PHARMACOLOGICAL EVALUATION OF LEAVES EXTRACT FROM *RIVEA ORNATA* ROXB.

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IN

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Submitted by

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LIST OF ABBREVIATIONS

WHO	World Health Organization
ISM	Indian Systems of Medicine
FA	Fatty Acids
TAG	Triacylglycerols
MAG	Monoacylglycerols
CD	Cluster Determinant
FABPs	Fatty Acids Binding Proteins
MTP	Microsomal Triglyceride Transfer Protein
PCTV	Prechylomicron Transport Vesicle
ER	Endoplasmic Reticulum
HMG CoA	3-Hydroxy 3 Methyl Glutaryl Co-enzyme
LPL	Lipoprotein Lipase
PPAR-α	Proliferator Protein Activated Receptor Alpha
VLDL	Very Low Density Lipoprotein
IDL	Intermediate Density Lipoprotein
LDL	Low Density Lipoprotein
HDL	High Density Lipoprotein
PC	Phosphatidylcholine
ATP	Adenosine Triphosphate
CETP	Cholesterol Ester Transfer Protein
RCT	Reverse Cholesterol Transport
APO	Apolipoprotein
LCAT	Lecithin Cholesterol Acyltransferase
AVD	Atherosclerotic Vascular Disease
eNOs	Endothelial Nitric oxide synthetase
APC	Activated Protein C

CHD	Congestive Heart Disease
FH	Familial Hypercholesterolemia
TC	Total Cholesterol
TG	Triglycerides
CAD	Coronary Artery Disease
SREBPs	Sterol Regulatory Element Binding Proteins
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
DM	Diabetes Mellitus
IHD	Ischemic Heart Disease
IDDM	Insulin Dependent Diabetes Mellitus
NIDDM	Non Insulin Dependent Diabetes Mellitus
HNF	Hepatocyte Nuclear Transcription Factor
JOD	Juvenile Onset Diabetes
GDM	Gestational Diabetes Mellitus
NPH	Neutral Protamine Hagedorn
DPP	Dipeptide Peptidase Inhibitor
NSAIDs	Non Steroidal Anti-Inflammatory Drugs
PGE2	Prostaglandin E2
TNF	Tumour Necrosis Factor
IL	Interleukin
PDGF	Platelet Derived Growth Factor
BFGF	Basic Fibroblast Growth Factor
TGFß	Transformin Growth Factor Beta
MERO	Methanolic Extract of *Rivea ornata*
VDCC	Voltage Dependent Calcium Channel
DRG	Dorsal Root Ganglion

PNs	Peripheral Nerves
FTIR	Fourier Transform Infrared Spectroscopy
HPTLC	High Performance Thin Layer Chromatography
SEM	Standard Error Mean
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
TS	Transverse Section
NS	Normal Saline
3D	3 Dimentional
OECD	Organization for Economic Co-operation and Development
IAEC	Institutional Animal Ethical Committee
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals

LIST OF SYMBOLS

p.o.	Per Oral
i.p.	Intra Peritoneal
s.c.	Subcutaneous
G	Gram
%	Percentage
µl	Microlitre
A	Alpha
B	Beta
C	Celsius
Hrs	Hours
Min	Minute
Nm	Nanometer
°	Degree
w/v	Weight by volume
w/w	Weight by weight
mMol	Millimole
Mm	Millimeter
M	Meter
Sec	Seconds
M	Meter
µg	Microgram
L	Litre
Mg	Milligram
Kg	Kilogram
Dl	Decilitre
Ml	Millilitre

INTRODUCTION

1. INTRODUCTION

1.1 Natural Products

Natural products signify large and diverse secondary metabolites with a comprehensive choice of biological activities those have established with their numerous practices, particularly in humans, veterinary and also in agriculture. The plant-derived Natural products are the products of secondary metabolism; the compounds which are not essential for existence in laboratory conditions but are certainly responsible for self-defense coordination in natural conditions.[1]

Herbal Medicine

The use of herbal medicines continues to expand rapidly across the world. Many people now take herbal medicines or herbal products for their health care in different national health-care settings. Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products. In some countries natural medicines may contain, by tradition, natural organic or inorganic active ingredients that are not of plant origin (e.g. animal and mineral materials).

Herbs include crude plant material, such as leaves, flowers, fruit, seeds, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered. Herbal materials include, in addition to herbs, fresh juices, gums, fixed oils, essential oils, resins and dry powders of herbs. In some countries, these materials may be processed by various local procedures, such as steaming, roasting or stir-baking with honey, alcoholic beverages or other materials.

Herbal preparations are the basis for finished herbal products and may include comminuted or powdered herbal materials, or extracts, tinctures and fatty oils of herbal materials. They are produced by extraction, fractionation, purification, concentration, or other physical or biological processes. They also include preparations made by steeping or heating herbal materials in alcoholic beverages and/or honey, or in other materials.

Finished herbal products consist of herbal preparations made from one or more herbs. If more than one herb is used, the term “mixture herbal product” can also be used. Finished herbal products and mixture herbal products may contain excipients in addition to the active ingredients. However, finished products or mixture herbal products to which chemically

defined active substances have been added, including synthetic compounds and/or isolated constituents from herbal materials, are not considered to be herbal.[2]

Pharmaceutical, insecticidal, and herbicidal importance have been driven form natural products discovery and been taken a significant role after the discovery of penicillin more than 85 years ago. Since then, numerous natural products have been isolated and characterized. However, throughout the ages, humans have relied on Mother Nature for the practice of herbal and phytonutrients treatment to fight against numerous diseases which are expanding across the world and about 80–85% or about 6 billion people worldwide trust herbal medication for the treatment of various diseases.[1]

Traditional Medicine

Traditional use of herbal medicines refers to the long historical use of these medicines. Their use is well established and widely acknowledged to be safe and effective, and may be accepted by national authorities.[2]

Traditional medicine is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness. The terms “complementary medicine”, “alternative medicine” and “nonconventional medicine” are used interchangeably with “traditional medicine” in some countries.[3]

Over the past 100 years, the development and mass production of chemically synthesized drugs have revolutionized health care in most parts of the word. However, large sections of the population in developing countries still rely on traditional practitioners and herbal medicines for their primary care. The World Health Organization (WHO) has also recognized the important role of traditional medicine in developing countries. WHO accepts that traditional systems will continue to play an important part in providing services to very large numbers of people, particularly in rural areas.[4] In India 70% and in Africa up to 90% of the population depend on traditional medicine to help meet their health care needs. In China, traditional medicine accounts for around 40% of all health care delivered and more than 90% of general hospitals in China have units for traditional medicine.[5]

The most common reasons for using traditional medicine are that it is more affordable, more closely corresponds to the patient’s ideology, allays concerns about the

adverse effects of chemical (synthetic) medicines, satisfies a desire for more personalized health care, and allows greater public access to health information. The major use of herbal medicines is for health promotion and therapy for chronic, as opposed to life-threatening, conditions. However, usage of traditional remedies increases when conventional medicine is ineffective in the treatment of disease, such as in advanced cancer and in the face of new infectious diseases. Furthermore, traditional medicines are widely perceived as natural and safe, that is, not toxic. This is not necessarily true, especially when herbs are taken with prescription drugs, over-the-counter medications, or other herbs, as is very common. In India herbal medicine is a common practice, and about 960 plant species are used by the Indian herbal industry, of which 178 are of a high volume, exceeding 100 metric tons per year.[6]

Modern medical doctors are too few in numbers in certain areas and are not always ready to live with the poor peoples in the slums, the high mountains, the desert areas, or the remote forests. Both Prime Ministers Jawaharlal Nehru and Indira Ghandi advocated the integration of the best of indigenous medicine with modern medicine in the regular practice. The government established a Central Council of Indian Medicine, a statutory body with a mandate to ensure conformity of standards of education and regulation of practice in respect to the traditional systems. To extend modern medical services to all sections of the population, particularly those living in backward and rural areas, would take a long time and require a large amount of funds. Because of the local availability and accessibility of herbs and other traditional medicines, treatment according to traditional medical systems is often cheaper[7].

Concepts and practices of different traditional medicinal systems in India are about several thousand years old. A large proportion of the Indian population still believes in and receives traditional medical care, which is based on the principles of three ancient codified Indian systems of medicine (ISMs): Ayurveda, Siddha, Unani and Homeopathy and therapies such as Yoga and Naturopathy. Though different chemicals, minerals, and animal products are also used in such system to prepare curative agents, but use of plants have been the basis of treatment in these system.[8, 9] Indian medical systems are found mentioned even in the ancient Vedas and other scriptures. The Ayurvedic concept appeared and developed between 2500 and 500 BC in India. The literal meaning of Ayurveda is “science of life,” because ancient Indian system of health care focused on views of man and his illness.[10]

Ayurveda

Ayurveda deals with the physical, mental, and spiritual world of mankind. It identifies man as an integral part of nature and stresses the necessity of maintaining harmony with all living and nonliving components of the surroundings (such as air, soil, and water). It is a prevention-oriented holistic science of natural healing developed by the great masters of India.[11]

The word 'Ayurveda' has derived out of fusion of two separate words- Áyu' i.e. life and 'veda' i.e. knowledge. Thus in literal meaning Ayurveda is the science of life. Ayurveda is a classical system of preventive, promotive and curative healthcare originating from the Vedas documented around 5000 years ago and currently recognized and practiced in India and many countries in the world. It is one of the most ancient healthcare systems having equal scientific relevance in the modern world, that take a holistic view of the physical, mental, spiritual and social aspects of human life, health and disease.

According to Ayurveda, health is considered as a basic pre-requisite for achieving the goals of life - Dharma (duties), Arth (finance), Kama (materialistic desires) and Moksha (salvation). As per the fundamental basis of Ayurveda, all objects and living bodies are composed of five basic elements, called the Pancha Mahabhootas, namely: Prithvi (earth), Jal (water), Agni (fire), Vayu (air) and Akash (ether). Ayurveda imbibes the humoral theory of Tridosha- the Vata (ether + air), Pitta (fire) and Kapha (earth + water), which are considered as the three physiological entities in living beings responsible for all metabolic functions. The mental characters of human beings are attributable to Satva, Rajas and Tamas, which are the psychological properties of life collectively terms as 'Triguna'. Ayurveda aims to keep structural and functional entities in a state of equilibrium, which signifies good health (Swasthya). Any imbalance due to internal or external factors leads to disease and the treatment consists of restoring the equilibrium through various procedures, regimen, diet, medicines and behavior change. Understanding of 'Functional Anatomy' i.e. Sharir is the unique contribution of Ayurveda to the modern science which has great potential for new discoveries in System Biology.[12]

Siddha

Siddha system of medicine is practiced in some parts of South India especially in the state of Tamilnadu. It has close affinity to Ayurveda yet it maintains a distinctive identity of its own. This system has come to be closely identified with Tamil civilization. The term 'Siddha' has come from 'Siddhi'- which means achievement. Siddhars were the men who achieved supreme knowledge in the field of medicine, yoga or tapa (meditation).[13]

It is a well-known fact that before the advent of the Aryans in India a well-developed civilization flourished in South India especially on the banks of rivers Cauvery, Vaigai, Tamiraparani etc. The system of medicine in vogue in this civilization seems to be the precursor of the present day Siddha system of medicine. During the passage of time it interacted with the other streams of medicines complementing and enriching them and in turn getting enriched. The materia medica of Siddha system of medicine depends to large extent on drugs of metal and mineral origin in contrast to Ayurveda of earlier period, which was mainly dependent upon drugs of vegetable origin.

According to the tradition eighteen Siddhars were supposed to have contributed to the development of Siddha medicine, yoga and philosophy. However, literature generated by them is not available in entirety. In accordance with the well-known self-effacing nature of ancient Indian Acharyas (preceptors) authorship of many literary work of great merit remains to be determined. There was also a tradition of ascribing the authorship of one's work to his teacher, patron even to a great scholar of the time. This has made it extremely difficult to clearly identify the real author of many classics.[14]

Homeopathy

Homeopathy is a distinct medical specialty being practiced across the world. It is a recognized medical system in India through the **Homeopathy Central Council Act, 1973.** The system has blended well into the ethos and traditions of the country that it has been recognized as one of the national systems of medicine.[15]

Homeopathic medicine, is a medical system that was developed in Germany more than 200 years ago. It's based on two unconventional theories:

- "Like cures like"—the notion that a disease can be cured by a substance that produces similar symptoms in healthy people

- "Law of minimum dose"—the notion that the lower the dose of the medication, the greater its effectiveness. Many homeopathic products are so diluted that no molecules of the original substance remain.[16]

Unani

The Unani medicine system was introduced to India about a thousand years ago by the Muslims and became indigenous to the country. It is now practiced in the Indo-Pakistan subcontinent. The Unani physicians who settled in India have added new drugs to the system and therefore the Unani system practiced in India is somewhat different from the original Greek form.[11]

Unani System of Medicine considers human body as a single unit, made by seven components known as Umoor-e-Tabiya. Based on Unani philosophy, the human body is made up of the four basic elements i.e. Earth, Air, water and fire which have different temperaments i.e. cold, hot, wet and dry respectively. After mixing and interaction of four elements a new compound having new Mizaj (temperament) comes into existence i.e. hot wet, hot dry, cold wet, and cold dry.[17, 18]

The body has the simple and compound organs, which receive their nourishment through four Akhlaat (Humors) i.e. Dam (Blood), Baigham (Phlegm), Safra (Yellow Bile) and Sauda (Black Bile). Each humor has its own temperament blood is hot and moist, phlegm is cold and moist, yellow bile is hot and dry and black bile is cold and dry[18,19]. Every person attains a temperament according to the preponderance of the humors in them body and it represents the person's healthy state. The temperament of a person may be sanguine, phlegmatic, choleric or melancholic.[19]

1.2 HYPERLIPIDAEMIA

Hyperlipidaemia is an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters and phospholipids and or plasma lipoproteins including very lowdensity lipoprotein and low-density lipoprotein, and reduced high-density lipoprotein levels.[20]

Intestinal Lipid Absorption

Growing bodies of evidences indicate, both in humans and animal models, that the small intestine is not only involved in the absorption of dietary lipids but actively regulates the production and secretion of CMs. The process of dietary lipid absorption is traditionally

divided into three components: (a) uptake into the enterocyte, (b) intracellular processing, and (c) transport into the circulation.[21]

Pancreatic lipase makes the first step possible through the hydrolysis of dietary fats, mostly triacylglycerols (TAG), within the lumen of the small intestine. Fatty acids (FA) and sn-2-monoacylglycerol (MAG) are the results of this enzymatic breakdown.[22] Hydrolysis products are then transported across the apical brush border membrane of the enterocyte by cluster determinant 36 (CD 36).[23]

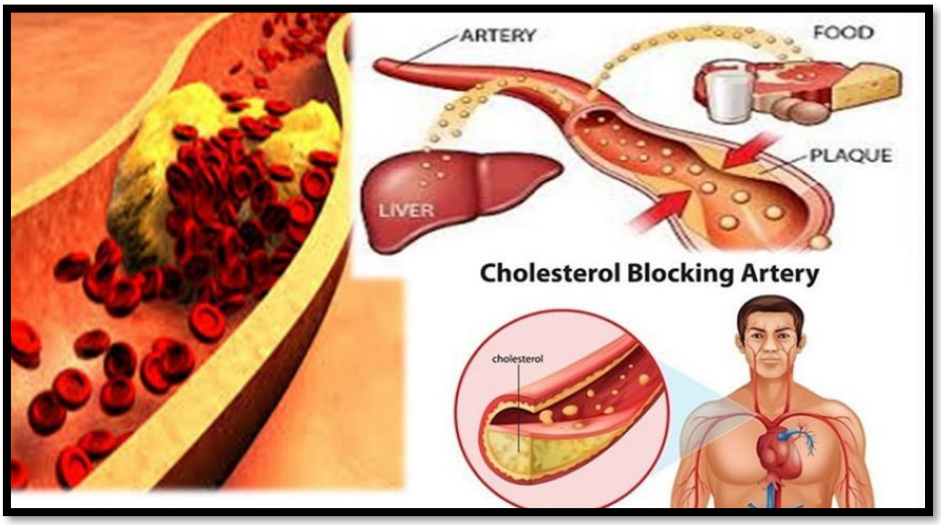


Fig. No. 1: Hyperlipidaemia Condition

The FA are then bound by FA binding proteins (FABPs) and targeted to microsomal compartments for re-esterification to triglycerides. De-novo lipogenesis represents another valid source of triglycerides useful for lipidation and this process is hormone-dependent.[21] CM assembly is a complex process that needs the activity of microsomal triglyceride transfer protein (MTP) to cotranslationally incorporate apoB-48 into a phospholipids-rich, dense, primordial chylomicron particle (prechylomicron).[24]

Then, prechylomicrons are included in a unique transport vesicle, the prechylomicron transport vesicle (PCTV), which is budded off the endoplasmic reticulum (ER) membrane and transported to the Golgi. Once into the Golgi compartment several chylomicrons fuse into another transport vesicle and are transported to the basolateral membrane for secretion in the circulation. Two different models have been proposed for CMs assembly. According to Hussain, the assembly of small nascent lipid poor CM particles and buoyant triglyceride-rich chylomicrons progress through independent pathways.[25] On the other hand the so called "core expansion" model, proposes that primordial chylomicrons and triglyceride-rich lipid droplets of various sizes join together to form lipoproteins of different size.[26]

Tab. No. 1: Regulators involved in the Lipoprotein Pathway

Enzymes	Function
HMG-CoA reductase	3-Hydroxy-3-methylglutaryl-coenzyme A reductase; the enzyme that catalyzes the rate-limiting step in cholesterol biosynthesis
Lipoprotein lipase (LPL)	An enzyme found primarily on the surface of endothelial cells that releases free fatty acids from triglycerides in lipoproteins; the free fatty acids are taken up into cells
Proliferator-activated receptor-alpha (PPAR-α)	Member of a family of nuclear transcription regulators that participate in the regulation of metabolic processes; target of the fibrate drugs and omega-3 fatty acids

Cholesterol

Cholesterol is a waxy fat molecule that the liver produces.[27] It is a major sterol in animal tissues, has a significant function in the human body. Cholesterol is a structural component of cell membranes and plays an integral role in membrane fluidity. Cholesterol is also important in the synthesis of lipid rafts which are needed for protein sorting, cellular signaling, and apoptosis.[28]

Cholesterol is derived both from the diet and by endogenous synthesis in the liver and it is a component of all cell membranes, a precursor of steroid hormones including estrogen, progesterone, testosterone, as well as vitamin D and bile salts, and of glycoproteins and quinones. The biochemistry and metabolism of cholesterol is complex. Cholesterol and other lipid fractions are transported in blood via lipoproteins of different densities.[29,30]

Triglycerides

Triacylglycerols (also called as triglycerides) are the most abundant lipids comprising 85-90% of body lipids. Most of the triglycerides (TG; also called neutral fat or depot fat) are stored in the adipose tissue and serve as energy reserve of the body. This is in contrast to carbohydrates and proteins which cannot be stored to a significant extent for energy purposes. Fat also acts as an insulating material for maintaining the body temperature of animals.[31]

Triglycerides are the most predominant storage form of energy. There are two main reasons for fat being the fuel reserve of the body

- Triglycerides (TG) are highly concentrated form of energy, yielding 9 Cal/g, in contrast to carbohydrates and proteins that produce only 4 Cal/g. This is because fatty acids found in TG are in the reduced form.

- The triglycerides are non-polar and hydrophobic in nature, hence stored in pure form without any association with water (anhydrous form).[32]

Lipoproteins

Lipoproteins are macro molecules aggregate composed of lipids and proteins; this structure facilitates lipids compatibility with the aqueous body fluids.[20] While in circulation, cholesterol, being a lipid, requires a transport vesicle to shield it from the aqueous nature of plasma. Complex, micelle-like amalgamations of various proteins and lipids achieve cholesterol transport through the vascular system. These particles, intuitively known as lipoproteins, are heterogeneous in size, shape, composition, function.[33]

Lipoproteins deliver the lipid components (cholesterol, triglycerides etc.) to various tissues for utilization.[34] Homeostasis of cholesterol is centered on the metabolism of lipoproteins, which mediate transport of the lipid to and from tissues.[33] Plasma lipoproteins are separated by hydrated density; electrophretic mobility; size; and their relative content of cholesterol, triglycerides, and protein into five major classes: chylomicrons, very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL).[35]

Tab. No. 2: Lipoprotein classes

Lipoprotein	Density (g/ml)	Size (nm)	Major Lipids	Major Apoproteins
Chylomicrons	<0.930	75-1200	Triglycerides	Apo B-48, Apo C, Apo E, Apo A-I, A-II, A-IV
VLDL	0.930- 1.006	30-80	Triglycerides	Apo B-100, Apo E, Apo C
IDL	1.006- 1.019	25-35	Triglycerides Cholesterol	Apo B-100, Apo E, Apo C
LDL	1.019- 1.063	18- 25	Cholesterol	Apo B-100
HDL	1.063- 1.210	5-12	Cholesterol Phospholipids	Apo A-I, Apo A-II, Apo C, Apo E

Chylomicrons

A small fat globule composed of protein and lipid. The chylomicrons are synthesized in the mucosa (the lining) of the intestine and are found in the blood and lymphatic fluid where they serve to transport fat from its port of entry in the intestine to the liver and to adipose tissue. After a fatty meal, the blood is so full of chylomicrons that it looks milky.[36]

Very Low Density Lipoproteins (VLDLs)

VLDLs are produced by the liver and are triglyceride rich. They contain apolipoprotein B-100, C-I, C-II, C-III, and E. Apo B-100 is the core structural protein and each VLDL particle contains one Apo B-100 molecule. Similar to chylomicrons the size of the VLDL particles can vary depending on the quantity of triglyceride carried in the particle,[47] but their triglyceride content is lower and cholesterol content higher than that of chylomicrons. Like chylomicrons, VLDLs are substrates for lipoprotein lipase-mediated triglyceride removal. Their function is to carry triglycerides synthesized in the liver and intestines to capillary beds in adipose tissue and muscle, where they are hydrolyzed to provide fatty acids that can be oxidized to produce adenosine triphosphate (ATP) for energy production. Alternatively, if not needed for energy production, they can be re-esterified to glycerol and stored as fat. After removal of their triglyceride, VLDL remnants (called IDLs) can be further metabolized to LDL. VLDLs serve as acceptors of cholesterol transferred from HDL. This transfer process is mediated by an enzyme called cholesterol ester transfer protein (CETP).[38]

Intermediate Density Lipoprotein (IDL)

Intermediate density lipoproteins (IDL) are also called as the VLDL remnants. These lipoproteins are less dense than LDL molecules but denser than VLDL particles. As the triglycerides on VLDL are broken down by the cells that need it, the particle becomes denser due to the change in the lipid to protein ratio. This results in VLDL being converted into IDL. Each native IDL particle consists of protein that encircles various fatty acids, enabling, as a water-soluble particle, these fatty acids to travel in the aqueous blood environment as part of the fat transport system within the body. IDL enable fats and cholesterol to move within the water-based solution of the bloodstream. Their size is, in general, 25 to 35 nm in diameter, and they contain primarily a range of triacylglycerols and cholesterol esters. They are cleared from the plasma into the liver by receptor-mediated endocytosis, or further degraded to form LDL particles.[39,40,41]

Low Density Lipoprotein (LDL)

These particles are derived from VLDL and IDL particles and they are even further enriched in cholesterol. LDL carries the majority of the cholesterol that is in the circulation. The predominant apolipoprotein is B-100 and each LDL particle contains one Apo B-100 molecule. LDL consists of a spectrum of particles varying in size and density. An abundance of small dense LDL particles are seen in association with hypertriglyceridemia, low HDL levels, obesity, type 2 diabetes (i.e. patients with the metabolic syndrome) and infectious and inflammatory states. These small dense LDL particles are considered to be more pro-atherogenic than large LDL particles for a number of reasons. Small dense LDL particles have a decreased affinity for the LDL receptor resulting in a prolonged retention time in the circulation. Additionally, they more easily enter the arterial wall and bind more avidly to intra-arterial proteoglycans, which traps them in the arterial wall. Finally, small dense LDL particles are more susceptible to oxidation, which could result in an enhanced uptake by macrophages.[37]

High Density Lipoprotein (HDL)

HDLs are heterogeneous particles regarding their size and composition. Compared with other lipoproteins, they have the highest relative density while being smallest in size. HDL have an important role in carrier in reverse cholesterol transport (RCT) and act as a carrier of cholesterol back to the liver. They effectively function in homeostasis and lipid metabolism.

HDL is mainly secreted by the liver and small intestines. The liver, which secretes ~70-80% of the total HDL in plasma, is the main source of HDL in the circulation. Apolipoprotein (apo)AI is the major structural protein and constitutes the framework of HDL to bear phospholipids and cholesterol. In addition to apoAI, several other apolipoproteins (for example, apoAII, apoAIV, apoB, apoCI and apoCII) contribute to the composition of HDL (1-3). HDL particles are highly uniform and can be divided into several sub-types based on their composition proteins or bulk density.[42]

Classification of HDL

Classification based on apoAII content:

In HDL, the content of apoAII content is lower than that of apoAI. HDL particles can be divided into two sub-types according to whether they contain apoAII. HDL of the LPAI category contain apoAI but not apoAII, while HDL of the LPAI: AII category contain apoAI as well as apoAII.

The difference between the two HDL subtypes regarding their function has remained to be fully elucidated. In human HDL, the small and dense apoAII-enriched HDL can stimulate paraoxonase1, platelet-activating factor acetylhyokolase, lipoprotein-associated phospholipase A2 and lecithin cholesterol acyltransferase (LCAT) activity and exert a higher anti-LDL-oxidative effect, as compared with HDL that does not contain apoAII.[43]

Classification based on buoyant density:[44]

Mature HDL can be divided into two subtypes, based on their buoyant density:

- HDL2 (1.063 g/ml<d<1.125 g/ml)
- HDL3 (1.125 g/ml<d<1.210 g/ml)

Using the method of gradient gel electrophoresis, they can be divided into five sub‑types:

- HDL2a (8.8-9.7 nm)
- HDL2b (9.7-12.9 nm)
- HDL3a (8.2-8.8 nm)
- HDL3b (7.8-8.2 nm)
- HDL3c (7.2-7.8 nm).

They can also be classified using non-denaturing two-dimensional gel electrophoresis:

- pre-β HDL/pre-β1HDL (d=5.6 nm)
- pre-β2HDL (d=12.0-14.0 nm)
- αHDL/α1HDL (d=11.0 nm)
- α2HDL (d=9.2 nm)
- α3HDL (d=8.0 nm)
- α4HDL (d=7.4 nm).

Functions of HDL

HDL acts as a carrier in Reverse Cholesterol Transport (RCT). A large number of epidemiological studies have found that low levels of high-density lipoprotein cholesterol (HDL-C) are an independent risk factor for atherosclerotic vascular disease (CVD). It is also having an anti-atherosclerotic effects.[45] According to the traditional view, HDL carries free cholesterol from peripheral cells, including macrophages and endothelial cells. Free cholesterol from HDL can be esterified into CE in the blood.[46]

HDL is also involved in the transport process of micro RNAs (miRNAs) in the cell. Biological studies have shown that HDL can combine with miRNAs by divalent cation binding.[47] HDL also has an anti-inflammatory role in macrophages and endothelial cells by inhibiting the expression of adhesion molecules.[48]

HDL exerts vascular protective effects by upregulating endothelial nitric oxide synthase (eNOs) expression and maintaining the caveolae lipid environment. HDL can boost the blood flow to resist thrombosis and inhibit platelet activation by inhibiting platelet-activating factor/cyclooxygenase A2. HDL can also lower anticoagulant activated protein C (APC) and thrombomodulin to reduce the formation of thrombin in endothelial cells and exerts an anti-thrombotic effect by inhibiting endothelial cell apoptosis and activities of tissue factors and endothelial cells.[49]

Classification and Hyperlipidaemia

Hyperlipoproteinemia[50,51]

Increased or decreased level of plasma lipoproteins is usually occurs due to abnormalities in the synthesis, degradation, and transport of their associated lipoprotein particles. Increased concentration of plasma lipids is etiologically related mainly to genetic disorders, dietary factors (such as ingestion of excessive calories, saturated fatty acids and cholesterol), or ingestion of drugs, or it may occur as a secondary phenomenon in a large variety of diseases. In any of these instances the elevation of the different plasma lipoproteins usually occurs in a number of combinations that have led to their classification into six different patterns or phenotypes.

