STUDY ON *IN VITRO* CHOLESTEROL ESTERASE INHIBITORY ACTIVITY AND *IN VIVO* ANTIHYPERLIPIDEMIC ACTIVITY OF *ANISOMELES MALABARICA* LEAF EXTRACT

A Dissertation submitted to
THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY
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In partial fulfillment of the requirements for the award of Degree of
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IN
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Submitted by
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CERTIFICATE

This is to certify that the M. Pharm, dissertation entitled **"Study on *in vitro* Cholesterol Esterase Inhibitory Activity and *in vivo* Antihyperlipidemic Activity of *Anisomeles Malabarica* Leaf Extract"** being submitted to The Tamil Nadu Dr. M.G.R Medical University, Chennai, in partial fulfillment of **Master of Pharmacy** programme in Pharmacology, carried out by **Mrs.Anandhi.B, (Register No.261525101**) in the Department of Pharmacology, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, under my direct supervision and guidance to my full satisfaction.

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CONTENTS

LIST OF ABBREVIATIONS

ALP	:	Alkaline Phosphatase
ALT	:	Alanine Aminotransferase
AMLE	:	Anisomeles Malabarica Leaf Extract
ANOVA	:	One-Way Analysis of Variance
Apo	:	Apolipoproteins
AST	:	Aspartate Aminotransferase
BMI	:	Body Mass Index
BSTFA	:	N, O-Bis(Trimethylsilyl) Trifluoroacetamide
CAT	:	Catalase
CEase	:	Cholesterol Esterase
CEL	:	Carboxyl Ester Lipase
CHD	:	Coronary-Heart Disease
CPK	:	Creatine Phosphokinase
CRI	:	Coronary Risk Index
CVD	:	Cardiovascular Diseases
FDA	:	Food and Drug Administration
GC-MS	:	Gas Chromatography Mass Spectroscopy
GIT	:	Gastrointestinal Tract

Gpx	:	Glutathione Peroxidase
GSH	:	Reduced Glutathione
GSSH	:	Glutathione Reductase
HCD	:	Hypercholesterolemic Diet
HDL	:	High Density Lipoproteins
HFD	:	High Fat Diet
HFD	:	High Fat Diet
HIV	:	Human Immunodeficiency Virus
HMG-CoA	:	3-Hydroxy-3-Methyl Glutaryl Coenzyme A
IDL	:	Intermediate Density Lipoproteins
LDH	:	Lactate Dehydrogenase
LDL	:	Low Density Lipoproteins
LP	:	Lipoprotein Lipases
MDA	:	Malondialdehyde
MS	:	Mass Spectrometry
NAD	:	Nicotinamide-Adenine Dinucleotide
NBT	:	Nitro Blue Tetrazolium Chloride
NICE	:	National Institute for Health and Care Excellence
NICE	:	National Institute for Health and Care Excellence
NPD	:	Normal Pellet Diet

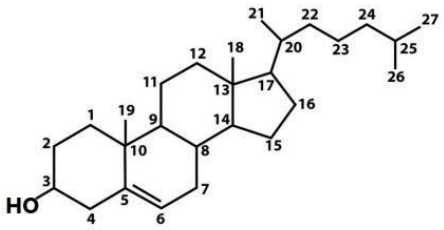
OECD	:	Organisation for Economic Co-Operation and Development
PMS	:	Phenazonium Methosulphate
Pnpb	:	P-Nitro Phenyl Butyrate
PPAR	:	Peroxisome Proliferator-Activated Receptor
PVD	:	Peripheral Vascular Disease
SOD	:	Superoxide Dismutase
TAG	:	Triacylglycerol
TBA	:	Thiobarbituric Acid
TC	:	Total Cholesterol
TCA	:	Trichloro Acetic Acid
TG	:	Triacylglycerol
TIA	:	Transient Ischaemic Attack
TIC	:	Total Ion Count
TLC	:	Thin Layer Chromatography
TMCS	:	Trimethyl Chlorosilane
TP	:	Total Protein
VLDL	:	Very Low Density Lipoprotein
WHO	:	World Health Organisation

