Hypolipidemic activity of *Pergularia daemia* leaves extract

On rats fed with high fat diet

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IN

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SUBMITTED BY

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Certificates

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"Hypolipidemic activity of *Pergularia daemia* leaves extract on rats fed with high fat diet"** submitted by the student bearing **Reg. No. 26103094** to "The Tamil Nadu Dr. M.G.R. Medical University", Chennai, in partial fulfillment for the award of degree of **MASTER OF PHARMACY** in **PHARMACOLOGY** was evaluated by us during the examination held on…….

Internal Examiner **External Examiner**

CERTIFICATE

This is to certify that the work embodied in this dissertation**, "Hypolipidemic activity of *Pergularia daemia* leaves extract on rats fed with high fat diet"** submitted to "The Tamil Nadu Dr. M.G.R. Medical University", Chennai, was carried out by **PAVAN KUMAR CH.L.V.S. [Reg. No. 26103094],** for the Partial fulfillment of degree of **MASTER OF PHARMACY** in Department of Pharmacology under my guidance and direct supervision, J.K.K. Nattraja College of Pharmacy, Komarapalayam, during the academic year 2011-2012.

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DECLARATION

The work presented in this dissertation entitled **"Hypolipidemic activity of *Pergularia daemia* leaves extract on rats fed with high fat diet",** was carried out by me, under the guidance and super vision of **Mr. RAJESH .V, M. Pharm., Assistant Professor and Head of Department of Pharmacology,** J.K.K. Nattraja College of Pharmacy, Komarapalayam.

I further declare that, this work is original and has not been submitted in part or full for the award of any other degree or diploma in any other university and the thesis is ready for evaluation.

PLACE: **PAVAN KUMAR CH.L.V.S.,**

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PAVAN KUMAR CH.L.V.S.,

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Dedicated to

Almighty

My Beloved Parents

My family members and friends

Contents

CONTENTS

LIST OF ABBREVIATIONS USED

Conc	-	Concentrated
Hb	-	Hemoglobin
GI	-	Gastro Intestinal
DNA	-	Deoxy ribo nucleic acid
TNF	-	Tumor Necrosis Factor
IF	-	Interferons
IL	-	Interleukins
NSAIDS	-	Non Steroidal Anti Inflammatory Drugs
RNA	-	Riboxy nucleic acid
CCl_4	-	Carbon tetrachloride
INH	-	Isoniazid
LFTs	-	Liver Function Tests
FBS	-	Fasting Blood glucose
TG	-	Triglyceride
TC	-	Total Cholesterol
LDL	-	Low Density Lipoprotein
VLDL	-	Very Low Density Lipoprotein
HDL	-	High Density Lipoprotein
AI	-	Atherogenic
CA	-	Coronary artery
WBC	-	White Blood Cells
HCL	-	Hydrochloric acid
H_2SO_4	-	Sulphuric acid
$NaNo_2$	-	Sodium nitrite
KOH	-	Potassium hydroxide
CPCSEA	-	Committee for the purpose of control and supervision on experimental animals
CMC	-	Carboxy Methyl Cellulose
µl	-	Micro litre
Wt	-	Weight
%w/w	-	Percent weight per weight

% v/v	-	Percent volume per volume
TP	-	Total Protein
OECD	-	Organization for Economic Co-operation and Economic Development
IU/L	-	International Units per Litre
g/dl	-	gram per deci litre
mg/dl	-	milli gram per deci litre
gms	-	grams
mg/kg	-	milli gram per kilo gram
nmol	-	nano mole
U/mg	-	Units per milli gram
%	-	Percentage
Kg	-	Kilogram
IP	-	Intra Peritoneal
SC	-	Subcutaneous
Kcal	-	Kilo Calorie
Kj	-	Kilo Joule
Vit	-	Vitamin
LCAT	-	Lecithin cholesterol acyl transferase
LDL-c	-	low density lipoprotein-cholesterol
HDL-c	-	High density lipoprotein-cholesterol
EDTA	-	Ethylene Diamine Tetra Acetic acid
CVD	-	Cardio Vascular Disease
CAD	-	Coronary Artery Disease

INTRODUCTION

CHOLESTEROL

Cholesterol is a fat (lipid) which is produced by the liver and is crucial for normal body functioning. Cholesterol exists in the outer layer of every cell in our body and has many functions. It is a waxy steroid and is transported in the blood plasma of all animals. It is the main sterol synthesized by animals - small amounts are also synthesized in plants and fungi.

The word "cholesterol" comes from the Greek word chole, meaning "bile", and the Greek word stereos, meaning "solid, stiff".

Functions of cholesterol

- It builds and maintains cell membranes (outer layer), it prevents crystallization of hydrocarbons in the membrane
- It is essential for determining which molecules can pass into the cell and which cannot (cell membrane permeability)
- It is involved in the production of sex hormones (androgens and estrogens)
- It is essential for the production of hormones released by the adrenal glands (cortisol, corticosterone, aldosterone, and others)
- It aids in the production of bile
- It converts sunshine to vitamin D
- It is important for the metabolism of fat soluble vitamins, including vitamins A, D, E, and K
- It insulates nerve fibers

There are three main types of lipoproteins

Cholesterol is carried in the blood by molecules called lipoproteins. A lipoprotein is any complex or compound containing both lipid (fat) and protein.

The three main types are:

LDL (low density lipoprotein)

People often refer to it as bad cholesterol. LDL carries cholesterol from the liver to cells. If too much is carried, too much for the cells to use, there can be a harmful buildup of LDL. This lipoprotein can increase the risk of arterial disease if levels rise too high. Most human blood contains approximately 70% LDL - this may vary, depending on the person.

HDL (high density lipoprotein)

People often refer to it as good cholesterol. Experts say HDL prevents arterial disease. HDL does the opposite of LDL - HDL takes the cholesterol away from the cells and back to the liver. In the liver it is either broken down or expelled from the body as waste.

Triglycerides

These are the chemical forms in which most fat exists in the body, as well as in food. They are present in blood plasma. Triglycerides, in association with cholesterol, form the plasma lipids (blood fat). Triglycerides in plasma originate either from fats in our food, or are made in the body from other energy sources, such as carbohydrates. Calories we consume but are not used immediately by our tissues are converted into triglycerides and stored in fat cells. When your body needs energy and there is no food as an energy source, triglycerides will be released from fat cells and used as energy - hormones control this process.

Normal cholesterol levels

The amount of cholesterol in human blood can vary from 3.6 mmol/liter to 7.8 mmol/liter. The National Health Service (NHS), UK, says that any reading over 6 mmol/liter is high, and will significantly raise the risk of arterial disease. The UK Department of Health recommends a target cholesterol level of under 5 mmo/liter.

Unfortunately, two-thirds of all UK adults have a total cholesterol level of at least five (average men 5.5, average women 5.6).

Below is a list of cholesterol levels and how most doctors would categorize them in mg/dl (milligrams/deciliter) and 5mmol/liter (millimoles/liter).

- Desirable - Less than 200 mg/dL
- Bordeline high - 200 to 239 mg/dL
- High - 240 mg/dL and above
- Optimum level: less than 5mmol/liter
- Mildly high cholesterol level: between 5 to 6.4mmol/liter
- Moderately high cholesterol level: between 6.5 to 7.8mmol/liter
- Very high cholesterol level: above 7.8mmol/liter

Dangers of high cholesterol levels

Atherosclerosis - narrowing of the arteries.

Higher coronary heart disease risk - an abnormality of the arteries that supply blood and oxygen to the heart.

Heart attack - occurs when the supply of blood and oxygen to an area of heart muscle is blocked, usually by a clot in a coronary artery. This causes your heart muscle to die.

Angina - chest pain or discomfort that occurs when your heart muscle does not get enough blood.

Other cardiovascular conditions - diseases of the heart and blood vessels.

Stroke and mini-stroke - occurs when a blood clot blocks an artery or vein, interrupting the flow to an area of the brain. Can also occur when a blood vessel breaks, Brain cells begin to die. If both blood cholesterol and triglyceride levels are high, the risk of developing coronary heart disease rises significantly.

Symptoms of high cholesterol (hypercholesterolaemia):

Symptoms of high cholesterol do not exist alone in a way a patient or doctor can identify by touch or sight. Symptoms of high cholesterol are revealed if you have the symptoms of atherosclerosis, a common consequence of having high cholesterol levels. These can include:

- Narrowed coronary arteries in the heart (angina)
- Leg pain when exercising - this is because the arteries that supply the legs have narrowed.
- Blood clots and ruptured blood vessels - these can cause a stroke or TIA (mini-stroke).
- Ruptured plaques - this can lead to coronary thrombosis (a clot forming in one of the arteries that delivers blood to the heart). If this causes significant damage to heart muscle it could cause heart failure.
- Xanthomas - thick yellow patches on the skin, especially around the eyes. They are, in fact, deposits of cholesterol. This is commonly seen among people who have inherited high cholesterol susceptibility (familial or inherited hypercholesterolaemia).

CAUSES FOR HIGH CHOLESTEROL

Lifestyle causes:

Nutrition - although some foods contain cholesterol, such as eggs, kidneys, eggs and some seafoods, dietary cholesterol does not have much of an impact in human blood cholesterol levels. However, saturated fats do! Foods high in saturated fats include red meat, some pies, sausages, hard cheese, lard, pastry, cakes, most biscuits, and cream (there are many more).

Sedentary lifestyle - people who do not exercise and spend most of their time sitting/lying down have significantly higher levels of LDL (bad cholesterol) and lower levels of HDL (good cholesterol).

Bodyweight - people who are overweight/obese are much more likely to have higher LDL levels and lower HDL levels, compared to people who are of normal weight.

Smoking - this can have quite a considerable effect on LDL levels.

Alcohol - people who consume too much alcohol regularly, generally have much higher levels of LDL and much lower levels of HDL, compared to people who abstain or those who drink in moderation.

TREATMENTS FOR HIGH CHOLESTEROL

Lifestyle

Most people, especially those whose only risk factor has been lifestyle, can generally get their cholesterol and triglyceride levels back to normal by:

- Doing plenty of exercise (check with your doctor)
- Eating plenty of fruits, vegetables, whole grains, oats, good quality fats
- Avoiding foods with saturated fats
- Getting plenty of sleep (8 hours each night)
- Bringing your bodyweight back to normal
- Avoiding alcohol
- Stopping smoking

Cholesterol-controlling medications

If your cholesterol levels are still high after doing everything mentioned above, your doctor may prescribe a cholesterol-lowering drug. They may include the following:

- **Statins (HMG-CoA reductase inhibitors)** - these block an enzyme in your liver that produces cholesterol. The aim here is to reduce your cholesterol levels to under 4 mmol/liter and under 2 mmol/liter for

your LDL. Statins are useful for the treatment and prevention of atherosclerosis. Side effects can include constipation, headaches, abdominal pain, and diarrhea. Atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin are examples of statins.

- **Aspirin** - this should not be given to patients under 16 years of age. Drugs to lower triglyceride levels - these are fibric acid derivatives and include gemfibrozil, fenofibrate and clofibrate.
- **Niacin** - this is a B vitamin that exists in various foods. You can only get very high doses with a doctor's prescription. Niacin brings down both LDL and HDL levels. Side effects might include itching, headaches, hot flashes (UK: flushes), and tingling (mostly very mild if they do occur).
- **Anti-hypertensive drugs** - if you have high blood pressure your doctor may prescribe Angiotensin-converting enzyme (ACE) inhibitors, Angiotensin II receptor blockers (ARBs), Diuretics, Beta-blockers, Calcium channel blockers.
- In some cases cholesterol absorption inhibitors (ezetimibe) and bile-acid sequestrants may be prescribed. They have more side effects and require considerable patient education to achieve compliance (to make sure drugs are taken according to instruction).

Total Cholesterol Levels

The sum of different cholesterol types in your blood is referred to as total cholesterol. This measurement, while good, can be occasionally misleading. Generally, more specific measurements are needed for better understanding the issue & choosing the right treatment, more so for those with history of diabetes or coronary artery disease. As a general rule, higher levels of cholesterol mean higher risk of coronary heart disease. Drop of cholesterol by 1 point achieves approximately 2% risk drop of heart disease**. (Christian Nordquist, 2009)**

Cholesterol Levels by The American Heart Association

Less than 200 mg/dL - Optimal

200 – 239 mg/dL- Borderline high

240 mg/dL or higher - High Cholesterol – over 200% the risk of coronary heart disease in comparison to someone with optimal levels

HDL Cholesterol Levels

High-density lipoproteins (HDLs), also known as good cholesterol, act like waste removal carriers. They move the cholesterol from your blood and artery walls to your liver for removal from your body. About 1/3 to 1/4 of blood cholesterol is carried by HDL. For this reason, obviously, higher HDL cholesterol levels are desirable.

Less than 40 mg/dL (men), 50 mg/dL (women)--Such HDL cholesterol levels are a major risk for heart.

60 mg/dL or above- Optimal – HDL cholesterol of 60 mg/dL & above is protective High triglyceride levels, physical inactivity, being overweight, obese, smoking, high carbohydrate intakes, type two diabetes, some medications as well as genetic factors can contribute to low HDL cholesterol levels.

LDL Cholesterol Levels

Low-density lipoproteins (LDLs), also known as bad cholesterol, keep blood cholesterol circulating in your bloodstream, leaving plaque on artery walls along the way. As this process develops over time, up goes atherosclerosis risk. Obviously, lower LDL cholesterol levels are desirable.

Less than 100 mg/dL - Optimal

100 – 129 mg/dL - Near optimal

130 – 159 mg/dL - Borderline high

160 – 189 mg/dL - High

190 mg/dL or above - Very high

Triglycerides Levels

High blood triglycerides generally mean lower HDL cholesterol, higher risk of heart attack & stroke. Additionally, underlying diseases or genetic disorders such as diabetes, high blood pressure, smoking, obesity & insulin resistance generally keep company to high triglycerides levels. The main therapy is a lifestyle change.

Triglycerides Levels by The American Heart Association

Less than 150 mg/dL - Normal

150 – 199 mg/dL - Borderline high

200 – 499 mg/dL - High

500 mg/dL or above - Very high

Antihyperlipidemic agents

Antihyperlipidemic agents promote reduction of lipid levels in the blood. Some antihyperlipidemic agents aim to lower the levels of low-density lipoprotein (LDL) cholesterol, some reduce triglyceride levels, and some help raise the high-density lipoprotein (HDL) cholesterol. By reducing the LDL cholesterol, they can prevent both the primary and secondary symptoms of coronary heart disease.

Cholesterol is found in each and every cell of our body. Cholesterol is a water repelling compound and so it does not get dissolved in the bloodstream. It is a waxy

steroid metabolite. It is required for the proper functioning of the body. It is required for the production of vitamin D, hormones and bile juices. Low-Density Lipoprotein (LDL) or bad cholesterol and High-Density Lipoprotein (HDL) or good cholesterol are the two types of cholesterol. Simple blood test helps measure cholesterol levels in the body. The ideal LDL:HDL ratio and the ideal cholesterol levels are same for all ages. As you are looking for cholesterol range by age, here is the required information regarding normal cholesterol range.

LDL Cholesterol Goals and Risk Factors

Maintaining low levels of LDL and high levels of HDL is essential if you want to lead a healthy and active life. High levels of LDL increase the risk of cardiovascular diseases, cancer and stroke. The ideal cholesterol range by age may vary slightly as your LDL cholesterol goal depends on how many other risk factors (diabetes, coronary heart diseases, obesity, etc.) you have. The risk of high cholesterol is ascertained by taking into account your age, sex, family history, weight, blood pressure and lifestyle habits. Along with aging, the influence of risk factors increases. Therefore healthy cholesterol levels by age may differ slightly. But there cannot be a fixed cholesterol chart by age or fixed numbers indicating cholesterol range by age. Children with diabetes or heart problems or obese children also need to watch for LDL, HDL levels.

Cholesterol Ratio

There cannot be cholesterol ratio by age. The desired cholesterol ratio may change slightly according to the risk factors, as explained above. Here follows

Total Cholesterol/HDL: The ideal cholesterol ratio of total cholesterol and HDL is 3.5:1.

HDL/LDL: 0.4:1 is the ideal cholesterol ratio for HDL/LDL.

LDL/HDL: The ideal cholesterol ratio of LDL/HDL is 2.5:1 **(kevin Belargo, 2008)**

LIPIDS

Lipids are a broad group of naturally occurring molecules which includes fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, phospholipids, and others. The main biological functions of lipids include energy storage, as structural components of cell membranes, and as important signaling molecules.**(Fahy E *et al*., 2009)**

Lipids may be broadly defined as hydrophobic or amphiphilic small molecules; the amphiphilic nature of some lipids allows them to form structures such as vesicles, liposomes, or membranes in an aqueous environment. Biological lipids originate entirely or in part from two distinct types of biochemical subunits or "building blocks": ketoacyl and isoprene groups. Using this approach, lipids may be divided into eight categories: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids and polyketides (derived from condensation of ketoacyl subunits); and sterol lipids and prenol lipids (derived from condensation of isoprene subunits).

Although the term lipid is sometimes used as a synonym for fats, fats are a subgroup of lipids called triglycerides. Lipids also encompass molecules such as fatty acids and their derivatives (including tri-, di-, and monoglycerides and phospholipids), as well as other sterol-containing metabolites such as cholesterol. **(Michelle *et al.*, 1993) (Subramaniam *et al.*, 1993)** Although humans and other mammals use various biosynthetic pathways to both break down and synthesize lipids, some essential lipids cannot be made this way and must be obtained from the diet.

CATEGORIES OF LIPIDS

- Fatty acyls
- Glycerolipids
- Glycerophospholipids
- Sphingolipids
- Sterol lipids
- Prenol lipids

- Saccharolipids
- Polyketides

BIOLOGICAL FUNCTIONS

- Membranes
- Energy storage
- Signaling
- Metabolism
- Biosynthesis
- Degradation
- Nutrition and health
- Lipid Metabolism

A few studies have suggested that total dietary fat intake is linked to an increased risk of obesity and diabetes. However, a number of very large studies, including the Women's Health Initiative Dietary Modification Trial, an eight year study of 49,000 women, the Nurses' Health Study and the Health Professionals Follow-up Study, revealed no such links. None of these studies suggested any connection between percentage of calories from fat and risk of cancer, heart disease or weight gain. The Nutrition Source, a website maintained by the Department of Nutrition at the Harvard School of Public Health, summarizes the current evidence on the impact of dietary fat: "Detailed research—much of it done at Harvard—shows that the total amount of fat in the diet isn't really linked with weight or disease.

Table 1. Apolipoproteins associated with lipoproteins

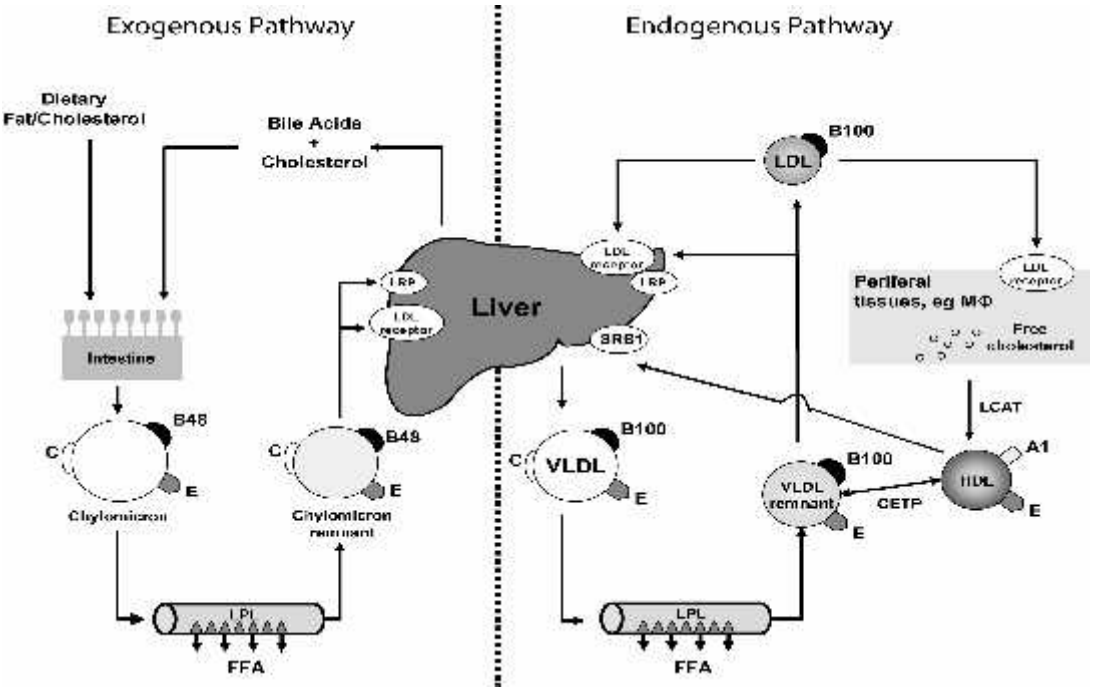
Apolipoproteins	**Lipoprotein association**	**Comments**
apoA1	Chylomicron, HDL	Major protein of HDL, activates LCAT
apoA2	Chylomicron, HDL	Enhances hepatic lipase activity
apoA4	Chylomicron, HDL	Co-factor for LPL activation, may have a role in chylomicron and VLDL secretion and

		catabolism, activates LCAT
apoA5	HDL	Activates LPL
apoB48	Chylomicron	Exclusively found in chylomicrons, alternative splice product of the apoB100 gene thereby lacking the LDL receptor-binding domain
apoB100	VLDL, IDL, LDL	Ligand for the LDL receptor
apoC1	Chylomicron, VLDL, IDL, HDL	Appears to modulate the interaction of apoE with β-VLDL and inhibit binding of β-VLDL to LRP
apoC2	Chylomicron, VLDL, IDL, HDL	Activates LPL
apoC3	Chylomicron, VLDL, IDL, HDL	Inhibits LPL
apoE	Chylomicron, VLDL, IDL, HDL	Ligand for LDL receptor and LRP

Based on their size, lipid and apolipoprotein composition, lipoproteins can be categorized into five classes: chylomicrons, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), and the high density lipoprotein (HDL). However, heterogeneity exists within each class because of the constant remodelling of their composition, shape, size, and surface charge, and exchange of protein and lipid constituents.