Tab. No. 3: Lipoprotein Patterns Resulting from Elevation of Different Plasma Lipid Fractions

Lipoprotein pattern	Increased lipid fraction	Predominant lipoprotein
Type I	Triglycerides	Chylomicrons
Type 2a	Cholesterol	LDL
Type 2b	Cholesterol and triglycerides	LDL and VLDL
Type 3	Triglycerides and cholesterol	Remnants
Type 4	Triglycerides	VLDL
Type 5	Triglycerides and cholesterol	VLDL and chylomicrons

Type 1-Hyperchylomicronemia

Criteria

- Chylomicrons present.
- VLDL (pre-/3-lipoproteins) normal or only slightly increased.

Type II-Hyper-ß-lipoproteinemia

Abnormal increase in LDL(ß) concentration.

Type IIa

Criteria

- Increase in LDL (ß).
- Normal VLDL (pre-ß) concentrations.

Type IIb

Criteria

- Increase in LDL (ß).
- Increase in VLDL (pre- ß).

Type III-"Floating ß" or "Broad ß" Pattern

Criteria

- Increase in VLDL
- Increase in Triglycerides
- Abnormal electrophoretic mobility ("floating ß, ß-VLDL").

Type IV-Hyperpre-ß-lipoproteinemia

Criteria

- Increased VLDL (pre-ß).
- No increase in LDL (ß).
- Chylomicrons absent.

Type V-Hyperpre-ß-lipoproteinemia and Chylomicronemia

Criteria

- Increased VLDL
- Chylomicrons present

Hypercholesterolemia[51]

Three primary disorders causing hypercholesterolemia have been identified. They are:

Polygenic hypercholesterolemia

Polygenic hypercholesterolemia is the term utilized to describe the most common primary disorder causing an increase in plasma cholesterol. It includes a group of related disorders in which multiple genes apparently interact to cause an elevation in LDL above the 95th percentile in the general population. Increased rate of formation of LDL, defective clearance of LDL, or both could be responsible for this elevation.

Familial hypercholesterolemia

Familial hypercholesterolemia is a common autosomal dominant disorder that affects approximately 1:500 persons in the general population. Its principal defect lies in the gene for the LDL receptor on the surface of cells so that clearance of LDL from plasma is delayed. Homozygotes are rare and usually attain a six- to eightfold increase in total plasma cholesterol due to an elevation in LDL; heterozygotes may have a two - to threefold elevation and can be diagnosed at birth with analysis of umbilical cord blood. The most important clinical characteristic of this disorder is the presence of premature and accelerated coronary artery disease.

A variant of familial hypercholesterolemia

Familial combined hyperlipidaemia is another common disorder that has an autosomal dominant inheritance. It can present clinically as hypercholesterolemia (type 2a), hypertriglyceridemia (type 4) or both (type 2b) and has also been called multiple-type hyperlipoproteinemia. It is characterized clinically by the absence of hyperlipoproteinemia during childhood, and its development occurs around puberty in association with variable and mild elevation in plasma lipid levels. There is no specific clinical or laboratory test to determine if an individual has this disorder, and family screening is needed in order to make the diagnosis.

Hypertriglyceridemia[51]

The primary disorders predominantly causing hypertriglyceridemia are:

Familial hypertriglyceridemia

Familial hypertriglyceridemia is a common autosomal dominant disorder characterized by increased plasma concentration of VLDL (type 4 lipoprotein pattern). Moderate elevations of triglycerides usually occur during early adulthood, and a triad of obesity, hyperglycemia, and hyperinsulinemia can be seen in affected individuals. In

individuals with moderate elevation in plasma triglycerides associated with a normal cholesterol level, the possibility of familial hypertriglyceridemia should be suspected.

Congenital deficiency of lipoprotein lipase

Congenital lipoprotein lipase deficiency is a rare autosomal recessive disorder secondary to absence or severe diminution in the activity of lipoprotein lipase. Affected individuals are homozygous for a mutation that prevents normal expression of lipoprotein lipase activity. The parents, although clinically normal, are obligate heterozygotes. This enzymatic disorder is reflected in a massive accumulation of chylomicrons in the plasma without elevation of VLDL (type 1 lipoprotein pattern). Triglycerides may reach levels of 2000 to 10,000 mg/dl. This disorder usually appears in childhood with recurrent bouts of abdominal pain secondary to pancreatitis.

Deficiency of apoprotein CII

Apoprotein CII deficiency is a rare autosomal recessive disorder caused by absence of apoprotein CII, a required cofactor for the activity of lipoprotein lipase. The ensuing functional deficiency in this enzyme leads to a clinical picture similar to that described above for congenital lipoprotein lipase deficiency.

Familial dysbetalipoproteinemias.

Familial dysbetalipoproteinemia, also called familial type 3 hyperlipoproteinemia, is a condition inherited through a single gene mechanism whose clinical presentation requires the presence of other genetic or environmental factors . Elevation of both plasma cholesterol and triglycerides occurs because of accumulation of remnant VLDL particles in the plasma. The metabolic defect in most patients occurs in apolipoprotein E. This has three common alleles, designated E^2, E^3, and E^4. Patients with this disorder have only apolipoprotein E^2 in VLDL, which is less effective in facilitating clearance of remnants than E^3 or E^4.

Causes of Hyperlipidaemia

Abnormal lipid profiles are generally a combination of abnormalities of the lipoprotein fractions. Hyperlipidaemia can broadly be classified as isolated elevation of cholesterol, isolated elevated TG and elevations of both. The cause may be genetic, environmental or both.[52]

Tab. No. 4: Causes and Clinical features of Hyperlipidaemia

CAUSES	CLINICAL FEATURES
Isolated cholesterol elevation	
Genetic Familial Hypercholesterolemia	relatively common (1 in 500 heterozygote); TC exceeds 300 mg/dL, family history of elevated TC common, associated with tendon xanthomas, premature (20 – 40 years old) CVD is common Homozygotes are rare, but have TC > 600 and if not treated usually die of MI prior to age 20.
Familial Defective Apolipoprotein B100	increases LDL and has a phenotype that is indistinguishable from that of FH, including increased susceptibility to CHD
Mutations Associated with Elevated LDL Levels	Rare and isolated; suspect if elevated LDL unresponsive to treatment
Elevated Plasma Lipoprotein(a)	Relationship to CVD unclear, studies contradictory.
Polygenic Hypercholesterolemia	No family history, no physical manifestations such as xanthomas, exact cause is unknown
Lp(X)	Associate with obstructive hepatic disease, CVD risk unclear
Sitosterolemia	rare; plant sterols absorbed in large amounts, tendon xanthomas develop in childhood, LDL levels normal to high
Cerebrotendinous Xanthomatosis	rare; associated with neurologic disease, tendon xanthomas, and cataracts in young adults
Elevated cholesterol and triglycerides	
Combined (Familial) Hyperlipidaemia	May occur randomly or with strong family history of hyperlipidaemia; type 2 diabetes and metabolic syndrome are associated and can make diagnosis more difficult
Familial Dysßlipoproteinemia (Type III Hyperlipoproteinemia)	severe hypertriglyceridemia and hypercholesterolemia (both often > 300mg/dL), associated with premature diffuse vascular disease, male predominance, Palmar xanthomas are pathognomonic
Hepatic Lipase Deficiency	Rare disorder with very high cholesterol and triglyceride concentrations, phenotypically similar to familial dysbetalipoproteinemia.
Isolated triglyceride elevations	

LPL deficiency	Results in elevated chylomicrons, which carry dietary fat; chylomicrons are generally not present after an overnight fast, so a creamy looking plasma in a fasting specimen should be a clue to the diagnosis, especially if seen in young children; extremely high triglycerides can lead to pancreatitis
ApoCII deficiency	This apolipoprotein is an activator of LPL; its absence causes a clinical picture identical to LPL deficiency
Familial hypertriglyceridemia	Autosomal dominant inheritance; Main defect is overproduction of VLDL triglycerides by the liver;

Secondary cause for Hyperlipidaemia:

Secondary causes of hyperlipidaemia are important to recognize. Some times hyperlipidaemia will be a clue to diagnose the underlying systemic disorders. It may greatly result in the risk of atherosclerosis with raised LDL concentration, triglyceride rich lipoprotein excess and also decrease in HDL concentration. Diagnosis of secondary causes is clue as to why the patient with suddenly developed worsening in lipid profiles.[53]

Diet

Foods which contain cholesterol, saturated fat, and Trans fats can raise your blood cholesterol level. These include: Cheese, Egg yolk, Fried and processed food, Ice cream, Pastries, Red meat.[54] Fish oil can also elevate LDL concentration when it is given to lower triglycerides in diabetics and patients with familial combined hyperlipidaemia.[55] Dietary factors that lower cholesterol include soluble fiber as well as substituting unsaturated fats or complex carbohydrates for saturated fats. Diets rich in unsaturated fats can lower HDL concentration slightly in men but not in women.[56] Alcohol can raise triglycerides as well as HDL concentration and can markedly aggravate hyperlipidaemia in patients with preexisting hypertriglyceridemia.[57]

Drugs

Drug-induced lipid and lipoprotein changes can clearly improve or aggravate atherogenic risk or heighten the risk of pancreatitis when they promote severe hypertriglyceridemia.[58]

Steroids

Steroid hormones can have a significant impact on lipid and lipoprotein concentrations. Cholesterol is the precursor of adrenocorticosteroids, androgens, estrogens, and progestins. Improper usage of these classes can convert a mild primary lipid abnormality into a clinically life-threatening situation.[59] A study of normal men and women showed that prednisone caused total cholesterol levels to be increased by 17.3%, triglyceride levels to be increased in women only, LDL-c to be increased by 10.9% (not significant), and HDL-c to be increased by 68%.[60]

Female Hormone Preparations

Estrogens raise triglycerides and HDL-c. These are elevated 1.5-fold to 2.5-fold in proportion to the potency.[61] LDL-c tends to be elevated with increasing estrogen potency in those on oral contraceptives. Progestins tend to lower triglycerides and HDL-c and in general have effects that are in the opposite direction of the estrogens. Medroxyprogesterone acetate is similar to progesterone and is used in combination with estrogen for postmenopausal estrogen replacement in women with an intact uterus. Norgestrel and norethindrone are derived from 19-nortestosterone and are used in birth control formulations with norgestrel more likely to raise LDL-c and lower HDL-c than norethindrone.[62]

Diuretics

In short-term studies, diuretics raise total cholesterol 5% to 8%, triglycerides 15% to 25%, and LDL concentration 8%.[63]

Alpha & Beta Blockers

These antihypertensives are associated with no change in LDL concentration and may cause increased HDL concentration. The mechanism is thought to be diminished clearance of apo Al HDL concentration.[64] Beta blockers raise triglycerides and lower HDL concentration.[65]

Hypothyroidism

Thyroid deficiency is also implicated in hypertriglyceridemia. Because thyroid deficiency can lead to a decrease in LPL activity, the hypertriglyceridemia of an underlying genetic triglyceride disorder can be exacerbated, and chylomicronemia can occur.[66]

Obesity

Obese subjects often have increased triglycerides and low HDL concentration. Obese subjects have increased synthetic rates for cholesterol and bile acids. They have increased turnover of apo LDL, but this is not necessarily associated with high LDL concentration levels.[67] Another way to look at lipid and lipoprotein changes in obesity is to consider what

happens when the obese undergo weight loss. With weight loss, triglycerides decrease early with a delayed effect on rise of HDL concentration.[68]

Diabetes Mellitus

In noninsulin-dependent diabetics, mild hypertriglyceridemia and low HDL-c are often seen and are due to both overproduction and removal defects. When a familial form of hypertriglyceridemia that causes enhanced production of triglyceride-rich VLDL and noninsulin-dependent diabetes coexist, removal mechanisms for dietary glyceride become saturated.[69]

Risks of Hyperlipidaemia

High cholesterol is associated with an elevated risk of cardiovascular disease. That can include coronary heart disease, stroke, and peripheral vascular disease. High cholesterol has also been linked to diabetes and high blood pressure.[70]

Strokes

Strokes (cerebrovascular accidents) are considered to be one of the most common causes of mortality and long term severe disability. There is a positive correlation between serum total cholesterol (TC) concentrations and ischaemic (thrombotic) stroke, and very low TC concentrations have been associated with an increased risk of haemorrhagic stroke. Raised low density lipoprotein cholesterol (LDL) or triglyceride (TG) concentrations, reduced high density lipoprotein cholesterol (HDL) concentrations, and a high TC to HDL ratio are associated with an increased risk of non-haemorrhagic stroke. There is evidence that lipoprotein (a) is a predictor of many forms of vascular disease, including premature coronary artery disease (CAD).[71,72]

Cardiovascular Disease (CVD)

According to the WHO, CVDs are the number 1 cause of death globally: more people die annually from CVDs than from any other cause. An estimated 17.9 million people died from CVDs in 2016, representing 31% of all global deaths. Of these deaths, 85% are due to heart attack and stroke. In low and middle income countries 37% of premature death was caused by the CVDs.[73] Coronary and peripheral artery diseases are caused by the hyperlipidaemic condition. The importance of total cholesterol for coronary artery disease (CAD) risk has been demonstrated in observational epidemiologic studies carried out over the last three decades.[74] The most common reason for the CAD is that build-up of fatty deposits on the inner walls of the blood vessels that supplies blood to the heart. Approximately 70% of cholesterol is transported in blood as low density lipoprotein (LDL) cholesterol. Much of the remaining cholesterol is transported from non-hepatic cells to the liver for synthesis into

lipoproteins, bile acids and steroids by high density lipoprotein (HDL), by a process known as reverse cholesterol transport. Disturbances in reverse cholesterol transport have been shown to enhance the deposition of LDL-cholesterol into the artery wall, resulting in atherosclerotic lesions.[75]

Hypertension

Raised blood pressure attributes to the leading risk factor for morbidity and mortality in India. Hypertension is attributable to 10.8% of all deaths in India.[76] Increased level of cholesterol in blood circulation may increase the risk of hypertension. The excess oily stuffs in cholesterol stick in to the walls of the arteries create a fatty build up, that eventually hardens and forming an inflexible plaque that damages the arteries and they become stiff and narrowed. The blood cannot able to flow easily through the blood vessels that lead to the hypertension.[77]

Antihyperlipidaemic drugs

These are drugs which lower the levels of lipids and lipoproteins in blood and have attracted considerable attention because of their potential to prevent cardiovascular disease by retarding the accelerated atherosclerosis in hyperlipidaemic individuals.[78]

Classification of Antihyperlipidaemic Drugs[78]

a) **HMG-CoA reductase inhibitors (Statins)**
   Lovastatin, Simvastatin, Pravastatin, Atorvastatin, Rosuvastatin, Pitavastatin
b) **Bile acid sequestrants (Resins)**
   Cholestyramine, Colestipol
c) **Lipoprotein lipase activator/ PPARα activators (Fibrates)**
   Fenofibrate, Bezafibrate, Clofibrate, Gemfibrozil
d) **Lipolysis and Triglyceride synthesis inhibitors**
   Nicotinic acid
e) **Sterol absorption inhibitor**
   Ezetimibe

HMG-CoA Inhibitors (Statins)

Statins were isolated from a mold, *Penicillium citrinum,* and identified as inhibitors of cholesterol biosynthesis. Subsequent studies established that statins act by inhibiting HMG-CoA reductase, which catalyzes an early, rate-limiting step in cholesterol biosynthesis. The first statin studied in humans was compactin, renamed mevastatin, which demonstrated the therapeutic potential of this class of drugs. The statins are the most effective and best-tolerated agents for treating dyslipidemia. Higher doses of the more potent statins (e.g.,

atorvastatin, simvastatin, and rosuvastatin) also can reduce triglyceride levels caused by elevated VLDL levels.[79]

Mechanism of action

Statins exert their major effect (reduction of LDL levels) through a mevalonic acid–like moiety that competitively inhibits HMG-CoA reductase. By reducing the conversion of HMG-CoA to mevalonate, statins inhibit an early and rate-limiting step in cholesterol biosynthesis, which results in increased expression of the LDL receptor gene. In response to the reduced free cholesterol content within hepatocytes, membrane-bound sterol regulatory element binding proteins (SREBPs) are cleaved by a protease and translocated to the nucleus. The transcription factors then bind the sterol-responsive element of the LDL receptor gene, enhancing transcription and increasing the synthesis of LDL receptors. Degradation of LDL receptors also is reduced. The greater number of LDL receptors on the surface of hepatocytes results in increased removal of LDL from the blood, thereby lowering LDL-C levels.[79]

Adverse effect

The major adverse effect of statin use is Myopathy. Hepatotoxicity is rarely observed. Gastrointestinal complaints and headache are usually mild.[78]

Bile acid sequestrants (Resins)

The bile-acid sequestrants or resins are among the oldest of the hypolipidemic drugs, and they are probably the safest, because they are not absorbed from the intestine. These resins also are recommended for patients 11-20 years of age. Because statins are more effective as monotherapy, the resins are most often used as second agents if statin therapy does not lower LDL-C levels sufficiently. When used with a statin, cholestyramine and colestipol usually are prescribed at submaximal doses. Maximal doses can reduce LDL-C by up to 25% but are associated with unacceptable gastrointestinal side effects. Colesevelam is a newer bile-acid sequestrant that is prepared as an anhydrous gel and taken as a tablet or as a powder that is mixed with water and taken as an oral suspension. It lowers LDL-C by 18% at its maximum dose.[79]

Mechanism of action

The bile-acid sequestrants are highly positively charged and bind negatively charged bile acids. Because of their large size, the resins are not absorbed, and the bound bile acids are excreted in the stool. Because more than 95% of bile acids are normally reabsorbed, interruption of this process depletes the pool of bile acids, and hepatic bile-acid synthesis increases. As a result, hepatic cholesterol content declines, stimulating the production of LDL receptors, an effect similar to that of statins. The increase in hepatic LDL receptors increases

LDL clearance and lowers LDL-C levels, but this effect is partially offset by the enhanced cholesterol synthesis caused by upregulation of HMG-CoA reductase. Inhibition of reductase activity by a statin substantially increases the effectiveness of the resins.[79]

Adverse effect

They are unpalatable in nature and inconvenient; have to be taken in large doses it causes flatulence and other gastrointestinal symptoms, interfere with absorption of many other drugs.

Lipoprotein lipase activators (Fibrates)

The fibrates are mainly used to treat two hyperlipidemias, familial hypertriglyceridemia (type IV) and dysbetalipoproteinemia (type III). They are also useful in the treatment of hypertriglyceridemia associated with type II diabetes (secondary hyperlipidemia). The fibrates are the drugs of choice in treating hypertriglyceridemias, particularly those associated with low levels of HDL cholesterol.The fibrates additionally appear to shift LDL particles to larger, hence less atherogenic species.[80]

Mechanism of action

The fibrates typically lower VLDL triglyceride by 40% or more and elevate plasma HDL cholesterol by 10 to 15%. The reduction of plasma triglycerides in humans appears due to increased lipoprotein lipase (LPL) activity. The fibrates activate a nuclear receptor (transcription factor) termed peroxisomal proliferation activated receptor (PPAR) that is a member of the steroid hormone receptor superfamily. PPAR increases transcription of the LPL gene and decreases transcription of the apolipoprotein CIII gene (apo CIII). Since LPL is responsible for catabolism of VLDL triglyceride and apo CIII is an inhibitor of LPL activity, the combined consequences of these changes are increased LPL activity and enhanced removal of triglyceride from the circulation.[80]

Adverse effect

Fibric acid compounds usually are well tolerated. Side effects may occur in 5-10% of patients but most often are not sufficient to cause discontinuation of the drug. Gastrointestinal side effects occur in up to 5% of patients. Other side effects are reported infrequently and include rash, urticaria, hair loss, myalgias, fatigue, headache, impotence, and anemia. Minor increases in liver transaminases and alkaline phosphatase have been reported.[79]

Lipolysis and Triglycerides synthesis inhibitors[79]

Niacin (nicotinic acid) is one of the oldest drugs used to treat dyslipidemia, favorably affects virtually all lipid parameters. Niacin is a water-soluble B-complex vitamin that functions as a vitamin only after its conversion to NAD or NADP, in which it occurs as an amide. Both niacin and its amide may be given orally as a source of niacin for its functions as a vitamin, but only niacin affects lipid levels. The hypolipidemic effects of niacin require larger doses than are required for its vitamin effects. Niacin is the best agent available for increasing HDL-C (30-40%); it also lowers triglycerides by 35-45% (as effectively as fibrates and the more effective statins) and reduces LDL-C levels by 20-30%. Niacin also is the only lipid-lowering drug that reduces Lp(a) levels significantly.

Mechanism of action

In adipose tissue, niacin inhibits the lipolysis of triglycerides by hormone-sensitive lipase, which reduces transport of free fatty acids to the liver and decreases hepatic triglyceride synthesis.

Adverse effect

Dyspepsia, Flushing and Hepatotoxicity. In patients with diabetes mellitus, niacin should be used cautiously because niacin-induced insulin resistance can cause severe hyperglycemia. Reversible side effects include toxic amblyopia and toxic maculopathy. Atrial tachyarrhythmias and atrial fibrillation have been reported, more commonly in elderly patients.[79]

Sterol absorption inhibitor[81]

Ezetimibe is the first compound approved for lowering total and LDL-C levels that inhibits cholesterol absorption by enterocytes in the small intestine. It lowers LDLC levels by 20% and is used primarily as adjunctive therapy with statins.

Mechanism of action

Ezetimibe inhibits luminal cholesterol uptake by jejunal enterocytes, by inhibiting the transport protein NPC1L.

Adverse effects

Other than rare allergic reactions, specific adverse effects have not been observed in patients taking ezetimibe.

1.3 Diabetes

As per the WHO, diabetes mellitus (DM) is defined as a heterogeneous metabolic disorder characterised by common feature of chronic hyperglycaemia with disturbance of carbohydrate, fat and protein metabolism. The number of individuals with diabetes is rising rapidly throughout the world. Both genetic and environmental factors contribute to its pathogenesis, which involves insufficient insulin secretion, reduced responsiveness to endogenous or exogenous insulin, increased glucose production, and/or abnormalities in fat and protein metabolism. The resulting hyperglycemia may lead to both acute symptoms and metabolic abnormalities.

DM is a leading cause of morbidity and mortality world over. It is estimated that approximately 1% of population suffers from DM. The incidence is rising in the developed countries of the world at the rate of about 10% per year, especially of type 2 DM, due to rising incidence of obesity and reduced activity levels. DM is expected to continue as a major health problem owing to its serious complications, especially end-stage renal disease, IHD, gangrene of the lower extremities, and blindness in the adults. It is anticipated that the number of diabetics will exceed 250 million by the year 2010. [82, 83]

Diabetes mellitus is a metabolic disorder of multiple etiologies. It is characterized by chronic hyperglycemia together with disturbances of carbohydrate, fat and protein metabolism resulting from defects of insulin secretion, insulin action or both. The relative contribution of these varies between different types of diabetes. These are associated with the development of the specific microvascular complications of retinopathy, which can lead to blindness, nephropathy with potential renal failure, and neuropathy. The latter carries the risk of foot ulcers and amputation and also autonomic nerve dysfunction. Diabetes is also associated with an increased risk of macrovascular disease. [84]

Classification

The older classification systems dividing DM into primary (idiopathic) and secondary types, juvenile-onset and maturity onset types, and insulin-dependent (IDDM) and non-insulin dependent (NIDDM) types, have become obsolete and undergone major revision due to extensive understanding of etiology and pathogenesis of DM in recent times. [81, 85]

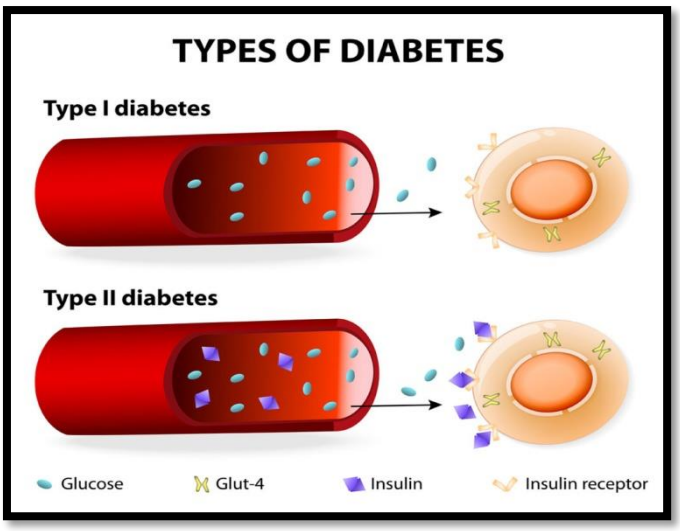


Fig. No. 2: Types of Diabetes Mellitus

I. **TYPE 1 DIABETES MELLITUS** (10%) (earlier called Insulin-dependent, or juvenile-onset diabetes)
Type IA DM: Immune-mediated
Type IB DM: Idiopathic

II. **TYPE 2 DIABETES MELLITUS** (80%) (earlier called non-insulin-dependent, or maturity-onset diabetes)

III. **OTHER SPECIFIC TYPES OF DIABETES** (10%)

- **a.** Genetic defect of β-cell function due to mutations in various enzymes (earlier called maturity-onset diabetes of the young or MODY) (e.g. hepatocyte nuclear transcription factor—HNF, glucokinase)
- **b.** Genetic defect in insulin action (e.g. type A insulin resistance)
- **c.** Diseases of exocrine pancreas (e.g. chronic pancreatitis, pancreatic tumours, post-pancreatectomy)
- **d.** Endocrinopathies (e.g. acromegaly, Cushing's syndrome, pheochromocytoma)
- **e.** Drug- or chemical-induced (e.g. steroids, thyroid hormone, thiazides, β-blockers etc)
- **f.** Infections (e.g. congenital rubella, cytomegalovirus)
- **g.** Uncommon forms of immune-mediated DM (stiff man syndrome, anti-insulin receptor antibodies)
- **h.** Other genetic syndromes (e.g. Down's syndrome, Klinefelter's syndrome, Turner's syndrome)

IV. GESTATIONAL DIABETES MELLITUS

TYPE 1 DM

It constitutes about 10% cases of DM. It was previously termed as juvenile-onset diabetes (JOD) due to its occurrence in younger age, and was called insulindependent DM (IDDM) because it was known that these patients have absolute requirement for insulin replacement as treatment. However, in the new classification, neither age nor insulin-dependence are considered as absolute criteria. Instead, based on underlying etiology, type 1 DM is further divided into 2 subtypes: [81]

Subtype 1A (immune-mediated) DM: This forms diabetes, which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulindependent diabetes or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the ß-cells of the pancreas. Markers of the immune destruction of the ß-cell include islet cell autoantibodies, autoantibodies to insulin, autoantibodies to GAD (GAD65), and autoantibodies to the tyrosine phosphatases IA-2 and IA-2ß. [86]

Subtype 1B (idiopathic) DM: Some forms of type 1 diabetes have no known etiologies. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. Only a minority of patients with type 1 diabetes fall into this category. Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes. This form of diabetes is strongly inherited, lacks immunological evidence for b-cell autoimmunity, and is not HLA associated.

Though type 1 DM occurs commonly in patients under 30 years of age, autoimmune destruction of β-cells can occur at any age. In fact, 5-10% patients who develop DM above 30 years of age are of type 1A DM and hence the term JOD has become obsolete. [86]

Gestational Diabetes

Gestational diabetes mellitus (GDM) is hyperglycemia first detected during pregnancy. This is distinct from women with diabetes undergoing pregnancy, who have diabetes in pregnancy rather than gestational diabetes. Plasma glucose levels, both fasting and post - prandial, are lower than normal in early pregnancy so that raised levels at this stage are almost certainly caused by previously undetected T2DM. Screening for GDM is generally undertaken at around 28 weeks. There is signifi cant morbidity associated with GDM including intrauterine fetal death, congenital malformations, neonatal hypoglycemia, jaundice, prematurity and macrosomia. Risk factors for GDM include certain ethnic groups,

those with previous GDM or abnormalities of glucose tolerance, age, obesity and previous large babies. [85]

Diagnosis [87]

Glycated hemoglobin (A1C) test.

This blood test, which doesn't require fasting, indicates your average blood sugar level for the past two to three months. It measures the percentage of blood sugar attached to hemoglobin, the oxygen-carrying protein in red blood cells.

