in Albino rats	110
20.	Effect of Ethanolic extract of *Withania coagulans* on Head dips in Albino rats	111

Table No.	Particulars	Page No.
21.	Effect of Ethanolic extract of *Withania coagulans* on Anxiety Parameters in Albino rats	111
22.	Effect of Ethanolic extract of *Withania coagulans* on Body Weight in Progesterone induced obesity in Albino rats	112
23.	Histopathological Studies of Progesterone Induced Obesity in Albino Rats (T.S of Liver)	112
24.	Effect of Ethanolic extract of *Withania coagulans* on Food consumption in Progesterone induced obesity in Albino rats	113
25.	Effect of Ethanolic extract of *Withania coagulans* on Ambulatory movements, Rearing and Grooming in Progesterone Induced Obesity in Albino rats	114
26.	Effect of Ethanolic extract of *Withania coagulans* on biochemical parameters in Progesterone induced obesity in Albino rats	115
27.	Effect of Ethanolic extract of *Withania coagulans* on biochemical parameters in Fructose induce hyperlipidemia in Albino rats	116
28.	Histopathological Studies of in Fructose induce hyperlipidemia in Albino rats (T.S of Liver)	117
29.	Analgesic activity of Ethanolic Extract of *Withania coagulans* against Tail Flick Test in Wistar Albino Rats	118
30.	Anthelmintic activity of Ethanolic Extract of *Withania coagulans* against *Pheretima posthuma*	119
31.	Antimicrobial screening of Ethanolic Extract of *Withania coagulans*	120

LIST OF FIGURES

Figure No.	Particulars
1.	Obesity & Health
2.	Medical Complications of Obesity
3.	Formation of Plaque in the coronary artery
4.	Floral diagram of *Withania coagulans* Dunal
5.	Plant showing flowers of *Withania coagulans* Dunal
6.	Dried flowers of *Withania coagulans* Dunal
7.	Fourier Transform Infra Red Spectrometer
8.	IR – Spectrum of the Ethanolic extract of *Withania coagulans*
9.	Photo Documentation Under UV (Alkaloids)
10.	3D Display @ 254nm (Alkaloids)
11.	Peak Display (10µl of test solution) (Alkaloids)
12.	Peak Display (20µl of test solution) (Alkaloids)
13.	Photo Documentation Under UV(Flavanoids)
14.	3D Display @ 254nm(Flavanoids)
15.	Peak Display (5µl of test solution) (Flavanoids)
16.	Peak Display (10µl of test solution) (Flavanoids)
17.	Photo Documentation Under UV(Glycosides)
18.	3D Display @ 254nm(Glycosides)
19.	Peak Display (5µl of test solution) (Glycosides)
20.	Peak Display (10µl of test solution) (Glycosides)
21.	Effect of Test compound on Body Weight in Acute oral toxicity in Albino rats
22.	Effect of Test compound on Biochemical parameters in Acute oral toxicity in Albino rats
23.	T.S of Kidney (Normal)
24.	T.S of Heart (Normal)

Figure No.	Particulars
25.	T.S of Liver (Normal)
26.	T.S of Pancreas (Normal)
27.	T.S of Kidney (Test)
28.	T.S of Heart (Test)
29.	T.S of Liver (Test)
30.	T.S of Pancreas (Test)
31.	Effect of Ethanolic extract of *Withania coagulans* on Time spent with the head dips
32.	Effect of Ethanolic extract of *Withania coagulans* on Head dips into the holes
33.	Effect of Ethanolic extract of *Withania coagulans* on Anxiety Parameters in Albino rats
34.	Effect of Ethanolic extract of *Withania coagulans* on Body Weight in Progesterone induced obesity in Albino rats
35.	T.S of Liver (Normal)
36.	T.S of Liver (Control)
37.	T.S of Liver (Standard)
38.	T.S of Liver (Test)
39.	Effect of Ethanolic extract of *Withania coagulans* on biochemical parameters in Progesterone induced obesity in Albino rats
40.	Effect of Ethanolic extract of *Withania coagulans* on Food consumption in Progesterone induced obesity in Albino rats
41.	Effect of Ethanolic extract of *Withania coagulans* on biochemical parameters in Fructose induce hyperlipidemia in Albino rats
42.	T.S of Liver (Normal)
43.	T.S of Liver (Control)
44.	T.S of Liver (Standard)
45.	T.S of Liver (Test)
46.	Analgesic activity of Ethanolic Extract of *Withania coagulans* against Tail Flick Test in Wistar Albino Rats

Figure No.	Particulars
47.	Anthelmintic activity of Ethanolic Extract of *Withania coagulans* against *Pheretima posthuma*
48.	Anthelmintic activity of Ethanolic Extract of *Withania coagulans* against *Pheretima posthuma* – Control
49.	Anthelmintic activity of Ethanolic Extract of *Withania coagulans* against *Pheretima posthuma* Standard
50.	Anthelmintic activity of Ethanolic Extract of *Withania coagulans* against *Pheretima posthuma* Test I
51.	Anthelmintic activity of Ethanolic Extract of *Withania coagulans* against *Pheretima posthuma* Test II
52.	Anthelmintic activity of Ethanolic Extract of *Withania coagulans* against *Pheretima posthuma* Test III
53.	Anthelmintic activity of Ethanolic Extract of *Withania coagulans* against *Pheretima posthuma* Test IV
54.	Zone of inhibition of Test drug 25, 50, 75 & 100mg against *Staphylococcus aureus*
55.	Zone of inhibition of Test drug 250mg against *Staphylococcus aureus*
56.	Zone of inhibition of Test drug 25, 50, 75 & 100mg against *Bacillus substilis*
57.	Zone of inhibition of Test drug 250mg against *Bacillus substilis*
58.	Zone of inhibition of Test drug 25, 50, 75 & 100mg against *Proteus vulgaris*
59.	Zone of inhibition of Test drug 250mg against *Proteus vulgaris*
60.	Zone of inhibition of Test drug 25, 50, 75 & 100mg against *Klebsiella aerogenes*
61.	Zone of inhibition of Test drug 250mg against *Klebsiella aerogenes*
62.	Zone of inhibition of Test drug 25, 50, 75 & 100mg against *Candida albicans*
63.	Zone of inhibition of Test drug 250mg against *Candida albicans*
64.	Zone of inhibition of Test drug 25, 50, 75 & 100mg against *Aspergillus niger*
65.	Zone of inhibition of Test drug 250mg against *Aspergillus niger*
66.	Antimicrobial screening of Ethanolic Extract of *Withania coagulans*

ABBREVATIONS

WHO	World Health Organization
CSIR	Council For Scientific And Industrial Research
NMITLI	New Millennium Indian Technology Leadership Initiative
SOP	Standard Operating Procedures
GAP	Good Agricultural Practice
GLP	Good Laboratory Practice
GSP	Good Supply Practice
GMP	Good Manufacturing Practice
AACE	American Association of Clinical Endocrinologists
ACE	American College Of Endocrinology
ABCD	Adiposity-Based Chronic Disease
BMI	Body Mass Index
MCP - 1	Monocyte Chemoattractant Protein–1
PAI – 1	Plasminogen Activator Inhibitor-1
Acyl-CoA	Acyl-Coenzyme A
ADRP	Adipocyte Differentiation–Related Protein
PPAR- γ	Peroxisome Proliferator-Activated Receptor–Gamma
EFRMD	Excessive Fat–Related Metabolic Diseases
GLP- 1	Glucagonlike Peptide-1
DXA	Dual-Energy Radiographic Absorptiometry
CT	Computed Tomography Scanning
MRI	Magnetic Resonance Imaging
NAFLD	Non-Alcoholic Fatty Liver Disease

GERD	Gastroesophageal Reflux Disease
MONICA	Multinational Monitoring of Trends and Determinants in Cardiovascular Disease
HbA_1c	Hemoglobin A_1c
DEXA	Dual-Energy Radiographic Absorptiometry
CCK	Cholecystikinin
GHRH	Growth Hormone Releasing Hormone
CRH	Corticotropin Releasing Hormone
HT	Hydroxy Tryptamine
DA	Dopamine
NA	Noradrenaline
ADR	Adverse Drug Reactions
LDL	Low Density Lipoproteins
VLDL	Very Low Density Lipoproteins
HDL	High Density Lipoproteins
TG	Tri Glycerides
TC	Total Cholesterol
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
OD	Once a Day
SSRI	Selective Serotonin Reuptake Inhibitors
MAOI	Mono Amino Oxidase Inhibitors
HMG- CoA	Hydroxy Methyl Glutaryl Co -Enzyme A Reductase
NYHA	New York Heart Association
HF	Heart Failure
NO	Nitric Oxide
CYP_3A_4	Cytochrome P3A450
(PPAR-α)	Peroxisome Proliferator Activated Receptor Alpha
(lpL)	Lipoprotein Lipase
IHD	Ischemic Heart Disease
PUFA	Poly Unsaturated Fatty Acids
BZD	Benzodiazepines
RAS	Renin Angiotensin System

Cl	Chloride
Ca^{2+}	Calcium
GABA	Gamma Amino Butyric Acid
cAMP	Cyclic Adenosine Mono Phosphate
K^{+}	Potassium
CNS	Central Nervous System
NSAID	Non Steroidal Anti Inflammatory Drugs
PG	Prostaglandins
HT	Hydroxy Tryptamine
SG	Substantia Gelatinosa
CSF	Cerebro Spinal Fluid
NAFLD	Nonalcoholic Fatty Liver Disease
IC	Inhibitory Concentration
LD	Lethal dose
MTT	DiMethyl Thiazol diphenyl Tetrazolium bromide
HPTLC	High Performance Thin Layer Chromatography
HPLC	High Pressure Liquid Chromatography
FTIR	Fourier Transform Infra Red Spectroscopy
CF	Chloroform Fraction
SD	Sprague Dawley
MIC	Minimum Inhibitory Concentration
OECD	Organisation for Economic Cooperation and Development
IAEC	Institutional Animal Ethical Committee
ANOVA	Analysis of Variance
CPCSEA	Committee for the Purpose of Control and Supervision of Experimental Animals
kg	Kilogram
mg	Milligram
ml	Milli Litres
mmol	Milli Mole
pH	Negative logarithm of hydrogen ion concentration
p.o.	Per Oral
i.p.	Intra Peritoneal

i.m.	Intra Muscular
i.v.	Intra Venous
s.c.	Sub Cutaneous
S.E.M	Standard Error Mean
DMSO	Di Methyl Sulphoxide

1. INTRODUCTION

1. 1. HERBAL MEDICINES

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Herbal medicines have been widely utilized as effective remedies for the prevention and treatment of multiple health conditions for centuries by almost every known culture. The first documented records of herbal medicine use date back 5,000 years in China. Similarly, India's Ayurvedic medicine tradition is thought to be more than 5,000 years old and herbal medicines remain an essential component of its practice. Herbal medicine is still the mainstay of about 75 - 80% of the world population, mainly in the developing countries, for primary health care. This is primarily because of the general belief that herbal drugs are without any side effects besides being cheap and locally available. According to the World Health Organization (WHO), the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times.[152, 153, 41, 59, 46, 35, 132]

Tribal healers in most of the countries, where ethnomedical treatment is frequently used to treat cut wounds, skin infection, swelling, aging, mental illness, cancer, asthma, diabetes, jaundice, scabies, eczema, venereal diseases, snakebite and gastric ulcer, provide instructions to local people as how to prepare medicine from herbal. They keep no records and the information is mainly passed on verbally from generation to generation. World Health Organization (WHO) has shown great interest in documenting the use of medicinal plants used by tribals from different parts of the world. Many developing countries have intensified their efforts in documenting the ethnomedical data on medicinal plants. Research to find out scientific evidence for claims by tribal healers on Indian herbs has been intensified. Once these local ethnomedical preparations are scientifically evaluated and disseminated properly, people will be better informed regarding efficacious drug treatment and improved health status.

Herbal medicines are currently in demand and their popularity is increasing day by day. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine. India is a vast repository of medicinal plants that are used in traditional medical treatments. The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments. The use of herbal medicine becoming popular due to toxicity and side effects of allopathic medicines. This led to sudden increase in the number of herbal drug manufactures. Herbal medicines as the major remedy in traditional system of medicine

have been used in medical practices since antiquity. The practices continue today because of its biomedical benefits as well as place in cultural beliefs in many parts of world and have made a great contribution towards maintaining human health.[6]

In India around 20,000 medicinal plant species have been recorded recently but more than 500 traditional communities use about 800 plant species for curing different diseases. Currently 80% of the world population depends on plant-derived medicine for the first line of primary health care for human alleviation because it has no side effects. Plants are important sources of medicines and presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources.

Herbal medicines are also in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects. They also offer therapeutics for age-related disorders like memory loss, osteoporosis, immune disorders, etc. for which no modern medicine is available. The strong historic bond between plants and human health began to unwind in 1897, when Friedrich Bayer and Co. introduced synthetic acetyl salicylic acid (aspirin) to the world. Aspirin is a safer synthetic analogue of salicylic acid, an active ingredient of willow bark, and was discovered independently by residents of both the New and Old worlds as a remedy for aches and fevers. Herbal medicine is the use of plants, plant parts, their water or solvent extracts, essential oils, gums, resins, exudates or other form of advanced products made from plant parts used therapeutically to provide proactive support of various physiological systems; or, in a more conventional medical sense, to treat, cure, or prevent a disease in animals or humans.[110]

About 25% of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use. Of the 252 drugs considered as basic and essential by the World Health Organisation (WHO), 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors. Examples of important drugs obtained from plants are digoxin from *Digitalis* spp., quinine and quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. India has had a rich, vibrant and diverse cultural history. An important component of this culture and tradition is that of health and healing. Thus there is a large health and healing related knowledge base present in all ethnic communities across the diverse ecosystems. However, over the last few centuries, this knowledge base has been diluted with increased influences from the

mainstream culture, which is derisive of local health traditions. It is important to urgently put in place effective documentation and assessment programs to revitalize local health traditions otherwise this great people's health culture will be irretrievably lost. This country is perhaps the largest producer of medicinal herbs and is rightly called the botanical garden of the world. There are very few medicinal herbs of commercial importance which are not found in this country. India officially recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine, representing about 75% of the medicinal needs of the Third World countries. It is estimated that there are over 7800 medicinal drug manufacturing units in India, which are estimated to consume about 2000 tons of herbs annually. Three of the ten most widely selling herbal medicines in the developed countries, namely preparations of *Allium sativum*, *Aloe arbedensis* and *Panax* sp. are available in India. There are about 7000 firms manufacturing traditional medicines with or without standardization.[110,52,123]

Thus, the modern social context and economic view of health services, the needs of the pharmaceutical market and the recognition that research on medicinal plants used in folk medicine represents a suitable approach for the development of new drugs have led to an increase in the number of publications in this field, and private and governmental institutions are now financially supporting research programmes worldwide. Even though is not the fruit of a fully scientific methodology, it is surprising to observe, simply typing the word. phytotherapy in one of the most used scientific data banks in the world, the growing use of this term by scientists**.** However, the potential use of higher plants as a source of new drugs is still poorly explored of the estimated 250,000. 500,000 plant species, only a small percentage has been investigated phytochemically and even a smaller percentage has been properly studied in terms of their pharmacological properties; in most cases, only pharmacological screening or preliminary studies have been carried out. It is estimated that 5000 species have been studied for medical use. [7,115,116]

Ayurveda, one of the major traditional forms of medical practice in India, has produced many useful leads in developing medications for chronic diseases. Almost 25 centuries ago, Hippocrates proclaimed, let food be thy medicine and medicine be thy food. Combining the strengths of the knowledge base of traditional systems such as ayurveda with the dramatic power of combinatorial sciences and High Throughput Screening will help in the generation of structure activity libraries. Ayurvedic knowledge and experiential database can provide new functional leads to reduce time, money and toxicity. These

records are particularly valuable, since effectively these medicines have been tested for thousands of years on people. Efforts are underway to establish pharmaco epidemiological evidence base regarding safety and practice of Ayurvedic
medicines. Development of standardized herbal formulations is underway as an initiative of the Council for Scientific and Industrial Research (CSIR) New Millennium Indian Technology Leadership Initiative (NMITLI). [31,18,43]

In 1991 WHO developed guidelines for the assessment of herbal medicine. Suggestions for herbal medicine standardization are out lined. The scenario and perception of herbal medicine are discussed. The publics belief that herbal and natural products are safer than synthetic medicines can only be ascertained by imposing regulatory standards on these products that should be manufactured using these Good Practices. Implementing standard operating procedures (SOP) leading to Good Agricultural Practice (GAP), Good Laboratory Practice (GLP), Good Supply Practice (GSP) and Good Manufacturing Practice (GMP) for producing these medicinal products from herbal or natural sources. [99,24]

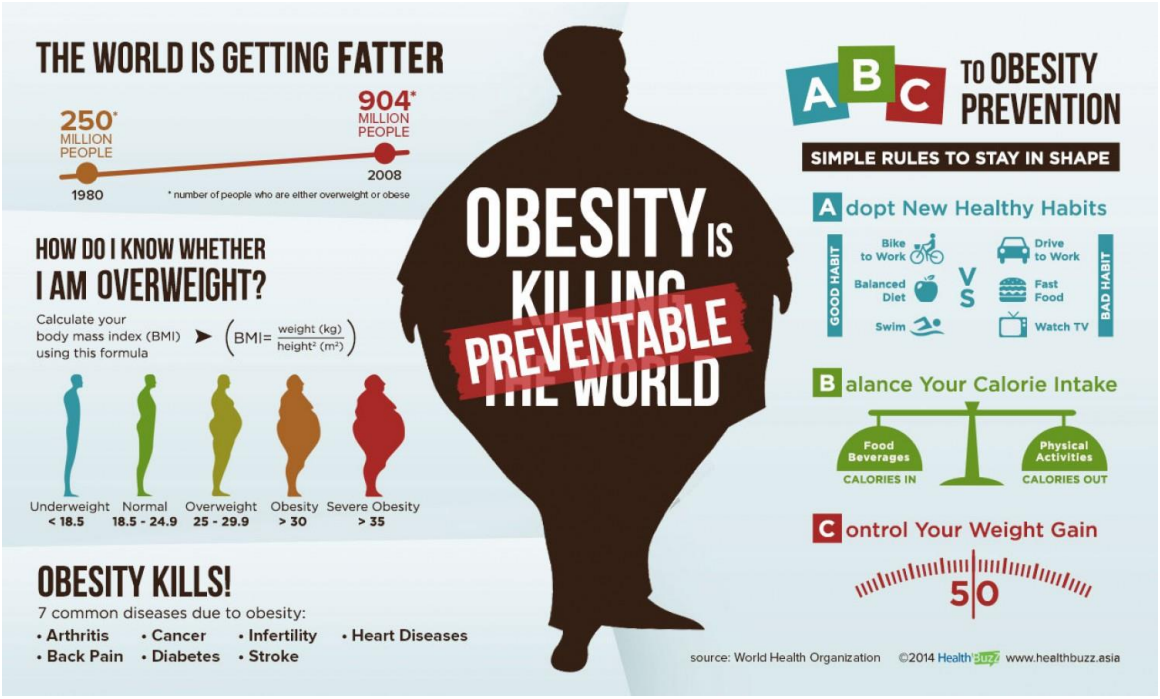
1. 2. OBESITY

Obesity is defined as "a condition of abnormal or excessive accumulation of adipose tissue, to the extent that health may be impaired. "People with abdominal fat distribution or **android** obesity are at greater risk to its consequences than those with the less serious **gynoid** fat distribution where fat is more evenly and peripherally distributed around the body.

Sedentary lifestyle of modern living, combined with the abundant availability of tempting, high-fat (junk) foods has caused almost an epidemic of obesity in the affluent nations. The current estimate indicates that the number of 'over weight' individuals in the world is almost the same as that of 'Underweight' ones. However, not everyone who partakes of such food becomes obese. The tendency to become obese is definitely inherited. The mortality rises exponentially with increasing bodyweight and the incidence of heart disease, hypertension, diabetes, sleep apnea, osteoarthritis and bowel cancer is higher in the obese than in the non-obese.

Obesity is a substantial public health crisis in the United States, and internationally, with the prevalence increasing rapidly in numerous industrialized nations. In 2009-2010, the prevalence of obesity among American men and women was almost 36%. [126]

Fig. No. 1: Obesity & Health



Background

The annual cost of managing obesity in the United States alone amounts to approximately $190.2 billion per year, or 20.6% of national health expenditures, according to a recent study.[68] Compared with a nonobese person, an obese person incurs $2741 more in medical costs (in 2005 dollars) annually. In addition, the annual cost of lost productivity due to obesity is approximately $73.1 billion,[17] and almost $121 billion is spent annually on weight-loss products and services.[84]

In a 2016 position statement, the American Association of Clinical Endocrinologists (AACE) and the American College of Endocrinology (ACE) proposed a new name for obesity, adiposity-based chronic disease (ABCD). The AACE/ACE did not introduce the name as an actual replacement for the term obesity but instead as a means of helping the medical community focus on the pathophysiologic impact of excess weight.[46]

Classification of obesity

Although several classifications and definitions for degrees of obesity are accepted, the most widely accepted classifications are those from the World Health Organization (WHO), based on BMI. The WHO designations include the following:

- Grade 1 overweight (commonly and simply called overweight) - BMI of 25-29.9 kg/m ^{2}

- Grade 2 overweight (commonly called obesity) - BMI of 30-39.9 kg/m ^{2}
- Grade 3 overweight (commonly called severe or morbid obesity) - BMI greater than or equal to 40 kg/m ^{2}

The cut-off for each grade varies according to an individual's ethnic background. For example, a BMI of 23 kg/m^2 or higher may define grade 1 overweight and 27.5 kg/m^2 or higher may define grade 2 overweight (obesity) in many Asian populations, in which the risk was shown to be high and extremely high for grade 1 and 2 overweight at these levels, respectively. Other BMI cutoffs identified as potential public health action points in these populations are 32.5 and 37.5 kg/m^2.[45]

The surgical literature often uses a different classification to recognize particularly severe obesity. The categories are as follows:

- Severe obesity - BMI greater than 40 kg/m ^{2}
- Morbid obesity - BMI of 40-50 kg/m ^{2}
- Super obese - BMI greater than 50 kg/m ^{2}

In children, a BMI above the 85th percentile (for age-matched and sex-matched control subjects) is commonly used to define overweight, and a BMI above the 95th percentile is commonly used to define obesity.

Pathophysiology

Hypertrophic versus hypercellular obesity

The Adipocytes, which is the cellular basis for obesity, may be increased in size or number in obese persons. Hypertrophic obesity, characterized by enlarged fat cells, is typical of android abdominal obesity. Hypercellular obesity is more variable than hypertrophic obesity; it typically occurs in persons who develop obesity in childhood or adolescence, but it is also invariably found in subjects with severe obesity.

Hypertrophic obesity usually starts in adulthood, is associated with increased cardiovascular risk, and responds quickly to weight reduction measures. In contrast, patients with hypercellular obesity may find it difficult to lose weight through nonsurgical interventions.

Adipocytes

Products

The Adipocytes is increasingly found to be a complex and metabolically active cell. At present, the adipocyte is perceived as an active endocrine gland producing several

peptides and metabolites that may be relevant to the control of body weight; these are being studied intensively.[66]

Many of the adipocytokines secreted by adipocytes are proinflammatory or play a role in blood coagulation. Others are involved in insulin sensitivity and appetite regulation. However, the function of many of these identified cytokines remains unknown or unclear. Proinflammatory products of the adipocyte include the following[180]

- Tumor necrosis factor–alpha
- Interleukin 6
- Monocyte chemoattractant protein–1 (MCP-1)

Other adipocyte products include the following

- Lipotransin
- Plasminogen activator inhibitor-1 (PAI-1) - Associated with cardiovascular risk
- Adipocyte lipid-binding protein
- Acyl-stimulation protein
- Prostaglandins - Coagulation role
- Adipsin
- Perilipins
- Lactate
- Leptin - Appetite regulator
- Adiponectin - Major role in insulin sensitivity
- Monobutyrin
- Phospholipid transfer protein

Metabolism and function

Critical enzymes involved in adipocyte metabolism and function include the following:

- Endothelial-derived lipoprotein lipase - Lipid storage
- Hormone-sensitive lipase - Lipid elaboration and release from adipocyte depots
- Acyl-coenzyme A (acyl-CoA) synthetases - Fatty acid synthesis

In addition, a cascade of enzymes is involved in beta-oxidation and fatty acid metabolism. The ongoing flurry of investigation into the intricacies of adipocyte metabolism has not only improved our understanding of the pathogenesis of obesity but has also offered several potential targets for therapy.

Development

Another area of active research is investigation of the cues for the differentiation of preadipocytes to adipocytes. The recognition that this process occurs in white and brown

adipose tissue, even in adults, has increased its potential importance in the development of obesity and the relapse to obesity after weight loss.

Among the identified elements in this process are the following transcription factors:

- Peroxisome proliferator-activated receptor–gamma (PPAR-gamma)
- Retinoid-X receptor ligands
- Perilipin
- Adipocyte differentiation–related protein (ADRP)
- CCAAT/enhancer-binding proteins (C/EBP) alpha, beta, and delta

PPAR-gamma agonists increase the recruitment, proliferation, and differentiation of preadipocytes (healthy fat) and cause apoptosis of hypertrophic and dysfunctional adipocytes (including visceral fat). This results in improved fat function and improved metabolic parameters associated with excessive fat–related metabolic diseases (EFRMD), including type 2 diabetes mellitus, hypertension, and dyslipidemia.[37]

Hormonal influences on appetite

In addition to neurotransmitters and neurogenic signals, many hormones affect appetite and food intake. Endocannabinoids, through their effects on endocannabinoid receptors, increase appetite, enhance nutrient absorption, and stimulate lipogenesis. Melanocortin hormone, through its effects on various melanocortin receptors, modifies appetite.

Several gut hormones play significant roles in inducing satiety, including glucagonlike peptide-1 (GLP-1), neuropeptide YY (PYY), and cholecystokinin. Leptin and pancreatic amylin are other potent satiety hormones. On the other hand, ghrelin, which is secreted from the stomach fundus, is a major hunger hormone.

Odor detection threshold

Smell plays an important role in feeding behavior. Differences in the odor detection threshold (ie, the lowest concentration of a substance detectable by the human olfactory sense) were found in a study that measured thresholds in 8 lean, fasted individuals before and during a 2-hour hyperinsulinemic euglycemic insulin clamp.[37]

Increased insulin led to reduced smelling capacity, potentially reducing the pleasantness of eating. Therefore, insulin action in the olfactory bulb may be involved in the process of satiety and may be of clinical interest as a possible factor in the pathogenesis of obesity.[31]

Leptin

Friedman and colleagues discovered leptin (from the Greek word *leptos*, meaning thin) in 1994 and ushered in an explosion of research and a great increase in knowledge about regulation of the human feeding and satiation cycle. Leptin is a 16-kd protein produced predominantly in white subcutaneous adipose tissue and, to a lesser extent, in the placenta, skeletal muscle, and stomach fundus in rats. Leptin has myriad functions in carbohydrate, bone, and reproductive metabolism that are still being unraveled, but its role in body-weight regulation is the main reason it came to prominence.

Since this discovery, neuromodulation of satiety and hunger with feeding has been found to be far more complex than the old, simplistic model of the ventromedial hypothalamic nucleus and limbic centers of satiety and the feeding centers of the lateral hypothalamus. Potentially, leptin sensitizers may assist in changing feeding habits.

The major role of leptin in body-weight regulation is to signal satiety to the hypothalamus and thus reduce dietary intake and fat storage while modulating energy expenditure and carbohydrate metabolism, preventing further weight gain. Unlike the Ob/Ob mouse model in which this peptide was first characterized, most humans who are obese are not leptin deficient but are instead leptin resistant. Therefore, they have elevated levels of circulating leptin. Leptin levels are higher in women than in men and are strongly correlated with BMI.[50]

Patients with night-eating syndrome have attenuation of the nocturnal rise in plasma melatonin and leptin levels and higher circadian levels of plasma cortisol. These individuals have morning anorexia, evening hyperphagia, and insomnia. In one study, patients with night-eating syndrome averaged 3.6 awakenings per night; 52% of these awakenings were associated with food intake, with a mean intake per ingestion of 1134 kcal. [38]

Genetics

Mutations resulting in defects of the leptin receptor in the hypothalamus may occur. These mutations result in early onset obesity and hyperphagia despite normal or elevated leptin levels, along with hypogonadotropic hypogonadism, and defective thyrotropin secretion.

Murray et al first reported on a sequence variant within the leptin gene that enhances the intrinsic bioactivity of leptin, but which was associated with reduced weight

rather than obesity.[62] This sequence variant within the leptin gene is also associated with delayed puberty.

Measurements of obesity

Obesity represents a state of excess storage of body fat. Although similar, the term overweight is puristically defined as an excess of body weight for height. Normal, healthy men have a body fat percentage of 15-20%, while normal, healthy women have a percentage of approximately 25-30%.[181] However, because differences in weight among individuals are only partly the result of variations in body fat, body weight is a limited, although easily obtained, index of obesity.

The body mass index (BMI), also known as the Quetelet index, is used far more commonly than body fat percentage to define obesity. In general, BMI correlates closely with the degree of body fat in most settings; however, this correlation is weaker at low BMIs.

An individual's BMI is calculated as weight/height^2, with weight being in kilograms and height being in meters (otherwise, the equation is weight in pounds ´ 0.703/height in inches^2). Online BMI calculators are available.

A person's body fat percentage can be indirectly estimated by using the Deurenberg equation, as follows

Body Fat Percentage = 1.2(BMI) + 0.23(age) - 10.8(sex) - 5.4

with age being in years and sex being designated as 1 for males and 0 for females.

This equation has a standard error of 4% and accounts for approximately 80% of the variation in body fat.

Although the BMI typically correlates closely with percentage body fat in a curvilinear fashion, some important caveats apply to its interpretation. In mesomorphic (muscular) persons, BMIs that usually indicate overweight or mild obesity may be spurious, whereas in some persons with sarcopenia (eg, elderly individuals and persons of Asian descent, particularly from South Asia), a typically normal BMI may conceal underlying excess adiposity characterized by an increased percentage of fat mass and reduced muscle mass.

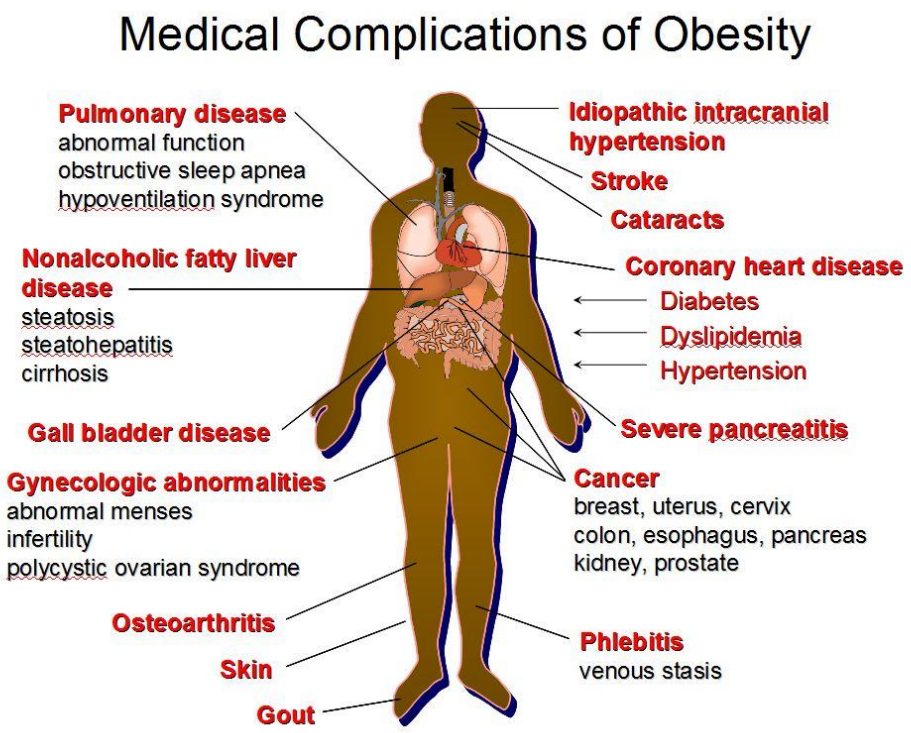
In view of these limitations, some authorities advocate a definition of obesity based on percentage of body fat. For men, a percentage of body fat greater than 25% defines obesity, with 21-25% being borderline. For women, over 33% defines obesity, with 31-33% being borderline.

Other indices used to estimate the degree and distribution of obesity include the 4 standard skin thicknesses (ie, sub scapular, triceps, biceps and suprailiac) and various anthropometric measures, of which waist and hip circumferences are the most important. Skinfold measurement is the least accurate means by which to assess obesity.

Dual-energy radiographic absorptiometry (DXA) scanning is used primarily by researchers to accurately measure body composition, particularly fat mass and fat-free mass. It has the additional advantage of measuring regional fat distribution. However, DXA scans cannot be used to distinguish between subcutaneous and visceral abdominal fat deposits.

The current standard techniques for measuring visceral fat volume are abdominal computed tomography (CT) scanning (at L4-L5) and magnetic resonance imaging (MRI) techniques. A simpler technique, using bioelectrical impedance, was recently introduced.[185]

Fig. No. 2: Medical complications of Obesity



Co morbidities of Obesity

Apart from total body fat mass, the following aspects of obesity have been associated with co morbidity

- Fat distribution
- Waist circumference
- Age of obesity onset
- Intra-abdominal pressure

Fat distribution

Accumulating data suggest that regional fat distribution substantially affects the incidence of co morbidities associated with obesity.[19] Android obesity, in which adiposity is predominantly abdominal (including visceral and, to a lesser extent, subcutaneous), is strongly correlated with worsened metabolic and clinical consequences of obesity.

Waist circumference

The thresholds used in the National Cholesterol Education Program Adult Treatment Panel III definition of metabolic syndrome[169] state that significantly increased cardiovascular risk (metabolic central obesity) exists in men with waist circumferences of greater than 94 cm (37 in) and in women with waist circumferences of greater than 80 cm (31.5 in), as well as waist-to-hip ratios of greater than 0.95 in men and of greater than 0.8 in women. Circumferences of 102 cm (40 in) in men and 88 cm (35 in) in women indicate a markedly increased risk requiring urgent therapeutic intervention.

These thresholds are much lower in Asian populations. After analyzing survey results of Chinese, Malay, and Asian-Indian cohorts, Tan and colleagues concluded that a waist circumference of greater than 90 cm in men and of more than 80 cm in women were more appropriate criteria for metabolic central obesity in these ethnic groups.[170]

Age of obesity onset

An elevated BMI during adolescence (starting within the range currently considered normal) is strongly associated with the risk of developing obesity-related disorders later in life, independent of adult BMI.[80] Increases in BMI during early adulthood (age 25-40 y) are associated with a worse profile of biomarkers related to obesity than are BMI increases during later adulthood.[186] This is consistent with most emerging data regarding timing of changes in BMI and later health consequences.

Intra-abdominal pressure

Apart from the metabolic complications associated with obesity, a paradigm of increased intra-abdominal pressure has been recognized. This pressure effect is most apparent in the setting of marked obesity (BMI ≥ 50 kg/m^2) and is espoused by bariatric surgeons.[48]

Findings from bariatric surgery and animal models suggest that this pressure elevation may play a role (potentially a major one) in the pathogenesis of comorbidities of obesity, such as the following[56]

- Pseudotumor cerebri
- Lower-limb circulatory stasis
- Ulcers
- Dermatitis
- Thrombophlebitis
- Reflux esophagitis
- Abdominal hernias
- Possibly, hypertension and nephrotic syndrome

Additional comorbidities

Overweight and obese individuals are at increased risk for the following health conditions:

- Metabolic syndrome
- Type 2 diabetes
- Hypertension
- Dyslipidemia
- Coronary heart disease
- Osteoarthritis
- Stroke
- Depression
- Non-alcoholic fatty liver disease (NAFLD)
- Infertility (women) and erectile dysfunction (men)
- Risk of stillbirth [24, 25]
- Gall bladder disease
- Obstructive sleep apnea
- Gastroesophageal reflux disease (GERD)
- Some cancers (eg, endometrial, breast, and colon)[184,155,71]
- Asthma

Pickwickian syndrome

The so-called Pickwickian syndrome is a combined syndrome of obesity-related hypoventilation and sleep apnea. It is named after Charles Dickens's novel *The Pickwick Papers*, which contains an obese character who falls asleep constantly during the day.

The hypoventilation in Pickwickian syndrome results from severe mechanical respiratory limitations to chest excursion, caused by severe obesity. The sleep apnea may be from obstructive and/or central mechanisms. Obstructive sleep apnea is common among men with collar size greater than 17 in (43 cm) and women with collar size greater than 16 in (41 cm).

Increased and decreased sleep duration

Sleep duration of less than 5 hours or more than 8 hours was associated with increased visceral and subcutaneous body fat, in a study of young African Americans and Hispanic Americans.[88] This association relates mostly to decreased leptin hormone and increased ghrelin hormone levels.[74]

Causes

The etiology of obesity is far more complex than simply an imbalance between energy intake and energy output. Although this view allows easy conceptualization of the various mechanisms involved in the development of obesity, obesity is far more than simply the result of eating too much and/or exercising too little (see the energy-balance equation, below).

Possible factors in the development of obesity include the following:

- Metabolic factors
- Genetic factors
- Level of activity
- Endocrine factors
- Race, sex, and age factors
- Ethnic and cultural factors
- Socioeconomic status
- Dietary habits
- Smoking cessation
- Pregnancy and menopause
- Psychological factors
- History of gestational diabetes
- Lactation history in mothers

International statistics

The prevalence of obesity worldwide is increasing, particularly in the industrialized nations of the Northern hemisphere, such as the United States, Canada, and most countries of Europe. Available data from the Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) project suggest that at least 15% of men and 22% of women in Europe are obese.[79,37]

Similar data are being reported in other parts of the world, including from many developing nations. Reports from countries such as Malaysia, Japan, Australia, New Zealand, and China have detailed an epidemic of obesity in the past 2-3 decades. Data from the Middle Eastern countries of Bahrain, Saudi Arabia, Egypt, Jordan, Tunisia, and Lebanon, among others, indicate this same disturbing trend, with levels of obesity often exceeding 40%.

Internationally, rates of obesity are higher in women than in men. A somewhat higher rate would be expected, given the biologically higher percentage of body fat in women.

Information from the Caribbean and from South America highlights similar trends. Although data from Africa are scant, a clear and distinct secular trend of profoundly increased BMIs is observed when people from Africa emigrate to the northwestern regions of the world. Comparisons of these indices among Nigerians and Ghanaians residing in their native countries with indices in recent immigrants to the United States show this trend poignantly.

Conservative estimates suggest that as many as 250 million people (approximately 7% of the estimated current world population) are obese. Two to 3 times more people than this are probably overweight. Although socioeconomic class and the prevalence of obesity are negatively correlated in most industrialized countries, including the United States, this correlation is distinctly reversed in many relatively undeveloped areas, including China, Malaysia, parts of South America, and sub-Saharan Africa.

Finucane et al conducted a comprehensive, constructive study that revealed growing global trends in BMI. This study may serve as wake-up call and initiate large-scale interventions in an effort to combat increasing body weight and associated adverse health consequences.[169]

Race-related demographics

Obesity is a cosmopolitan disease that affects all races worldwide. However, certain ethnic and racial groups appear to be particularly predisposed. The Pima Indians of Arizona and other ethnic groups native to North America have a particularly high prevalence of obesity. In addition, Pacific islanders (eg, Polynesians, Micronesians, Maoris), African Americans, and Hispanic populations (either Mexican or Puerto Rican in origin) in North America also have particularly high predispositions to the development of obesity.

Secular trends clearly emphasize the importance of environmental factors (particularly dietary issues) in the development of obesity. In many genetically similar cohorts of high-risk ethnic and racial groups, the prevalence of obesity in their countries of origin is low but rises considerably when members of these groups emigrate to the affluent countries of the Northern Hemisphere, where they alter their dietary habits and activities. These findings form the core concept of the thrifty gene hypothesis espoused by Neel and colleagues.[48]

The thrifty gene hypothesis posits that human evolution favored individuals who were more efficient at storing energy during times of food shortage and that this historic evolutionary advantage is now a disadvantage during a time of abundant food availability.

Age-related demographics

Children, particularly adolescents, who are obese have a high probability of becoming adults who are obese; hence, the bimodal distribution of obesity portends a large-scale obesity epidemic in the next few decades. Taller children generally tend to be more obese than shorter peers, are more insulin-resistant, and have increased leptin levels. [64]

Adolescent obesity poses a serious risk for severe obesity during early adulthood, particularly in non-Hispanic black women. This calls for a stronger emphasis on weight reduction during early adolescence, specifically targeting groups at greater risk.[85]

Diagnosis

Laboratory studies

- Fasting lipid panel
- Liver function studies
- Thyroid function tests

- Fasting glucose and hemoglobin A1c (HbA1c)

Evaluation of degree of body fat

BMI calculation, waist circumference, and waist/hip ratio are the common measures of the degree of body fat used in routine clinical practice. Other procedures that are used in few clinical centers include the following:

- Caliper-derived measurements of skin-fold thickness
- Dual-energy radiographic absorptiometry (DEXA)
- Bioelectrical impedance analysis
- Ultrasonography to determine fat thickness
- Underwater weighing

Physical Examination

In the clinical examination, measure anthropometric parameters and perform the standard, detailed examination required in evaluating patients with any chronic, multisystem disorder, such as obesity.