The liver plays a central role in lipid metabolism. Lipoprotein trafficking from and to the liver proceeds along two pathways: the endogenous and the exogenous pathway cholesterol transport from peripheral tissues to the liver is often considered as the third pathway.

Figure No.1 Exogenous and Endogenous pathway



The exogenous pathway involves the uptake of dietary lipids by the intestine followed by their transport through the body via lipoproteins. After the lipid intake, the triglycerides and the cholesteryl esters will be hydrolyzed in the intestine and subsequently assembled into chylomicrons. These large triglyceride-rich chylomicrons containing apoA1, apoA2, apoA4, and apoB48 are secreted into the lymph in order to transport entrapped lipids to the circulation. In the bloodstream, the chylomicrons exchange apoA1 and associated apoA4 for apoC1, apoC2, apoC3, and apoE with HDL which subsequently results in the formation of the more cholesterol-rich chylomicron remnants due to loss of triglycerides to peripheral tissue. ApoC2 is an activator of the lipolytic enzyme lipoprotein lipase (LPL), which is associated with the capillary endothelium of skeletal muscle, cardiac muscle, and adipose tissue. ApoC2 aided interaction of chylomicrons with LPL allows the lipolysis of its core triglycerides leading to the formation of free fatty acids which are taken up by surrounding tissue for further use. Lipolysis gradually becomes less efficient due to depletion of the LPL co-activator apoC2. Eventually the chylomicron remnants, enriched in cholesteryl esters, apoB48, and apoE, are eliminated from the circulation by the liver mainly via the uptake by the LDL-

receptor which specifically recognizes apoE and apoB100, and by the LDL-receptor related protein (LRP).

The liver produces the triglyceride-rich lipoprotein, VLDL, which is introduced into the endogenous pathway. Some of the VLDL contained triglycerides will be derived from internalized chylomicron remnants, but the bulk of the VLDL triglycerides are synthesized de novo in the liver from fatty acids produced from dietary carbohydrate derived Acetyl-CoA. The formation of VLDL starts with the transfer of lipids towards the major structural protein of VLDL, apolipoprotein B100 (apoB100). This transfer is catalyzed by the microsomal triglyceride transfer protein (MTP), making this enzyme a key factor in the assembly of VLDL. Subsequently before secretion into the blood circulation, the pre-VLDL particle acquires apoC1, apoC2, apoC3, and ApoE. ApoE plays a critical role when it comes to secretion of VLDL and clearance of VLDL remnants. Similar to chylomicrons, VLDL transports triglycerides to skeletal muscle, cardiac muscle, and adipose tissue and also serves as substrate for LPL lipolysis in the circulation. As a result, VLDL particles gradually reduce in size due to hydrolysis of the triglycerides leading to the formation of the more cholesterol-rich IDL. Small sized VLDL and IDL are also referred to as VLDL remnants. These remnants are partially cleared by the liver through an apoE-mediated process while the remainder can be converted to LDL. The conversion to LDL is accompanied not only by a further loss in triglycerides but also by the depletion of apoE and the apoC's. With apoB100 as sole apolipoprotein constituent of LDL, LDL can be cleared by the liver or taken up by peripheral tissue via the LDL receptor. High levels of LDL and/or VLDL remnants are considered as important risk factors for atherosclerosis, and are therefore also termed atherogenic lipoproteins.

Reverse cholesterol transport

While the first two pathways focuses mainly on the transport of triglycerides from intestine and liver to peripheral tissue via apoB-containing lipoproteins followed by the clearance of cholesterol-rich lipoprotein remnants, the third pathway involves the net transport of (excess) cholesterol from peripheral

tissue back to the liver where it can be removed from the body via the bile by conversion to bile acids or as biliary cholesterol. The reverse transport of cholesterol is mediated by the lipoprotein HDL, of which the metabolism is complex and only partially understood. HDL is a heterogeneous class of lipoprotein particles existing of different sub fractions that differ in apolipoprotein and lipid composition, size, density, and even physiologic function. However, apoA1 plays a key role in the biogenesis and function of all HDL subclasses. Generation of HDL starts with the intestinal and hepatic synthesis and secretion of lipid-poor apoA1. This apoA1 'particle' acquires phospholipids and unesterified cholesterol from cell membranes and plasma lipoprotein to convert into a discoidal preβ-HDL particle. Both nascent pre-HDL particles are potent cholesterol acceptors and take up free cholesterol from peripheral cells and, importantly from a cardiovascular point of view, macrophages. This cholesterol efflux is mediated by the integral membrane protein ATP-binding cassette transporter A-1 (ABCA1), which actively transports cellular cholesterol (and phospholipids) to the nascent HDL. The importance of ABCA1 in reverse cholesterol was established in patients with Tangier disease, who are characterized by HDL deficiency. This disease is caused by a dysfunctional ABCA1 and results in massive accumulation of cholesterol in tissue macrophages and prevalent atherosclerosis. Cholesterol taken up by the nascent HDL particles will be esterified by the enzyme lecithin: cholesterol acyltransferase (LCAT), transforming nascent preβ-HDL into larger spherical HDL-particles. Subsequently, the latter HDL particles are subjected to remodelling after interaction with other plasma lipoproteins or tissues. For instance, the cholesteryl ester transfer protein (CETP) catalyzes the exchange of cholesteryl esters for triglycerides with apoB-containing lipoproteins. The uptake of HDL cholesterol by the liver is mainly mediated by the scavenger receptor class B1 (SR-B1). Once in the liver, cholesterol can be re-used for lipoprotein assembly, used as substrate for bile acid synthesis, or secreted directly as free cholesterol into the bile.

Although during the last years classifications of the different stages of HDL have become as intricate as the particle itself due to the various compositions, sizes, functions, etc., it is becoming increasingly clear that HDL plays a significant role in

cholesterol homeostasis. Particularly the role of HDL in reverse cholesterol transport and the athero-protective characteristics of this particle are widely accepted

Genetic factors in dyslipidemia

The expression of lipid-related genes under normal conditions is tightly coordinated in order to maintain lipid homeostasis. Unfortunately, in western societies and in emerging economies a large part of the population suffer from a perturbed lipid homeostasis resulting in unbalanced plasma levels of the different lipoproteins and an increased incidence of coronary events and cardiovascular deaths. Although factors such as high cholesterol consumption and increasing age are important, undesirable lipoprotein profiles are also determined by genetic factors such as dysfunctional genes.

Familial hypercholesterolemia (FH), one of the most common causes of hypercholesterolemia, was the first genetic disease of lipid metabolism to be characterized. Patients suffer from elevated plasma levels of LDL-cholesterol caused by mutations in the LDL-receptor gene. To date, well over 500 mutations of the LDL-receptor have been identified. The severity of the hypercholesterolemia depends on the nature of the molecular defect and whether the mutation is homo- or heterozygous. Patients with homozygous FH develop significant atherosclerotic heart disease at a relative early age. The clinical prognosis of patients with heterozygous FH is related not only to the magnitude of the elevation in plasma LDL-cholesterol levels, but also to the presence of other cardiovascular risk factors.

A disease that shows similarity with FH in that it also results in a reduced uptake of LDL by the LDL-receptor is familial defective apoB100 (FDB). This disease is caused by several mutations in apoB100 at the postulated binding site to the LDL-receptor. However, the phenotype of patients homozygous for FDB generally is less severe than that of FH patients. The latter is the result of a reduced formation rate of LDL particles in the plasma, since FDB patients are still able to clear the LDL precursor particles (VLDL, IDL, and their remnants) via apoE mediated uptake Polymorphisms in lipid related genes, such as apoE, also

predispose to cardiovascular diseases. Three major apoE isoforms, E2, E3, and E4, are encoded by three common alleles at the apoE gene locus: E2, E3, and E4. The apoE2 isoform is associated with lower and that of apoE4 with elevated total plasma- and LDL-cholesterol levels than the more common apoE3 isoform. These three isoforms differ in their affinity for the LDL receptor which can influence the serum profile and levels of apoE containing lipoproteins. For example, the majority of patients with type III hyperlipidemia, which is characterized by elevated levels of cholesteryl ester enriched remnant particles; appear to be homozygous for apoE2. The apoE4 allele, which is receiving great attention due to its linkage to Alzheimer's disease, is associated with an increased prevalence for cardiovascular disease especially in populations consuming diets rich in saturated fat and cholesterol.

Many other, mostly rare, single lipid-related gene mutations such as the ABCA1 mutations found in patients with Tangier disease discussed earlier have been described. However, these and the aforementioned examples are of monogenic nature and, with the exception of FH, show a low frequency. Dyslipidemia being a multifactorial process, the major portion of this predisposition is polygenic, reflecting cumulative effects of multiple genetic sequence variants. These hereditary factors are difficult to identify since possible dyslipidemia phenotypes are the result of complex interactions between genetic and environmental factors.

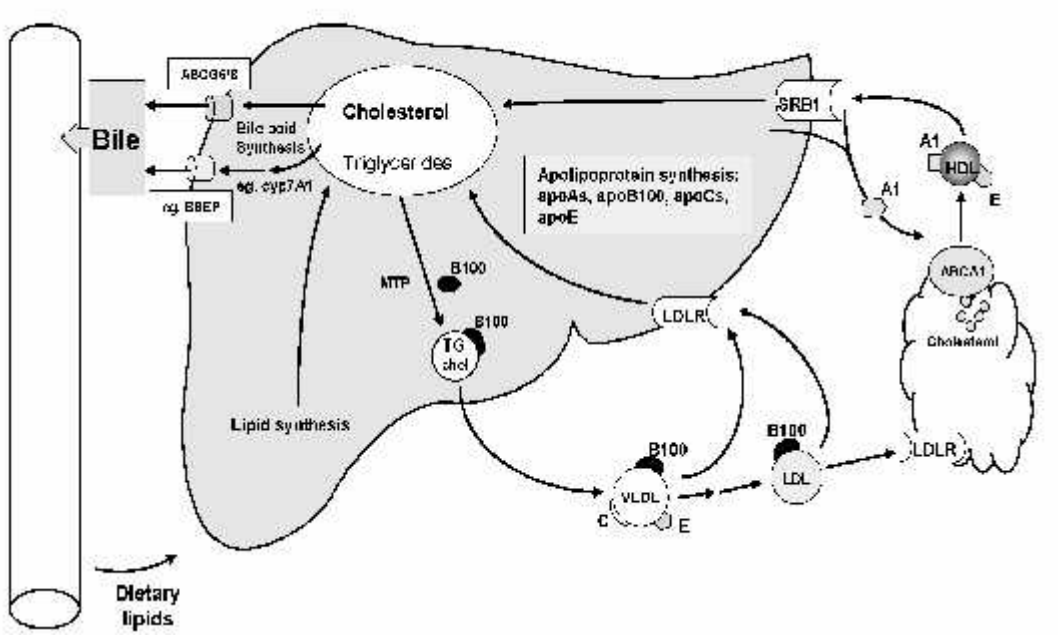
Central role of the liver in lipid metabolism

As indicated above, maintenance of lipid homeostasis is the sum of numerous complex physiological processes both at an organ, and at a cellular level. The organ that plays a key role in the synthesis and catabolism of lipids is the liver. It expresses enzymes that are responsible for synthesis (e.g. fatty acid synthetase and HMG-CoA reductase) of triglycerides and cholesterol (esters). Excess of cholesterol can be metabolized in the liver via enzymes such as cholesterol-7-α-hydroxylase and sterol 27-hydroxylase (cyp7A1 and cyp27, respectively), and removed from the body via bile acid synthesis. Further, it is involved in assembly and secretion of the main lipoproteins, i.e. VLDL and HDL, and the elimination of serum lipoproteins via lipoprotein receptors. Most, if not all, apolipoproteins involved in the

metabolism of lipoproteins 16 General Introduction is expressed in the liver. In order to prevent high lipid levels, which correlate with increased risk of cardiovascular disorders, it is essential that the expression of lipid-related genes in the liver is well orchestrated. Several aspects and processes will be highlighted below

Figure No 2.

Intestine Liver Peripheral Tissue



Lipoprotien synthesis:

Via the formation of triglycerides-rich lipoproteins in the liver, lipids are distributed throughout the body. Hepatic assembly and secretion of apoB-containing VLDL requires a temporary pool of lipids to form the monolayer surface (mainly phosphatidyl choline and cholesterol) and the neutral lipid core, the production of the structural protein apoB, and the rate limiting enzyme MTP. The lipid pool used for VLDL synthesis is fed by two sources: uptake of dietary lipids and de novo synthesis. Important rate limiting enzymes for the synthesis of fatty acids are acetyl-CoA carboxylase and fatty acid synthase, while the enzymes HMG-CoA synthase and HMG-CoA reductase are major check points in cholesterol synthesis. The

availability of lipids and rate of lipid biosynthesis, lipogenesis, influence the rate of VLDL assembly and secretion.

The structural protein of VLDL is apoB. ApoB exists in two forms: the full length apoB100 and a truncated protein that contains only 48% of the full length version, apoB48. ApoB48 is the result of post-transcriptional modification of apoB100 mRNA by a cytidine deaminase (apobec1) containing enzyme complex. In humans, apobec1 is solely active in the intestine, and unlike rodents, no hepatic apobec1 expression can be found. Hence, VLDL particles synthesized in human liver only contain apoB100. Although apoB100 is the major apolipoprotein of VLDL, a significant portion of newly synthesized apoB is degraded intracellular via several possible proteosomal and non-proteosomal processes prior to secretion. Studies showing that an increased apoB100 secretion is not accompanied by a detectable change in apoB mRNA, support the notion that apoB100 production is regulated at a post-transcriptional level by means of degradation. The underlying mechanisms of degradation and its regulatory control have not been completely elaborated, but it has become clear that premature degradation can be prevented by translocation across the endoplasmic reticulum (ER) and subsequent lipidation of the nascent apoB100. The protein crucial for these processes is MTP, which resides in the lumen of the ER as a heterodimer with the ubiquitous ER-enzyme, protein-disulfide isomerase. The latter seems to be required by MTP for its solubility, ER retention, and for lipid-transfer-activity of MTP. In the ER, MTP binds to newly synthesized lipid-poor apoB and facilitates the transfer of triglycerides, cholesterol esters, and phospholipids. Recent studies indicate that during the lipidation process the affinity of MTP for apoB decreases, allowing at a certain point the dissociation of MTP from ApoB, and the formation of a secretion-competent primordial VLDL. Based on findings with conditional liver-specific MTP gene knockout mice, specific MTP inhibitors, and hepatic MTP over expression in mice, MTP is considered to be the rate-limiting enzyme in the VLDL assembly/secretion pathway.

Hepatic uptake and removal of cholesterol

As described above, cholesterol-rich remnants and HDL are cleared from the plasma via hepatic receptors. The main receptors responsible for the uptake of chylomicron- and VLDL-remnants, and LDL are the LDL receptor and LRP. Given the importance of the reverse cholesterol pathway in lipid homeostasis, the identification of the HDL-receptor, scavenger receptor class B type 1 (SR-B1) was a breakthrough. SR-B1 is a cell-surface transmembrane protein which functions as a high affinity receptor for HDL, but also can bind a wide variety of other ligands including LDL and VLDL. Not only does SR-B1 bind HDL, but it also mediates selective lipoprotein cholesterol uptake. This selective uptake involving cholesterol delivery from plasma HDL to the liver without particle degradation does not require co-factors. Upon cholesterol uptake, the cholesterol can be stored in the liver after conversion to cholesteryl esters by acyl-CoA: cholesterol acyltransferase (ACAT). The intracellular cholesterol pool can be re-used for assembly of VLDL or eliminated from the liver via bile directly or via bile acid synthesis.

Direct secretion of cholesterol into the bile proceeds through two ATP-binding cassette (ABC) transporters, ABCG5 and ABCG8. These ATP dependent sterol transporters function as obligate heterodimers to promote sterol excretion into the bile. Conversion of cholesterol into bile acid involves a cascade of reactions and almost 20 different enzymes have been identified. Basically, at present two pathways exist for bile acid synthesis. The classical neutral pathway, which is the main route, involves the rate-limiting enzyme cholesterol 7α-hydroxylase (cyp7A1), and leads to the production of mainly cholic acid. The alternative pathway or acidic pathway is initiated by the enzyme sterol 27-hydroxylase (cyp27) which preferentially produces chenodeoxycholic acid. Both bile acids can be transported into the bile after which the bile acids are secreted into the intestine to leave the body or to be re-absorbed. **(Douglas Adams., 1971)**

CHOLESTEROL

Cholesterol is a chemical compound that is naturally produced by the body and is structurally a combination of lipid (fat) and steroid. Cholesterol is a building block for cell membranes and for hormones like estrogen and testosterone. About 80% of the body's cholesterol is produced by the liver, while the rest comes from our diet. The main sources of dietary cholesterol are meat, poultry, fish, and dairy products. Organ meats, such as liver, are especially high in cholesterol content, while foods of plant origin contain no cholesterol. After a meal, dietary cholesterol is absorbed from the intestine and stored in the liver. The liver is able to regulate cholesterol levels in the blood stream and can secrete cholesterol if it is needed by the body.

LDL AND HDL CHOLESTEROL

LDL cholesterol is called "bad" cholesterol, because elevated levels of LDL cholesterol are associated with an increased risk of coronary heart disease, stroke, and peripheral artery disease. LDL lipoprotein deposits cholesterol along the inside of artery walls, causing the formation of a hard, thick substance called cholesterol plaque. Over time, cholesterol plaque causes thickening of the artery walls and narrowing of the arteries, a process called atherosclerosis, which decreases blood flow through the narrowed area.

HDL cholesterol is called the "good cholesterol" because HDL cholesterol particles prevent atherosclerosis by extracting cholesterol from the artery walls and disposing of them through the liver. Thus, high levels of LDL cholesterol and low levels of HDL cholesterol (high LDL/HDL ratios) are risk factors for atherosclerosis, while low levels of LDL cholesterol and high levels of HDL cholesterol (low LDL/HDL ratios) are desirable and protect against heart disease and stroke.

Total cholesterol is the sum of LDL (low density) cholesterol, HDL (high density) cholesterol, VLDL (very low density) cholesterol, and IDL (intermediate density) cholesterol.

HDL (Good) Cholesterol

HDL cholesterol particles prevent atherosclerosis by extracting cholesterol from the artery walls and disposing of them through the liver. Thus, high levels of LDL cholesterol and low levels of HDL cholesterol (high LDL/HDL ratios) are risk factors for atherosclerosis, while low levels of LDL cholesterol and high level of HDL cholesterol (low LDL/HDL ratios) are desirable making them known as good cholesterol. Both heredity and diet have a significant influence on a person's LDL, HDL and total cholesterol levels.