Fasting blood sugar test.

A blood sample will be taken after an overnight fast. A fasting blood sugar level less than 100 mg/dL (5.6 mmol/L) is normal. A fasting blood sugar level from 100 to 125 mg/dL (5.6 to 6.9 mmol/L) is considered prediabetes. If it's 126 mg/dL (7 mmol/L) or higher on two separate tests, you have diabetes.

Oral glucose tolerance test

For this test, you fast overnight, and the fasting blood sugar level is measured. Then you drink a sugary liquid, and blood sugar levels are tested periodically for the next two hours. A blood sugar level less than 140 mg/dL (7.8 mmol/L) is normal. A reading of more than 200 mg/dL (11.1 mmol/L) after two hours indicates diabetes. A reading between 140 and 199 mg/dL (7.8 mmol/L and 11.0 mmol/L) indicates prediabetes.

Management of Diabetes [87]

Depending on the type of diabetes blood sugar monitoring, insulin and oral hypoglycemic therapy, healthy diet and maintaining the healthy body weight in regular life play a major role in the management of diabetes.

Healthy diet

There is no specific diet for diabetes but adding more fruits, vegetables, lean proteins and whole grains in regular diet, foods which are higher in nutrition and fiber and low in fat and calories helps to control the blood sugar level. Patient with diabetes have to avoid saturated fatty food, refined carbohydrates and sweets.

Physical activity

Every one need regular aerobic exercise, in patient with diabetes mellitus helps to promote the transport of sugar into the cells where it is used for energy. Exercise increases the sensitivity of the insulin, which means body needs less insulin to transport sugars into the cells.

Monitoring of blood sugar

Careful monitoring is the only way to make sure that the blood sugar level remains within the normal range. Depend on the treatment, periodic monitoring of blood glucose level in essential to assure the safety of the patients. Treatment with oral hypoglycemic drugs and insulin some time leads to the hypoglycemic level, periodic measure of blood sugar level helps to avoid such a conditions.

Insulin Therapy

Insulin is a peptide hormone produced by beta cells of the pancreatic islets; it is considered to be the main anabolic hormone of the body. [88] Insulin is responsible for allowing glucose in the blood to enter cells, providing them with the energy to function. The pancreas is an organ behind the stomach that is the main source of insulin in the body. Clusters of cells in the pancreas called islets produce the hormone and determine the amount based on blood glucose levels in the body. The higher the level of glucose, the more insulin goes into production to balance sugar levels in the blood. Insulin also assists in breaking down fats or proteins for energy. [89]

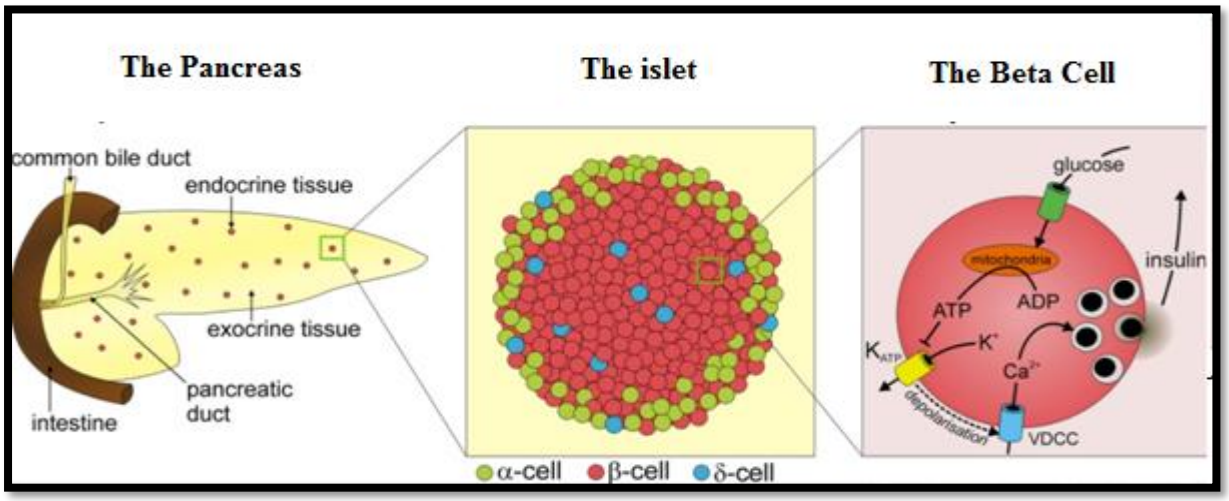


Fig. No. 3: Secretion and Release of Insulin

Types

Rapid Acting

The body absorbs this type into the bloodstream from the subcutaneous tissue extremely quickly.

- Insulin Lispro, Insulin Aspart, Insulin Glulisine

Short acting

It starts working approximately 30 minutes after injection and peaks at approximately 2 to 3 hours but will continue to work for three to six hours.

- Regular (soluble) insulin

Intermediate Acting

This type insulin enters the bloodstream at a slower rate but has a longer-lasting effect. It is most effective at managing blood sugar overnight, as well as between meals.

- Insulin Zinc Suspension or Lente, Neutral Protamine Hagedorn (NPH)

Long-acting insulin

Long-acting insulin is slow to reach the bloodstream and has a relatively low peak, it has a stabilizing "plateau" effect on blood sugar that can last for most of the day.

- Protamine zinc insulin, Insulin glargine

Oral hypoglycemic therapy [90]

Oral hypoglycemic drugs are chemical agents which lowers the blood glucose level.

Classification

Sulfonylurea

- **1st Generation**
  Tolbutamide, Tolazamide, Chlorpropamide, Acetohexamide
- **2nd generation**
  Glipizide, Glyburide, Glimepride, Gliclazide

Biguanides

- Metformin

Meglitinides

- Repaglinde, Nataglinide

Thiazolidiones or Glitazones

- Pioglitazones, Troglitazone, Rosiglitazone

Alpha-glycosidase inhibitor

- Acarbose, Miglitazone

Dipeptide peptidase inhibitor (DPP-G)

- Vidagliptin, Sitagliptin

Amylin analogue

- Pramlintide

Incretin mimetic (Glucagon like peptide analogue)

- Exenatide, Liraglutide

1.4 Analgesia (Pain)

An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Pain is a conscious experience that results from brain activity in response to a noxious stimulus and engages the sensory, emotional and cognitive processes of the brain. In general terms we can distinguish two dimensions or components of pain: (a) sensory-discriminative and (b) affective-emotional.

Nociception is the process by which information about a noxious stimulus is conveyed to the brain. It is the total sum of neural activity that occurs prior to the cognitive processes that enable humans to identify a sensation as pain. Nociception is necessary but not sufficient for the experience of pain. [91]

Nociceptors

Nociceptors are special receptors which are activated through various noxious stimuli. They present at the free nerve endings of the primary afferent neuron. Each nociceptor has its own receptive field. This means one nociceptor will transduce the signal of pain when a particular region is skin is stimulated. The size of receptive fields are vary throughout the body and there is often overlap with neighbouring fields. [92]

Nociceptors can be found in the skin, muscle, joints, bone and organs (other than the brain) and can fire in response to a number of different stimuli. Three types of nociceptors exist:

- Mechanical nociceptors – detects sharp, pricking pain
- Thermal and mechano-thermal nociceptors – detects sensations which elicit pain which is slow and burning, or cold and sharp in nature
- Polymodal nociceptors – detects mechanical, thermal and chemical stimuli

Pathway of pain

Signals from mechanical, thermal and mechano-thermal nociceptors are transmitted to the dorsal horn of the spinal cord predominantly by Aδ fibres. These myelinated fibres have a low threshold for firing and the fast conduction speed means they are responsible for transmitting the first pain felt.

In addition, Aδ fibres permit for the localisation of pain and form the afferent pathway for the reflexes elicited by pain. Aδ fibres predominantly terminate in Rexed laminae I where they mainly release the neurotransmitter glutamate.

Polymodal nociceptors transmit their signals into the dorsal horn through C fibres. C fibres are unmyelinated and a slow conduction speed. For this reason, C fibres are responsible for the secondary pain we feel which is often dull, deep and throbbing in nature. These fibres typically have large receptive fields and therefore lead to poor localisation of pain. [92]

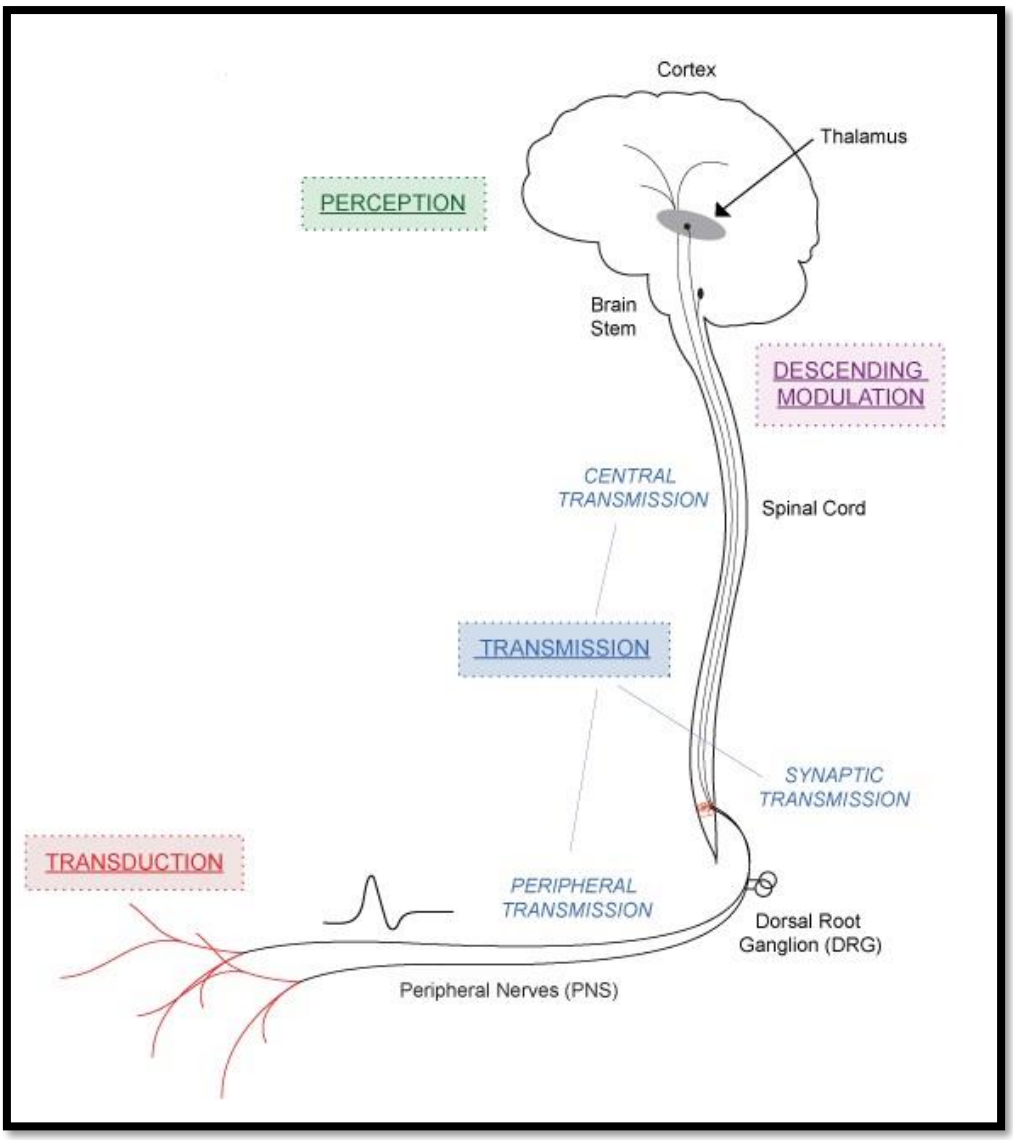


Fig. No. 4: Pathway of Pain

TYPES OF PAIN

Somatic Pain

Pain arising from the skin and integumental structures, muscles, bones and joints is known s Somatic pain. It is usually caused by inflammation and is well-defined or sharp pain.

Visceral Pain

Pain arising from the viscera is vague, dull- aching type, difficult to pinpoint to a site and is known as visceral pain. It may be accompanied by autonomic responses like sweating, nausea and hypotension. It may be due to spasm, ischaemia or inflammation.

Referred Pain

When pain is referred to a cutaneous area which receives nerve supply from the same spinal segment as that of the affected viscera, it is known as referred pain, e.g. cardiac pain referred to the left arm. [93]

Analgesics

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

Classification

- Opioid analgesics
- Non opioid analgesics

Opioid analgesics

Within the central nervous system there are three types of opioid receptors which regulate neurotransmission of pain signals. These receptors are called mu, delta and kappa opioid receptors. The drugs which bind to this receptors and relieves the pain are called as opioid receptors. They are all G protein coupled receptors and their activation leads to a reduction in neurotransmitter release and cell hyperpolarisation, reducing cell excitability.

Mechanism of action

Opioid analgesics bind with opioid receptors and reduce the neurotransmitter release from the first order neuron, and causes hyperpolarisation of the second order neuron. Together, this reduces the firing of action potentials in the second order neuron, blocking the transmission of pain signals. [92]

Classification of opioid analgesics

Agonists

- Morphine, codeine and its derivatives such as pethidine, methadone, levorphanol and tramadol.

Partial agonists

- Buprenorphine and meptazinol.

Mixed agonist

- Nalbuphine, pentazoxine and butorphanol

Non opioid analgesics

Non-opioid analgesics can be classified due to their chemical characteristics as acid (NSAIDs = Non-Steroidal Anti-Inflammatory Drugs) and non-acid. The common mechanism of action of these substances is their effect on prostaglandin synthesis. [94]

Classification

Acid/NSAIDs

i. Salicylates
   - Aspirin (Acetyl salicylic acid)

ii. Acetic acids
   - Diclofenac, ketorolac, indomethacin

iii. Propionic acids
   - Ibuprofen, naproxen, ketoprofen

iv. Oxicams
   - Piraxicam, tenoxicam, meloxicam

v. Coxibs
   - Celecoxib, rofecoxib, etoricoxib

Non acids

i. Anilines
   - Paracetamol

ii. Pyrazolones
   - Mitamizole

1.5 Pyrexia (Fever)

Fever is a complex physiologic response triggered by infectious or aseptic stimuli. Elevations in body temperature occur when concentrations of prostaglandin E2 (PGE2) increase within certain areas of the brain. These elevations alter the firing rate of neurons that control thermoregulation in the hypothalamus. Although fever benefits the nonspecific immune response to invading microorganisms, it is also viewed as a source of discomfort and is commonly suppressed with antipyretic medication. [95]

Pathogenesis of fever

The critical "endogenous pyrogens" involved in producing a highly regulated inflammatory response to tissue injury and infections are polypeptide cytokines. Pyrogenic cytokines, such as interleukin-1b (IL-1b), tumor necrosis factor (TNF), and interleukin-6 (IL-6), are those that act directly on the hypothalamus to produce a fever response. [96] Exogenous pyrogens, such as microbial surface components, evoke pyrexia most commonly through the stimulation of pyrogenic cytokines. The gram-negative bacteria's outer membrane lipopolysaccharide (endotoxin), however, is capable of functioning at the level of the hypothalamus, in much the same way as IL-1b. [97]

Antipyretics

The drugs which reduce the elevated body temperature are called as the antipyretic agents.

Classification

Salicylates

- Aspirin (acetylsalicylic acid), choline salicylate, magnesium salicylate and sodium salicylate

Acetaminophen

- Paracetamol

NSAIDs

- Ibuprofen, naproxen and ketoprofen

1.6 Wound [98]

Wound is an injury involving an external or internal break in body tissue, usually involving the skin.

Types

Abrasion

An abrasion occurs when your skin rubs or scrapes against a rough or hard surface. Road rash is an example of an abrasion. There's usually not a lot of bleeding, but the wound needs to be scrubbed and cleaned to avoid infection.

Laceration

A laceration is a deep cut or tearing of your skin. Accidents with knives, tools, and machinery are frequent causes of lacerations. In the case of deep lacerations, bleeding can be rapid and extensive.

Puncture

A puncture is a small hole caused by a long, pointy object, such as a nail or needle. Sometimes, a bullet can cause a puncture wound. Punctures may not bleed much, but these wounds can be deep enough to damage internal organs. If you have even a small puncture wound, visit your doctor to get a tetanus shot and prevent infection.

Avulsion

An avulsion is a partial or complete tearing away of skin and the tissue beneath. Avulsions usually occur during violent accidents, such as body-crushing accidents, explosions, and gunshots. They bleed heavily and rapidly.

Wound Healing [99]

Wound is an injury to living tissue caused by a cut, blow, or other impact, typically one in which the skin is cut or broken. Healing is the body response to injury in an attempt to restore normal structure and function. Healing involves 2 distinct processes:

- **Regeneration** when healing takes place by proliferation of parenchymal cells and usually results in complete restoration of the original tissues.
- **Repair** when healing takes place by proliferation of connective tissue elements resulting in fibrosis and scarring. At times, both the processes take place simultaneously.

Regeneration

Some parenchymal cells are short-lived while others have a longer lifespan. In order to maintain proper structure of tissues, these cells are under the constant regulatory control of their cell cycle. Regeneration of any type of parenchymal cells involves the following two processes:

i. Proliferation of original cells from the margin of injury with migration so as to cover the gap.

ii. Proliferation of migrated cells with subsequent differentiation and maturation so as to reconstitute the original tissue.

Repair

Repair is the replacement of injured tissue by fibrous tissue.

Two processes are involved in repair:

- Granulation tissue formation
- Contraction of wounds.

Repair response takes place by participation of mesenchymal cells (consisting of connective tissue stem cells, fibrocytes and histiocytes), endothelial cells, macrophages, platelets, and the parenchymal cells of the injured organ.

Granulation Tissue Formation

The term granulation tissue derives its name from slightly granular and pink appearance of the tissue. Each granule corresponds histologically to proliferation of new small blood vessels which are slightly lifted on the surface by thin covering of fibroblasts and young collagen. The following three phases are observed in the formation of granulation tissue.

Phase of inflammation

Following trauma, blood clots at the site of injury. There is acute inflammatory response with exudation of plasma, neutrophils and some monocytes within 24 hours.

Phase of clearance

Combination of proteolytic enzymes liberated from neutrophils, autolytic enzymes from dead tissues cells, and phagocytic activity of macrophages clear off the necrotic tissue, debris and red blood cells.

Phase of ingrowth of granulation tissue

This phase consists of two main processes

1. angiogenesis or neovascularisation,
2. fibrogenesis.

- **Angiogenisis**

  Formation of new blood vessels at the site of injury takes place by proliferation of endothelial cells from the margins of severed blood vessels. Initially, the proliferated endothelial cells are solid buds but within a few hours develop a lumen and start carrying blood. The newly formed blood vessels are more leaky, accounting for the oedematous appearance of new granulation tissue. Soon, these blood vessels differentiate into muscular arterioles, thin-walled venules and true capillaries. The process of angiogenesis is stimulated with proteolytic destruction of basement membrane. Angiogenesis takes place under the influence of following factors:

  a) Vascular endothelial growth factor (VEGF) elaborated by mesenchymal cells while its receptors are present in endothelial cells only.
  b) Platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), basic fibroblast growth factor (BFGF) and surface integrins are all associated with cellular proliferation.

- **Fibrogenesis**

  The newly formed blood vessels are present in an amorphous ground substance or matrix. The new fibroblasts originate from fibrocytes as well as by mitotic division of fibroblasts. Some of these fibroblasts have combination of morphologic and functional characteristics of smooth muscle cells (myofibroblasts). Collagen fibrils begin to appear by about 6th day. As maturation proceeds, more and more of collagen is formed while the number of active fibroblasts and new blood vessels decreases. This results in formation of inactive looking scar known as cicatrisation.

Contraction of Wounds

The wound starts contracting after 2-3 days and the process is completed by the 14th day. During this period, the wound is reduced by approximately 80% of its original size. Contracted wound results in rapid healing since lesser surface area of the injured tissue has to be replaced.

Treatment

Wound healing can be accomplished in one of the following two ways: [99]

- Healing by first intention (primary union)
- Healing by second intention (secondary union).

Healing by First Intention (Primary Union)

This is defined as healing of a wound which has the following characteristics:

- ✓ Clean and uninfected
- ✓ Surgically incised
- ✓ Without much loss of cells and tissue; and
- ✓ Edges of wound are approximated by surgical sutures.

Healing by Second Intention (Secondary Union)

This is defined as healing of a wound having the following characteristics:

- ✓ Open with a large tissue defect, at times infected
- ✓ Having extensive loss of cells and tissues; and
- ✓ The wound is not approximated by surgical sutures but is left open.

Drugs used on wound healing [100]

NSAIDs

- Ibuprofen

Effect: Affects inflammatory phase by inhibiting cyclo-oxygenase production; reduces tensile strength of wound.

- Colchicine

Effect: Affects inflammatory phase; affects proliferative phase by decreasing fibroblast proliferation; affects remodelling phase by degrading newly formed extracellular matrix.

Corticosteroids

- Prednisolone

Effect: Affects haemostatic phase by decreasing platelet adhesion; affects inflammatory phase by affecting phagocytosis; affects remodelling phase by reducing fibroblasts activity and inhibiting collagen synthesis.

Antiplatelets

- Aspirin

Effect: Affects haemostatic phase by inhibiting platelet aggregation; inhibits inflammation mediated by arachidonic acid metabolites.

Anticoagulants

- Heparin

Effect: Affects haemostatic phase by its effect on fibrin formation; can lead to thrombus formation by causing thrombocytopaenia (white clot syndrome).

- Warfarin

Effect: Affects haemostatic phase by its effect on fibrin formation; can cause tissue necrosis and gangrene by release of atheromatous plaque emboli in form of microcholesterol crystals (blue toe syndrome)

Vasoconstrictors

- Nicotine, cocaine, adrenaline

Effect: Affects proliferative phase by inhibiting neovascularisation and decreasing granulation tissue formation; impairs microcirculation and increases graft rejection and ulcer necrosis**.**

LITERATURE REVIEW

LITERATURE REVIEW

1. **Hammeso, W. W. *et. al.,* (2019)** reported that the leaf latex of *A. megalacantha* possessed significant antihyperglycemic and antihyperlipidemic potential which may prove the claimed use of the plant in amelioration of diabetes and associated complications in Ethiopian folk medicine.[101]
2. **NT Abdullah *et. al.,* (2019)** evaluated the antidiabetic activity of methanolic extract of *Simarouba glauca* in STZ induced diabetes in rats for type I diabetic mellitus.[102]
3. **Sushma Gour *et. al.,* (2019)** evaluated that the antipyretic activity of hydroalcoholic leaf extract of *Morus Alba* Linn using Brewer's yeast induced pyrexia model in wistar strain albino rats.[103]
4. **Kannika Inchab *et. al.,* (2019)** evaluated the anti-inflammatory, analgesic, and antipyretic activities of water extract from fresh leaves of *Pseuderanthemum palatiferum* (WEPP). Antipyretic activity was evaluated by yeast-induced hyperthermia model.[104]
5. **Shanti Vasudevan CN *et. al.,* (2019)** investigated that the antipyretic activity of its heart wood aqueous extract of *Pterocarpus santalinus* L. using brewer's yeast induced pyrexia method.[105]
6. **Neha Kaila *et. al.,* (2019)** ascertained the analgesic and antipyretic activities of rhizome extracts of *Picrorhiza kurrooa*. antipyretic activity was evaluated in Wister rats by brewer's yeast induced pyrexia method.[106]
7. **Sabina Evan Prince *et. al.,* (2019)** analyzed the activity of *Bacopa monnieri* as an antipyretic, analgesic and anti-ulcerogenic agent. The antipyretic activity was evaluated by a yeast solution which was used to induce fever before administering the drugs.[107]
8. **Mansour Sobeh *et. al.,* (2019)** investigated the antioxidant, anti-inflammatory, analgesic, and antipyretic activities of the extract of *Albizia anthelmintica.* Pyrexia was induced by intraperitoneal injection of Brewer's yeast.[108]
9. **M. G. Minoué Kuum *et. al.,* (2019)** investigated that the anti-inflammatory, analgesic and antipyretic potential of aqueous extract of *Albizia ferruginea* stem bark. The antipyretic activity of A. ferruginea extract was assessed on brewer's yeast induced pyrexia.[109]
10. **Farjana Akhter *et. al.,* (2019)** studied that the phytochemical constituents and to evaluate the antibacterial, antioxidant, antidiarrheal and antipyretic activities of different extracts of Duabanga grandiflora.[110]

11. **Swapnil Y. Chaudhari *et. al.*, (2018)** studied that an Ayurvedic herbo-metalo-mineral formulation of *Hridayarnava Rasa* possessed significatnt Antihyperlipidemic activity against high fat diet induced hyperlipidemia in Charles Foster albino rats.[111]
12. **B. H. M. R. K. Guptha *et. al.*, (2018)** evaluated the antihyperlipidemic activity of *Chloroxylon swietenia* in triton WR1339 induced hyperlipidemia in rats.[112]
13. **Venkateswarlu G *et. al.*, (2018)** studied the antipyretic activity of methanolic whole plant extracts of Limnophila repens (L. repens) and Argyreia cymosa (A. cymosa) using Brewer's yeast-induced pyrexia model.[113]
14. **Rajendra Kumar Swain *et. al.*, (2018)** evaluated the analgesic activity of hydroalcoholic extract of *Cinnamomum zeylanicum* (HAECZ) bark in albino rats.[114]
15. **Doan, H. V. *et. al.*, (2018)** evaluated that the antidiabetic activity of the extract from *C. cainito* stem bark and the possible mechanisms underlying this activity against alloxan induced diabetic mice.[115]
16. **Belayneh, Y. M. *et. al.*, (2018)** studied the hydromethanolic extract of *Calpurnia aurea* leaves possesses signifcant antihyperglycemic activity against streptozotocin induced hyperglycemia model in mice and justifying the traditional use of the plant for diabetes.[116]
17. **Hussain, S. N. *et al.*, (2018)** studied *C. sophera* extract possesses antidiabetic activity against alloxan induced rats using glibenclamide as reference standard. This lends some support for the traditional use of this herb in the management of diabetes.[117]
18. **Dr. Sam Pavan Kumar Gandamalla *et. al.*, (2017)** investigated the analgesic and anti-inflammatory effects of the leaves of *Toona ciliata* in laboratory animals. The ethanol extract of the leaves of *Toona ciliata* was used to investigate the acute effect on analgesia by Hot-plate test in mice and on inflammation in rats using carrageenan-induced rat paw edema model.[118]
19. **Charles Chatterjee *et. al.*, (2017)** evaluated the analgesic activity of methanolic extraction of leaves of *Bougainvillea spectabilis* by tail immersion method in Mice.[119]
20. **G. Vijay Kumar *et. al.*, (2016)** evaluated that the Leaf Extracts of *Leucas aspera* Linn. Having significant inhibition against dexamethasone-induced hyperlipidemia in rats by maintaining the serum levels of cholesterol, TGs and near to the normal levels.[120]
21. **Nagar, H. K. *et. al.*, (2016)** the wound healing effect of ethanolic extract of Cestrum nocturnum (L.) leaves (EECN) using excision and incision wound model. Methods.[121]