Waist and hip circumference are useful surrogates in estimating visceral fat; serial tracking of these measurements helps in estimating the clinical risk over time. Neck circumference is predictive of a risk of sleep apnea, and its serial measurement in the individual patient is clinically useful for risk stratification.[169]

Examination of organ systems should include the following:

- Cutaneous - Search for intertriginous rashes from skin- on- skin friction; also search for hirsutism in women, acanthosis nigricans, and skin tags, which are common with insulin resistance secondary to obesity
- Cardiac and respiratory - Exclude cardiomegaly and respiratory insufficiency
- Abdominal - Attempt to exclude tender hepatomegaly, which may suggest hepatic fatty infiltration or NASH, and distinguish the striae distensae from the pink and broad striae that suggest cortisol excess

When examining the extremities, search for joint deformities (eg, coxa vara), evidence of osteoarthritis, and any pressure ulcerations. Localized and lipodystrophic fat distribution should also be identified, because of their common association with insulin resistance.

Management

Treatment of obesity starts with comprehensive lifestyle management (ie, diet, physical activity, behavior modification).[62] The 3 major phases of any successful weight-loss program are as follows:

- Preinclusion screening phase
- Weight-loss phase
- Maintenance phase - This can conceivably last for the rest of the patient's life but ideally lasts for at least 1 year after the weight-loss program has been completed

Surgery

Among the standard bariatric procedures are the following:

- Roux-en-Y gastric bypass
- Adjustable gastric banding
- Gastric sleeve surgery
- Vertical sleeve gastrectomy
- Horizontal gastroplasty
- Vertical-banded gastroplasty
- Duodenal-switch procedures
- Biliopancreatic bypass
- Biliopancreatic diversion

Medications

Currently, the 3 major groups of drugs used to manage obesity are as follows:

- Centrally acting medications that impair dietary intake
- Medications that act peripherally to impair dietary absorption
- Medications that increase energy expenditure

1. 2. 1. Anorexiants and Treatment of Obesity[147, 139, 118]

Regulation of energy balance

Obesity is a disorder of energy balance. Hypothalamus is a major centre that regulates food consumption by modulating hunger and satiety. It intergrates neural, hormonal and nutrient messages from elsewhere and sends signals to higher centres, leading to the feeling of hunger or satiety, Hypothalamus also controls the energy expenditure via the adrenergic nervous system and the pituitary hormones.

The central control of body weight is a complex phenomenon, not well understood, regulated by Multiple brain nuclei and many neurotransmitters of these **neuropeptide Y** is the most potent appetite stimulant and is abundantly present in the hypothalamus. It also suppresses sympathetic activity and thus reduces energy expenditure.

Neuropeptides affecting energy balance:

- **Increase food intake**
  Noradernaline, GHRH Neuropeptide Y, Ghrelin, Opioids,Endogenous Cannabinoids.

- **Inhibit food intake**
  5-HT, Dopamine, Cholecystikinin (CCK), CRH, Neurotensin, Bombesin and Glucagon-like peptide, a MSH

Treatment of Obesity

Principles of treatment of Obesity

- Nutritionally adequate, low calorie diet, containing at least 1 gm of protein/kg of desirable body weight.
- Fat intake below 25% of total calories prescribed.
- Liberal use of fiber- containing foods
- Regular exercise
- Behavioural Therapy for change in life-style
- Anorectic drugs – as adjunctive therapy

In an obese individual if an organic cause such as an endocrine disease is present, it should be treated. Commonly obesity is due to excessive eating and lack of adequate excerise. The patient must be made to appreciate this at the beginning of the treatment and explained that *diet is the mainstay of treatment of obesity;* he/she adequately motivated by the concept of 'positive health'. It must also be emphasized that dietary treatment of obesity consists of a lifelong reorientation of his eating habits (and often those of his family) and not merely of a 'course' of dieting .Those who look upon dieting as a course of treatment are bound to regain the lost weight.

There are many diets for obesity. It has, however, been established that nothing about the diet matters except its calorie content. Hence, the best diet is one which is low in calorie content (1000-1500 per day), contains all the essential nutrients, provides a variety

and is simple for the patient to follow for prolonged periods. Drastic one-step calorie reduction may not be tolerated by patient; it is better to reduce the daily calorie intake progressively in steps of 500 calories.

The results of therapy are likely to be more satisfactory in hypertrophic obesity (enlarged, distended fat cells, with usual onset in adult life) than in the hyper plastic variety (more numerous fat cells, with usual onset in childhood).

The patient should be told at the onset of therapy that an average weekly loss of 0.5-0.8 kg is adequate and would result in annual loss of about 25 kg. This can be achieved by consuming 500-1000 fewer calories per day.

Classification of drugs used for obesity

I Centrally acting, appetite Suppressants (Anorexiants)

- **Adrenergic agents** eg : Benzamphetamine, Phentermine, Mazindol, Phendimetrazine , Diethylpropion
- **5-HT agonists** eg. Fenfluramine, Dexfenfluramine, Fluoxetine, Lorcaserin.
- **Drugs acting on both adrenergic and 5 – HT systems** eg Sibutramine
- **Cannabinoid receptor antagonists** eg Rimonabant

II Drugs acting in the GI tract

- **Bulk anorexiants** eg. Dietary fibre, Methylceelulose, Guargum.
- **Non-absorbable fat substitutes eg**. Olestra
- **Lipase inhibitors** eg. Orlistat

III Drugs combinations

Eg: Phenteramine + Topiramate, Bupropion + Naltrexone

IV Miscellaneous Eg: Metformin

Anorexiants

Amphetamine and related compounds:

Their anorexiant effect appears to be related to central relase of DA and NA from the adrenergic cells. They also inhibit the reuptake of NA and DA. Their major drawbacks are drug dependence and sympathomimetic ADR like hyper tension, tachycardia, insomnia

and nervousness. Hence, sympathomimetics are rarely recommended in the treatment of obesity **Methylamphetamine is highly addictive and is extensively abused.**

Other related drugs are phenteramine, phendimetrazine, mazindol and diethylpropion.

Fenfluramine: This drug, once used extensively, acts by increasing the brain synaptic 5-HT by accelerating it's release and reducing its re-Uptake. However, it was found to cause cardiac valvular injury and primary pulmonary hypertension. It has been withdrawn from the market.

Fluoxetine: This 5-HT reuptake inhibitor antidepressant , in the dose of 60mg/day, is associated with weight loss ; but its long term use should be avoided in view of the experience with fenfluramine.

Lorcaserin: This is a selective central $5HT_2$ receptor agonist. It suppreses the appetite. It has a t1/2 of 11 hours and is extensively metabolised by the liver. Headache, nausea,dizziness and euphoria (at doses > 40mg) are the frequently reported adverse effects. Lorcaserin inhibits CYP 2D6 and hence increases the concentration of other drugs metabolised by this enzyme (eg.dextromethorphan).

It took over 30years to detect the serious adverse reaction to fenfluramine. This emphasies the vital importance of continued and vigilant post-marketing adverse drug reaction surveillance. Similar long term toxicity is likely to occur following the use of oter drugs acting as 5-HT agonists.

Sibutramine and rimonabant are no more used because of their cardio vascular and neuropsychiatric toxicity respectively.

Limitations of centrally acting anorexiants

- Short term efficacy
- Relapse of weight gain
- Possible toxicity during long term therapy;
- Weight cycling as a result of drug holiday, leading to adverse outcomes such as coronary heart disease; and
- Inability to prevent obesity- related illnesses and to prolong life
- Poor compliance

Drugs acting in the GI tract

Bulk anorexiants

Methylcellulose, a non-digestible polysaccharide, when ingested, swells and adds to the bulk in the diet. Though used as an appetite satiator, it is no more effective than the high residue low caloric diet for the obvious reason that an obese person is interested in eating good food and not something that would merely distend the stomach. It forms an important, cheap constitutent of many 'costly' commercial preparations advertised for the treatment of obesity. **Guar gum, Karaya gum** (Sterculia gum, Kanormal) and **glucomannan** are the other bulking agents.

Digestion inhibitors

Olestra

This is a mixture of sucrose – fatty – acid esters that is neither digested nor absorbed from the GI tract. When used as a component of diet, it increases the bowel function and stool bulk. It is recommended as a fat substitute in cooking. Long term effects on the health are yet not fully known. It is expensive.

Orlistat

Orlisatat (Tetrahydrolipostatin) is related to a lipase inhibitor, lipostatin produced by the mould, *streptomyces toxytricini*. It acts by inhibiting pancreatic and other lipases including phospholipase A_2. It is not absorbed and the effects are confined to the GI tract, where it prevents the lipase-catalysed breakdown (GI lipase inhibiton) of TG and subsequent absorption of about $1/3^{rd}$ of the dietrary fat. The additional benefit is a modest reduction in the plasma levels of total cholesterol and LDL cholesterol but not of TG. It is modestly effective and causes weight loss over 1-4 years. It is almost completely excreted unchanged in feces. The common adverse effects are abdominal pain, flatulence,increased defecation of oily /fatty stools with anal leaking ,which can be inconvenient and socially embarrassing.

Drug Combinations

(a)Phenteramine in combination with **topiramate,** an antiepileptic, given as OD cause dose dependent weight loss. However because of ADR, the discontinuation rate is nearly 40%. Topiramate is also a carbonic anhydrase inhibitor and can cause metabolic acidosis.

(b)Bupropion , a week antidepressant , also stimulates hypothalamic neurons to produce anorexigenic effects. However, these neurons also secrete beta endorphin, an endogenous opioid, which counters this action. Hence, bupropion is combined with **naltrexone,** an opioid antagonist. The combinations are reported to cause weight loss but the effect may not be sustained.

Miscellaneous: The antidiabetic, metformin induces weight loss in diabetic and some nondiabetic obese subjects.

Experts recommend that drugs may be considered in adults with a BMI of over 27 in the presence of an obesity-related complication and in those with a BMI of over 30 even in the absence of such a complication. For persons with a BMI of over 40,bariatric surgery may be considered. As Obesity is a chronic disease and as relapse is common after cessation of drug therapy, prolonged, lifelong, drug therapy may be desirable. However, none of the available drugs is safe enough for prolonged use. They also fail to maintain weight loss over 10% in a year. Evidence suggests that combined drug therapy is no better than drug monotherapy. Irrespective of the drugs used, if significant weight loss does not occur within 4-6 months of therapy, the drug is discontinued. Drug should not be used simply for cosmetic weight control.

Thus the treatment of obesity mainly depends on permanent lifestyle modifications such as change in eating habits and regular physical excerise, supported by behavioural therapy.

Some drugs causing anorexia

- Aminophylline
- Amphetamines and related drugs
- Antimicrobials: Metronidazole, Fluoroquinolones, Erythromycin , Nitrofurantion, Tetracycline
- Antimalarials
- Metformin

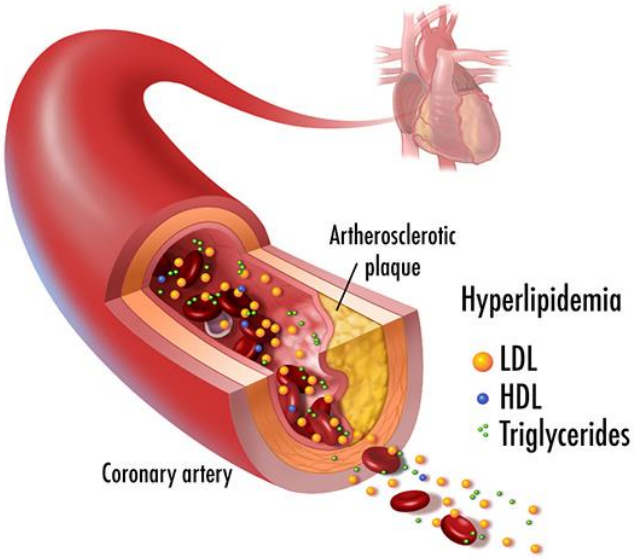
- Digitalis
- Levodopa

Drugs which may cause weight gain

- Antipsychotics, especially Olanzapine
- Antidepressants: Tricyclics, SSRI,MAOI, Mirtazapine
- Hormones: Glucocorticoids , Insulin Progestogens, Oral Contaceptives and Danazol
- Beta Blockers
- Oral Hypoglycemic agents : SulfonylUreas, Glitazones
- Anticonvulsants: Phenytoin , Sodium Valproate
- Antihistaminics, especially the first generation agents Pizotifen

1. 3. ANTIHYPERLIPIDEMIC DRUGS[172, 148,135,119]

Fig. No. 3: Formation of Plaque in the Coronary artery



Antihyperlipidemic Drugs

These drugs act predominantly on:

- **Elevated Cholesterol**, e.g HMG-CoA reductase inhibitos (statins), Cholestyramine resin, Ezetimibe Fibre:
- **Elevated triglycerides**,eg Fibric acid derivatives (fibrates),Fish oil or
- **Both**, eg; Nicotinic acid

STATINS (HMG – CoA - reductase inhibitors)

Table No. 1: Statins and dose recommended

Statins	**High intensity statin therapy (mg/day)**	**Moderate intensity statin therapy (mg/day)**
Atorvastatin	40 to 80	10 to 20
Rosuvastatin	20 to 40	5 to 10
Simvastatin	-	20 to 40
Pravastatin	-	40 to 80
Lovastatin	-	40
Fluvastatin	-	80 as extended release; if 40 twice a day
Pitavastatin	-	2 to 4

Mechanism of action

Normally, about 70-75% of plasma LDL is removed by hepatocytes, by receptor – mediated endocytosis. Cholesterol esters from LDL molecules are hydrolysed in the liver to free cholesterol. The liver also produces cholesterol by de now synthesis by a pathway involving formation of mevalonic acid by the enzyme Hydroxy Methyl Glutaryl Co - enzyme A reductase (HMG -CoA reductase). Statins inhibit this rate -limiting enzyme. This decreases hepatic cholesterol synthesis, which leads to increased synthesis of high affinity LDL receptors (up regulation) on the surface of liver cells. This results into increased clearance (uptake) of cholesterol-rich plasma LDL with subsequent.

Hepatic regulation of synthesis of Cholesterol and bile acids

Site of action of statins;(II) Site of action of resins and ezetimibe reduction in plasma LDL cholesterol This action is dose dependent and is observed in 10 days. Full effect is generally seen within 6 weeks. Effect of statins on HDL-C is variable.

Absorption, fate and excretion

Given orally, the absorption of all the statins (except fluvastatin) varies between 40% and 90% .Fluvastatin is absorbed almost completely.Lovastatin and simvastatin are prodrugs and are hydrolysed in the GI tract to the active metabolites. Atorvastatin,

fluvastatin and rosuvastatin are fluorinated compounds that are active as such. All undergo first pass metabolism and most of the dose is excreted in the bile; only about 5-20% is excreted in the urine.

Adverse reactions

Generally statins are well tolerated. ADR are mostly mild and dose dependent. They may cause reversible rise in hepatic aminotransferase levels. Small rise in the plasma creatine kinase level is not uncommon. Rarely it may be marked and accompanied by muscular pains (myositis) and even myopathy. Rhabdomyolysis is rare.

Rarely, Statins may cause impotence, gynecomastia, peripheral neuropathy, memory loss, insomnia, mood changes and depression.

Clinical trials demonstrate that statins may increase the risk of new-onset diabetes but incidence is not as much as they reduce the cardiovascular risk.

Statins are contraindicated in pregnancy and in a woman planning to become pregnant, during breast-feeding, in children and in patients with severe liver disease.

Drug interactions

Combination of a statin with a fibric acid derivative and nicotinc acid potentiates the rise in plasma CPK level.

Lovastatin, Simvastatin and atorvastatin undergo extensive first pass metabolism by CYP3A4 and their toxicity can be increased by the concurrent use of hepatic microsomal enzyme inhibitors. Fluvastatin and rosuvastatin are metabolized by hepatic CYP2C9. Inhibitors of this enzyme such as ketoconazole, metronidazole and cimetidine can increase the plasma levels of these statins.

Maximum dose of simvastatin recommended is 10mg in patients taking amiodarone,diltiazem or verapamil and 20mg/day in those on amlodipine and ranolazine.

Therapeutic uses

Statins are useful in both primary and secondary prophylaxis of hypercholesterolemia. Their main indications are

- MI or any cardiovascular event

- Patients less than 70 years with known IHD
- Familial hypercholesterolemia
- Diabetes mellitus
- Subjects with strong family history of premature cardiovascular disease.

Statins used alone in maximum doses, can lower LDL level by 30-50%. Statin therapy can reduce the 5 year incidence of major coronary events, and stroke significantly, largely irrespective of the initial lipid level.

In general statins are more effective in subjects with high risk factors than a particular LDL cholesterol level. Currently they are indicated in :

(i) Individuals with clinical evidence of **atherosclerotic cardiovascular disease** (ASCVD) eg IHD,MI

(ii) Subjects with primary elevation of LDL cholesterol >190mg%

(iii) Subjects with age 40-75 years with diabetes mellitus and LDL cholesterol between 70-189mg % without clinical evidence of ASCVD

(iv) Subhects with age 40-75 years, having LDL cholesterol between 70-189 mg% with no evidence of diabetes mellitus or ASCVD but having estimated 10 years ASCVD risk of 7.5% or higher. This risk is calculated based on the presence of risk factors in a given patient.

Patients with clinical evidence of ASCVD and those with LDL cholesterol>190mg% are treated with high intensity statin therapy, which reduces LDL-cholesterol by >50%. Others are treated with high intensity statin therapy,wherein a decrease by 30 to 50 % is expected

There is no unanimity about what can be considered as an “ideal” level of serum cholesterol and hence currently importance is given to the clinically relevant risk factors rather than chasing a particular target level for LDL cholesterol. Routine use of statin in subject with>75 years and without evidences of ASCVD and those with NYHA class II, III and IV HF do not seem to be benefitted.

Statins also possess other lipid-independent effects. These are

- Decrease in platelet aggregation and in fibrinogen levels.
- Improvement in endothelial function and increase in local NO production

- Decrease in macrophage infiltration into the vessel wall
- Decrease in the arterial muscle proliferation
- Retardation of the progression of hypertrophy of the vessel wall; and
- Decrease LDL oxidation in the vessel wall

These effect contribute to anti-inflammatory potential of statins,

Choice of Statin

Therapeutically there are no clear differences among various statins. Hence the choice of statins depends upon the degree of LDL elevation, drug pharmacokinetics, drug interactions, presence of hepatic/renal disease and cost. Atorvastatin and simvastatin are more efficacious than others, and therefore may be preferred. Atorvastatin and fluvastatin need no dose adjustment in patients with renal impairment. Pravastatin and fluvastatin are not metabolized by CYP3A4 and are likely to cause few drug interactions. Atorvastatin and rosuvastatin jave long $t_{1/2}$ (14 and 19 hours, respectively) and can be taken at any time of the day

Since most cholesterol synthesis occurs during the night the short t1/2 compounds are best administered at night as a single dose.

Table No. 2: Doses of commonly used statins in Asia Population

Statins	Starting/Max.Dose (mg/day)
Atorvastatin	10/40
Rosuvastatin	2.5/20
Simvastatin	5/20
Pravastatin	10/20
Lovastatin	10/80
Fluvastatin	20/60

Note: Atorvastatin may be given at any time of the day; Paravstatin and Fluvastatin at bedtime; others with evening meal**.**

Ezetimibe

This prodrug 2-azetidinone, is converted in intestine to an active metabolise ezetimibe glucuronide (t1/2-22hr). It binds to a intestinal mucosal transporter and decreases delivery of dietary and biliary cholesterol to the liver. Thus, it inhibits absorption of cholesterol by the small intestine. Reduction of hepatic cholesterol stores casuses

increases in LDL receptors on the hepatocytes (up-regulartion) and an increased LDL cholesterol clearance from the circulation. The plasma total and LDL cholesterol decreases with minimal increase in HDL-C table 40.1.It also interrupts the entero-hepatic cycling of cholesterol. It is 90% bound to plasma proteins and is largely excreted in the faeces.

Adverse reactions are fatigue, dizziness, headache, abdominal discomfort, diarrhea, arthralgia and hypersensitivity reactions.

Therapuetic uses- It can be used as monotherapy in the dose of 5-10mg day. However, it acts synergistically when combined with statins and is particularly useful in patients who do not tolerate large doses of statins. However, this decreases the progress of atherosclerosis. It should be avoided in children and in pregnant/lactating women.

Cholestyramine Resin

This insoluble chloride salt of a basic anion exchange resin, has a strong affinity for bile salts (cholates) in the intestine. It bind the cholates into an insoluble complex which is excreted in faeces(fig 40.1) The result is decreased absorption of exogenous cholesterol and increased metabolism of endogenous cholesterol in to bile acids in the liver. This lead to increased LDL receptors on liver cells and increased removal of LDL from the circulation. The drug, however, does not significantly lower the plasma levels of triglycerides. The dose is 12-24g per day in a single or divided doses.

Adverse Reactions

These include nausea,vomiting,heartburn and constipation. Large doses may interfere with absorption of fats and fat soluble vitamins, causing steatorrhoea. It also interferes with the absorption of thyroid hormones, tetracyclines, warfarin, digoxin and phenobarbitone.

It is now rarely used to treat hyperlipidemia It may be useful in relieving pruritus associated with biliary cirrhosis and cholestatic jaundice.

Colestipol and Colesevalam like cholestyramine are bile acid sequestrants.

Gugulipid- This is the steroidal fraction derived from the plant *commiphora mukul* and has moderate and variable hypocholesterolemic activity. It is well tolerated. It is available as 25 mg tablets.

Fibre - Soluble fibres (pectins in citrus fruits, mucilages in psyllium seeds, gums in oat products and in beans) have a modest effect in lowering serum cholesterol. Psyllium seeds (Isapgol) is administered in the dose of 2.5-5g twice a day; oat bran is used in the dose of 50-150g a day. Insoluble fibre such as is present in wheat bran is not effective in lowering serum cholesterol

Fibrates are the derivatives of fibric (isobutyric) acid and include **gemfibrozil,bezafibrate and fenofibrate.** Clofibrate is no more used

Mechansim of action

These drugs stimulate the nuclear transcription receptor, **Peroxisome proliferator Activated Receptor alpha (PPAR-α)** that controls the expression of genes, which mediate Tg metabolism. They increase lipoprotein lipase (lpL) activity and the hydrolysis of TG and promote HDL production. They reduce the incorporation of fatty acid into VLDL in the liver, this inhibiting its synthesis and secretion. The plasma TG declines by 50% and cholesterol by 10-15% HDL increases by 20%. They are claimed to reduce plasma fibrinogen levels to increase fibrinolysis and to reduce abnormal platelet stickiness.

Absorption, fate and excretion- They are almost completely absorbed from the gut, are highly protein bound (more than 90%) They are largely excreted unchanged in the urine.

Adverse reactions- Fibrates are generally well tolerated although, they occasionally produce allergic reactions,nausea and diarrhoea. Though rare, potentially serious effects on the skeletal (myositis) and cardiac muscle have been reported. Fibrates should be avoided in patients with renal or hepatic damage and in alcoholics (with hypertriglyceridemia) who are predisposed to myositis. Long term therapy is associated with an increase in gall stone formation. *The* combination of a fibrate and a stain increases the chance of rhabdomyolysis and myopathy. These drugs displace acidic drugs from their plasma protein binding and may enhance the action of warfarin and sulfonylureas.

Preparations and dosage

(i) Fenofibrate: 67,134,200 mg single dose capsules. Dose: one capsule OD with a meal.

(ii) Gemfibrozil 300 mg capsules. Dose 600 mg twice a day 30 minutes before meals.

(iii) Bezafibrate: 200 mg tablets. Doses: 200 mg three times a day with meals.

Therapeutic Uses

These drugs are effective in reducing mainly plasma TG Fenofibrate is preferred to gemfibrozil.

Fish oil

Epidemiological evidence suggests that fatty fish eating communities have a lower incidence of IHD than non-fish-eaters. **Omega 3 PUFA** in the fish oil decreses hepatic production of TG and increases its clearance. Thus they reduce plasma TG but have little effect on HDL and LDL cholesterol and can sometimes increase total cholesterol upto 50% They also lead to synthesis of leukotrienes and prostaglandins of series 1 and 3 , which are less pro inflammatory than those of series 2. Unlike gemfibrozil and niacin, omega-3-PUFAs do not interact with statins to enhance the incidence of myositis. They may decerase the risk of cardiac death in recent MI. But, in chronic IHD there is a possibility of a proarrhythmic effect.

Adverse effects

These include eructions, dyspepsia and unpleasant after taste. Large doses of omega 3 – PUFA can inhibit platelet aggregation and may worsen glycemic control in diabeties.

Eating fatty fish may be beneficial in healthy people but there is no convincing evidence supporting the prevention of cardio vascular disease in the general population. The drug is contraindicated in patients with type Ila hyperlipidemia. The dose is five 1g capsule bid.

Omega-3 fatty acids are also prevent in nuts (especially walnuts) dark green leafy vegetables,soya beans,flax seeds and flax seed oil and hemp seeds.

Icosapent ethyl, the ethyl ester of eicosapentanoic acid is available as 1 gram capsule. A dose, 2 gm bid with food can lower serum TG without increasing LDL cholesterol.

Table No. 3: Choice of lipid lowering drugs

Drug	Hypercholesterolemia (elevated LDL-C+ TG<200 mg/dl)	Combined hyperlipidemia (elevated LDL-C+ TG 200-400 mg/dl)	Hypertriglyceridemia (TG>400 mg/dl)
Statins	Drugs of choice	Effective	-
Fibrates	-	Effective in high doses	Drugs of choice
Nicotinic acid	Effective – usefulness limited by adverse effects of full doses	Drug of choice	Effective But Poorly Tolerated

Note: Statins can be combined with (a) nicotinic acid, or (b) a fibrate, in resistant cases.

Nicotinic Acid

This vitamin in large doses effectively and rapidly reduces plasma TG by lowering VLDL levels; LDL levels diminish more slowly, whereas HDL level rise during therapy Nicotinamide lacks these actions.

Mechanism of action

In the adipose tissue, nicotinic acid inhibits adenylyl cyclise, and prevents lipolysis by hormone sensitive lipase. This reduce the transport of fatty acid to the liver. In the liver, it reduces both synthesis and esterification of fatty acids. The end result is reduction in the hepatic production of VLDL and in plasma TG, VLDL cholesterol and LDL cholesterol.

Adverse reactions

In pharmacologicl doses, nicotinic acid produces intense cutaneous flushing and pruritus by increasing the local prostaglandin levels. This can be minimized by starting with a small dose and gradually building it up to the full dose, and by taking the drug with meals; further , one tablet of aspirin, taken 30 minutes earlier, markedly reduces, the flush. Other adverse reactions are nausea, vomiting, diarrhoea; abnormalities of liver function, clinical jaundice; hyperuricemia, hyperglycemia; and potentation of hypotension casused by antihypertensives

Therapeutic Uses

Nicotinic acid is usefull in all forms of hyperlipoproteinemias except type I and is drug of choice in type V hyper-lipoproteinemia.The usual dose is 2-8 g/day, in divided doses, with meals. It is advisable to start with 100 mg tid with meals and to build up the dose in 2-4 weeks.

Folic Acid Supplements are used to prevent homocysteinemia

1.4. Anxiety and Anxiolytics[171,146,138,117]

Anxiety is actually an adaptive response which prepares a person to face the challenges of life. Anxiety is characterised by psychologic symptoms (tension, fear, apprehension, lack of concentration etc) as well as by sympathetic plus somatic symptoms (tachycardia, tremors, sweating, GIT distress etc). Fatigue and sleep disturbances are also common. If anxiety persists it impairs person's ability to perform the job and often lead to visceral organ dysfunction and neurological problems.

Classification of Anxiolytics

1. Benzodiazepines

This is the most important group with multifarious uses and may be divided according to their duration of action.

Short Acting

- Triazolam, Oxazepam, Midazolam

Intermediate Acting

- Alprazolam, Estazolam, Temazepam, Lorazepam, Nitrazepam

Long Acting

- Diazepam, Flurazepam, Clonazepam, Chlordiazepoxide

2. Non Benzodiazepine Hypnotics

Zolpidem , Zaleplon, Zopiclone, Eszopiclone

3. Atypical Anxiolytics

Buspirone, Ipsapirone, Gepirone

4. β-Adrenoceptor Antagonists (Non-Sedating Anxiolytics)

Propranolol

5. Barbiturates

Long Acting

Phenobarbital, Mephobarbital

Short Acting

Pentobarbital, Amobarbital

Ultra-Short Acting

Thiopental, Methohexital

6. Miscellaneous Group

Melatonin, Ramelton, Triclophos, Hydroxyzine and Promethazine

Benzodiazepines (BZDs)

Site of Action

BZDs exhibit relatively greater depressant action on limbic system (which regulates thoughts and mental functions) compared to midbrain ascending RAS (which maintains Wakefullness). Hence they reduce anxiety at a dose which has little depressant effects on RAS, i.e., they elicit anxiolytic action with less sedative effects. At higher doses, however, they depress RAS and induce sedative-hypnotic action but this property differs qualitatively with individual drug. Muscle relaxation is produced by a medullary site of action, by inhibiting polysynaptic reflexes in the spinal cord. Ataxia is due to their action on cerebellum.

Mechanism of Action

Normally a balance between excitatory inputs (mostly glutamatergic) and the inhibitory inputs (mainly GABAergic) determines the prevailing level of neuronal activity in limbic system and RAS of the brain. If the balance swings in favour of GABA, nervousness and anxiety are reduced while sedation, amnesia, muscle relaxation and ataxia appear. In contrast, a reduction in GABAergic activity elicits arousal, anxiety, insomnia and restlessness. GABA acts on two distinct classes of receptors.

- GABA A receptors which are located mainly post-synaptically. These are directly linked with Cl ion channels, opening of which causes hyper-polarisation and reduction in membrane excitability

- GABA B receptors which are G-Protein coupled receptors. Their activation decreases cAMP formation. They cause pre and postsynaptic inhibition by inhibiting ca^{2+} channel opening and increasing K^{+} conductance

GABA receptor has several different binding sites which include:

- The neurotransmitter GABA-binding site
- The modulatory sites to bind benzodiazepines, their antagonists like flumazenil and inverse agonists such as B-carbolines
- The modulatory as well as blocking site at chloride ion channel

GABA A receptors are the target for several important centrally acting drugs like benzodiazepines, barbiturates, neurosteroids and picrotoxin. BZDs which have powerful sedative and anxiolytic effects, selectively bind, with high affinity, to the modulatory site on GABA A receptor in such a way that the binding of GABA to GABA A receptor is facilitated. This modulatory site of GABA A receptor is distinct from the GABA binding site and is specific for BZDs; hence it is also referred to as "Benzodiazepine receptor" BZDs neither substitute for GABA, nor activate GABA A receptor but rather enhance binding of GABA to the GABA binding site of GABA A receptor . They appear to increase the frequency (rather than duration) of GABA gated chloride channel opening. A model of hypothetical GABA-BZD-CT ion channel receptor complex with sites of action for agonists, antagonists, inverse agonists channel blockers and channel modulators

BZD Inverse Agonists and Antagonists

Inverse agonists (e.g., β-carboline) exert opposite effects to that of conventional BZDs producing signs of anxiety and convulsions BZD inverse agonists have no therapeutic utility except as research tools. BZD antagonist flumazenil blocks the actions of BZD and BZD agonists and is however used clinically. It undergoes significant first pass metabolism in liver, hence not used orally. On I.V injection, the effect starts spontaneously and lasts for 1- 2 hrs. Plasma half life i.e. 1- 2 hrs. Its main use is to reverse BZD anaesthesia (0.5 mg I.V) or to treat BZD overdose toxicity (0.2mg/ min; maximum 5 mg; till patient regains consciousness). Side effects are rate except anxiety, tremors and discomfort.

Pharmacokinetic Characteristics

There are marked pharmacokinetic variations among BZDs. Except for midazolam (given I.V or I.M), all can be given orally (though absorption varies). Although some of BZDs (diazepam, oxazepam, and chlordiazepoxide) are more than 90% protein bound no significant drug displacement reactions occur because of their marked volume of distribution. They cross placental barrier (caution in Pregnancy). Most BZDs are metabolised by Phase I metabolic processes and the resultant metabolites are subsequently conjugated (phase II reactions)to form water – soluble metabolities that are excreted in urine. However, many phase I metabolites are pharmologically active which extend the half-life (and hence duration of action) of parent drug. These include midazolam (which acts through its active metabolite, hydroxymethyl midazolam); diazepam (active metabolite oxazepam and nordiazepam also called clorazepate); flurazepam (active metabolite desmethylflurazepam and hydroxymethylflurazepam); chlordizepoxide (active metabolite desmethyldiazepam and oxazepam) and alprazolam (active metabolite, alfa-hydroxyalprazolam),

Accumalation,with multiple dosing, is not clinically significant with short-acting BZDs but is significant with intermediate acting and long acting BZDs. Because of longer t1/2, withdrawal effects are milder with long-acting BZDs.

Drugs Interactions

- BZDs potentiate the effects of other CNS depressants, such as alcohol, hypnotics and neuroleptics
- Smoking decreases the activity of BZDs
- Aminophylline antagonises sedative effects of BZDs
- Enzyme inhibitors like cimetidine and Ketoconazole enhance BZD action

Non-Benzodiazepine Hypnotics

Several non-benzodiazepines discussed below are more selective in their central actions as they act as agonist at the modulatory site of GABA receptor to which BZDs bind. Two types of BZD receptor have been suggested: BZD receptor type I (BZ) found throughout the brain and in large concentrations in the cerebellum and BZD receptor type II (BZ2) found mainly in the cerebral cortex, hippocampus and spinal cord. It is the BZ1 receptor that is thought to be responsible for antianxiety, sedative and hypnotic action

while BZ2 receptor appears to be associated with muscle relaxation, anticonvulsant action and amnesia. Most BZDs act on both BZ1 and BZ2 receptors. Non-Benzodiazepines such Zolpidem, Zaleplon, Zopiclone and Eszopiclone act on BZ1 receptor only; while 1-5 – benzodiazepines such as clobazam act preferably at BZD receptor to elicit muscle relaxant and anticonvulsant actions.

Zolpidem, Zopiclone, Eszopiclone and Zaleplon

These drugs act on BZ1 receptors and have hypnotic actions. As hypnotic, Zolpidem (t1/2 2-3 hrs) and Zaleplon (t1/2 3-4 hrs) have faster onset of action with shorter duration of hypnotic effects while Zopiclone (t1/2 6-8 hrs) and eszopiclone (t1/2 6 hrs) are slightly longer acting. In therapeutic doses they hardly alter REM sleep pattern and have minimal daytime residual sedation. The problem of rebound insomnia (after withdrawal of the drug) is also minimal. Zolpidem (10 -20 mg) and Zaleplon (10-20 mg) at bed time are used to treat short-term insomnia. While Zopiclone (7.5mg) and eszopiclone (1-3 mg) at bed time are used to treat short term insomnia. Their effects can be blocked by flumazenil, which is antagonist at both BZ1 and BZ@ receptors.

These drugs have negligible muscle relaxant and anticonvulsant action. The risk of tolerance and dependence is much less after their withdrawal as compared to BZDs. Side effects and safety in overdoses are almost similar to BZDs. Dose reduction is needed in hepatic disease and in elderly patients. Headache, daytime drowsiness and nightmares can occur with higher doses.

Atypical Anxiolytics

Buspirone, Ipsapirone and Gepirone

These drugs act through non-GABAergic systems and have low propensity of side effects by acting as a partial agonist primarily at brain 5-HT receptors. Hence, by selective activation of the inhibitory presynaptic 5-HT receptor, they suppress 5-HT neurotransmission through neuronal system. The drug buspirone (5-10 mg TDS) is used in anxiety states. However, the useful anxiolytic effects of buspirone are delayed for more than two weeks, which makes it unsuitable for management of acute anxiety states. It has no muscle relaxant, or anticonvulsant or hypnotic action.

Buspirone has minimal abuse liability and elicits no withdrawal reactions like rebound anxiety and insomnia etc., on abrupt discontinuation. Buspirone causes lesser

impairment of psychomotor skills and does not potentiate the CNS depressant effects of ethanol (non-sedating anxiolytic drug). However, it can cause tachycardia, nervousness, GIT distress and paresthesias. Although mechanism is unknown, it causes dose-dependent pupillary constriction.

Barbiturates

Mechanism of Action

Barbiturates act on the channel modulatory site of GABA a receptor and potentiate the GABA mediated inhibitory effects by increasing the duration of chloride channel opening. At higher concentrations, barbiturates directly increase chloride ion conductance i.e. they exhibit a GABA mimetic action and not a GABA facilitatory action (as exhibited by BZDs)

Pharmacokinetics

- The rate of absorption of barbiturates depends on their lipid solubility. The pH of solutions of their sodium salts is usually alkaline and therefore cannot be given by I.M or S.C routes for fear of necrosis and pain at the site of injection. But these can be given slow I.V., orally and at times rectally. These are weakly acidic drugs. Hence, they remain unionised in acidic pH and fully ionized at alkaline pH
- These are widely distributed depending upon their lipid solubility and the regional blood flow to the different part of the body. The action of the ultra-short-acting barbiturates such as thiopental is terminated primarily as a result of redistribution from brain to lean tissues muscles and then fat and adipose tissue.
- These are metabolized by both phase-I (liver microsomal oxidation) and phase-II (glucuronyl conjugation) processes. Their prolonged use leads to an induction of liver microsomal enzymes, which results in the development of metabolic tolerance.
- Although excreted through urine, these get readily reabsorbed from renal tubules. Alkalinisation increases their ionization and therefore increases their renal excretion

Adverse Effects

- Repeated use of barbiturates leads to the development of metabolic tolerance due to enzymatic induction. This leads to various drug interactions because of the accelerated metabolism of concomitantly administered drugs along with barbiturates.
- Barbiturates have considerable abuse liability and exhibit both psychic as well as physical dependence on withdrawal after prolonged use. Withdrawal symptoms include tremors, insomnia, headache, restlessness and delirium
- They cause hangover, impairment of judgement and "drug automatism"
- They cause respiratory depression, laryngeal oedema and hypersensitivity reactions (skin rash , swelling of lips and eyelids)

Drug Interactions

Barbiturates reduce the effectiveness of various concomitantly administered drugs due to induction of their metabolism. Such drugs include oral contraceptives, anticoagulants, tolbutamide and theophylline.

Contraindications

These are contraindicated in liver dysfunctions, Kidney disease and sever pulmonary insufficiency e.g., emphysema

Patients having family history of porphyria or in cases of acute intermittent porphyria because barbiturates cause induction of ALA - synthetase enzyme in mitochondria. This leads to increased synthesis of porphyrins and hence porphyria and neurotoxicity.

1.5. Analgesics[173,149,136,120]

Pain - Introduction

Pain is an unpleasant sensory and emotional experience, associated with actual or potential tissue damage or described in terms of such damage. It is a subjective experience which cannot be objectively defined or qualified satisfactorily. Pain act as a warning signal against disturbances in the body and thus has a protective function. However, on many occasions pain seems pointless, only contributing to the discomfort in the subject. As a symptom, pain demands instant relief and in practice its dramatic relief highly impresses a layman.

Pain receptors are disturbed throughout the body. Clinically, pain can be considered as

- Superficial or cutaneous pain
- Deep non-visceral pain from muscles, joints, ligaments and bones
- Visceral pain
- Referred pain
- Deafferentiation or neuropathic pain and
- Psychogenic or functional pain

Pain arising from the skin and from the deep non-visceral structures like muscles, bones and joints is also termed as **somatic pain**. It is usually well defined and is generally caused by an inflammatory reaction in the tissues; it may be accompanied by contraction of the surrounding skeletal muscles as in patients with rheumatoid arthritis. However, other causes such as direct irritation of a nerve as in trigeminal neuralgia, herpes zoster, increased pulsation of the intracranial arteries as in migraine, or vascular insufficiency as in thromboangitis obliterans are also incriminated in the genesis of somatic pain.

Pain arising from the skin and superficial mucous membrane or nerves is felt as pricking, if brief, and stinging, smarting or burning if prolonged.

Deep nonvisceral (Skeletomuscular) pain usually has a dull character and it may be accompanied by a sickening sensation dut to an autonomic response. Sometimes it spreads to other areas and may even occur as referred pain. BP and pulse,however, are nott much affected,unless the pain is acute and severe.

Visceral Pain, unlike the somatic pain, is diffuse, less easily localised and ofter 'referred'. It is dull-aching or colicky in character and is often accompanied by sweating, nausea, fall in BP and even shock. In addition, muscle rigidity and hyperaesthesia are common accompaniments. In practice, visceral pain may be due to spasm (renal or biliary colic), ischemia (myocardial infarction), inflammation (appendicitis,pancreatitis) or stimulation of the sensory nerve endings (peptic ulcer).

Deep pain, whether visceral or somatic in origin , may be misinterpreted as coming from some part of the body other than the actual site of stimulation. This is called **referred pain.**

Thus, cardiac pain is commonly referred to the left arm and diaphragmatic pain to the shoulder. Usually, the pain is referred to a cutaneous area which receives its nerve supply form the same spinal segment as the affected viscus.

Although various theories have been prosposed to explain the pain mechanism none can explain all its aspects. The assimilation of sensory pain at the level of consciousness depends on various factors such as the nature of sensory receptors, the intensity of the impulses transmitted to the CNS, their intergration and finally their modulation by other sensory information. The conscious appreciation of pain appears to depend upon the widespread activity of the entire cortex; and individuals differ widely in their reactions to similar painful experiences.

Deafferentiation pain is caused by partial damage to axons and nerve membranes, which become very sensitive to mechanical and chemical stimuli. Such pain may either be of burning, superficial (dysathetic) type; or of stabbing (lancinating) character. It has a peculiarly unpleasant quality about it and may not respond to opioids nor to NSAID.

Psychogenic or functional pain is usually a vague pain which follows no definite anatomical pattern of distribution. Such pain is usually continuous from day to day and involves more than one part of the body. It however, does not disturb sleep. Psychogenic pain is often preceded by a phase of exhaustion while organic pain brings about exhaustion. However, psychic element is present in all types of pain.

Pain pathways

Painful stimuli may primarily be physical stimuli such as pressure or heat, or they may be chemical stimuli from the product of inflammation.