INTRODUCTION

CHOLESTEROL

Cholesterol is a waxy, fat-like substance that's found in all cells of the body. All of them have a similar cyclic nucleus resembles the phenanthrene rings (rings A, B, C) to which a cyclopentanone ring is attached. The parent nucleus is better designated as cyclopentano perhydrophenanthrene. They are divided as sterols, bile acids, sex hormones, etc., Our body needs some cholesterol to make hormones, vitamin D, and substances that help us to digest foods. Quantity of cholesterol will be 140 gms in the body of a man weighing 70 kg. Greater part of the cholesterol in the body is synthesized whereas 0.3 gram per day is provided by the average diet. Normal concentration of cholesterol in the blood is 140-220 mg per 100 ml of blood. Cholesterol travels through the bloodstream in small packages called lipoproteins.Two kinds of lipoproteins carry cholesterol throughout our body: low-density lipoproteins (LDL) and high-density lipoproteins (HDL). Having healthy levels of both types of lipoproteins is important. LDL cholesterol is called "bad" cholesterol. A high LDL level leads to a build up of cholesterol in the arteries. HDL cholesterol is called "good" cholesterol. This is because it carries cholesterol from other parts of the body back to the liver, which removes the cholesterol from our body.[1]

Chemistry:



Cholesterol

- It is a white, waxy, solid associated with fats and chemically different from them.
- It has apparent nucleus which is said to be cyclopentanoperhydro phenanthrene nucleus.
- It has a hydroxyl group at C_3, an unsaturated bond at C_5-C_6, two methyl groups at C_{10} and C_{13} and 8 carbon paraffin side chains attached to C_{17}.
- It is an alcohol.
- It occurs free and combined with fatty acids by ester linkage at the hydroxyl group.
- Cholesterol in ester form often reffered as 'bound' cholesterol esters (CE).These are normally rich in linoleic acid.[2]

FUNCTIONS OF CHOLESTEROL

Cholesterol serves a variety of functions in the human body. This includes: manufacture of steroids, or cortisone-like hormones, including vitamin D and the sex hormones testosterone, estrogen and cortisone. This in turn controls a myriad of bodily functions.[2]

- Assisting the liver in the manufacture of bile acids, which is essential for digestion and absorption of fat-soluble vitamins such as vitamin A, D, E and K.
- Formation of the myelin sheath, a neuron consisting of fat-containing cells that insulate the axon from electrical activity. This ensures proper function of our brain cells by aiding route of electrical impulses. The absence of cholesterol might lead to loss of memory and difficulty in focusing.
- As a cell to interconnect "lipid molecules", which are needed to stabilize our cell membranes.
- As a source of energy.
- Maintenance of our body temperature

- Protection of internal organs
- Modulation the fluidity of cell membranes

Factors Affecting Blood Cholesterol Levels

Many factors determine whether your blood cholesterol level is high or low. The following are the most important: (2)

Heredity

Your genes partly determine the amount of cholesterol your body makes, and high blood cholesterol can run in families.

Diet

There are two nutrients in the foods you eat that can increase your blood cholesterol level: saturated fat and cholesterol. Saturated fat is a type of fat found mostly in foods that come from animals. Cholesterol comes only from animal products. Saturated fat raises your cholesterol level more than anything else in the diet. Reducing the amounts of saturated fat and cholesterol you eat is an important step in reducing your blood cholesterol levels.

Weight

Excess weight tends to increase blood cholesterol levels. If you are overweight and have high blood cholesterol, losing weight may help you lower it.

Exercise

Regular physical activity may help to lower LDL-cholesterol and raise desirable HDL-cholesterol levels.

Age and Gender

Before menopause, women have total cholesterol levels that are lower than those of men the same age. Pregnancy raises blood cholesterol levels in many women, but blood cholesterol levels should return to normal about 20 weeks after

delivery. As women and men get older, their blood cholesterol levels rise. In women, menopause often causes an increase in their LDL-cholesterol levels. Some women may benefit from taking estrogen after menopause, because estrogen lowers LDLs and raises HDLs.

Alcohol

Intake increases HDL-cholesterol. It is not known whether it also reduces the risk of heart disease. Drinking too much alcohol can certainly damage and liver and heart muscle and cause other health problems. Because of these risks, you should not drink alcoholic beverages to prevent heart disease.