Lowering your LDL cholesterol is easier to do than raising your HDL cholesterol. However, there is great benefit in bringing your HDL numbers up and even greater benefit by doing both - lowering LDL/raising HDL.

For a long time, focus was primarily on LDL and the need to bring numbers down. However, researchers and physicians have now identified that bringing the HDL level up is just as beneficial and a natural way of fighting off bad cholesterol.

Although more difficult to accomplish, there are definite steps you can take to help raise your HDL:

- Weight Loss
- Exercise
- B3 (Niacin)

Some studies have shown that when antioxidants are coupled with cholesterol-reducing medications such as Statin-type drugs along with Niacin, there was some level of benefit. Further research suggests that in women with high plasma levels of HDL, the risk of heart attack becomes reduced.

The higher your HDL levels the better. Today, the average for women is between 50 and 55 mg/dL and for men 40 to 45 mg/dL. Again, getting this level over 60 is a very effective start toward improving overall cholesterol ratios.

LDL (Bad) Cholesterol

LDL cholesterol is called "bad" cholesterol, because elevated levels of LDL cholesterol are associated with an increased risk of coronary heart disease (CHD). Lipoprotein deposits cholesterol on the artery walls forming a hard layer called the plaque which obstructs the flow of blow of blood to the heart. This cholesterol plaque causes thickening of the artery walls and narrowing of the arteries, a process called atherosclerosis.

The liver not only manufactures and secretes LDL cholesterol into the blood; it also Apart from removing LDL cholesterol from the blood the liver also produces and secretes LDL cholesterol into the blood. A high number of active LDL receptors on the liver surfaces is associated with the rapid removal of LDL cholesterol from the blood and low blood LDL cholesterol levels. High LDL cholesterol blood levels are often associated with the deficiency of these LDL receptors.

Lowering LDL cholesterol is the primary focus in preventing atherosclerosis and heart attacks. The benefits of lowering LDL cholesterol include:

- Reducing or stopping the formation of new cholesterol plaques on the artery walls;
- Removing or reduction of existing cholesterol plaques on the artery walls;
- Opening up of the narrowed arteries;
- Avoid rupturing the cholesterol plaques, which facilitate formation of blood clots.
- Decreasing the risk of heart attacks; and
- Decreasing the risk of strokes.

Lowering LDL (Bad) Cholesterol

Various studies have shown that chances of having a heart attack are actually decreased by as much as 25% for every 10% drop in cholesterol level. Lowering LDL cholesterol is a key factor in bringing total cholesterol down to a safe level.

Lowering LDL: Advantages

- Chances of heart attack and/or stroke are decreased
- Formation of new cholesterol plaques is reduced
- Existing plaques are removed
- Rupture of existing plaques is prevented

A particular study confirmed risks of heart attack/stoke and even deaths are reduced considerably by lowering LDL. In this study, more than 4,000 people with confirmed heart disease were given either a cholesterol-lowering drug called statin or a placebo. Results showed that for the people taking the Statin, the total cholesterol levels were reduced 25%, LDL was lowered 35%, and death occurring from heart disease was reduced by a staggering 42%.

Daily calorie intake of fat should be cut down to less than 30%. Consuming too many calories from all kinds of food, whether it is carbohydrates, proteins, or fats, the body will absorb the food and turn it into triglycerides which are then circulated in the bloodstream to be later stored as fat.

Lowering LDL levels has a great impact on their health of people suffering from heart disease. The more the LDL level the higher is the risk of a heart attack. If you have high LDL levels, better to consult a physician and follow his advice to bring them down.

Triglycerides (VLDL Cholesterol)

Triglycerides are a particular form of fat that is transported through your blood to the tissue. The majority of your body's fat tissue is made up of triglycerides. However, high level of triglyceride in the blood can be a risk for heart disease.

Triglycerides is usually measured when you have your LDL cholesterol is checked by your physician. The optimal number for your triglycerides would be anything 150 or less.

Serum triglycerides come from two sources. The first source is the foods that you eat. If you consume a meal containing a lot of fat, your intestine will package some of those fats and transport them to your liver. The second source is your actual liver. Once the fats are received by the liver, it then takes fatty acids released by your fat cells and bundles them up as triglycerides, which are then sent out to the rest of your body to use as fuel.

There is some controversy relating around high triglyceride levels and if they alone are the cause for heart disease. The reason for this debate is that many people having high LDL and low HDL also have high triglyceride levels. Other health conditions related to high triglycerides include high blood pressure, obesity, diabetes, chronic kidney and liver circulatory disease, and hypothyroidism.

Research shows that you can lower your triglycerides without the use of medication although that is an option if needed or prescribed by your doctor.

Triglycerides (VLDL) Treatment

Triglyceride is a fatty substance that is composed of three fatty acids. Like cholesterol, triglycerides in the blood either comes from the diet or the liver. Also, like cholesterol, triglyceride cannot dissolve and circulate in the blood without combining with a lipoprotein. Thus, after a meal, the triglyceride and cholesterol that are absorbed into the intestines are packaged into round particles called chylomicrons before they are released into the blood circulation. A chylomicron is a collection of cholesterol and triglyceride that is surrounded by a lipoprotein outer coat. (Chylomicrons contain 90% triglyceride and 10% cholesterol.)

The liver removes triglyceride and chylomicrons from the blood, and it synthesizes and packages triglyceride into VLDL (very low-density lipoprotein) particles and releases them back into the blood circulation.

The elevated triglyceride levels in the blood lead to atherosclerosis and heart attacks remains controversial. While most doctors now believe that an abnormally high triglyceride level is a risk factor for atherosclerosis, it is difficult to conclude that

elevated triglycerides by themselves cause atherosclerosis. However, it is increasingly recognized that elevated triglyceride is often associated with other conditions that increase the risk of atherosclerosis, including obesity, low levels of HDL- cholesterol, insulin resistance and poorly controlled diabetes mellitus, and small, dense LDL cholesterol particles.

In some people, abnormally high triglyceride levels (hypertriglyceridemia) are inherited. Examples of inherited hypertriglyceridemia disorders include mixed hypertriglyceridemia, familial hypertriglyceridemia, and familial dysbetalipoproteinemia.

Hypertriglyceridemia can often be caused by non-genetic factors such as obesity, excessive alcohol intake, diabetes mellitus, kidney disease, and estrogen- containing medications such as birth control pills.

Treating Elevated Blood Triglyceride Levels

The first step in treating hypertriglyceridemia is a low fat diet with a limited amount of sweets, regular aerobic exercise, loss of excess weight, reduction of alcohol consumption, and stopping cigarette smoking. In patients with diabetes mellitus, meticulous control of elevated blood glucose is also important.

When medications are necessary, fibrates (such as Lopid), nicotinic acid, and statin medications can be used. Lopid not only decreases triglyceride levels but also increases HDL cholesterol levels and LDL cholesterol particle size. Nicotinic acid lowers triglyceride levels, increases HDL cholesterol levels and the size of LDL cholesterol particles, as well as lowers the levels of Lp (a) cholesterol.

The statin drugs have been found effective in decreasing triglyceride as well as LDL cholesterol levels and, to a lesser extent, in elevating HDL cholesterol levels. A relatively new medicine, fenofibrate (Tricor), shows promise as an effective agent in lowering serum triglyceride levels as well as raising HDL levels, particularly in patients who have had suboptimal responses to Lopid. In some patients, a combination of Lopid or Tricor with adjunctive statin therapy (see below) may be

prescribed. While this combination is often effective in patients with complex lipid disorders, the potential for side effects may be increased and such patients should be under strict medical supervision.

Blood Cholesterol Levels:

In order for your doctor to know your cholesterol level, a blood sample must be taken from your finger or your arm. The blood sample will be tested for total cholesterol and HDL cholesterol levels.

If your total cholesterol is less than 200 mg/dl and your HDL-cholesterol ("good" cholesterol) is 40 mg/dL.

Cholesterol Levels - Risk Factors:

A plethora of things can affect cholesterol levels. Some of them which you have control over cholesterol levels include: Diet, Weight, & Physical Activity.

Diet: Cholesterol in the food and the saturated diet you eat make your blood cholesterol level go up. Saturated fat is the primary concern but cholesterol in foods also matters. The amount of saturated fat and cholesterol in your diet helps lower your blood cholesterol level.

Weight: Being overweight is a risk factor for heart disease. It also tends to increase your cholesterol. Losing weight can help lower your LDL and total cholesterol levels, as well as raise your HDL and lower your triglyceride levels.

Physical Activity: Physical inactivity is ingredient to develop heart disease. Consistent physical activity can help lower LDL (bad) cholesterol and raise HDL (good) cholesterol levels. It also helps you lose weight. A minimum of 30 minutes of physical exercise can be deemed sufficient if done on all days.

Cholesterol levels: Things beyond your control

Age and Gender: Cholesterol levels rise as women and men get older. Before the age of menopause, women have lower total cholesterol levels than men of the same age. LDL levels tend to rise in women passing their age of menopause.

Heredity: High blood cholesterol can run in families. Your genes decide how much amount of cholesterol your body makes.

In general, the higher your LDL level and the more risk factors you have (other than LDL), the higher the LDL level, more risk factors you have and greater are your chances of developing heart disease or having a heart attack. People having heart disease are vulnerable for a heart attack. A combination of risk factors and people with diabetes's are prone to heart attack.

Medications to Lower Cholesterol

Statins

- Atorvastatin
- Fluvastatin
- Lovastatin
- Pravastatin
- Rosuvastatin
- Simvastatin

Bile Acid Resins

- Cholestyramine
- Colestipol
- Colesevelam

Nicotinic Acid

- Niacin (Vitamin B3)

Fibrates

- Clofibrate
- Fenofibrate
- Gemfibrozil

Lowering Cholesterol Levels

Drug therapy seems inevitable to people who after following a balance diet and exercising to keep themselves fit, still suffer from high cholesterol levels. Although we have medicines claiming to lower cholesterol, it still remains a matter of debate among the various kinds of drugs.

People who need modern day medicine to try and stop high cholesterol levels, the drugs of first choice for elevated LDL cholesterol are the HMG CoA reductase inhibitors, e.g., atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin. Statin drugs are very effective for lowering LDL cholesterol levels and have few immediate short-term side effects.

Drug consideration can be accounted for people with LDL cholesterol of 190 mg/dl and without coronary heart disease with two or more other risk factors. The objective is to bring it down to 130mg/dl. The same process is applied to persons with LDL Cholesterol of 160mg/dl without coronary heart disease and with two three other risk factors .The objective is to lower it down to 130mg/dl.

For persons with coronary heart disease with LDL Cholesterol of 130mg/dl, the objective is to bring it down to 100mg/dl.

Precautionary Measures

In men less than 35 years of age and premenopausal women with LDL cholesterol levels of 190 to 219 mg/dL, drug therapy should be delayed except in high-risk patients such as those with diabetes. In coronary heart disease patients with LDL

cholesterol levels of 100 to 129 mg/dL, the physician should exercise clinical judgment in deciding whether to begin drug treatment.

In some cases, a physician may decide that using cholesterol-lowering drugs at lower LDL cholesterol levels is justified. On the other hand, drug therapy may not be appropriate for some patients who meet the above criteria. This may be true for elderly patients.

Factors Influencing Cholesterol Lowering Drugs

- The presence of other coronary heart disease risk factors influences the use of cholesterol-lowering drugs:
- Age (for men, 45 years or older; for women, 55 years or older OR premature menopause)
- High blood pressure (140/90 mm Hg or higher)
- HDL cholesterol less than 40 mg/dL
- Family history of premature CHD (a father, brother or son with a history of CHD before age 55, OR a mother, sister or daughter with CHD before age 65)
- Smoking, living or working every day with people who smoke
- Diabetes (fasting blood sugar of 126 mg/dL or higher)

Managing Cholesterol Levels

Managing your cholesterol levels leads to a very healthy lifestyle. A few things have to be done to get the desired results:

Exercise : Cutting down between 1,200 and 1,500 calories each week doing aerobic exercise can have dramatic results. Exercising is a wonderful way to keep your heart healthy, it also helps lose weight, which is an additional benefit. By losing excess weight, you see significant increases in your HDL cholesterol."

Avoid Trans Fatty Acids: Avoid Trans fatty acid containing foods such as French fries, cookies, cakes and many of the fried fast foods.

Minimize Carbohydrates : Avoid sugar, flour, potatoes and white rice thus cutting down on carbohydrates.. HDL level drops dramatically when blood sugar is spiked by carbohydrates. Avoid Cholesterol Foods: Reduce food sources of cholesterol such as egg yolk, liver, kidney, brains, etc.

Stop Smoking : According to a study, within just one week of quitting smoking, HDL levels raised by seven points.

HYPERLIPIDEMIA

More than 650,000 people die every year of coronary heart disease (CHD) in the US alone. In 1984 it was demonstrated for the first time that there exists a link between serum cholesterol levels and risk to CHD. A 1% drop in serum cholesterol reduces the risk for CHD by 2%. Positive risk factors for CHD include Age (men>45 yrs, women>55 yrs); family history of premature CHD; smoking; hypertension (>140/90 mm Hg); low HDL cholesterol (<35 mg/dl); obesity (>30% overweight); diabetes; and high LDL (>160 mg/dl). Negative risk factors include high HDL levels (>60 mg/dl). Major lipids found in blood stream are triglycerides, phospholipids, cholesterol and cholesterol esters and free fatty acids. The function of cholesterol is to help carry fat in the body, because fat being insoluble in water cannot travel on its own in the blood stream. Cholesterol associates with fat and protein and comes out of the liver as lipoprotein.

There are several types of lipoproteins for the transport of fatty material in the body such as chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), intermediate density lipoproteins (IDL) and high density lipoproteins (HDL). Each has a different function in the transport system. Hyperlipidemia, the elevation of lipid concentration in plasma, is the manifestation of a disorder in the synthesis and degradation of plasma lipoproteins. Primary type hyperlipidemia can be treated with drugs but the secondary type originating from

diabetes, renal lipid nephritis or hypothyroidism demands the treatment of original disease rather than hyperlipidemia. **(Joel *et al.*, 2001, and Sharma *et al.*, 1997)**

Classification of hyperlipidemia

Type I hyperlipidemia: It is characterised by high concentration of blood chylomicrons. Currently there are no drugs available for treating this type.

Type II hyperlipidemia: It is sub divided into Type II-A hyperlipidemia and Type II-B hyperlipidemia. Type II A hyperlipidemia is characterized by high LDL and cholesterol levels with a slight increase in blood triglycerides. Type II B hyperlipidemia is characterized by the elevation of triglycerides, serum cholesterol, LDL and VLDL.

Type III hyperlipidemia: This condition results when there are elevated levels of triglycerides and IDL. A blockade in the normal conversion of VLDL to LDL results in accumulation of IDL. Controlled diet is the treatment of this type of hyperlipidemia.

Type IV hyperlipidemia: It is the sequel to high concentration of triglycerides and VLDL and often faulty carbohydrate metabolism. Both diet and drug therapy is recommended for this type of hyperlipidemia.

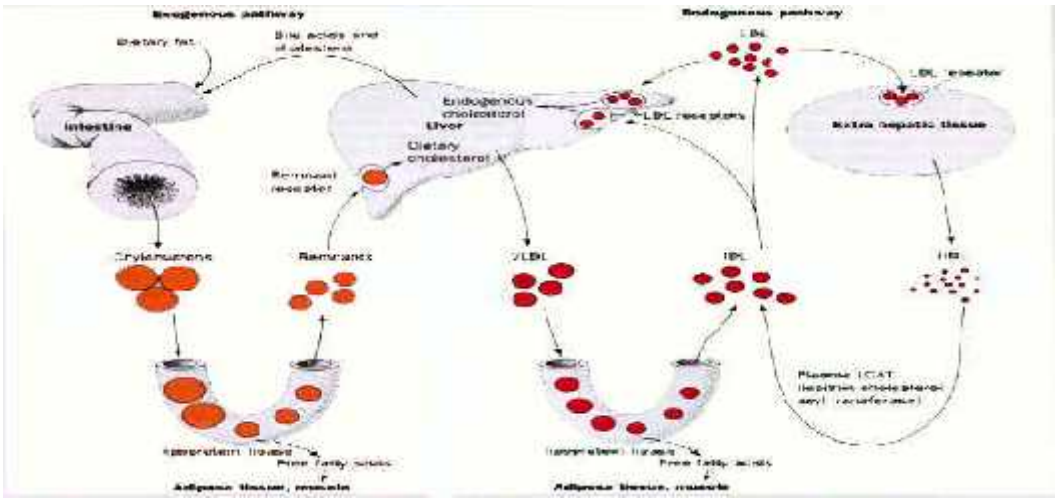
Type V hyperlipidemia: It shows elevated levels of chylomicrons, VLDL and triglycerides resulting from faulty carbohydrate metabolism.

A major concern in patients with hyperlipidemia is the increased risk of atherosclerosis resulting in heart diseases. The aim of treating the patients with hyperlipidemia is to reduce serum cholesterol and/or improve the HDL cholesterol by maintaining a high ratio of HDL to LDL cholesterol level thereby reducing the risk of developing heart diseases. **(Nanjaian Mahadevan *et al.*, 2007)**

Mechanism of Lipid Transport

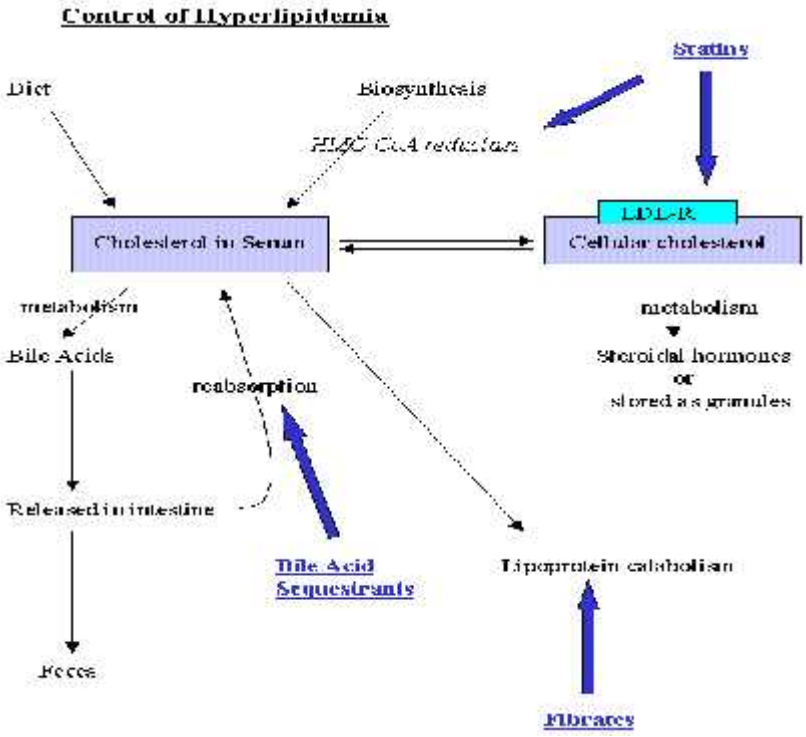
Dietary fat including cholesterol and triglycerides are absorbed in the intestine and released in the blood stream as chylomicrons. These are least dense particles having very high proportion of triacylglycerides. Lipoprotein lipase acts on these particles to release some free fatty acids that deposit in adipose tissues. The remnants of chylomicrons are picked up by the liver which has a receptor specific to chylomicron remnants. After further clean up liver releases particles called the very low density lipoproteins (VLDL) in the blood. These have lower triacyl glycerides than chylomicrons. Once again LPL works on these VLDL particles releasing more free fatty acids and changing the content of the particles to IDL and LDL. There are LDL receptors on the cell membranes of the extra hepatic cells which can pick up the LDL particles. This is how cholesterol reaches the interior of normal cells. Within cells, LDL particles are repackaged. Excess cholesterol is esterified and stored. Excess cholesterol suppresses the biosynthesis of LDL-receptors so that intake of cholesterol decreases. It also suppresses cholesterol biosynthesis. Repackaged LDL particles called HDL particles are then released into the blood stream. These particles are sensed by the liver through the HDL-receptors. Thus the liver gets constant information as to how much LDL and HDL are present in the blood.**(Umesh R Desai, 2000)**

Figure No. 3



Strategies for treating Hyperlipidemia

Figure No. 4



Statins

Statins inhibit the rate-limiting step in the biosynthesis of cholesterol - HMG -CoA reductase. Decreased cholesterol biosynthesis steps up the levels of the LDL-receptor resulting in the positive cycle for lowered cholesterol levels in serum. For patients who have familial hypercholesterolemia due to defective LDL-receptor genes these drugs are not effective. Statins are most effective cholesterol lowering drugs. Statins lower total cholesterol and LDL particles. These are competitive inhibitors. The HMG-CoA has a conformation similar to the lactone moiety of statins resulting in binding at the same site without any productive effect.