A Variety of naturally occurring compound, can elicit pain response in experimental animals, e.g. histamine, acetylcholine, bradykinin, PGs, 5-HT and substance P. These substances are present oin venoms and products of inflammation.

Nociception is a physiological process by which pain is perceived. The specialised peripheral neurons responsible for this are called **nociceptors.** Their cell bodies are located in posterior horns of the spinal cord.

It appears that tactile sensation is transmitted by large diameter (L), Fast conducting nerve fibres and pain via small diameter (S), slow conducting nerves fibres (nociceptors). Impluses from the nociceptors, on reaching the spinal cord, activate the first transmission cell and also the collateral cells in the substantia gelatinosa (SG). Anatomically, these nerve fibres are carried in the dorsal nerve root and end in the SG ried in the dorsal gray horn. The SG cells inhibit the passage of signals and thus decrease the output reaching the higher centres. If,however, pain stimulus is more intense, then the SG cells are inhibited, releasing the dorsal horm cell from inhibiton, resulting in higher outpur reaching in the higher centres, leading to perception of pain. This **gate control** mechanism allows the sensory input to be decreased or augmented depending on the relative activity of L fiberes and S fibres.

Activation of nociceptor casuses release of various neuro transmitters leading to activation of secondary axons. The secondary axons arising from the dorsal horn travel through the opposite spinothalamic tract, which terminate in the thalamus that projects to the post central gyrus which is mainly responsible for localisation of pain. Although the thalamus is responsible for perception of pain, the cerebral cortex is essential for its discriminative, bral cortex is essential for its discriminative,exact and meaningful interpretation and for some of it emotional components. The other intermingled fibres which form an ascending multisynaptic pathway terminate in the thalamus and from there project to frontal and limbic systems,and the hypothalamus. This system is concerned with the emotional concomitants of pain.

Higher centres, through their central inhibitory and facilitatory mechanisms exert modulating influence on the gating mechanism. Thus clinically the sensation of pain has several components including the emotional psychic reaction.

Analgesics are the drugs which relieve pain without causing loss of consciousness.

Classification

Analgesics are classified into:

1. Opioid
2. Non-Opioid

Opioid analgesics

The word **opiates** refers to the products obtained from the opium poppy. The term **Opioid** (opiate-like) is used to denote all naturally occurring, semi-synthetic and synthetic drugs which have a morphine like tha action viz relief from pain and depression of the CNS, both reversed by naloxone. These drugs wereformerly called 'narcotic' analgesics because some of them (such as morphine) induce sleep. The term 'narcotic' is no longer applied to opioids but is restricted in the legal sense to drugs capable of producing dependence.

The opioids are further sub classified as

- **Agonists** such as morphine and compounds which resemble it in most of their actions, viz. derivatives of morphine, codeine and its derivatives synthetic compounds such as pethidine, methadone, levorphanol and tramadol
- **Partial agonists e.g.** buprenorphine and meptazinol. They have partial agonist action only on the mu receptors
- **Mixed agonist-** antagonists which act as agonists at one type of opiod receptors and as competitive antagonists at another type of receptors e.g. nalbuphine, pentazoxine, and butorphanol. Patients who have received repeated doses of morphine - like drug to the point of physical dependence may experience an opioid withdrawal reaction when given a mixed agaonist-antagonist.

Opioid antagonists, by themselves, produce few effects unless an opioid are activated as in shock or stress, an opioid antagonist does produce visible eggects.

Opioid receptors are part of family of G-protein coupled receptors. They have been classified in to **mu** (μ1, μ2) **delta** (δ1, δ2), **kappa** (κ1, κ2, κ3) and nociceptin (orphamin) types, when activated they

(i) Open K^+ channels to inhibit post-synaptic neurons and

(ii) Close Ca^{++} channels on the presynaptic neurons to inhibit release of the neurotransmitters from nonciceptive nerve teriminals. These actions reduce neuronal excitability

Opioid receptors are also present in the peripheral nerves where they respond to peripherally applied opiods and locally released endogenous peptides during inflammation.

The pharmacological effects associated with theses receptor subtypes and selectivity of the various opioid drugs for these receptors are summarised.

The vast majority of opioid drugs used as analegesics are agonistat mu receptors. Similarly the opioid antagonists naloxone and naltrexone, show a high selectivity for mu receptors. Drug with moxed agonist-antagonist properties bind to more than one receptors class at the usual clinical doses.

1.6. Anthelmintic Drugs[175,150,137,121]

Worm infestation (helminthiasis) is one of the major global public health problems, more so in tropical countries. Besides the environmental conditions peculiar to tropics, poverty, illiteracy, lack of adequate sanitary facilities and of pure water supply make total eradication of this problem very difficult. The commonest parasites observed are round worms, hookworms, thread worms, tape worms, filarial worms and schistosomes.

Worms can cause various GI and general symptoms. In addition, some of them cause blood loss, nutritional deficiencies, urticaria and other allergic manifestations, intestinal obstruction and hepatosplenomegaly. Roundworms have been implicated in the pathogenesis of bronchial spasm in endemic areas. Peripheral blood eosinophilia occurs in all nematode infestations (except enterobiasis). A careful examinations of stool may often spare the unnecessary removal of teeth and tonsils, usually blamed for ‘spectic foci’, in cases with ‘ resistant urticaria’

The helminths are multicellular organisms possessing three germ layers and exhibiting a bilateral symmetry.They are classified into two major phyla (1) **Phylum nemathelminthes** (roundwo**rms; nematodes)** and **(2) phylum platyhelminthes** (flat worms : **cestodes and trematodes). Anthelmintics** are drug used in the treatment of helminthiasis.

An anthelmintic drug which kills the worm is called **vermicidal,** while that which affects the worm in such a way that it is easily expelled is known as **vermifuge.**

Drug Therapy of Roundworms

Mebendazole - This broad spectrum anthelmintic is a benzimidazole derivative. Given orally it is poorly (<10%) absorbed. It inhibits microtubule polymerisation by binding to beta-tubulin.

It is highly effective in ascariasis, enterobiasis, trichuriasis and in hookworm infestation. *It is the drug of choice in enterobiasis and in trichuriasis.* It also has some action against *S.stercoralis*. The drug is slow acting and it may take 2-3 days for parasitic clearance from the gut. It also adversely affects the ova of the trichuris and the hookworm. It is effective in vio against the larve of *Trichinella spiralis* and exerts a lethal effect on the germinal membrane of the larvae of *Echinococcus granulosus* (Hydaid worm).

Adverse reactions

These are usually mild and consist of abdominal pain, nausea and diarrhoea. Large oral doses, may cause vertigo, dizziness,headache and arthalgia. Benzimidazoles are embryotoxic and teratogenic in animals, and should be avoided in pregnancy.

Preparation and Dosage

It is available as 100 mg tablets and liquid suspension

Threapeutic Uses

- **Enterobiasis:** A single dose of 100 mg repeated after one week.
- **Hookworm and round worm infestation:** 100mg bid for 3 days.
- **Taenia infestation:** 300 mg tid for 3 days.

- **Hydatid eysts of the liver:** 400 -600 mg tid for 21-30 days for regression of hydatid cysts. Such treatment, though rarely, may cause bone narrow aplasia. Hence *albendazole* is preferred.

Albendazole

This broad spectrum benzimidazole has actions similar to those of mebendazole. It also has larvicidal actions in hydatid disease, ascariasis and ankylostomiasis and ovicidal properties in ascariasis, ankylostomiasis and trichuriasis.

Given orally, it is rapidly absorbed and has better bioavailability than mebendazole. After absorption, it undergoes first pass metabolism in the liver to its active metabolite,albendazole sulfoxide. It is well distributed in tissues, bile CSF and hydatid cyst. It has plasma t1/2 8-12 hours. Its major advantage is that its effective against many common intestinal worms in a single dose and is cost effective.

The drug is well tolerated and adverse reactions are mild, mainly GI disturbances. When used in hydatid disease for long term therapy it may cause alopecia, liver damage and bone marrow depression.

Threapeutic Uses

- **Ascariasis, ankylostomiasis and trichuriasis -** Usually 400 mg single dose. Hookworm and trichuriasis may need a repeat dose.
- **Enterobiasis -** 400 mg single dose,repeated after 4 weeks.
- **Hydatid disease -** 400 mg bid for one months, repeated if necessary.
- **Strongylodiasis -** 400 mg twice daily for 3 days, repeated after 3 weeks if necessary.
- **Trichinella spiralis -** 400 mg bid for 8-14 days.
- **Cysticercosis -** It is considered the drug of choice because of short course, less toxicity than praziquantel, better penetration into CSF and cost. It is given 400 mg bid for 21 days. A Glucocorticoid is stared prior to albendazole to reduce the intensity of the inflammatory reactions to the dead parasites.

Pyrantel Pamoate

This drug, a tetrahydropyrimidine derivative, is highly effective against round worms and E.vermicularis and a little less effective against hook worms. It has a depolarising, neuromuscular blocking action and causes spastic paralysis of the worms. It is not much absorbed from the gut.

In ascariasis and enterobiasis, it is usually given in a single dose (10 mg/kg upto a maximum of 1 g); for hookworm infestation, the same dose is repeated on the three successive days. The dose can be repeated after 2 weeks, if needed. It can be used during pregnancy.

Adverse reactions

These are usually mild and include GI disturbances, abdominal pain, headache, drowsiness and skin rashes.

Oxantel pamote, an analogue of pyrantel, it also claimed to be useful in trichuriasis. It is given in a single dose.

Piperazine

This drug was extensively used in the therapy of ascariasis and enterobiasis.

Mechanism of action

It act as a GABA agonist and causes hyperpolarization of ascaris muscle resulting in faccid paralysis of the worms which are then easily expelled by peristaltic movements. This eliminates the danger of worm migration.

Absorption, fate and excretion

Piperazine is absorbed from the gut, to the extent of about 30% The drug is partly metabolised in the body and partly excreted unchanged in the urine.

Adverse reactions

Piperazine has a wide margin of safety. Adverse effects are uncommon. They include nausea, vomiting, diarrhoea and urticaria. Neurotoxic effects, observed rarely; include vertigo, muscular incoordination, hypotonia, ataxia of cerebellar type (‘worm-

wobble'), paraesthesiae, blurring of vision and very rarely ,seizures. *The drug appears to be safe during pregnancy.*

Levamisole

Levamisole is I-tetramisole and accounts for most of the antehelmintic activity of the racemic mixture, tetramisole. Like pyrantel, it casuses sustained contracture of somatic muscles of the worm by an irreversible non-competitive, depolarisation type of neuromusucular block. It is rapidly and considerably absorbed and about 60% is excreted in the urine, mostly as metabolities, within 24 hours.

Levamisole also has **immunostimulant properties**.

Adverse reactions

Reported are usually mild and include nausea, vomiting, abdominal pains, diarrhoea, giddiness and drowsiness.

These drugs are not active against larvae and hence follow up re-evaluation of treated patients is necessary.

2. LITERATURE SURVEY

1. Rajiv P *et. al.*, (2017) studied Screening for Phytochemicals and FTIR Analysis of *Myristica dactyloids* Fruit Extracts and the results reveals that the alkaloids, steroids, flavonoids, phenolic compounds, proteins, carbohydrates, cardio glycosides and saponins were present in methanolic extract when compared to other solvent extracts. FT-IR analysis shows the presence of different functional groups such as carboxylic acids, aromatics, alkanes, alcohols, phenols, aliphatic amines, alkenes and amine groups in the fruit extracts. [114]

2. Kadam AB *et. al.*, (2017) carried out work on Assessment of Acute Oral Toxicity of Synergistic Formulation Extract of Traditional Contraceptive Plants. It was a single high extract dose of 2000mg/kg administered and the effects on mortality, behavioral pattern as well as spontaneous locomotors activity were evaluated. The limit dose of 2000 mg/kg did not cause any mortality. The findings suggested that the LD50 value of aqueous and petroleum ether was found to be greater than 2000 mg/kg. Hence, synergistic extract of plants is nontoxic. [58]

3. Qian Chen *et. al.*, (2017) studied Effects of Natural Products on Fructose-Induced Nonalcoholic Fatty Liver Disease (NAFLD) and as a sugar additive, fructose is widely used in processed foods and beverages. Excessive fructose consumption can cause hepatic steatosis and dyslipidemia, leading to the development of metabolic syndrome. Recent research revealed that fructose-induced nonalcoholic fatty liver disease (NAFLD) is related to several pathological processes, including: (1) augmenting lipogenesis; (2) leading to mitochondrial dysfunction; (3) stimulating the activation of inflammatory pathways; and (4) causing insulin resistance. [109]

4. Neelam B Bare *et. al.*, (2017) reported Pharmaceutical Importance of *Withania Coagulans* in Health and Diseases. The berries of the shrub are used for milk coagulation. They are also used in dyspepsia, flatulent colic and other intestinal infections. *Withania coagulans* (Stocks) Dunal is used to treat nervous exhaustion, disability, insomnia, wasting diseases, failure to thrive in children, impotence. Its fruits are used for liver complaints, asthma and biliousness. In particular withanolide isolated from the plants are considered to have Antimicrobial, antiinflammatory, antiumor,

hepatoprotective, antihyperglycemic, cardiovascular, immunosuppressive, free radical scavenging, antimutagenic and central nervous system depressant activities of the plant have been reported. [89]

5. Avinash Shankar *et. al.*, (2016) carried out work on *Withania Coagulans* in Management of Diabetes Mellitus. *Withania coagulans* fruit extract orally early morning and evening at bed time as an adjuvant with continuing anti diabetics in old cases and with OHA in fresh cases progressively established normoglycemic level both fasting and pp and spared the dose of continuing anti diabetic drug and patients taking insulin become completely free of insulin prick. It also bioregulated lipid profile, alleviated diabetic complication and reduced the dose of OHA without any alteration in hemato, hepatic and renal function.[13]

6. Chavan RT *et. al.*, (2016) reported HPTLC fingerprint analysis and antimicrobial activity of leaf extracts of *Cassia fistula* L. The phytochemical screening showed the presence of alkaloids, carbohydrates, glycosides, saponins, triterpenes, tannins, flavonoids, photobatalin and anthraqunies in methanol and aqueous extracts of leaf. The analysis of methanolic extract of leaves by HPTLC confirmed the presence of flavonoids(Peak 4) and alkaloids(Peak 3).[20]

7. Sandhiya V *et. al.*, (2016) performed studies on Effect of Chloroform Fraction of *Withania coagulans* Bud on the Regulation of GLUT4 and PPAR γ-Expressions Levels in Diabetic L6 Myotubes. The free radical scavenging activity of chloroform fraction (CF) of a crude drug shows 510µg/ml of scavenging activity. The IC50 value for MTT assay was found to be 84.7µg/ml. The GLUT4 study shows significant uptake of glucose. PPAR gamma activity regulation of glucose disposal and insulin sensitivity in the skeletal muscles shows concentration dependence response using standard Pioglitazone. The bud of *Withania coagulants* will be a promising medicine for more ailments.[130]

8. Nandagoapalan V *et. al.*, (2016) carried out work on Phytochemical Analysis of Some Traditional Medicinal Plants and in the study, principal phytoconstituents of 25 traditional medicinal plants were identified in order to relate their presence with bioactivities of the plants. Screening of the plants was performed using standard methods and resulted in the detection of the presence of tannins, flavonoids, phenolics, saponins,

steroids, cardiac glycosides and alkaloids. Flavonoids were present in 19 of 25 plants while alkaloids were present in sixteen plants. The presence of these phytochemicals can be correlated with medicinal potential of these plants.[86]

9. Salem Mohamed Edrah *et. al.*, (2016) reported Qualitative and quantities analysis of phytochemicals of various extract for *Ephedra altissima* from Libya. The chemical constituents (Qualitative and Quantities analysis) all most presented in crude extracts of plant (Tannins, Saponins, Flavonoids, Cardiac glycosides and Alkaloids) formerly Steroids; Terpenoids and Anthraquinones not presented in some crude extracts were considered. [129]

10. Nguyen Le Bao Duy *et. al.*, (2016) suggested Preliminary Phytochemical, Acute Oral Toxicity and Anticonvulsant Activity of the Seed Extract of *Brassica juncea* and the phytochemical study showed the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, and phenolic compounds in the seeds of B. juncea. The Acute oral toxicity study indicated that the extract was safe and non-toxic to mice up to 5000 mg/kg body weight. [91]

11. Idris Bello *et. al.*, (2016) concluded the Acute and Sub-Acute Toxicity Evaluation of the Methanolic Extract of *Alstonia scholaris* Stem Bark. The results demonstrate that, while a single dose and short term oral intake of *Alstonia scholaris* bark extract caused no toxicity up to a dose of 2000 mg/kg b.w., toxic effects manifested in the long term treatment at the highest dose (500 and 1000 mg/kg). The long-term toxic effect was found to be associated with alterations in hematological compositions and end-organ damage to the liver. [51]

12. Shah MA *et. al.*, (2016) studied Toxicity Study of *Brassica oleracea* Var. Italica Extracts in Sprague Dawley (SD) Rats and there was no mortality or any significant changes noticed in the SD rats after the administration of tested plant extract of 300, 2000 and 4000 mg/kg body weight respectively. The experimental animals did not showed any drug related changes in behavior, breathing, skin effects, water consumption, impairment in food intake and temperature. Furthermore *B. oleracea* Var. Italica extract

did not produce any remarkable change in biochemical and hematological parameters following the administration of tested crude plant extract of 400 and 8000 mg/kg body weight for 28 consecutive days. [142]

13. Shandesh Bhattarai *et. al.*, (2016) Studied Antibacterial Activity of Selected Ethnomedicinal Plants of Sagarmatha Region of Nepal. Among 15 extracts examined, 13 (86 %) extracts showed antibacterial property against *Staphylococcus aureus* followed by 8 (53 %) extracts against *Escherichia coli* and 6 (40 %) extracts each against *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. Plant extracts were more likely to inhibit Gram-positive bacteria, *Staphylococcus aureus*. With respect to Gram-negative bacteria, it was more common for plant extracts to inhibit *Escherichia coli* than *Pseudomonas aeruginosa* or *Klebsiella pneumonia*. [145]

14. Bahmani Nasrin *et. al.*, (2016) reported Evaluation of antibacterial effects of *Withania coagulans* and *Cynara cardunculus* extracts on clinical isolates of *Brucella* strains and among the tested antibiotics there was only 10% resistance to rifampin. Examination for plant extracts showed the mean zone of inhibition growth for *C.cardunculus* and *W.coagulans* were 28 and 17mm (in 40mg/ml) respectively by disk diffusion method and the highest Minimum inhibitory concentration (MIC) were 10.81µg/ml for *C.cardunculus* and 43.24µg/ml for *W.coagulans*. The present study showed *C.cardunculus* extracts possess compounds with antibacterial properties, therefore can be used as antimicrobial agents in new drugs for therapy of brucellosis.[14]

15. Maryam Sarbishegi *et. al.*, (2016) studied Neuroprotective effects of *Withania coagulans* root extract on CA1 hippocampus following cerebral ischemia in rats . WCE showed neuroprotective activity by significant decrease in MDA level and increase in the SOD, CAT and GPx activity in pretreated groups as compared to I/R groups ($p<0.001$). The number of intact neurons was increased while the number of TUNEL positive neurons in CA1 hippocampal region in pretreated groups were decreased as compared to I/R group ($p<0.001$). [72]

16. Rajagopal PL *et. al.*, *(2016)* studied Anthelmintic activity of the flowers of *Sesbania grandiflora* Pers. Three concentrations (100, 150, 200 mg/ml) of each extract were studied. This study is mainly concerned with the determination of time of paralysis

and time of death of the worms. When there was a gradual increase in the dose, a gradual increase in the anthelmintic activity was observed. The ethanolic extract of the flower showed a significant anthelmintic activity at highest concentration of 200 mg/ml.[111]

17. Dilpesh Jain *et. al.*, (2016) studied Evaluation of Anti-obesity Effect of Fattolin, a Polyherbal Formulation in Progesterone Induced Obesity in Mice and Fattolin treatment (200 and 400 mg/kg) significantly decreased food intake, body weight and liver weight. A significant increase in number of ambulation and rearing and decrease in grooming was also observed. Moreover it significantly decreased levels of blood glucose, triglycerides, total cholesterol, LDL, VLDL and liver enzymes; however more significant difference was observed with Fattolin 400 mg/kg treatment. Structural abnormality of hepatocytes like mild congestion and focal necrosis induced by progesterone administration was markedly improved with Fattolin treatment at both doses.[29]

18. Nishesh Sharma *et. al.*, (2015) studied a review on Biological properties and conservation of critically endangered plant *Withania coagulans* - Indian Rennet. *W. coagulans* possesses several medicinal properties and is used in treatment of various diseases. Plant is known to possess many bioactive compounds responsible for its biological and pharmacological activities, withanolides being the main acitive biochemical constituent. The plant has become endangered due to unrestricted collection from wild stands for both traditional as well as medicinal purposes. Low germination rate and reproductive failure have also contributed towards the present endangered status of the plant. [94]

19. N. Durga Maha Lakshmi *et. al.*, (2015) carried out work on Phytochemical Screening and FTIR Analysis of *Clitoria Ternatea* Leaves. The phytochemical analyses showed presence of proteins, carbohydrates, glycosides, resins, alkaloids, steroids, tannins, and phenols. The FTIR spectra analyses confirmed the presence of different functional groups with a peak value of Phenols at 3389.57, Alkanes at 2925.41 and 2856.66, Primary amines at 1632.33, Aromatic amines at 1409.06, Carboxylic acids at 1057.61, Alkenes at 926.50, Primary and Secondary Amines at 869. 33

20. Nandha Kumar S *et. al.*, (2015) studied Phytochemical screening and characterization of the bioactive compounds from the leaves of *Hyptis suaveolens* and *Spathodea campanulata*. The crude extracts were scanned in the wavelength ranging from 200-800 nm and the characteristic peaks were detected. FTIR method was performed in mid infrared region 4000-400 cm-1 which was used to detect the characteristic peak values and their functional groups. The maximum compounds were found in the methanolic extract of *H. suaveolens* and the aqueous extract of *S.campanulata* with the presence of primary amines, alkanes, carboxylic acid, aromatics, alcohols, ketones and phenols. [87]

21. Florence AR *et. al.*, (2015) studied FTIR and GC-MS spectral analysis of *Gmelina asiatica* L. Leaves. The FTIR spectroscopic studies revealed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines. The results of the GC-MS analysis provide different peaks determining the presence of 50 phytochemical compounds in the extracts. The major phytoconstituents are Ethyl α-D-glucopyranoside (21.86%); 2-Hexadecen-1-OL, 3,7,11,15- Tetramethyl-, [R-(R*,R*-(E)] (14.96%); 9,12,15-Octadecatrienoic acid (14.96%); Pentadecanoic acid (10.71%); Ethyl (9Z, 12Z) -9,12-Octadecadienoate (7.12%). [39]

22. Aparna Saraf *et. al.*, (2015) demonstrated HPTLC Fingerprint Profile and Antimicrobial Activity of Leaves of *Achyranthes Aspera* Linn. The in vitro antibacterial activity was performed on extracts of leaf of *Achyranthes aspera* Linn in petroleum ether, chloroform, acetone, ethanol, methanol and water against multi drug resistance organisms such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. The organic extracts of leaves of the plant at a concentrations of 0.005%, 0.007% and 0.01% were taken and their activities were measured. The results revealed that all the extracts had variable degree of antibacterial activity. [10]

23. Mohamed El Hasan Shayoub *et. al.*, (2015) performed Phytochemical analysis of leaves extract of *Eucalyptus camaldulensis* Dehnh and the result of the phytochemical screening showed the presence of tannins, sterol, triterpenoids , saponins, flavonoids, and

phenolic compounds. There were a complete absence of alkaloids, Anthraquinone glycoside and cyanogenic glycoside. [76]

24. Muhammad Ahmed *et. al.*, (2015) studied Acute Toxicity (Lethal Dose 50 Calculation) of Herbal Drug Somina in Rats and Mice and at the end of the study, all the animals in all the dose groups were sacrificed and the internal organ-body was compared with values from the control group. The LD50 was found to be >10,000 mg/kg body weight upon oral administration in mice and rats as no mortality was observed after single dose administration. According to Hodge and Sterner toxicity scale, the obtained value of LD 50 > 10,000 mg/kg classified the Somina as Practically non-toxic herbal medicine.[81]

25. Wataru Hiruma *et. al.*, (2015) carried out work on Antitumor Effects and Acute Oral Toxicity Studies of a Plant Extract Mixture Containing Rhus verniciflua and Some Other Herbs. The single dose toxicity study of Rv-PEM01 did not result in any deaths or abnormalities in daily behavior, body weight gain, or anatomical observations at necropsy. Thus, so we could not calculate the 50% lethal dose (LD50) in mice, but it would be higher than 5.0 g/kg. Treatment with Rv-PEM01 at a dose of 2.5 g/kg tended to show antitumor activities on mice bearing Colon26 tumors compared with the control group. It was concluded that the formula was a safe antitumor agent with no side effects on mouse physiological function as judged by survival and organ weight.[182]

26. Alowanou Goué G *et. al.*, (2015) performed Acute oral toxicity activity of aqueous extract of *Combretum glutinosum* Perr. ex De leaves in wistar rats. A single dose of 2000 mg/kg bw were given in one single administration by gavage female rats. No animal died and no behavioral signs of acute toxicity were observed.. It was concluded that the aqueous extract of C. glutinosum is safe for oral use at single dose of 2000 mg/kg bw. On condition of chronic evaluation of the toxicity of the aqueous extract of C. glutinosum leaves, could therefore be continuously used by small breeders to control small ruminant helminthes.[8]

27. Vidya Sabbani *et. al.*, (2015) demonstrated Acute Oral Toxicity Studies of Ethanol Leaf Extracts of Derris Scandens & Pulicaria Wightiana in Albino Rats. No mortality

and no significant changes were observed in body weight and wellness parameters at 175, 550 and 2000 mg/kg body wt. doses of both Derris scandens and Pulicaria wightiana which reveal the safety of these plants in the doses up to 2000 mg/kg body weight. [178]

28. Preeti *et. al.*, (2015) reported Medicinal plants possessing anxiolytic activity: A brief review. There are very few anti-anxiety remedies available for the management of anxiety. Thus urgent need for new anti-anxiety drugs is a global concern. Therefore, the demand of herbal medicines is increasing due to their wide application and therapeutic efficacy with least side effects. The review collected the information of anti-anxiety potential plants with emphasis their botanical source, common name and other biological activities, which are helpful to develop new anti-anxiety herbal formulations.[106]

29. Shadab Ahmed *et. al., (*2015) found out Analgesic Activities of Methanol Extract of *Terminalia chebula* Fruit and different doses (300, 500 and 1000 mg/kg) were assessed for analgesic activity by tail immersion technique and acetic acid induced writhing test. Results of both tail flick method and acetic acid induced writhing test revealed that *T. chebula* fruit extract possessed varying degree of analgesic activity significant at 300 mg/kg and highly significant at 500 and 1000 mg/kg in comparison to control.[141]

30. Suresh Kumar dev *et. al., (2015)* studied Analgesic and anti-nociceptive activity of hydroethanolic extract of *Capparis deciduas* (Forssk.) Edgew. and the maximum effect was observed at 60 min at a dose of 200 mg/kg p.o., which was higher than the standard drug morphine sulfate (1.5 mg/kg i.p.) while in the tail flick model, effect was comparable with morphine sulfate. In formalin-induced paw licking model, administration of CDHE completely abolished the early phase at 100 and 200 mg/kg p.o. and in the late phase, the result of CDHE (200 mg/kg p.o.) was superior than indomethacin (10 mg/kg p.o.). CDHE was effective in both non-narcotic and narcotic models of nociception, signifying its possible action via peripheral and central mechanism. [165]

31. Debasmita Dutta Pramanick *et. al.*, (2015) studied Pharmacognostic evaluation of *Withania coagulans* Dunal (Solanaceae) - an important ethnomedicinal plant**.** Physico-chemical and phyto-chemical screening of drug material are done for determination of

quality/purity of crude drug and for detection of plant constituents respectively. The plant is characterized by shrubby habit with dioecious and polygamous flowers; fruits (berries) enclosed in persistent leathery calyx; seeds ear-shaped, with fruity smell. Fruit pedicel with branched and unbranched trichomes, massive collenchymatous cortex, intra-xylary phloem and hollow pith; calyx with spongy parenchyma; pericarp with exocarp, mesocarp and endocarp; seeds with highly lignified sclerenchyma cells and strongly thickened endosperm. The plant is rich in alkaloids, esterase, carbohydrates, steroids, phenolic compounds, tannins, free amino acids and organic acids. [26]

32. Avijit Chatterjee *et. al.*, (2015) carried out work on Anti-inflammatory and Analgesic Activity of Methanolic Extract of Medicinal Plant *Rhodiola rosea l*. Rhizomes. The orally administered methanolic extract of *Rhodiola rosea* demonstrated a significant analgesic and anti-inflammatory in animal model. The findings in the study suggest that the methanolic extract of the herb *Rhodiola rosea* possesses analgesic and anti-inflammatory activities. [12]

33. Trini Suryowati *et. al.*, (2015) carried out work on Antihyperlipidemic Activity of Torbangun Extract (*Coleus amboinicus* Lour) on Diabetic Rats Induced by Streptozotocin. Oral administration with graded doses T1 and T2 of torbangun leaf ethanol extract exhibited antihyperglycemic and antihyperlipidemic activity in streptozotocin-induced diabetic rats. Data were analyzed with test followed Post-Hoc test with 95% significance level. The daily oral treatment with torbangun leaf ethanol extract showed a significant reduction in blood glucose. There were decreased serum contents of triglycerides and significantly total cholesterol, whereas HDL-cholesterol was increased.[170]

34. Claudia I Gamboa*et. al.*, (2015) identified Plants with Potential use on Obesity and its Complications and explained the anti-obesity potential and reported mechanisms of action of diverse plants such as: *Camellia sinensis, Hibiscus sabdariffa*, *Hypericum perforatum, Persea americana, Phaseolus vulgaris, Capsicum annuum, Rosmarinus officinalis, Ilex paraguariensis, Citrus paradisi*, C*itrus limon, Punica granatum, Aloe vera, Taraxacum officinale* and *Arachis hypogaea* is summarized.[22]

35. Sushmitha J *et. al.*, (2015) suggested on Evaluation of Anti-Obesity Activity of *Hordeum vulgaregrains* in Albino Rats. The successful management of obesity is possible through lifestyle changes in diet and physical activity. *Hordeum vulgare* is traditionally used as weight losing remedy so, present study selected grains of *Hordeum vulgare* plant for evaluation of anti-obesity activity by using high fat diet induced, anti-psychotic drug induced obesity in rats.[166]

36. Adnyana IK *et. al.*, (2014) performed studies on Anti-Obesity Effect of the Pomegranate Leaves Ethanol Extract (*Punica Granatum* l.) in High-Fat Diet Induced Mice. The pomegranate leaves ethanol extract at a dose 50 mg/kg and 100 mg/kg b.w. showed a significant decrease of body weight, faeces index, total fat index, food index, and lee's index compared to control mice. Furthermore, significantly inhibitory activity also showed from *in vitro* assay.[5]

37. Souravh Bais *et. al.*, (2014) was undertaked Antiobesity and Hypolipidemic Activity of *Moringa oleifera* Leaves against High Fat Diet-Induced Obesity in Rats and indicate that the rats treated with *Moringa oleifera* (MO) have significantly attenuated the body weight without any change in the feed intake and also elicited significant thermogenic effect and to act as hypolipidemic and thermogenic property in obesity related disorders.[158]

38. Pankti P Dalwadi *et. al.*, (2014) evaluated Anti hyperlipidemic activity of *Tephrosia purpurea* plant extracts in poloxomer 407 induced hyperlipidemic rats and Estimation of lipid profile shows that *Tephrosia purpurea* stem extract (500mg/kg), *Tephrosia purpurea* leaves extract (400mg/kg), *Tephrosia purpurea* whole plant extract (300mg/kg) shows the less significant antihyperlipidemic activity. While *Tephrosia purpurea* whole plant extract at the dose of 600mg/kg shows potent antihyperlipidemic activity. It decreases TC, TG, LDL, VLDL and increases HDL levels. *Tephrosia purpurea* whole plant extract 600 mg/kg shows significant antihyperlipidemic activity as standard drug Atorvastatin. [98]

39. Ashok Kumar Gupta *et. al.*, (2014) studied Protective effect of *Ficus infectoria* plant extract against fructose induced hyperlipidemia and hyperglycemia in rats and

explored the antihyperlipidemic and hypoglycemic potential of the methanolic extract of *Ficus infectoria* in Wistar rats. Hyperlipidemia and hypoglycemia in rats were induced by fructose solution (10% w/v, p.o., ad libitum) for 3rd and 8th weeks respectively. These activities were measured by estimating the triglyceride, total cholesterol, LDL, VLDL, HDL and serum glucose levels. *F. infectoria* at 200mg/kg and 400mg/kg showed significant effect. [11]

40. Rajesh Asija *et. al.*, (2014) carried out work on Anti Inflammatory and Analgesic Activity of Medicinal Plants - A Review. The various plants have a potential medicinal implication. Medicinal plants are considered as imperative therapeutic aid. Therapy of classical NSAIDs and the opoids in the management of inflammatory and pain stipulation are major problems. The conservative drug available in the marketplace treat inflammation and analgesia produces various side effects. For conquer this problems medicinal plants play a major role to alleviate many diseases related with inflammation and analgesia. [112]

41. Meda Ramesh *et. al.*, (2014) performed a Review on Anxiolytic Activity of Some Herbal Plants. These herbal plants extraction processes used to different solvents present in phytochemical constituents. They need further evidence base via clinical studies. Anxiety disorders are commonly researched but the efficacy of herbal medicines in these disorders needs to be studied further. The review addresses herbal therapy, safety issues and future areas of application in the field.[73]

42. Shan P Mohammed *et. al.*, (2014) studied Evaluation of Anxiolytic Activity of *Ixora coccinea* Linn. Ethanolic Extract in Swiss Albino Mice and the anxiolytic activity of Ixora coccinea ethanolic extract. The methods used for this study are elevated plus maze paradigm test and Hole board test. The ethanolic extract ICEE shows a significant ($P<0.01$) anxiolytic effect during the elevated plus maze test and the hole board test in a dose dependent manner when compared with the standard dose of diazepam.[144]

43. Muhammad Riaz *et. al.*, (2014) studied Neuropharmacological effects of methanolic extracts of Rubus fruticosus L.All extracts were found to be anxiolytic in nature, while no muscle relaxing activity or sedative effect was observed. The order of central nervous system (CNS) depressant effect for various parts of R. fruticosus was fruit > root > leaves > stem.[82]

44. Priyadarshini L *et. al.*, (2014) performed Acute Toxicity and Oral Glucose Tolerance Test of Ethanol and Methanol Extracts of Antihyperglycaemic Plant Cassia Alata Linn . Mice appeared to be normal and no mortality was observed in the acute toxicity test after treated with an extract up to a concentration of 3000mg/kg body weight. It ensures that the plant as safe for use as a medicinal plant. The glucose utilization (OGTT) was evaluated using STZ induced diabetic mice. The study provides remarkable evidence about the ability of the extracts in reducing blood glucose level. The % glycaemic change of ethanol and methanol extract is 18.74 and 8.35 respectively after 180min of glucose load, which is lesser than the DC (diabetic control) which showed a % glycaemic change of 132.94. [107]

45. Manjulika Yadav *et. al.*, (2014) carried out work on Preliminary Phytochemical Screening of Six Medicinal Plants Used In Traditional Medicine and carbohydrates, glycosides and coumarins were present in all the selected plants except P. dactylifera and R. sativus. Alkaloids were present in all the selected plants except F. religiosa, P. dactylifera and R. sativus. Proteins were present only in F. religiosa and S. chirata. Whereas emodins, anthraquinones, anthocyanins and leucoanthocyanins were absent in all the selected six plants.[70]

46. Snehlata Pandey *et. al.*, (2014) performed Phytochemical Screening of Selected Medicinal Plant Cinnamon Zeylanicum bark extract, Area of research; Uttarakhand, India. Extracts of this medicinal plant was utilized the standard screening method (Guevarra, et al, 2005) for the detection of secondary metabolites. The phytochemicals are important in human health this is because they display different biological activities such as antifungal, antibacterial activities. Quantitative phytochemical analysis of this plant confirms the presence of various phytochemicals like alkaloids. Flavonoids, tannins, saponins, steroid and glycosides in their six solvents cold, hot, warm water, acetone, ethanolic and methanolic bark extracts. [157]

47. Mohanty C *et. al.*, (2014) studied Development of phytochemical fingerprint of an Indian medicinal plant Chitrak (Plumbago zeylanica L) using High Performance Thin Layer Chromatography (HPTLC). Root and leaf extracts by ethanol, chloroform and acetone showed higher antibacterial activity against E. coli as compared to standard

Kanamycin (MIC-100 μg/ml). The high performance thin layer chromatography (HPTLC) fingerprints were used for the quantitation of two bioactive markers: gibberellic acid and quinol R in the plant powder of different organs. Maximum content of gibberellic acid was found in acetone extract of the root (59.74%, Rf 0.79) followed by methanol root extract (53.01%). [77]

48. Karthika K *et. al.*, (2014) performed TLC and HPTLC Fingerprint Profiles of Different Bioactive Components from the Tuber of Solena amplexicaulis. The profiles of various individual secondary metabolites were made and developed for authentication. The methanolic tuber extract showed the presence of 5 alkaloids, 6 flavonoids, 2 glycosides, 10 saponins and 7 terpenoids. The development of such fingerprint can be used in differentiation of the species from the adulterant in terms of phytochemical constituents and hence act as biochemical markers in the pharma industry and plant systematic studies.[60]

49. Preethi MP *et. al.*, (2014) performed work on Principal Component Analysis and HPTLC Fingerprint of *In Vitro* and Field Grown Root Extracts of *Withania Coagulans.* The HPTLC system was standardized and was found out that roots of *Withania coagulans*, AUF Wc 024 and AUF Wc 025 had maximum withanolide A accumulation (1.17mg/g). The extractive value was found to be high for AUF Wc 021 (392.4 mg/g). Toluene: Ethyl acetate: Formic acid (5:5:1) has been standardized as the best solvent system for HPTLC analysis of withanolides.[105]

50. Rashmi Tambe *et. al.*, (2014) studied Phytochemical screening and HPTLC fingerprinting of leaf extracts of Psidium guajava Linn. and Preliminary phytochemical screening of the extracts showed the presence of alkaloids, triterpenes, tannins, saponins, glycosides, phenolic compounds and flavonoids. The proximate analysis showed satisfactory result with respect to foreign matter, moisture content, ash value and extractive values. HPTLC finger printing of methanol extract of leaf powder revealed presence of three polyvalent phytoconstituents with their Rf value 0.95, 1.11, 1.41 at 220nm. Component number 3 at Rf 1.41 showed maximum concentration and presence of total five components with their Rf value 0.18, 0.91, 1.21, 1.42, 1.52 at 450nm.Component number 4 at Rf 1.41 showed maximum concentration. Aqueous

extract of leaf powder showed total six components with their Rf value 0.29, 0.74, 0.85, 0.96, 1.31 at 220nm.Component number 4 at Rf 0.96 showed maximum concentration. [122]

51. Manickam Murugan *et. al.*, (2014) performed Phytochemical, FT-IR and antibacterial activity of whole plant extract of Aerva lanata (L.) Juss. Ex. Schult. Qualitative phytochemical analysis of the methanol and ethanol extracts prepared from Aerva lanata whole plant revealed the presence of alkaloids, anthraquinones, catechins, coumarins, flavonoids, phenols, quinones, saponins, steroids, sugar, glycosides, tannins and xanthoproteins. The FT-IR spectrum confirmed the presence of alkyl group, methyl group, alcohol group, ethers, esters, carboxylic acid and anhydrides. [69]

52. Nidhi Agarwal *et. al.*, (2014) reported Paneer Doda *(Withania coagulans* Dunal): A Promising Therapeutic Agent. The amino acids present are proline, hydroxyproline, valine, tyrosine, aspartic acid, glycine asparagin, cysteine and glutamic acid. A variety of withanolides have been found in the plant which are responsible for its therapeutic properties. It is widely used in treating diabetes mellitus, nervous exhaustion, disability, insomnia, wasting diseases and failure to thrive in children. The fruits of the plant are reported to be sedative, emetic, alterative and diuretic. They are also helpful in liver complaints, asthma and biliousness.[93]

53. Geethu MG *et. al.*, (2014) studied Fourier-Transform Infrared Spectroscopy Analysis of Different Solvent Extracts of Water Hyacinth (Eichhornia Crassipes Mart Solms.) An Allelopathic Approach. Eichhornia crassipes samples extracted with different solvents continuously was subjected to FTIR to determine whether the plant can be discriminated on the basis of biochemical profiles using solvents. The extraction factor was superior in water rich in polar molecules. The FTIR signal at 900, 1500, 1714, 3000, 3100cm-1 were considered as an indicator of polyphenols. The functional groups of each extract were identified.[42]

54. Rajeshwari Sahu *et. al.*, (2014) performed studies on Ultraviolet-Visible and Fourier Transform Infrared Spectroscopic Studies on Non-Conventional Species of Curcuma. FTIR method was performed on a Perkin Elmer spectrophotometer system, which was used to detect the characteristic peak values and their functional groups. The

UV-VIS profile of C. caesia. rhizome methanolic extract showed the peaks at 256.00 nm, 288.00 nm and 330.00 nm with the absorption 0.617,1.235 and 0.557respectively.[56]

55. Neha Sahu *et. al.*, (2013) studied Phytochemical Analysis of Bougainvillea Glabra Choisy by FTIR and UV-VIS Spectroscopic Analysis. FTIR method was performed on a Perkin Elmer spectrophotometer system, which was used to detect the characteristic peak values and their functional groups. As the result UV-VIS profile showed the peaks at 324.00nm and 290.00nm for flavonoid and FTIR spectra showed the peak at 3364.58cm-1 for OH group.[90]