Stress

Over the long term, stress has not been shown to raise blood cholesterol levels. The real problem with stress may be how it affects your habits. For example, when some people are under stress they console themselves by eating fatty foods. The saturated fat and cholesterol in these foods probably cause higher blood cholesterol, not the stress itself.

Synthesis of cholesterol:

Liver is the principle organ for cholesterol synthesis. Other tissues like skin, ovary, intestine and testis are also capable for cholesterol synthesis. The microsomal and cytosol fraction of the cell are involved in the cholesterol synthesis. One interesting point is that brain of new born child can synthesize cholesterol but adult brain cannot.(2)

Body cholesterol is primarily of endogenous origin and its homeostasis involves the movement of cholesterol between peripheral tissues and liver.

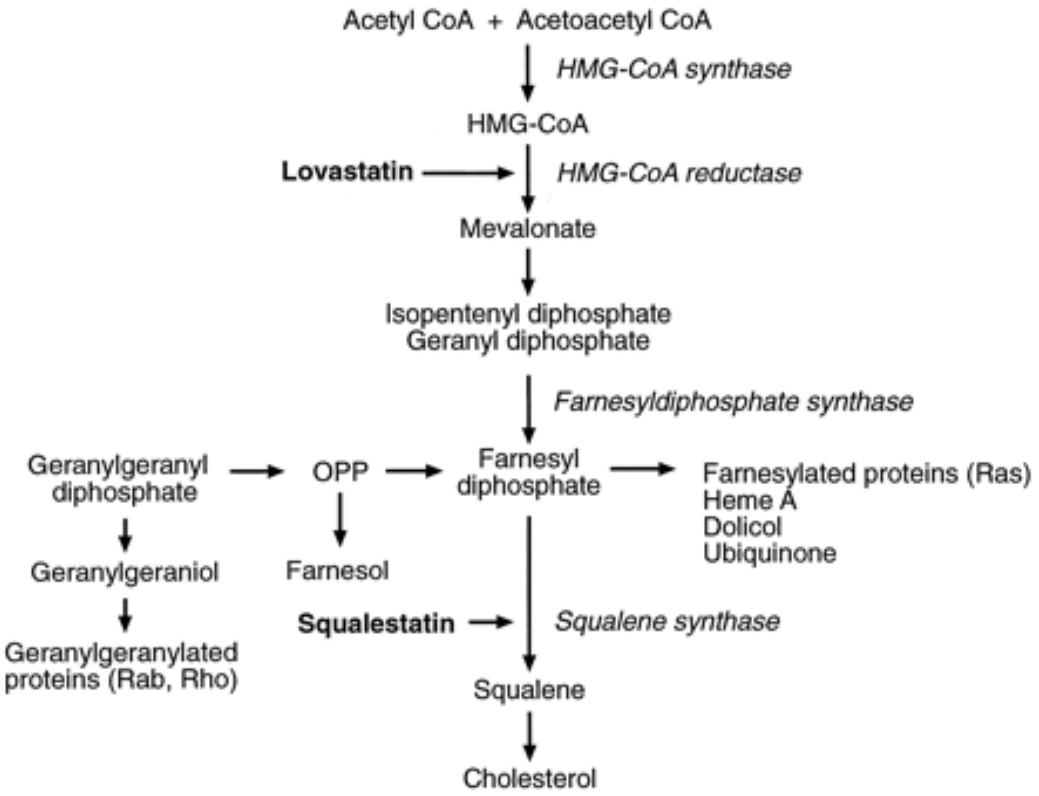
The liver regulates

- *de novo* synthesis of cholesterol
- Excretion of cholesterol into bile directly or in the form of bile salts.
- Cholesterol discharge into blood as very low density lipoproteins (VLDL).
- Modulation of receptor-mediated cholesterol uptake.
- Formation of cholesterol esters and the storage of cholesterol.

Intestine controls cholesterol absorption and excretion through faeces.

Biosynthesis of cholesterol can occur in five stages

- Six carbon compound, mevalonate, is synthesized from acetyl-CoA
- Formation of isoprenoid units (five membred ring structures) occurs from mevalonate by the loss of CO_2.
- Six of these units combine to form a thirty carbon compound named squalene ($C_{30}H_{50}$)
- Cyclization of squalene occurs and forms a parent steroid called lanosterol.
- After many steps cholesterol ($C_{27}H_{46}O$) is formed from lanosterol with the loss of three methyl groups.