All statins are highly protein bound (95-98%) except for pravastatin (50%, due to carboxylate moiety). Most statins have a short half-life of about 1-3 hr except for atorvastatin which has a 1/2 of about 14 h. A 15-30 point drop in LDL could be reasonably expected with most statins after a therapy of about 1 month. A

combination therapy (with bile acid sequestering agents) is helpful for particularly difficult cases. Although possible, statins typically do not affect the concentrations of steroid hormones in circulation.

Fibrates

Gemfibrozil was introduced in 1981 and remains the second most useful antilipidemic agent. It primarily decreases serum triglycerides. Newer drugs including beclofibrates, ciprofibrates, fenofibrates, are more effective in lowering serum LDL cholesterol. However, the fibrates are almost never used alone. They are mostly used in combination with bile acid sequestering agents.

The antitriglycedemic effect of clofibrate in human is related to the increased catabolism of serum TG-rich proteins (VLDL and VLDL remnants), but not to any effect on hepatic TG or VLDL synthesis and release from liver. The action of clofibrate is related to an increase in adipose tissue or muscle LPL activity which accelerates the rate of intravascular catabolism of VLDL to IDL and LDL.

Clofibrate is metabolized to chlorophenoxyisobutyric acid (CPIB) which is the active form of the drug. The drug is high protein bound with a half-life of around 15 hrs.

Bile Acid Sequestering (BAS) Agents

Colestipol and cholestyramine are anion exchange resins that are approved in 1970s for the reduction of elevated serum cholesterol in patients with hypercholesterolemia. These resins are water insoluble, inert to digestive enzymes in the intestinal tract and are not absorbed. Both resins are quaternized at stomach pH and exchange anions for bile acids dramatically reducing the reabsorption of bile acids. The liver senses that bile acid concentrations have gone down and hence turns on cholesterol metabolism. Serum HDL and TG levels remain unchanged. LDL levels are found to decrease.

The fall in LDL concentration is apparent in 4 to 7 days. The decline in serum cholesterol is usually evident by 1 month. When the resins are discontinued, the serum cholesterol usually returns to baseline within a month. When bile acid secretion is partially blocked, serum bile acid concentration rises. For these patients, cholestyramine reduces bile acid deposits in the dermal tissues. One of greatest advantage of these polymeric agents is that they can be safely used for pregnant women. However, exercise caution for nursing women because presence of these cationic polymeric agents in the GI tract might lower the absorption of vitamin D. BAS agents may also lower the amount of anticoagulants (warfarin, Coumadin) absorbed due to sequestration.

Nicotinic Acid

Pharmacologic doses of nicotinic acid reduce serum cholesterol and TG levels in types II, III, IV, and V hyperlipoproteinemias. TG and VLDL are reduced by 20 to 40% in 1 to 4 days; LDL reduction may be seen in 5-7 days. The decrease in LDL is usually greater if niacin is used with a BAS resin. HDL is increased by 20%. The exact mechanism is unknown. It is known that niacin decreases lipolysis in adipose tissue, decreases TG esterification in the liver and increase LPL activity. Niacin is rapidly absorbed. **(Umesh R Desai, 2000)**

Plant remedies for Hyperlipidemia

A number of plant preparations such as Allium sativum, Cicer arientinum, Inula recemosa, Terminalia arjuna, Trigonella foenum graecum, Commiphora mukul, green tea and curcumin have been reported to have hypolipidemic actions. Few of these also, possess certain other beneficial properties like antianginal and antiplatelet actions. Plant preparations contain many compounds that work synergistically on multiple parts of the body. For example, garlic is not only antibacterial, but antifungal, and helps to lower cholesterol. This synergy of chemicals helps to balance the overall activity of the herb. Since the chemicals in herbs are non-specific and unconcentrated, there are generally fewer side effects from herbs than from manufactured drugs. Further, according to a study published in the April 15, 1998

issue of the Journal of the American Medical Association, the fifth leading cause of death in the United States in 1994 was adverse drug reactions of modern medicine, an excess of 100,000 deaths. By contrast, there have been less than 100 adverse reactions and only one death attributed to herbs in Canada since 1990. Most reactions to herbs have to do with an individual allergic reaction to the herb or to an interaction with prescription drugs.

Table no. 2

Sl.No	Name of Plant	Family	Common/ Indian vernacular names	Plant parts
1	*Aegle marmelos*	Rutaceae	Beal fruit, bilwa	Fruits
2	*Agave Veracruz*	Amaryllidaceae	American aloe, barakhawar	Roots, leaves, gum
3	*Allium cepa*	Lilliaceae	Onion, piyaj, palandu	Bulbs
4	*Aloe barbadensis*	Lilliaceae	Ghee kumar, gwarpatha	Leaves
5	*Bambusa arundunacea*	Graminae	Bamboo vamsha	Leaves
6	*Bosswellia serrata*	Burserraceae	Salai guggal	Gum
7	*Brassicavercapitata*	Cruciferae	Cabbage	Oil
8	*Cajanus cajan*	Fabaceae	Red gram	Seeds
9	*Capparis decidua*	Capparaceae	Karli, tint	Leaves, fruits and stems
10	*Capsicum frutescens*	Solanaceae	Chillies	Fruits
11	*Carum capaticum*	Umbelliferae	Jowan, ajowan	Fruits, roots
12	*Celastrus paniculatus*	Celastraceae	Khunjri, kusur	Seed oil, barks, roots and fruits
13	*Curcuma amada*	Zingiberaceae	Mango ginger, haridra	Rhizomes
14	*Cyamopsis tetragonoloba*	Leguminosae	Guar, gwar	Seeds
15	*Emblica officinalis*	Euphorbiaceae	Amla, amlki	Dried fruits,

				Seeds, leaves
16	*Eugenia cumini*	Myrtaceae	Jamun	Seeds
17	*Inula racemosa*	Compositae	Puskarmul	Roots
18	*Juglans regia*	Juglandaceae	Walnut, akhrot	Kernel, oil
19	*Medicago sativum*	Papilionaceae	Alfalfa	Seeds
20	*Momordica charantia*	Cucurbitaceae	Bitterground,	Fruits
21	*Musa saspientum*	Musaceae	Banana, kela	Roots,Stems, Flowers, Fruits
22	*Nepeta hindostana*	Labiatae	Billiola, badranj boya	Whole plant
23	*Phaseolus aureus*	Fabaceae	Green gram	Seeds
24	*Phaseolus mungo*	Fabaceae	Black gram	Seeds
25	*Pergularia daemia*	Asclepidaeceae	Veliparutti, Uttravani	Whole plant
26	*Piper nigrum*	Piperaceae	Golmirch, kalimich	Leaves
27	*Pisum sativum*	Papilionaceae	Gardenpea, matar	Seeds
28	*Pterocarpus marsupium*	Papilionaceae	Indian malabarkino	Gum and leaves
29	*Saussuraea lappa*	Asteraceae	Kustha, Kut	Roots
30	*Terminalia arjuna*	Combretaceae	Arjun	Barks

FLAVONOIDS, LIPIDS AND LIPOPROTEINS

The lipid lowering properties of flavonoids have been evaluated. Dyslipidemia is characterized by hepatic over secretion of Apo BIOO-containing lipoproteins, hypertriglyceridemia, delayed clearance of LDL and low levels of HDL. Dietary studies in humans have demonstrated the beneficial effects of many flavonoids including those contained in soy protein isolate and green tea, both of which lower LDL cholesterol. However, studies with anthocyanidins, black tea, chocolate, red wine and grape demonstrated no beneficial effects on LDL-C or HDL-C in human studies. However, both animal and human studies have

demonstrated that mixtures of specific bioactive flavonoids can decrease LDL-C. Treatment of fructose-induced, insulin-resistant hamsters with a mixture of citrus polymethoxylated flavones improved dyslipidemia and glucose tolerance. However, the mechanism was not elucidated.

Berberine, an alkaloid isolated from Chinese herbs, up-regulated LDL receptor expression in cultured hepatocytes through a MAPKerk dependent mechanism and reduced plasma lipids in a hyperlipidemic hamster model. Administration of berberine to hypercholesterolemic patients reduced LDL cholesterol by 25%, demonstrating the cholesterol lowering potential of berberine in humans.

FLAVONOIDS AND ATHEROSCLEROSIS

The early stages of atherosclerosis are characterized by endothelial dysfunction and macrophage foam cell formation. Administration of pomegranate juice, rich in anthocyanidins and proanthocyanidins to apolipoprotein E-deficient (apoE1') mice resulted in dramatic reductions in lipid peroxides and macrophage CE accumulation, without significantly affecting plasma cholesterol. Following 3 months of supplementation, atherosclerosis was significantly reduced. Administration of pomegranate by-product (PBP) to apoE^mice also attenuated atherosclerosis development, as a result of decreased cellular uptake of oxidized LDL and macrophage oxidative stress. Using the same apoE1' mouse model, 4 month supplementation of a low fat diet with the polyphenolic compound resveratrol led to a reduction in total plasma cholesterol and LDL-C and an increase in HDL-C. The mechanism for the reduction in plasma cholesterol was through a reduction in hepatic cholesterol synthesis, which may have stimulated LDL receptor mediated uptake of LDL from plasma. Resveratrol also prevents lipid peroxidation and increases the cholesterol efflux from macrophages through these mechanisms, resveratrol significantly reduced atherosclerotic plaque development in the aortic arch of apoE1' mice.

REVIEW OF LITERATURE

PLANT PROFILE

Classification

Kingdom - Plantae – Plants

Subkingdom - Tracheobionta – Vascular plants

Division - Magnoliophyta – Flowering plants

Class - Magnoliopsida – Dicotyledons

Order - Gentianales

Family - Asclepiadaceae – Milkweed family

Genus - Pergularia L. – pergularia

Species - Pergularia daemia (Forssk.) Chiov. – Pergularia

Synonyms : Asclepias daemia, Daemia extensa, Cynanchum extensum

Vernacular names

English : Hariknot plant

Hindi : Utaran, Sagovani, Aakasan, Gadaria Ki bel, Jutak

Marathi : Utarn

Tamil : Uttamani, Seendhal kodi

Malayalam : Veliparatti

Telugu : Dustapuchettu, Jittupaku

Kannada : Halokoratige, Juttuve, Talavaranaballi, Bileehatthi balli

Sanskrit : Uttamarani, Kurutakah, Visanika, Kakajangha

Description

Pergularia is a perennial twining herb, foul-smelling when bruised and with much milky juice, stem hairy. Leaves are thin, broadly ovate, heart-shaped or nearly circular, hairless above, velvety beneath. Greenish yellow or dull white, and sweet-scented flowers are borne in lateral cymes which are at first corymb-like, afterwards raceme-like. The five petals are hairy and spreading outwards. Corona outer and inner, outer truncate, inner curved high over the staminal column, spur acute. Fruit is a follicle, with soft spines all over and a long beak. Seeds are densely velvety on both sides.

Flowering: August-February.

ORIGIN

The cultivated form is thought to be African in origin, from where it was introduced to practically all temperate regions of the world.

PARTS USED

Leaves, latex from the plant, roots and whole plant are used.

ACTIVE INGREDIENTS

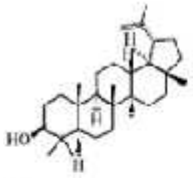
The active ingredients in Pergularia daemia are cardenolides, alkaloids, triterpenes and saponins.

PHARMACOLOGICAL EFFECTS

The plant has been demonstrated to possess multiple pharmacological activities such as antiinflammatory, hepatoprotective, anticancer, antidiabetic, antioxidant, antibacterial, antifungal, analgesic, antiinfertility and central nervous system depressant activity. This review highlights on the existing information particularly on the phytochemistry and various pharmacological properties of Pergularia daemia which may provide incentive for proper evaluation of the plant as a medicinal agent.

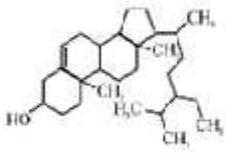
Structures and activities of some active compounds from Pergularia daemia

Lupeol



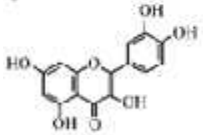
Responsible for antihepatotoxicity, antitumor, anti-inflammatory (Shirwaikar *et al*., 2004; antiarthritic (Agarwal and Rangari, 2003) antiacetical/chemopreventive agent (Suda, 2003) and antimicrobial (Siddiqui *et al*., 2008), antiedemic, antihyperglycemic, anticonvulsant, cytotoxic and hypotensive, antiedemic and antiperoxidant activities (Sunitha, *et al*., 2001)

β-sitosterol



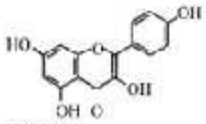
Responsible for antidiabetic, antioxidant (Li *et al*., 2007), atherosclerosis,prostate enlargement, antimicrobe, candidicide, spermicide, ulcerogenic, antihypercholesterolemia antifungal, antidiabetic, anticancer (Imanaka *et al*., 2008), antiedemic, antiinflammatory, anticephalic, antifeedant, Antigonado-trophic, antihyperlipoproteinaemic and antiprostatitic

Quercetin



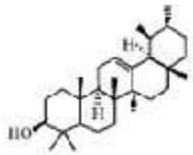
Responsible for anti-inflammatory (Laura *et al*., 2008), antihistamine, antioxidant, anticancer activities and to prevent prostatitis, heart disease, cataracts, allergies and respiratory diseases such as bronchitis, asthma and hives

Kaempferol



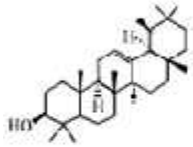
Responsible for antidepressant (Habibzadeh *et al*., 2008), anti-cancer (Nguyen *et al*., 2008) and inhibit fertility, epilepsy, anti-inflammatory, anti-oxidants, spasm, anti-ulcer, lidan diuretics and cough

α-Amyrin



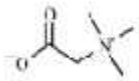
Responsible for anti-inflammatory activity, antioxidant, anti protease, antinociceptive effect (Otuki *et al*., 2005), antiedemic antitumor, cytotoxic, hepatoprotective, insecticide and antiedemic activities

Beta-Amyrin



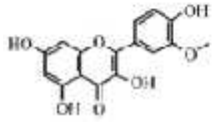
Responsible for anti elastase activity, antifungal, antidepressant (Subarnas *et al*., 1993), antiedemic, antiinflammatory hepatoprotective, mosquitocide and antiedemic activities

Betaine



Treat high levels of homocysteine, heart disease and liver Disease, rheumatoid arthritis, thyroid problems, tic colour vitiglio, gall stones, indigestion (Smart, 2004)

Isorhamnetin



Responsible for anti adipogenic (Jongtung *et al*., 2009), antitumor (Tang *et al*., 2006), antigenotoxic and antioxidant activities (Boukhlel *et al*., 2009)

MEDICINAL USES

In ayurvedic system of medicine this plant is used for delayed child birth, amenorrhea, asthma, snake bite, rheumatic swellings and also to treat post-partum hemorrhage. The decoction of the plant (10-20 mL) is also applied on white spots (leucoderma). Leaf decoction is an uterine tonic and is taken orally up to 20 mL day-1. The stem and root bark extract is taken against fever and diarrhea in infants. The leaves are specially used as a condiment for soup and porridge yam. Fruits are digestive and thermogenic. Plant extract is useful in uterine and menstrual disorders and in facilitating parturition. Pergularia has been used in folk medicine for the treatment of liver disorders. Traditionally used in treating various ailments for human beings. Some of the folklore people used this plant to treat jaundice, anthelmintic, laxative, anti-pyretic, expectorant and also used in infantile diarrhoea.

LITERATURE SURVEY

- **Lokesh T Nikajoo *et al.*, 2009** investigated the analgesic activity of aqueous and alcohol root extracts of Pergularia daemia (Forsk.) Chiov. For the evaluation of analgesic activity of analgesic activity of aqueous and alcohol root extracts of Pergularia daemia (Forsk.) Chiov. Using eddy's hot plate and heat conduction method. In eddy's hot plate method the aqueous extract showed significant analgesic activity at the doses of 500 mg/kg ($p<0.01$) and 1000 mg/kg ($p<0.001$) and alcohol extract showed significant analgesic activity at the doses of 500 and 1000 mg/kg ($p<0.001$). In heat conduction method both extracts showed significant analgesic activity at the doses of 500 & 1000 mg/kg ($p<0.001$) as compared to control group, when analysed statistically by Tukey Kramer Multiple Comparison Test. The result obtained show that the aqueous and alcohol root extracts of Pergularia daemia (Forsk.) Chiov. Possesses significant analgesic activity.

- **Raman R Chandak *et al.*, 2010** investigated the Antibacterial activity of Chloroform and Ethanol extracts of Whole Plant of Pergularia daemia Linn. Were tested cup-plate agar diffusion method against Gram positive and Gram negative bacteria. It was observed that all extracts have inhibitory effect, water extract being most effective.

- **Golam sadik *et al.*, 2001** investigated the antifertility activity of ethonalic and its steroidal fraction of Pergularia daemia (Forsk.) Chiov. Both the ethanol extract and the steroidal fraction showed significant antifertility activity in the pre implantation stage in female mice. The ethanol extract showed late abortificient effect.

- **Suresh M *et al.*, 2010** investigated the antifungal activity of the salts from five Indian medicinal plants against three human pathogenic fungi. Antifungal assay was done using agar disc diffusion method. The result showed that the plat salt of Indian medicinal plants i.e. Acalypha indica, Centella asiatica, Aerva lanata, Clerodendrum inerme, Pergularia daemia, Solanum melongena exhibited significant antifungal activity against one or more test organism.

- **S.Venkataraman *et al.*, 2010** investigated the analgesic, antipyretic and anti-inflammatory of Petroleum ether and Chloroform extract of plant of Pergularia daemia Forsk were studied .The analgesic activity was found out by eddy's hot plate method by using standard Diclofenac sodium . The antipyretic activity was found out by yeast induced pyrexia method by using standard Paracetamol. The anti-inflammatory activity was found out by Carragenan induced paw edema method by using standard Diclofenac sodium. In carrageenan induced paw odema method the chloroform extract of Pergularia daemia showed more significant inhibition than petroleum ether extract. The results were found to be highly significant ($p<0.01$) in comparison to the control. In eddy's hot

plate method the chloroform extract of Pergularia daemia exhibited significant analgesic activity than petroleum extract. In antipyretic activity the chloroform and pet ether both extracts having significant activity.

- **V. M. Shastry *et al.*, 2011** investigated the acute and chronic anti-inflammatory activity of petroleum ether extract (PDPE), ethanolic extract(PDEE) and aqueous extract(PDAE) of whole plant of Pergularia daemia (100, 200 and 400 mg/kg) have been investigated in carrageenan- and formalin- induced paw edema and turpentine oil-induced granuloma pouch in rats. Oral administration of PDEE (200 and 400 mg/kg) significantly reduced the paw volume ($P < 0.001$) at 3h in carrageenan model. The treatment of PDEE (200 and 400 mg/kg) significantly reduced the volume of exudates ($P < 0.001$) in turpentine oil-induced granuloma pouch dose dependently. Chronic inflammation induced by formalin injection was significantly ($P < 0.001$) inhibited by PDEE (400 mg/kg) as compared to the control rats. Diclofenac (10 mg/kg) was used as reference drug. Thus, the present study shows that whole plant of Pergularia daemia possess significant anti-inflammatory activity and supports the claim in traditional medicine for the treatment of inflammatory conditions.

- **Hariram V Bhaskar *et al.*, 2009** investigated the antioxidant and free radical scavenging properties of the ethanol and aqueous extracts from roots of Carissa carandas and Pergularia daemia in various in vitro systems. Reducing power, DPPH radical, superoxide radical, nitric oxide radical and hydrogen peroxide radical scavenging assays were carried out to evaluate the antioxidant potential of the ethanol and aqueous extracts from roots of C. carandas and P. daemia. Total phenolic content and total flavonoids were also evaluated. In the DPPH radical scavenging assay theIC50 values were 178.84 and 169.39 µg/ml for ethanol extract of C. carandas and P. daemia respectively. In the nitric oxide radical scavenging assay the ethanol extract of P. daemia showed maximum percentage

inhibition (65.14±0.0115), while the aqueous extract of P. daemia exhibited least percentage inhibition(62.69±0.0461) on superoxide radical scavenging, when compared with other extracts. The aqueous extract of C. carandas and P. daemia exhibited 50%scavenging activity at 208.07 and 243.09 µg/ml on H2O2 radical. The reducing power of the extracts increased dose dependently. All the extracts exhibited significant antioxidant ($p < 0.01$) activity. The extracts of the roots of C. carandas and P. daemia possess antioxidant properties and could serve as free radical inhibitors or scavengers.