22. **Sneha D. Borkar *et. al.*, (2015)** studied the leaves of *R. hypocrateriformis* contain secondary metabolites such as tannin and possess mild antioxidant properties. Nutritional analysis indicates the presence of energy in highest amount, carbohydrates, proteins, fats, calcium, phosphorous, zinc, and magnesium.[122]
23. **Asie Shojaii *et. al.*, (2015)** evaluated the anti-inflammatory and analgesic activities of the hydro-alcoholic extract of the pods of *Astragalushamosus* (HAAH), a plant used in Iranian traditional medicine, and antinociceptive effects of different fractions in animal models.[123]
24. **S.S. Godipurge *et. al.*, (2015)** investigated the potential pharmacological activities of the ethanolic extract of aerial parts of *Rivea hypocrateriformis* (EERH) and also measured their phenolic and flavonoid contents.[124]
25. **Pal Chandrashekhar R *et. al.*, (2015)** evaluated the antihyperlipidemic activity of methanolic extract of Gloriosa superb (MEGS) in two different experimental models of hyperlipidemia namely Poloxamer-407 induced hyperlipidemia and Cholesterol and cholic suspension induced hyperlipidemia.[125]
26. **Mukesh Singh Sikarwar *et. al.*, (2015)** demonstrated ethanolic extract fraction of *Hibiscus rosa-sinensis* Linn. possessed significant antihyperlipidemic activity.[126]
27. **Syed Safiullah Ghori *et. al.*, (2015)** studied that the antihyperlipidemic activity of Ethanolic Extact Of *Glycosmis pentaphylla* against hyperlipidemia induced by Triton X 100 and also High Fat Diet induced experimental models.[127]
28. **Jain P and Surana (2015)** Studied the hypolipidemic activity of traditional medicines from different regions of India.[128]
29. **Mahesh Anand Goudar *et. al.*, (2014)** evaluated the antidiabetic activity of petroleum ether (PETP) and ethanolic (EtTP) extracts of bark of Terminalia paniculata Roth (Family: Combretaceae) in normal and Streptozotocin induced diabetic rats. The extracts of Terminalia paniculata was investigated for antidiabetic activity in Streptozotocin induced diabetic rats by oral administration of extract 100 mg.kg body weight for single-dose one day and multiple-dose seven days.[129]
30. **Dinesh Dhingra *et. al.*, (2014)** evaluated the antihyperlipidemic activity of dried pulp of *Aloe succotrina* leaves in Wistar albino rats.[130]
31. **M S. Sikarwar *et. al.*, (2014)** demonstrated chloroform extract of *P. pinnata* leaves possessed significant antihyperlipidemic activity hence it could be a potential herbal medicine as adjuvant with existing therapy for the treatment of hyperlipidemia.[131]

32. **Hardik Mori *et. al.,* (2014)** evaluated the antihyperlipidemic activity of rhizome of *Tectaria coadunata* against high fat diet induced hyperlipidemia in wistar albino rats.[132]
33. **Ritu Mishra *et. al.,* (2014)** studied that methanolic **extracts of** *Trichosanthes anguina* as significant anti-hyperlipidemic effects on Triton induced hyperlipidemia in rats.[133]
34. **Panneer selvam Vijayaraj *et. al.,* (2013)** evaluated that Ethanolic extract of *Cassia auriculata* has a beneficial effect in treating hyperlipidemia and ROS without any side effects at the dosage and duration.[134]
35. **Kittana Mäkynen *et. al.,* (2013)** evaluated that antioxidant and antihyperlipidemic activity of Citrus grandis Linn. In experimental animals.[135]
36. **VJ Sharma *et. al.,* (2013)** studied that pharmacognostical characters and anti-oxidant effect of methanolic extract obtained from the plant *Rivea ornata*. Anti-oxidant activity was studied by DPPH scavenging assay method. The extract was administered orally in dose of 200 and 400 mg/kg/body weight to healthy animals. These extract showed a greater anti anti-oxidant effect comparative to standard drug vitamin C.[136]
37. **Najat T Hwisa *et. al.,* (2013)** studied that *Rivea ornata* (Roxb.) is a potential medicinal plant used topically in haemorrhagic diseases and piles. It also possess to contain antiinflammatory activity. The leaves are given after parturition. Owing to its medicinal and industrial importance, the macroscopic and microscopic characters of the leaves were also studied.[137]
38. **Pawar, R. *et. al.,* (2013)** evaluated the ethanolic extract ointment of SCL effectively stimulates wound contraction; increases tensile strength of excision, incision and burn wounds.[138]
39. **Murthy, S. *et. al.,* (2013)** studied the wound healing effects of 50% ethanol extract of dried whole plant of *Bacopa monniera* (BME) on wound models in rats. BME (25 mg/kg) was administered orally, once daily for 10 days (incision and dead space wound models) or for 21 days or more (excision wound model) in rats.[139]
40. **Mukesh S. Sikarwar *et. al.,* (2012)** studied that the alcoholic extract of *Salacia chinensis* roots possessed significant antihyperlipidemic activity and hence it could be a potential herbal medicine as adjuvant with existing therapy for the treatment of hyperlipidemia.[140]
41. **Bhaskar, A. *et. al.,* (2012)** studied that the wound-healing activity of H. rosa sinensis (5 and 10% w/w) on wistar albino rats was studied using three different models viz., excision, incision and dead space wound.[141]

42. **Kiessoun Konaté *et. al.,* (2012)** investigated that the toxicological effects of aqueous acetone extracts of *Sidaacuta* Burn f. and *Sidacordifolia* L. Furthermore, their analgesic capacity was assessed, in order to assess the efficiency of the traditional use of these two medicinal plants from Burkina Faso.[142]
43. **Naveed Muhammad *et. al.,* (2012)** evaluated that methanolic extract of *Viola betonicifolia* has marked Antipyretic, Analgesic and Anti-inflammatory activity.[143]
44. **Kumbhar ST *et. al.,* (2012)** evaluated that the analgesic activity of a *Ficusglo merata* leaf extract in mice by tail immersion method.[144]
45. **Amberkar mohanbabu vittalrao *et. al.,* (2011)** Studied that alcoholic extract of *Kaempferia glanga* in rats by tail flick and hot plate method.[145]
46. **Yashwant Kumar, A. *et. al.,* (2011)** investigated that the alcoholic (AlcE) and aqueous (AqsE) extracts of stem bark of Erythrina indica (Papilionaceae) for hypoglycaemic effects in normal and diabetic rats. Diabetes was induced in rats by a single dose administration of alloxan (120 mg/kg, i.p.) or by injecting dexamethasone (10 mg/kg, i.p.) for 10 days.[146]
47. **Garg, V. *et. al.,* (2011)** evaluated that the the ethanolic and aqueous extracts of F. benghalensis have properties that render them capable of promoting accelerated wound-healing activity compared with placebo control.[147]
48. **P.R. Rachh *et. al.,* (2010)** studied that Antihyperlipidemic activity of *Gymenma sylvestre* R. Br. Leaf Extract on high cholesterol diet induced hyperlipidemia in rats.[148]
49. **AHM Zulfiker *et. al.,* (2010)** evaluated that centrally acting analgesic potential using hot plate and peripheral pharmacological actions using acetic acid induced writhing test in mice.[149]
50. **Bassey S. Antia *et. al.,* (2010)** evaluated that the *P. maximum* leaves extract possesses the antidiabetic activity against alloxan induced hyperglycemia model in rats and confirms the folkloric use of this plant as antidiabetic agent.[150]
51. **M. A. Khayum *et. al.,* (2008)** assessd that the influence of lisinopril on the glipizide action in healthy and streptozotocin induced diabetic albino rats using single and repeated treatments of selected doses (2.5mg/kg, 5mg/kg, 10mg/kg and 12mg/kg) of lisinopril on the glucose lowering effect of glipizide.[151]
52. **G. W. Staples *et. al.,* (2007)** studied that a synopsis of *Rivea* Choisy (*Convolvulaceae*) accounts for all names published in the genus in a concise nomenclatural review. Three species are recognized; a key is provided to identify them. Excluded species are referred

to the currently accepted names and an index of numbered specimens examined will allow curators to correctly name material in herbaria.[152]

53. **KN Reddy *et. al.*, (2007)** documented that the traditional wild food plants used by tribal people in Andhra Pradesh. A Total of 156 species were documented as wild plants used for food purposes. Among these species, 56 species are herbs followed by 55 trees, 27 shrubs and 18 climbers. Mostly, herbs are used as leafy vegetables. It has been observed that the traditional knowledge on wild food plants is on sharp decline.[153]
54. **S. S. Jalalpure *et. al.*, (2006)** evaluated that the antidiabetic potential of the bark of Holarrhena antidysenterica Linn after single dose (acute study) and prolonged treatment (chronic study) in alloxan induced diabetic albino rats.[154]
55. **James *et. al.*, (2004)** investigated the anti-pyretic and analgesic activity of ethanol (EEO) and aqueous (AEO) extracts of Emblica officinalis fruits in several experimental models.[155]
56. **Kallappa M *et. al.*, (2000)** found that *Rivea ornata* seed oil was contain 12,13-epoxy-octadec-cis-9-enoic acid (vernolic acid, 22.0%) along with the other normal fatty acids like palmitic acid(24.2%), stearic acid (8.9%), oleic acid (17.1%) and linoleic acid (27.8%).[156]
57. **A.Hattaa *et. al.*, (1998)** studied that the anti-nociceptive effect of ethanolic extract of 11 traditionally used Jordanian plants by using the acetic acid-induced writhing and hot-plate test in mice.[157]
58. **Elaine Elisabetsky *et. al.*, (1995)** evaluated that analgesic activity of extracts of *P. colorata*, using the formalin, writhing and tail-flick methods.[158]

AIM & OBJECTIVE

3. AIM AND OBJECTIVE

The universal role of plants in the treatment of disease is exemplified by their employment in all the major systems of medicine irrespective of the underlying philosophical premise. As examples, we have Western medicine with origins in Mesopotamia and Egypt, the Unani and Ayurvedic systems centred in western Asia and the Indian subcontinent and those of the Orient (China, Japan, Tibet, etc.). How and when such medicinal plants were first used is, in many cases, lost in pre-history, indeed animals, other than man, appear to have their own materia medica.

One of these plants which is used to treat various disease is *Rivea ornata* choisy. Traditionally they were used to treat hemorrhage and piles.

Rivea ornata are the most widely used medicinal plant of Ayurveda and siddha and has well descripted pharmacological activities such as antimicrobial activity, antioxidant activity, anti inflammatory activity.

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 sub acute oral toxicity
- To study the antilipidemic activity
- To study the anti-diabetic activity
- To study the analgesic activity
- To study the antipyretic activity
- To study the wound healing activity

PLAN OF THE WORK

4. PLAN OF THE WORK

- Collection of the *Rivea ornata* leaves
- Authentication of the plant
- Extraction with suitable solvent
- Phytochemical screening of the extract
- Analytical studies
    - ✓ FTIR spectral analysis
    - ✓ HPTLC method
- Pharmacological Studies
    - ✓ Acute oral toxicity test – Validation
        - OECD guidline 423
    - ✓ Sub acute toxicity test
        - OECD guideline 407
    - ✓ Antihyperlipidemic activity
        - Triton X 100 induced hyperlipidemia in rats
    - ✓ Anti-diabetic activity
        - Alloxan induced diabetes in rats
    - ✓ To study the analgesic activity
        - Tail immersion test in rats
        - Hot plate method
    - ✓ To study the antipyretic activity
        - Brewer’s yeast-induced hyperpyrexia in rats
    - ✓ To study the wound healing activity
        - Excision wound model in rats

PLANT PROFILE

5. PLANT PROFILE

Authentication Certificate

Dr. V. NANDAGOPALAN
Controller of Examinations
Associate Professor in Botany

NATIONAL COLLEGE (Autonomous)
Nationally Re-Accredited with A+ Grade by NAAC
College with Potential for Excellence by UGC
Tiruchirappalli – 620 001, Tamil Nadu, India
e-mail: veenan05@gmail.com

Since 1919

DATE: 09-05-2019

PLANT AUTHENTICATION CERTIFICATE

This is to certify that, the given plant sample by **Mr.NAHOOR MEERAN** doing **M.Pharm.,** Periyar College of Pharmaceutical science, Tiruchirappalli – 21 is ***Rivea ornate(Roxb.)*** **Choisy in Hook.** belongs to the family ***Convolulaceae***.

(V.NANDAGOPALAN)

Description of the plant[137,159,160]

A climbing or straggling shrub, Leaves orbicular, glabrous above, white silky beneath, white flowers, fragrant, 3 or more flowered axillary cyme, fruit capsule, subglobose, brown.

Fig. No. 5: Leaves of *Rivea ornata*

Fig. No. 6: Flower of *Rivea ornata*

Taxonomical Classification of *Rivea ornata*

Kingdom	:	Plantae
Subkingdom	:	Trachebionta
Infrakingdom	:	Tracheophyta
Superdivision	:	Spermatophyte
Division	:	Magnoliophyta
Class	:	Magnolipsida
Subclass	:	Asteridae
Order	:	Solanales
Family	:	Convolvulaceae
Subfamily	:	Rivea

Synonyms

Argyreia ornate (Roxb.) Sweet (synonym)

Convolvulus ornatus (Roxb.) Wall. (ambiguous synonym)

Convolvulus tarita Buch.-Ham. ex Wall. (synonym)

Lettsomia ornata Roxb. (synonym)

Rivea clarkeana Craib (synonym)

Rivea laotica Ooststr. (synonym)

Rivea ornata var. griifithii Clarke (synonym)

Other common names

Tamil	:	Muchuttai, Musnttai
Gujarati	:	Phangya
English	:	Midnapore Creeper
Hasada	:	Baisjamburunari, Bhaisjamburunari
Marathi	:	Phand, Phanji, Sanjwel
Naguri	:	Tonangutuara
Sanskrit	:	Aparajitil, Ajanlsi, Ajara, Jirna, Jirnadaru, Padma, Phanji, Phanjika, Sukshmapatra, Supushpika
Telugu	:	Bodditige

Habit

Erect shrub or scan dent from woody rootstock; stems 1–2.5 m tall, whitish hairy.

Leaves

Leaves orbicular to reniform, 10–15 by 6–20 cm, base cordate, apex rounded or emarginate, upper side glabrous, underside densely whitish tomentose, glabrescent; lateral veins 5 or 6 per side; petiole 3–12.5 cm long, hairy.

Macroscopy

Leaves are simple, alternate, petiolate and exstipulate and reniform-ovate, cordate in shape with a shortly acuminate, or caudate to 4x5 cm, orbicular with a long petiole. Leaves are sparsely grey in color and usually silky beneath.

Flowers

Flower sepals subequal, oblong to elliptic-oblong, 14–17 by 8–9 mm, apex obtuse, rounded, or emarginate, outer 3 sepals appressed hairy outside, margins glabrous, inner sepals glabrous; corolla tube c. 5 cm long, limb c. 7–8 cm diam., subentire, white with 5 greenish bands hairy outside; stamens included, filaments inserted at middle of tube or below, anthers c. 7 mm long; pistil included, disc cupular, ovary glabrous, style c. 25 mm long, stigma lobes c. 4 mm long.

Fruits

Fruit subglobose, c. 20–30 mm diam., glossy brown, glabrous; fruiting calyx woody, eventually reflexed.

Chemical Constituent

Rivea ornata seed oil was found to contain 12, 13- epoxy-octadec-cis-9-enoic acid (vernolic acid, 22.0%) along with the other normal fatty acids like palmitic acid (24.2%), stearic acid (8.9%), oleic acid (17.1%) and linoleic acid (27.8%).

Leaves contains carbohydrates, phytosterols, phenolic compounds, alkaloid, triterpenoids, fixed oil and tannins.

Seeds

Seeds c. 7–9 mm by 4–6 mm, brown, embedded in crumbly crust.

Distribution

India, Nepal, Myanmar, Thailand, Laos.

Ecology

Open jungle, deciduous dipterocarp forest, mixed forests, on sandy loam; altitude: c. 10–500 meter

Phenology

Flowering: September, October; fruiting: September, November.

METHODOLOGY

6. METHODOLOGY

6.1. EXTRACTION[136,137]

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.

Leaves of *Rivea ornata* (Roxb.) were collected; Shade dried; dried material was ground into the coarse powder which was used for further study to extract with methonal using soxhlet apparatus.

Fig. No. 7: 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 methanol.

Methonolic 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 the identification of constituents by phytochemical screening and for pharmacological studies.

6.2. PHYTOCHEMICAL SCREENING[136,137]

Preliminary phytochemical screening is done before resorting. Qualitative analysis is thus a part of preliminary standardization. Phytochemical evaluation is used to determine the nature of phyto constituents present in the plant by using suitable chemical tests. It is essential to study the pharmacological activities of the plant.

Detection of Flavonoids

- **Shinoda test**

  To the solution of extract, few piece of magnesium turnings and concentrated HCl was added drop wise, pink to crimson red, occasionally green to blue color appears after few minutes indicates the presence of flavonoids.

- **Alkaline reagent test**

  To the test solution, few drops of sodium hydroxide solution was added, intense yellow color is formed which turns to colorless on addition of few drops of dilute HCl indicate presence of flavonoids.

Detection of Alkaloids

- **Dragendorff's reagent**

  The substance was dissolved in 5ml of distilled water, to this 5ml of 2M HCl was added until an acid reactions occurs, then 1ml of Dragendorff's reagent was added and examined for an immediate formation of an orange red precipitate.

- **Mayer's reagent**

  The substance was mixed with little amount of Dilute Hydrochloric acid and Mayer's reagent and examined for the formation of white precipitate.

- **Wagner's reagent**

  The test solution was mixed with Wagner's reagent and examined for the formation of reddish brown precipitate.

- **Hagne's reagent**

  The test solution was mixed with few drops of Hydrochloric acid and filtered and then Hagne's reagent was added and examined for the formation of yellow precipitate.

Detection of Carbohydrates

- **Molisch's test**

  To the test solution, few drops of alcoholic alpha naphthol and few drops of Conc. Sulphuric acid were added through the sides of test tube, purple to violet color ring appears at junction.

- **Fehling's test**

  The test solution was mixed with Fehling's I and II and heated and examined for the appearance of red coloration for the presence of sugar.

Tannins

- Small quantities of test substances was treated with Ammonium Hydroxide then few drops of Potassium ferricyanide solution was added and examined for the formation of transited red colour.

Detection of Proteins

- **Biuret test**

  The sample was treated with sodium hydroxide and 5-8 drops of 10% w/v Copper sulphate solution, violet color was formed.

- **Millon's test**

  The sample was treated with Millon's reagent, a white precipitate was formed.

Detection of fats and fixed oils

- Small quantities of extracts were pressed between two filter papers. An oily stain on filter paper indicates the presence of fixed oils and fats.

Detection of Steroids

- **Libermann Burchards Test**

  The powdered drug was treated with few drops of acetic anhydride, boiled and cooled. Conc.sulphuric acid was added on the sides of the test tube, brown ring was formed at the junction of two layers and upper layer turns green which shows presence of steroids.

Detection of Terpenoids

- The extract was treated with caustic soda, a deep red color was formed, indicates presence of tri terpenoids.

Detection of Glycosides

- **Borntrager's test**

  The extract was boiled with 1ml of sulphuric acid in a test tube for five minutes. Filtered while hot, cooled and shaken with equal volume of chloroform. The lower layer of solvent was separated and shaken with half of its volume of dilute ammonia. A rose pink to red colour was produced in the ammonical layer.

Detection of Phenols

- **Ferric chloride test**

  A small quantity of substance were dissolved with 2ml distilled water and a few drops of 10% aqueous ferric chloride solution was added and observed for the appearance of blue or green color.

Detection of Saponins

- A drop of sodium bicarbonate solution was added to the sample and the mixture was shaken vigorously and left for 3 minutes. Development of any honey comb like froth was examined.

6.3. INFRARED SPECTROSCOPY[137,162]

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.

Tab. No. 5: Specifications of FTIR Spectrophotometer

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

6.4. HPTLC ANALYSIS[136,137,162]

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.

Test Solution : Methanolic Extract of *Rivea ornata* (MERO)

Stationary Phase : Merck, TLC Plates Silica gel 60 F-254

Mobile Phase : Toluene : Ethyl acetate : Acetone (2:4:4 v/v/v)

Tank : TTC 10×10

Saturation time : 20 Minutes

Procedure

The samples (5µl, 10µl, 15µl, 20µl) 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 20cm × 10cm 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 254 nm and white light using photo documentation chamber. Finally, the plate was fixed in scanner stage and scanning was done at 254 nm. The plate was kept in photo-documentation chamber and captured the images under white light, UV light at 254 nm. Densitometric scanning was performed on Camag TLC scanner III and operated by Server Win software (Version 2.5.18053.1).

6.5. ANIMAL EXPERIMENTATION

Pharmacological evaluation of the methanolic extract of *Rivea ornata* (Leaves) 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 Pharmacological 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/005/2019. The animals were maintained at a well ventilated, temperature controlled 30°C ± 1°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.

6.5.1. Validation of Acute toxicity[136]

Acute Toxic Class method: Guideline number 423

Principle

The test substance will be administered orally to a group of experimental animals at one of the defined doses. The substance will test 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, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous systems, somatomotar activity and behavior pattern. Attention was directed to observations or tremors, convulsions, salivation diarrhea, lethargy, sleep and mortality. In addition, behavioural changes body weight, histopathological studies and biochemical parameters were also observed.

6.5.2. Sub acute toxicity[163]

Repeated dose oral toxicity method: Guideline number 407

The methanolic extract of *Rivea ornata* was orally administered daily in dose of 200 mg/kg to group of experimental animals for a period of 28 days. During the period of administration the animals are observed closely, each day for signs of toxicity. Animals that die or are euthanised during the test are necropsied and at the conclusion of the test surviving animals are euthanised and necropsied. A 28 day study provides information on the effects of repeated oral exposure and can indicate the need for further longer term studies. It can also provide information on the selection of concentrations for longer term studies. The data derived from using the Test Guideline (TG) should allow for the characterization of the test substance toxicity, for an indication of the dose response relationship and the determination of the No-Observed Adverse Effect Level (NOAEL).

Wistar albino rats of 6 animals from each sex selected with the weight variation of ±20% of the mean weight of each sex divided into the two groups. The experimental room was maintained at the temperature of 22°C (±3°C) and the relative humidity of 50-60% with 12 hours artificial dark and light cycle. Animals were fed with conventional laboratory diet with an unlimited supply of water.

Group I **:** Normal saline (5mL/kg, b.w., *p.o.*)

Group II **:** Methanolic Extract of *Rivea ornata* (MERO) (200mg/kg, bw., *p.o.*)

Experimental design

The animals from group I were administered with normal saline (5ml/kg, bw., *p.o.*) and group II with test substance (MERO 200mg/kg, bw., *p.o.*) by gavage, daily, for 28 consecutive days. At the end of the experiment the animals were sacrificed to collect their blood and organs (Heart, liver, kidney and pancreas) for biochemical, hematological and histological analyzes.

Blood analysis

Biochemical analyzes included liver function markers (AST, ALT), total cholesterol, and nephrotic marker urea. Hematologic parameters included red and white blood cells, hemoglobin, platelets, Erythrocite sedimentation rate.

Histopatholigical examinations

The organs were fixed in 10% concentrated formalin, underwent several liquor baths, before passing into toluene and being included in the liquid paraffin. The paraffin blocks are mounted on a microtome to make the cuts. Soaked in aqueous dyes (Hematoxylin and Eosin) and observed under microscopes.

Statistical analysis

The results are expressed are Mean ± SEM (n=6) two way ANOVA using a Graph pad and PRISM software version 8.2.1 (441). *** $P<0.001$, ** $P<0.01$ and *$P<0.05$ were considered as statistically significant.

6.5.3. Antihyperlipidemic activity[20,111,112]

Triton X 100 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 intraperitoneal administration of Triton X 100 in 100mg/kg.

Principle

Triton X-100 acts as surfactant & suppresses the action of lipases to block the uptake of lipoproteins from circulation by extra-hepatic tissue resulting in to increased blood lipid concentration.

Treatment

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

Group I : Normal diet water *ad libitum*

Group II : Normal diet and Triton X 100 (100mg/kg) (positive control)

Group III : Normal diet and Triton X 100 (100mg/kg) + Methanolic extract of *Rivea ornata* (200mg/kg, p.o.)

Group IV : Normal diet & Triton X 100 (100mg/kg) +Atrovastatin (10mg/kg, p.o.)

Experimental Design

Hyperlipidemia was induced by single intraperitoneal injection of freshly prepared solution of Triton X-100 (100 mg/ kg) in physiological solution after overnight fasting for 18 hrs. After 72 hours drugs were administered to the respective groups in predetermined dose for 7 days.

Biochemical analysis

On the 8th day, blood was collected by cardiac 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 was assayed for total cholesterol, triglycerides, high density lipoprotein (HDL), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL).

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 a Graph pad and PRISM software version 8.2.1 (441). *** $P<0.001$, ** $P<0.01$ and *$P<0.05$ were considered as statistically significant.

6.5.4. Antidiabetic activity[101,102]

Principle

Alloxan is an acidic compound obtained by the oxidation of uric acid and isolated as an efflorescent crystalline hydrate. It is a toxic glucose analogue, which selectively destroys insulin-producing cells (beta cells) in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus in these animals, with characteristics similar to type I diabetes in humans.

Wistar Albino Rats weighing 150-200g were divided into 4 groups and kept under the temperature of 25°C and the relative humidity of 50-60% with 12 hours dark and light cycle. Animals were fasted for 12 hours before and after injection of alloxan.

Group I **:** Normal saline (5mL/kg, b.w., *p.o.*)

Group II **:** Alloxan (150mg/kg, b.w., *p.o.*)

Group III **:** Alloxan (150mg/kg, b.w., *p.o.*) + Glibenclamide (10mg/kg, b.w., *p.o.*)

Group IV **:** Alloxan (150mg/kg, b.w., *p.o.*) + Methanolic Extract of *Rivea ornata* (200mg/kg, bw., *p.o.*)

Experimental Design

Alloxan is used to induce diabetes. The dose of alloxan for diabetes control group is 150mg/kg of body weight. It was prepared by dissolving in normal saline and administered intraperitonially. Rats were fasted 12 hours before and after injection of alloxan, unfed animals are more susceptible for alloxan induction diabetes. One week after treatment, animals with moderate diabetes (blood glucose level above of 150 mg /dl) were used for the experiment. Test and standard drugs were administered to the respective group in predetermined dose for 14 days.

Evaluation

Blood Glucose level

After 0 minutes, 1 hour, 2 hours, 4 hours, 7^{th} day and 14^{th} day from the administration of standard and test drug, the blood glucose level was estimated by glucometer.

Histopathology of Pancreas

After the Blood glucose estimation was over the animals were sacrificed for histopathology studies. The pancreas of each animal from all four groups were isolated for histopathology studies. The isolated pancreas 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 a Graph pad and PRISM software version 8.2.1 (441). *** $P<0.001$, ** $P<0.01$ and *$P<0.05$ were considered as statistically significant.

6.5.5. Analgesic Activity[114,119,123]

a) Tail Immersion Test in Rats

Wistar albino rats of either sex weighing 18-22g were divided into three 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 I **:** Control - Normal Saline (5ml/kg, b.w., *p.o.*)

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

Group III **:** Test (Methanolic extract of *Rivea ornata* 200mg/kg, *p.o.*)

Experimental Design

The procedure is based on the observation that NSAID 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 was recorded before and at 30, 45, 60 and 90 minutes after the administration of the treatments. The cut off time is 15 seconds.

Evaluation

Tail withdrawal reflex

Statistical analysis

The results are expressed are Mean ± SEM (n=10) two way ANOVA using a Graph pad and PRISM software version 8.2.1 (441). *** P<0.001, ** P<0.01 and *P<0.05 were considered as statistically significant.

b) Hot plate method

Wistar albino mice of either sex weighing 20-25g were divided into three 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 I : Control - Normal Saline (5ml/kg, b.w., *p.o.*)

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

Group III : Test (Methanolic extract of *Rivea ornata* 200mg/kg, *p.o.*)

The mice were placed on a hot plate maintained at 55°C within the restrainer. The reaction time (in seconds) or latency period was determined as the time taken for the mice to react to the thermal pain by licking their paws or jumping. The reaction time was recorded before and at 30, 45, 60 and 90 minutes after the administration of the treatments. The maximum reaction time was fixed at 45 sec to prevent any injury to the tissues of the paws. If the reading exceeds 45 sec, it would be considered as maximum analgesia.

Statistical analysis

The results were expressed as Mean ± SEM (n=6) two way ANOVA using a Graph pad and PRISM software version 8.2.1. (441). *** $P<0.001$, ** $P<0.01$ and *$P<0.05$ were considered as statistically significant.

6.5.6. Antipyretic activity[95,103,143]

Yeast-induced hyperpyrexia in rats

Wistar albino rats of either sex weighing 150-200g were divided into three groups of six animals each. They were housed individually in standard laboratory environment for 7 days of period, fed with common laboratory diet and water *ad libitum*. The dosage of the drugs administered to the different groups as follows

Group I **:** 12.5% w/v yeast suspended in a 0.5% w/v normal saline (10ml/kg, b.w., *sc*.) + Normal saline at dose of (5ml/kg, b.w., *sc*.)