56. Priyanka Pandey *et. al.*, (2013) concluded the Physico-chemical and preliminary phytochemical screening of Psoralea corylifolia. The presence of alkaloids, glycosides, carbohydrates, steroids, polyphenol, saponins and terpenoids were indicated by the test conducted. The three Rf value (0.82; 0.63; 0.45) of ethanol extracts and two Rf value (0.72; 0.65) of aqueous extracts were found in TLC plate. HPLC method was developed for the fingerprinting of bakuchiol, psoralen, and angelicin present in Psoralea corylifolia extract. [108]

57. Suman Kumar R *et. al.*, (2013) studied Phytochemical Screening of some compounds from plant leaf extracts of Holoptelea integrifolia (Planch.) and Celestrus emarginata (Grah.) used by Gondu tribes at Adilabad District, Andhrapradesh, India. . The phytochemical analysis of leaf extracts in aqueous, methanol, acetone, petroleum ether and chloroform extracts of indigenous medicinally important plants of Holoptelea integrifolia and Celestrus emarginata were investigated. The phytochemical analysis revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, coumarins, quinines, cardiac glycosides, Xanthoproteins, glycosides, steroids, phenols, resins, carboxylic acid group in varying concentrations. [162]

58. Librado A Santiago *et. al.*, (2013) carried out work on Acute Oral Toxicity Study Of The Crude Ethanolic Leaf Extract Of *Ficus Pseudopalma* Blanco (Moraceae) In Sprague Dawley Rats. Acute oral toxicity of the crude ethanolic leaf extract of F. pseudoplama was performed according to the guidelines set by OECD 425 on six 8-12 week old female Sprague Dawley rats weighing from 160-210g. One rat was treated with normal saline solution that served as the control. Toxicological and pharmacological

observations were completed for 14 days. On day 14, all test animals were sacrificed via cervical dislocation and subjected to gross necropsy; liver samples were subjected to histopathological examination. Gross examination of the rodent's organs was all normal and regarded as unremarkable. [67]

59. Sabeeha Shafi *et. al.*, (2013) concluded the Acute Oral Toxicity And Hypoglycaemic Study Of Ethanolic Extract Of Portulaca Oleracea (Whole Plant) In Swiss Albino Mice . In acute oral toxicity study, the animals showed behavioural changes also.50% of the animals died at the dose level of 500 mg/kg b.w and 100% animals died at the dose levels of 1000,1500 and 2000mg/kg b.w, thereby indicating that the dose below than 500mg.kg b.w is safe for further studies. In hypoglycemic activity the dose of 400mg/kg b.w showed a highly significant reduction of serum glucose levels. [127]

60. Nurul Husna R *et. al.*, (2013) reported Acute Oral Toxicity Effects of Momordica Charantia in Sprague Dawley Rats. The extract was administered orally at two different doses of 300 mg/kg and 2000 mg/kg of body weight. The toxicity signs were recorded within the first 24 hours after forced feeding. Both of the treated groups showed dizziness and depression during the first 30 minutes. No significant difference of feeding patterns which included water, food intake and body weight gain were observed. Haematological evaluations did not show significant differences in white blood cells count (WBC), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) levels.[95]

61. Vedhanarayanan P *et. al.*, (2013) concluded the Antimicrobial activity and phytochemical screening of Wrightia tinctoria (Roxb.) R.Br. The findings showed potential antibacterial properties of the extracts against the organisms tested. Among the three solvents tested, ethanol extract of leaf showed higher inhibition zone. Ethanol extract of Wrightia tinctoria exhibits maximum zone of inhibition against Escherichia coli (29 mm), Bacillus subtilis (24 mm) Staphylococcus aureus (30 mm) and Pseudomonas aeruginosa (24 mm). Preliminary phytochemical analysis of Wrightia tinctoria showed the presence of alkaloids, flavonoids, phenols, saponins, steroids and tannins.[176]

62. Shashank Matthew *et. al.*, (2013) studied Analgesic and Anti-Inflammatory Activity of *Kalanchoe Pinnata* (Lam.) Pers. and investigated ethanol and aqueous extracts of dried stem of plant *'Kalanchoe pinnata* (lam.) Pers.' against with Anti-inflammatory and in rats and Analgesic activity in mice.[151]

63. Prashanta KR Deb *et. al.*, (2013)carried out work on In-Vitro Anthelmintic Activity of *Acorus Calamus* Leaves and investigation of the anthelmintic potential of crude MeOH extract, 50% EtOH and aqueous extract of the leaves of *Acorus calamus* on Indian earth-worm (*Pheretima posthuma*). Albendazole in same concentration as those of extract was included as standard reference and normal saline water with 1% CMC as control. All the extracts exhibited significant anthelmintic activity at a concentration of 100 mg/ml. Peak activity was exhibited by the MeOH extract at a concentration of 100 mg/ml.[104]

64. Jiju V *et. al.*, *(2013) performed* Evaluation of anthelmintic activity of methanolic extract of *Asystasia gangeticum.* The parameters like the time of paralysis and the time of death were determined by using the extract at the concentrations of (10-100 mg/ml). The extract exhibited significant anthelmintic activity at highest concentration of 100 mg/ml as compared with piperazine citrate (10 mg/ml) as standard reference and distilled water as control.[57]

65. Madhavi E *et. al.*, (2013) studied a Review on Medicinal Plants with Potential Hypolipidemic Activity. More than 70 medicinal plants have been documented to have significant hypolipidemic action. This reviews these reports published in literatures in the last 5 years. This review indicates that the research has stopped with just reporting the effect of plant derivates and the findings are not translated into clinical research. Taking these finding forward is mandatory to develop new drugs in this area. Hence further research into identifying the active principle, conducting preclinical studies & if possible clinical studies is needed.[177]

66. *Ankur Datta et. al.*, (2013) reported the Antidiabetic and antihyperlipidemic activity of hydroalcoholic extract of *Withania coagulans* Dunal dried fruit in experimental rat

models and it may be concluded that the WCDF extract can be considered as an adjuvant in the treatment of type 2 DM which can possibly lower the dose requirement of standard oral hypoglycemic agents like glipizide. The hydroalcoholic WCDF extract was effective and comparable to atorvastatin in controlling lipid profile in high‑cholesterol diet‑induced hyperlipidemia in albino rats.[9]

67. Shirin Hasani-Ranjbar *et. al.,* (2013) studied a systematic review of anti-obesity medicinal plants - an update and explained that studies with Nigella Sativa, Camellia Sinensis, Crocus Sativus L, Seaweed laminaria Digitata, Xantigen, virgin olive oil, Catechin enriched green tea, Monoselect Camellia, Oolong tea, Yacon syrup, Irvingia Gabonensi, Weighlevel, RCM-104 compound of Camellia Sinensis, Pistachio, Psyllium fibre, black Chinese tea, sea buckthorn and bilberries show significant decreases in body weight. [154]

68. Suneetha D *et. al.,* (2013) was undertaked study on Evaluation of Anti-Obesity Activity of Methanolic Extract of *Sapindus emarginatus* by Progesterone Induced Obesity on Albino Mice and evaluation of anti obesity activity the organisms used were albino mice. The standard drug used was orlistat. The two test doses of methanolic extract of pericarps of flowers of *Sapindus emarginatus* were prepared. The total evaluation period was 28 days.[164]

69. Tejendra Bhakta et. al., (2013) carried out work on *In-Vitro* Anthelmintic Activity of *Acorus Calamus* Leaves. Three concentrations (25, 50, 100 mg/ml) of each extract were studied in activity which involved the determination of time of paralysis (vermifuge) and time of death (vermicidal) of the worms. Albendazole in same concentration as those of extract was included as standard reference and normal saline water with 1% CMC as control. Results: All the extracts exhibited significant anthelmintic activity at a concentration of 100 mg/ml. Peak activity was exhibited by the MeOH extract at a concentration of 100 mg/ml.[104]

70. Vijusha M *et. al.,* (2013) studied Screening of Behavioural, Muscle Co-Ordination & Anxiolytic Activities of Methanolic Extract of *Tabebuia rosea* (Bertol). The behavioural muscle coordination and anxiolytic activities were evaluated by using various models of CNS using mice. Diazepam was used as standard drug for muscle coordination and

anxiolytic studies. The extracts were administered orally at 500 mg/kg. The results of the present study indicates that the methanolic extract of *Tabebuia rosea* leaves are effective in inducing a significant protection against behavioural, muscle coordination and anxiolytic activities, as evidenced by various CNS models with respect to control. This study confirmed the behavioural, muscle coordination and anxiolytic activities of this plant as it is used in traditional medicine.[61]

71. *Vandana Gupta et. al.*, (2013) suggested a review of *Withania Coagulans Dunal.* (Paneer Doda). Withanolides are steroidal lactones having significant pharmacological activities. In various studies it has been seen that the *Withania coagulans* posses several medicinal properties such as hepatoprotective, antiinflammatory, antihyperglycaemic, free radical scavenging, hypolipidaemic, antimicrobial, cardiovascular, central nervous system depressant, immunomodulating, antitumour and cytotoxic activities. [175]

72. Jhansee Mishra *et. al.*, (2013) studied *Withania Coagulans* in Treatment of Diabetics and Some Other Diseases: A Review. The berries of the shrub are used for milk coagulation. It is popularly known as Indian cheese maker. In Punjab, the fruits of W. Coagulans are used as the source of coagulating enzyme for clotting the milk which is called "paneer".[55]

73. Prakash Chandra Gupta *et. al.*, (2012) studied *Withania Coagulans* Duna*l*- An Overview. The fruits of the plant are reported to be sedative, emetic, alterative and diuretic. Further, they are used for liver complaints, asthma and biliousness. The active compounds, in particular, withanolides isolated from the plant are considered to have antimicrobial, anti-inflammatory, antitumor, hepatoprotective, anti-hyperglycemic, cardiovascular, immuno-suppressive, free radical scavenging and central nervous system depressant activities. [101]

74. Kirtikar Shukla *et. al.*, (2012) carried out work on the Aqueous Extract of Withania coagulans Fruit Partially Reverses Nicotinamide/Streptozotocin-Induced Diabetes Mellitus in Rats. Daily treatment with aqueous W. coagulans at 250mg/kg of body weight for 30 days restored plasma glucose, HbA1c, tissue glycogen, and glucose metabolic enzymes to nearnormal ranges in bothMD and SD animals. The results of this

study reveal that the regular administration of aqueous W. coagulans extract for 30 days significantly improved glycemic status and nearly normalized plasma glucose concentrations. [63]

75. Abidemi J Akindele *et. al.*, (2012) studied Anxiolytic activity of aerial part hydroethanolic extract of Allium ascalonicum Linn. (Liliaceae) in mice and in the hole-board test, A. ascalonicum significantly ($p<0.05$, 0.01) increased the number/duration of head dips and number of sectional crossings. In the elevated plus maze test, A. ascalonicum significantly ($p<0.05$) increased the number of entries into the open arm withcorresponding reduction in number of entries into the closed arm. In the light/dark exploration test, A. ascalonicum significantly ($p<0.05$, 0.01) increased the latency of entry into the dark box, time spent in the light box, and number of rearing and assisted rearing. [3]

76. Naga Kishore R *et. al.*, (2012) was undertaken Evaluation Of Anxiolytic Activity Of Ethanolic Extract Of Foeniculum Vulgare In Mice Model and the study was designed to investigate the anxiolytic activity of ethanolic extracts of Foeniculum vulgare fruit. The anxiolytic activity was evaluated by elevated plus maze, rota rod, open field test, and hole board models. The efficacy of extract (100-200mg/kg) was compared with standard anxiolytic drugs diazepam (1mg/kg). Extract administered animals showed exploratory behavior with all tests similar to diazepam. The results showed that the extract significantly increased the number of entries and time spent in the open arm in the elevated plus maze apparatus.[85]

77. Chetan Salwaan *et. al.*, (2012) studied the Investigation of the Pharmacognostical, Phytochemical and Antioxidant Studies of Plant *Withania coagulans* Dunal. It was observed that 50% ethanol extract of *Withania coagulans* contains carbohydrates, proteins, glycosides, steroids and sterols, anthraquinones and triterpenoids. The antioxidant activity of *Withania coagulans* Dunal was studied by DPPH and Nitric oxide method and it was observed that it has antioxidant activity. It showed more activity in DPPH method than Nitric oxide method. Preliminary Phytochemical study of 50% ethanolic extract of the root parts is found to contain carbohydrates, protein, some steroids, anthraquinone, flavonoids, tannin, phenolic compounds and triterpenoids are

present. The antioxidant activity was determined and the plant extract showed low activity nitric oxide free radical inhibition method and moderate activity by DPPH method. The activity was compared with rutin and ascorbic acid.[21]

78. Sangh Partap *et. al.*, (2012) carried out work on *In-Vitro* Anthelmintic Activity of *Luffa cylindrica* Leaves in Indian Adult Earthworm and Different extracts of *L. cylindrica* were taken for anthelmintic activity against Indian earthworm *Pheretima posthuma*. Two concentrations (50 and 100 mg/ml) of various extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. Albendazole (20 mg/ml) was used as reference standard and carboxy methyl cellulose (0.5%) as a control group. Dose dependent activity was observed in the plant extracts but methanolic extract exhibited more activity as compared to others. The anthelmintic activity of *Luffa cylindrical* leaves extract has therefore been demonstrated for the first time.[131]

79. Abhishek B *et. al.*, (2012) studied Anthelmintic activity of *Cynodon dactylon* and the phytochemical tests were done to find the presence of the active chemical constituents such as alkaloids, glycosides, terpenoides, Steroids, saponins, flavonoids, tannins, carbohydrates, proteins and fixed oils. Standardization of *Cynodon dactylon* was carried out to check the extractive value, loss on drying, ash value etc. Anthelmintic activity was evaluated on adult Indian earthworm Pheretima Posthuma by using albendazole as a standard drug. The aqueous extract of *Cynodon dactylon* shows anthelmintic activity as compared with the standard drug.[2]

80. Selvamohan T *et. al.*, (2012) reported Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. The methanol, ethanol and aqueous extracts of seven medicinal plants were evaluated for activity against medically important bacteria such as *Staphylococcus* sp., *Escherichia coli, Klebsiella* sp., *Pseudomonas* sp. The ethanolic and aqueous extracts showed minimum antimicrobial activity when compared to methanolic extracts. The methanolic extract *Phyllanthus niruri* (stone breaker) showed the maximum activity against *Staphylococcus* sp. [140]

81. Saswata Banerjee *et. al.*, (2012) carried out work on Evaluation of Analgesic Activities of Methanolic Extract of Medicinal Plant *Juniperus Communis* Linn. The

extract showed a dose dependent and significant (P<0.01) inhibition of writhing response. . This has been observed that the plant showed significant activity as anti nociceptive agent and was proved to act both peripherally and centrally.[134]

82. Sai Mangala Divi *et. al.*, (2012) performed a Comparative Study **on** Evaluation of Antidiabetic and Antihyperlipedemic Potential of Aqueous Extract of Moringa oleifera in Fructose Fed Insulin Resistant and STZ Induced Diabetic Wistar Rats. Administration of aqueous extract of Moringa oleifera for 60 days restored all the alterations to normal/ near normal. The study clearly reveals that aqueous extract of Moringa oleifera leaf possesses potent antihyperglycemic and antihyperlipedemic effect in both Insulin resistant and Insulin deficient rat models.[128]

83. Mehrana Jafari *et. al.*, (2012) performed studies on Remedial Use of Withanolides from Withania Coagolans (Stocks) Dunal and the biological activities of withanolieds from Withania coagulans described. Anti-inflammatory effect, anti cancer and alzheimer's disease and their mechanisms, antihyperglycaemic, hypercholes-terolemic, antifungal, antibacterial, cardiovascular effects and another activity are defined. [32]

84. Rohit Gundamaraju *et. al.*, (2012) studied Evaluation of Anti-Obesity Activity of *Lantana camara* Var Linn. by Progesterone Induced Obesity on Albino Mice and the presence of phyto constituents such as steroids , flavinoids , alkaloids, etc. are resulted in the reduction of the body weight. [124]

85. Sudhanshu Kumar Bharti *et. al.*, (2012) reported the Antidiabetic effect of aqueous extract of *Withania coagulans* flower in Poloxamer-407 induced type 2 diabetic rats. A significant increase in blood glucose, glycosylated haemoglobin (HbA1c), and serum insulin levels were observed in control rats. Treatment with *W. coagulans* extract reduced the elevated levels of blood glucose, HbA1c, and insulin in T2DM rats. In oral glucose tolerance test, we found a significant improvement in glucose tolerance in the rats treated with *W. coagulans* extract. The insulin sensitivity, that is, both peripheral and hepatic insulin resistance was assessed. *W. coagulans* extract treatment significantly improved insulin sensitivity index (*KITT*) that was decreased in control rats. [161]

86. Rohit Jain *et. al.*, (2012) studied the review of Phytochemistry, pharmacology, and biotechnology of Withania somnifera and Withania coagulans and presents a consolidated account of the phytochemistry, pharmacology and biotechnology involving *in vitro* propagation, genetic transformation and metabolite profiling in *W. somnifera* and *W. coagulans.*[125]

87. Abhijeet R Borate *et. al.*, (2011) studied Antihyperlipidemic Effect of Protocatechuic Acid in Fructose Induced Hyperlipidemia in Rats. Herbal treatment for hyperlipidemia has fewer side effects. Protocatechuic acid (PCA), which is predominantly present in the flowers of *Hibiscus sabdariffa*, possesses antihyperlipidemic and free radical scavenging activity. Taking this into consideration PCA at the dose of 25 and 50 mg/kg were evaluated against fructose induced hyperlipidemia in rats and it has showed a significant decrease ($p<0.05$, $p<0.01$ respectively) in the levels of serum TC, TG and LDL and HDL was significantly increased ($p<0.05$, $p<0.01$ respectively) in serum when compared to fructose control group. Thus Protocatechuic acid has anti-hyperlipidemic activity which may be due to increased uptake of LDL cholesterol by hepatic LDL receptor or may be due to its effect on enzymes involved in metabolism and excretion of cholesterol.[1]

88. Debasis Mishra *et. al.*, (2011) carried out work on an Experimental Study of Analgesic Activity of Selective Cox-2 Inhibitor with Conventional NSAIDs and the study was carried out to investigate the analgesic activity of Etoricoxib (10 mg) for individual drug therapy and etoricoxib (5 mg) for combination therapy with diclofinac potassium (10 mg) using Acetic acid induce writhing, Hot plate and Tail immersion methods. The test and standard drugs significantly ($p<0.001$) reduced the number of abdominal constriction and stretching of hind limb induce by the injection of acetic acid in a dose dependent manner. The Hot plate and Tail immersion test useful in the elucidating centrally mediated antinociceptive responses, which focused mainly on changes above the spinal cord level. All the test and standard drugs significantly ($p<0.001$) reduced the pain as compare to the control group. [25]

89. Tahira Mughal *et. al.*, (2011) performed studies on Antifungal Studies of *Withania Coagulans* and *Tamarix aphylla. In vitro* studies were carried out to evaluate the antifungal activity of methanolic , pet ether and dichloromethane extract of aerial parts of

Withania coagulans and *Tamarix aphylla* against seven fungal strains (*Trichoderma viridis ,Aspergillus flavus* , *Fusarium laterifum* , *Aspergillus fumigatus* , *Candida albicans* , *Trichophyton mentogrophytes* and *Microsporum canis*) and having better antifungal efficacy. [167]

90. Girija K *et. al.*, (2011) carried out work on Anti-hyperlipidemic activity of methanol extracts of three plants of Amaranthus in triton-WR 1339 induced hyperlipidemic rats and the test extract possessed better anti- hyperlipidemic activity against triton-WR 1339 induced Hyperlipidemia and obtained the better results when compared with the standard drug (atrovastatin treated).[44]

91. Lakshmi BVS *et. al.*, (2011) demonstrated Antihyperlipidemic activity of Bauhinia purpurea extracts in hypercholesterolemic albino rats and there was a significant increase in high density lipoprotein levels after the treatment with *Bauhinia purpurea* extracts. Ethanol extract of leaves showed a marked effect over body weight reduction and also had a significant effect on the lipoprotein profile. There is a lowered atherogenic index, TC: HDL-c and LDL: HDL-c ratios in the extract treated groups. [65]

92. Pezeshki A *et. al.*, (2011) suggested the Influence of *Withania coagulans* Protease as a Vegetable Rennet on Proteolysis of Iranian UF White Cheese. Extraction of protease from *Withania coagulans*' fruits and the effect on proteolysis of Iranian UF white cheese in comparison with pure chymosin and fungi rennet (fromase) were investigated during ripening. The results indicated that, except for pH which was significantly ($P< 0.05$) lower in cheeses made with *Withania coagulans*, there was no significant difference observed among the cheeses produced with different rennet preparations as in moisture, fat and salt contents during ripening. [100]

93. Das R *et. al., (2011)* *performed In Vitro* Anthelmintic Activity of Leaves of *Juglans Regia L* against *Pheretima Posthuma and* attempt to evaluate anthelmintic activity of different extracts of leaves of *J. regia L*. Different extracts of the plant material were tested against adult Indian earthworms *Pheretima posthuma* (Pheritimidae) as test worms. The bioassay involved determination of the time of paralysis and time of death control. Piperazine citrate (10 mg/mL) was used as standard reference drug. [23]

94. Prashant Tiwari1 *et. al., (2011)* *studied* Comparative Anthelmintic Activity of Aqueous and Ethanolic Stem Extract of *Tinospora Cordifolia and* aqueous and Ethanolic extracts of stem of *Tinospora cordifolia* using *Eisenia foetida* at four different concentrations (10, 25, 50 and 100 mg/ml) respectively. The study involved the determination of time of paralysis (P) and time of death (D) of the worms. At the concentration of 100 mg/ml both the ethanolic and the aqueous extracts exhibited very significant activities as compared to the standard drug piperazine citrate (10 mg/ml). [103]

95. Deepika Mathur *et. al.,* (2011) studied Evaluation of *in vivo* antimutagenic potential of fruits extracts of *Withania coagulans*. The results confirmed that a single i.p administration of W. coagulans fruit extract at the dose of 500, 1000 and 1500 mg/kg body weight prior to 24 hours have significantly prevented the micronucleus formation in dose dependent manner in bone marrow cells of mice as compared to cyclophosphamide group.[27]

96. Deo SS *et. al.,* (2011) studied Antimicrobial Activity and HPLC Fingerprinting of Crude Ocimum Extracts. The crude aqueous extracts of Ocimum sanctum showed strong antimicrobial activity against S.aureus and moderate against others. Whereas the crude aqueous extracts of Ocimum kilimandsacharicum showed moderate activity against the gram positive and gram negative organisms and strong activity against C. albicans at higher concentration, same as that shown by the standard for C. albicans.[28]

97. Simona Zavoi *et. al.,* (2011) worked on a Comparative Fingerprint and Extraction Yield of Medicinal Herb Phenolics with Hepatoprotective Potential, as Determined by UV-Vis and FT-MIR Spectroscopy.. The value of the MIR signal at 1743 cm-1 may be considered a good indicator of phenolics concentration in such extracts. Combined UV-Vis and FTIR spectroscopy are recommended as rapid and reliable tools to investigate the fingerprint and to predict the composition of medicinal plants or to evaluate the quality and authenticity of different standardized formulas.[156]

98. Onasanwo SA *et. al.,* (2010) studied Antidepressant and Anxiolytic Potentials of Dichloromethane Fraction from Hedranthera barteri and was investigated in animal models of depression and anxiety in mice. Graded doses (25-200mg/kg p.o. bw) of

DMHBR reduced the immobility time with significant effects produced by 50mg/kg (43.7%), 100mg/kg (45.6%) and 200mg/kg (31.5%) in the tail suspension test (TST) and by 100mg/kg (66.3%) in forced swimming test (FST), indicating a possible antidepressant-like activity when compared with standard antidepressant drug, imipramine.[97]

99. Prasad SK *et. al.*, (2010) reported Pharmacognostical Standardization of Withania coagulans Dunal. The study includes macroscopical and microscopical evaluation along with estimation of its physicochemical parameters such as ash and extractive values, preliminary phytochemical screening and fluorescence analysis. It also includes quantification of some of the active constituents such as withanolides (withaferin-A) by HPTLC, total phenolic, tannin, flavonoids and flavonols. [102]

100. Janak Dabheliya *et. al.*, (2010) carried out work on Diuretic Potential of Aqueous Extract of Fruits of *Withania Coagulans* Dunal in Experimental Rats. The diuretic activity of the aqueous extract of fruits of *Withania coagulans* was studied by *invivo* Lipschitz test model with slight modifications using furosemide as a standard. The volumes of urine, urinary concentration of sodium, potassium and chloride ions were the parameters of the study. The results indicated significant ($P < 0.001$) increase in the urine volume by 79.12 % and 71.02 % in 500 mg/kg and 750 mg/kg doses respectively, when compared to control group. Urinary electrolyte excretions were increased with both the doses when compared to control. [54]

101. Sumathi P *et. al.*, (2010) suggested Antimicrobial activity of some traditional medicinal plants**.** The results showed that the minimum inhibitory concentration (MIC) of P. niruri leaf extract was 50 µg/ml against Salmonella typhi and Staphylococcus aureus, whereas, the MICs of T. bellerica fruit extract against Escherichia coli and S. aureus were 50 and 200 µg/ml respectively. However, the leaf extracts of the Andrographis paniculata, T. Chebula and V. negundo have not shown any antimicrobial activity in the tested concentrations**.**[163]

102. Zulfiker AHM *et. al.*, (*2010*) studied *In vivo* analgesic activity of ethanolic extracts of two medicinal plants - *Scoparia dulcis* L. and *Ficus racemosa* Linn. The crude extracts of both the plants were found to have significant ($p<0.001$) analgesic activity at

the oral dose of 100 & 200 mg/kg b. wt., in the tested models. In hot plate test *S. dulcis* showed increased latency period than *F. racemosa* whereas in acetic acid induced writhing test *F. racemosa* showed reduced number of writhes than *S. dulcis* at two dose levels which are significant ($p<0.001$) compared to control. [188]

103. Eliana H Akamine *et. al.*, (2010) carried out work on Obesity induced by high-fat diet promotes insulin resistance in the ovary and the ovary from high-fat-fed female rats showed a reduction in the insulin receptor substrate/phosphatidylinositol 3-kinase/AKT intracellular pathway, associated with an increase in FOXO3a, IL1B, and TNFa protein expression. These changes in the insulin signaling pathway may have a role in the infertile state associated with obesity.[34]

104. Islam MR *et. al.*, (2010) obeserved Analgesic, anti-inflammatory and antimicrobial effects of ethanol extracts of mango leaves and analgesic bioassay, oral administration of the ethanol leaves extract significantly ($P<0.01$) reduced the writhing response. The degree of inhibition of leaves extract was 55.8% compared to the effect of standard analgesic drug, Diclofenac Sodium (75.28%). On the other hand, though leaves extract reduce paw edema but they did not show any significant effect.[53]

105. Yash Prashar *et. al.*, (2010) carried out work on Anti-Obesity Activity of *Bauhinia Variegata* Linn. in High Fat Diet Induced Obestity in Female Rats and emphasized to explore the effect of AEBV 200 &400mg/kg, p.o on energy balance disorders like, obesity, hyperphagia, hyperglycaemia and hyperlipidemia. They predicted that *Bauhinia variegata* root extracts exerted significant anti-obese activity due to its hypophagic, hypoglycaemic and hypolipidemic effect in rats fed on high fat diet. [186]

106. Yonghua Zhanga *et. al.*, (2009) studied Progesterone metabolism in adipose cells and reported induction of preadipocyte differentiation increased expression levels of AKR1C1 and modified the pattern of progesterone metabolism substantially, leaving 20-hydroxyprogesterone as the main metabolite generated. On the other hand, progesterone itself showed no consistent effect on adipocyte differentiation. They concluded, preadipocytes and lipid-storing, mature adipocytes efficiently generate progesterone metabolites in women, which is consistent with rather modest effects progesterone on abdominal fat cell differentiation.187

107. Oliveira LMB *et. al.*, (2009) studied Anthelmintic activity of Cocos nucifera L. against sheep gastrointestinal nematodes. The in vitro assay was based on egg hatching (EHT) and larval development tests (LDT) with Haemonchus contortus. The concentrations tested in the EHT were 0.31, 0.62, 1.25, 2.5 and 5 mg/ml, while in the LDT they were 5, 10, 20, 40 and 80 mg/ml. The extract efficacy in the EHT and LDT, at the highest concentrations tested, was 100% on egg hatching and 99.77% on larval development. The parameters evaluated in the controlled test were not statisticallydifferent, showing that despite the significant results of the in vitro tests, the LGCHF ethyl acetate extract showed no activity against sheep gastrointestinal nematodes.[96]

108. Wasswa Peter *et. al.*, *(2006)* carried out work on the In-Vitro Ascaricidal Activity of Selected Indigenous Medicinal Plants Used in Ethno Veterinary Practices in Uganda. The research findings showed that *Tetradenia riparia*, *Cassia occidentalis*, *Carica papaya*, *Momordica foetida* and *Erythrina abysinnica* may be of value in the treatment of helminthiasis; whereas *Moringa oleifera* and *Cannabis sativa* are probably ineffective or of limited value for the same purpose.[116]

3. OBJECTIVE OF THE WORK

The plants are the key source of medicine in Ayurveda for treatment and prevention of diseases and maintenance of healthy life. The plants are used in medicine since antiquity. Much of the medicinal plants are documented in the Ancient Ayurvedic classics and these plants are still used successfully to treat different ailments. One of these plants which is used to treat various disease is *Withania coagulans* Dunal. The shrub is important for the property of coagulating milk, possessed by its berries; they are used for this purpose in North-West India and adjoining country.

Withania coagulans are the most reputed medicinal plants of Ayurveda and has well-descript pharmacological activities such as physiological and metabolic restoration, anti-arthritic, anti-aging, cognitive function, improvement in geriatric states and recovery from neurodegenerative disorders.[75, 132]

Based on the literature review, it was planned to carry out the biological screening as outlined below

- To validate acute oral toxicity
- To study the behavioral coordination in rats
- To study the antiobesity activity in progesterone induced obese rats
- To study the antihyperlipidemic activity in fructose induced hyperlipidemic rats
- To study the analgesic activity
- To evaluate the antimicrobial activity and anthelmintic activity

4. PLAN OF THE WORK

- ✓ Collection of the *Withania* flowers
- ✓ Authentication of the flowers
- ✓ Extraction with the suitable solvent – Ethanolic extraction
- ✓ Phytochemical screening of the extract

- **Analytical studies**
  - ➢ FTIR spectral analysis
  - ➢ HPTLC method

- ***Invivo* studies**

❖ Acute oral toxicity test - Validation

- ➢ OECD guideline 423

❖ Behvioural coordination study

- ➢ Hole board test in rats

❖ Antiobesity activity

- ➢ Progesterone induced obesity in rats

❖ Antihyperlipidemic activity

- ➢ Fructose induced hyperlipidemia in rats

❖ Analgesic activity

- ➢ Tail immersion test in rats

- ***Invitro* studies**

❖ Antimicrobial activity

- ➢ Disc diffusion method

❖ Anthelmintic activity

- ➢ Study of paralysis and death time in various worms

5. PLANT DESCRIPTION

PLANT AUTHENTICATION

Since 1919

NATIONAL COLLEGE
(Autonomous)
Nationally Re-accredited with 'A' Grade by NAAC
College with Potential for Excellence
TIRUCHIRAPALLI - 620 001
TAMILNADU
Email: veenan05@gmail.com

Dr. V. Nandagopalan, *M. Sc., M. Phil., Ph.D.,S.L.S.T.*
Dean of Sciences
Associate Professor in Botany

Phone No: 0431- 2482995
FAX: 0431 - 2481997

30-05-2017

AUTHENTICATION CERTIFICATE

This is to certify that the Plant specimen (Dry flowers) given by **Ms. R.SARATHA**, presently studying in M.Pharm., at Periyar College of Pharmaceutical Sciences, Tiruchirappalli- 620 021 is ***Withania coagulans*** (L.). Dunal belongs to the family **Solanaceae**.

Dean of Sciences 30/5/17

Dr. V.NANDAGOPALAN, M.Sc.,M.Phil.,Ph.D.,S.L.S.T.
Dean of Science
Associate Professor, Dept. of Botany
National College (Autonomous)
Tiruchirappalli-620 001.

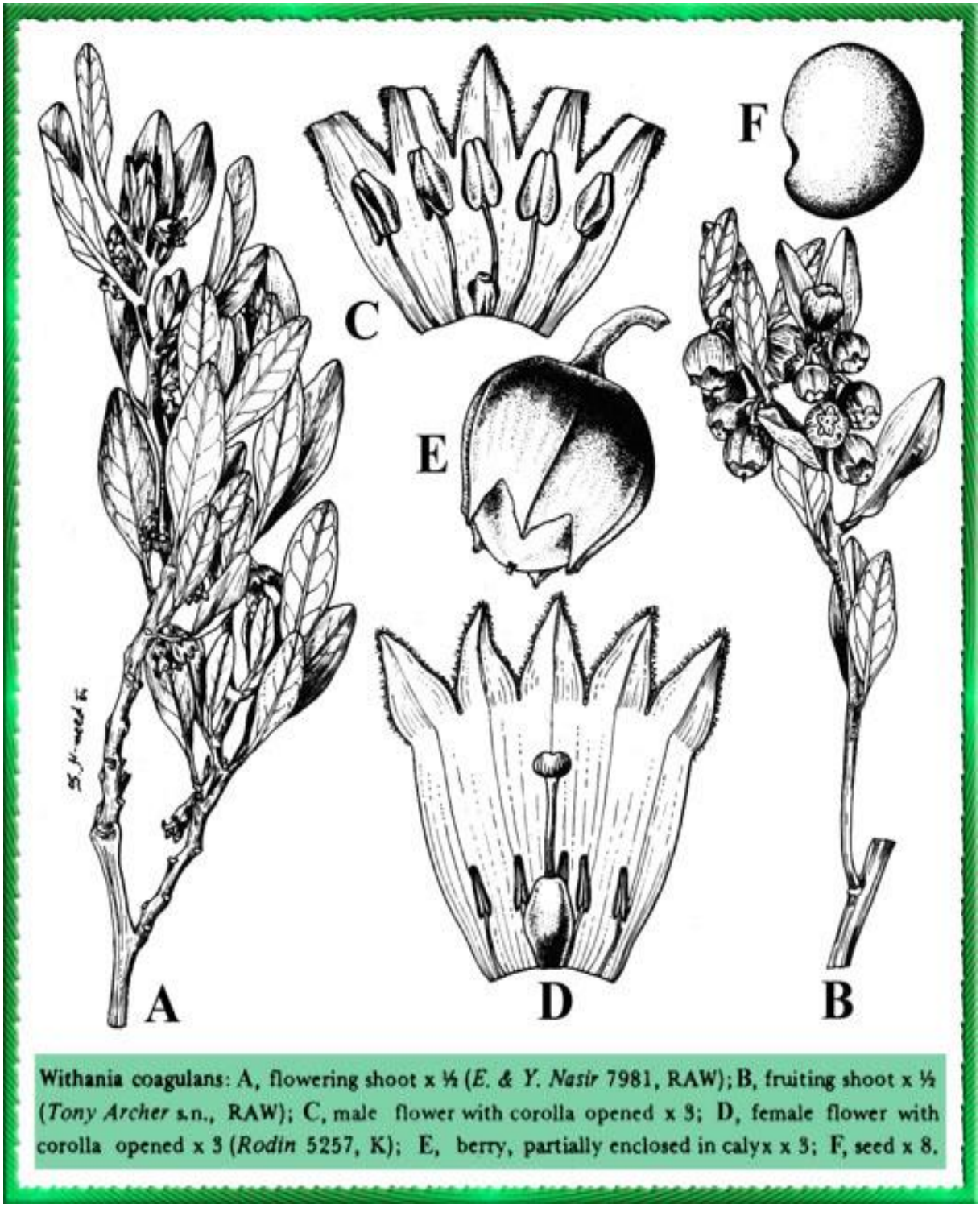


Fig. No. 4: Floral diagram of *Withania coagulans* Dunal

***Withania coagulans* Dunal (Flower bud)**

The dried flower buds of *Withania coagulans* Dunal was collected in Chennai from an authenticated dealer on March 2017.

Fig. No. 5: Plant showing flowers of *Withania coagulans* Dunal

Fig. No. 6: Dried flowers of *Withania coagulans* Dunal

Taxonomical classification of *Withania coagulans* Dunal[21, 101]

Table No. 4: Taxonomical classification of *Withania coagulans* Dunal

Rank	Scientific Name and Common Name
Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Asteridae
Order	Solanales
Family	Solanaceae
Genus	*Withania*
Species	*coagulans* (L.) Dunal

Other common names[132]

Table No. 5: Common names of *Withania coagulans* Dunal

Tamil	Panir poo
Hindi	Akri, Punir
English	Indian cheese maker, Vegetable rennet
Punjabi	Spin bajja, panir
Marathi	Kaknaj
Sindhi	Punirjafota, Punirband
Persian	Kaknajehindi, Punirbad
Arabic	Javzulmizaja, Kaknajehindi
Telugu	Panneru-gadda
Urdu	Hab kaknaj

Botanical description and Characteristic feature[101]

Macroscopical

A rigid, grey under shrub, 60-120 cm high

A rigid grey- tomentose undershrub 0.3-0.9 m. high, branches terete, clothed with dense grey or yellowish white tomentum

Leaves

2.5-5.7 by 1-2.2 cm., lanceolate-oblong, obtuse, entire, clothed with a persistent not easily detachable greyish tomentum, of a uniform colour on both sides, thick, more or less rugose, base acute, running down into an often obscure petiole; petiole 6 mm. long but often indistinct

Flowers

Dioecious, in axillary clusters; pedicles 0-6mm. long, deflexed, slender

Calyx 6 mm. long, campanulate, clothed with fine stellate grey tomentum; teeth triangular, 2.5 mm. long.

Corolla 8 mm. long, stellately mealy outside, divided about 1/3 the way down; lobes ovateoblong, subacute

Male flowers

Stamens about level with the top of the corolla- tube; filaments 2 mm. long, glabrous; anthers 3-4 mm. long. Ovary ovoid, without style or stigma

Female flowers

Stamens scarcely reaching ½ way up the corolla-tube; filaments about 0.85 mm. long; anthers smaller than in the male flowers, sterile

Ovary

Ovoid, glabrous; style glabrous; stigma mushroom- shaped, 2- lamellate

Fruits

Berry 6-8 mm. diam., globose, smooth, closely girt by the enlarged membranous calyx which is scurfy pubescent outside

Seeds

2.5-3mm diam., dark brown, ear shaped, glabrous

Flowering period

From January to April and berries ripen during January to May

The natural regeneration is from seed

Habitat

It grows as short shrub (35-75 cm) with central stem. This shrub is common Afghanistan and East Indian. It has milk coagulating property. It is also found in North West India, in Punjab and in Pakistan. It is also known as Pakistani herb.

Chemical Constituents

Berries contain milk coagulating enzyme esterase, free amino acids, fatty oil, essential oil and alkaloids. The essential oil was active against Micrococcus pyogenes var. aureus and also shows anthelmintic activity.

The withanolides, withacoagin, coagulan and withasomidienone have been isolated from plant along with other withanolides and withaferin. 3- β-hydroxy-2, 3-dihydrwithanolide E isolated from plant showed significant hepatoprotective activity and anti-inflammatory activity equal to hydrocortisone.

Uses

- It is used as Emetic, Diuretic and Anti Diabetic agent
- Ripe fruits used as sedative, CNS depressant and anti-inflammatory
- Also used in chronic liver trouble
- Dried fruits used as carminative, dyspepsia and flatulence
- Leaves used as alterative and febrifuge

6. METHODOLOGY

6. 1. Extraction[21]

Extraction is defined as the process of isolation of material from an insoluble residue which may be liquid or solid, by treatment with a solvent on the basis of the physical nature of crude drug to be extracted, that is liquid or solid.

Flower buds of *Withania coagulans Dunal* were collected; dried material was ground into coarse powder which was used for further study to extract with ethanol using Soxhlet apparatus.

Petroleum ether extract

About 500gm of dried coarse powder was extracted with 2.5 litre of petroleum ether by (60-80°C) continuous hot percolation using Soxhlet apparatus. The extraction was continued for 24 hours and defatting was done using petroleum ether. After completion of extraction, the petroleum ether extract was filtered and solvent was removed by distillation under reduced pressure. A dark green coloured residue was obtained. Then the extract was stored in a dessicator. The marc was stored for further extraction with ethanol.

Ethanolic Extract

Marc obtained from the petroleum ether extract was dried and finally extracted with 2.5 litre of ethanol. The extraction was continued for 24 hours. After completion of extraction, the extract was filtered and solvent was removed by distillation under reduced pressure. A brown coloured residue was obtained. Then it was stored in dessicator.

The extracts were used for identification of constituents by phytochemical screening and for pharmacological studies.

6. 2. Identification of Plant Constituents by Preliminary Phytochemical Tests[21, 101]

The plant extracts were subjected to preliminary phytochemical screening for the detection of various plant constituents present. The term qualitative analysis refers to the establishing and proving the identity of a substance. The active ingredients, after isolation, can be incorporated into the modern medicine system for the development of newer formulation for therapeutic ailments. Systematic investigation of the plant material for its phytochemical behavior involves four different stages.

- Procurement of raw material and quality control
- Extraction, isolation, purification and characterization of the constituents of interest
- Investigation of biosynthetic pathways of the particular compound
- Quantitative evaluation

Qualitative Phytochemical Analysis

The ethanolic extract of Flower buds of *Withania coagulans* Dunal were subjected to the following chemical tests separately for the identification of various active constituents.

Tests for Alkaloids

1. **Dragendorff's Test**: To 1 mL of the extract, added 1 mL Dragendorff's reagent, and orange red precipitate indicated the presence of alkaloids.