- **Te Khorombi *et al.*, 1997** investigated the methanol–dichloromethane (1:1 v/v) extract of the whole plant Pergularia daemia inhibited the growth of the cancerous cells when tested both at the CSIR and NCI. Repeated flash chromatography of the organic extract afforded the isolation of five compounds that were characterized as β-sitosteryl glucoside, β-sitosterol, α-amyrin, 3-O-acetyl-α-amyrin and a disaccharide, sucrose. These compounds showed no significant anti-cancer activity against the CSIR's three cell lines, except α-amyrin that exhibited low potency. Furthermore, substitution of the hydroxyl group at position 3 of α-amyrin with the acetate functionality resulted in loss of activity. This finding suggests that the presence of the hydroxyl group at position 3 is important for the inhibition of the growth of cancer cells.

- **Manickam Pavunraj *et al.*, 2010** investigated the leaves of milkweed, Pergularia daemia (Forssk) Choiv., on the antifeedant activity against two important lepidopteran pests, Helicoverpa armigera (Hub.) and Spodoptera litura (F.), were studied. Maximum antifeedant activity was recorded in ethyl acetate crude extract against H. armigera (70.3%) and S. litura (71.82%) at 1% concentration. Ethyl acetate crude extract was further subjected to column chromatography, which was performed using hexane as initial solvent and then by increasing the polar strength using

ethyl acetate. Fractions collected at hexane and ethyl acetate (80:20) yielded 6-(4,7-hydroxy-heptyl) quinone, a novel compound which showed significant antifeedant activity against H. armigera (80.22% at 2000 ppm) and S. litura (68.31% at 2000 ppm).

- **BA Vyas1 *et al.,* 2011** investigated The whole-plant, Pergularia daemia (Family: Asclepediaceae), extract (50% alcohol) for its antiurolithiatic and diuretic activity. Ethylene glycol (0.75% in water) feeding resulted in hyperoxaluria as well as increased renal excretion of calcium and phosphate. Alcoholic extract (400 mg/kg) of P. daemia was given orally in curative and preventive regimens over a period of 28 days. Supplementation with extract significantly ($P < 0.001$) lowered the urinary excretion and kidney retention levels of oxalate, calcium and phosphate. Furthermore, high serum levels of urea nitrogen, creatinine and uric acid were significantly ($P < 0.001$) reduced by the extract. The results were comparable with the standard drug, cystone (750 mg/kg). The reduction of stone-forming constituents in urine and their decreased kidney retention reduces the solubility product of crystallizing salts such as calcium oxalate and calcium phosphate, which could contribute to the antiurolithiatic property of the extract. The extract exhibited significant diuretic activity at dose of 400 mg/kg body weight as evidenced by increased total urine volume and the urine concentration of Na +, and K + . These findings affirm assertions made regarding the effectiveness of the extract of this plant against urinary pathologies in the Indian folk medicine.

- **G.Ramu *et al.,* 2009** investigated the Root starch (14.0% w/w) obtained from Pergularia daemia was employed as a disintegrant to paracetamol tablets at concentrations of 2.5-10.0 % w/w in the present study. The limit tests, loss on drying, ash value and microbial load were well within the official limits. The starch was also evaluated for various parameters as per

Indian Pharmacopoeia. The granules prepared by wet granulation technique were evaluated for percentage of fines, bulk and tapped densities and flow properties. Tablet properties including thickness, content uniformity, average weight and weight variation, hardness, friability, disintegration time and drug dissolution were evaluated. The disintegrant efficiency of extracted starch was compared with that of the corn starch in tablets prepared using magnesium stearate, aerosil, microcrystalline cellulose and gelatin as lubricant, glidant, diluent and binder respectively. The disintegration time for tablet formulations prepared using 10 % w/w extracted starch was less (166 s) than that of the tablet formulations prepared using corn starch as a disintegrant (270 s). Dissolution studies showed that the drug release from the tablet containing 2.5 to 10 % w/w was more than 60 % in 1hr. Studies indicated that Pergularia daemia starch is a good disintegrant in tablet formulation.

- **Suresh kumar *et al.,* 2007** investigated the hepatoprotective activity of ethonalic and its ethanol fraction of Pergularia daemia plant aerial parts exhibited significant hepatoprotective effect against CCl4 induced hepatotoxicity in rats. Glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, total bilirubin, total cholesterol, total protein and albumin in serum indicated hepatoprotective effect of the ethanol extract and its ethanol fraction. Histopathological examination of liver sections confirmed that, pre-treatment with ethanol extraction and its ethonalic fraction prevented hepatic damage induced by CCl4. The results were comparable with the standard hepatoprotective drug Silymarin. The extract and its fraction showed no signs of toxicity up to a dose level of 2000 mg/kg. It is suggested that, the presence of flavonoids in ethanol extraction and its ethonalic fraction may be responsible for hepatoprotective properties. HPTLC profile of flavonoids of bio active extracts was developed using quercetin-3-glycoside as marker. Results indicate hepatoprotective properties of ethanol extract of Pergularia daemia.

- **Varsha Dhulasavant *et al.,* 2011** investigate the hypolipidemic effect of Cinnamomum tamala Nees. Leaves extracts in high cholesterol diet induced hyperlipidemia. Aqueous and ethanolic extracts of leaves of Cinnamomum tamala Nees. were administered in doses of 400mg/kg /day p.o. each for 10 days. Simultaneous administration of Cinnamomum tamala Nees. leaves extracts significantly($p<0.001$) prevent the rise in serum levels of total cholesterol, triglyceride, LDL-C,VLDL-C and atherogenic index whereas significant ($p<0.01$) increases in the level of HDL-C.

- **W.C. Lee *et al.,* 2001** investigated the Effects of high-cholesterol diet on the interendothelial clefts and the associated junctional complexes in rat aorta. The arterial endothelial intercellular cleft (AEC) and its associated junctional complex (JC) are the determinants of permeability to macromolecules. This study analyzed frequencies of AEC and JC profile types in the rat thoracic aorta at 1 and 12 months after feeding the animals with a normal or a high-cholesterol diet. Rats on either a normal diet or high-cholesterol diet for 12 months showed more of the simple 'end to end' or 'overlap' types (PB0.01) but fewer complex 'interdigitating' type (PB0.01) of AEC compared to the 1 month group. With regard to JC, the frequencies of gap junctions were decreased (PB0.01) while the tight junctions and the normal junctionless complex were increased (PB0.01) after 12 months of normal diet as compared with 1 month on the normal diet. These changes in frequencies for gap junction and tight junction were even greater for the high-cholesterol diet than for the normal diet treatment. Moreover, the incidence of open junctions was also noticeably increased after 12 months of high-cholesterol diet. These findings suggest that the proportions of the AEC and JC were highly responsive to aging whereas those of JC were more susceptible to the high-cholesterol diet treatment.

- **Bhalodia Yagnik *et al.*, 2009** investigated the antihyperlipidemic and antioxidant activity of methanolic fruit extract of Benincasa cerifera in high fat diet induced hyperlipidemic rat. Wistar albino rats were fed with high fat diet containing 2% cholesterol and 20% coconut oil for 4 weeks and serum cholesterol level were measured every week. At the end of third week methanolic extract of Benincasa cerifera fruit (500 mg/kg/day) was administered orally for 1 week. At the end of fourth week, the serum lipid metabolites such as total cholesterol, triglycerides and high density lipoproteins were determined. In order to determine antioxidant activity of extract, renal tissues were homogenized in ice cold saline buffer and the assay of lipid peroxidation, superoxide dismutase, reduced glutathione and catalase were performed in control, hyperlipidemic and treated rats. High fat diet feeding rat for 4 weeks shows significant difference in lipid metabolites, lipid peroxidation and antioxidant enzymes level when compared with normal control rat. Oral administration of Benincasa cerifera for one week resulted in a significant reduction in total cholesterol and triglyceride level, and significant improvement in lipid peroxidation, superoxide dismutase and reduced glutathione in renal tissues of hyperlipidemic rats when compared with hyperlipidemic control rat. The Benincasa cerifera showed significant antihyperlipidemic and antioxidants activity in hyperlipidemic rat.

- **Si-Yuan Pan *et al.*, 2009** investigated the Effects of the ethanol extract of Fructus Schisandrae (EtFSC) on serum and liver lipid content in mice fed with high fat/cholesterol (HFC) diet for 8 or 15 days. The induction of hypercholesterolemia by HFC diet caused significant increases in serum and hepatic total cholesterol (TC) levels (up to 62% and 165%, resp.) and hepatic triglyceride (TG) levels (up to 528%) in mice. EtFSC treatment (1 or 5 g/kg/day for 7 days; from Day 1 to 7 or from Day 8 to 14, i.g.) significantly decreased the hepatic TG level (down to 35%) and slightly increased the hepatic index (by 8%) in hypercholesterolemic mice. Whereas fenofibrate treatment (0.1 g/kg/day for 7 days, i.g.) significantly

lowered the hepatic TG level (by 61%), it elevated the hepatic index (by 77%) in hypercholesterolemic mice. Acute toxicity test showed that EtFSC was relatively non-toxic, with an LD50 value of 35.63 ± 6.46 g/kg in mice. The results indicate that EtFSC treatment can invariably decrease hepatic TG in hypercholesterolemic mice, as assessed by both preventive and therapeutic protocols, suggesting its potential use for fatty liver treatment.

- **Vincenzo *et al.*, 2010** investigated Bergamot juice produces hypolipemic activity in rats though the mechanism remains unclear. Here we investigated on the effect of bergamot extract (BPF) in diet-induced hyperlipemia in Wistar rats and in 237 patients suffering from hyperlipemia either associated or not with hyperglycaemia. BPF, given orally for 30 days to both rats and patients, reduces total and LDL cholesterol levels (an effect accompanied by elevation of cHDL), triglyceride levels and by a significant decrease in blood glucose. Moreover, BPF inhibited HMG-CoA reductase activity an enhanced reactive vasodilation thus representing an efficient phytotherapeutic approach in combating hyperlipemic and hyperglycaemic disorders.

- **Gloria A. Otunola1 *et al.*, 2010** investigated the effects of a high dietary soybean oil and cholesterol on serum total cholesterol, low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), and triglycerides were investigated. Total protein, albumin, glucose, gamma glutamyl transferase (GGT), aspartate aminotransferase (AST) and alanine amino transferase (ALT) activities were also investigated in weanling female Wistar rats for eight weeks. Two groups of weanling Wistar rats were use in this study. The first group of rats were fed with a control diet made up of the normal rat chow (C), while the second group was given a hypercholesterolemic diet (HPC) enriched with 25% soybean oil and 1% cholesterol for eight weeks. The dietary intake of the HPC diet

significantly increased the level of total cholesterol, LDL-C and triglycerides in the serum of animals fed the (HPC) diet. GGT, AST and ALT activities were also markedly elevated in rats fed with the HPC diet. While total protein and glucose level of the animals fed with the HPC diet was remarkably reduced, there was no significant difference in the HDL-C and albumin contents of both groups. This study established that hypercholesterolemia is induced by high soybean oil/cholesterol diet, despite the fact that soybean oil has high content of poly unsaturated fatty acids. Also, the HPC diet led to weight loss in the rats and injury to both heart and liver of the rats.

- **Mukesh Sikarwar *et al.*, 2008** investigated the possible antihyperlipidemic effect of petroleum ether (60-800C), chloroform, ethanol and aqueous extracts of Nerium indicum leaves in triton (400mg/kg b.w.) induced and atherogenic diet induced hyperlipidemic rats. A comparison was also made between the action of Nerium indicum leaves extracts and a known antihyperlipidemic drug simvastatin (10mg/kg body wt.). Oral administration of 500 mg/kg body wt. of the chloroform extract of Nerium indicum leaves exhibited a significant reduction ($p<0.01$) in serum lipid parameters like total cholesterol, triglycerides, low density lipoprotein (LDL), very low density lipopreotein (VLDL) and increase in high density lipoprotein (HDL) in hyperlipidemic rats in comparison with hyperlipidemic control in both models.

- **R P Umbare *et al.*, 2009** investigated antihyperlipidemic effect of plant Phyllanthus amarus Schumach against cholesterol diet induced hyperlipidemia in Wister rats. Hydro-alcoholic extract of leaves of Phyllanthus amarus Schumach (HAEPAS) was studied for its in-vivo anti-hyperlipidemic potential using cholesterol diet induced

hyperlipidemia model in rats. The result of study indicated that HAEPAS possess significant hypolipidemic activity at doses 300 and 500 mg/kg.

AIM AND OBJECTIVE

The main aim of the study is to evaluate the methonalic extract of *Pergularia daemia* on rats fed with high fat diet.

Coronary artery disease (CAD) is one of the most important causes of death all over the world. CAD remains the most common cause of morbidity and mortality all over the world. In depending countries, the incidence of cardiovascular disease is increasing alarmingly. India is on the verge of cardiovascular epidemics. It is expected that the circulatory system disorders are going to be the greatest killer in India by the end of year 2015. It is well established increased levels of blood cholesterol especially low density lipoprotein cholesterol (LDL-c) is an important risk factor for cardiovascular complications since it favours lipid deposition in tissues including blood vessels. Evidences from lipid lowering trials have clearly established that reduction of total cholesterol or LDL-c is associated with decreased risk of atherosclerosis and coronary heart disease. Furthermore, epidemiological studies have also shown an inverse correlation between HDL cholesterol level and the risk of CVD. A large number of allopathic hypolipidemic drugs are available in the market but these lag behind the desired properties such as efficacy and safety on long term use, cost and simplicity of administration.

Plant and plant products are being used as a source of medicine since long. According to World Health Organization (WHO) more than 80% of the world's population, mostly in poor and less developed countries depend on traditional plant based medicines for their primary healthcare needs. The efficacy and safety of herbal medicine have turned the major pharmaceutical population towards medicinal plant's research. Owing to the global trend towards improved 'quality of life', there is considerable evidence of an increase in demand from medicinal plant. A number of medicinal plants have shown their beneficial effect on the cardiovascular disease (CVD) by virtue of their lipid lowering, antianginal, antioxidant and cardioprotective effects. The risk of hyperlipidemia would be reduced by consumption of flavonoids and glycosides, supported by abundant studies. For instance, the flavonoids extracted from

gingko, soyabean, and some other plants have been beneficial to hyperlipidemia patients. (**Jing jing chen and Xian grong, 2007)**

Traditionally the plant *P.daemia* is used as anthelmintic, laxative, antipyretic and expectorant, also used to treat infantile diarrhoea and malarial intermittent fevers. Latex of this plant used for toothache. Stem bark remedy for cold and fever. Aerial parts of this plant reported the various harmacological activities like hepatoprotective, antifertility, anti-diabetic, analgesic, antipyretic and anti-inflammatory.

Pergularia daemia leaves contains various nutrient elements, such as vitamins, minerals, carbohydrates, edible fibres and phytochemicals like cardenolides, alkaloids and saponins and phenolic compounds. The plant was found to contain various triterpenes and steroidal compounds.

Phenolic compounds from *Pergularia daemia* attracted the attention of scientists to define their chemical composition and their properties for human health. The reported evidences of beneficial health effects of phenolic compounds include inhibiting some neurodegenerative diseases such as CVD, diabetes, certain types of cancer. Considering the health benefits of phenolic compounds in *Pergularia daemia*, it is planned to evaluate the lipid lowering effect on rat fed with high fat diet (hypercholestreolemic rats).

PLAN OF WORK

Collection

Collection of plant (*Pergularia daemia*) leaves and shade dried.

Extraction

Extraction of dried leaf powder using methanol in soxhlet apparatus for 72 hours.

Phytochemical investigation of methanol extract of *Pergularia daemia*.

Acute oral toxicity study of methanol extract of *Pergularia daemia* leaves.

Evaluation of anti-hyperlipidemic activity of Pergularia daemia leaf extract in rats fed with high fat diet.

Screening method

Cholesterol Diet induced Hyperlipidemia in rats. (**Rumi Ghosh *et al*., 2010**)

Biochemical estimation of serum lipid parameters

- Total cholesterol
- Serum HDL
- Serum LDL
- Serum VLDL
- Triglycerides

1. **Histopathological study of liver.**

2. **Statistical analysis.**

One way ANOVA followed by standard Dunnett's test.

MATERIALS AND METHODS

5.1 PLANT MATERIAL

The fresh leaves of *Pergularia daemia* were collected from local areas at komarapalayam, Tamilnadu. The material was taxonomically identified, confirmed and authenticated by Botanical survey of India (BSI) at TN agricultural university, Coimbatore.

With authentication no **BSI/SRC/5/23/2011-12/Tech.-1203** and the voucher specimen was retained in our laboratory for further reference. The collected leaves were shade dried and the dried material was crushed to coarse powder with mechanical grinder. The powder was stored in air- tight container which was used for extraction.

5.2 PREPARATION OF EXTRACT

140 grams of powdered leaf material was defatted with petroleum ether (60^0-80^0C) and then extracted with 95% methanol for 72 hrs using soxhlet apparatus. The extract obtained was concentrated to dryness under reduced pressure and the percentage yield was calculated.

5.3 CHEMICALS

Petroleum ether – Nice chemicals. Pvt Ltd, Cochin**.**

Methanol – Cheme pure Laboratory, Chennai**.**

CMC- Reachem Laboratory Chemicals Pvt Ltd, Chennai**.**

Cholesterol – Loba chemi laboratory Pvt Ltd, Mumbai.

Sodium cholate -- Loba chemi laboratory Pvt Ltd, Mumbai.

❖All other chemicals and reagents were of the analytical grades.

5.4 PHYTOCHEMICAL SCREENING

The extract obtained was subjected to Priliminary Phytochemical screening.

5.4.1 Test for alkaloids

0.5gm of extract was dissolved in 10ml of dilute HCl (0.1N HCl) and filtered. The filtrate was used to test the presence of alkaloids.

❖ **Mayer's test**

Filtrate was treated with Mayer's reagent. Formation of yellow cream precipitate indicates the presence of alkaloids.

❖ **Dragendroff's test**

Filtrate was treated with Dragendroff's reagent. Formation of red colored precipitate indicates the presence of alkaloids.

❖ **Hager's test**

Filtrate was treated with Hager's reagent. Formation of yellow colored precipitate indicates the presence of alkaloids.

❖ **Wagner's Test**

Filtrate was treated with wagner's reagent. Formation of brown (or) reddish brown precipitate indicates the presence of alkaloids. **(Rosenthalar, 1930)**

5.4.2 Detection of Phytosterols and Triterpenoids

0.5gm of extract was treated with 10ml of chloroform and filtered. The filtrate was used to test the presence of phytosterols and Triterpenoids.

❖ **Libermann's Test**

To 2ml filtrate in hot alcohol, few drops of acetic anhydride was added. Formation of brown precipitate indicates the presence of sterols.

❖ **Libermann's Burchard Test**

100mg of extract was treated with 2ml of chloroform and filtered. To the filtrate few drops of acetic anhydride was added, boiled and cooled. Conc H_2So_4 was added through the sides of the test tube. Formation of brown ring at the junction indicates the presence of steroidal saponins.

❖ **Salkowaski Test**

To the test extract solution few drops of Conc H_2So_4 was added, shaken and allowed to stand, lower layer turns red indicates the presence of sterols. **(Peach and Tracey)**

5.4.3 Detection of Flavoniods

❖ **Shinoda Test**

To 100mg of extract, few fragments of magnesium metal were added in a test tube, followed by dropwise addition of Conc Hcl. Formation of magenta colour indicates the presence of flavonoids.

❖ **Alkaline Reagent Test**

To 100mg of extract, few drops of sodium hydroxide solution was added in a test tube. Formation of intense yellow color that becomes colorless on addition of few drops of dilute acid (Hcl) indicates the presence of flavanoids. **(Shellard, 1957)**

5.4.4 Detection of Saponins

- **Foam test**

The extract was diluted with 20ml of distilled water and it was shaken in a graduated cylinder for 15 minutes. A 1cm layer of foam indicates the presence of Saponins.