Pathway for cholesterol synthesis

Transportation of cholesterol:

Lipids are transported in the circulation packaged in lipoproteins. The clinical relevance of blood lipid levels is that abnormal levels of lipids in certain lipoproteins are linked to an increase risk of atherosclerosis. Atherosclerosis is a cardiovascular disease in which lipids and inflammatory cells accumulate in plaques within the walls of blood vessels. As a result, vessel walls are narrowed and clots may form, impeding blood flow and oxygen delivery and causing tissue injury. Heart disease occurs because the coronary arteries supplying the heart are a major site where atherosclerotic plaques form.[3]

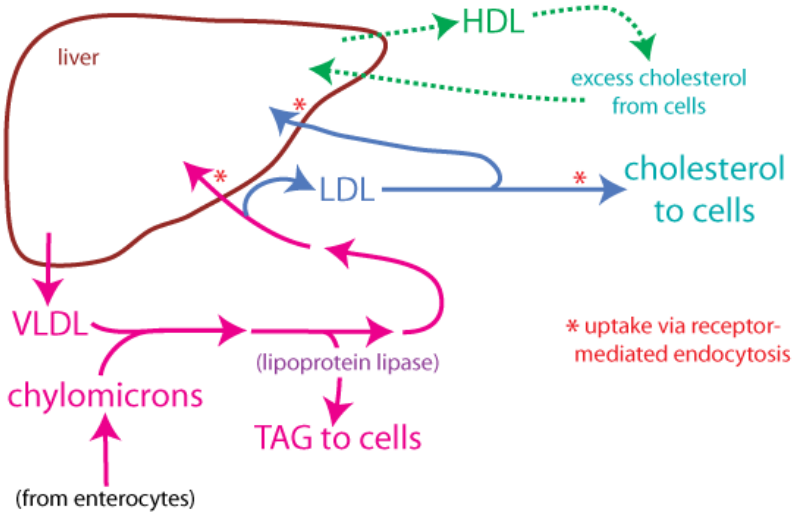
The liver is central to the regulation of cholesterol levels in the body. Not only does it synthesize cholesterol for export to other cells, but it also removes cholesterol from the body by converting it to bile salts and putting it into the bile where it can be eliminated in the feces. Furthermore, the liver synthesizes the various lipoproteins involved in transporting cholesterol and other lipids throughout the body. Cholesterol synthesis in the liver is under negative feedback regulation. Increased cholesterol in a hepatocyte leads to decreased activity of HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis.

Types of Lipoproteins

Lipoproteins are particles that contain triacylglycerol, cholesterol, phospholipids and amphipathic proteins called apolipoproteins. Lipoproteins can be differentiated on the basis of their density, but also by the types of apolipoproteins they contain. The degree of lipid in a lipoprotein affects its density—the lower the density of a lipoprotein, the more lipid it contains relative to protein. The four major types of lipoproteins are chylomicrons, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL).[4]

The figure below summarizes the fates of lipoproteins produced by the liver.

Fate of lipoproteins in Liver.



Chylomicrons and VLDL deliver TAG to cells in the body

Two types of lipoproteins are triglyceride-rich: the chylomicrons and VLDL. Chylomicrons are synthesized by enterocytes from lipids absorbed in the small intestine. VLDL is synthesized in the liver. The function of these lipoproteins is to deliver energy-rich triacylglycerol (TAG) to cells in the body (pink pathway). TAG is stripped from chylomicrons and VLDL through the action of lipoprotein lipase, an enzyme that is found on the surface of endothelial cells. This enzyme digests the TAG to fatty acids and monoglycerides, which can then diffuse into the cell to be oxidized, or in the case of an adipose cell, to be re-synthesized into TAG and stored in the cell.

LDL delivers cholesterol to cells in the body

As VLDL particles are stripped of triacylglycerol, they become more dense. These particles are remodeled at the liver and transformed into LDL. The function of LDL is to deliver cholesterol to cells, where it is used in membranes, or for the synthesis of steroid hormones (blue pathway). Cells take up cholesterol by receptor-mediated endocytosis. LDL binds to a specific LDL receptor and is internalized in

an endocytic vesicle. Receptors are recycled to the cell surface, while hydrolysis in an endo lysosome releases cholesterol for use in the cell.