2. **Wagner's Test**: To 1 mL of the extract, added 2 mL of Wagner's reagent. The formation of a reddish brown precipitate indicated the presence of alkaloids

3. **Mayer's Test**: To 1 mL of the extract, added 2 mL of Mayer's reagent, a dull white precipitate revealed the presence of alkaloids.

4. **Hager's Test**: To 1 mL of the extract, added 3 mL of Hager's reagent, the formation of yellow precipitate confirmed the presence of alkaloids.

Tests for Carbohydrates

1. **Molisch Test**: To 2 mL of the extract, added 1mL of α - naphthol solution, and concentrated sulfuric acid through the sides of test tube. Purple or reddish violet color at the junction of the two liquids revealed the presence of carbohydrates.
2. **Fehling's Test**: To 1 mL of the extract, added equal quantities of Fehling's solution A and B, upon heating formation of a brick red precipitate indicated the presence of carbohydrates.

3. **Benedict's Test**: To 5 mL of Benedict's reagent added 1 mL of extract solution and boiled for 2 minutes and cooled. Formation of a red precipitate showed the presence of carbohydrates.

Tests for Proteins and Amino Acids

1. **Biuret Test**: To 1 mL of the extract added 1mL of 40% sodium hydroxide solution and 2 drops of 1% copper sulfate solution. Formation of violet color indicated the presence of proteins.
2. **Xanthoprotein Test**: To 1 mL of the extract added 1 mL of concentrated nitric acid. A white precipitate was formed, it was boiled and cooled. Then, 20% of sodium hydroxide or ammonia was added. Orange color indicated the presence of aromatic amino acids.

3. **3. Lead Acetate Test**: To the extract, 1 mL of lead acetate solution was added. Formation, of proteins.

Tests for Steroids

1. **Liebermann Burchard Test**: Dissolved the extract in 2 mL of chloroform in a dry test tube. Added 10 drops of acetic anhydride and 2 drops of concentrated sulfuric acid. The solution became red, then blue and finally bluish green, indicated the presence of steroids.

2. **Salkowski Test**: Dissolved the extract in chloroform and added equal volumes of concentrated sulfuric acid. Formation of bluish red to cherry red color in chloroform layer and green fluorescence in the acid layer represented the steroid components in the tested extract.

3. **Ninhydrin Test**: Added two drops of freshly prepared 0.2% ninhydrin reagent to then extract solution and heated. Development of blue color revealed the presence of proteins, peptides or amino acids.

Tests of Glycosides

1. **Legal's Test**: Dissolved the extract in pyridine and added sodium nitroprusside solution to make it alkaline. The formation of pink red to red color showed the presence of glycosides.

2. **Baljet Test**: To 1 mL of the test extract added 1 mL sodium picrate solution and the yellow to orange color revealed the presence of glycosides.

3. **Borntrager's Test**: Added a few mL of dilute sulfuric acid to 1 mL of the extract solution.Boiled, filtered and extracted the filtrate with chloroform. The chloroform layer was treated with 1 mL of ammonia. The formation of red color showed the presence of anthraquinone glycosides.
4. **Keller Kiliani Test**: Dissolved the extract in acetic acid containing traces of ferric chloride and transferred to a test tube containing sulfuric acid. At the junction, formation of a reddish brown color, which gradually became blue, confirmed the presence of glycosides.

Test for Flavonoids

1. **Shinoda Test:** To 1 mL of the extract, added magnesium turnings and 1-2 drops of concentrated hydrochloric acid. Formation of red color showed the presence of flavonoids.

5. 3. Infrared Spectroscopy[143]

Fourier Transform –Infra Red Spectrophotometer

Fourier transform infrared (FTIR) spectroscopy is a measurement technique that allows one to record infrared spectra. Infrared light is guided through an interferometer and then through the sample (or vice versa). A moving mirror inside the apparatus alters the distribution of infrared light that passes through the interferometer. The signal directly recorded, called an "interferogram", represents light output as a function of mirror position.

A data-processing technique called Fourier transform turns this raw data into the desired result (the sample's spectrum): Light output as a function of infrared wavelength (or equivalently, wavenumber). As described, the sample's spectrum is always compared to a reference..

Fig. No. 7: Fourier Transform Infra Red Spectrometer

Table No. 6: Specifications of FTIR Spectrophotometer

Model	Spectrum RX I
Make	Perkin Elmer
Range	$4000cm^{-1}$ to $400cm^{-1}$
Specimen	Solids, Liquids

5. 4. HPTLC Fingerprinting[47]

CAMAG HPTLC system equipped with Linomat 5 applicator, TLC scanner 3, repro star 3 with 12 bit CCD camera for photo documentation, controlled by WinCATS-4 software were used. All the solvents used for HPTLC analysis were obtained from MERCK. A total of 100 mg extract was dissolved in 5 mL of ethanol and used for HPTLC analysis as test solution.

The samples (10 µl) were spotted in the bands of width 8 mm with a Camag microlitre syringe on pre-coated silica gel glass plate 60 F- 254. The sample loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase and the plate was developed up to 83 mm in the respective mobile phase.

Linear ascending development was carried out in 20 cm X 10 cm twin trough glass chamber saturated with the mobile phase and the chromatoplate development with the same mobile phase to get good resolution of phytochemical contents. The optimized chamber saturation time for mobile phase was 30 min at room temperature. The developed plate was dried by hot air to evaporate solvents from the plate.

The plate was photo-documented at UV 366 nm and white light using photo documentation chamber. Finally, the plate was fixed in scanner stage and scanning was done at 366 nm. The plate was kept in photo-documentation chamber and captured the images under white light, UV light at 254 and 366 nm. Densitometric scanning was performed on Camag TLC scanner III and operated by CATS software (V 3.15, Camag).

5. 4. 1. HPTLC Fingerprinting – Alkaloids

TLC Details T1, T2-10, 20 µL of Test Solution

Identity Test

Test Solution: Weigh about 0.5g of sample and shaken for 15min with 15mL of 0.1N sulphuric acid and then filtered. The filtrate is washed with 0.1N sulphuric acid to a volume of 20mL; 1mL of concentrated ammonia is added. The mixture is shaken with chloroform; separated chloroform layer is passed over anhydrous sodium sulphate, and evaporated to dryness. The dried residue is dissolved in methanol used for TLC analysis.

Stationary phase: Silica Gel 60 F254

Mobile phase: Toluene: Ethyl Acetate: Diethylamine (7: 2: 1)

Procedure: Applied 10, 20µL of test solutions spotted on a precoated silica gel 60 F254 HPTLC plate (E. Merck) of uniform thickness 0.2mm using Linomat5 sample applicator. Developed the plate in the solvent system to a distance of 8cm. Observed the plate under UV light at 254nm & 366nm using CAMAG REPROSTAR3.

5. 4. 2. HPTLC Fingerprinting – Flavanoids

TLC Details - T1, T2-5, 10µL of Test solution.

Identity Test

Test Solution: About 100mg of extract was dissolved in ethanol used for TLC analysis.

Stationary phase: Silica Gel 60 F254

Mobile phase: Toluene: Ethyl acetate: Formic acid (5: 4: 1)

Procedure: Applied 5, 10µL of test solutions spotted on a precoated silica gel 60 F254 HPTLC plate (E. Merck) of uniform thickness 0.2mm using Linomat5 sample applicator. Developed the plate in the solvent system to a distance of 8cm. Observed the plate under UV light at 254nm & 366nm using CAMAG REPROSTAR3.

5. 4. 3. HPTLC Fingerprinting – Glycosides

TLC Details - T1, T2-5, 10 µL of T.S.

Identity Test

Test Solution: About 0.5g of sample refluxed with 25mL of 7.5% hydrochloric acid for 15min.After cooling, the mixture is extracted with ether. The ether phase was concentrated to dryness and the collected residue was dissolved in methanol was used for TLC analysis.

Stationary phase: Silica Gel 60 F254

Mobile phase: Ethyl Acetate: Methanol: Water (100: 13.5: 10)

Procedure: Applied 5, 10µL of test solutions spotted on a precoated silica gel 60 F254 HPTLC plate (E. Merck) of uniform thickness 0.2mm using Linomat5 sample applicator. Developed the plate in the solvent system to a distance of 8cm. Observed the plate under UV light at 254nm & 366nm using CAMAG REPROSTAR3.

5.5. ANIMAL EXPERIMENTATION

Pharmacological evaluation of the ethanolic extract of *Withania coagulans* Dunal (Flower Buds) was carried out in the Department of Pharmacology, Periyar college of Pharmaceutical Sciences, Tiruchirappalli, Tamilnadu, India. Animal facility of this institute is approved by CPCSEA. The experimental protocols for the antiobesity and antihyperlipidemic activities have been approved by the Insititutional Animal Ethics Committee and conducted according to the guidelines of Indian National Sciences Academy for the use and care of experimental animals. IAEC approved this proposal with approval number PCP/IAEC/002/2017. The animals were maintained at a well ventilated, temperature controlled $30^{o}C \pm 1^{o}C$ animal room for 7days prior to the experimental period and provided with food and water *ad libitum*. The animals were acclimatized to laboratory conditions before the test. Each animal was used only once.

5. 5. 1. Toxicity Studies – Validation[4]

Validation of Acute Toxicity

Acute Toxic Class method: Guideline number- 423

The test substance will be administered orally to a group of experimental animals at one of the defined doses. The substance will be tested using a stepwise procedure, each step using three animals of a single sex (normally female). Absence or presence of compound related mortality of the animals dosed at one step will determine the next step i.e.

- no further testing is needed
- dosing of three additional animals, with the same dose
- dosing of three additional animals at the next higher or the next lower dose level

Healthy young rats were used. The test substance was administered in a single dose (2000 mg/kg, b.w., *p.o.*) by gavage using a stomach tube.

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days.

Observed changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and mortality. In addition, behavioral changes, body weight, histopathological studies and biochemical parameters were also observed.

5. 5. 2. Behavioural Activity[61]

Hole Board Test

Behavioural activity was evaluated by using Hole-board model.

Wistar albino rats of either sex weighing 150-200g were divided into four groups of six animals each. The animals were fasted overnight for 18h. The dosage of the drugs administered to the different groups was as follows

Group-1: Control (Normal Saline 5mL/kg, b.w., *p.o.*).

Group-2: Standard drug (Diazepam 1mg/kg, b.w., *i.p.*).

Group-3: Test (Ethanolic extract of *Withania coagulans* 200mg/kg, b.w., *p.o.*).

Hole board test is a generally used method for screening the potential anxiolytic character of the drugs.

Dimensions of the hole board

The Hole board consists of wooden chamber (60×60×45cm) with 16 holes (diameter of 5 cm) on the floor, elevated from the ground so that the rat could peep through the holes. Each rat will be placed individually in the apparatus for recording the anxiolytic parameters.

Experimental Design

The Ethanolic extract of *Withania coagulans* was freshly dissolved in a suitable amount of distilled water. Diazepam (1mg/kg, b.w., *i. p.*) was dissolved in distilled water. Control groups received only normal saline.

One hour after oral administration of the test extract and standard drug, the animals were submitted to the Hole board test. The cut off time is 5 minutes.

Evaluation

The various evaluation tests for Hole board test like latency to the first head dips, no. of head dips in the holes, total time spent with the head dips, no. of rearings and no. of defecation units.

Statistical analysis

The results are expressed are Mean ± SEM (n=6) two way ANOVA using Graph pad PRISM software version. *** $P<0.001$, ** $P<0.01$ and * $P<0.05$ were considered as statistically significant.

5. 5. 3. Antiobesity Activity[29, 164]

Progesterone Induced Obesity

Principle

Progesterone is a steroidal female sex hormone playing an important role in menstrual cycle and pregnancy. Progesterone impairs fat metabolism and induces obesity due to fat accumulation induced by the absorption of lipase from the GIT. Progesterone induced obesity is a well-known model for screening the anti-obesity effect of drugs.

Wistar albino rats of either sex weighing 150-200g were divided into four groups of six animals each. The animals were fasted overnight for 18h. The dosage of the drugs administered to the different groups was as follows

Group 1	**:**	Normal Saline (5mL/kg, b.w., *p.o.*).
Group 2	**:**	Obesity control (10mg/kg Progesterone b.w., *s.c.*).
Group 3 (Standard)	**:**	Progesterone (10mg/kg b.w., *s.c.*) + Orlistat (10mg/ kg b.w. *p.o.*)
Group 4 (Test)	**:**	Progesterone (10mg/kg b.w., *s.c.*)+ Ethanolic extract of *Withania coagulans* (200mg/kg b.w., *p.o.*)

Experimental Design

Progesterone is the obesity control used to induce obesity. The dose of obesity control is 10mg/kg of body weight. It was prepared by dissolving in distilled water and a dose of 10 mg/kg was administered subcutaneously in the dorsal neck region for 28 days. The test drugs were injected 30 min before progesterone administration.

The extract and standard Orlistat were soluble in distilled water and administered by gavage using a stomach tube.

The total time period for the evaluation of anti-obesity activity was 28days. Body weight, food consumption behavior and behavioral changes for every week were examined and recorded. After completion of 28 studies the animals were sacrificed and before that the blood is collected from each group for biochemical estimation.

Evaluation

Body weight

The body weights every rat (g) were recorded for every week for 28 days in each group just before dosing by using electronic weighing balance.

Rearing, grooming and ambulatory moments were studied.

Assessment of food consumption behavior in rats

It is very important to assess the food consumption behavior of all groups so the consumption behavior was studied on days 1, 7, 14, 21, and 28. On experimental days, 30 min after last drug administration, 10 g of sweetened chow was presented to groups of mice in glass Petri dishes and food intake was recorded at 0.5, 1, and 2 h time intervals. Nearest to 0.1 g with correction for spillage and the amount of food consumed/ 20 g body weight was calculated.

Biochemical parameters

On 29th day the blood is collected from all the mice by retro orbital puncture method, and subjected for centrifugation at 3000 rpm immediately. The serum samples of all the blood samples were carefully collected and preserved for bio chemical estimation. Serum glucose levels, total cholesterol, LDL- C, HDL- C, VLDL- C, Triglycerides, SGOT, and SGPT were estimated.

Histopathology of Liver

After the biochemical examination was over the animals were sacrificed for histopathology studies .The liver of each animal from all four groups were isolated for histopathology studies. The isolated livers were carefully kept in 10% formalin solution in order to prevent the damage.

Statistical analysis

The results are expressed are Mean ± SEM (n=6) two way ANOVA using Graph pad PRISM software version. *** $P<0.001$, ** $P<0.01$ and * $P<0.05$ were considered as statistically significant.

5. 5. 4. Antihyperlipidemic Activity[01]

Fructose Induced Hyperlipidemia

Male Wistar albino rats weighing 200-250g, used for the study were fed on pellet diet and water ad libitum. Hyperlipidemia was induced by supplementing their drinking water with 10% fructose.

Priniciple

Fructose has been reported to induce hypertriglyceridemia associated with insulin resistance, hyperinsulinemia and hypertension. After absorption in GIT, Fructose is transported *via* portal circulation to the liver, where it enters hepatocytes via the glucose transporter GLUT- 5 independently of insulin and is rapidly metabolised. Fructose is metabolised into "glycerol-3-phosphate" and "acetyl CoA". These two intermediate metabolites are then used as substrates for glycerides synthesis, contributing to VLDL-TG production in liver. The exposure of liver to such large quantities of fructose leads to rapid stimulation of lipogenesis and TG accumulation which in turn contributes to reduced insulin sensitivity and hepatic insulin resistance/glucose intolerance

Treatment

Rats were divided into the following 4 groups of 6 each:

Group I **:** Normal diet water *ad libitum*

Group II **:** Normal diet and 25% Fructose in water (Positive Control)

Group III **:** Normal diet and 25% Fructose in water + Ethanolic extract of *Withania coagulans* (200 mg /kg, *p.o.*)

Group IV **:** Normal diet and 25% Fructose in water + Atrovastatin (10mg/kg, *p.o.*)

Experimental Design

Each group of animals were administered vehicle or drugs daily for 3 weeks by gavage using oral feeding needle. Feeding of animals by fructose and extracts were done simultaneous from the beginning. At the end of the 3 weeks period, animals were kept for overnight fasting and the blood samples were collected from the tail vein in the centrifuging tubes.

Biochemical analysis

On the 21st day, blood was collected by retro orbital sinus puncture, under mild ether anaesthesia. The collected samples were centrifuged for 10 minutes at 3000rpm. Then serum samples were collected and used for various biochemical experiments. The serum and liver extract were assayed for total cholesterol, triglycerides, high density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and serum blood glucose.

Histopathological studies

At the end of the treatment period, the animals were sacrificed for histopathology studies .The livers of each animal from all four groups were isolated for histopathology studies. The isolated livers were carefully kept in 10% formalin solution in order to prevent the damage.

Statistical analysis

The results are expressed are Mean ± SEM (n=6) two way ANOVA using Graph pad PRISM software version. *** P<0.001, ** P<0.01 and * P<0.05 were considered as statistically significant.

5. 5. 5. Analgesic Activity[25]

Tail Immersion Test in Mice

Wistar albino rats of either sex weighing 150-200g were divided into four groups of six animals each. The animals were fasted overnight for 18h. The dosage of the drugs administered to the different groups was as follows

Group-1: Control - Normal Saline (5mL/kg, b.w., *p.o.*).

Group-2: Standard (Diclofenac sodium 10mg/kg in 10mL of normal saline, *p.o.*)

Group-3: Test (Ethanolic extract of *Withania coagulans* 200mg/kg, p.o.)

Experimental Design

The procedure is based on the observation that morphine-like drugs are selectively capable of prolonging the reaction time of the typical tail-withdrawal reflex in rats induced by immersing the end of the tail in warm water of 55 °C.

The lower 5 cm portion of the tail is marked. This part of the tail is immersed in to the water bath of exactly 55 °C. Within a few seconds the rat reacts by withdrawing the tail. After each determination the tail is carefully dried. The reaction time is determined before and periodically after oral administration of the test and standard substance. The cut off time is 15sec.

Evaluation

Tail withdrawal reflex

Statistical analysis

The results are expressed are Mean ± SEM (n=6) two way ANOVA using Graph pad PRISM software version. *** P<0.001, ** P<0.01 and * P<0.05 were considered as statistically significant.

5. 5. 6. Anthelmintic Acitivity[2, 104]

The dosage of the drugs administered to the different groups was as follows

Group-1: Control (Normal saline 10mL)

Group-2: Standard drug (Albendazole 20mg/ mL)

Group-3: Test (Ethanolic extract of *Withania coagulans* 25mg/ mL)

Group-4: Test (Ethanolic extract of *Withania coagulans* 50mg/ mL)

Group-5: Test (Ethanolic extract of *Withania coagulans* 75mg/ mL)

Group-6: Test (Ethanolic extract of *Withania coagulans* 100mg/ mL)

Experimental worms

All the experiments were carried out in Indian adult earthworms (***Pheretima posthuma***) due to its anatomical resemblance with the intestinal roundworm parasites of human beings. They were collected from moist soil and washed with water to remove all fecal matters.

Administration of Albendazole

Albendazole (20 mg/mL) was prepared by using 10 mL of Normal Saline as a vehicle.

Administration of extract

The suspension of Ethanolic extract of *Withania coagulans* at different concentration (25, 50, 75 & 100mg/ 10mL) were prepared by using 10 mL of Normal Saline as a vehicle and final volume was made up to 10 mL for respective concentration. Albendazole was used as standard. Groups of approximately equal size worms consisting of six earthworms individually in each group were released into in each 10 mL of desired concentration of drug and extracts in the petridish.

Experimental Design

On adult Indian earth worm *Pheretima posthuma* as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings. Pheretima posthuma was placed in petridish containing different concentrations (25, 50, 75 & 100 mg/ 10 mL) of Ethanolic extract of *Withania coagulans*. Each petridish was placed with 6 worms and observed for paralysis or death. Mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. The test results were compared with Reference compound Albendazole (20 mg/ mL) treated samples. The time period of the study is 3 hours.

Statistical analysis

The results are expressed are Mean ± SEM (n=6) two way ANOVA using Graph pad PRISM software version. *** $P<0.001$, ** $P<0.01$ and * $P<0.05$ were considered as statistically significant.

5. 5. 7. Anti- Microbial Screening[15]

Principle

Known concentration of Ciprofloxacin and Nystatin antibiotic disc was placed on agar plate that has been inoculated or seeded uniformLy over the entire plate with a culture of the bacterium to be tested. The test compound was inoculated for 18 - 24 hour at 37^0C. During this period the antibacterial agents diffuses through the agar and may prevent the growth of organism. Effectiveness of susceptibility is proportional to the diameter of zone

of inhibition around the disc, organism which grows up to the edge of the disc are resistant.

Material and methods

Medias

Media for bacteria - Nutrient agar

Media for fungi - Sabouraud Dextrose Agar (SDA)

Organisms Used

Bacteria

Gram- positive

- *Staphylococcus aureus* (NCIM 2079)
- *Bacillus substilis* (NCIM 2063)

Gram- negative

- *Proteus vulgaris* (NCIM 2027)
- *Klebsiella species* (NCIM 2098)

Fungi

- *Aspergillus niger* (NCIM 105)
- *Candida albicans* (NCIM 3102)

Antibiotic disc used

- Ciprofloxacin (5µg/ disc)- Bacteria
- Nystatin (100 units/ disc)- Fungi

were used repectively as standards for bacterial and fungal strains.

Test Sample

- Ethanolic ectract of *Withania coagulans* Dunal (25, 50, 75, 100 & 250mg/ disc)

Table No. 7: Composition of Nutrient Agar Medium

S.No	Ingredients	g/l
1	Yeast extract	5g
2	Meat Extract	10g
3	.pectin	5g
4	Sodium chloride	5g
5	Agar	20g

Suspended 2g agar in 100 mL distilled water and added all ingredients. Mix well and heat to dissolve the medium completely. Sterilized by autoclaving at 15 lbs pressure (121^0c) for 15 min.

Table No. 8: Composition of Sabouraud Dextrose Agar Medium

S.No	Ingredients	g/l
1	Glucose	4%
2	Peptone	1%
3	Water	100 mL

For sabouraud dextrose agar medium 2% agar powder was mixed with above constituents pH was maintained at 5.4 for fungal media. This media was sterilized by autoclaving at 121^0C at 15 lbs pressure for 20 min.

Determination of Zone of Inhibition[15]

The disc diffusion method was used to determine the antimicrobial activities of the newly synthesized compounds. Muller Hington Agar media was prepared, sterilized and used as the growth medium for bacteria culture. 20 mL of the sterilized medium was poured into each sterilized petri dish, covered and allowed to solidify. The plates were then seeded with the test organism (bacterial culture) by sterile cotton swabs

For fungal culture Saboraud Dextrose Agar was prepared and transferred into sterile petri dishes and solidified. The sterilised paper discs were soaked in prepared solution of synthesized compounds and were dried at 50ºC. The dried paper disc was then placed on both plates (Muller Hington and Saboraud Dextrose Agar) seeded with test micro organisms.

The plates were then incubated for bacterial culture for 37 ºC for 24 hours and for fungus the plates were incubated at room temperature for 48 hours and the zone of inhibition were measured.

6.1.RESULTS

Table No. 9: Preliminary Phytochemical Studies

S.No	Plant Constituents	Test / reagent	Ethanolic extract of *Withania coagulans*
1.	Steroids	Salkovaski Liberman Burchard Test	+
2.	Alkaloids	Dragendroff's Hager's test Mayer's test Wagner's test	+
3.	Saponin	Forms test Haemolysis test	-
4.	Fat and oils	Filter paper test	-
5.	Tanins and phenolic compounds	Ferric chloride test Lead acetate test Pot. Dichromate Bromine water	-
6.	Flavonoids	Shinoda test Lead acetate test	+
7.	Carbohydrates	Molisch test Fehling's test Barfoed's test	+
8.	Proteins	Millons test Biuret test Xanthoprotein test	+
9.	Amnio acid test	Ninhydrine test	+
10.	Glycosides	Legal's test Baljet test Bontrager's test Keller Kiliani test	+

Table No. 10: IR Spectral Data of the Ethanolic extract of *Withania coagulans*

S. No	Wavenumber (cm^{-1})	Interpretation
1.	3418.82 & 3382.21	Polymeric association of alcohols and phenols indicates presence of miscellaneous chromophoric groups
2.	2974.21	Chromophoric hydrocarbon - Alkene
3.	2934.88	Chromophoric hydrocarbon - Alkene
4.	2112.11	Alkene monosubstituted
5.	1722.28	Saturated 6-membered and higher ring
6.	1662.94	α, β - unsaturated bending- acyclic
7.	1575.63	C-NO_2 aromatic nitro compounds
8.	1414.44	Phenolic OH bending
9.	1215.63	Aliphatic CN vibrations
10.	1049.29	Aromatic C-C multiple bond stretching
11.	925.15	CH bending; Alkene monosubstituted
12.	879.63	One Hydrogen atom bending
13.	814.00	Two adjacent hydrocarbon atoms
14.	777.55	C-X stretching
15.	648.58	C-X stretching
16.	621.68	C-H stretching

HPTLC Fingerprinting - Alkaloids

Table No. 11: Chromatogram Data of 10µl of test solution

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %
1.	0.04	1.7	0.06	17.0	3.27	0.08	0.1	243.1	1.49
2.	0.09	0.1	0.10	14.5	2.78	0.13	0.3	197.7	1.21
3.	0.13	0.3	0.15	13.9	2.67	0.17	8.2	246.7	1.51
4.	0.17	8.7	0.20	36.5	7.03	0.22	3.1	685.2	4.19
5.	0.22	3.4	0.23	11.6	2.24	0.24	0.1	63	0.39
6.	0.34	6.0	0.42	178.3	34.31	0.46	36.9	6266.6	38.34
7.	0.46	37.2	0.49	62.7	12.06	0.54	6.0	1804.0	11.04
8.	0.57	9.7	0.58	17.9	3.44	0.62	3.2	506.6	3.10
9.	0.64	3.7	0.67	39.1	7.52	0.71	17.2	1115.9	6.83
10.	0.71	17.01	0.74	42.7	8.21	0.84	0.1	1894.0	11.59
11.	0.87	0.3	0.92	85.6	16.47	0.99	0.1	3320.0	20.31

Table No. 12: Chromatogram Data of 20µl of test solution

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %
1.	0.0	16.6	0.01	20.0	1.68	0.02	4.2	161.0	0.41
2.	0.03	4.2	0.04	18.7	1.57	0.05	2.5	198.7	0.51
3.	0.06	2.6	0.07	22.6	1.90	0.09	1.0	268.1	0.68
4.	0.09	0.2	0.11	17.0	1.43	0.13	0.2	257.5	0.66
5.	0.14	0.3	0.16	17.6	1.48	0.17	12.7	288.7	0.74
6.	0.18	12.9	0.20	48.8	4.10	0.24	3.4	1086.0	2.77
7.	0.32	2.3	0.43	268.1	22.53	0.47	64.0	9999.9	25.49
8.	0.47	64.2	0.50	103.2	8.67	0.56	7.5	3295.0	8.40
9.	0.56	8.8	0.59	19.7	1.66	0.63	1.3	596.1	1.52
10.	0.64	0.1	0.68	72.2	6.07	0.72	30.4	2061.3	5.25
11.	0.72	30.9	0.75	64.5	5.42	0.83	0.2	2871.8	7.32
12.	0.84	0.1	0.92	517.8	43.51	0.99	0.8	18144.7	46.25

HPTLC Fingerprinting - Glycosides

Table No. 13: Chromatogram Data of 5µl of test solution

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %
1.	0.00	0.1	0.03	22.1	2.57	0.08	5.9	713.0	1.06
2.	0.08	6.0	0.12	30.3	3.53	0.18	15.0	1391.0	2.06
3.	0.19	16.0	0.24	46.7	5.43	0.30	13.5	1948.9	2.89
4.	0.33	10.2	0.34	10.5	1.22	0.38	0.8	244.2	0.36
5.	0.57	3.8	0.70	168.5	19.59	0.71	162.7	6200.5	9.19
6.	0.71	163.3	0.82	582.1	67.67	0.97	3.6	56939.5	84.43

Table No. 14: Chromatogram Data of 10µl of test solution

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %
1.	0.05	0.4	0.08	23.5	3.08	0.09	17.7	423.0	0.57
2.	0.10	18.4	0.16	44.4	5.84	0.18	37.0	1565.5	2.10
3.	0.22	38.2	0.27	79.3	10.43	0.39	1.6	4321.2	5.79
4.	0.57	0.3	0.82	613.2	613.2	0.97	2.6	68369.5	91.55

HPTLC Fingerprinting - Flavanoids

Table No. 15: Chromatogram Data of 5µl of test solution

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %
1.	0.01	16.4	0.01	21.2	2.94	0.03	0.2	219.9	1.05
2.	0.20	1.9	0.25	35.3	4.91	0.28	8.4	1011.6	4.84
3.	0.31	11.3	0.35	30.4	4.23	0.39	14.1	1080.5	5.17
4.	0.40	12.7	0.48	402.1	55.86	0.53	51.4	13030.4	62.37
5.	0.53	51.6	0.54	53.3	7.40	0.57	17.3	944.5	4.52
6.	0.57	17.4	0.60	40.1	5.57	0.62	25.6	1003.6	4.80
7.	0.62	25.8	0.65	56.4	7.84	0.70	15.1	2029.9	9.72
8.	0.70	15.2	0.73	22.4	3.12	0.78	3.0	715.4	3.42
9.	0.92	0.3	0.95	18.9	2.63	0.96	15.7	395.8	1.89
10.	0.97	15.9	0.98	39.7	5.51	0.99	25.3	459.5	2.20

Table No. 16: Chromatogram Data of 10µl of test solution

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %
1.	0.01	17.8	0.01	23.3	2.00	0.03	0.2	223.7	0.62
2.	0.07	0.2	0.11	10.6	0.91	0.16	0.0	295.8	0.82
3.	0.20	0.2	0.24	46.8	4.03	0.28	10.3	1267.0	3.52
4.	0.30	13.9	0.35	45.7	3.93	0.40	18.1	1892.7	5.25
5.	0.40	18.2	0.48	479.2	41.19	0.52	86.0	16772.4	46.54
6.	0.52	86.9	0.53	98.4	8.46	0.56	39.1	1973.3	5.47
7.	0.57	39.4	0.59	83.4	7.17	0.61	60.3	2059.3	5.71
8.	0.61	60.5	0.64	113.3	9.74	0.69	50.5	4328.8	12.01
9.	0.70	50.9	0.73	112.6	9.66	0.78	61.6	4693.1	13.02
10.	0.78	61.5	0.80	65.5	5.63	0.87	0.0	1700.7	4.72
11.	0.96	0.8	0.98	84.4	7.25	0.99	49.9	835.7	2.32

Pharmacological Evaluation

Toxicity Studies - Acute Oral Toxicity

Table No. 17: Behavioral Changes in Acute Oral Toxicity in Albino rats

S.No.	Symptoms	Control (Normal Saline 5ml/kg, *p.o.*)	Ethanolic extract of *Withania coagulans* (2000mg/kg, *p.o.*)
1	Death	--	--
Autonomous Nervous System			
2	Head movements	--	--
3	Scratching	--	--
4	Altered reactivity to touch	--	++
5	Loss of righting reflex	--	--
6	Loss of corneal reflex	--	--
7	Defecation/Diarrhea	--	--
8	Salivation	--	++
9	Lacrimation	--	--
10	Myosis/ Mydriasis	--	--
11	Loss of traction	--	--
Central Nervous System			
12	Convulsions	--	--
13	Tremor	--	--
14	Straub tail	--	--
15	Sedation	--	--
16	Excitation	--	--
17	Jumping	--	++
18	Abnormal gait	--	--
19	Motor in-coordination	--	--
23	Akinesia	--	--

S.No.	Symptoms	Control (Normal Saline 5ml/kg, *p.o.*)	Ethanolic extract of *Withania coagulans* (2000mg/kg, *p.o.*)
24	Catalepsy	--	--
25	Loss of balance	--	--
26	Fore-paw treading	--	--
27	Writhing	--	--
28	Stereotypy	--	--
29	Altered fear	--	--
30	Altered respiration	--	--
31	Aggression	--	--
32	Analgesia	--	--
33	Body Temperature	--	--

Table No. 18: Effect of Test compound on Body Weight in Acute oral toxicity in Albino rats

S. No	Group	Body weight (gm)	
		Initial	At the end of the study
1.	**Control** (Normal Saline 5ml/kg)	245.66 ± 6.09	249.50 ± 4.43
2.	**Test drug** (Ethanolic extract of *Withania coagulans* 2000mg/mL)	241.66 ± 5.57	245.83 ± 7.70

n = 6 Values are expressed as ± S.E.M.

Table No. 19: Effect of Test compound on Biochemical parameters in Acute oral toxicity in Albino rats

S. No	Biochemical Parameters	Control (Normal Saline 5ml/kg)	Test Drug (Ethanolic extract of *Withania coagulans* 2000mg/ml)
1	Glucose (mg/dl)	156.33 ± 1.08	137.66 ± 0.49
2	Blood Urea(mg/dl)	15.34 ± 0.06	15.16 ± 0.30
4	Total cholesterol(mg/dl)	81.00 ± 0.25	80.66 ± 0.33
5	SGOT (U/l)	112.00 ± 0.85	115.00 ± 0.25
6	SGPT(U/l)	74.83 ± 0.30	76.00 ± 1.12
7	Albumin (gm/dl)	3.32 ± 0.06	3.50 ± 0.09

n = 6 Values are expressed as ± S.E.M.

Behavioural Coordination Study – Hole Board Test

Table No. 20: Effect of Ethanolic extract of *Withania coagulans* on Head dips in Albino rats

S. No	Groups	Total time spent with the head dips		No. of head dips in the holes			
		Before Treatment	After Treatment	30 min	60 min	90 min	120 min
1	**Control** (Normal Saline 5mL/kg, b.w., *p.o.*)	67 ± 6.87	67 ± 7.58	15 ± 2.85	19 ± 0.49	13 ± 0.24	16 ± 0.92
2	**Standard** (Diazepam 1mg/kg, b.w., *i.p.*)	62 ± 6.17	179 ± 27.57	34 ± 1.84	34 ± 0.28	35 ± 0.92	39 ± 0.37
3	**Test** Ethanolic Extract of *Withania coagulans* (200mg/kg, *p.o.*)	66 ± 6.52	155 ± 11.71***	32 ± 0.9**	29 ± 0.98**	30 ± 0.47**	34 ± 0.74**

n = 6 Values are expressed as ± S.E.M. Values are Mean ± SEM (n=6) two way ANOVA. Where, *** P<0.001, ** P<0.01 and * P<0.05

Table No. 21: Effect of Ethanolic extract of *Withania coagulans* on Anxiety Parameters in Albino rats

S. No	Groups	No. of rearings	No. of defecation units	No. of first head dips to each of the hole
1.	**Control** (Normal Saline 5mL/kg, b.w., *p.o.*)	30 ± 0.96	7±0.28	9±0.92
2.	**Standard** (Diazepam 1mg/kg, b.w., *i.p.*)	25±0.85**	5±0.49***	14±0.24***
3.	**Test** Ethanolic Extract of *Withania coagulans* (200mg/kg, *p.o.*)	22±0.39**	4±0.98**	16±0.47**

n = 6 Values are expressed as ± S.E.M. Values are Mean ± SEM (n=6) two way ANOVA. Where, *** P<0.001, ** P<0.01 and * P<0.05

Antiobesity Activity - Progesterone Induced Obesity

Table No. 22: Effect of Ethanolic extract of *Withania coagulans* on Body Weight in Progesterone induced obesity in Albino rats

S. No.	Group	1st day	7th day	14th day	21st day	28th day
1.	**Normal** (Normal saline 5ml/kg *p.o.*)	225.66 ± 0.33	224.50 ± 0.34	230.83 ± 0.40	244.83 ± 0.30	225.33 ± 0.21
2.	**Positive control** (Progesterone 10mg/kg, *i.p.*)	227.83 ± 0.30	350.50 ± 0.34	375.16 ± 0.30	435.33 ± 0.21	495.00 ± 0.25
3.	**Test** (Ethanolic Extract of *Withania coagulans* 200mg/kg, *p.o.*)	228.33 ± 0.33	270.66 ± 0.33***	285.16 ± 0.30***	298.66 ± 0.49***	310.50 ± 0.34***
4.	**Standard** (Orlistat 10mg/kg, *p.o.*)	235.66 ± 0.33***	286.00 ± 0.63***	297.50 ± 0.34***	324.83 ± 1.01***	357.00 ± 0.51***

n = 6 Values are expressed as ± S.E.M. Values are Mean ± SEM (n=6) two way ANOVA. Where, *** P<0.001, ** P<0.01 and * P<0.05

Table No. 23: Histopathological Studies of Progesterone Induced Obesity in Albino Rats (T.S of Liver)

S. No	Group	Description
1.	Normal	Normal architecture
2.	Positive Control	Presence of the necrotic lesions in the liver cells and the presence fatty liver was found
3.	Standard	Normal architecture regained with the drug treatment
4.	Test	Focal necrosis and slight hepatic toxicity. Fatty deposition got reduced

Table No. 24

Effect of Ethanolic extract of *Withania coagulans* on Food consumption in Progesterone induced obesity in Albino rats

S. No.	Group	Food Consumption (g)														
		0.5 hr					1 hr					2 hr				
		1st day	7th day	14th day	21st day	28th day	1st day	7th day	14th day	21st day	28th day	1st day	7th day	14th day	21st day	28th day
1.	**Normal** (Normal saline 5ml/kg *p.o.*)	3.27 ± 0.12	3.25 ± 0.35	3.66 ± 0.33	3.54 ± 0.35	3.65 ± 0.16	3.10 ± 0.12	3.90 ± 0.16	3.87 ± 0.10	3.58 ± 0.33	3.55 ± 0.27	3.55 ± 0.21	3.45 ± 0.28	3.55 ± 0.23	3.65 ± 0.45	3.57 ± 0.75
2.	**Positive control** (Progesterone 10mg/kg, *i.p.*)	3.26 ± 0.16	7.81 ± 0.22	8.55 ± 0.35	8.68 ± 0.37	8.97 ± 0.33	3.27 ± 0.17	7.65 ± 0.33	6.65 ± 0.10	8.35 ± 0.22	8.55 ± 0.27	3.63 ± 0.23	6.66 ± 0.33	7.65 ± 0.22	8.88 ± 0.17	8.45 ± 0.16
3.	**Test** (Ethanolic Extract of *Withania coagulans* 200mg/kg, *p.o.*)	3.80 ± 0.12**	6.55 ± 0.27**	6.54 ± 0.24**	7.54 ± 0.27**	5.55 ± 0.29 **	3.97 ± 0.13 **	7.54 ± 0.15 **	6.51 ± 0.32 **	5.55 ± 0.35 **	5.31 ± 0.27 **	3.78 ± 0.18 **	5.55 ± 0.21 **	5.54 ± 0.31 **	5.64 ± 0.58**	5.32 ± 0.27**
4.	**Standard** (Orlistat 10mg/kg, *p.o.*)	3.98 ± 0.21**	5.55 ± 0.32**	4.54 ± 0.25**	4.56 ± 0.39**	4.57 ± 0.15 **	3.82 ± 0.23 **	5.55 ± 0.27 **	5.65 ± 0.26 **	5.63 ± 0.55 **	6.45 ± 0.65 **	3.77 ± 0.12 **	5.53 ± 0.36 **	5.35 ± 0.33 **	4.54 ± 0.87 **	4.65 ± 0.95 **

n = 6 Values are expressed as ± S.E.M. Values are Mean ± SEM (n=6) two way ANOVA. Where, *** P<0.001, ** P<0.01 and * P<0.05

Table No. 25

Effect of Ethanolic extract of *Withania coagulans* on Ambulatory movements, Rearing and Grooming in Progesterone Induced Obesity in Albino rats

S. No.	Group	Ambulatory movements					Rearing					Grooming				
		1st day	7th day	14th day	21st day	28th day	1st day	7th day	14th day	21st day	28th day	1st day	7th day	14th day	21st day	28th day
1.	**Normal** (Normal saline 5ml/kg *p.o.*)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	**Positive control** (Progesterone 10mg/kg, *i.p.*)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.	**Test** (Ethanolic Extract of *Withania coagulans* 200mg/kg, *p.o.*)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4.	**Standard** (Orlistat 10mg/kg, *p.o.*)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

n = 6 Values are expressed as ± S.E.M. Values are Mean ± SEM (n=6) two way ANOVA. Where, *** P<0.001, ** P<0.01 and * P<0.05

Table No. 26

Effect of Ethanolic extract of *Withania coagulans* on biochemical parameters in Progesterone induced obesity in Albino rats

S.No	Treatment	Glucose (mg/dl)	Total Cholesterol (mg/dl)	TG (mg/dl)	HDLC (mg/dl)	LDLC (mg/dl)	VLDL - C(mg/dl)	SGOT (U/l)	SGPT (U/l)
1	**Normal** (Normal saline 5ml/kg *p.o.*)	118.31 ± 3.6	105.6 ± 4.3	29.61 ± 2.00	58.24 ± 0.8	14.2 ± 0.9	79.30 ± 1.30	128.54 ± 1.60	65.15 ± 1.5
2	**Positive control** (Progesterone 10mg/kg, *i.p.*)	186.02 ± 4.3	142.6 ± 2.5	76.75 ± 1.2	74.04 ± 1.5	22.23 ± 0.18	152.9 ± 1.6	190.02 ± 0.7	90.2 ± 1.45
3	**Test** (Ethanolic Extract of *Withania coagulans* 200mg/kg, *p.o.*)	150.0 ± 4.0***	128.9 ± 2.43***	27.14 ± 0.4***	60.4 ± 1.08***	18.02 ± 1.5***	120.2 ± 2.4***	150.5 ± 4.15***	65.9 ± 1.11***
4	**Standard** (Orlistat 10mg/kg, *p.o.*)	125.8 ± 4.4***	120.6 ± 1.5**	24.05 ± 2.62**	40.2 ± 2.8**	11.11 ± 0.12***	90.6 ± 2.0**	134.7 ± 1.65**	52.04 ± 2.1**

n = 6 Values are expressed as ± S.E.M. Values are Mean ± SEM (n=6) two way ANOVA. Where, *** P<0.001, ** P<0.01 and * P<0.05

Antihyperlipidemic Activity – Fructose Induced Hyperlipidemia

Table No. 27

Effect of Ethanolic extract of *Withania coagulans* on biochemical parameters in Fructose induce hyperlipidemia in Albino rats

S. No	Treatment	Glucose (mg/dl)	Total Cholesterol (mg/dl)	TG (mg/dl)	HDLC (mg/dl)	LDLC (mg/dl)	VLDL - C(mg/dl)	SGOT (U/l)	SGPT (U/l)
1	**Normal** (Normal saline 0.5ml/kg *p.o.*)	111.21 ± 3.7	102.6 ± 4.3	30.61 ± 2.00	54.24 ± 0.8	16.2 ± 0.9	89.30 ± 1.30	126.54 ± 1.60	64.15 ± 1.5
2	**Positive control** (25% Fructose in water)	182.02 ± 4.3	152.6 ± 2.5	76.75 ± 1.2	24.04 ± 1.5	42.23 ± 0.18	152.9 ± 1.6	180.02 ± 0.7	91.2 ± 1.45
3	**Test** (Ethanolic Extract of *Withania coagulans* 200mg/kg, *p.o.*) + 25% Fructose in water	122.0 ± 4.0***	125.9 ± 2.43***	25.14 ± 0.4***	60.4 ± 1.08***	21.02 ± 1.5***	115.2 ± 2.4***	145.5 ± 4.15***	69.9 ± 1.11***
4	**Standard** (Atrovastatin 10mg/kg, *p.o.* + 25% Fructose in water)	126.8 ± 4.4***	120.6 ± 1.5**	28.05 ± 2.62**	63.2 ± 2.8**	19.11 ± 0.12***	95.6 ± 2.0**	126.7 ± 1.65**	62.04 ± 2.1**

n = 6 Values are expressed as ± S.E.M. Values are Mean ± SEM (n=6) two way ANOVA. Where, *** P<0.001, ** P<0.01 and * P<0.05

Table No. 28

Histopathological Studies of in Fructose induce hyperlipidemia in Albino rats
T.S of Liver

S. No	Group	Description
1.	Normal	Normal architecture
2.	Positive Control	Presence of the fatty changes in the liver cells and the presence of necrosis and fatty liver was found
3.	Standard	Mild necrosis was found and fatty deposition got reduced
4.	Test	Focal necrosis and slight hepatic toxicity

Analgesic Activity - Tail Immersion Test in Mice

Table No. 29: Analgesic activity of Ethanolic Extract of *Withania coagulans* against Tail Flick Test in Wistar Albino Rats

S. No	Groups	Basal reaction time (Secs.)	Reaction Time (Secs.)			
			60mins.	90mins.	120mins.	180mins.
1.	**Group 1 Control** (Administered With 1mL Of Distilled Water Orally)	3.18 ± 0.03	3.40 ± 0.05	3.83 ± 0.04	3.76 ± 0.05	3.83 ± 0.04
2.	**Group2 Standard Drug** (Pentazocin10mg/Kg In 10ml Of Normal Saline)	3.25 ± 0.03	6.60 ± 0.03***	7.81 ± 0.03***	8.53 ± 0.11***	7.87 ± 0.02***
3.	**Group 3 Test** (Ethanolic Extract Of *Withania Coagulans* 200mg/Kg, *p.o.*)	3.29 ± 0.06	7.76 ± 0.04***	7.82 ± 0.07***	8.36 ± 0.06***	7.79 ± 0.07***

n = 6 Values are expressed as ± S.E.M. Values are Mean ± SEM (n=6) two way ANOVA. Where, *** P<0.001, ** P<0.01 and * P<0.05

Anthelmintic Acitivity

Table No. 30: Anthelmintic activity of Ethanolic Extract of *Withania coagulans* against *Pheretima posthuma*

S. No	Group	Concentration (Mg/Ml)	*Pheretima posthuma*	
			Paralysis (min)	Death (min)
1.	**Control** (1% CMC in 10ml of Normal Saline)	10 ml of 5% CMC	180.40 ± 0.24	244.50 ± 0.22
2.	Standard (Albendazole + 1% CMC in 10ml of Normal Saline)	20mg/10ml	8.00 ± 0.25***	22.66 ± 0.16***
3.	**Test** (Ethanolic Extract Of *Withania Coagulans* + 1% CMC in 10ml of Normal Saline)	25 mg/10ml	15.00 ± 0.25**	19.66 ± 0.21**
		50 mg/10ml	13.00 ± 0.25**	14.83 ± 0.21**
		75 mg/10ml	11.00 ± 0.25**	12.16 ± 0.16**
		100 mg/10ml	7.33 ± 0.21***	21.16 ± 0.16***

n = 6 Values are expressed as ± S.E.M. Values are Mean ± SEM (n=6) two way ANOVA. Where, *** P<0.001, ** P<0.01 and * P<0.05

Anti- Microbial Screening

Table No. 31: Antimicrobial screening of Ethanolic Extract of *Withania coagulans*

S. No	Test Organism	Zone of Inhibition (mm)						
		Solvent	Standard	Test				
				25 mg	50mg	75mg	100mg	250mg
1.	*Bacillus subtilis*	0.08	34.66	8.66	2.66	10.00	22.00	32.66
2.	*Staphyloccus aureus*	0.06	40.50	8.66	10.50	15.66	20.00	38.33
3.	*Proteus vulgaris*	0.08	30.50	2.00	7.83	10.50	10.00	32.66
4.	*Klebsiella pneumoniae*	0.08	31.00	2.83	7.16	7.58	10.00	33.16
5.	*Candida albicans*	2.16	32.33	2.83	12.66	14.33	16.00	33.50
6.	*Aspergillus niger*	1.33	35.00	2.50	10.33	12.16	20.00	37.83

6.2. DISCUSSION

6.2.1. Extraction of plant material

The Flower buds of *Withania coagulans* Dunal was collected, dried and extracted using ethanol and prior to the ethanolic extraction the plant is milled and defatted using petroleum ether. The yield of the Ethanolic extract is 60%.