Group II **:** 12.5% w/v yeast suspended in a 0.5% w/v normal saline (10ml/kg, b.w., *sc*.) + Methanolic extract of *Rivea ornata* (MERO) (200mg/kg, b.w., *p.o.*)

Group III **:** 12.5% w/v yeast suspended in a 0.5% w/v normal saline (10ml/kg, b.w., *sc*.) + Paracetamol (150mg/kg, b.w., *p.o.*)

Experimental Design

The body temperature of each albino Wistar albino rats was recorded by measuring rectal temperature at predetermined intervals. The rats are trained to remain quiet in a restraint cage. A thermister probe is inserted 3 to 4 cm into the rectum and fastened to the tail by adhesive tape. Temperature is recorded on a thermometer. After measuring the basal rectal temperature, animals are given subcutaneous injections of 10 ml/kg of 12.5% w/v yeast suspended in a 0.5% w/v normal saline (10ml/kg, b.w., *sc*.). At the 19th hr after yeast injection the rectal temperature of the rats were recorded then Normal saline at dose of 5ml/kg was injected to the group I. Methanolic extract of *Rivea ornata* was administered with dose 200mg/kg to group II animals. The group III rats received the standard drug paracetamol at the dose of 150 mg/kg at the 19th hr after yeast injection. Subcutaneous injection of yeast suspension markedly increased the rectal temperature 19th hr after its administration. Rectal temperature of all the rats was recorded again on the 20th, 21st and 22nd hr after yeast injection.

Statistical analysis

The results are expressed are Mean ± SEM (n=10) two way ANOVA using a Graph pad and PRISM software version 8.2.1 (441). *** P<0.001, ** P<0.01 and *P<0.05 were considered as statistically significant.

6.5.7. Wound Healing Activity[121,138,139]

Excision Wound Model

Wistar albino rats of either sex weighing 200-250g were divided into three groups of six animals each. They were housed individually in standard laboratory environment for 7 days of period, fed with common laboratory diet and water *ad libitum*. The dosage of the drugs administered to the different groups as follows

Group I **:** Control – Simple ointment base

Group II **:** 10% methanolic extract ointment of *Rivea ornata* (MERO)

Group III **:** Povidone Iodine cream (10%)

Experimental Design

Three groups of animals containing six rats in each group were anesthetized by bottle method with anesthetic ether. The rats were depilated on the back and a predetermined area of 300 mm^2 full thickness skins was excised in the dorsal interscapular region. Rats were left undressed to the open environment. The formulation ointment and standard drug were applied daily until the complete healing. In this model, wound contraction and epithelialization period was monitored. Wound contraction was measured as percent contraction in each 3 days after wound formation.

Evaluation

Measurement of Wound Contraction

An excision wound margin was traced after wound creation by using transparent paper and area measured by graph paper. Wound contraction was measured in each 5 days interval, until complete wound healing and expressed in percentage of healed wound area. The evaluated surface area was then employed to calculate the percentage of wound contraction, taking initial size of wound, 300 mm^2, as 100%, by using the following formula as:

$$\% \text{ of wound contraction} = \frac{(\text{Initial wound size} - \text{Specific day wound size}) \times 100}{\text{Initial wound size}}$$

Statistical analysis

The results are expressed are Mean ± SEM (n=10) two way ANOVA using a Graph pad and PRISM software version 8.2.1 (441). *** P<0.001, ** P<0.01 and *P<0.05 were considered as statistically significant.

RESULTS & DISCUSSION

7. RESULTS AND DISCUSSION

7.1 Results

Tab. No. 6: Preliminary Phytochemical Analysis

S.No	Plant Constituents	Test / reagent	Methanolic extract of *Rivea ornata*
1	**Flavonoids**	Shinoda test	--
		Alkaline reagent test	
2	**Alkaloids**	Dragendorff's reagent	++
		Mayer's reagent	
		Wagner's reagent	
		Hagne's reagent	
3	**Carbohydrates**	Molisch's test	++
		Fehling's test	
4	**Tannins**	Ammonium Hydroxide	++
5	**Protein**	Biuret test	--
		Millon's test	
6	**Fat and Fixed oils**	Filter paper test	++
7	**Steroids**	Libermann Burchards Test	++
8	**Terpenoids**	Caustic soda	--
9	**Glycosides**	Borntrager's test	++
10	**Phenols**	Ferric chloride test	++
11	**Saponins**	Form test	++

Analytical Determination

1. FTIR Analysis

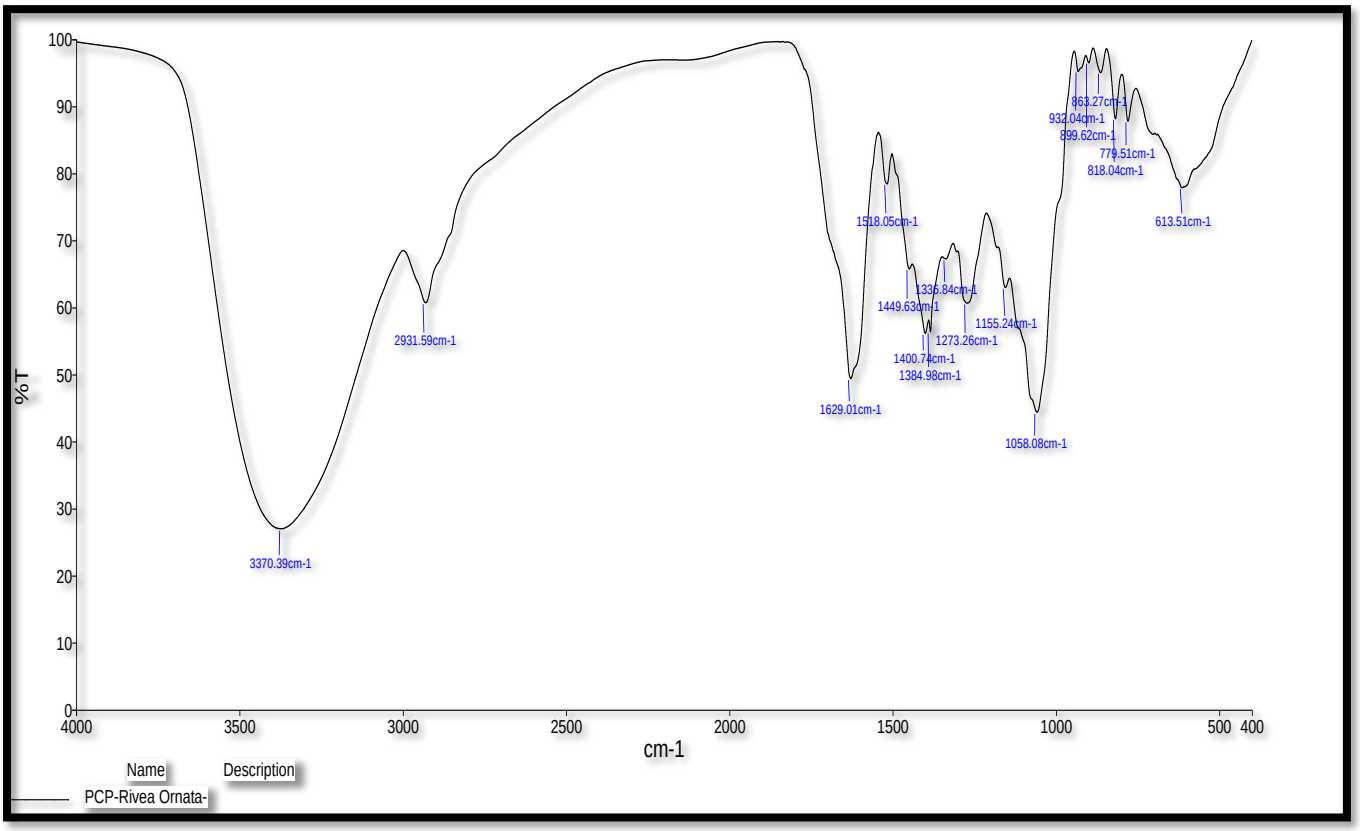


Fig. No. 8: FTIR Spectrum of Methanolic Extract of *Rivea ornata*

Tab. No. 7: FTIR Interpretation of the Methanolic Extract of *Rivea ornata*

S. No	Wavenumber (cm-1)	Functional Group
1	3370.39	O-H stretching
2	2931.59	C-H stretching
3	1629.01	C=C stretching
4	1518.05	N-O stretching
5	1449.63	C-H bending
6	1400.63	O-H bending
7	1384.98	Phenolic O-H bending
8	1273.26	C-N stretching
9	932.04	C=C bending
10	899.62	C-H bending

HPTLC Analysis

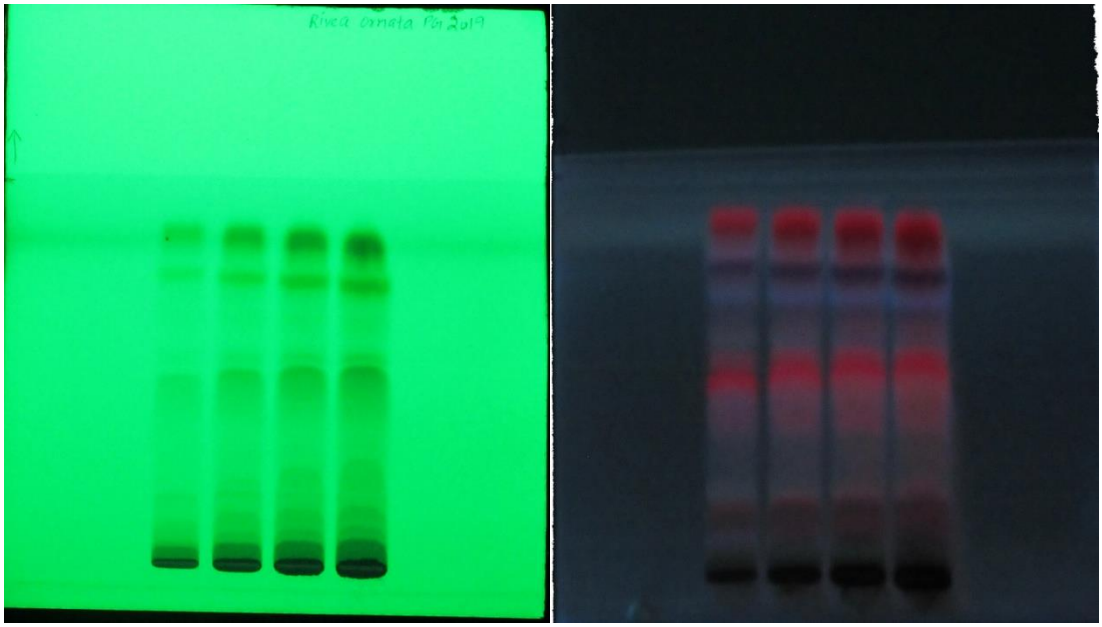


Fig. No. 9: HPTLC Chromatogram of MERO

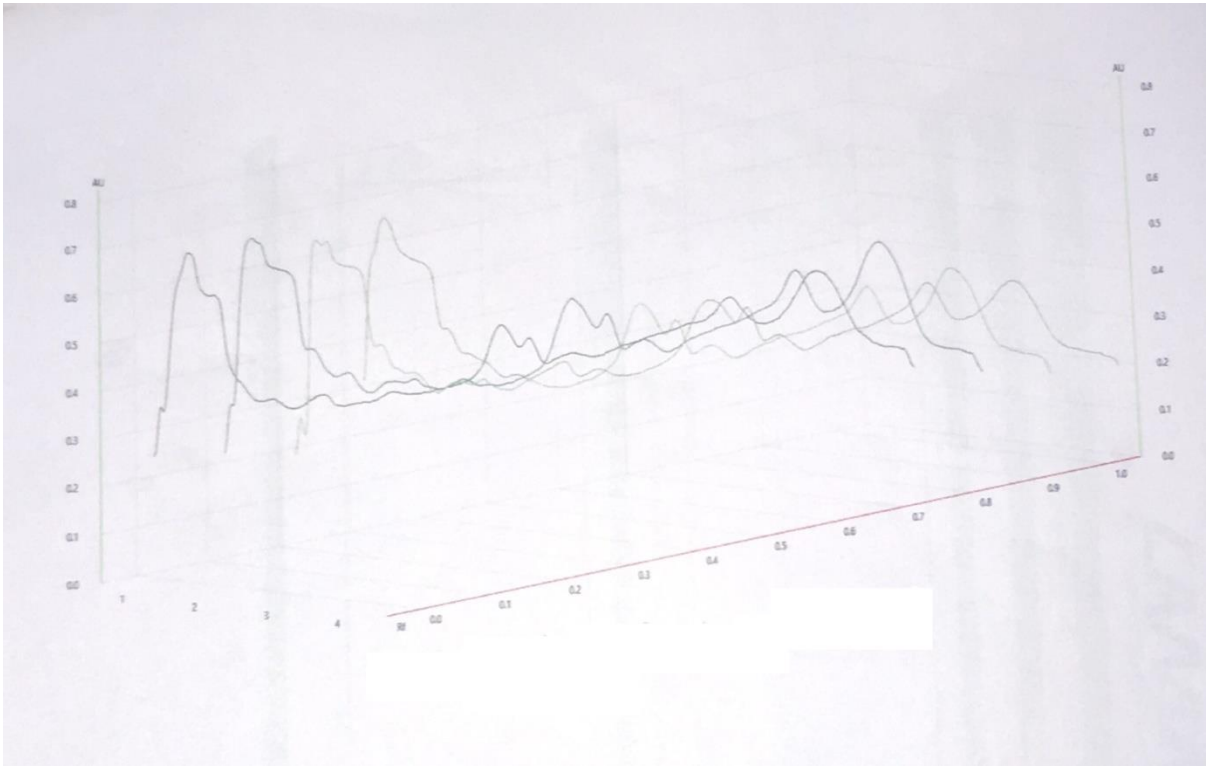


Fig No. 10: HPTLC chromatogram of MERO (3D)

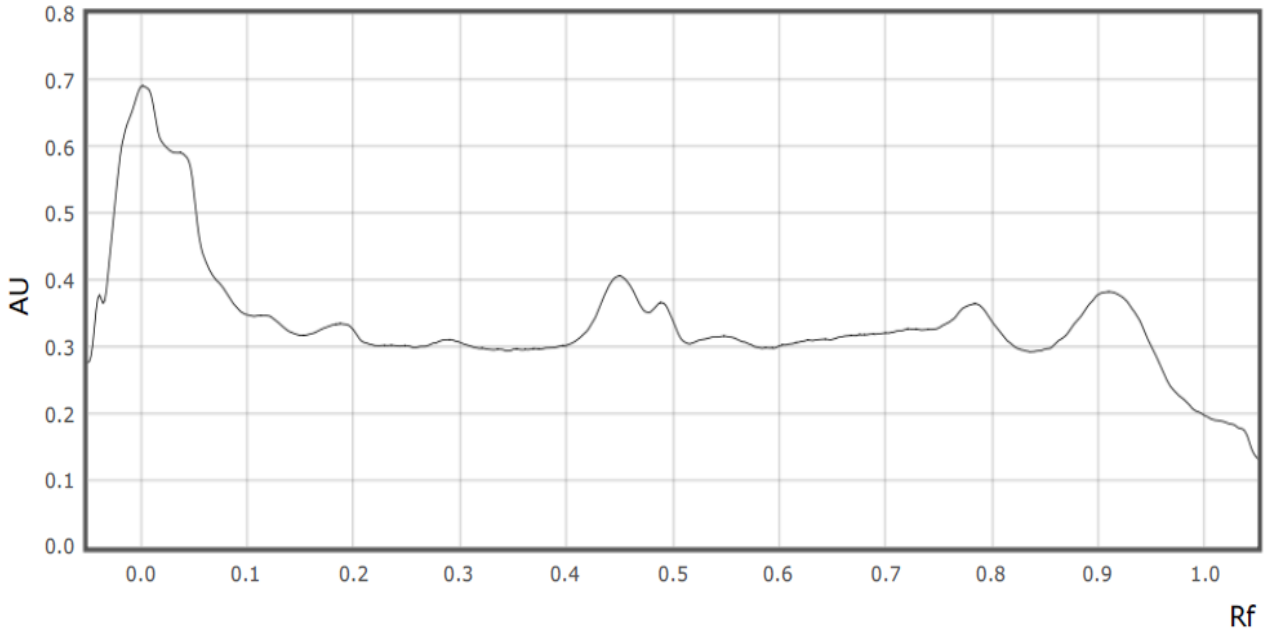


Fig. No. 11: HPTLC Chromatogram of MERO at 5µl Concentration

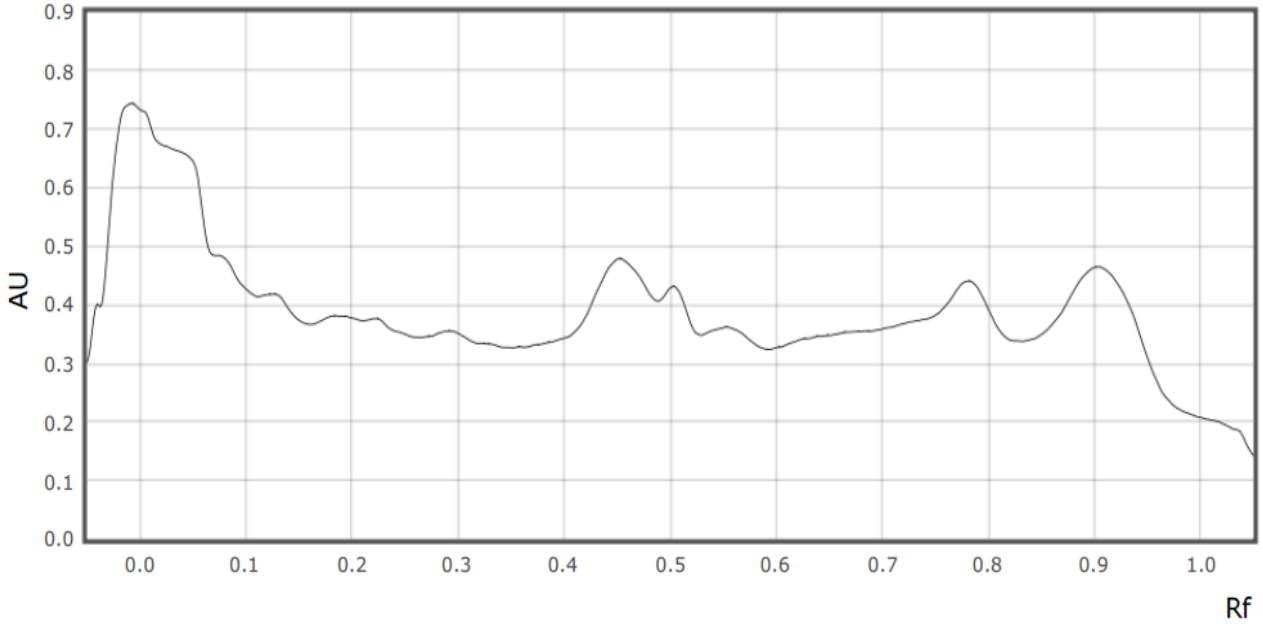


Fig. No. 12: HPTLC Chromatogram of MERO at 10µl Concentration

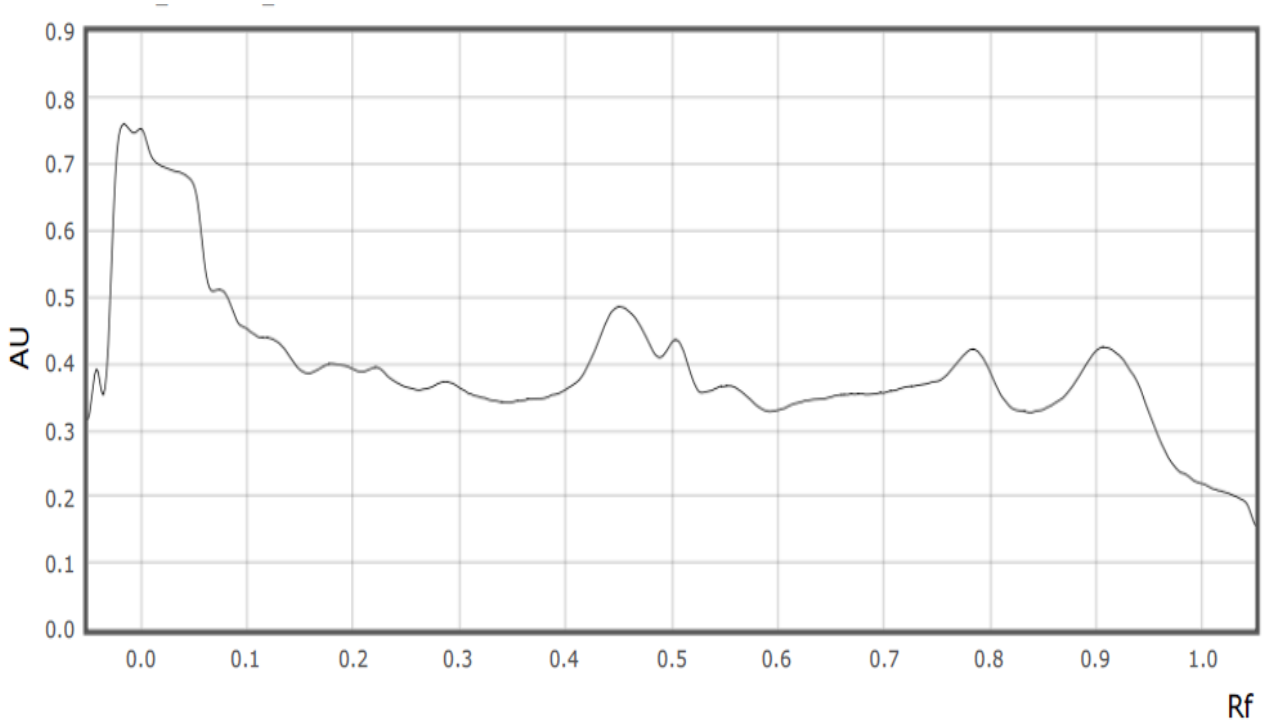


Fig. No. 13: HPTLC Chromatogram of MERO at 15µl Concentration

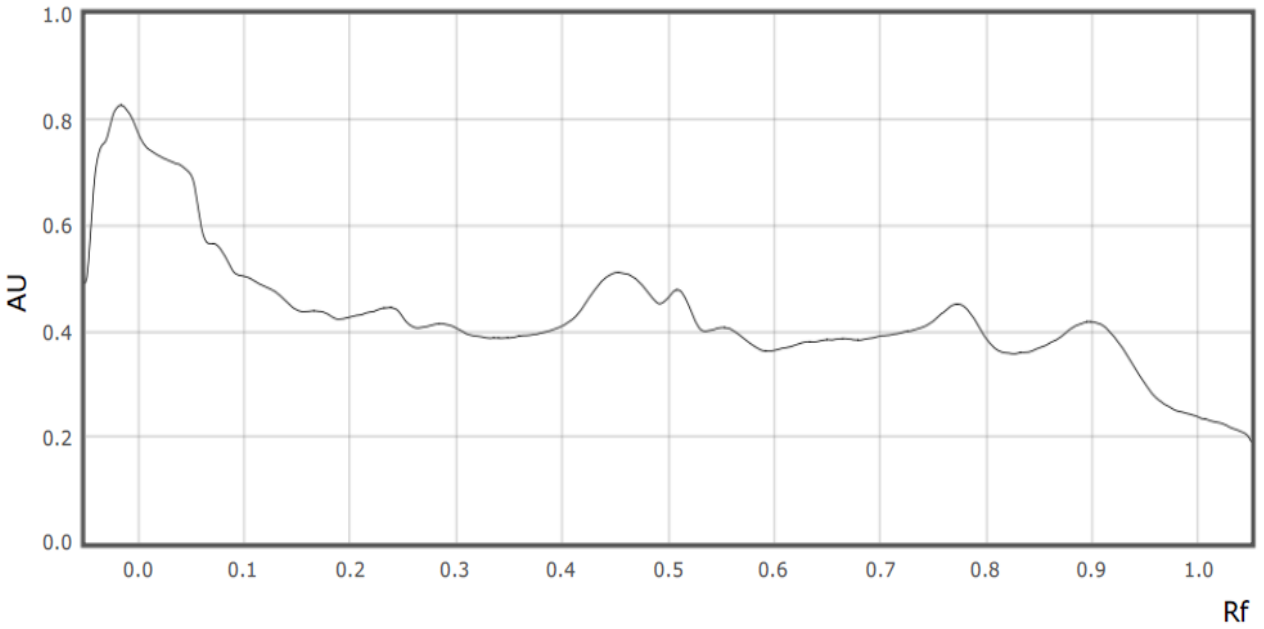


Fig. No. 14: HPTLC Chromatogram of MERO at 20µl Concentration

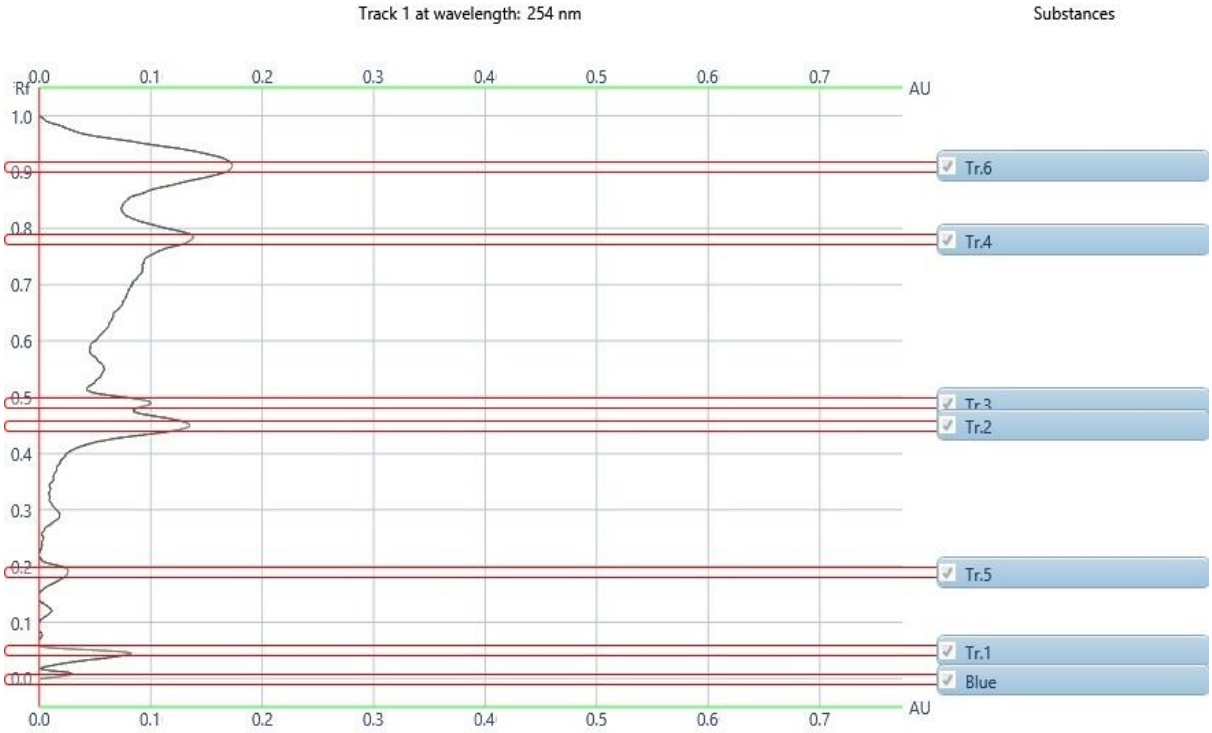


Fig. No. 15: HPTLC peak at System suitability test

Tab. No. 8: Rf values from HPTLC Chromatogram of MERO

S. No.	Track No.	R_f Value
1.	Tr. 1	0.050
2.	Tr. 2	0.450
3.	Tr. 3	0.490
4.	Tr. 4	0.780
5.	Tr. 5	0.190
6.	Tr. 6	0.910

PHARMACOLOGICAL EVALUATION

Toxicity Studies - Acute Oral Toxicity (Validation)

Table No. 9: Behavioral Changes in Acute Oral Toxicity in Albino rats

S.No.	Symptoms	Control (Normal Saline 5ml/kg, p.o.)	Methanolic Extract of *Rivea ornata* (2000mg/kg, *p.o.*,)
1	Death	--	--
Autonomic 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 Co ordination	--	--
20	Akinesia	--	--
21	Catelepsy	--	--
22	Loss of Balance	--	--
23	Fore-paw treading	--	--
24	Writhing	--	--
25	Stereotypy	--	--
26	Altered fear	--	--
27	Altered respiration	--	--
28	Aggression	--	--
29	Analgesia	--	++
30	Body temperature	--	--