HDL is involved in reverse cholesterol transport

Excess cholesterol is eliminated from the body via the liver, which secretes cholesterol in bile or converts it to bile salts. The liver removes LDL and other lipoproteins from the circulation by receptor-mediated endocytosis. Additionally, excess cholesterol from cells is brought back to the liver by HDL in a process known as reverse cholesterol transport (green pathway). HDL (or really, the HDL precursor) is synthesized and secreted by the liver and small intestine. It travels in the circulation where it gathers cholesterol to form mature HDL, which then returns the cholesterol to the liver via various pathways.

Cholesterol esterase:

Dietary cholesterol comprised of free and esterified cholesterol.In diets rich in meats, significant percent of cholesterol is esterified. Hydrolysis of cholesteryl ester in the lumen is catalysed by cholesterol esterase (CEase).

Cholesterol esterase is an acid lipase, which is synthesized in the pancreas, catalyses the hydrolytic cleavage of cholesterol, sterol esters and triglycerides.

The name pancreatic cholesterol esterase is ascribed to the only enzyme in the pancreas that hydrolyzes cholesterol esters to unesterified cholesterol and free fatty acids. However, investigations over a period of more than 30 years revealed that a protein with similar properties can also be purified from homogenates of several other tissues and body fluids and that enzyme is a nonspecific lipase capable of hydrolysing cholesteryl esters, vitamin esters, triacylglycerol, phospholipids, and lysophospholipids.[5]

At the onset of these investigations, it was not clear whether these various

enzyme activities were properties of the same protein. Thus, this enzyme was named nonspecific lipase, phospholipase A_1 lysophospholipase, bile-salt-stimulated lipase, bile salt-dependent lipase, carboxyl ester lipase, and carboxyl ester hydrolase.(5)

Sequence comparison with other protiens also revealed that this enzyme is responsible for the lipoamidase activity in milk, which may account for its ability to hydrolyse the physiological lipoamide substrate ceramide.

Nomenclature of this enzyme was made based on the various substrates of this enzyme.

- Most commonly used name carboxyl ester lipase (CEL), based on the general reactivity of with lipids containing carboxyl ester bonds.
- Cholesterol esterase or cholesterol ester lipase, due to its documented physiological function as a cholesteryl ester hydrolase.
- Bile-salt-stimulated or bile salt dependent lipase, based on unique bile salt dependency.

Cholesterol esterase has received most attention as having a potential role in cholesterol absorption. CEase has broad substrate specificity, hydrolysing tri-, di- and mono-glycerides and phospholipids *in vitro*. It also hydrolyzes cholesterol esters, which form a part of dietary cholesterol and cannot be engrossed without prior hydrolysis to free cholesterol. As such, it is one of the essential enzymes that mediate absorption of dietary lipids through the intestinal wall into the blood stream. A number of studies have recommended a possible role for CEase in the absorption of free cholesterol at the brush border membrane of the small intestine, through a CEase gene.(5)

Synthesis of cholesterol esterase

Major tissues for synthesis of this enzyme are acinar cells of exocrine

pancreas and lactating mammary glands.

- Enzyme synthesized by the pancreas is hoard in zymogen granules and is secreted with the pancreatic juice in a process stimulated by the gastric hormones such as cholecystokinin, secretin and bombesin. CEase mixes with the bile salt in the lumen of digestive tract and becomes active enzyme which catalyses nutrient digestion and absorption through GIT.
- Enzyme produced from the lactating mammary glands secreted as a major constituent of milk proteins and reaches the digestive tract of the infants, which plays a role in nutrient digestion and absorption in them.
- Low but significant levels are also synthesized in other tissues like liver, eosinophils, endothelial cells and macrophages. Physiological function of this enzyme synthesized outside digestive tract is unknown.[5]

Diseases caused due to high cholesterol levels

Hyperlipidemia is a main cause of atherosclerosis and atherosclerosis associated conditions such as ischemic cerebro-vascular disease, peripheral vascular disease (PVD) and coronary-heart disease (CHD).Although the prevalence of these atherosclerosis associated events has decreased in United States, these conditions still account for the majority of fatality and morbidity between middle-aged and older people. The occurrence and complete number of annual events will raise over the next decade because of the aging and epidemic of obesity of the U.S. population.