6.2.2. Preliminary Phytochemical studies

As a part of the preclinical study, the ethanolic extract of Flower buds of *Withania coagulans* Dunal was subjected to qualitative chemical test and confirmed the presence of carbohydrates, alkaloids, steroids, flavanoids, glycosides, proteins and amino acids shown in **Table No.9**

6.2.3. IR Spectral Data of the Ethanolic extract of *Withania coagulans*

The ethanolic extract of Flower buds of *Withania coagulans* Dunal was subjected to the Infra Red Spectroscopy for their possible functional group **(Table No. 10).** The ethanolic extract of Flower buds of *Withania coagulans* Dunal showed the possible stretching and bending shows the presence of the functional group.

6.2.4. HPTLC Fingerprinting

The HPTLC fingerprinting of Ethanolic extract of Flower buds of *Withania coagulans* Dunal was studied. The HPTLC fingerprinting was done for confirming the presence of alkaloids, Flavanoids and glycosides. These are done after the identification tests and indicate the presence of alkaloids, Flavanoids and glycosides. The stationary and the mobile phases are selected based on the identification test. Three different chromatograms are obtained and photo documentation was done **(Fig. Nos. 9, 13& 17).** The 3D display of the chromatogram was obtained from 10 µl and 20µl of the test solution **(Fig. Nos. 10, 14 &18)** and the Peak display at 10 µl and 20 µl of the test solution was shown (**Fig. Nos. 11, 12, 15, 16, 19 & 20.** The interpreted data of the Peak display at 10 µl and 20 µl of the test solution was shown in **Table Nos. 11-16.**

6.2.5. Pharmacological Screening

6.2.5.1. Toxicity Studies – Validation

Validation of Acute Toxicity

In acute oral toxicity studies, ethanolic extract of Flower buds of *Withania coagulans* Dunal did not produce mortality at a dose of 2000mg/kg body weight in rats and hence $1/10^{th}$ of LD_{50} (i.e.) 200mg/kg was considered as the dose level for further pharmacological screening. The parameters observed were behavioral changes, biochemical parameters, body weight, histopathological studies and mortality.

Behavioural Changes

Behavioral changes observed were death, abnormal gait, aggression, akinesia, altered fear, altered muscle tone, altered respiration, analgesia, body temperature, catalepsy, convulsions, excitation, fore paw treading, jumping, loss of balance, motor in-coordination, sedation, stereotypy, straub tail, tremor, writhing, altered reactivity to touch, defecation/diarrhoea, head movements, lacrimation, loss of corneal reflux, loss of righting reflux, loss of traction, miosis/mydriasis, salivation and scratching. Among the behavioral parameters observed the animal showed positive response for altered fear, catalepsy, excitation and stereotypy **(Table No. 17)**.

Biochemical Parameters and Body Weight

The biochemical parameters observed in animals treated with 2000 mg/kg of ethanolic extract of Flower buds of *Withania coagulans* Dunal were albumin, blood urea, glucose, SGOT, SGPT, total cholesterol. All the biochemical parameters were in normal range. The increase in level of SGOT, SGPT, blood urea and decrease in level of Uric acid were observed **(Table No. 19) (Fig. No. 22).** The body weights of the groups are studied on the first day of the administration of the drugs and at the end of the 14 days of study. There is no change in the body weight of the rats as shown in **Table No. 18 & Fig. No. 21.**

Histopathological Studies

Fig. Nos. 23-30 represents the transverse sections of Heart, Kidney, Liver and Pancreas showing normal histology upon administration of 2000mg/kg of Test Compound and the histopathology of the test group was compared with the control group. It shows the normal architecture and there is no change in the cells indicates the absence of toxicity

6.2.5.2. Behavioural Activity

Hole Board Test

The various evaluation tests for Hole board test like latency to the first head dips, no. of head dips in the holes, total time spent with the head dips, no. of rearings and no. of defecation units as shown in **Table Nos. 20 & 21 & Fig. Nos. 31-33.** The increase in the number of head dips and the total time spent with the head dips and the decrease in the no. of rearings, defecation units indicates the anxiolytic property of the test drug when compared with the standard drug.

6.2.5.3. Antiobesity Activity

Progesterone Induced Obesity

The body weight of every rat (g) were recorded for every week for 28 days (**Table No. 22 & Fig. No. 34)** and the food consumption behavior was studied on days 1, 7, 14, 21, and 28. The food intake was recorded at 0.5, 1, and 2 h time intervals **(Table No. 24 & Fig. No. 40).**

Rearing, grooming and ambulatory moments were studied **(Table No. 25)**.

Serum glucose levels, total cholesterol, LDL- C, HDL- C, VLDL- C, Triglycerides, SGOT, and SGPT were estimated (**Table No. 26 & Fig. No. 39).**

Table Nos. 23 & Fig. Nos. 35-38 represents the transverse sections Liver obtained from various groups such as Normal, Control, Standard and Test groups.

6.2.5.4. Antihyperlipidemic Activity

Fructose Induced Hyperlipidemia

Daily administration of 25 % of fructose in drinking water for 21 days significantly increased ($P < 0.01$) hyperlipidemia in rats by increasing the serum TC, TG, LDL- C, glucose levels and serum HDL-C levels was significantly decreased ($P < 0.01$) in fructose control group when compared to the normal control group. The effect of Ethanolic extract of *Withania coagulans* on serum lipid profile levels was showed in Table 1. Treatment with Ethanolic extract of *Withania coagulans* significantly reduced ($P < 0.05$, $P < 0.01$ respectively) the serum TC, TG, LDL-C levels, glucose and serum HDL-C levels was significantly increased ($P < 0.01$) when compared to the fructose control group. The change in lipid levels in test groups combinations were comparable with group of individual test drug treated rats. At 21st day, two hours after the final treatment, animals were anaesthetized and blood from each animal was withdrawn from retro-orbital plexus.The serum were assayed for total cholesterol, triglycerides, high density lipoprotein

(HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and serum blood glucose **(Table No. 27 & Fig. No. 41).**

Table No. 28 & Fig. Nos. 42-45 represents the transverse sections of liver obtained from various groups such as Normal, Control, Standard and Test groups and the histology of liver was studied

6.2.5.5. Analgesic Activity

The analgesic activities of the ethanolic extract of *Withania coagulans* was evaluated by employing the tail flick method. The result of analgesic activity of Ethanolic extract of *Withania coagulans* in **Table No. 29** respectively. The results are also graphically illustrated in **Fig. No. 46**

6.4.5.6. Anthelmintic Activity

The data reveals that of Ethanolic extract of *Withania coagulans* showed paralysis and death at different concentrations (25, 50, 75 & 100mg/ml) **(Table No. 30 & Fig. Nos. 47-53)**. The effects are comparable with that of the effects produced by the standard drug albendazole (10mg/ml). The activity reveals concentration dependent nature of different extracts. The activity of the extracts was found to be inversely proportional to the time taken for paralysis/death of the worms and due to the presence of alkaloids, flavanoids and glycosides present in the extracts.

6.5.5.7. Antimicrobial Activity

The antimicrobial activity of the Ethanolic extract of of *Withania coagulans* Dunal have been tested against *Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris, Klebseilla species, Candida albicans* and *Asperigellus niger* and compared with the standard drugs such as Ciprofloxacin (5µg/disc) for Bacteria and Nystatin (100 units/disc) for Fungi **(Table No. 31).**

Selective Toxicity is the property of an ideal antimicrobial agent. It means that the drug is harmful to a parasite but not to the host. Usually the effects is relative rather absolute, i.e. a drug in a concentration tolerated by the host may damage an infecting microorganism. The different mechanisms are Inhibition of cell wall synthesis, Inhibition/ Alteration of cell wall membrane, Inhibition of Protein synthesis, Inhibition of nucleic acid

synthesis. Alteration of cell membrane function, Polymyxin, Amphotericin B, Imidazole are examples.

These drugs act by disruption of the functional integrity of the cytoplasmic membrane of bacteria and fungi has a structure different from that of animal cells, and can be more readily disrupted by certain chemotherapeutic agents. Ethanolic extract of *Withania coagulans* Dunal exhibited appreciable activity against the *Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris, Klebseilla species, Candida albicans* and *Asperigellus niger* The Ethanolic extract of of *Withania coagulans* Dunal was exhibiting competent biological activity both gram negative & gram positive bacterial species and also fungus (**Fig. Nos. 54-65**). The results are also graphically illustrated in **Fig. No. 66**

Analytical Determination
IR - Spectroscopy

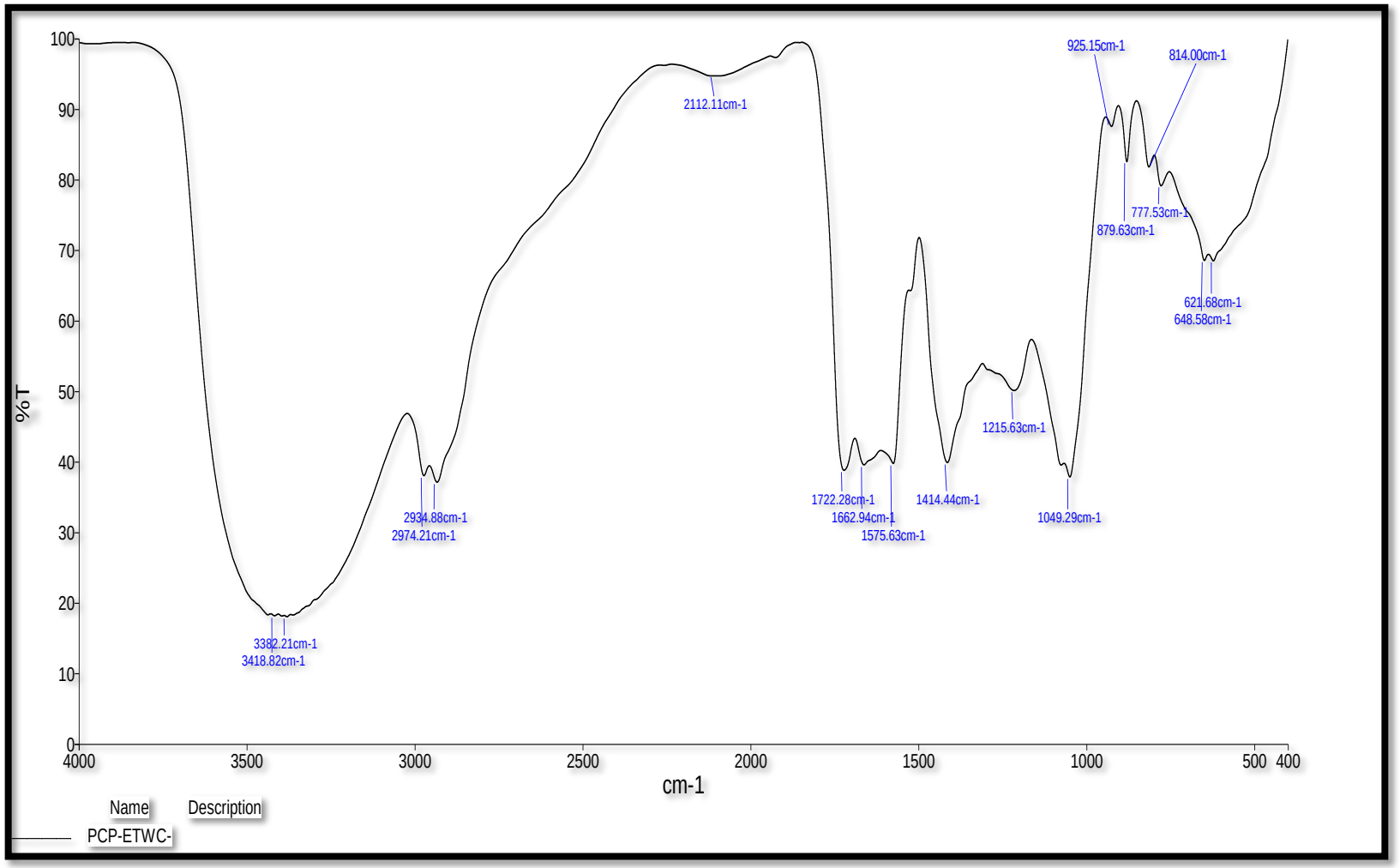
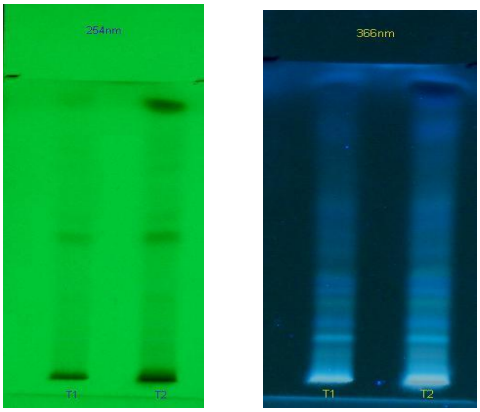


Fig. No. 8: IR – Spectrum of the Ethanolic extract of *Withania coagulans*

HPTLC fingerprinting profile EEWC - Alkaloids

Fig. No. 9: Photo Documentation Under UV

AT 254nm **AT 366nm**



T1 T2 **T1 T2**

Fig. No. 10: 3D Display @ 254nm

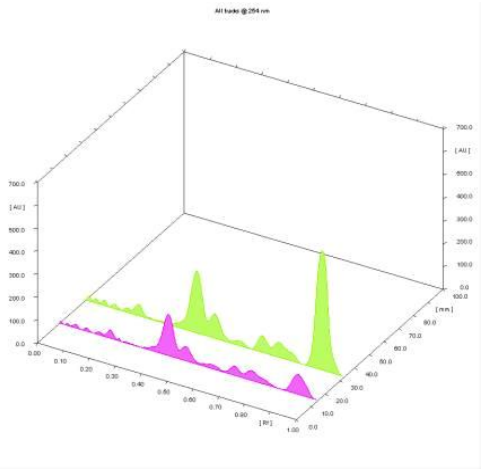


Fig. No. 11: PEAK DISPLAY (10µl of test solution)

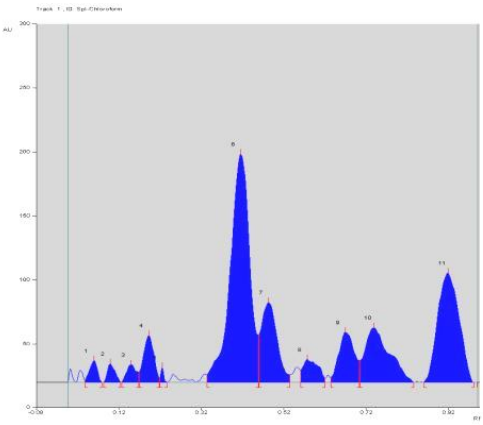
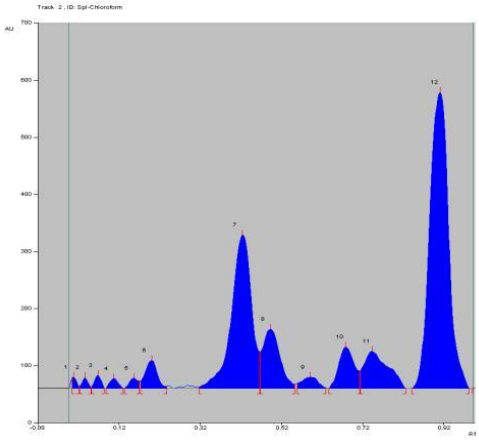


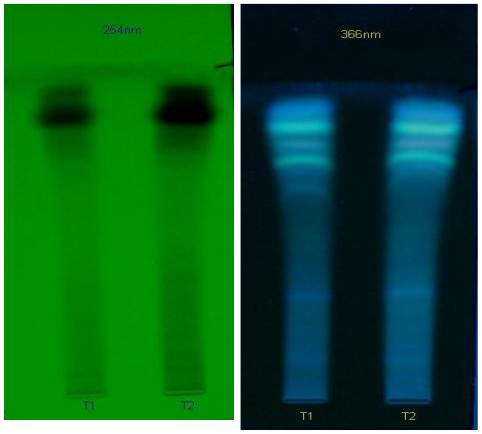
Fig. No. 12: PEAK DISPLAY (20µl of test solution)



HPTLC fingerprinting profile of *EEWC* - Glycosides

Fig. No. 13: Photo Documentation Under UV

AT 254nm **AT 366nm**



T1 T2 **T1 T2**

Fig. No. 14: 3D Display @ 254nm

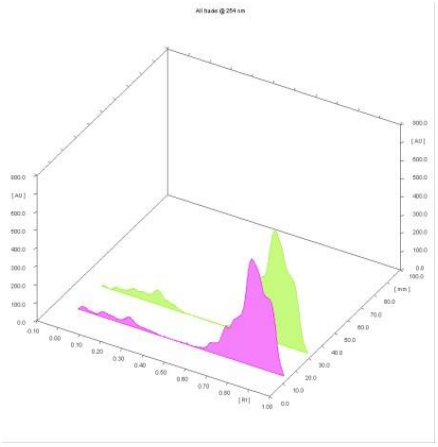


Fig. No. 15: PEAK DISPLAY (05µl of test solution)

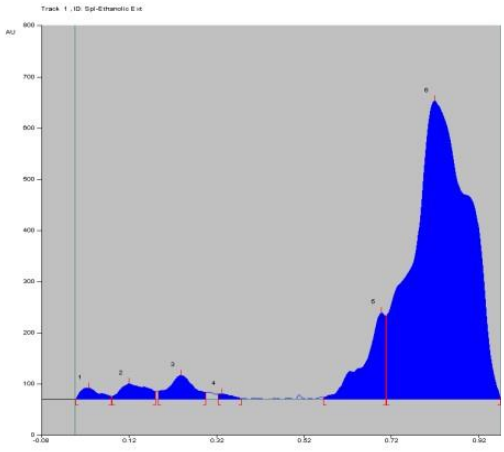
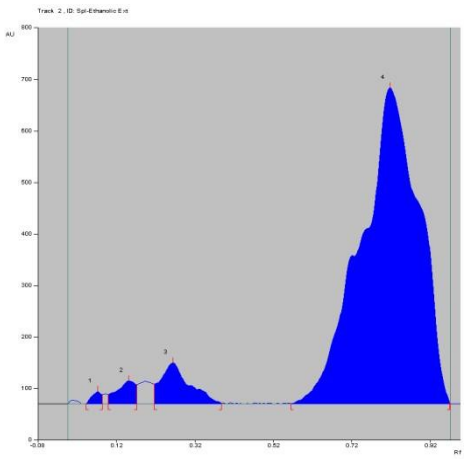


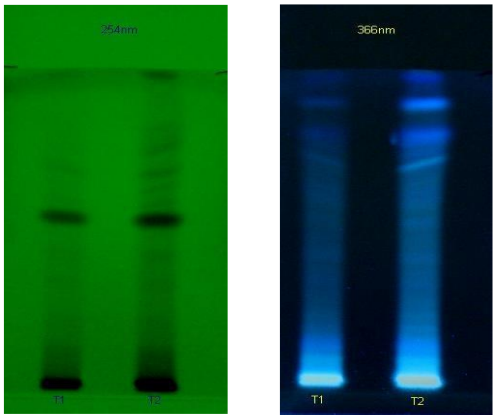
Fig. No. 16: PEAK DISPLAY (10µl of test solution)



HPTLC Fingerprinting Profile of EEWC – Flavanoids

Fig. No. 17: Photo Documentation Under UV

AT 254nm **AT 366nm**



T1 T2 **T1 T2**

Fig. No. 18: 3D Display @ 254nm

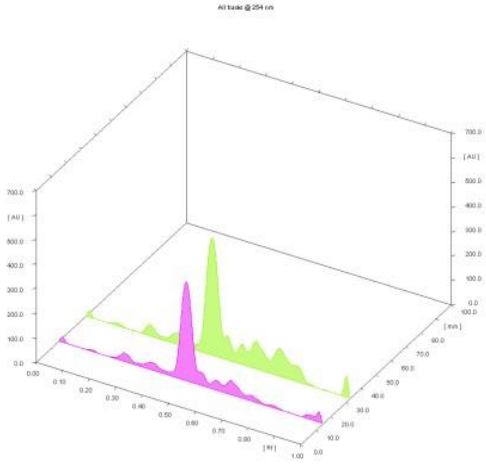


Fig. No. 19: PEAK DISPLAY (05µl of test solution)

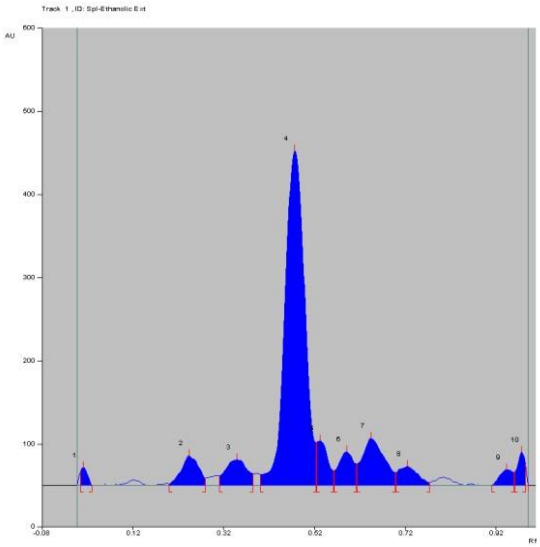


Fig. No. 20: PEAK DISPLAY (10µl of test solution)

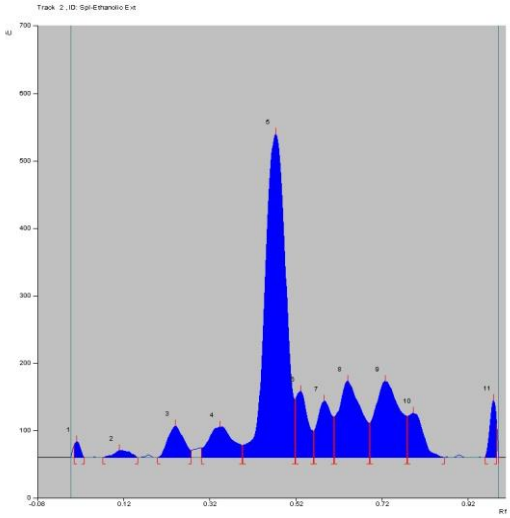


Fig. No. 21: Effect of Test compound on Body Weight in Acute oral toxicity in Albino rats

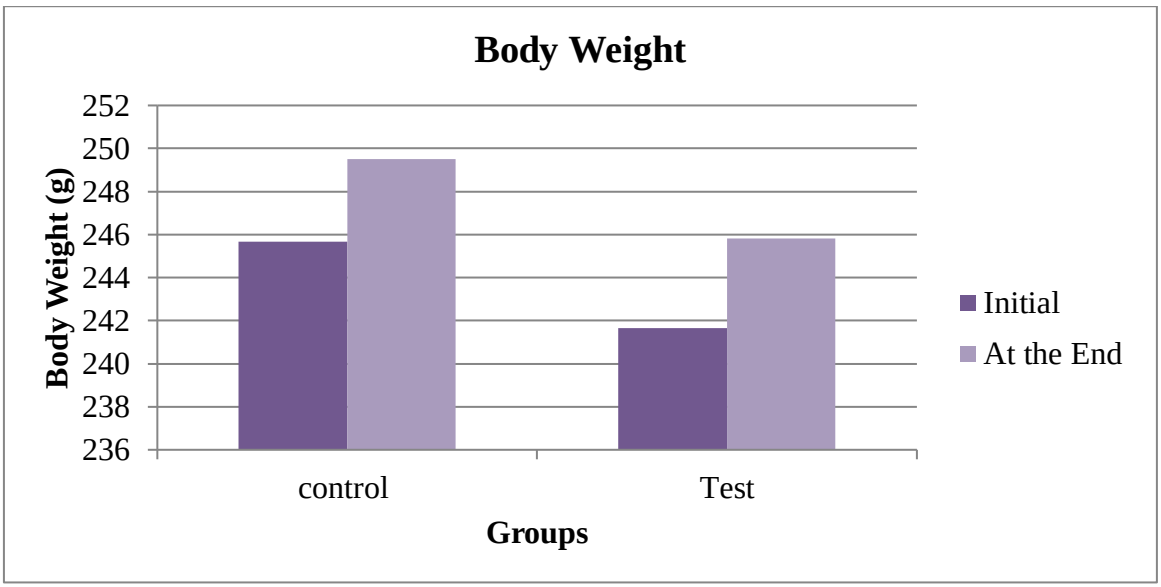
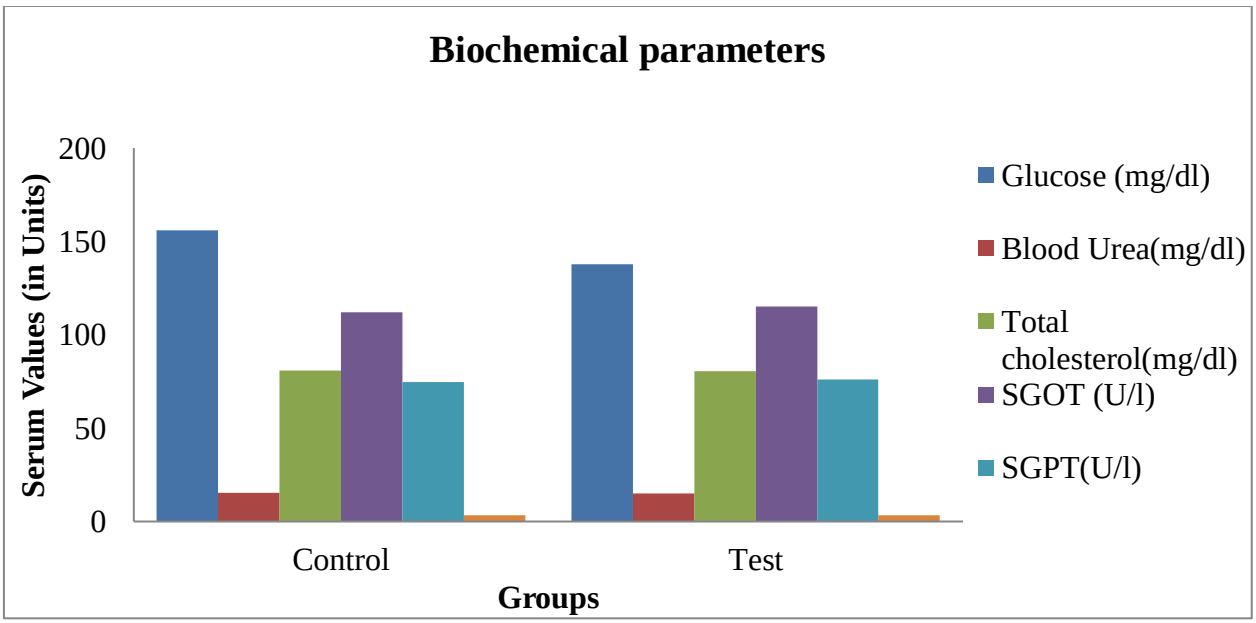


Fig. No. 22: Effect of Test compound on Biochemical parameters in Acute oral toxicity in Albino rats



Histopathological Studies of Acute Oral Toxicity

Control

Fig. No. 23: T.S of Kidney

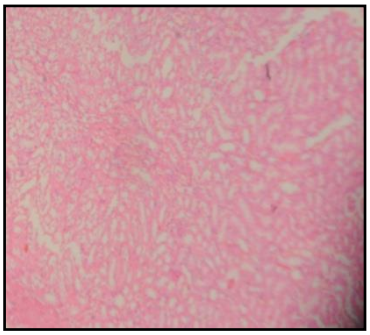


Fig. No. 24: T.S of Heart

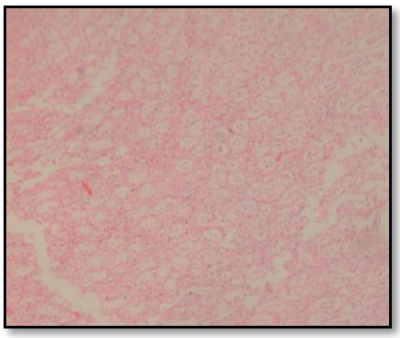


Fig. No. 25: T.S of Liver

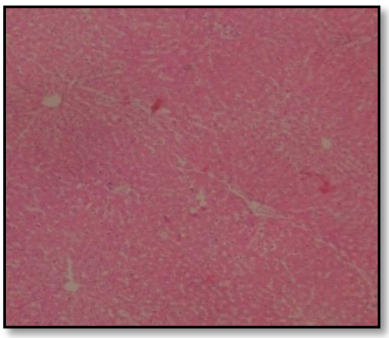
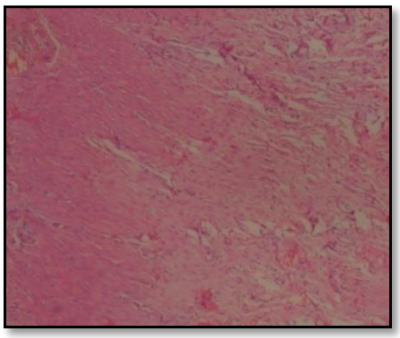


Fig. No. 26: T.S of Pancreas



Control - Normal saline 5 ml/kg, *p.o*

Test

Fig. No. 27: T.S of Kidney

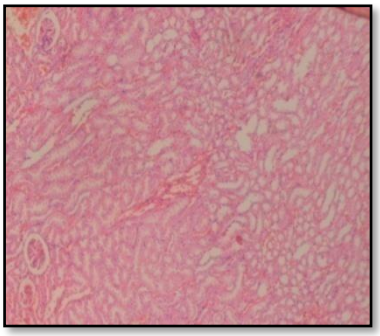


Fig. No. 28: T.S of Heart

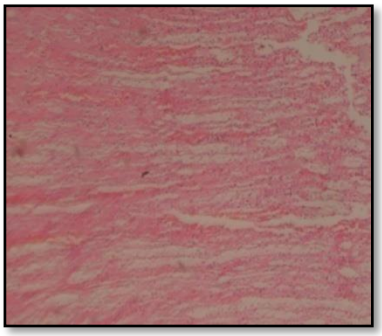


Fig. No. 29: T.S of Liver

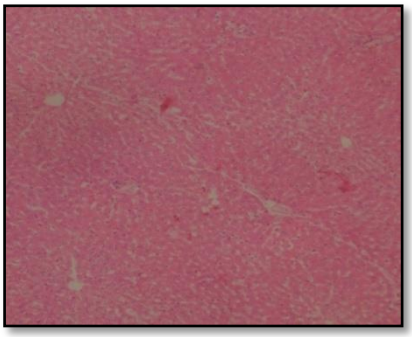
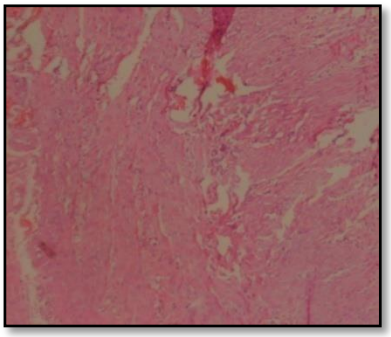


Fig. No. 30: T.S of Pancreas



Test drug - Ethanolic extract of *Withania coagulans* (2000 mg/kg, *p.o.*)

Fig. No. 30: Effect of Ethanolic extract of *Withania coagulans* on Time spent with the head dips

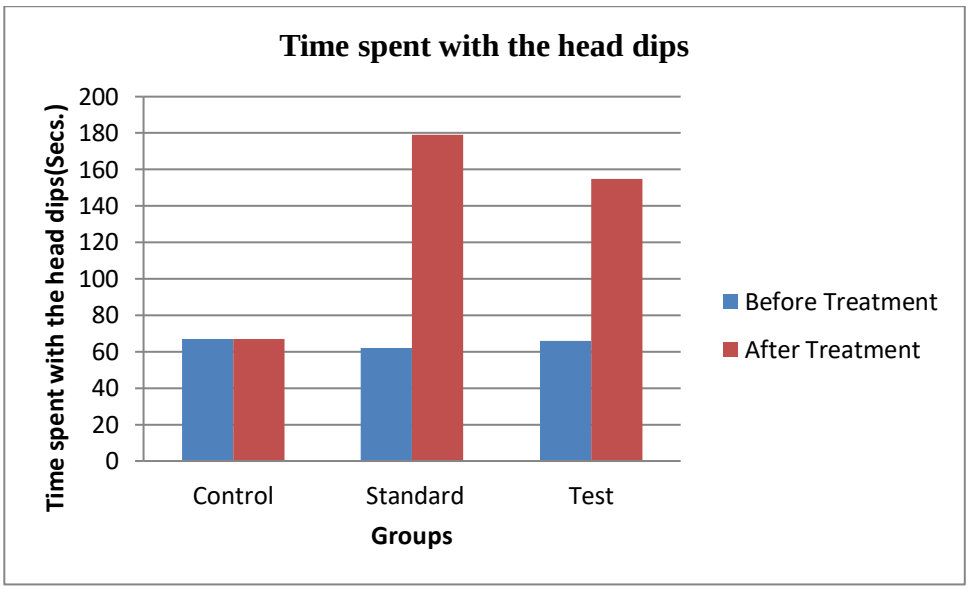


Fig. No. 32: Effect of Ethanolic extract of *Withania coagulans* on Head dips into the holes

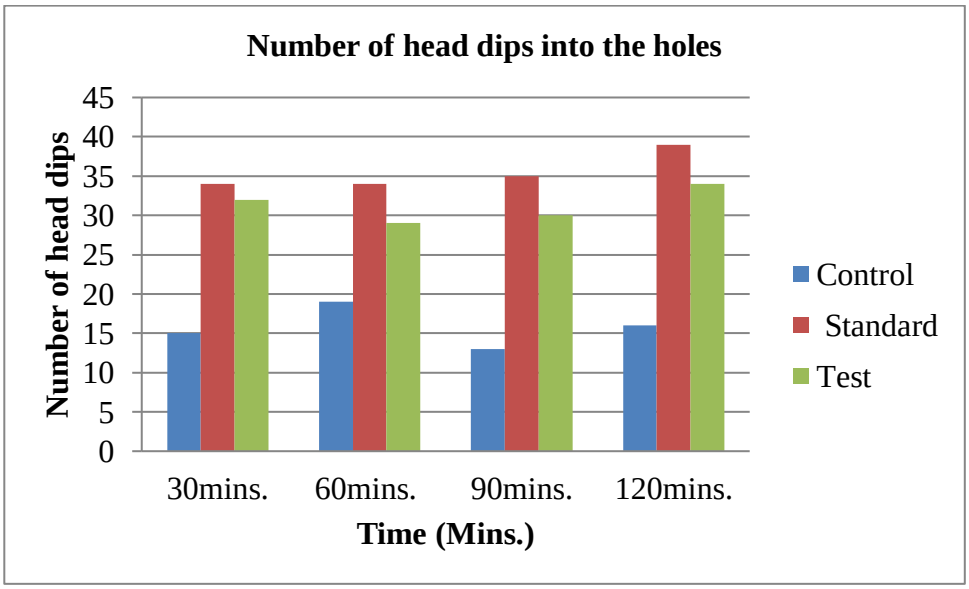


Fig. No. 33: Effect of Ethanolic extract of *Withania coagulans* on Anxiety Parameters in Albino rats