Tab. No. 10: Effect of Test compound on Body Weight in Acute oral toxicity in Albino rats

S. No.	Group	Body weight	
		Initial	At the end of the study
1	**Control** (Normal Saline 5ml/kg)	212.045 ± 2.209	212.738 ± 1.775
2	**Test Drug** (MERO 2000mg/kg)	213.100 ± 2.154	205.686 ± 2.170

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

Tab. No. 11: 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 (MERO 2000mg/kg)
1	Glucose (mg/dl)	149.795 ± 0.547	139.213 ± 0.687
2	Blood Urea (mg/dl)	15.401 ± 0.173	15.06 ± 0.089
3	Total cholesterol (mg/dl)	81.867 ± 0.425	76.108 ± 0.723
4	SGOT (U/I)	113.035 ± 0.424	112.672 ± 0.226
5	SGPT (U/I)	75.092 ± 0.338	74.928 ± 0.360
6	Albumin (gm/dl)	3.397 ± 0.0723	3.555 ± 0.090

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

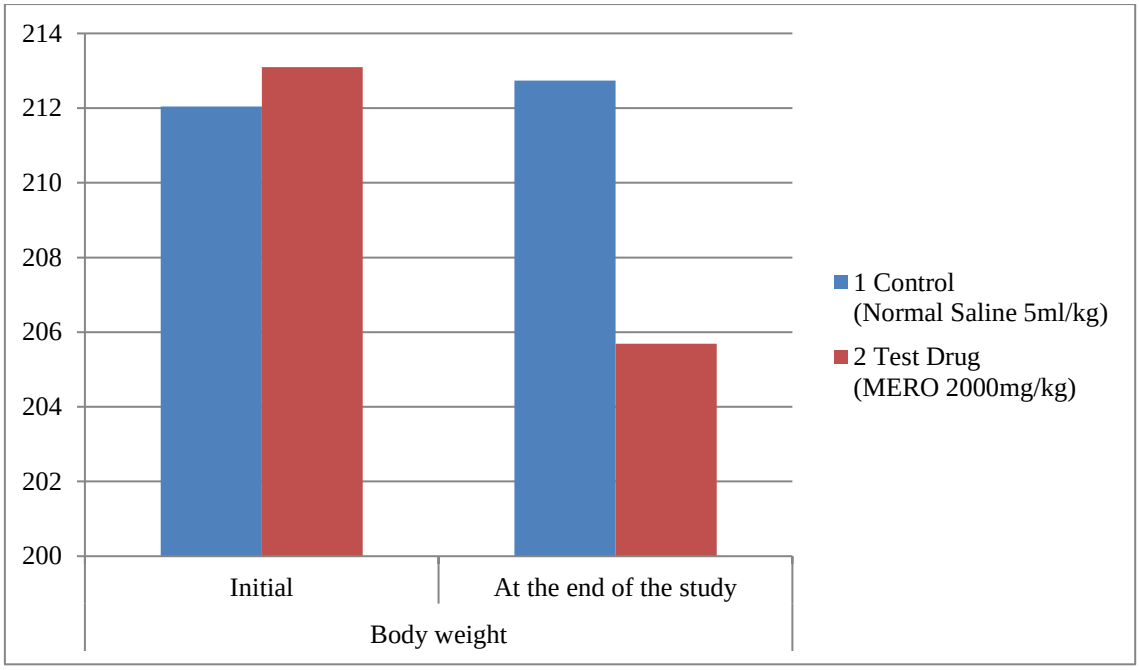


Fig. No. 16: Effect of Test compound on Body Weight in Acute oral toxicity in Albino rats

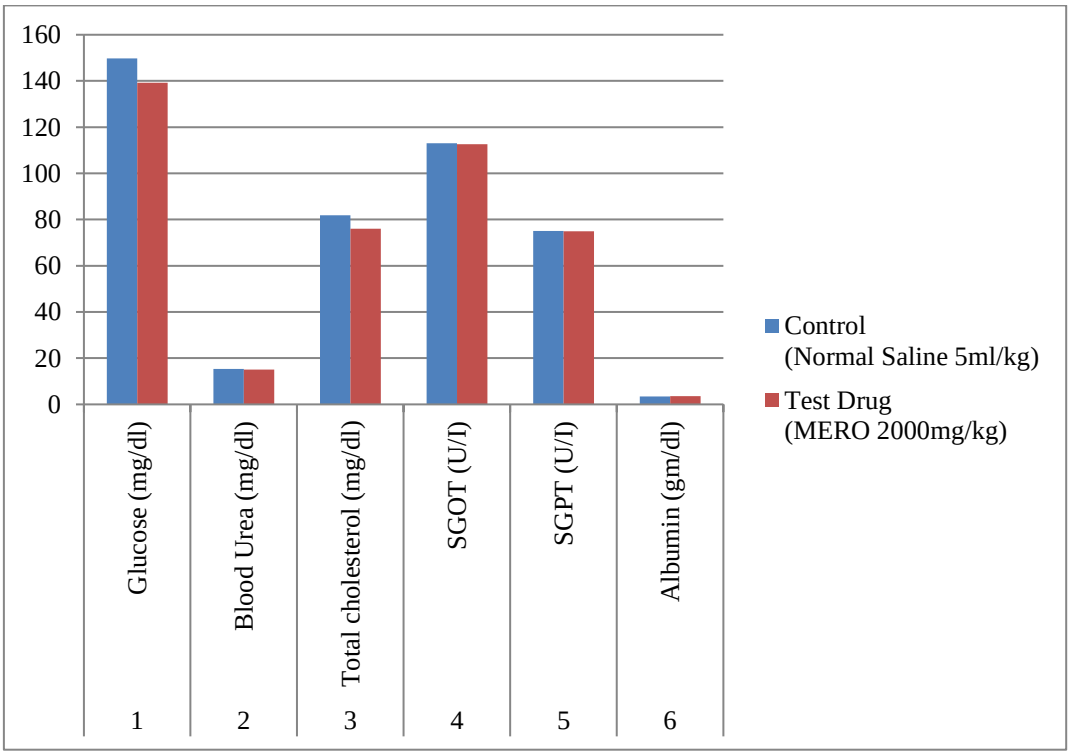


Fig. No. 17: Effect of Test compound on Biochemical parameters in Acute oral toxicity in Albino rats

Histopathological Studies of Acute Oral Toxicity

Control – Normal Saline (5ml/kg, *p.o*)

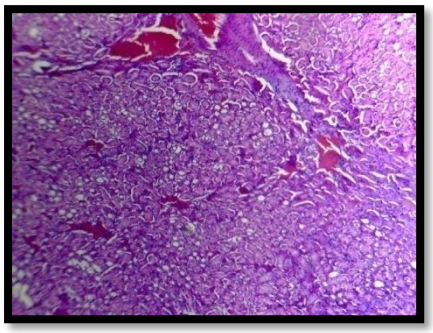


Fig. No. 18: T.S of Heart

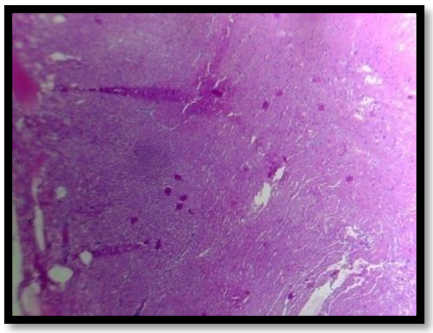


Fig. No. 19: T.S of Kidney

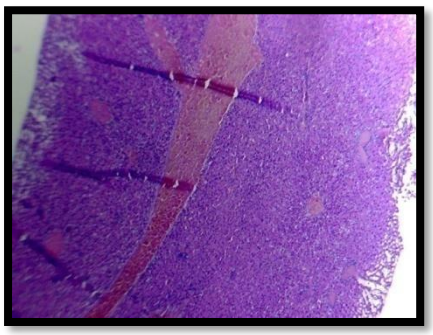


Fig. No. 20: T.S of Liver

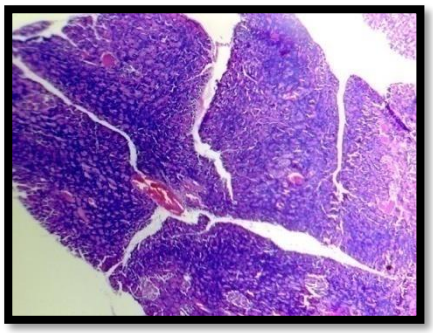


Fig. No. 21: T.S of Pancreas

Test – Methanolic Extract of *Rivea ornata* (2000 mg/kg, *p.o*)

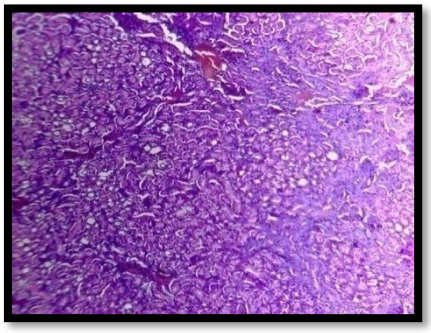


Fig. No. 22: T.S of Heart

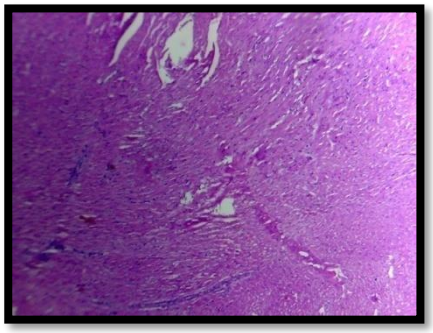


Fig. No. 23: T.S of Kidney

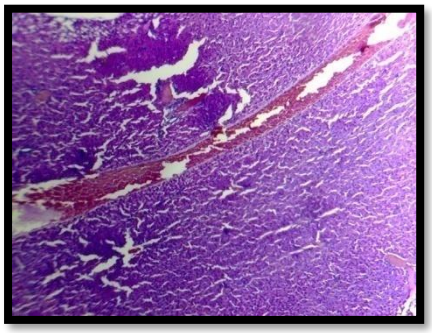


Fig. No. 24: T.S of Liver

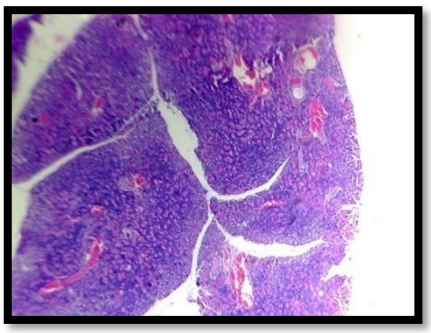


Fig. No. 25: T.S of Pancreas

Toxicity Studies - Subacute Oral Toxicity (Validation)

Table No. 12: Behavioral Changes in Subacute Oral Toxicity in Albino rats

S.No.	Symptoms	Control (Normal Saline 5ml/kg, p.o.)	Methanolic Extract of Rivea ornata (200mg/kg, *p.o.*,)
1	Death	--	--
Autonomic 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 Co ordination	--	--
20	Akinesia	--	--
21	Catelepsy	--	--
22	Loss of Balance	--	--
23	Fore-paw treading	--	--
24	Writhing	--	--
25	Stereotypy	--	--
26	Altered fear	--	--
27	Altered respiration	--	--
28	Aggression	--	--
29	Analgesia	--	++
30	Body temperature	--	--

Tab. No. 13: Effect of Test compound on Body Weight in Subacute oral toxicity in Albino rats

S. No.	Group	Body weight	
		Initial	At the end of the study
1	**Control** (Normal Saline 5ml/kg)	201.29 ± 0.94	203.57 ± 0.69
2	**Test Drug** (MERO 200mg/kg)	201.22 ± 0.55	190.08 ± 1.17

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

Tab. No. 14: Effect of Test compound on Biochemical parameters in Subacute oral toxicity in Albino rats

S. No.	Biochemical Parameters	Control (Normal Saline 5ml/kg)	Test Drug (MERO 200 mg/kg)
1	Glucose (mg/dl)	148.48 ± 0.41	138.27 ± 0.33
2	Blood Urea (mg/dl)	16.19 ± 0.13	13.72 0.11
3	Total cholesterol (mg/dl)	82.28 ± 0.11	73.77 ± 0.09
4	SGOT (U/I)	113.61 ± 1.28	112.15 ± 0.25
5	SGPT (U/I)	75.28 ± 0.07	74.41 ± 0.32
6	Albumin (gm/dl)	3.26 ± 0.03	3.52 ± 0.02

n=6, Values are expressed as ± S.E.M

Tab. No. 15: Effect of Test compound on Haematological parameters in Subacute oral toxicity in Albino rats

S. No.	Group	Hb (gm%)	RBC (10^6/cu.mm)	Total WBC (10^3/cu.mm)	ESR (mm/1st hr)	Platelets
1	**Control** (Normal Saline 5ml/kg)	14.43 ± 0.13	7.32 ± 0.03	7.22 ± 0.02	3.18 ± 0.02	400 ± 9.26
2	**Test Drug** (MERO 200mg/kg)	14.13 ± 0.06	6.88 ± 0.03	7.25 ± 0.02	3.19 ± 0.02	393 ± 12.76

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

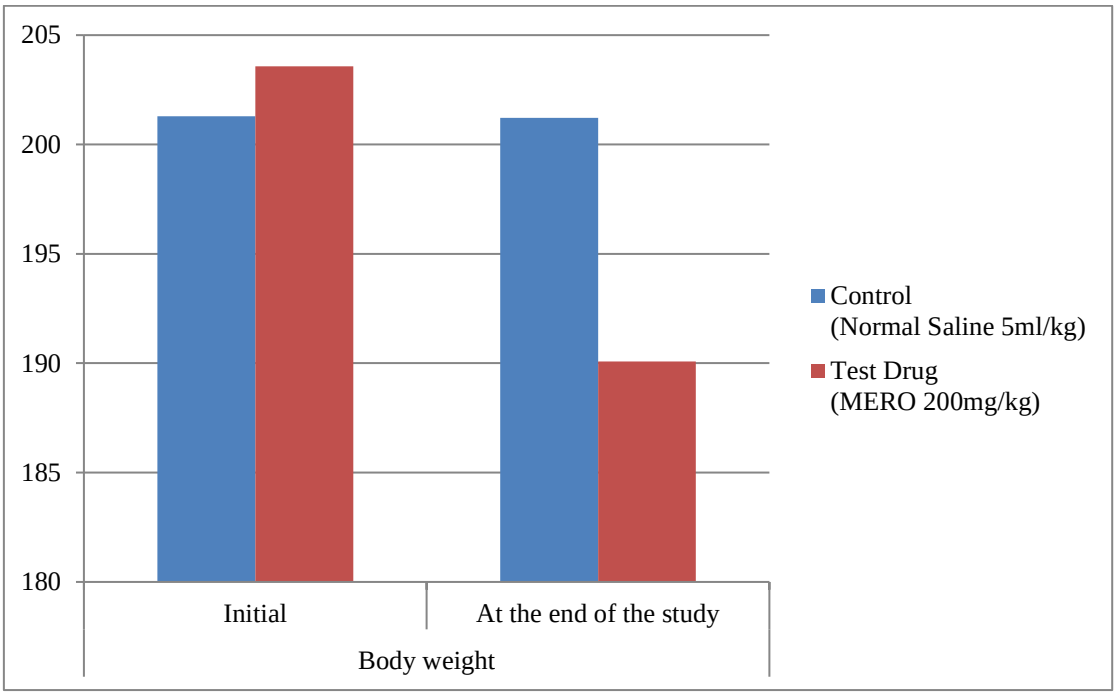


Fig. No. 26: Effect of Test compound on Body Weight in Subacute oral toxicity in Albino rats

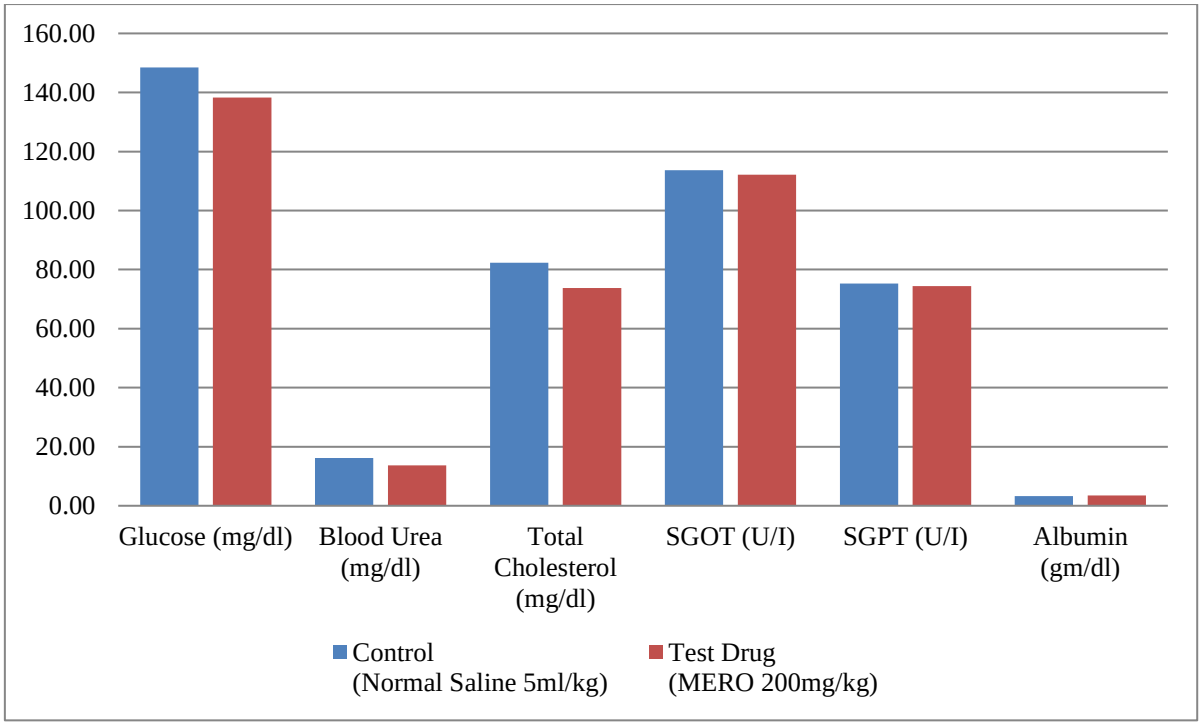


Fig. No. 27: Effect of Test compound on Biochemical parameters in Subacute oral toxicity in Albino rats

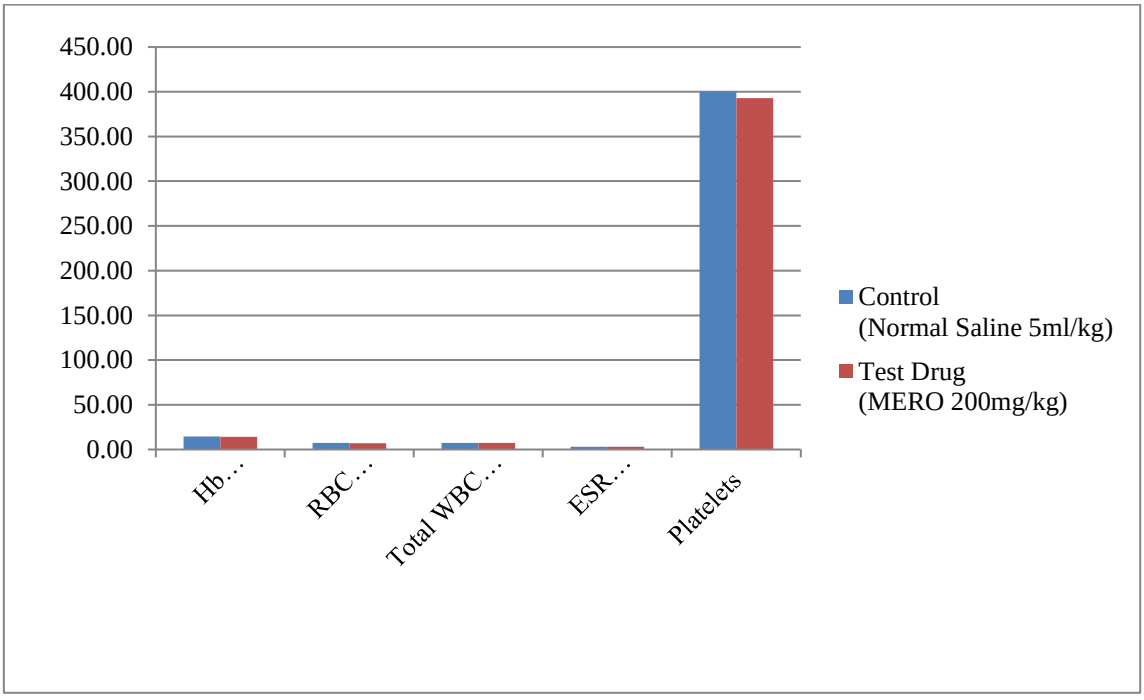


Fig. No. 28: Effect of Test compound on Biochemical parameters in Subacute oral toxicity in Albino rats

Histopathological Studies of Subacute Oral Toxicity

Control – Normal Saline (5ml/kg, *p.o*)

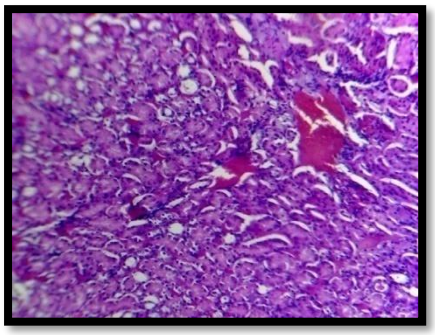


Fig. No. 29: T.S of Heart

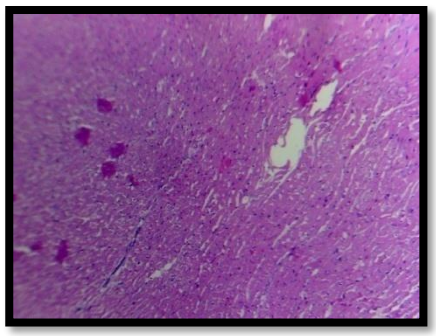


Fig. No. 30: T.S of Kidney

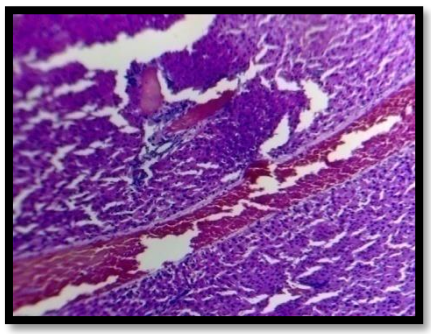


Fig. No. 31: T.S of Liver

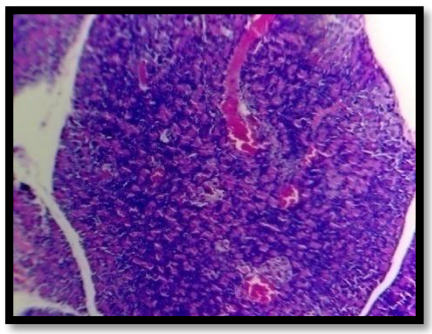


Fig. No. 32: T.S of Pancreas

Test – Methanolic Extract of *Rivea ornata* (200 mg/kg, *p.o*)

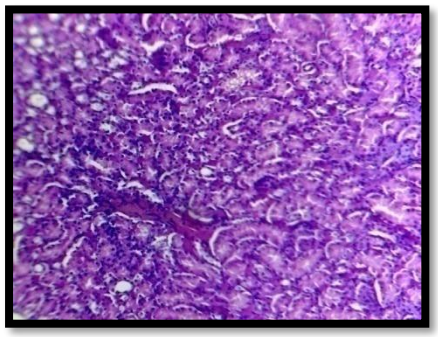


Fig. No. 33: T.S of Heart

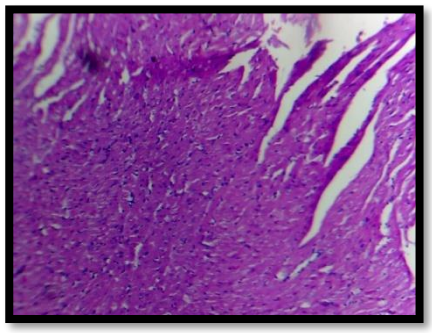


Fig. No. 34: T.S of Kidney

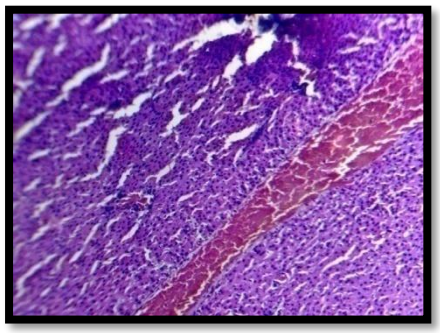


Fig. No. 35: T.S of Liver

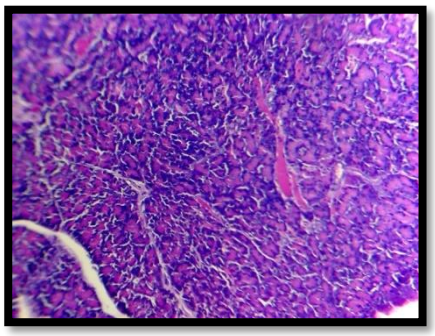


Fig. No. 36: T.S of Pancreas

Antihyperlipidemic activity – Triton X 100 induced hyperlipidemia

Tab. No. 16: Body weight changes in Antihyperlipidemic activity of MERO

S.No.	Group	Body Weight (g)		
		1st Day	4th Day	10th Day
1	Normal Control	209.56 ± 0.39	210.55 ± 0.39	211.68 ± 0.26
2	Toxic Control	210.72 ± 0.43***	220.2 ± 0.30***	226.22 ± 0.38***
3	Standard	209.00 ± 0.51***	221.59 ± 0.61***	212.66 ± 0.56***
4	Test	210.98 ± 0.32***	221.40 ± 0.57***	216.78 ± 0.52***

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

Tab. No. 17: Antihyperlipidemic activity of MERO

S. No.	Groups	Serum Lipid Parameter (mg/dl)				
		Total Cholesterol	Total Triglycerides	HDL - C	LDL - C	VLDL – C
1	**Normal Control** (Normal Saline 5ml/kg)	75.54 ± 0.91	83.40 ± 0.57	35.89 ± 0.53	24.22 ± 0.24	15.69 ± 0.40
2	**Toxic Control** (Triton X 100 100mg/kg)	121.52 ± 0.62	148.73 ± 0.57	28.41 ± 0.55	88.94 ± 0.47	33.01 ± 0.30
3	**Standard** (Atorvastatin 10mg/kg)	86.80 ± 0.62***	73.64 ± 0.60***	37.09 ± 0.51***	44.13 ± 0.70***	22.11 ± 0.44***
4	**Test** (MERO 200mg/kg)	93.20 ± 0.58***	85.28 ± 0.57***	34.55 ± 0.35***	51.10 ± 0.64***	26.70 ± 0.26***