5.4.5 Detection of Proteins and Amino acids

100mg of extract was taken in 10ml of water and filtered. The filtrate was used to test the presence of protein and amino acids.

- ✓ **Millon's Test**

2ml of filtrate was treated with 2ml of millon's reagent in a test tube and heated in a water bath for 5 minutes, cooled and few drops of $NaNo_2$ was added. Formation of white precipitate, which turns to red upon heating, indicates the presence of proteins and amino acids.

- ✓ **Ninhydrin Test**

2ml of filtrate, 0.25% ninhydrin reagent was added in a test tube and boiled for 2 minutes. Formation of blue color indicates the presence of amino acids.

- ✓ **Biuret Test**

2ml of filtrate was treated with 2ml of 10% sodium hydroxide in a test and heated for 10 minutes. A drop of 7% copper sulphate solution was added in the above mixture. Formation of purplish violet indicates the presence of Proteins. **(Finar, 1959) (Hawk, 1954)**

5.4.6 Detection of Fixed oils and Fats

- **Oil spot test:**

One drop of extract was placed on filter paper and the solvent was evaporated. An oily stain of filter paper indicates the presence of fixed oil. **(Rosenthalar, 1930)**

5.4.7 Detection of Phenolics and Tannins

100mg of extract was boiled with 1ml of distilled water and filtered. The filtrate was used of the test.

- **Ferric chloride Test**

To 2ml of filtrate, 2ml of 1% ferric chloride was added in a test tube. Formation of bluish black color indicates the presence of Phenolic nucleus.

- **Lead acetate Test**

To 2ml of filtrate, few drops of lead acetate solution was added in a test tube. Formation of yellow precipitate indicates the presence of Tannins.

5.4.8 Detection of Carbohydrate

500mg of extract was dissolved in 5ml of distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

- **Molisch's test**

To one ml of filtrate, two drops of Molisch's reagent was added in a test tube and 2ml of Conc H_2So_4 was added carefully along the side of the test tube. Formation of violet ring at the junction indicates the presence of carbohydrates.

❖ **Fehling's test**

To one ml of filtrate, 4ml of Fehling's reagent was added in a test tube and heated for 10 minutes in a water bath. Formation of red precipitate indicates the presence of reducing sugar.

❖ **Benedicts Test**

Filtrate was treated with Benedict's reagent and heated on water bath. Formation of orange red precipitate indicates the presence of reducing sugars. **(Rosenthalar, 1930)**

5.4.9 Detection of Glycosides

0.5gm of extract was hydrolyzed with 20ml of dilute Hcl (0.1N) and filtered. The filtrate was used to test the presence of glycosides.

◆ **Modified Borntrager's test**

1ml of filtrate 2ml of 1% ferric chloride solution was added in a test tube and heated for 10 minutes in boiling water bath. The mixture was cooled and shaken with equal volume of benzene. The benzene layer was separated and treated with half its volume of ammonia solution. Formation of rose pink or cherry color in the ammonical layer indicates the presence of anthranol glycoside.

◆ **Legal's test**

To 1ml of filtrate, 3ml of sodium nitropruside in pyridine and methanolic alkali (KoH) was added in a test tube. Formation of pink to blood red color indicates the presence of cardiac glycoside.

- **Keller Killiani Test**

Small portion from the extract was shaken with 1ml of Glacial acetic acid containing trace of ferric chloride. 1ml of Conc H_2So_4 was added carefully by the sides of the test tube. A blue color in the acetic acid layer and red color at the junction of two liquids indicate the presence of glycosides. **(Hawk, 1954)**

4.5 ACUTE ORAL TOXICITY STUDY OF METHANOL EXTRACT OF *Pergularia Daemia.* LEAVES

Animals

Swiss albino mice of female sex weighing 20-25gms were used for the study. The animals were obtained from Agricultural University, Mannuthy, Thrissur, kerala (328/99/CPCSEA) and were housed in polypropylene cages. The animals were maintained under standard laboratory conditions ($25^0 \pm 2^0$C; 12hr light and dark cycle). The animals were fed with standard diet and water *ad-libitum*. Ethical clearance (for handling of animals and the procedures used in study) was obtained from the Institutional Animal Ethical Committee (IAEC) before performing the study on animals.

Acute oral toxicity study for methanol extract of *Pergularia daemia.* a leaf was carried out as per OECD guidelines 425 (Up and Down procedure). The test procedure minimizes the number of animals required to estimate the acute oral toxicity. The test allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity. Animals were fasted (food but not water was with held overnight) prior to dosing. The fasted body weight of each animal was determined and the dose was calculated according to the body weight.

- **Limit test at 2000mg/kg**

The drug was administered in the dose of 2000mg/kg body weight orally to one animal. If the test animal survived. Then four other animals were dosed sequentially; therefore, a total of five animals were tested. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hour), and daily thereafter, for a total of

14 days. After the experimental period, the animals were weighed and humanely killed and their vital organs including heart, lungs, liver, kidneys, spleen, adrenals, sex organs and brain were grossly examined. **(OECD Guidance; 2000)**

Evaluation of hypolipidemic activity of methanol extract of *Pergularia daemia* leaves.

Healthy Albino rats (Wistar strain) of either sex weighing 150-200g were used for the study. The animals were obtained from Agricultural University, Manuthy, Thrissur, Kerala.

The animals were housed in polypropylene cages and were maintained under standard laboratory conditions ($25^{0} \pm 2^{0}$c, 12 hr light and dark cycle). The animals were fed with standard pellet diet and water *ad libitum.*

Ethical clearance (for handling of animals and the procedures used in study) was obtained from Institutional Animal Ethical Committee (IAEC) before performing the study on animals. (20MP01DEC10)

Cholesterol Diet:

Rats were made hyperlipidemic by the oral administration of high cholesterol diet (regular diet mixed with 2% w/w cholesterol, 1% w/w sodium cholate, 2.5% w/w coconut oil) to healthy rats for 30 days. (**Pandya *et al.*, 2006**), (**Rumi Ghosh *et al.*, 2010**).

Experimental Design:

The rats with elevated cholesterol level were divided into 3 groups of 6 animals each and given drug/ vehicle treatment for 15 days. A group of 6 normal animals were included in the study.

Group I animals served as normal control fed with normal diet.

Group II animals served as a **Hypercholesterolemic Control (HC- C)**. Animals received vehicle (CMC 0.5%) 2 ml/kg P.O.

Group III Hypercholesterolemic animals received methanol extract of *Pergularia daemia* 200 mg/kg P.O once daily orally for 15 days.

Group IV Hypercholesterolemic animals received methanol extract of *Pergularia daemia* 400 mg/kg P.O once daily orally for 15 days.

At the end of experimental period, the animals were fasted overnight and fasting blood samples were collected by retro orbital vein puncture technique in clot activator tube. The samples were centrifuged at 4000-5000rpm to separate serum, which was subjected for the estimation of lipid profile.

Estimation of lipid profiles

The total cholesterol in plasma was estimated by cholesterol oxidase enzymatic method using Agappe Diagnostic kit **(Siedel *et al.*, 1981).** Plasma triglyceride (TGL) was estimated by Autoanalyser using enzymatic-GPO method **(Rifai *et al.*, 1999).** High density lipoprotein (HDL) was estimated by selective precipitation followed by cholesterol oxidase enzymatic method using HDL-cholesterol phosphotungstic acid of Erba diagnostic Mannheim gmbh kit **(Burstein *et al.*, 1970).** Low density lipoprotein (LDL) was estimated by direct measurement with the homogeneous method performed with the reagent LDL-C Select FS (DiaSys) **(Caio Mauricio Mendes de Cordova *et al.*, 2004)**. Very low density lipoprotein (VLDL) was calculated using the formula TGL/5 **(Vijayabaskar *et al.*, 2008).** Atherogenic Index (AI), which is a measure of the atherogenic potential of an agent, was calculated using the following formula and the results were tabulated **(Rekha and Ekambaram, 2010).**

Atherogenic Index (AI), which is a measure of atherogenic potential, was calculated using the following formula

$$\text{Atherogenic Index} = \frac{\text{Total serum HDL}}{\text{Total serum cholesterol}}$$

$$\text{Percentage protection} = \frac{\text{AI of control} - \text{AI of treated group}}{\text{AI of control}} \times 100$$

ESTIMATION OF BIOCHEMICAL PARAMETERS

Total Cholesterol, HDL, LDL, VLDL, triglycerides and phospholipids were estimated in serum. All the biochemical parameters were estimated using semi-autoanalyser (photometer, Germany) with enzymatic kits.

Estimation of Serum Total cholesterol (TC)

Principle

Determination of cholesterol was done after enzymatic hydrolysis and oxidation. The colorimetric indicator is quinoneimine, which is generated from 4-aminoantipyrine and phenol by hydrogen peroxidase under the catalytic action of peroxidase (Trinder's reaction).

$$\text{Cholesterol ester} + H_2 \xrightarrow{\text{CHO}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHO}} \text{Cholesterol-3-one} + H_2O_2$$

$$2H_2O_2 + \text{Amino antipyrine} + \text{phenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + H_2O_2$$

Method

CHOD-PAP: enzymatic photometric test

Table 3 Reagents

1	**goods buffer(pH 6.7)**	**50mmol/I**
2	**Phenol**	**5mmol/I**
3	**4-amino antipyrine**	**0.3mmol/I**
4	**Cholesterol esterase**	**>200 U/I**

5	**Cholesterol oxidase**	**>100U/I**
6	**Peroxidase**	**3 KU/I**
7	**Standard**	**5.2 mmol/I**

Assay procedure

a. 1ml (1000 µl) of reagent-1 has taken in a 5ml test tube.

b. Added 0.01 ml (10 µl) of serum.

c. Mixed well and incubated at 37^0C for 5 min.

d. The sample was tested by using photometer.

NORMAL RANGE: <200 mg/dl in serum.

Estimation of Serum Triglycerides (TG)

Principle: Determination of triglycerides (TG) alters enzymatic splitting with lipioprotein lipase. Indicator is quinoneimine which is generated from aminoantipyrine and 4-chlorophenol by hydrogen peroxidase under the catalytic action of peroxidase.

$$\text{Triglycerides} \xrightarrow{\text{LPL}} \text{Glycerol + fatty acid}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate + ADP}$$

$$\text{Glycerol-3-phosphate + } O_2 \xrightarrow{\text{GPO}} \text{Dihydroxy acetone phosphate + } H_2O_2$$

$$2H_2O_2\text{+4-amino antipyrine+4-chlorophenol} \xrightarrow{\text{POD}} \text{Quinonelimine+HCL+4 } H_2O_2$$

Method

Colorimetric enzymatic test using glycerol-3-phosphate-oxidase (GPO).

Reagents

Components and concentrations in the test goods buffer pH 7.2, 50 mmol/I

Table. 4 Reagents

4 chloroPhenol	**4mmol/l**
ATP	**2mmol/l**
Mg^{2+}	**15mmol/l**
Glycerokinase	**>0.4 Kμ/l**
Peroxidise	**>2 Kμ/l**
Lipoprotein lipase	**>4 Kμ/l**
4-aminoantipyrine	**0.5 mmol/l**
Glycerol-3-phosphate-oxidase	**>1.5 Kμl/l**
Standard	**(2.3 mmol/l)**

Assay procedure

a. 1 ml (1000ml) of reagent-1 has taken in a 5 ml test tube.

b. Added 0.01 ml (10μl) of serum.

c. Mixed well and incubated at 37^0C for 15min.

d. The sample was tested by using photometer.

NORMAL RANGE: <200 mg/dl in serum.

Estimation of serum HDL

Principle

Chylomicrons, VLDL, and LDL are precipitated by adding phosphotungstic acid and magnesium ions to the sample. Centrifugation leaves only the HDL in the supernant. The cholesterol content in it is determined enzymatically.

Method

Phosphotungstic acid precipitation method.

Table. 5 Reagents

Phosphotungstic acid	**0.55 mmol/l**
Magnesium chloride	**25 mmol/l**

Assay procedure

Preparation of supernatant for the HDL estimation

A. Added 200 µl of serum to the 500 µl of HDL-Cholesterol precipitating reagent (from HDL kit) in 1.5 ml centrifuge tube and mixed well and centrifuged the above solution at 4000rpm for 10 min.

B. Preparation of test sample for the estimation of HDL-cholesterol
   a. Taken 1000 µl of reagent-1 (from cholesterol kit) in a 5ml test tube.

   b. Added, 100 µl of supernatant from above centrifuged solution.

   c. The sample was tested by using photometer.

Estimation of serum low-density lipoprotein cholesterol (LDL)

Using the data obtained including total cholesterol, HDL cholesterol and VLDL, the LDL cholesterol levels were calculated using the empirical equation of Friede Wald Calculation:

Serum LDL cholesterol = Total cholesterol -(HDL cholesterol+ VLDL cholesterol)

Estimation of serum very low-density lipoprotein cholesterol (VLDL)

Using the data obtained including triglycerides, the VLDL cholesterol levels were calculated using empirical equation of Friede Wald calculation:

Serum cholesterol VLDL= Triglycerides/5

HISTOPATHOLOGICAL TECHINIQUES

Immediately after collection of blood the animals were sacrificed by cervical decapitation. The liver was separated, washed with pH 7.4 buffer, blotted with dry filter paper and the organ weight was recorded.

Histopathology

A small portion of the liver tissues from all the groups was excised immediately after sacrifice and fixed in 10% neutral formalin. The washed tissues were dehydrated in descending grades of isopropanol and cleared in xylene. The tissues were then embedded in molten paraffin wax. Sections were cut at 5 μm thickness and stained with hematoxylin and eosin and then viewed under light microscope for histopathological changes.

Statistical analysis

Results are expressed as mean ± SEM (standard error of mean). Results were analysed statistically by one-way ANOVA followed by Dunnett's test using Prism (Graphpad Software Inc., La Jolla, CA Trial version). The criterion for statistical significance was set at $p < 0.05$.

Phytoconstituents detected in methanol extract of *Pergularia daemia* leaves

Table. 6

S. No.	Test	Inference
1.	**Test for Alkaloids** a. Mayers test b. Wagner's test c. Dragen draft's test	 + + +
2.	**Test for Phytosterols and Triterpenoids** a. Leibermann's test c. Salkowaski test	 + +
3.	**Test for Flavonoids** a. Alkaline reagent test b. Lead acetate test	 + +
4.	**Test for saponins** Foam test	 +
5.	**Test for Proteins and Aminoacids** a. Millon's test b. Ninhydrin test c. Biuret test	 - - -
6.	**Test for fixed oils and fats** Oily spot test	 +
7.	**Test for Phenolics and Tannins** Ferric chloride test	 +
8.	**Test for carbohydrates** a. Molisch's test b. Fehling's test c. Benedict's test	 + + +
9.	**Test for Gylcosides** a. Modified Borntrager's test b. Legal's test c. Keller-Killiani test	 + + +

(+) Present, (-) Absent.

Acute oral Toxicity study of methonalic extract of *Pergularia daemia* leaves. (Guideline425)

Observations

Table. 7

RESPIRATORY BLOCKAGE IN NOSTRIL	
Dyspnoea	Nil
Apnoea	Nil
Tachypnea	Nil
Nostril discharge	Nil
MOTOR ACTIVITIES	
Locomotion	Normal
Somnolence	Nil
Loss of righting reflex	Nil
Anaesthesia	Nil
Catalepsy	Nil
Ataxia	Nil
Toe walking	Nil
Prostration	Nil
Fasciculation	Nil
Tremor	Nil
CONVULSION (INVOLUNTRAY CONTRACTION)	
Clonic/tonic/tonic-clonic convulsion	Nil
Asphyxial convulsion	Nil
Opistotones (titanic spasm)	Nil
REFLEXES	
Corneal	Normal
Eyelid closure	Normal
Righting	Normal
Light	Normal
Auditory and sensory	Normal

OCULAR SIGNS	
Lacrimation	Nil
Miosis	Nil
Mydriasis	Nil
Ptosis	Nil
Chromodacryorrhea	Nil
Iritis	Nil
Conjunctivitis	Nil
SALIVATION	
Saliva secretion	Nil
PILOERECTION	
Contraction of erectile tissue	Nil
ANALGESIA	
Decrease in reaction to induced pain	Nil
MUSCLE TONE	
Hypo or hypertonia	Nil
GIT SIGN	
Solid dried / watery stool	Nil
Emesis	Nil
Red urine	Nil
SKIN	
Oedema	Nil
Erythema	Nil

Table 8: **Effect of *Pergularia daemia* methonalic extract on body weight in high fat diet induced hypercholesterolemic rats**

Groups	Treatment	Initial weight gm(s)	Final weight gm(s)	Weight gain	% increase in Body weight
Group I	Normal diet	121.7±11.88	130.9 ±1.33	9.23±0.649	7.58 %
Group II	Extract 200mg/kg + Normal diet	135.7±12.16^{nsa}	140.8 ±5.509^{nsa}	5.16 ±1.218nsa	3.82 %
Group III	Extract 400mg/kg + Normal diet	130.0 ±6.952^{nsa}	138.8 ±7.463^{nsa}	8.862±1.254**^{a}	6.81 %
Group IV	High fat diet(HFD)	165.0 ±5.77^{nsb}	195.8 ±5.449^{nsb}	30.81 ±1.217***^{b}	18.67 %
Group V	Extract 200mg/kg +HFD	158.3 ±6.67^{nsc}	167.5 ±8.827^{nsc}	9.216±0.654***^{c}	5.81 %
Group VI	Extract 400mg/kg +HFD	144.2 ±8.7^{nsc}	148.5 ±5.881^{nsc}	4.350±0.427***^{c}	3.01 %

All the values are expressed as mean ± SEM, n = 6 in each group.

[a]Values are significantly different from group I. Non significant (ns); *p < 0.05; **p < 0.01; ***p < 0.001

[b]Values are significantly different from group I. Non significant (ns); *p < 0.05; **p < 0.01; ***p < 0.001

[C]Values are significantly different from group IV. Non significant (ns); *p < 0.05; **p < 0.01; ***p < 0.001

Figure No 5.

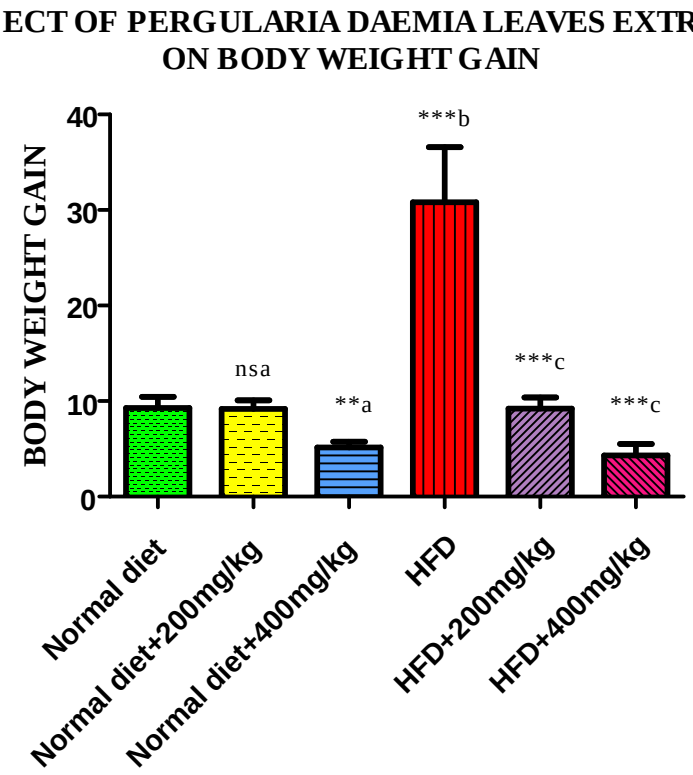

EFFECT OF PERGULARIA DAEMIA LEAVES EXTRACT ON BODY WEIGHT GAIN
BODY WEIGHT GAIN
40
30
20
10
0
nsa
**a
***b
***c
***c
Normal diet
Normal diet+200mg/kg
Normal diet+400mg/kg
HFD
HFD+200mg/kg
HFD+400mg/kg

Table 9: Effect of methonalic extract of *Pergularia daemia* leaves on serum lipid profiles in rats fed with high fat diet

Group	Treatment	Total cholesterol mg / dl	HDL mg / dl	LDL mg / dl	Triglyceride mg / dl	VLDL mg / dl
Group I	Normal diet	175.0±8.851	27.83 ±8.851	73.17 ±5.294	110.0 ±10.25	19.33±1.476
Group II	High fat diet (HFD)	212.5 ±8.839**[b]	13.33±0.988***[b]	100.8 ±5.23**[b]	167.5 ±14.82***[b]	23.33±2.246[nsb]
Group III	Normal diet + 200mg/kg	150.0 ± 7.303[nsa]	16.17±1.424**[a]	70.50 ±3.97[nsa]	108.7 ±3.180[nsa]	18.0±0.9309[nsa]
Group IV	Normal diet + 400 mg/kg Extract	139.2 ±6.509**[a]	22.50±2.895[nsa]	65.83 ±4.16[nsa]	95.83 ± 4.16[nsa]	16.33±1.382[nsa]
Group V	Extract 200mg/kg + HFD	150.8 ±7.12***[c]	20.17±2.786[nsc]	74.0 ±6.583**[c]	102.5 ±4.425***[c]	17.5±1.607*[c]
Group VI	Extract 400 mg/kg + HFD	117.8 ±5.002***[c]	27.17 ±1.641***[c]	67.83±4.324***[c]	97.50 ± 3.81***[c]	15.17±0.833**[c]

All the values are expressed as mean ± SEM, n = 6 in each group.