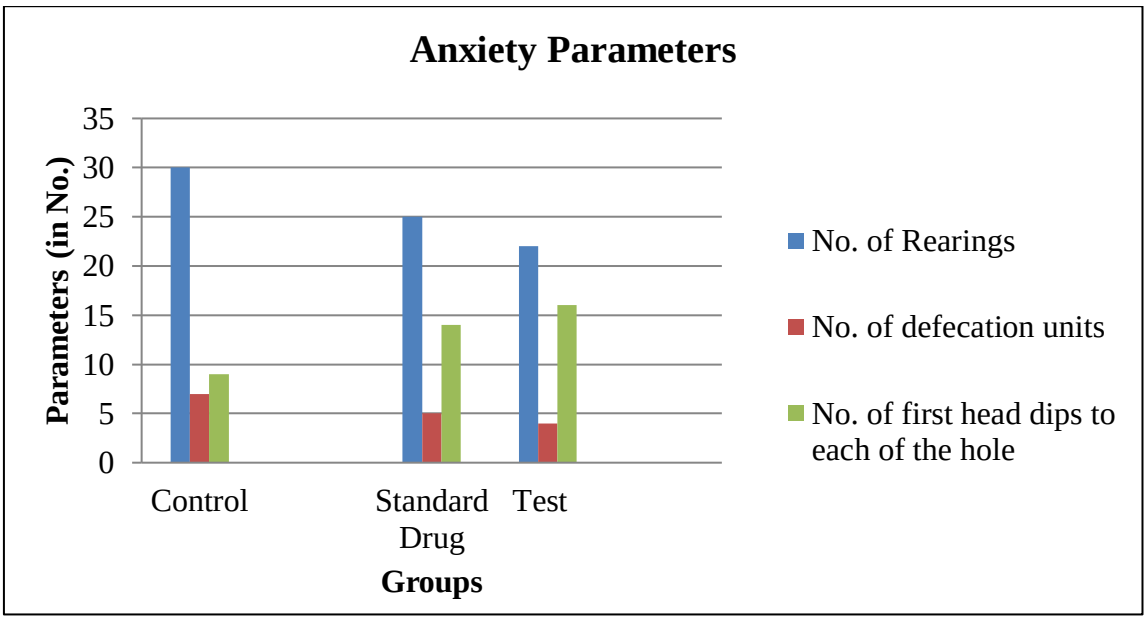
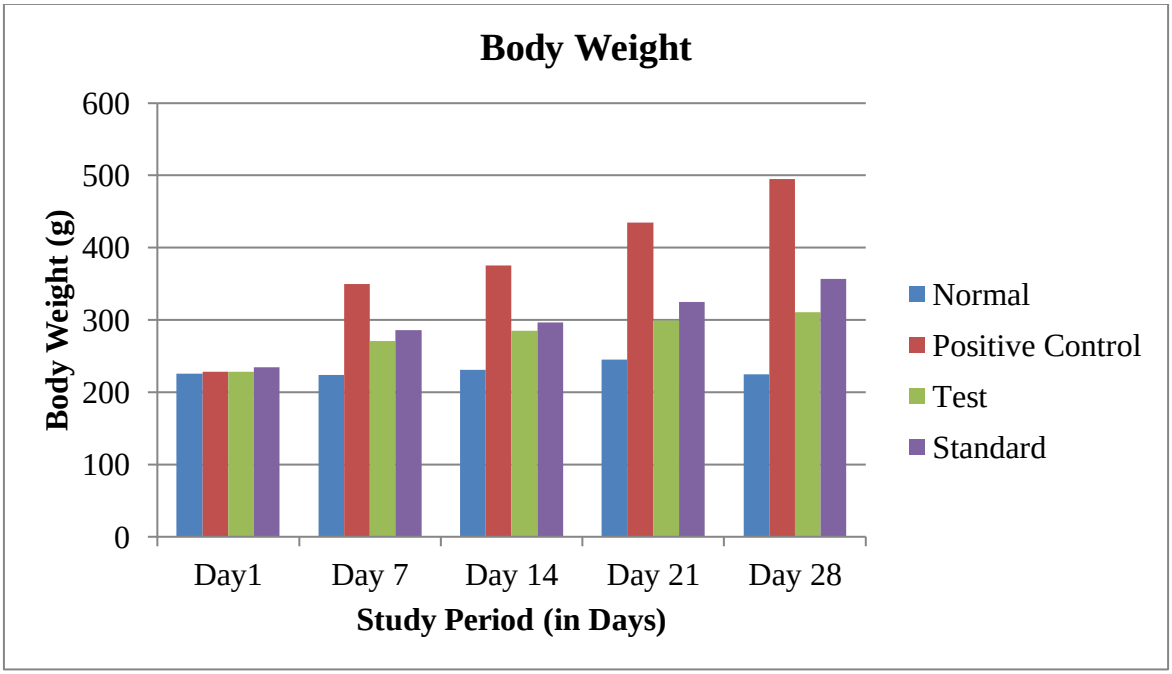


Fig. No. 34: Effect of Ethanolic extract of *Withania coagulans* on Body Weight in Progesterone induced obesity in Albino rats



Histopathological Studies of Progesterone Induced Obesity in Albino Rats

T.S of Liver

Fig. No. 35: Normal

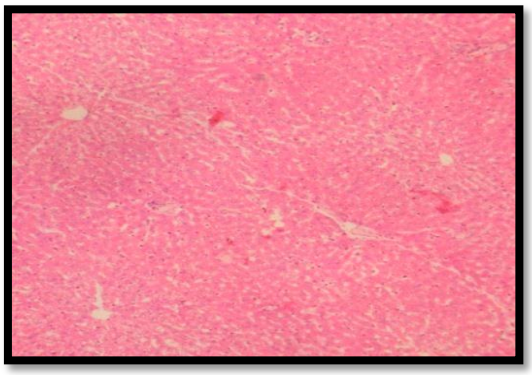


Fig. No. 36: Obesity control

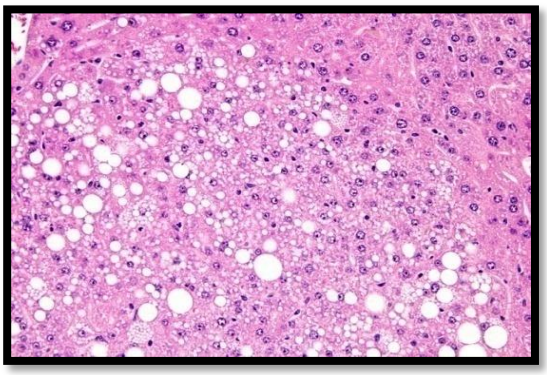


Fig. No. 37: Standard

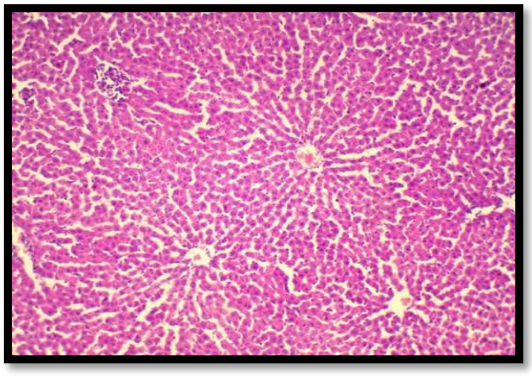
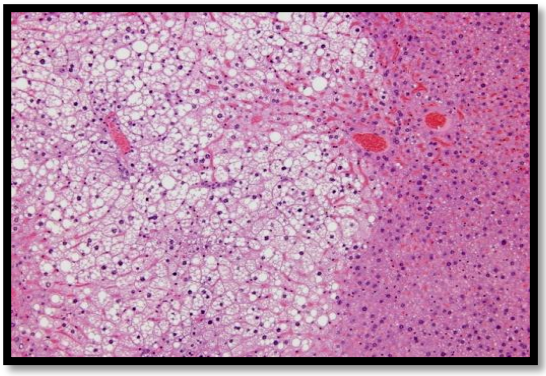


Fig. No. 38: Test



Normal - Normal Saline (5ml/kg *p.o.*)

Obesity Control - Progesterone (10mg/kg, *s.c.*)

Standard – Orlistat (10mg/kg, *p.o.*) + Progesterone (10mg/kg b.w., *s.c.*)

Test - Ethanolic Extract of *Withania coagulans* (200mg/kg, *p.o.*) + Progesterone (10mg/kg, b.w., *s.c.*)

Fig. No. 39: Effect of Ethanolic extract of *Withania coagulans* on biochemical parameters in Progesterone induced obesity in Albino rats

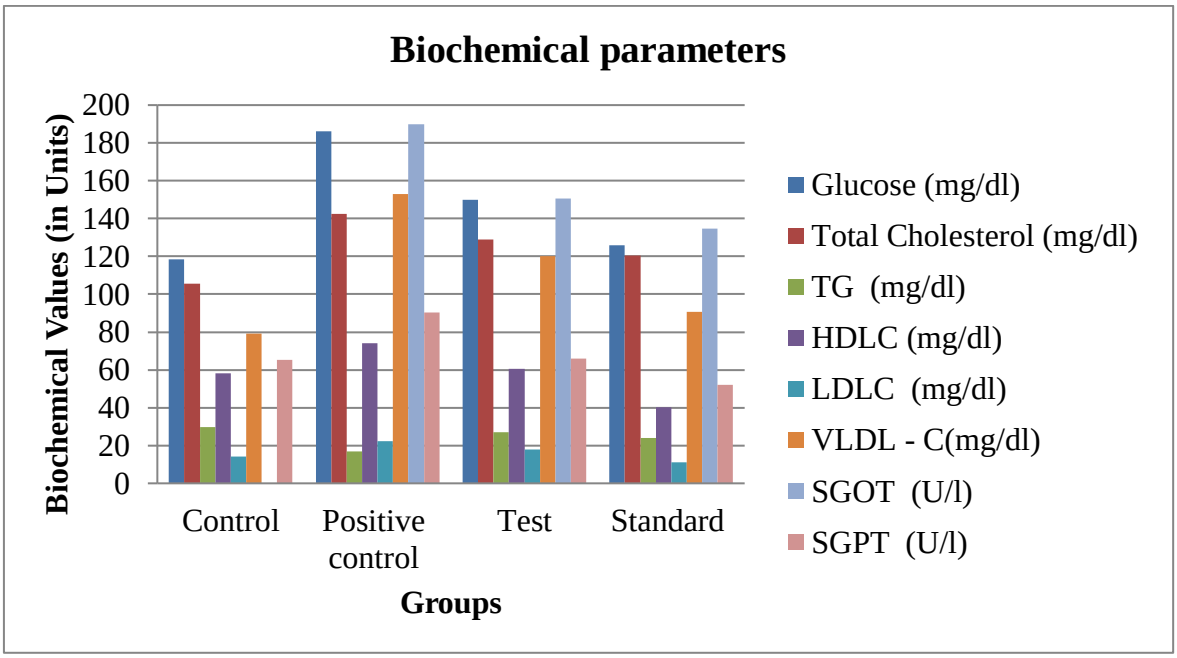


Fig. No. 40: Effect of Ethanolic extract of *Withania coagulans* on Food consumption in Progesterone induced obesity in Albino rats

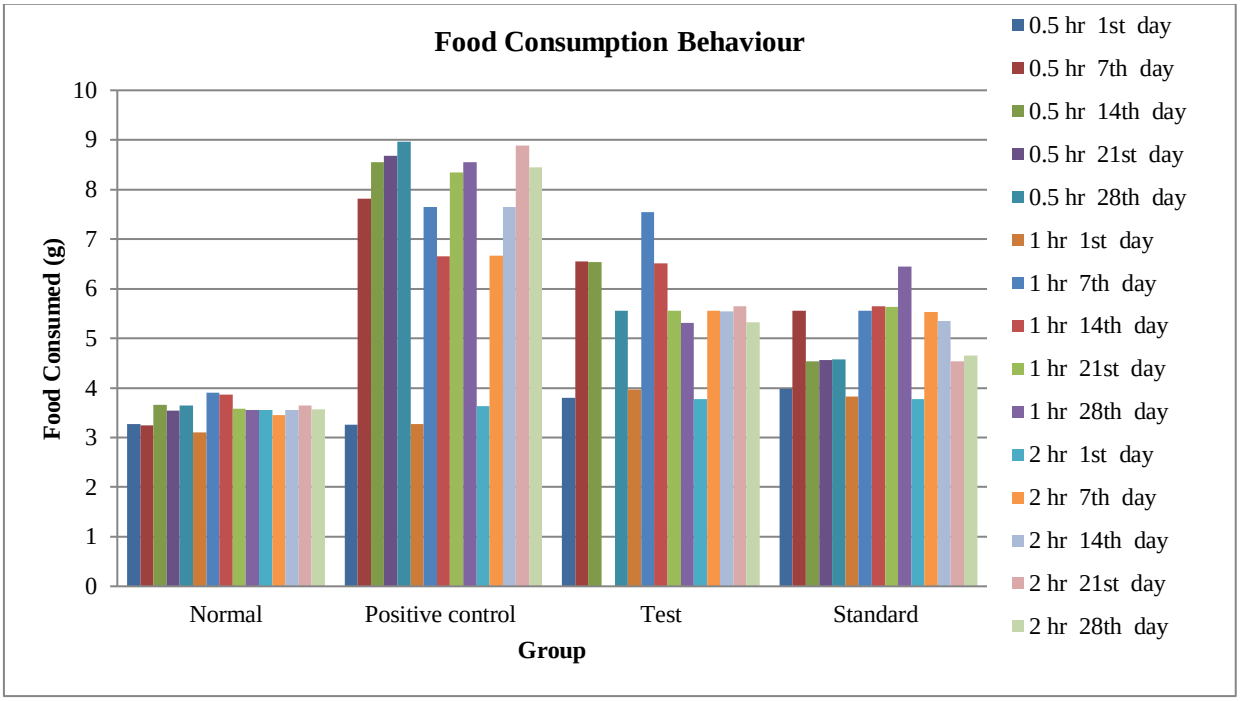
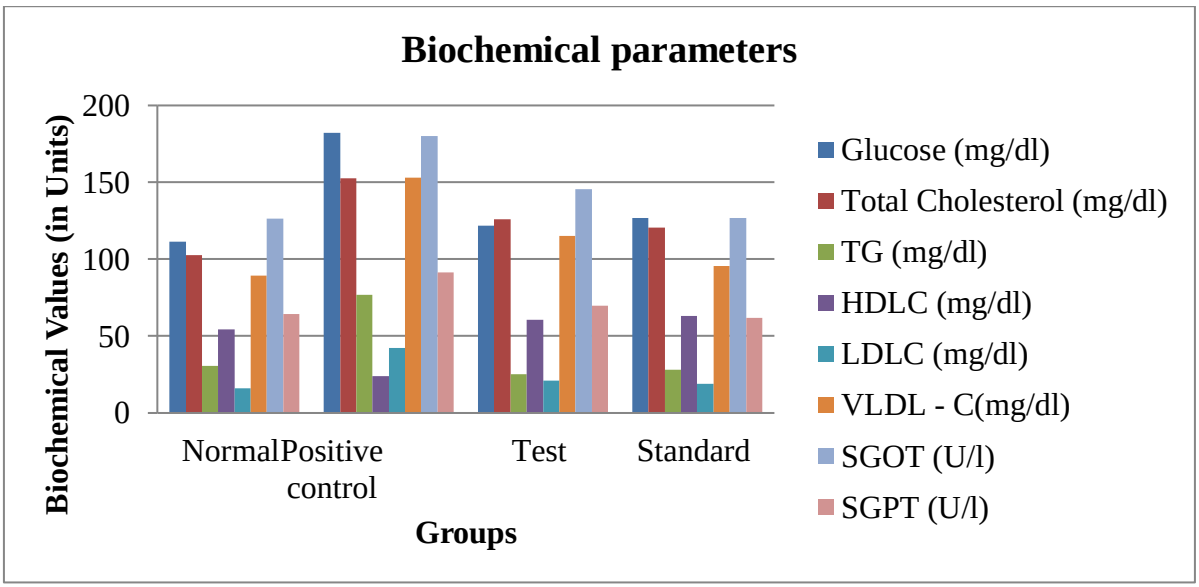


Fig. No. 41: Effect of Ethanolic extract of *Withania coagulans* on biochemical parameters in Fructose Induced Hyperlipidemia in Albino rats



Histopathological Studies of in Fructose induce hyperlipidemia in Albino rats
T.S of Liver

Fig.No. 42 : Normal

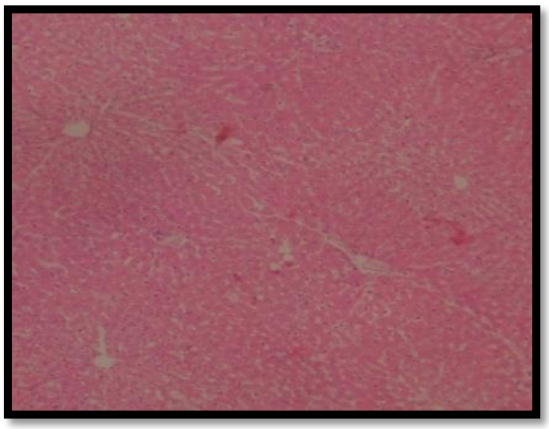


Fig.No. 43: Lipidemic Control

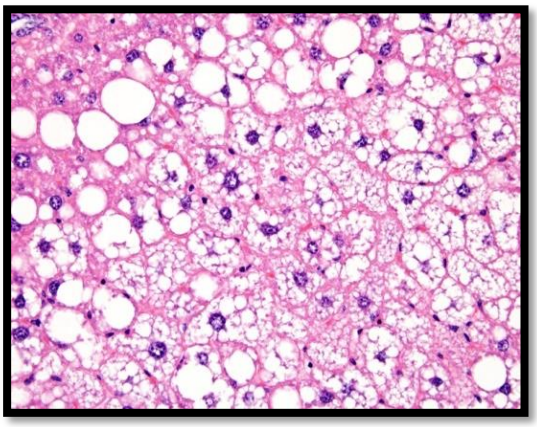


Fig.No. 44: Standard

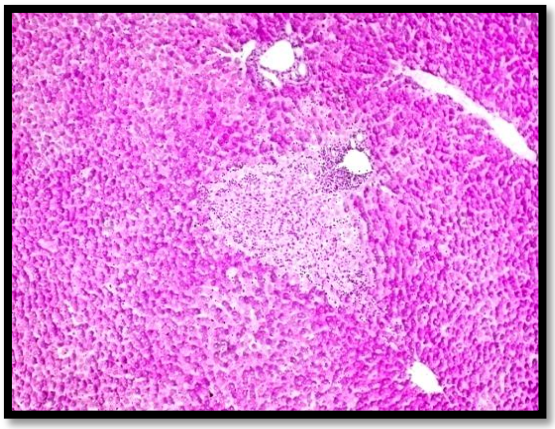
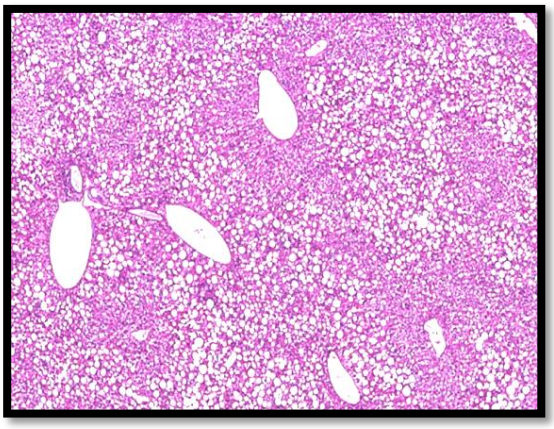


Fig.No. 45: Test



Normal - Normal Saline (5ml/kg *p.o.*)
Lipidemic Control - 25% Fructose in water
Standard – Orlistat (10mg/kg, *p.o.*) + 25% Fructose in water
Test - Ethanolic Extract of *Withania coagulans* (200mg/kg, *p.o.*) + 25% Fructose in water

Fig. No. 46: Analgesic activity of Ethanolic Extract of *Withania coagulans* against Tail Flick Test in Wistar Albino Rats

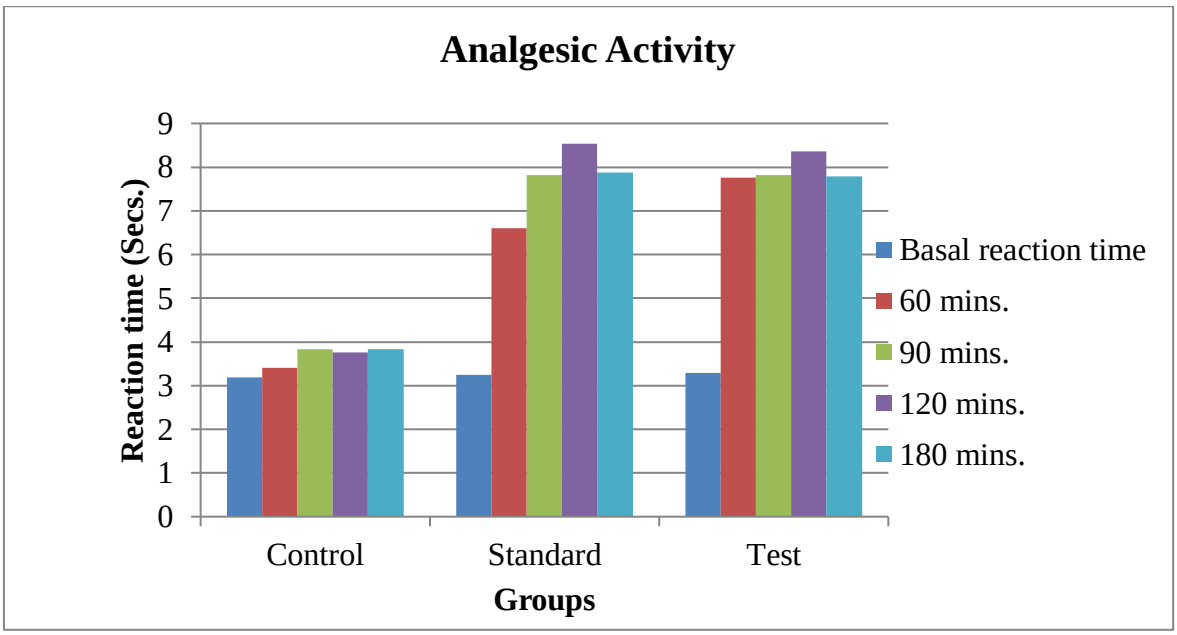
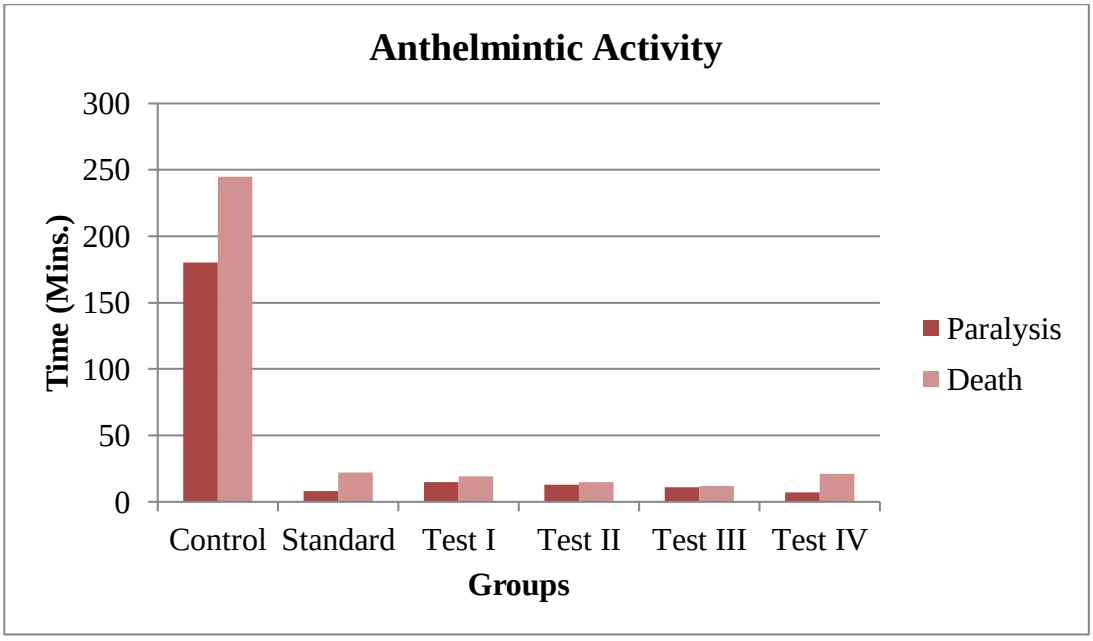


Fig. No. 47: Anthelmintic activity of Ethanolic Extract of *Withania coagulans* against *Pheretima posthuma*



Anthelmintic activity of Ethanolic Extract of *Withania coagulans* against *Pheretima posthuma*

Fig. No. 48: Control

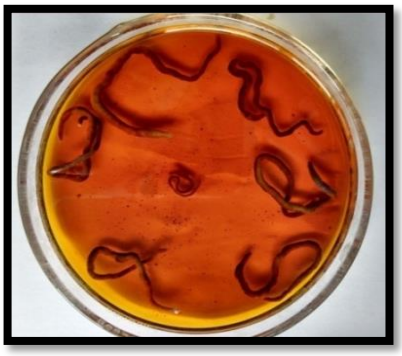
Fig. No. 49: Standard

Fig. No. 50: Test I

Fig. No. 51: Test II

Fig. No. 52: Test III

Fig. No. 53: Test IV

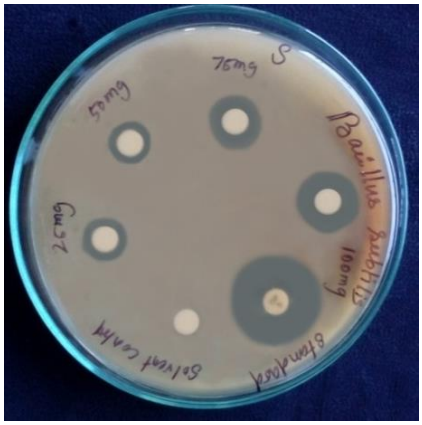


Control - 1% CMC in 10ml of Normal Saline
Standard - Albendazole
Test I - 25 mg/ml of Ethanolic Extract of *Withania Coagulans*
Test II - 50 mg/ml of Ethanolic Extract of *Withania Coagulans*
Test III - 75 mg/ml of Ethanolic Extract of *Withania Coagulans*
Test IV - 100 mg/ml of Ethanolic Extract of *Withania Coagulans*

Fig. No. 54: Zone of inhibition of Test drug 25, 50, 75 & 100mg against *Staphylococcus aureus*

Fig. No. 55: Zone of inhibition of Test drug 250mg against *Staphylococcus aureus*

Fig. No. 56: Zone of inhibition of Test drug 25, 50, 75 & 100mg against *Bacillus substilis*

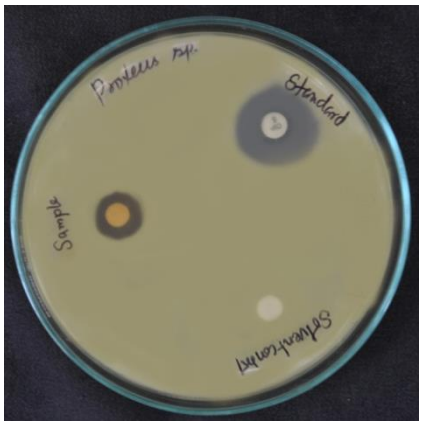


Test drug: Ethanolic Extract of *Withania coagulans* (25, 50, 75, 100 & 250mg/ disc)
Standard: Ciprofloxacin (5µg/ disc) – Bacteria & Nystatin (100 units/ disc) – Fungi
Solvent: DMSO

Fig. No. 57: Zone of inhibition of Test drug 250mg against *Bacillus substilis*

Fig. No. 58: Zone of inhibition of Test drug 25, 50, 75 & 100mg against *Proteus vulgaris*

Fig. No. 59: Zone of inhibition of Test drug 250mg against *Proteus vulgaris*



Test drug: Ethanolic Extract of *Withania coagulans* (25, 50, 75, 100 & 250mg/ disc)
Standard: Ciprofloxacin (5µg/ disc) – Bacteria & Nystatin (100 units/ disc) – Fungi
Solvent: DMSO

Fig. No. 60: Zone of inhibition of Test drug 25, 50, 75 & 100mg against *Klebsiella aerogenes*

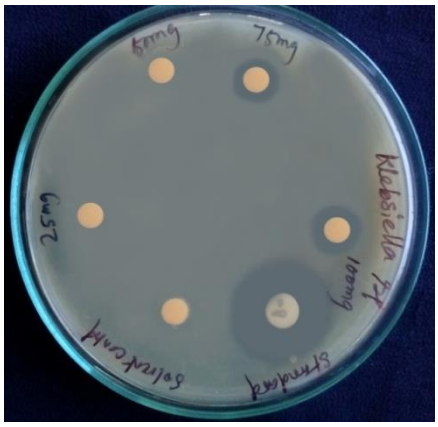


Fig. No. 61: Zone of inhibition of Test drug 250mg against *Klebsiella aerogenes*

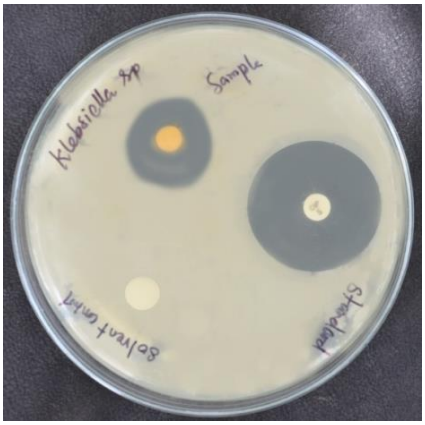
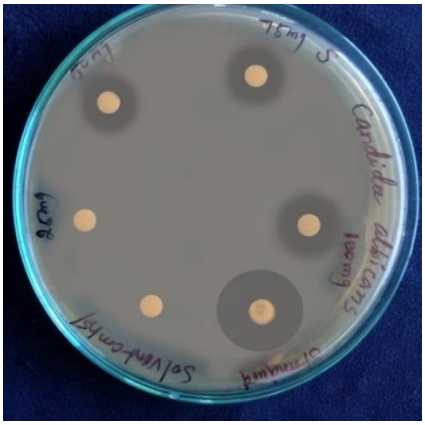


Fig. No. 62: Zone of inhibition of Test drug 25, 50, 75 & 100mg against *Candida albicans*



Test drug: Ethanolic Extract of *Withania coagulans* (25, 50, 75, 100 & 250mg/ disc)
Standard: Ciprofloxacin (5µg/ disc) – Bacteria & Nystatin (100 units/ disc) – Fungi
Solvent: DMSO

Fig. No. 63: Zone of inhibition of Test drug 250mg against *Candida albicans*

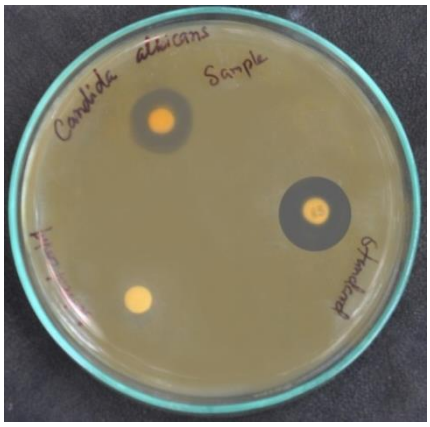
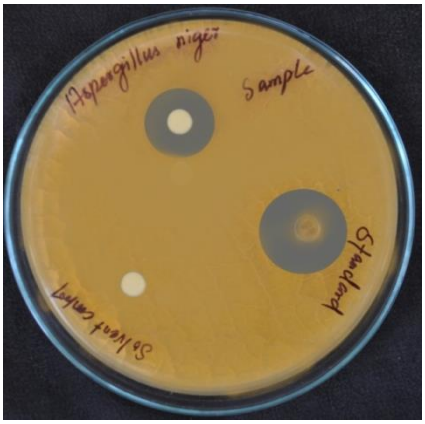


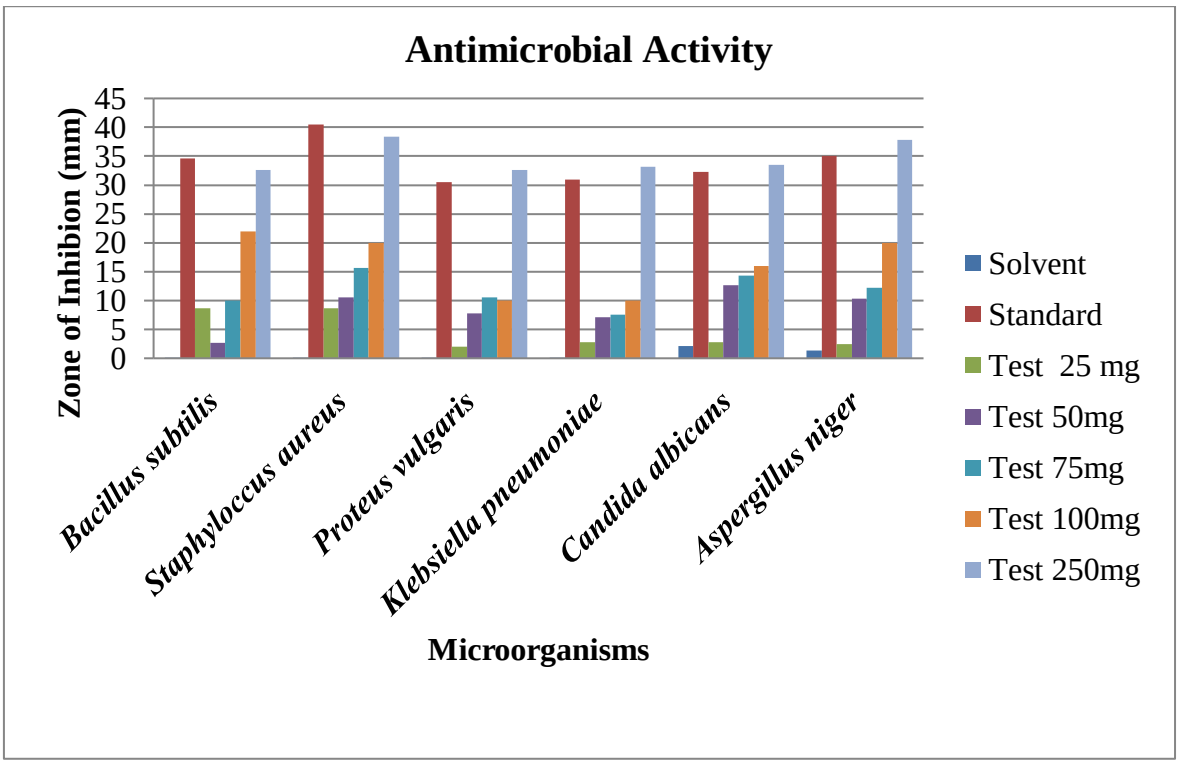
Fig. No. 64: Zone of inhibition of Test drug 25, 50, 75 & 100mg against *Aspergillus niger*

Fig. No. 65: Zone of inhibition of Test drug 250mg against *Aspergillus niger*



Test drug: Ethanolic Extract of *Withania coagulans* (25, 50, 75, 100 & 250mg/ disc)
Standard: Ciprofloxacin (5µg/ disc) – Bacteria & Nystatin (100 units/ disc) – Fungi
Solvent: DMSO

Fig. No. 66: Antimicrobial screening of Ethanolic Extract of *Withania coagulans*



8. CONCLUSION

From the study entitled "Pharmacological Evaluation of *Withania coagulans* Dunal (Flower buds), the following conclusion could be drawn

- The present study has thus duly supported the traditional use of Ethanolic Extract of *Withania coagulans* Dunal have scientifically proved the behavioral coordination, antiobesity, antihyperlipidemic, analgesic, anthelmintic, and antimicrobial activity

- Apart from the suggested actions listed in discussion part absence of acute toxicity may also offer a new hope for safe treatment in future

- Preliminary phytochemical study ethanolic extract of *Withania coagulans* Dunal was found to contain Carbohydrates, Protein, Steroids, Flavanoids, Alkaloids, Glycosides and Amino Acids are present

- Presence of Alkaloids, Flavanoids and Glycosides in the ethanolic extract of *Withania coagulans* Dunal was concluded by IR & HPTLC analysis. Though present in small quantities, it was found to produce considerable effects

- The results of the present study indicate that the ethanolic extract of *Withania coagulans* was non-toxic up to dose level of 2000mg/kg body weight in albino rats as per acute oral toxicity studies. $1/10^{th}$ of the LD_{50} Dose is 200mg/kg is used for Pharmacological screening

- Evaluation of anti-obesity activity of ethanolic extract of *Withania coagulans* showed significant anti obesity property by the obtained significant results against Progesterone induced obesity. From the results observed in the Progesterone induced obesity, it may be concluded that the test compound at the dose of 200mg/kg body weight displays a significant anti- obesity activity compared to standard drug Orlistat

- The ethanolic extract of *Withania coagulans* at a dose of 200mg/kg exhibited significant hypolipidemic activity in Fructose induced hyperlipidemic rats. This is showed by the reduction of serum lipid parameters such as triglycerides, total cholesterol, LDL, VLDL, SGOT and SGPT with an increase in HDL concentration in the group treated with 200mg/kg of Ethanolic Extract of *Withania coagulans.* It is found that there is a significant reduction in the Serum glucose

- The ethanolic extract of *Withania coagulans* was subjected to the Tail Flick Analgesic test, showed a significant inhibitory effect

- The ethanolic extract of *Withania coagulans* was subjected to the Anthelmintic activity against *Pheritima posthuma*, showed a significant inhibitory effect at higher doses 75mg/10ml of Normal Saline & 100mg/10ml of Normal Saline

- The ethanolic extract of *Withania coagulans* was subjected to the antimicrobial test, showed a significant inhibitory effect on both gram positive, gram negative bacteria and also fungi

- In future, further investigation might provide an insight to identify the functional groups in the ethanolic extract of *Withania coagulans* active responsible for the behavioral coordination, antiobesity, antihyperlipidemic, analgesic, anthelmintic, and antimicrobial effect and to elucidate the exact mechanism of action, which is responsible for the observed significant activity with low toxicity and better therapeutic index

8. BIBILIOGRAPHY

1. **Abhijeet R Borate, Anupama A Suralkar, Smita S Birje, Pravin V Malusare & Pritam A Bangale.** Antihyperlipidemic Effect of Protocatechuic Acid in Fructose Induced Hyperlipidemia in Rats. *International Journal of Pharma and Bio Sciences* 2011, 2 (4): 456-460.

2. **Abhishek B, Anita Thakur.** Anthelmintic activity of *Cynodon dactylon. Journal of Pharmacognosy and Phytochemistry* 2012,1 (3): 1- 3**.**

3. **Abidemi J Akindele, Hakeem A Sanni, & Pamela C Edeh.** Anxiolytic Activity of Aerial Part Hydroethanolic Extract of *Allium ascalonicum* Linn. (Liliaceae) in Mice. *Functional Foods in Health and Disease* 2012, 2 (11): 448-459.

4. Acute oral Toxicity Studies OECD – Guidelines number – 423.

5. **Adnyana IK, Elin Y.Sukandar,Ari Yuniarto,Finna S.** Anti-Obesity Effect of the Pomegranate Leaves Ethanol Extract(*Punica granatuml*.) in High-Fat Diet Induced Mice. *International Journal of Pharmacy and Pharmaceutical Sciences* 2014, 6(4): 626-631.

6. **Agarwal A**, *Pharma Times*. 2005, 37 (6): 9-11.

7. **Agrawal OP, Raju PS. In: Abdin MZ, Abroi YP.** Global market of herbal products: Opportunities for India. Traditional system of medicine. Narosa Publishing House: New Delhi, India. 2006, 5-10.

8. **Alowanou Goué G, Olounladé Abiodoun P, Azando Erick Virgile B, Dossou Tobi R, Dedehou Vidjinnangni Fifamè Grâce N, Daga Florian D, Adenilé Adam D, Hounzangbé-Adoté Sylvie M.** Acute Oral Toxicity Activity of Aqueous Extract of *Combretum glutinosum* Perr. Ex De Leaves in Wistar Rats. *Int. J. Pure App. Biosci.* 2015, 3 (4): 72-78.

9. **Ankur Datta, Chiranjib Bagchi, Saibal Das, Achintya Mitra, Anuradha De Pati, Santanu Kumar Tripathi.** Antidiabetic and Antihyperlipidemic Activity of Hydroalcoholic Extract of *Withania coagulans* Dunal Dried Fruit in Experimental Rat Models. *Journal of Ayurveda & Integrative Medicine* 2013, 4 (2): 99-107.

10. **Aparna Saraf & Aruna Samant.** HPTLC Fingerprint Profile and Antimicrobial Activity of Leaves of *Achyranthes aspera* Linn. *World Journal of Pharmacy and Pharmaceutical Sciences Lume* 2015, 4 (3): 879-899.

11. Ashok Kumar Gupta, Mansi Verma, Gajraj Singh Lodhi. Protective Effect of *Ficus infectoria* Plant Extract against Fructose Induced Hyperlipidemia and Hyperglycemia in Rats. *The Journal of Phytopharmacology* 2014, 3 (6): 431-435.

12. Avijit Chatterjee, Biswarup Sen, Sumanta Das, Tapan Kumar Chatterjee. Anti-Inflammatory and Analgesic Activity of Methanolic Extract of Medicinal Plant *Rhodiola rosea* L. Rhizomes. *International Journal of Pharma Research & Review* 2015, 4 (2):1-8.

13. AvinashShankar. *Withania coagulans* in Management of Diabetes Mellitus. *International Journal of Clinical Chemistry and Laboratory Medicine* 2016, 2 (1): 22-29.

14. Bahmani Nasrin, Naseri Zahra, Alikhani Mohammad Yosef, Karami Manoochehr & Kalvandi Ramazan. Evaluation of Antibacterial Effects of *Withania coagulans* and Cynara Cardunculus Extracts on Clinical Isolates of Brucella Strains. *International Journal of Medical Research &Health Sciences* 2016, 5 (9): 90-95.

15. Bauer AW, Kirby WMM, Sherris JC & Turck M. Antibiotic Susceptibility Testing by a Standerdised Single Disk Method. *American Journal of Clinical Pathology* 1996, 45: 493 – 496.

16. Bays H, Blonde L, Rosenson R. Adiposopathy: how do diet, exercise and weight loss drug therapies improve metabolic disease in overweight patients? *Expert Rev Cardiovasc Ther*. 2006, 4 (6):871-895.