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

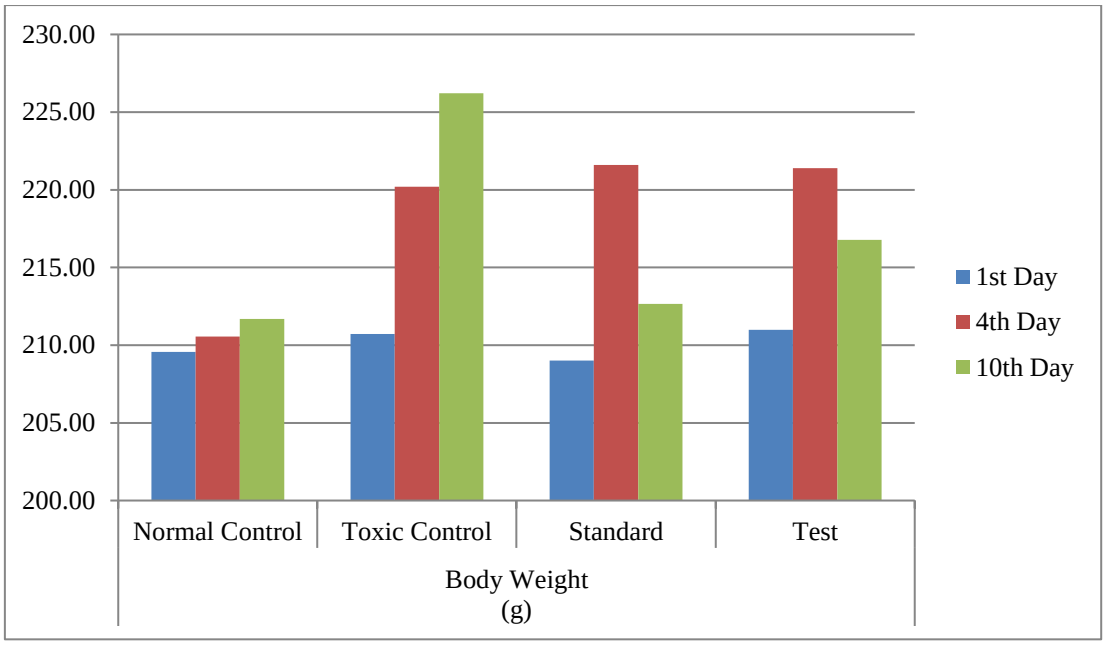


Fig. No. 37: Body weight changes in Antihyperlipidemic activity of MERO

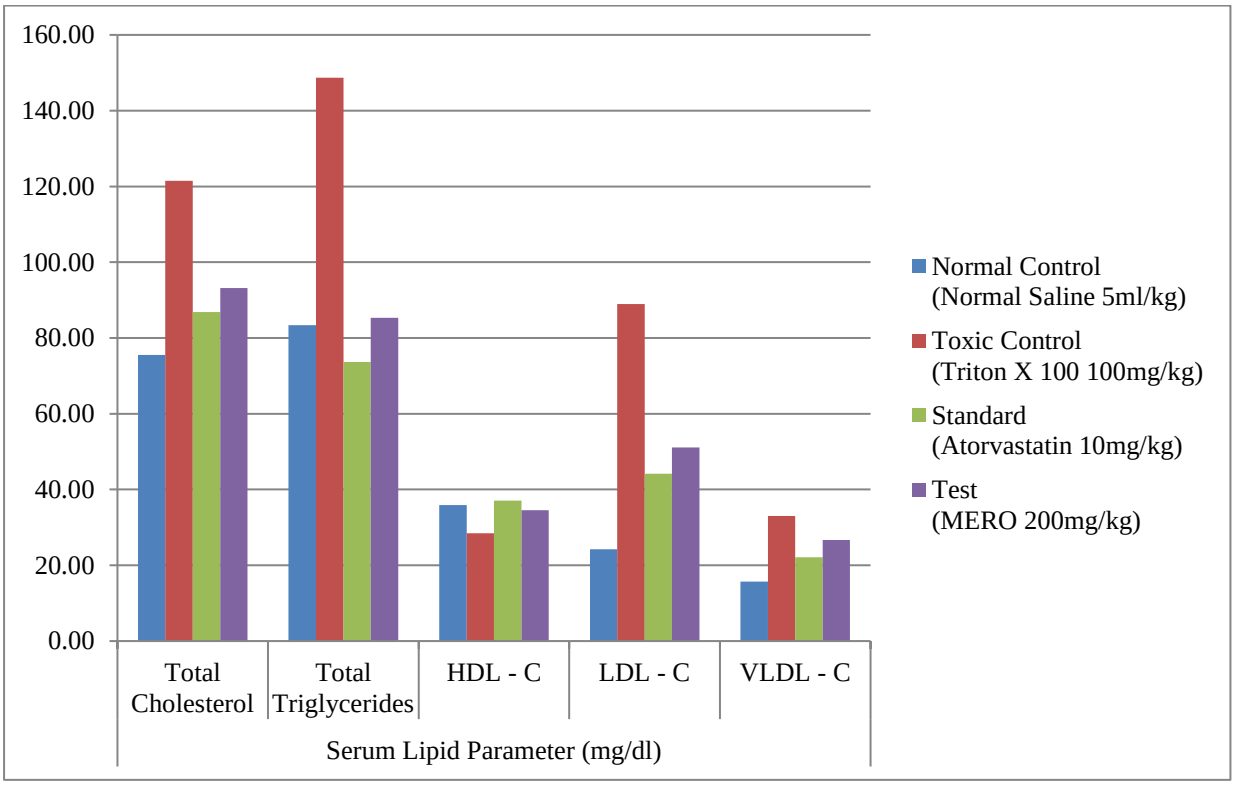


Fig. No. 38: Serum Lipid parameters in Antihyperlipidemic activity of MERO

Histopathological study in Antihyperlipidemic Activity of *Rivea ornata*

Histopathology of Liver

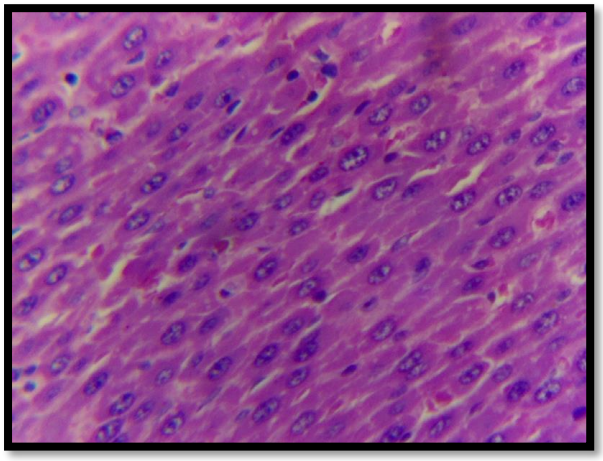


Fig. No. 39: Normal Control

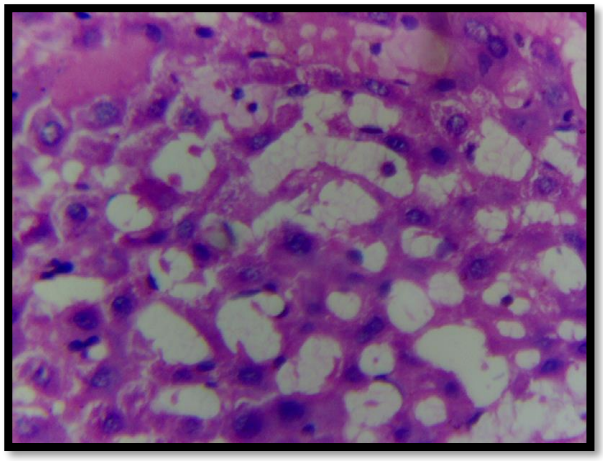


Fig. No. 40: Toxic Control

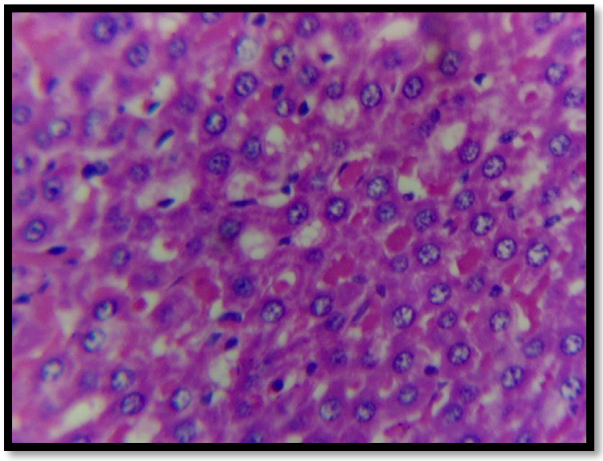


Fig. No. 41: Standard

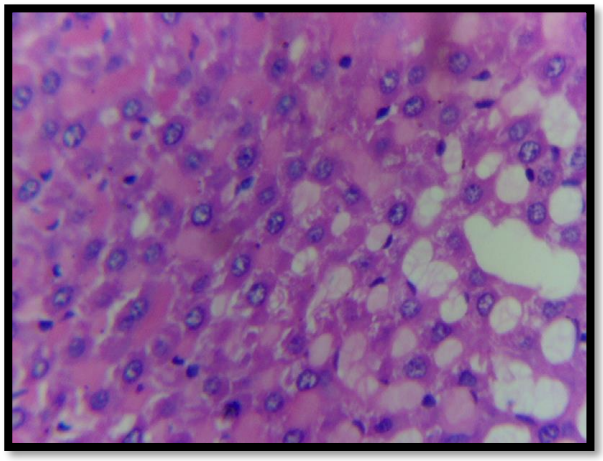


Fig. No. 42: Test

Normal Control - Normal Saline (5ml/kg)

Toxic Control - Triton X 100 (100mg/kg)

Standard - Atorvastatin (10mg/kg)

Test - Methanolic Extract of *Rivea ornata* (200mg/kg)

Tab. No. 18: Histopathological study in Antihyperlipidemic Activity of *Rivea ornata*

T.S of Liver

S. No	Group	Description
1	Normal Control	Normal architecture
2	Toxic 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

Antidiabetic activity – Alloxan Induced Diabetes

Tab. No. 19: Antidiabetic activity of Methanolic Extract of *Rivea ornata*

S.No.	Drug/Dose	Blood Glucose Level						
		0 Hour	**1 Hour**	**2 Hours**	**3 Hours**	**4 Hours**	**7th day**	**14th day**
1	**Normal Control** Normal saline (5ml/kg)	108.33 ± 1.58	105.67 ± 2.29	106.00 ± 1.29	107.50 ± 2.14	108.33 ± 2.20	108.33 ± 1.89	107.17 ± 1.54
2	Diabetic Control Alloxan (150mg/kg)	191.17 ± 1.54	193.50 ± 1.91	192.00 ± 2.17	192.17 ± 1.56	192.33 ± 1.41	191.83 ± 1.54	191.83 ± 1.92
3	**Standard** Glibenclamide (10mg/kg)	192.5 ± 1.26***	147.17 ± 1.01***	138.17 ± 1.08***	125.5 ± 0.85***	119.00 ± 0.73***	120.33 ± 0.88***	117.33 ± 0.49***
4	**Test** MERO (200mg/kg)	192.50 ± 2.04***	150.17 ± 0.70***	149.17 ± 0.95***	132.00 ± 0.73***	164.50 ± 1.28***	150.33 ± 0.99***	139.50 ± 0.67***

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

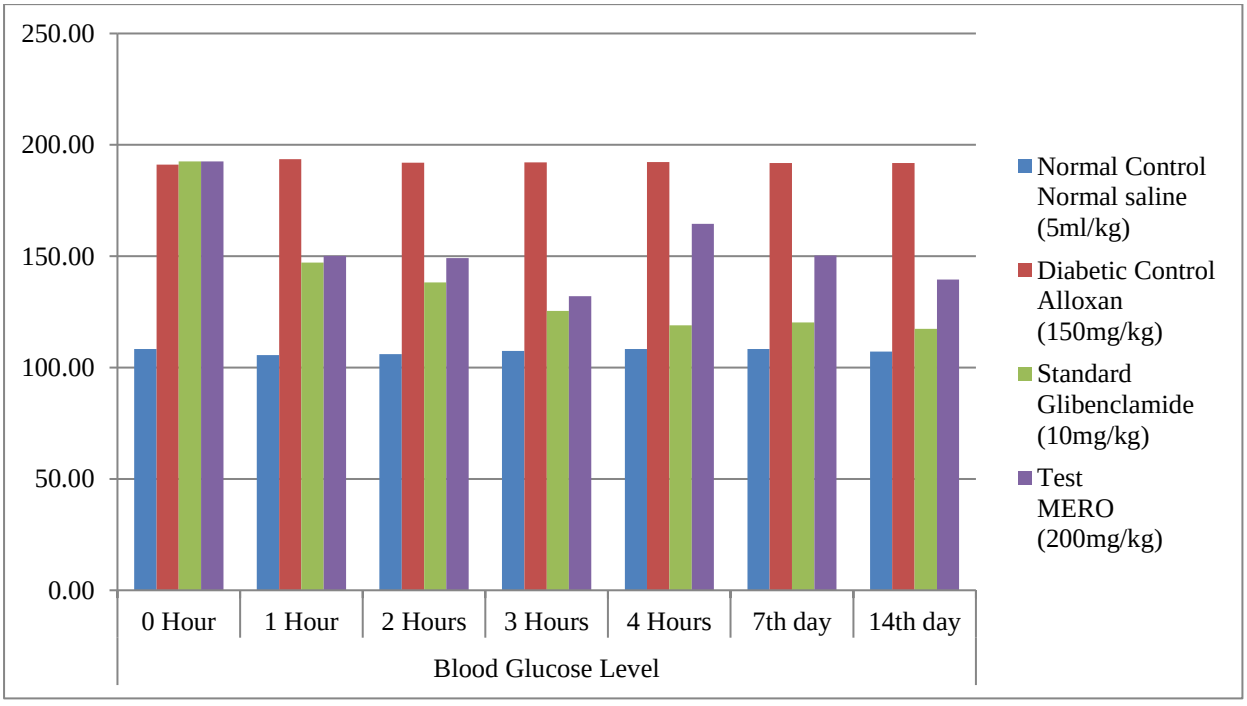


Fig. No. 43: Antidiabetic activity of Methanolic Extract of *Rivea ornata*

Histopathological Study in Antidiabetic activity of MERO

Histopathology of Pancreas

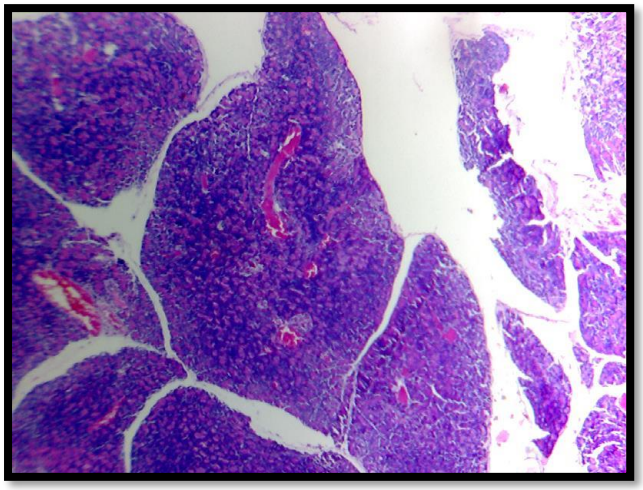


Fig. No. 44: Normal Control

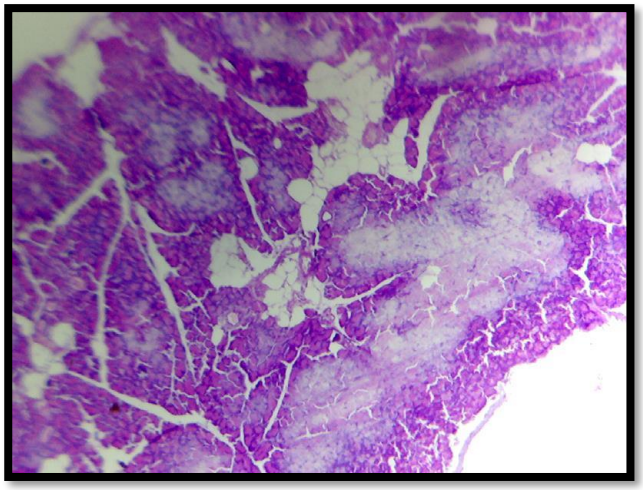


Fig. No. 45: Toxic Control

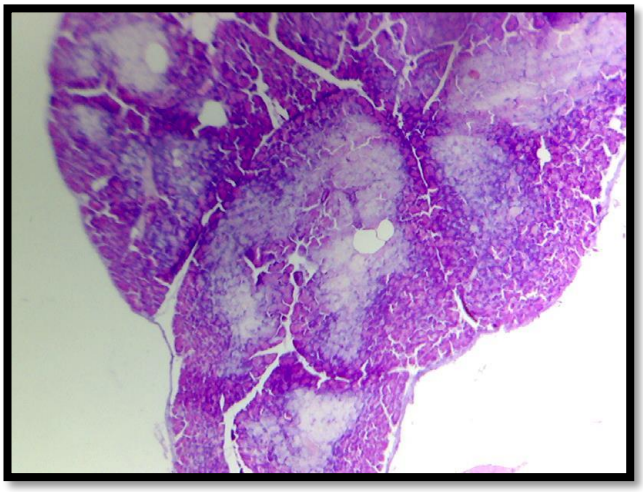


Fig. No. 46: Standard

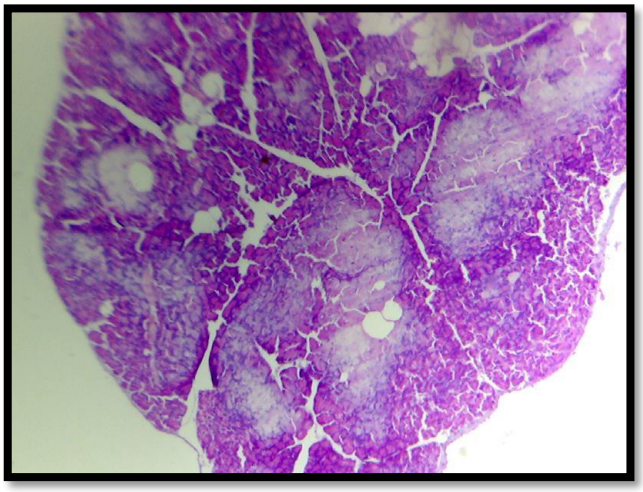


Fig. No. 47: Test drug

Normal Control - Normal Saline (5ml/kg)

Toxic Control - Alloxan (150mg/kg)

Standard - Alloxan (150mg/kg) + Glibenclamide (10mg/kg)

Test - Alloxan (150mg/kg) + Methanolic Extract of *Rivea ornata* (200mg/kg)

Tab. No. 20: Histopathological Study in Antidiabetic activity of MERO

T.S of pancreas

S. No	Group	Description
1	Normal Control	Normal architecture of pancreatic islet cells
2	Toxic Control	Expanded and dilated architecture of pancreatic islet cells
3	Standard	Absence of dilatation, prominent hyperplastic pancreatic islets.
4	Test	Prominent hyperplastic pancreatic islets with moderate expansion and slight dilatation.

Analgesic activity

Tab. No. 21: Analgesic activity of MERO - Tail Immersion Method

S.No.	Drug/Dose	Reaction time in seconds				
		0 minute	**30 minutes**	**45 minutes**	**60 minutes**	**90 minutes**
1	**Control** Normal saline (5ml/kg)	1.72 ± 0.06	1.81 ± 0.04	1.8 ± 0.02	1.85 ± 0.04	1.81 ± 0.02
2	**Standard** Diclofenac (10mg/kg)	1.74 ± 0.02***	3.1 ± 0.02***	5.7 ± 0.02***	7.6 ± 0.01***	9.49 ± 0.02***
3	**Test** MERO (200mg/kg)	1.81 ± 0.04***	2.93 ± 0.02***	4.61 ± 0.02***	6.95 ± 0.02***	8.37 ± 0.02***

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

Tab. No. 22: Analgesic activity of MERO – Hot plate Method

S.No.	Drug/Dose	Reaction time in seconds				
		0 minute	**30 minutes**	**45 minutes**	**60 minutes**	**90 minutes**
1	**Control** Normal saline (5ml/kg)	4.18 ± 0.06	4.1 ± 0.05	4.16 ± 0.04	4.15 ± 0.02	4.12 ± 0.04
2	**Standard** Diclofenac (10mg/kg)	4.22 ± 0.06***	10.19 ± 0.04***	11.55 ± 0.02***	12.15 ± 0.05***	12.21 ± 0.03***
3	**Test** MERO (200mg/kg)	4.04 ± 0.04***	8.77 ± 0.02***	10.01 ± 0.02***	10.14 ± 0.02***	10.27 ± 0.02***

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

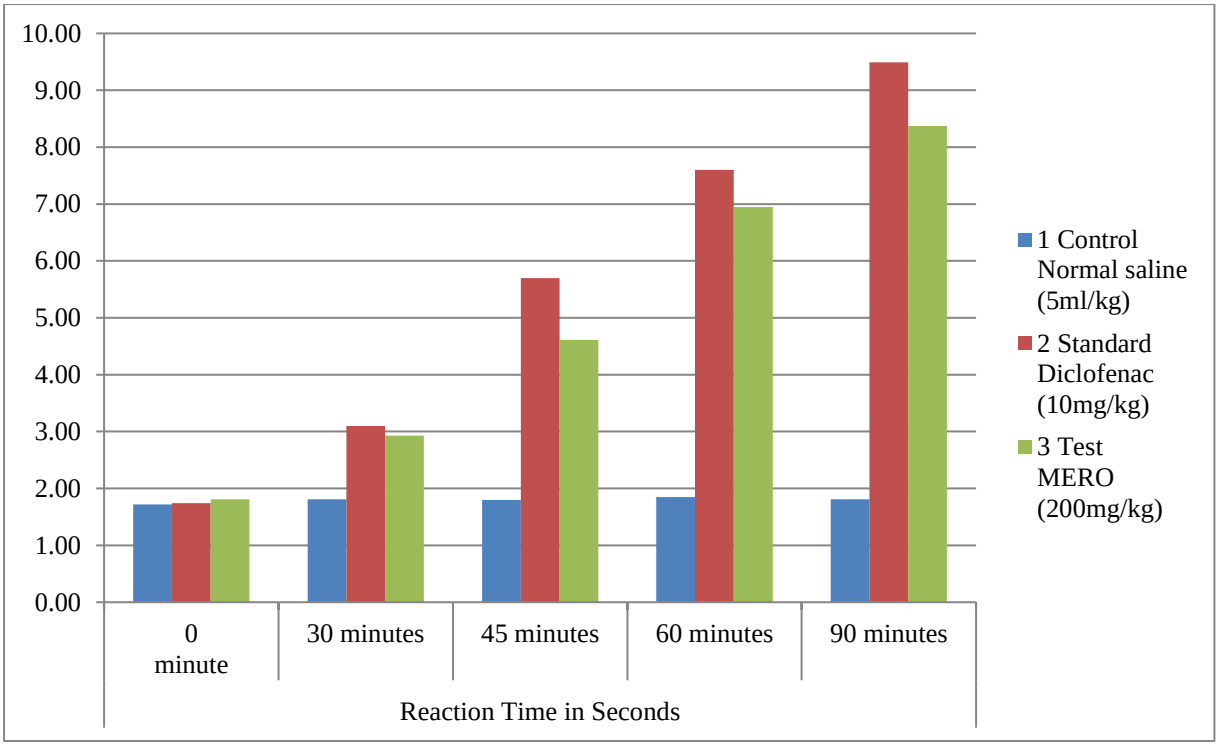


Fig. No. 48: Analgesic activity of MERO - Tail immersion method

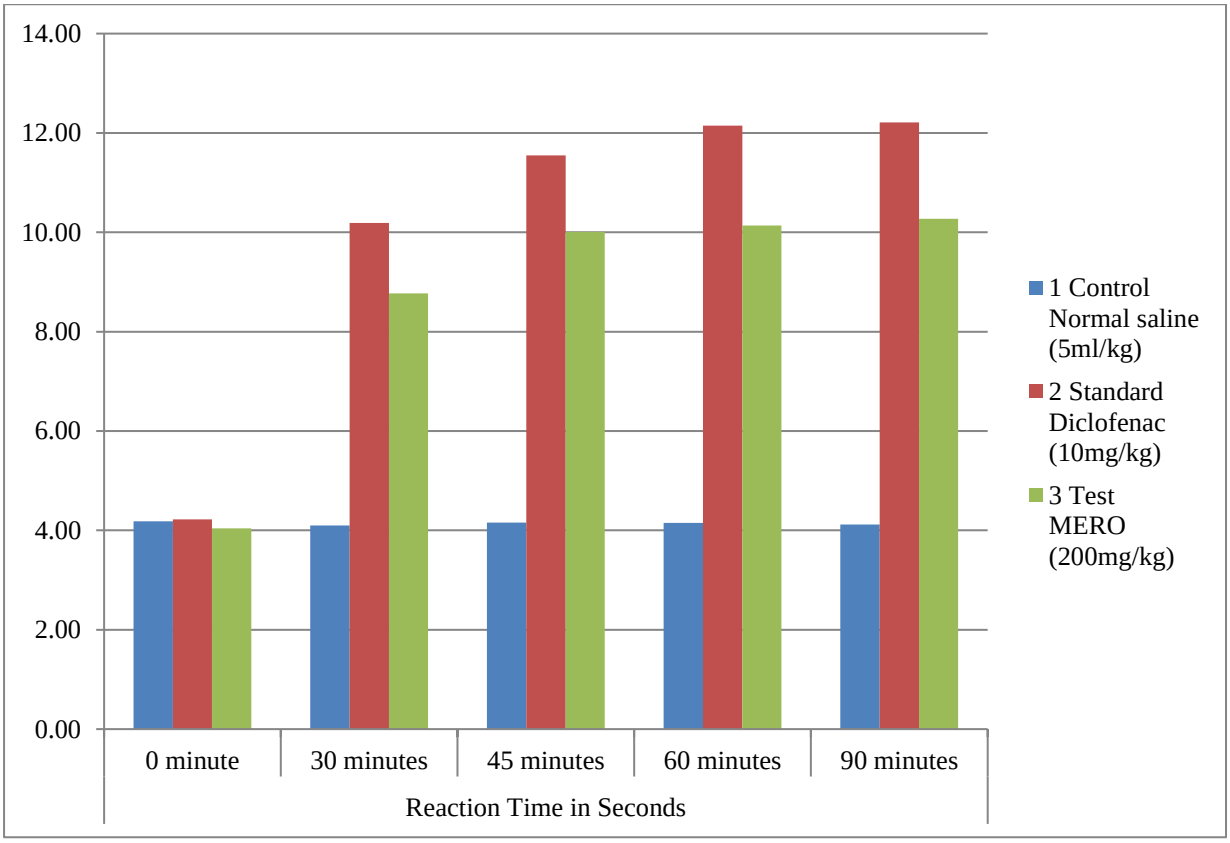


Fig. No. 49: Analgesic activity of MERO – Hot plate Method

Antipyretic activity

Tab. No. 23: Antipyretic activity of MERO – Brewer's Yeast Induced Hyperpyrexia

Group	Basal Temperature (before yeast administration) (F)	Pre-Drug control (before drug administration) (F)	Rectal temperature after drug administration (F)				
			1 Hour	2 Hours	3 Hours	4 Hours	5 Hours
Control Normal Saline (5ml/kg)	97.54 ± 0.19	102.44 ± 0.05	102.42 ± 0.06	102.42 ± 0.10	102.39 ± 0.09	102.37 ± 0.08	102.34 ± 0.10
Standard Paracetamol (150mg/kg)	97.49 ± 0.14***	102.21 ± 0.08***	99.66 ± 0.04***	99.15 ± 0.03***	98.57 ± 0.03***	97.82 ± 0.03***	97.48 ± 0.03***
Test MERO (200mg/kg)	97.56 ± 0.13***	102.06 ± 0.19***	99.81 ± 0.03***	99.27 ± 0.05***	98.8 ± 0.04***	98.19 ± 0.03***	97.77 ± 0.03***