[a]Values are significantly different from group I. ns; *p < 0.05; **p < 0.01; ***p < 0.001

[b]Values are significantly different from group I. ns; *p < 0.05; **p < 0.01; ***p < 0.001

[c]Values are significantly different from group IV. ns; *p < 0.05; **p < 0.01; ***p < 0.001

Figure No. 6

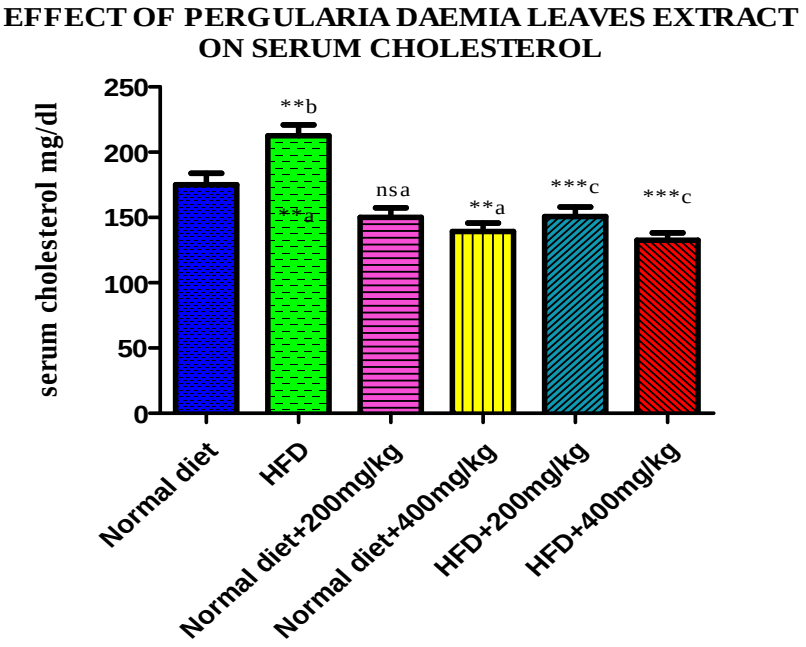


Figure No. 7

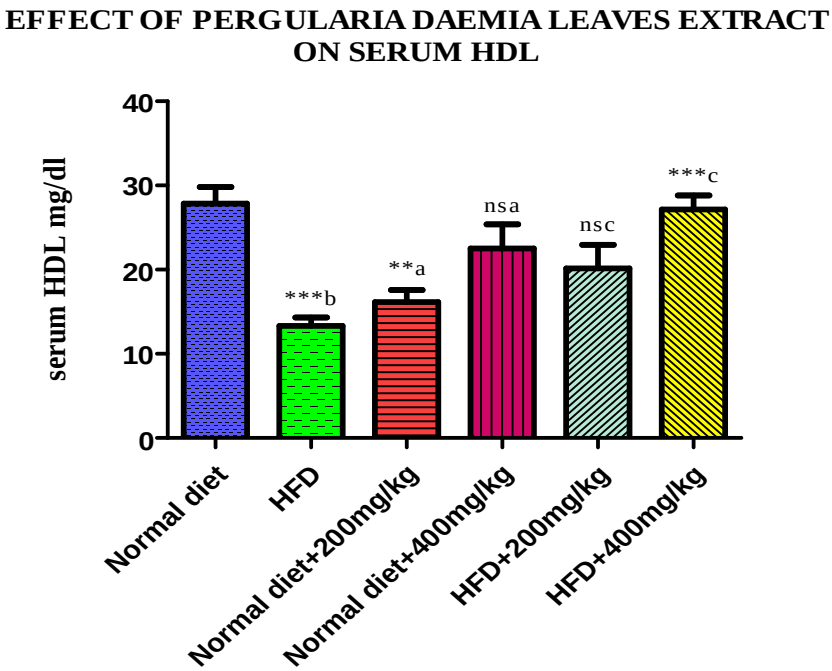


Figure No. 8

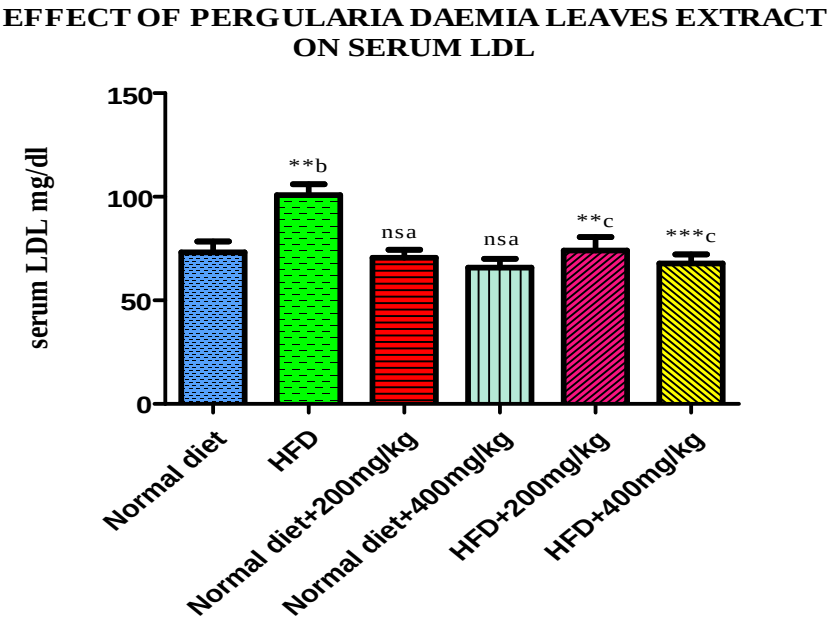


Figure No. 9

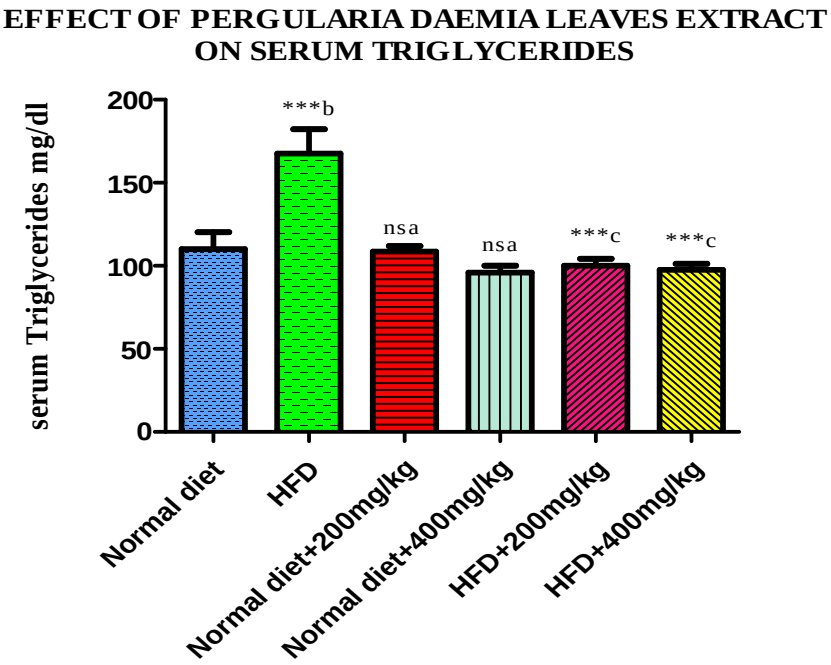


Figure No. 10

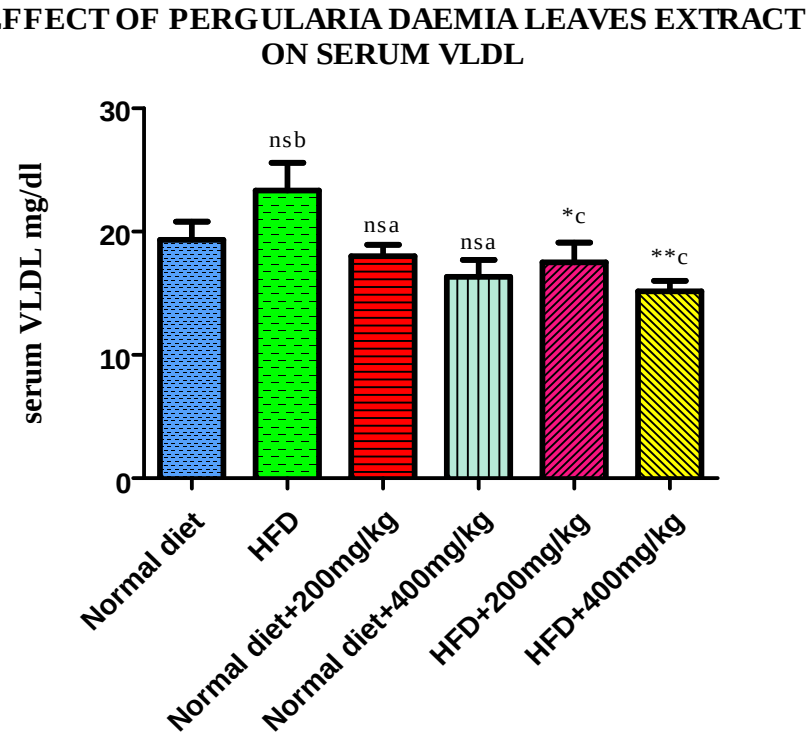

EFFECT OF PERGULARIA DAEMIA LEAVES EXTRACT ON SERUM VLDL
serum VLDL mg/dl
30
20
10
0
nsb
nsa
nsa
*c
**c
Normal diet
HFD
Normal diet+200mg/kg
Normal diet+400mg/kg
HFD+200mg/kg
HFD+400mg/kg

Table 10: Atherogenic index of *Pergularia daemia* methonalic extract

Group	Treatment	Atherogenic index	Percentage (%) Protection
Group I	Normal diet	3.95 ±0.328	--
Group II	High fat diet (HFD)	12.56 ± 0.921***[b]	--
Group III	Extract (200 mg/kg) +Normal diet	6.722 ± 0.527[nsa]	33.56%
Group IV	Extract (400 mg/kg)+ Normal diet	4.25 ± 0.358[nsa]	55.23%
Group V	Extract (200 mg/kg)+HFD	5.08 ± 0.625***[c]	52.70%
Group VI	Extract (400 mg/kg)+HFD	3.58 ± 0.268***[c]	70.62%

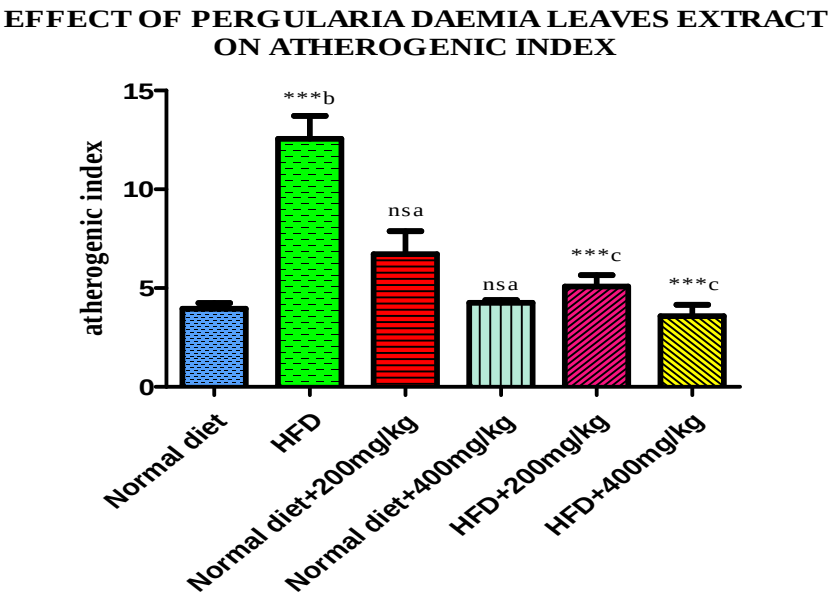
All the values are expressed as mean ± SEM, n = 6 in each group.

[a]Values are significantly different from group I. ns;*p<0.05;**p < 0.01;***p<0.001

[b]Values are significantly different from group I. ns;*p <0.05;**p<0.01;***p< 0.001

[c]Values are significantly different from group IV. ns;*p <0.05;**p<0.01;***p< 0.001

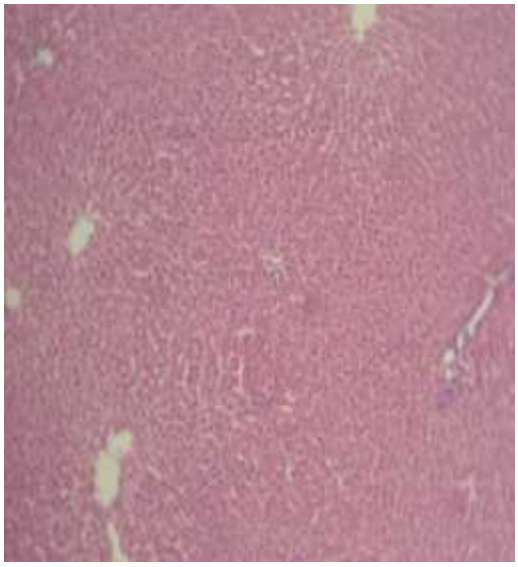
Figure No. 11

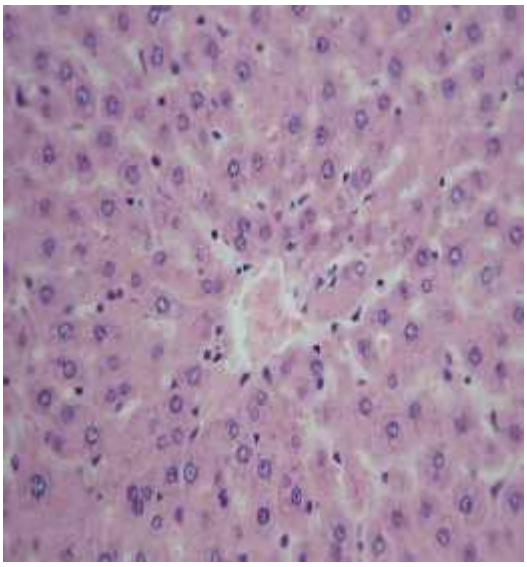

EFFECT OF PERGULARIA DAEMIA LEAVES EXTRACT ON ATHEROGENIC INDEX
atherogenic index
15
10
5
0
***b
nsa
nsa
***c
***c
Normal diet
HFD
Normal diet+200mg/kg
Normal diet+400mg/kg
HFD+200mg/kg
HFD+400mg/kg

Histopathological examination Of Liver

Figure No. 12

(a) Section of liver of control group rat on normal diet. (H&E 10x)
Normal liver with intact lobular architecture, normal central veins and sinusoids





(b) Normal portal tracts without inflammation

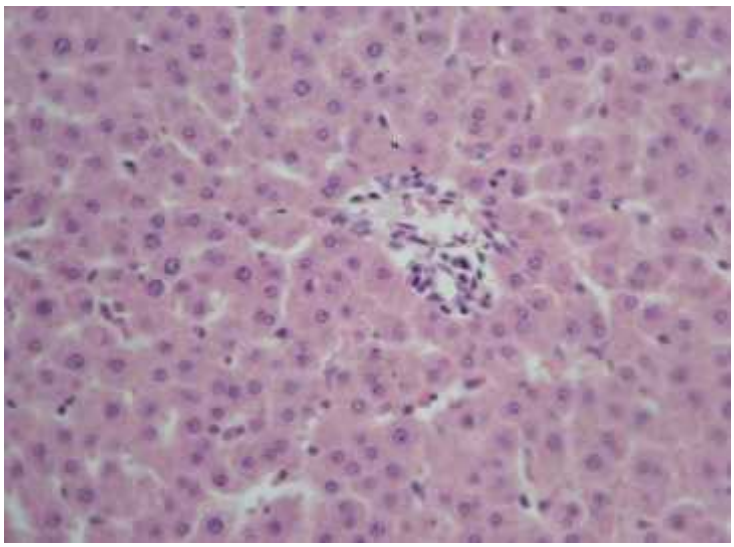
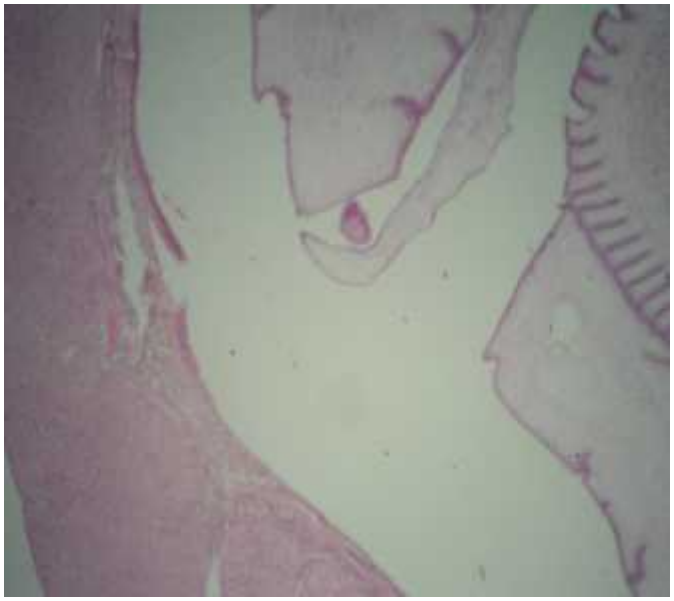


Figure No. 13

(a) Section of liver of rat fed with high fat diet showing large dilated space with fibrous wall and amorphous material. (H&E 10x)



(b) Section of liver of rat fed with high fat diet showing normal portal tracts and lobular inflammation.(40X)

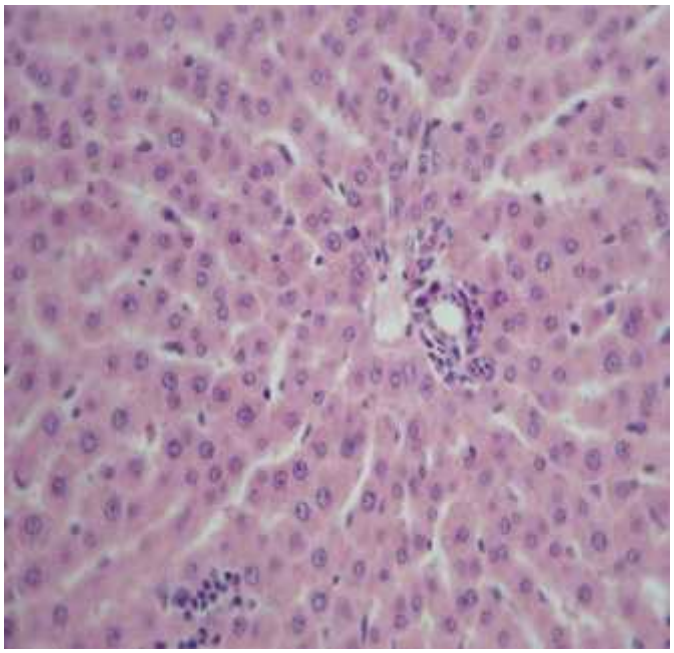
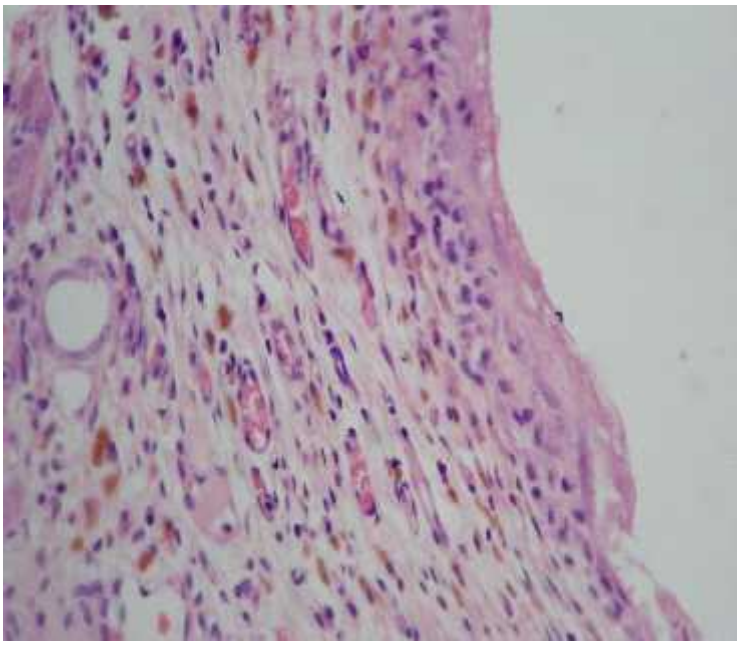