17. Birketvedt GS, Florholmen J, Sundsfjord J, Osterud B, Dinges D, Bilker W. Behavioral and neuroendocrine characteristics of the night-eating syndrome. *JAMA*. 1999, 282 (7): 657-663.

18. Calixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Braz J Med Biol Res.* 2000, 33: 179-189.

19. Cawley J, Meyerhoefer C. The medical care costs of obesity: an instrumental variables approach. *J Health Econ.* 2012, 31 (1): 219-30.

20. Chavan R TA, Kadam AS & Kote JR. HPTLC Fingerprint Analysis and Antimicrobial Activity of Leaf Extracts of *Cassia fistula* L. *Scholars Research Library Der Pharmacia Lettre* 2016, 8 (3):100-104.

21. Chetan Salwaan, Amrinder Singh, Anuj Mittal, Prabhsimran Singh. Investigation of the Pharmacognostical, Phytochemical and Antioxidant Studies of Plant *Withania coagulans* Dunal. *Journal of Pharmacognosy and Phytochemistry* 2012, 1 (3): 32 – 38.

22. Claudia Gamboa-Gómez I, Nuria Rocha-Guzmán E, Alberto Gallegos-Infante J, Martha Moreno-Jiménez R, Blanca Vázquez-Cabral D, Rubén González-Laredo F. Plants With Potential Use on Obesity and its Complications. *Excli Journal* 2015, 14: 809-831.

23. Das R, Mehta DK & Gupta A. *In Vitro* Anthelmintic Activity of Leaves of Juglans Regia L against *Pheretima posthuma*. *Sci. Revs. Chem. Commun* 2011, 1 (1): 78-82.

24. David J Newman, Gordon M Cragg and Kenneth M Snader. Natural Products as Sources of New Drugs over the Period 1981- 2002. *J. Nat. Prod.* 2003, 66: 1022-1037.

25. Debasis Mishra, Goutam Ghosh, Sudhir Kumar P & Prasanna Kumar Panda. An Experimental Study of Analgesic Activity of Selective Cox-2 Inhibitor with Conventional NSAIDs. *Asian Journal of Pharmaceutical and Clinical Research* 2011, 4 (1): 78 – 81.

26. Debasmita Dutta Pramanick & Srivastava SK. Pharmacognostic Evaluation of Withania *coagulans* Dunal (Solanaceae) - An Important Ethnomedicinal Plant. *Bioscience Discovery* 2015, 6 (1): 06-13.

27. Deepika Mathur & Agrawal RC. Evaluation of *In Vivo* Antimutagenic Potential of Fruits Extracts of *Withania coagulans*. *Scholars Research Library Der Pharma Chemica* 2011, 3 (4): 373-376.

28. Deo SS, Inam F & Mahashabde RP. Antimicrobial Activity and HPLC Fingerprinting of Crude Ocimum Extracts. *E-Journal of Chemistry* 2011, 8 (3): 1430-1437.

29. Dilpesh Jain,Preeti Bavage,Akansha Sable,Mitali Gokhale. Evaluation of Anti-Obesity Effect of Fattolin, a Polyherbal Formulation in Progesterone Induced Obesity in Mice. *Indian Journal of Pharmaceutical Education and Research* 2016, 50 (3): 391-396.

30. Dolly Jaiswal, Prashant Kumar Rai & Geeta Watal. Antidiabetic Effect of Withania *coagulans* in Experimental Rats. *Indian Journal of Clinical Biochemistry* 2009, 24 (1): 88-93.

31. Dubey NK, Rajesh Kumar & Pramila Tripathi. Global promotion of herbal medicine: India's opportunity. *Current Science* 2004, 86 (1): 37-41.

32. Dunal Maryam Khodaei,Mehrana Jafari, Mitra Noori. Remedial use of Withanolides from *Withania coagulans* (Stocks). *Advances in Life Sciences* 2012, 2 (1): 6-19.

33. Durga Maha Lakshmi N, Mahitha B, Madhavi T & John Sushma N. Phytochemical Screening and FTIR Analysis of *Clitoria ternatea* Leaves. *International Journal of Scientific & Engineering Research* 2015, 6 (2): 287-290.

34. Eliana H Akamine,Anderson C Marcal, Joao Paulo Camporez, Mara S Hoshida,Luciana C Caperuto, Estela Bevilacqua and Carla R O Carvalho. Obesity Induced by High-Fat Diet Promotes Insulin Resistance in the Ovary. *Journal of Endocrinology* 2010, 206: 65–74.

35. Evans M. A guide to herbal remedies. Orient Paperbacks. 1994.

36. Finkelstein EA, DiBonaventura Md, Burgess SM, Hale BC. The costs of obesity in the workplace. *J Occup Environ Med.* 2010, 52 (10): 971-6.

37. Finucane MM, Stevens GA, Cowan MJ, Danaei G, Lin JK, Paciorek CJ. National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9·1 million participants. *LANCET* 2011, 377 (9765): 557-567.

38. Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA.* 2012, 307 (5): 491-7.

39. Florence AR & Jeeva S. FTIR and GC-MS Spectral Analysis of *Gmelina asiatica* L. Leaves. *Science Research Reporter* 2015, 5 (2): 125-136.

40. Gallagher D, Heymsfield SB, Heo M, Jebb SA, Murgatroyd PR, Sakamoto Y. Healthy percentage body fat ranges: an approach for developing guidelines based on body mass index. *Am J Clin Nutr*. 2000, 72 (3): 694-701.

41. Garodia P, Ichikawa H, Malani N, Sethi G, Aggarwal BB. From ancient medicine to modern medicine: Ayurvedic concepts of health and their role in inflammation and cancer. *J Soc Integr Oncol.* 2007, 5: 25-37.

42. Geethu MG, Suchithra PS, Kavitha CH, Aswathy JM, Dinesh Babu, Murugan K. Fourier-Transform Infrared Spectroscopy Analysis of Different Solvent Extracts of Water Hyacinth (*Eichhornia crassipes* Mart Solms.) an Allelopathic approach. *World Journal of Pharmacy and Pharmaceutical Sciences* 2014, 3 (6): 1256-1266.

43. Gioacchino Calapai & Achille P. Caputi. Herbal Medicines: Can We Do Without Pharmacologist? *eCAM* 2007, 4 (S1): 41-43.

44. Girija K, Lakshman K. Anti-Hyperlipidemic Activity of Methanol Extracts of Three Plants of Amaranthus in Triton-WR 1339 Induced Hyperlipidemic Rats. *Asian Pacific Journal of Tropical Biomedicine* 2011, 5(1): 62-65.

45. Grundy SM, Brewer HB, Cleeman JI, Smith SC, Lenfant C. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation*. 2004, 109(3): 433-8.

46. Gupta LM & Raina R. Side effects of some medicinal plants. *Current Science.* 1998, 75: 897-900.

47. H. Wagner. Plant Drug Analysis. 1983: 53.

48. Hackethal V. Obese women may have 25% increased risk for stillbirth. *Medscape Medical News.* 2014.

49. Hairston KG, Bryer-Ash M, Norris JM, Haffner S, Bowden DW, Wagenknecht LE. Sleep duration and five-year abdominal fat accumulation in a minority cohort: the IRAS family study. *Sleep*. 2010, 33(3): 289-95.

50. Hamdy O. The role of adipose tissue as an endocrine gland. *Curr Diab Rep*. 2005, 5(5): 317-9.

51. Idris Bello, Abdulmenem Suliman Bakkouri, Yasser M. Tabana , Bassel Al-Hindi , Majed Ahmed Al-Mansoub, Roziahanim Mahmud & Mohd. Zaini Asmawi. Acute and Sub-Acute Toxicity Evaluation of the Methanolic Extract of *Alstonia scholaris* Stem Bark. *Med. Sci.* 2016, 4(4): 1-14.

52. Ilya Raskin, David M. Ribnicky, Slavko Komarnytsky, Nebojsa Ilic, Alexander Poulev, Nikolai Borisjuk, Anita Brinker, Diego A. Moreno, Christophe Ripoll, Nir Yakoby, Pastor & Bertold Fridlender. Plants and human health in the twenty-first century. *Trends in Biotechnology* 2002, 20 (12): 522-531.

53. Islam MR, Mannan MA, Kabir MHB, Islam A & Olival KJ. Analgesic, Antiinflammatory and antimicrobial effects of ethanol extracts of mango leaves. *J. Bangladesh Agril. Univ.* 2010, 8 (2): 239–244.

54. Janak Dabheliya, Shoeb Ali Khan, Manan Joshipura, Manoj Vasoya, Sanjay Patel,Vijaya S. Diuretic Potential of Aqueous Extract of Fruits of *Withania coagulans* Dunal in Experimental Rats. *International Journal of Pharmacy and Pharmaceutical Sciences* 2010, 2 (4): 51-53.

55. Jhansee Mishra, Alok Kumar Dash, Shailesh Nandan Mishra, Ashish Kumar Gupta. *Withania coagulans* in Treatment of Diabetics and Some Other Diseases: A Review. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 2013, 4 (2): 1251-1258.

56. Jiao L, Berrington de Gonzalez A, Hartge P, Pfeiffer RM, Park Y, Freedman DM. Body mass index, effect modifiers, and risk of pancreatic cancer: a pooled study of seven prospective cohorts. *Cancer Causes Control* 2010, 21 (8): 1305-14.

57. Jiju V, Megha Gorantla & Chamundeeswari D. Evaluation of Anthelmintic Activity of Methanolic Extract of *Asystasia gangeticum. Int. J. Of Pharm. & Life Sci.* 2013, 4 (6): 2727-2730.

58. Kadam AB, Gaykar BM. Assessment of Acute Oral Toxicity of Synergistic Formulation Extract of Traditional Contraceptive Plants. *International Journal of Pharmacognosy and Phytochemical Research* 2017, 9 (3): 313-318.

59. Kamboj VP. Herbal Medicine. *Current Science* 2000, 78: 35-9.

60. Karthika K, Jamuna S, Paulsamy S. TLC and HPTLC Fingerprint Profiles of Different Bioactive Components from the Tuber of *Solena Amplexicaulis. Journal of Pharmacognosy and Phytochemistry* 2014, 3 (1): 198-206.

61. Kavya Sree K, Vijusha M, Rajani A, Hemamalin K & Ratna Sundari EG. Screening of Behavioural, Muscle Co-Ordination & Anxiolytic Activities of Methanolic Extract of *Tabebuia rosea* (Bertol). *Asian J Pharm Clin Res.* 2013, 6 (5): 187-190.

62. Ketterer C, Heni M, Thamer C, Herzberg-Schäfer SA, Häring HU, Fritsche A. Acute, short-term hyperinsulinemia increases olfactory threshold in healthy subjects. *Int J Obes (Lond)*. 2011, 35 (8): 1135-1138.

63. Kirtikar Shukla, Piyush Dikshit, Rimi Shukla, & Jasvinder K. Gambhir. The Aqueous Extract of *Withania coagulans* Fruit Partially Reverses Nicotinamide/ Streptozotocin - Induced Diabetes Mellitus in Rats. *Journal of Medicinal Food* 2011, 1829 (9): 1 – 9.

64. Kyrgiou M, Kalliala I, Markozannes G. Adiposity and cancer at major anatomical sites: umbrella review of the literature. *BMJ*. 2017, 356: 477.

65. Lakshmi BVS, Neelima N, Kasthuri N, Umarani V, Sudhakar M. Antihyperlipidemic Activity of *Bauhinia Purpurea* Extracts in Hypercholesterolemic Albino Rats. *International Journal of Pharmtech Research* 2011, 3 (3): 1265-1272.

66. Li C, Ford ES, Zhao G, Croft JB, Balluz LS, Mokdad AH. Prevalence of self-reported clinically diagnosed sleep apnea according to obesity status in men and women: National Health and Nutrition Examination Survey, 2005-2006. *Prev Med*. 2010, 51 (1): 18-23.

67. Librado A Santiago, Valerio VLM, & Yolo RT. Acute Oral Toxicity Study of the Crude Ethanolic Leaf Extract of *Ficuspseudopalma blanco* (Moraceae) in Sprague Dawley Rats. *International Journal of Research and Development in Pharmacy and Life Sciences* 2013, 2 (6): 674-679.

68. Lieb W, Sullivan LM, Harris TB, Roubenoff R, Benjamin EJ, Levy D. Plasma leptin levels and incidence of heart failure, cardiovascular disease, and total mortality in elderly individuals. *Diabetes Care*. 2009, 32 (4): 612-616.

69. Manickam Murugan, Veerabahu Ramasamy Mohan. Phytochemical, FTIR and Antibacterial Activity of Wholeplant Extract of *Aerva lanata* (L.) Juss. Ex. Schult. *Journal of Medicinal Plants Studies* 2014, 2 (3): 51-57.

70. Manjulika Yadav, Sanjukta Chatterji, Sharad Kumar Gupta & Geeta Watal. Preliminary Phytochemical Screening Of Six Medicinal Plants Used In Traditional Medicine. *International Journal of Pharmacy and Pharmaceutical Sciences* 2014, 6 (5): 539-542

71. Martinelli CE, Keogh JM, Greenfield JR, Henning E, van der Klaauw AA, Blackwood A. Obesity due to melanocortin 4 receptor (MC4R) deficiency is associated with increased linear growth and final height, fasting hyperinsulinemia, and incompletely suppressed growth hormone secretion. *J Clin Endocrinol Metab.* 2011, 96 (1): E181-188.

72. Maryam Sarbishegi, Zahra Heidari, Hamidreza Mahmoud zadeh-Sagheb, Moharram Valizadeh, Mahboobeh Doostkami. Neuroprotective Effects of *Withania coagulans* Root Extract on Ca1 Hippocampus Following Cerebral Ischemia in Rats. *AJP* 2016, 6 (4): 399 – 409.

73. Meda Ramesh & Jaya Sankar Reddy V. A Review on Anxiolytic Activity of Some Herbal Plants. *International Journal of Innovative Pharmaceutical Research* 2014, 5 (2): 389-394.

74. Metcalf BS, Hosking J, Frémeaux AE, Jeffery AN, Voss LD, Wilkin TJ. BMI was right all along: taller children really are fatter (implications of making childhood BMI independent of height) EarlyBird 48. *Int J Obes (Lond).* 2011, 35 (4): 541-547.

75. Mishra L, Lal P, Sangwan RS, Sangwan NS, Uniyal GC, Tuli R. Unusually sulphated oxygenated steroids from *Withania somnifera*. *Phytochemistry* 2005, 66: 2702–2707.

76. Mohamed El Hasan Shayoub, Azza Dawoud Hussien Dawoud, Mona AM Abdelmageed, Ali M Ehassan, Ahmad M Ehassan. Phytochemical Analysis of Leaves Extract

of Eucalyptus *Camal dulensis* Dehnh Omdurman. **Journal of Pharmaceutical Science** 2015, 2 (1): 64-71.

77. Mohanty IC, Vishnu K. Soni & Sahu GR. Development of Phytochemical Fingerprint of an Indian Medicinal Plant Chitrak (*Plumbago zeylanica* L) using High Performance Thin Layer Chromatography (HPTLC). *Journal of Medicinal Plant Research* 2014, 8 (18): 669-685.

78. Molarius A, Seidell JC, Sans S, Tuomilehto J, Kuulasmaa K. Varying sensitivity of waist action levels to identify subjects with overweight or obesity in 19 populations of the WHO. *J Clin Epidemiol* 1999, 52 (12): 1213-1224.

79. Molarius A, Seidell JC, Sans S, Tuomilehto J, Kuulasmaa K. Waist and hip circumferences, and waist-hip ratio in 19 populations of the WHO. *Int J Obes Relat Metab Disord*. 1999, 23 (2): 116-125.

80. Montonen J, Boeing H, Schleicher E, Fritsche A, Pischon T. Association of changes in body mass index during earlier adulthood and later adulthood with circulating obesity biomarker concentrations in middle-aged men and women. *Diabetologia* 2011, 54 (7): 1676-1683.

81. Muhammad Ahmed. Acute Toxicity (Lethal Dose 50 Calculation) of Herbal Drug Somina in Rats and Mice. *Pharmacology & Pharmacy* 2015, 6: 185-189.

82. Muhammad Riaz L, Muhammad Zia-Ul-Haq, Najm Ur-Rahman, Mansoor Ahmad. Neuropharmacological Effects of Methanolic Extracts of *Rubus fruticosus*. *Turkish Journal of Medical Sciences* 2014, 44: 454-460. **L**

83. Mulcahy N. 'Strong evidence': obesity tied to 11 cancers. *Medscape Medical News* 2017, 28.

84. Murray PG, Read A, Banerjee I, Whatmore AJ, Pritchard LE, Davies RA. Reduced appetite and body mass index with delayed puberty in a mother and son: association with a rare novel sequence variant in the leptin gene. *Eur J Endocrinol*. 2011, 164 (4): 521-527.

85. Naga Kishore R, Anjaneyulu N, Naga Ganesh M & Sravya N. Evaluation of Anxiolytic Activity of Ethanolic Extract of *Foeniculum vulgare* in Mice Model. *International Journal of Pharmacy and Pharmaceutical Sciences* 2012, 4 (3): 584-586.

86. Nandagoapalan V, Doss A & Marimuthu C. Phytochemical Analysis of Some Traditional Medicinal Plants. *Bioscience Discovery* 2016, 7 (1): 17-20.

87. Nandha Kumar S & Nivetha Thampi. Phytochemical Screening and Characterization of the Bioactive Compounds from the Leaves of *Hyptis suaveolens* and *Spathodea campanulata*. *Journal of Chemical and Pharmaceutical Research* 2015, 7 (7): 840-850.

88. Neel JV. The "thrifty genotype" in 1998. *Nutr Rev*. 1999, 57 (5): S2-S9.

89. Neelam B, Bare,Pratima S, Jadhav. Pharmaceutical Importance of *Withania coagulans* in Health and Diseases. *International Journal of Advances in Science Eng and Tech.* 2017, 5 (1-3): 41-44.

90. Neha Sahu, Jyoti Saxena. Phytochemical Analysis of Bougainvillea Glabra Choisy by FTIR and UV-Vis Spectroscopic Analysis. *Int. J. Pharm. Sci.* 2013, 21 (1): 196-198.

91. Nguyen Le Bao Duy & Dao Thi Diem Trang. Preliminary Phytochemical, Acute Oral Toxicity and Anticonvulsant Activity of the Seed Extract of *Brassica juncea*. *European Journal of Medicinal Plants* 2016, 14 (1): 1-9.

92. Nidhi Agarwal, Pramod Kumar Raghav, Richa Singh & Renu Singh. Paneer Doda (*Withania coagulans* Dunal): A Promising Therapeutic Agent. *International Journal of Green and Herbal Chemistry* 2014, 3 (2): 701-711.

93. Nidhi Sharma, Preeti Sharma, Nakuleshawar Dut Jasuja & Suresh Chand Joshi. Hypocholesterolemic and Antioxidant Potentials of Some Plants and Herbs: A Review. *RRJZS* 2013, 1 (2): 26-42.

94. Nishesh Sharma, Durgesh, Varnika, Eapen P Koshy, Manjul Dhiman. Biological Properties and Conservation of Critically Endangered Plant *Withania coagulans* - Indian Rennet; A Review. *International Journal of Advanced Research in Biological Sciences* 2015, 2 (10): 24–31.

95. Nurul Husna R, Noriham A, Nooraain H , Azizah AH , & Farah Amna O. Acute Oral Toxicity Effects of Momordica Charantia in Sprague Dawley Rats. *International Journal Of Bioscience, Biochemistry and Bioinformatics* 2013, 3 (4): 408-410.

96. Oliveira LMB, Bevilaqua CML, Costa CTC, Macedo ITF, Barros RS, Rodrigues ACM, Camurc ALF¸ Vasconcelos A, Morais SM, Lima YC , Vieira LS , Navarro AMC. Anthelmintic Activity of *Cocos nucifera* L. against Sheep Gastrointestinal Nematodes. *Veterinary Parasitology* 2009, 159: 55–59.

97. Onasanwo SA, Chatterjee M & Palit G. Antidepressant and Anxiolytic Potentials of Dichloromethane Fraction from *Hedranthera barteri.* Afr. *J. Biomed Res. 2010*, 13: 76 – 81.

98. Pankti P Dalwadi & Pragnesh V Patani. Anti Hyperlipidemic Activity of *Tephrosia Purpurea* Plant Extracts in Poloxomer 407 induced Hyperlipidemic Rats. *International Journal of Pharmacological Research* 2014, 4 (4): 187-193.

99. Payne G, Bringi V, Prince C, Shuller M. The quest for commercial production of chemicals from plant cell culture, Plant Cell and Tissue Culture in Liquid Systems. *Oxford University Press, Oxford.* 1991.

100. Pezeshki, Hesari J, Ahmadi Zonoz A & Ghambarzadeh B. Influence of *Withania coagulans* Protease as a Vegetable Rennet on Proteolysis of Iranian UF White Cheese. *J. Agr. Sci. Tech.* 2011, 13: 567-576.

101. Prakash Chandra Gupta. *Withania coagulans* Dunal- an Overview. *International Journal of Pharmaceutical Sciences Review and Research* 2012, 12 (2): 68- 73.

102. Prasad SK, Singh PN, Wahi AK, Hemalatha S. Pharmacognostical standardization of *withania coagulans* Dunal. *Phcog. J* 2010, 2 (11): 386- 395.

103. Prashant Tiwari, Bimlesh Kumar, Manoj Kumar, Mandeep Kaur, Jiban Debnath, Pardeepsharma. Comparative Anthelmintic Activity of Aqueous and Ethanolic Stem Extract of *Tinospora cordifolia*. *International Journal Of Drug Development & Research* 2011, 3 (1): 70-83.

104. Prashanta KR Deb, Ranjib Ghosh, Sankari Das, Tejendra Bhakta. *In-Vitro* Anthelmintic Activity of *Acorus calamus* Leaves. *Asian J Pharm Clin Res* 2013, 6 (3): 135-137.

105. Preethi MP, Sangeetha U, Pradeepa D, Valizadeh M, Kalaiselvi Senthil. Principal Component Analysis and HPTLC Fingerprint of *In Vitro* and Field Grown Root Extracts of *Withania coagulans*. *International Journal of Pharmacy and Pharmaceutical Sciences* 2014, 6 (5): 480-488.

106. Preeti, Milind Parle, Monu & Kailash Sharma. Medicinal Plants Possessing Anxiolytic Activity: A Brief Review. *Pelagia Research Library Der Pharmacia Sinica* 2015, 6 (5):1-7.

107. Priyadarshini L, Mazumder PB & Choudhury MD. Acute Toxicity and Oral Glucose Tolerance Test of Ethanol and Methanol Extracts of Antihyperglycaemic Plant *Cassia alata* Linn. *IOSR Journal of Pharmacy and Biological Sciences* 2014, 9 (2): 43-46.

108. Priyanka Pandey, Rakesh Mehta & Ravi Upadhyay. Physico-Chemical and Preliminary Phytochemical Screening of *Psoralea corylifolia. Archives of Applied Science Research* 2013, 5 (2): 261-265.

109. Qian Chen , Tingtingwang , Jian Li , Sijianwang , Feng Qiu , Haiyang Yu , Yi Zhang & Tao Wang. Effects of Natural Products on Fructose-Induced Nonalcoholic Fatty Liver Disease. *Nutrients* 2017, 9 (96): 1-12.

110. Raghavendra HL, Yogesh HS, Gopalakrishna B, Chandrashekhar VM, Sathish kumar BP, & Vadlapudi Kumar. An Overview of Herbal Medicine. *Int. J. Ph. Sci.* 2009, 1 (1):1-20.

111. Rajagopal PL, Premaletha K, Sreejith KR. Anthelmintic Activity of the flowers of *Sesbania grandiflora* Pers. *Journal of Innovations in Applied Pharmaceutical Sciences* 2016, 1 (2): 8-11.

112. Rajesh Asija, Radhey Shyam Kumawat, Sangeeta Asija, Divya Sharma, Himanshu Baheti. Anti Inflammatory and Analgesic Activity of Medicinal Plants - A Review. *International Journal of Pharmaceutical Erudition* 2014, 4 (3): 22-32.

113. Rajeshwari Sahu, Jyoti Saxena. Ultraviolet-Visible and Fourier Transform Infrared Spectroscopic Studies On Non-Conventional Species of Curcuma. *Indian Journal of Advances in Chemical Science* 2014, 2 (4): 300-302.

114. Rajiv P, Deepa A, Vanathi P, Vidhya D. Screening for Phytochemicals and FTIR Analysis of *Myristica dactyloids* Fruit Extracts. *International Journal of Pharmacy and Pharmaceutical Sciences* 2017, 9(1): 315-318.

115. Rajshekharan PE. Herbal medicine in World of Science. *Employment News* 2002, 3: 21-27.

116. Ramakrishnappa K. Biodiversity and the Ecosystem Approach in Agriculture, Forestry and Fisheries. New York, USA. Impact of Cultivation and Gathering of Medicinal Plants on Biodiversity: Case studies from India. *United Nations Publications* 2003, 210-5.

117. Rang HP, Pale MM, Ritter JM, Flower RJ, Henderson. Pharmacology. 7th edition, Churchill Livingstone 2011: 531 – 539.

118. Rang HP, Pale MM, Ritter JM, Flower RJ, Henderson. Pharmacology. 7th edition, Churchill Livingstone 2011: 385 – 392.

119. Rang HP, Pale MM, Ritter JM, Flower RJ, Henderson. Pharmacology. 7th edition, Churchill Livingstone 2011: 284 – 291.

120. Rang HP, Pale MM, Ritter JM, Flower RJ, Henderson. Pharmacology. 7th edition, Churchill Livingstone 2011: 503 – 522.

121. Rang HP, Pale MM, Ritter JM, Flower RJ, Henderson. Pharmacology. 7th edition, Churchill Livingstone 2011: 668 – 672.

122. Rashmi Tambe, Singhal RG, Kiran Bhise, Maushumi Kulkarni. Phytochemical Screening and HPTLC Fingerprinting of Leaf Extracts of *Psidium guajava*. *Journal of Pharmacognosy and Phytochemistry* 2014, 3 (1): 52-56.

123. Rates SMK. Plants as source of drugs.*Toxicon.* 2001, 39: 603-613.

124. Rohit Gundamaraju, Sartaj Banu Mulaplli, Ramesh.C. Evaluation of Anti-Obesity Activity of *Lantana camara* Var Linn. by Progesterone Induced Obesity on Albino Mice. *International Journal of Pharmacognosy and Phytochemical Research* 2012, 4 (4): 213-218.

125. Rohit Jain, Sumita Kachhwaha & Kothari SL. Phytochemistry, Pharmacology, and Biotechnology of *Withania somnifera and Withania coagulans*: A Review. *Journal of Medicinal Plants Research* 2012, 6 (41): 5388-5399.

126. Roundtable on Obesity Solutions, Food and Nutrition Board, Health and Medicine Division, National Academies of Sciences, Engineering, and Medicine. Obesity in the Early Childhood Years: State of the Science and Implementation of Promising Solutions: Workshop Summary. 2016.

127. Sabeeha Shafi, Nahida Tabassum. Acute Oral Toxicity and Hypoglycaemic Study of Ethanolic Extract of *Portulaca oleracea* (Whole Plant) in Swiss Albino Mice. *International Journal of Pharmacy and Pharmaceutical Sciences* 2013, 5 (4): 389- 393.

128. Sai Mangala Divi , Ramesh Bellamkonda, Sarala Kumari Dasireddy. Evaluation of Antidiabetic and Antihyperlipedemic Potential of Aqueous Extract of *Moringa oleifera* in Fructose Fed Insulin Resistant and STZ Induced Diabetic Wistar Rats: A Comparative Study. *Asian Journal of Pharmaceutical and Clinical Research* 2012, 5 (1): 67- 72.

129. Salem Mohamed Edrah, Atega Aljenkawi, Afifa Omeman, Fouzy Alafid. Qualitative and Quantities Analysis of Phytochemicals of Various Extract for *Ephedra altissima* from Libya. *Journal of Medicinal Plants Studies* 2016, 4 (3): 119- 121.

130. Sandhiya V, Shree Krishna M. Effect Of Chloroform Fraction of *Withania Coagulans* Bud on the Regulation of GLUT4 & PPAR -Expressions Levels in Diabetic L6 Myotubes. *Int. J. Life. Sci. Res.* 2016, 2 (3): 285- 289.

131. Sangh Partap, Saurabh Kumar, Amit Kumar, Neeraj K. Sharma, Jha. *In-Vitro* Anthelmintic Activity of *Luffa cylindrica* Leaves in Indian Adult Earthworm. *Journal of Pharmacognosy and Phytochemistry* 2012, 1 (2): 27 – 31.

132. Sangwan RS, Chaurasia ND, Mishra LN, Lal P, Uniyal GC, Sharma R. Phytochemical variability in commercial 83 herbal products and preparation of *Withania somnifera* (Ashwagandha). *Curr Sci.* 2004, 86: 461- 465.

133. Sanjoy kumar pal, Yogeshwar shukla. Herbal medicine: Present and future. *Asian Pacific Journal of Cancer prevention* 2003, 4: 281-288.

134. Saswata Banerjee, Antara Mukherjee, Tapan Kumar Chatterjee. Evaluation of Analgesic Activities of Methanolic Extract of Medicinal Plant *Juniperus communis* Linn. *Int J Pharm Sci* 4 (5): 547-550.

135. Satoskar, Nirmala Rege, Bhandarka SP. Pharmacology & Pharmacotherapeutics. 24th edition, Elseiver Health science 2015: 584 – 593.

136. Satoskar, Nirmala Rege, Bhandarka SP. Pharmacology & Pharmacotherapeutics. 24th edition, Elseiver Health science 2015: 149 - 168.

137. Satoskar, Nirmala Rege, Bhandarka SP. Pharmacology & Pharmacotherapeutics. 24th edition, Elseiver Health science 2015: 815 – 826.

138. Satoskar, Nirmala Rege, Bhandarka SP. Pharmacology & Pharmacotherapeutics. 24th edition, Elseiver Health science 2015: 208 – 230.

139. Satoskar, Nirmala Rege, Bhandarka SP. Pharmacology & Pharmacotherapeutics. 24th edition, Elseiver Health science 2015: 577 – 584.

140. Selvamohan T & Ramadas S, Shibila Selva Kishore. Antimicrobial Activity of Selected Medicinal Plants against Some Selected Human Pathogenic Bacteria. *Pelagia Research Library Advances in Applied Science Research* 2012, 3 (5): 3374-3381.

141. Shadab Ahmed, Auroj Naved, Rafeeq Alam Khan, Shahida Siddiqui. Analgesic Activities of Methanol Extract of *Terminalia chebula* Fruit. *Pharmacology & Pharmacy* 2015, 6: 547- 553.

142. Shah MA, Sarker MMR, Gousuddin M. Toxicity Study of *Brassica oleracea* Var. Italica Extracts in Sprague Dawley (SD) Rats. *International Journal of Pharmacognosy and Phytochemical Research* 2016, 8 (5): 735- 741.

143. Shameer. H, Nageswara Rao .R, Vijay kumar MMJ, Jayachandran, Sreenivasa GM. Synthesis, Characterization and Anti- inflammatory activity of novel *N*- Subtituted-5-oxa-Imidazole. *Indian J. of Research in Pharmacy and Biotechnology* 1: 2320- 3471

144. Shan P Mohammed, Nasiya Latheef & Sri Ganeshan P. Evaluation of Anxiolytic Activity of *Ixora coccinea* Linn. Ethanolic Extract in Swiss Albino Mice. *Clinical & Experimental Pharmacology* 2014, 4 (1): 1- 3.

145. Shandesh Bhattarai, Om Basukala. Antibacterial Activity of Selected Ethnomedicinal Plants of Sagarmatha Region of Nepal. *International Journal of Therapeutic Applications* 2016, 31: 27- 31.

146. Sharma HL & Sharma KK. Principles of Pharmacology. 2nd edition, Paras Publications 2012: 442 – 450.

147. Sharma HL & Sharma KK. Principles of Pharmacology. 2nd edition, Paras Publications 2012: 899 – 901.

148. Sharma HL & Sharma KK. Principles of Pharmacology. 2nd edition, Paras Publications 2012: 324 – 335.

149. Sharma HL & Sharma KK. Principles of Pharmacology. 2nd edition, Paras Publications 2012: 490 – 501.

150. Sharma HL & Sharma KK. Principles of Pharmacology. 2nd edition, Paras Publications 2012: 797 – 808.

151. Shashank Matthew, Ajay Kumar Jain, Manoj James, Cathrin Matthew, Debjit Bhowmik. Analgesic and Anti-Inflammatory Activity of *Kalanchoe pinnata* (Lam.) Pers. *Journal of Medicinal Plants Studies* 2013, 1(2): 24-28.

152. Sheetal Verma & S.P. Singh. Current and future status of herbal medicines. *Veterinary World* 2008, 1 (11): 347- 350.

153. Shen-nung pen ts'ao ching (Divine Husbandman's Materia Medica).**2** G

154. Shirin Hasani-Ranjbar, Zahra Jouyandeh & Mohammad Abdollahi. A Systematic Review of Anti-Obesity Medicinal Plants - An Update. *Journal of Diabetes & Metabolic Disorders* 2013, 12 (28): 1- 10.

155. Shiwaku K, Anuurad E, Enkhmaa B, Kitajima K, Yamane Y. Appropriate BMI for Asian populations. *LANCET*. 2004, 363 (9414): 1077.

156. Simona Zavoi , Florinela Fetea , Floricuta Ranga , Raluca M. Pop , Anca Baciu , Carmen Socaciu. Comparative Fingerprint and Extraction Yield of Medicinal Herb Phenolics with Hepatoprotective Potential, As Determined by UV-Vis and FTIR Spectroscopy. *Horti Agrobo.* 2011, 39 (2): 82- 89.

157. Snehlata Pandey , Rajshree Pandey, Rita Singh. Phytochemical Screening of Selected Medicinal Plant *Cinnamon zeylanicum* bark extract, Area of research; Uttarakhand, India. *International Journal of Science and Research Publication* 2014, 4 (6): 1 - 6.

158. Souravh Bais, Guru Sewak Singh, & Ramica Sharma. Antiobesity and Hypolipidemic Activity of Moringa *oleifera* Leaves Against High Fat Diet-Induced Obesity in Rats. *Advances in Biology* 2014, 162 (914):1- 9.

159. Spiegel K, Tasali E, Penev P, Van Cauter E. Brief communication: Sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite. *Ann Intern Med.* 2004, 141 (11): 846- 50.

160. Srikanth Ineedi, Vikas Kumar. Anti-Hyperlipidemic Activity of Hyperforin:An *in Vivo* Study in Rats. Pharmacologyonline 2009, 2: 133-139.

161. Sudhanshu Kumar Bharti, Amit Kumar, Neeraj Kumar Sharma, Supriya Krishnan, Ashok Kumar Gupta & Shree Ram Padamdeo. Antidiabetic Effect of Aqueous Extract of *Withania coagulans* Flower in Poloxamer-407 Induced Type 2 Diabetic Rats. *Journal of Medicinal Plants Research* 2012, 6 (45): 5706- 5713.

162. Suman Kumar R, Venkateshwar C, Samuel G, Gangadhar S RA. Phytochemical Screening of Some Compounds From Plant Leaf Extracts of *Holoptelea integrifolia* (Planch.) and *Celestrus emarginata* (Grah.) used by Gondu Tribes at Adilabad District, Andhra Pradesh, India. *International Journal of Engineering Science Invention* 2013, 2 (8): 65- 70.

163. Sumathi P & Parvathi A. Antimicrobial Activity of Some Traditional Medicinal Plants. *Journal of Medicinal Plants Research* 2010, 4 (4):316- 321.

164. Suneetha D, S.Divya Teja.Banda, Ramesh C, Firasat Ali. Evaluation of Anti-Obesity Activity of Methanolic Extract of *Sapindus emarginatus* by Progesterone Induced Obesity on Albino Mice. *International Journal of Pharmaceutical Sciences Review and Research* 2013, 23(2): 164-169.

165. Suresh Kumar Dev, Ajay Shukla , Choudhury PK , Singh GK. Analgesic and Anti-Nociceptive Activity of Hydroethanolic Extract of *Capparis deciduas* (Forssk.) Edgew. *Asian Journal of Pharmacy and Pharmacology* 2015, 1 (1): 40- 44.

166. Sushmitha J, Sumalatha G, Yogitha Reddy, Alekhya CHV, Jyothirmai CH. A Research Article on Evaluation of Anti-Obesity Activity of *Hordeum vulgaregrains* in Albino Rats. *Asian Journal of Phytomedicine and Clinical Research* 2015, 3 (3): 78 - 87.

167. Tahira Mughal, Samina Shahid & Sadia Qureshi. Antifungal Studies of *Withania coagulans* and *Tamarix aphylla*. *Journal of Applied Pharmacy* 2011, 03 (03): 289-294.

168. Tan CE, Ma S, Wai D, Chew SK, Tai ES. Can we apply the National Cholesterol Education Program Adult Treatment Panel definition of the metabolic syndrome to Asians?. *Diabetes Care* 2004, 27 (5): 1182- 6.

169. Tirosh A, Shai I, Afek A, Dubnov-Raz G, Ayalon N, Gordon B. Adolescent BMI trajectory and risk of diabetes versus coronary disease. *N Engl J Med.* 2011, 364 (14): 1315-1325.

170. Trini Suryowati, Rimbawan, Rizal Damanik, Maria Bintang, Ekowati Handharyani. Antihyperlipidemic Activity of Torbangun Extract (*Coleus amboinicus* Lour) on Diabetic Rats Induced by Streptozotocin. *IOSR Journal of Pharmacy* 2015, 5 (5): 50-54.

171. Tripathi KD. Essential of medical Pharmacology. 7th edition, Jaypee Brothers medical publishers 2013: 439 – 454.

172. Tripathi KD. Essential of medical Pharmacology. 7th edition, Jaypee Brothers medical publishers 2013: 612 – 620.

173. Tripathi KD. Essential of medical Pharmacology. 7th edition, Jaypee Brothers medical publishers 2013: 453 – 468.

174. Tripathi KD. Essential of medical Pharmacology. 7th edition, Jaypee Brothers medical publishers 2013: 808 – 818.

175. Vandana Gupta, Bipin Bihari Keshari. *Withania coagulans* Dunal. (Paneer Doda): A Review. *International Journal of Ayurvedic & Herbal Medicine* 2013, 3 (5): 1330- 1336.

176. Vedhanarayanan RBRP, Unnikannan P, Sundaramoorthy P. Antimicrobial Activity and Phytochemical Screening of *Wrightia tinctoria* (Roxb.). *Journal of Pharmacognosy and Phytochemistry* 2013, 2 (4): 123- 125.

177. Venu Gopala Rao Konda, Madhavi E, Ruckmani A & Venkataramana Y. A Review on Medicinal Plants with Potential Hypolipidemic Activity. *Int J Pharm Bio Sci* 2013, 4 (4): 729 – 740.

178. Vidya Sabbani, Alluri Ramesh & Satla Shobharani. Acute Oral Toxicity Studies of Ethanol Leaf Extracts of *Derris scandens & Pulicaria wightiana* in Albino Rats. *IJPR* 2015, 5 (1): 12-17.

179. Wadden TA, Webb VL, Moran CH, Bailer BA. Lifestyle modification for obesity: new developments in diet, physical activity, and behavior therapy. *Circulation* 2012, 125 (9): 1157-70.

180. Ward LC. Segmental bioelectrical impedance analysis: an update. *Curr Opin Clin Nutr Metab Care* 2012, 15 (5): 424-9.

181. Wasswa Peter & Olila Deogracious. The *In-Vitro* Ascaricidal Activity of Selected Indigenous Medicinal Plants Used in Ethno Veterinary Practices in Uganda. *Afr. J. Trad. Cam* 2006, 3 (2): 94 – 103.

182. Wataru Hiruma, Kohei Suruga, Kazunari Kadokura, Tsuyoshi Tomita, Ayaka Miyata, Yoshihiro Sekino, Masahiko Kimura, Nobuo Yamaguchi,Yasuhiro Komatsu, Tony Buffington CA, Nobufumi Ono. Antitumor Effects and Acute Oral Toxicity Studies of a Plant Extract Mixture Containing *Rhus verniciflua* and Some Other Herbs. *Open Journal Of Immunology* 2015, 5: 39- 49.

183. Weight Loss Markets for Products and Services. BCC Research. Available at http://www.bccresearch.com/report/weight-loss-markets-products-services-fod027c.html. Accessed: April 23, 2012.

184. Wijga AH, Scholtens S, Bemelmans WJ, de Jongste JC, Kerkhof M, Schipper M. Comorbidities of obesity in school children: a cross-sectional study in the PIAMA birth cohort. *BMC Public Health* 2010, 10: 184.

185. Yao R, Ananth CV, Park BY, Pereira L, Plante LA. for the Perinatal Research Consortium. Obesity and the risk of stillbirth: a population-based cohort study [abstract]. Presented at: The 2014 SMFM Annual Meeting; February 3-8, 2014; New Orleans, LA. *Am J Obstet Gynecol.* 2014, 210.

186. Yash Prashar & Saravana Kumar A. Anti-Obesity Activity of *Bauhinia variegata* Linn. in High Fat Diet Induced Obestity in Female Rats. *Pharmacology online* 2010, 2: 1008-1016.

187. Yonghua Zhang,Mélanie Nadeau, Frédérick Faucher,Odette Lescelleur,Simon Biron,Marleen Daris,Caroline Rhéaume,Van Luu-The, André Tchernof. Progesterone Metabolism in Adipose Cells. *Molecular and Cellular Endocrinology* 2009, 298: 76- 83.

188. Zulfiker AHM, Mahbubur Rahman M, Kamal Hossain M. *In Vivo* Analgesic Activity of Ethanolic Extracts of Two Medicinal Plants *Scoparia Dulcis L & Ficus Racemosa* Linn. *Biology and Medicine* 2010, 2 (2): 42-48.