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

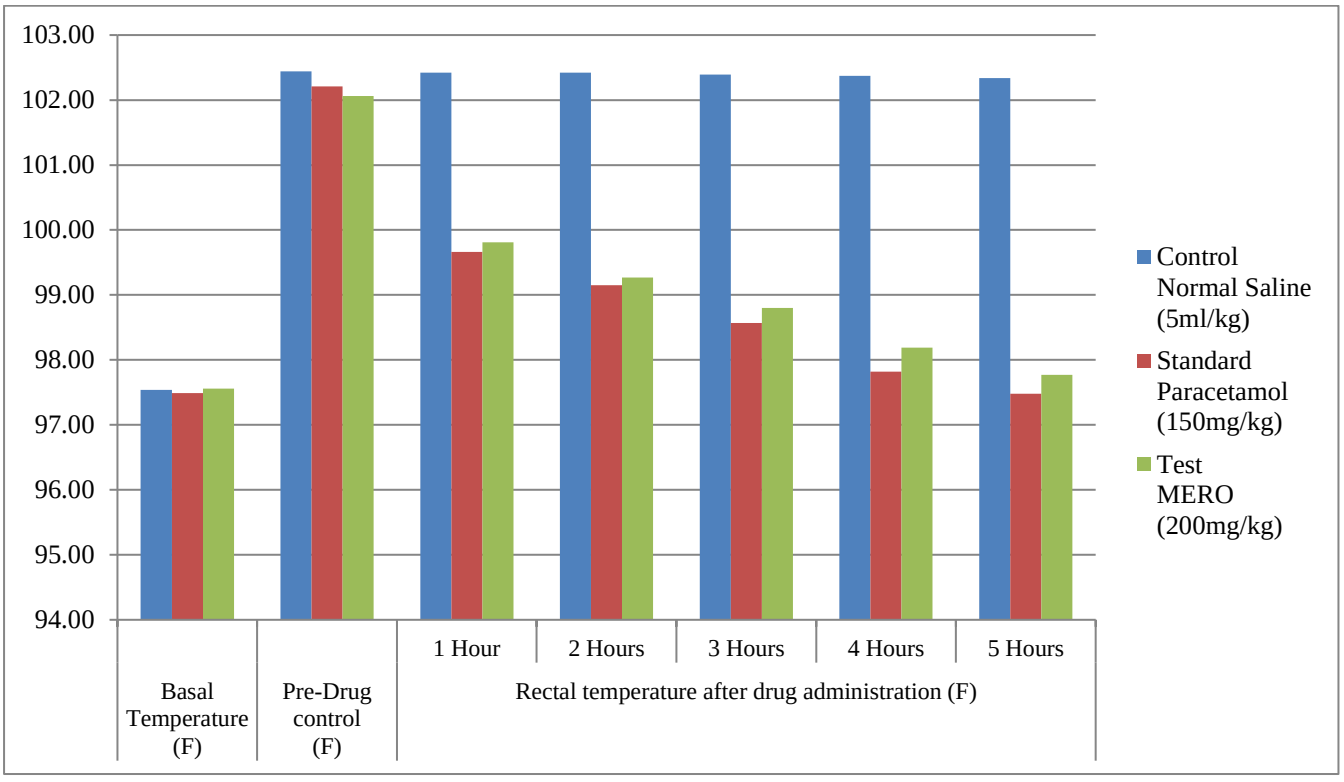


Fig. No. 50: Antipyretic activity of MERO – Brewer's Yeast Induced Hyperpyrexia

Wound Healing Activity – Excision Wound Model

Tab. No. 24: Wound Healing activity of Methanolic Extract of *Rivea ornata*

S. No	Wound Area (mm^2)			
	Day 0	**Day 3**	**Day 7**	**Day 12**
Control (Normal Saline)	310.9 ± 2.68	242.26 ± 1.70	123.34 ± 1.65	32.5 ± 0.62
Standard (Povidone iodine Cream)	311.98 ± 4.18***	222.47 ± 2.53***	99.18 ± 3.09***	15.25 ± 0.08***
Test (MERO Cream)	317.2 ± 3.55***	200.16 ± 2.39***	72.93 ± 2.14***	0.02 ± 0.01***

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

Tab. No. 25: Period of Epithelialization in Wound Healing activity of MERO

S. No	Period of Epithelialization
Control (Normal Saline)	14.5 ± 0.43
Standard (Povidone iodine)	13.5 ± 0.22
Test (MERO)	11.83 ± 0.31

Tab. No. 26: Wound Contraction percentage in Wound Healing activity of MERO

S. No.	Group	Wound Contraction (%)			
		0 Day	3rd Day	7th Day	12th Day
1	**Control** (Normal Saline)	0	22.05 ± 0.91	49.09 ± 0.62	73.59 ± 0.85
2	**Standard** (Povidone Iodine Cream)	0	28.59 ± 1.64	55.45 ± 1.03	84.47 ± 1.17
3	**Test** (MERO Cream)	0	36.83 ± 1.35	63.51 ± 1.34	99.98 ± 0.01

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

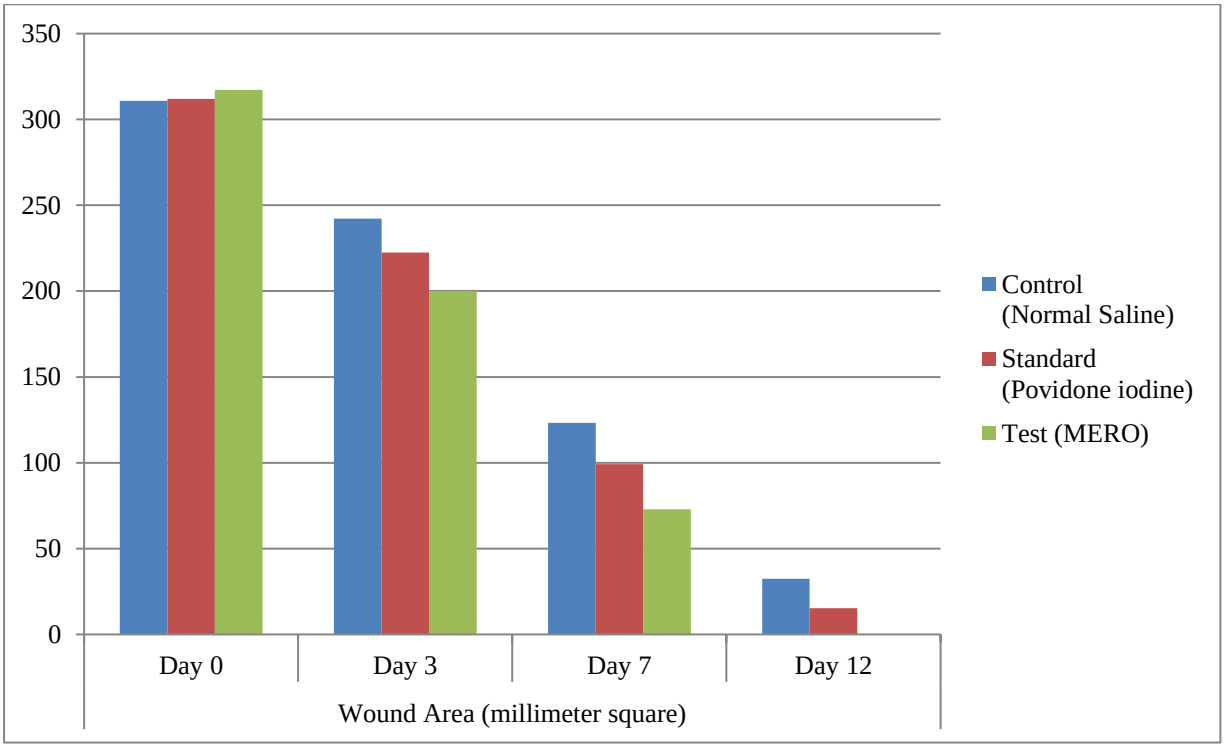


Fig. No. 51: Wound Healing activity of Methanolic Extract of *Rivea ornata*

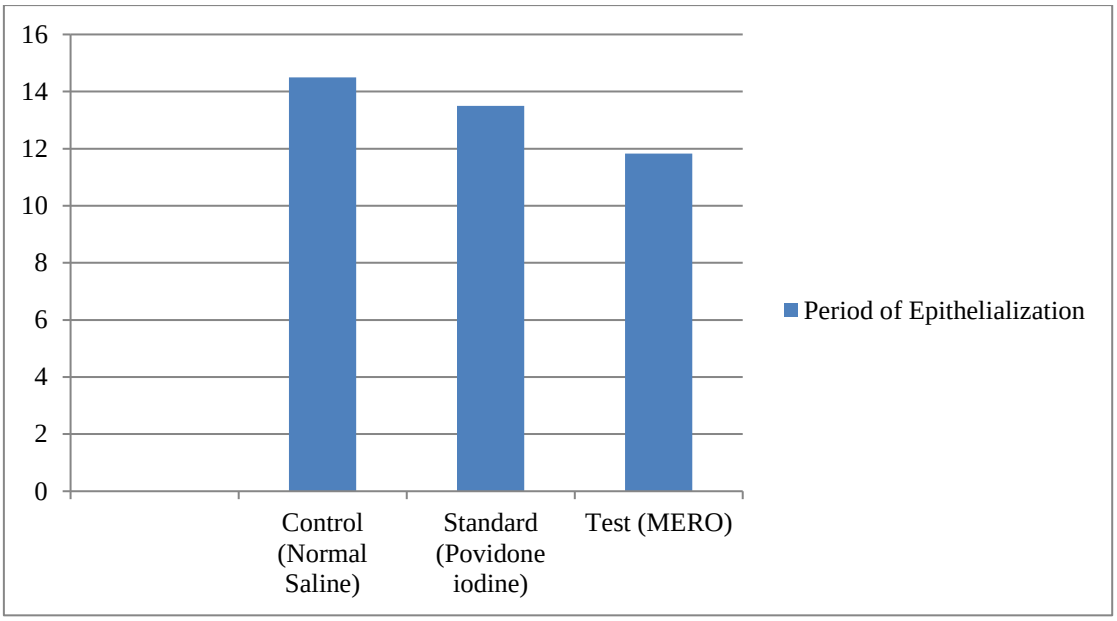


Fig. No. 52: Period of Epithelialization in Wound Healing activity of MERO

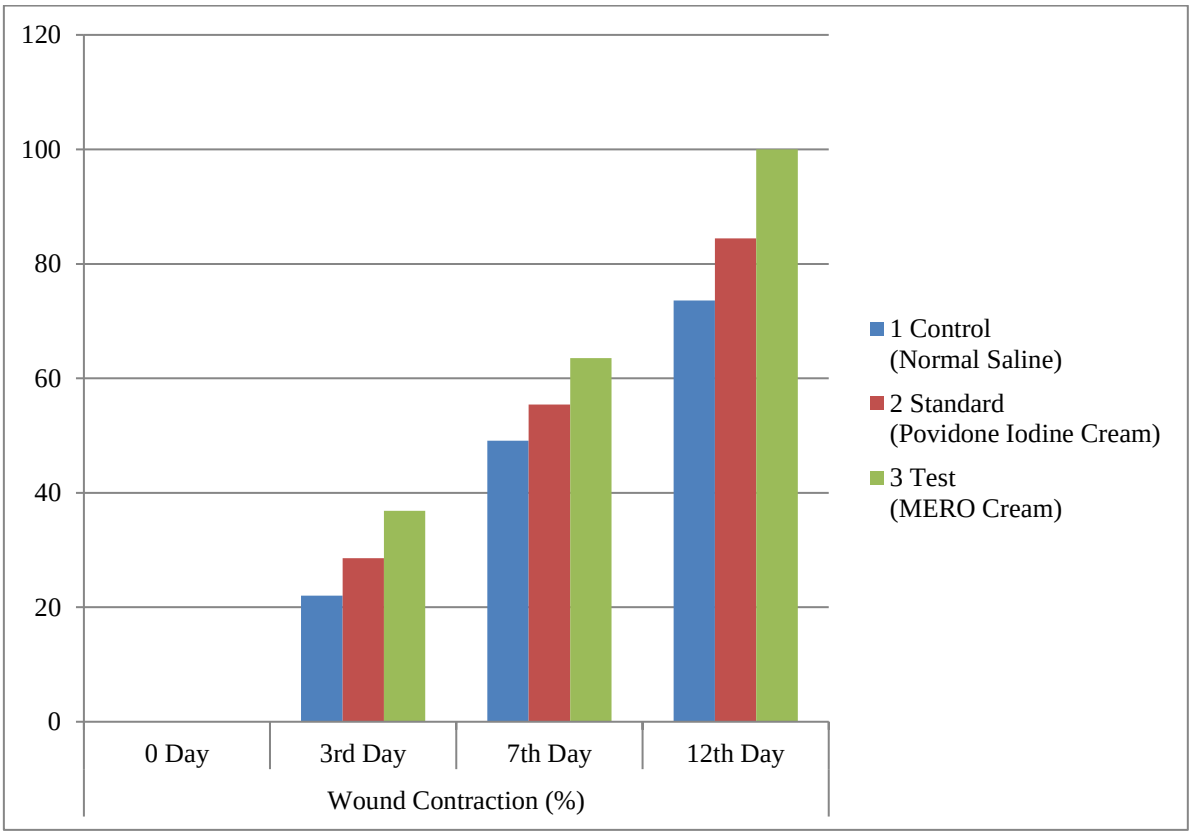


Fig. No. 53: Wound Contraction Percentage in Wound Healing activity of MERO

Wound Healing activity of Methanolic Extract of *Rivea ornata*

O Day

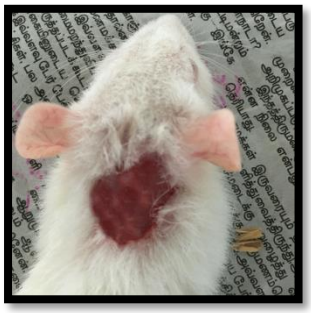


Fig. No. 54: Control

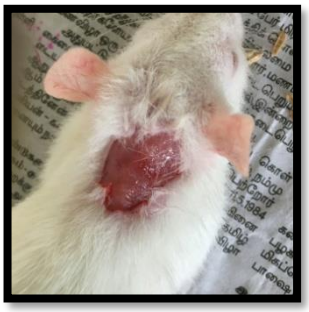


Fig. No. 55: Standard

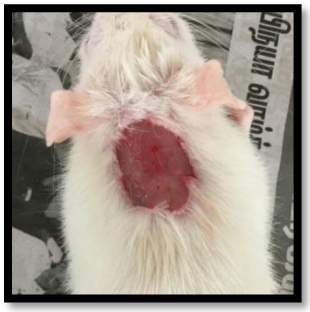


Fig. No. 56: Test

3rd Day

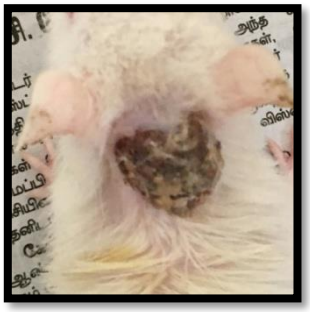


Fig. No. 57: Control

Fig. No. 58: Standard

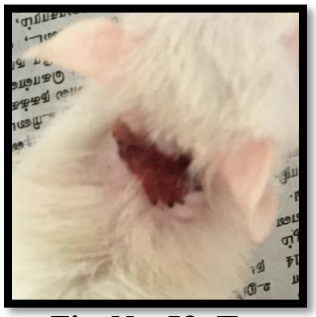


Fig. No. 59: Test

7th Day

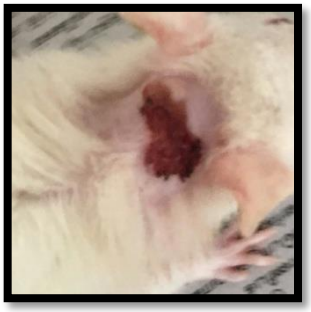


Fig. No. 60: Control

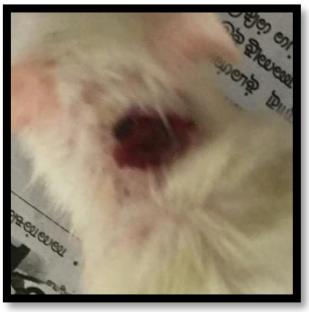


Fig. No. 61: Standard

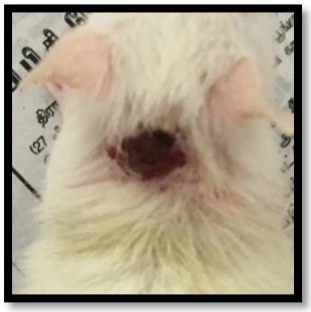
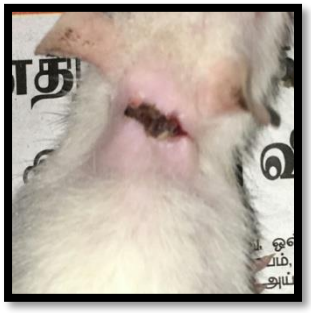
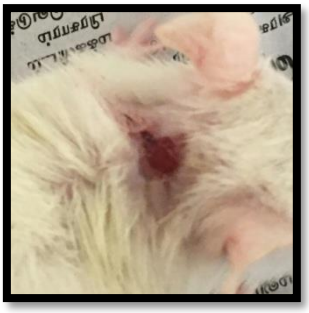


Fig. No. 62: Test

12th Day





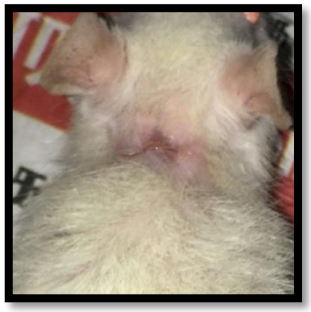


Fig. No. 63: Control **Fig. No. 64: Standard** **Fig. No. 65: Test**

7.2. Discussion

7.2.1. Extraction of Plant material

The leaves of *Rivea ornata* Roxb. was collected, dried and extracted using methanol and prior to the ethanolinic extraction the plant was coarsely milled and defatted using petroleum ether. The yield of the methanolic extract was 60%.

7.2.2. Preliminary Phytochemical Analysis

As a part of the preclinical study, the methanolic extract of leaves of *Rivea ornata* Roxb. was subjected to qualitative chemical test and confirmed the presence of carbohydrates, alkaloids, steroids, glycosides, tannins, saponins and phenolic compounds shown in **Table No. 6**.

7.2.3. IR Spectral Data of the Ethanolic extract of *Rivea ornata*

The methanolic extract of leaves of *Rivea ornata* Roxb. was subjected to the Infra Red Spectroscopy for their possible functional group **(Table No. 7).** The spectrum was obtained and photo documentation was done **(Fig. No. 8)**. The methanolic extract of leaves of *Rivea ornata* roxb. showed the possible stretching and bending shows the presence of the functional group.

7.2.4. HPTLC

The HPTLC fingerprinting of Methanolic extract of leaves of *Rivea ornata* Roxb. was studied. The HPTLC fingerprinting was done. The 3D display of the chromatogram was obtained and photo documentation was done **(Fig. No. 10)** and four different chromatograms were obtained and photo documentation was done **(Fig. Nos. 11, 12, 13 and 14).** The Peaks are viwed at system suitability was shown **(Fig. No. 15)**.

7.2.5. Pharmacological Screening

7.2.5.1. Toxicity Studies – Validation of Acute Toxicity.

In acute oral toxicity studies, methanolic extract of leaves of *Rivea ornata* Roxb. did not produce mortality at a dose of 2000mg/kg body weight in rats. Hence 1/10th of LD50 (i.e.) 200mg/kg was considered as the dose level for further pharmacological screening. The parameters observed were behavioral changes, hiochemical 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 reactivity to touch and analgesia. **(Table No. 9)**.

Biochemical Parameters and Body Weight

The biochemical parameters observed in animals treated with 2000 mg/kg of methanolic extract of leaves of *Rivea ornata* Roxb. 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. 11) (Fig. No. 17).** 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 **(Table No. 10 & Fig. No. 16).** Histopathological Studies **(Fig. Nos. 18 - 25)** 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.

7.2.5.2. Toxicity Studies – Subacute Toxicity.

In subacute oral toxicity studies, methanolic extract of leaves of *Rivea ornata* Roxb. did not produce mortality at a dose of 200mg/kg body weight in rats for 28 days. 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 move ments, lacrimation, loss of corneal reflex, loss of righting reflex, loss of traction, miosis/mydriasis, salivation and scratching. Among the behavioral parameters observed the animal showed positive response for altered reactivity to touch and analgesia. **(Table No. 12)**.

Biochemical Parameters and Body Weight

The biochemical parameters observed in animals treated with 2000 mg/kg of methanolic extract of leaves of *Rivea ornata* Roxb. 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. 14) (Fig. No. 27).** 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 **(Table No. 13 & Fig. No. 26).** Histopathological Studies **(Fig. Nos. 18 - 25)** represents the transverse sections of Heart, Kidney, Liver and pancreas showing normal histology upon administration of 200mg/kg of Test Compound for 28 days 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.

7.2.5.3. Antihyperlipidemic activity

Triton X 100 Induced Hyperlipidemia

Intraperitoneal administration of Triton X 100 (100mg/kg, b.w.) increased the level of serum total cholesterol, triglycerides, LDL, VLDL and decrease in the level of good cholesterol carrier HDL by inhibiting the lipase activity. Elevated level of blood cholesterol especially LDL was the major risk factor for the coronary heart disease and HDL as cardio protective protein. Treatment with methanolic extract of leaves of *Rivea ornata* (200 mg/kg) significantly decreased the level of cholesterol, triglycerides, VLDL and LDL as compared to hyperlipidemic control. There was significant increase in HDL as compared to control.

The body weight of the animal was measured at 1^{st}, 4^{th} and 10^{th} day during the study **(Tab.No. 16) (Fig. No. 37)**

The blood was collected on 10^{th} day by cardiac puncture under mild anesthesia, the serum was assayed for total cholesterol, total triglycerides, HDL-C, LDL-C and VLDL-C. **(Tab. No. 17) (Fig. No. 38)**

The liver was collected on 10^{th} day and transverse sections were obtained from various groups such as normal control, toxic control, standard and test **(Fig. Nos. 39 – 42)**

Triton X 100 induced hyperlipidemia is associated with alteration in the activities of enzyme responsible for cholesterol transport and metabolism. Triton X 100 cause significant increases in triglycerides level by inhibiting the capillary lipoprotein lipase which is responsible for plasma triglycerides hydrolysis and produce significant increase in serum cholesterol by the stimulation of 3-hydroxy-3-rmethylglutaryl-Co-enzyme A (HMG-CoA) reductase activity in the liver.

Treatment with methanolic extract of *Rivea ornata* (200 mg/kg) showed the significant antihyperlipidemic activity. Reduction in the triglycerides level may be due to increased lipoprotein lipase activity and reduction in total cholesterol level may be due to inhibition of HMG CoA reductase activity.

7.2.5.4. Antidiabetic Activity

Alloxan Induced Hyperglycemia

Intraperitoneal administration of alloxan (150mg/kg, b.w.) increased the blood glucose level by degrading the beta cells of pancreas. Treatment with methanolic extract of leaves of *Rivea ornata* (200mg/kg) was decrease the blood glucose level and shows antidiabetic index upto 3 hours. The blood glucose level was measured at 0 hour, 1 hour, 2 hour, 3 hour, 4 hour, 7^{th} day and 14^{th} day **(Tab. No. 18) (Fig. No. 43).**

The pancreas was collected on 14^{th} day and transverse sections were obtained from various groups such as normal control, toxic control, standard and test **(Fig. No. 44 – 47)**

Alloxan has two distinct pathological effects: it selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase (The glucose sensor of the beta cell), and it causes a state of insulin-dependent diabetes. Alloxan also induce ROS (Reactive Oxygen Species) formation, resulting in the selective necrosis of beta cells.

The preliminary phytochemical analysis showed that the methanolic extract of *Rivea ornata* contains well known antioxidant phytochemicals such as alkaloids, glycosides and polyphenols which act as free radical scavengers. The presumed mechanism of action of these antioxidants was because of insulin mimetic effect on the peripheral tissues by either stimulation of regeneration process or release of pancreatic secretion of insulin from existing beta cells.

7.2.5.5. Analgesic Activity

The analgesic activity of methanolic extract of leaves of *Rivea ornata* was evaluated by tail immersion and hot plate method. The result of analgesic activity of methanolic extract of leaves of *Rivea ornata* was shown in **Tab. No. 19, Fig. No. 48** and **Tab. No. 20, Fig. No. 49** respectively.

Tests of analgesic drugs commonly measure the nociception and involve the reaction of animals to pain stimuli. The stimulus may be Thermal (Tail flick and Hot plate), Chemical (Acetic acid induced writhing) or mechanical (Tail or paw pressure tests). Tail immersion and Hot plate model induce pain by thermal stimuli method. The analgesic effect of Rivea

ornata may be mediated through peripheral pain mechanism or through suppression of prostaglandin pathway.

7.2.5.6. Antipyretic activity

Brewer's yeast induced hyperpyrexia

Subcutaneous injection of 12.5% w/v Brewer's yeast solution increased the body temperature in rats. Treatment with methanolic extract of leaves of *Rivea ornata* (200mg/kg) was decrease the body temperature and that was compared with the reference standard paracetamol (150mg/kg, b.w.) and shown in **Tab. No. 21** and **Fig. No. 50.**

Normally, the infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediators (cytokines, such as interleukin-1ß, interleukin-α, interleukin-ß, and TNF-α), which increase the synthesis of prostaglandin E2 (PgE2) near hypothalamic area and thereby trigger the hypothalamus to elevate the body temperature. Brewer's yeast induces both TNF-α and prostaglandin synthesis.

The mechanism of action of antipyretic effect of methanolic extract of *Rivea ornata* may be due to the inhibition of prostaglandin synthesis or by inhibiting the formation of inflammatory mediators.

7.2.5.7. Wound Healing activity

The wound healing activity of methanolic extract of leaves of *Rivea ornata* Roxb. was evaluated by the excision wound model. About area of wound with 300mm^2 was produced by excision made at the neck part of the rat. Application of ointment of methanolic extract of leaves of *Rivea ornata* greatly reduce the area of wound was compared with control and standard group **(Tab. No. 22) (Fig. No. 53 – 64).** The wound contraction percentage was calculated and shown in **Tab. No. 23 and Fig. No. 52.**

Wound healing is a complex process that can be divided into at least 3 continuous and overlapping processes: an inflammatory reaction, a proliferative process leading to tissue restoration, and, eventually, tissue remodeling. Wound healing processes are regulated by multiple growth factors and cytokines released at the wound site. The shorter period of epithelialization and faster wound area contraction could be due to the ability of *Rivea ornata* leaf extract to enhance collagen synthesis, induction of cell proliferation, and antimicrobial activities of bioactive constituents such as alkaloids, steroids and glycosides.

CONCLUSION

8. CONCLUSION

From the study entitled "Pharmacological Evaluation of Leaves Extract from *Rivea ornata Roxb.*, the following conclusion could be drawn

- The present study has thus duly supported the traditional use of methanolic Extract of *Rivea ornata Roxb.* have scientifically proved the antihyperlipidemic, antidiabetic, analgesic, antipyretic and wound healing activity
- Apart from the suggested actions listed in discussion part absence of acute toxicity and sub acute toxicity may also offer a new hope for safe treatment in future
- Preliminary phytochemical study of methanolic extract of *Rivea ornata Roxb.* was found to contain Carbohydrates, Steroids, Alkaloids, Glycosides, Tannins, Phenolic compounds, Gums and mucilage are present
- Presence of Alkaloids, Tannins and Glycosides in the methanolic extract of *Rivea ornata Roxb.* was observed in IR & HPTLC analysis. Though present in small quantities, it was found to produce considerable effects
- The results of acute toxicity study indicate that the methanolic extract of *Rivea ornata* was non-toxic up to dose level of 2000mg/kg body weight in albino rats as per acute oral toxicity studies. 1/10th of the LD50 Dose is 200mg/kg is used for Pharmacological screening
- The results of subacute toxicity study indicate that the methanolic extract of *Rivea ornata* was non-toxic in chronic administration
- The methanolic extract of *Rivea ornata* at a dose of 200mg/kg exhibited significant hypolipidemic activity in Triton X 100 induced hyperlipidemic rats. This is showed by the reduction of serum lipid parameters such as triglycerides, total cholesterol, LDL, VLDL with an increase in HDL concentration in the group treated with 200mg/kg of methanolic Extract of *Rivea ornata*
- The methanolic extract of *Rivea ornata* at a dose of 200mg/kg showed significant antidiabetic activity in Alloxan induced hyperglycemic rats. This is showed by the reduction of blood glucose level in the group treated with 200mg/kg of methanolic extract of *Rivea ornata*

- The methanolic extract of *Rivea ornata* was subjected to the Tail immersion and hot plate method Analgesic test, showed a significant analgesic effect
- The methanolic extract of *Rivea ornata* was subjected to the Antipyretic activity against Brewer's yeast induced hyperpyrexia showed a significant reduce in body temperature of the animals treated with 200mg/kg of methanolic extract of *Rivea ornata*
- The methanolic extract of *Rivea ornata* was subjected to the wound healing activity, showed a significant wound healing effect on excision wound in rats
- In future, further investigation might provide an insight to identify the functional groups in the methanolic extract of *Rivea ornata* responsible for the antihyperlipidemic, antidiabetic, analgesic, antipyretic and wound healing activity and to elucidate the exact mechanism of action, which is responsible for the observed significant activity with low toxicity and better therapeutic index

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