Figure No. 14

(a) Section of liver of rat given methonal extract of *Pergularia daemia* 200mg/kg along with high fat diet showing fibrous wall with inflammation and macrophages (H&E 10x)



(b) Section of liver of rat given methonal extract of *Pergularia daemia* 200mg/kg along with high fat diet showing dilated central vein (H&E 10x)

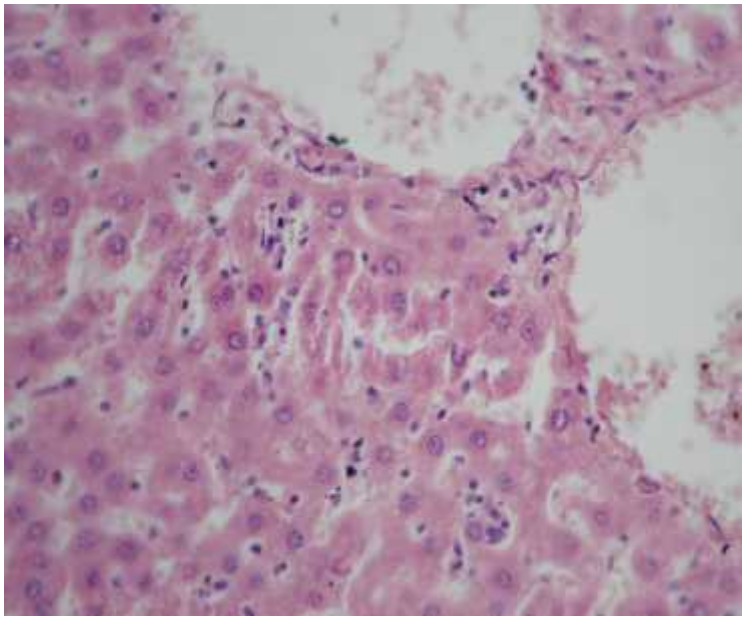
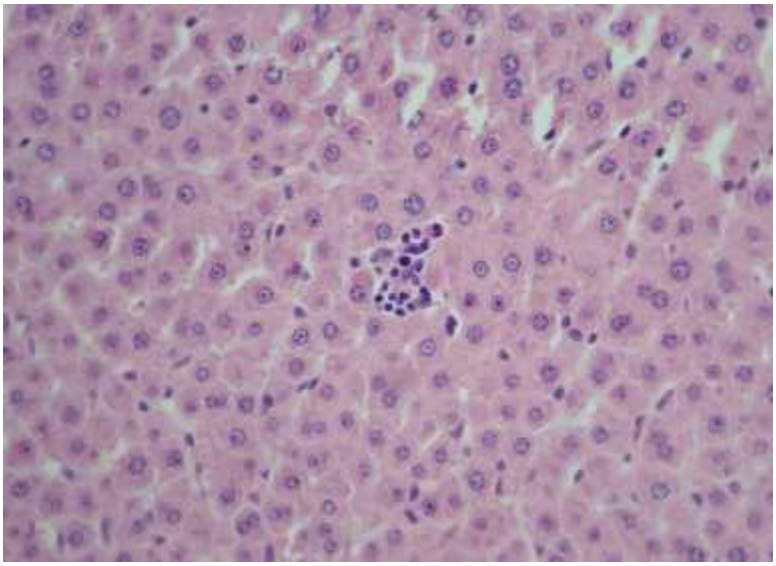


Figure No. 15

(a) Section of liver of rat given methonal extract of *Pergularia daemia* 400mg/kg along with high fat diet showing lobular inflammation (H&E 40x)



(b) Section of liver of rat given methonal extract of *Pergularia daemia* 400mg/kg along with high fat diet showing portal tract with mild inflammation (H&E 40x)

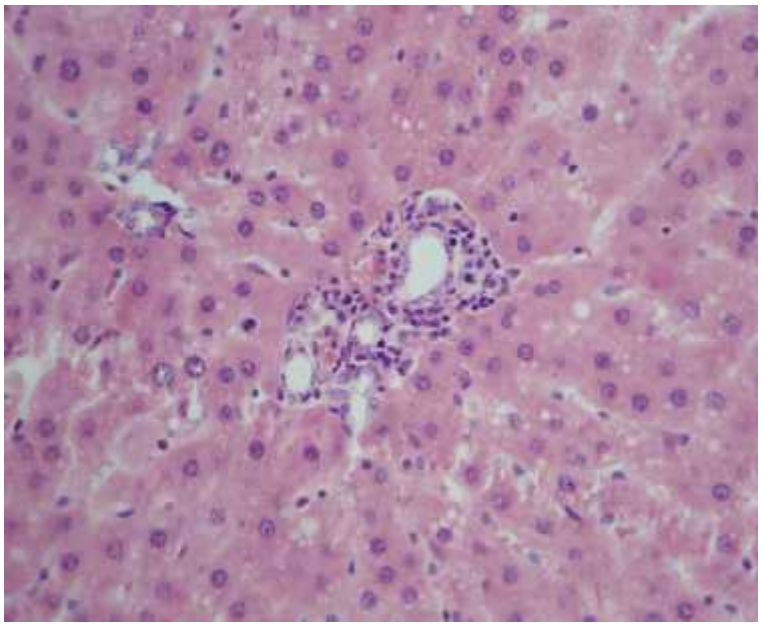


Figure No. 16

(a) Section of liver of rat given methonal extract of *Pergularia daemia* 200mg/kg along with normal diet showing normal sinusoids and central vein (H&E 40x)

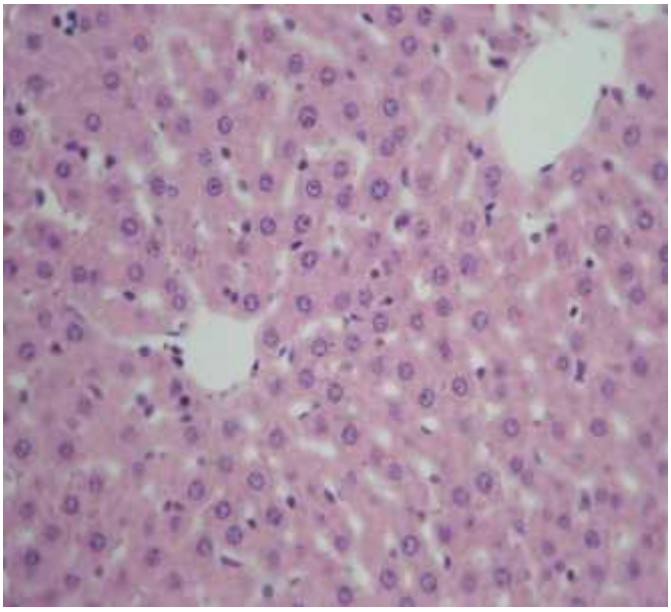
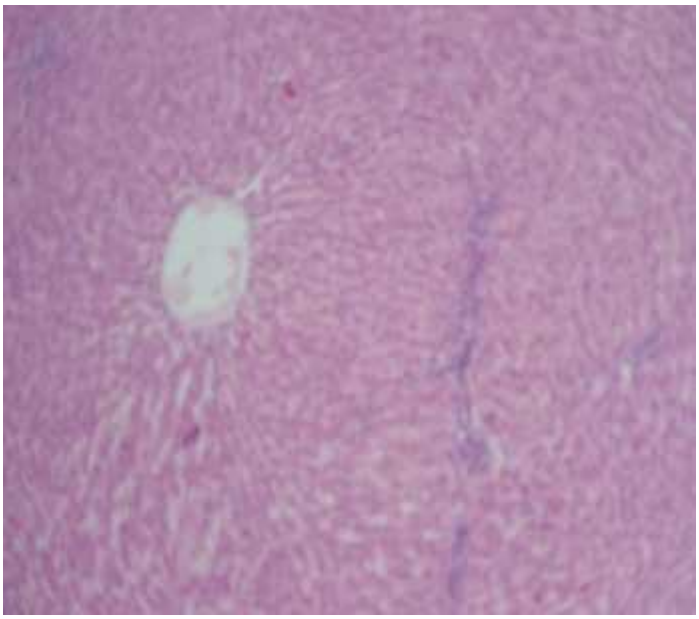


Figure No. 17

(a) Section of liver of rat given methonal extract of *Pergularia daemia* 400mg/kg along with normal diet showing dilated central vein (H&E 40x)



Preliminary phytochemical study of *Pergularia daemia* extract showed the presence of phytosterols, triterpenoids, flavonoids, carbohydrates, glycosides, phenolic compounds, tannins, and fixed oils, fats. The results are shown in **Table 6**.

The effect of *Pergularia daemia* extract on body weight of rats with high fat diet is shown in **Table 8**. A significant increase (P<0.001) of weight gain (30.812 ±1.217) was found in rats fed with cholesterol diet compared to rats fed with normal diet (9.23±0.649). Administration of *Pergularia daemia* extract once daily orally for 15 days for rats fed with high fat diet significantly prevented the body weight gain. A dose level of 400mg/kg *Pergularia daemia* extract was found to be highly significant (P<0.001) when compared to cholesterol fed animals. The animals treated with 400mg/kg *Pergularia daemia* extract showed increase in average weight gain of 4.350 gms from the initial weight.

The effect of *Pergularia daemia* extract on lipid profile in control and rats fed with high fat diet is shown in **Table 9**. Total cholesterol levels were significantly increased (P<0.001), (212.5 ±8.839) in high fat diet fed rats compared to animals fed with normal diet (175.0±8.851). Administration of *Pergularia daemia* extract 200 mg/kg once daily orally for 15 days show significant change (150.8 ±7.120) in cholesterol level compared to rats fed with high fat diet. The results were non-significant (ns). The animals treated with 200 mg/kg and 400 mg/kg *Pergularia daemia* extract once daily for 15 days are also showed a significant decrease (P<0.001), (150.8 ±7.120 and 117.8 ±5.002) in cholesterol level compared to rats fed high fat diet.

The animals on high fat diet showed a significant fall (P<0.001) (13.33±0.988) in HDL level compared to animals fed with normal diet (27.83 ±8.851). Administration of *Pergularia daemia* extract once daily orally for 15 days showed increase in HDL level in high fat diet fed animals. Both the dose levels (200 mg/kg and 400 mg/kg), (20.17±2.7860 and 27.17 ±1.641) were showed dose dependent effect.

The animals fed with high fat diet showed a significant increase (P<0.01) in LDL level (100.8 ±5.23) compared to animals on normal diet (73.17 ±5.294). Administration

of *Pergularia daemia* extract once daily for 15 days showed a significant decrease (P<0.001) in LDL level (74.0 ±6.583 and 67.83±4.324) compared to animals fed with high fat diet. Both the dose levels (200 mg/kg and 400 mg/kg) were found to be effective in lowering the LDL level. The results showed the *Pergularia daemia* extract is highly effective in reducing the LDL cholesterol level.

The triglyceride levels in animals fed with fat diet were significantly increase (P<0.001) in triglyceride levels (167.5 ±14.82) compared to animals fed with normal diet (110.0 ±10.25). Administration of *Pergularia daemia* extract showed a significant decrease (P<0.001) in triglyceride level compared to animals fed with high fat diet. A dose dependent decrease in triglyceride level was noted in *Pergularia daemia* extract treated animals. Both the dose levels (200 mg/kg and 400 mg/kg), (102.5 ±4.425 and 97.50 ± 3.81) were found to be effective in reducing the triglyceride levels.

A significant increase in VLDL (23.33±2.246) level was noted in rats fed with high fat diet compared to rats fed with normal diet (19.33±1.476). Administration of *Pergularia daemia* extract 400 mg/kg daily for 15 days showed significant (P<0.01), (15.17±0.8330) decrease in VLDL compared to animals fed with high fat, but the results of VLDL in animals treated with 200 mg/kg *Pergularia daemia* extract was less significant(P<0.05). The results showed the *Pergularia daemia* extract at a dose level of 200 mg/kg and 400mg/kg was effective in lowering VLDL level.

A significant increase in HDL-cholesterol which was related to a significant reduction of atherogenic index was observed **(Table 10).** The atherogenic index was significantly reduced (P<0.001) in *Pergularia daemia* extract treated group to high fat diet fed group. The percentage protection against the hyperlipidemia was found to be 52.70 % with 200 mg/kg *Pergularia daemia* extract and 70.62 % with 400 mg/kg *Pergularia daemia* extract.

Histopathological examination

Liver

Histopathological examination of liver of control group rat on normal diet showed normal structure and architecture. There is no evidence of changes in degeneration, no lobular inflammation, granuloma or fibrosis, Fatty change or congestion in liver section. The hepatocytes are unremarkable. The central vein and the sinusoids are normal.

Section of liver of rat fed with high fat diet showed largely dilated duct with fibrotic wall that show inflammation. There is amorphous material in the lumen of dilated duct seen. The central veins and sinusoids mildly dilated. There is no lobular inflammation, granuloma or fibrosis. The central vein and sinusoids are dilated. Inflammatory with fatty changes were noted.

Section of liver of rat given *Pergularia daemia* extract 200 mg/kg along with high fat diet showed largely dilated duct with fibrotic wall that show inflammation. There is amorphous material in the lumen of dilated duct seen. The central veins and sinusoids are mildly dilated. Inflammatory and fatty changes were observed. *Pergularia daemia* extract 200 mg/kg showed only a mild restoration of disrobed structure.

Section of liver of rat received 400 mg/ kg *Pergularia daemia* extract along with high fat diet showed a marked decrease in inflammatory and fatty changes. The central vein and sinusoids are mildly dilated. The portal tracts show mild inflammation. The liver section showed normal lobular architecture and architecture compared to the section of liver of rats on normal diet. The section showed inflammatory and fatty changes. The section showed loss of distinct liver characteristic configuration.

Section of liver treated with *Pergularia daemia* extract 200 mg/kg along with normal diet showed normal lobular architecture. There is no lobular inflammation, granuloma or fibrosis. The portal tracts, hepatic parenchyma, the central veins and sinusoids are mildly dilated.

Section of liver treated with *Pergularia daemia* extract 400 mg/kg along with normal diet showed normal lobular architecture. There is no lobular inflammation, granuloma or fibrosis. The portal tracts are normal. The hepatic parenchyma is unremarkable. The central veins are mildly dilated.

From the results, it was observed that *Pergularia daemia* extract 400 mg/kg was highly effective in protection the liver function in hyperlipidemic animals.

DISCUSSION

Hyperlipidemia is a well-known risk factor for cardiovascular diseases, especially to cause atherosclerotic coronary artery disease (CAD). Plaque formation, thrombosis, and vessel occlusion can follow, leading to CAD. CAD involves one or more specific cardiovascular pathologies, including myocardial infarction, ischemia, and angina. Between 13 and 14 million people in the United States are believed to suffer from this complex and life-threatening condition, and over 25 million people worldwide are expected to die from cardiovascular-related pathologies by the year 2020.It has been established that nutrition plays an important role in the etiology of hyperlipidemia and atherosclerosis.

Cholesterol feeding has often used to evaluate serum or tissue cholesterol levels to assess hypercholesterolemia related metabolic disturbances in different animal models. Rats were fed with diet supplemented with cholesterol, 1% w/w sodium cholate and 2.5% w/w coconut oil served as a experimental model to screen anti hyperlipidemic activity. According to these previous findings reports (**Rumi ghosh, *et al*., 2010**) showed that feeding rats with high cholesterol diet for 7 days was induced hyperlipidemia. Similar results have been reported feeding rats with a high cholesterol diet for 7 consecutive days resulted in marked hyperlipidemia. The mechanism of sodium cholate is twofold an increase of cholesterol absorption and a concomitant suppression of cholesterol 7 α-hydroxylase activity that results in decreased cholesterol excretion. Sodium cholate (Na^+ salt or cholic acid) improves cholesterol absorption by its emulsifying property. Supplementation of cholesterol in diet results in a marked increase in the production of cholestryl ester rich-VLDL by the liver and intestine and a number as well as rate of cholesterol by the hepatic LDL receptors. Consequently serum levels of LDL-cholesterol and VLDL-cholesterol is increased. Increase in LDL cholesterol has been pointed out as one of the risk factors for the development of atherosclerosis and related cardiovascular diseases.

From the obtained results it was observed that the animal on high fat diet significantly increased the total cholesterol, triglyceride, LDL-cholesterol and VLDL in serum compared to rats on normal diet. The HDL level was significantly reduced. When high fat diet was co-administrated with *Pergularia daemia* extract, the elevated levels of total cholesterol, triglyceride and LDL-C has shown considerable decline suggesting beneficial modulator influence on cholesterol metabolism and turnover. It was noted that total cholesterol,

triglyceride, LDL-cholesterol and VLDL lowering activity of methanol extract of *Pergularia daemia* 400 mg/kg was more significant compared to 200 mg/kg *Pergularia daemia* extract. The notable changes in the HDL levels in serum were not statistically influenced by *Pergularia daemia* extract in rats fed with high fat diet. Elevated serum triglyceride is considered as independent risk factor for cardiovascular disease.

A significant decline in serum triglyceride level observed in *Pergularia daemia* extract treated rats supports the cardiovascular protective influence. Increase in LDL cholesterol has been pointed out as one of the risk factors for the development of atherosclerosis and related cardiovascular diseases. *Pergularia daemia* extract lowered the LDL cholesterol, which can afford a beneficial role in reducing cardiovascular complications. One of the possible mechanisms of lowering body weight by *Pergularia daemia* extract is by decreased adipocytic lipogenesis excreted by phenolic glycosides. *Pergularia daemia* contains plenty of different flavonoid glycosides e.g. quercetin-3-glycoside, quercetin-3-galactoside, kaempferol-3-galactoside, kaempferol-3-glycoside, kaempferol-3-malonylhexoside, isorhamnetin-3-glycoside and isorhamnetin-3- malonylhexoside, phenolic acid derivatives and other compounds. These evidences strongly support that the *Pergularia daemia* extract shows hypolipidaemic activity through multiple mechanisms.

CONCLUSION

The deleterious effects of high blood cholesterol and beneficial effects of lowering blood cholesterol in reducing morbidity and mortality from cardiovascular diseases are well established. Non pharmacological measures like dietary restriction and exercise may help in lowering blood cholesterol levels. When such therapy fails, and in patients with abnormally high blood cholesterol levels, drug therapy is indicated. The available drugs like statins, fibrates and nicotinic acid though very effective, have a spectrum of adverse effects and are costly.

A growing attention has been recently focused on the improvement of human health by consumption of herbal plant like *Pergularia daemia*, which has excellent historical health benefits due to presence of plenty of phytoconstituents. A myriad of nutritional benefits has been attributed to these phytochemicals. Since phytoconstituents are concentrated largely in leaves and considering it as a health benefits in treating cardiovascular diseases, a study was carried out to evaluate the effect of *Pergularia daemia* extract on rats fed with high fat diet.

The results of the study demonstrated that oral administration of *Pergularia daemia* extract evokes a beneficial effect on the hyperlipidemia. This finding supports its use for the treatment and management of cardiovascular diseases. This implies that *Pergularia daemia* leaves extract consumption can prevent or be helpful in reducing the complication of dyslipidemia associated disorders.

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