Hyperlipidemia (hypercholesterolemia), Dyslipidemias and low levels of HDL cholesterol are main basis of increased atherogenic risk. Both genetic disorders and lifestyle (sedentary behavior, high calorific diets) add to the dyslipidemias perceived in developed countries around the world. Drug therapy like,3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitors,

the statins which are mainly efficient and best tolerated drugs currently in the use for treating dyslipidemia, nicotinic acid (niacin), bile acid-binding resins, fibric acid derivatives and the cholesterol absorption inhibitor 'ezetimibe' were the other drugs used for this treatment.(2)

OBESITY

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have a negative effect on health. People are generally considered obese when their body mass index (BMI), a measurement obtained by dividing a person's weight by the square of the person's height, is over 30 kg/m^2, with the range 25–30 kg/m^2 defined as overweight. Some East Asian countries use lower values. Obesity increases the likelihood of various diseases and conditions, particularly cardiovascular diseases, type 2 diabetes, obstructive sleep apnea, certain types of cancer, osteoarthritis and depression.(6)

Obesity is most commonly caused by a combination of excessive food intake, lack of physical activity, and genetic susceptibility. A few cases are caused primarily by genes, endocrine disorders, medications, or mental disorder. The view that obese people eat little yet gain weight due to a slow metabolism is not generally supported. On average, obese people have a greater energy expenditure than their thin counterparts due to the energy required to maintain an increased body mass.

Obesity is mostly preventable through a combination of social changes and personal choices. Changes to diet and exercising are the main treatments. Diet quality can be improved by reducing the consumption of energy-dense foods, such as those high in fat and sugars, and by increasing the intake of dietary fiber. Medications may be taken, along with a suitable diet, to reduce appetite or decrease fat absorption. If diet, exercise, and medication are not effective, a gastric balloon or surgery may be performed to reduce stomach volume or bowel length, leading to

feeling full earlier or a reduced ability to absorb nutrients from food.

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health.It is defined by body mass index (BMI) and further evaluated in terms of fat distribution via the waist–hip ratio and total cardiovascular risk factors.BMI is closely related to both percentage body fat and total body fat. In children, a healthy weight varies with age and sex. Obesity in children and adolescents is defined not as an absolute number but in relation to a historical normal group, such that obesity is a BMI greater than the 95th percentile. The reference data on which these percentiles were based date from 1963 to 1994, and thus have not been affected by the recent increases in weight.BMI is defined as the subject's weight divided by the square of their height and is calculated as follows.

$$B=m/h^2$$

where *m* and *h* are the subject's weight and height respectively.

BMI is usually expressed in kilograms per square metre, resulting when weight is measured in kilograms and height in metres. To convert from pounds per square inch multiply by 703 $(kg/m^2)/(lb/sq\ in)$.

The most commonly used definitions, established by the World Health Organization (WHO) in 1997 and published in 2000, provide the values listed in the table.

Table-1 Body Mass Index

BMI (kg/m^2)		**Classification**
From	**up to**	
<18.5	<18.5	Underweight

18.5	18.5	normal weight
25.0	25.0	Overweight
30.0	30.0	class I obesity
35.0	35.0	class II obesity
40.0	40.0	class III obesity

Some modifications to the WHO definitions have been made by particular organizations. The surgical literature breaks down class II and III obesity into further categories whose exact values are still disputed.

- Any BMI ≥ 35 or 40 kg/m^2 is *severe obesity*.
- A BMI of ≥ 35 kg/m^2 and experiencing obesity-related health conditions or ≥40–44.9 kg/m^2 is *morbid obesity*.
- A BMI of ≥ 45 or 50 kg/m^2 is *super obesity*.

Causes and mechanisms of obesity

Energy output:

The usual cause of obesity is an unbalance between energy intake and output. There are indeed intricate weight-regulating mechanisms that act upon the Body's energy stores. The latter can in effect be 'destored' when the body needs them, thereby compensating for a lack of energy intake compared to one's needs.

The daily energy output is made up of three entities:

Basic metabolism:

This is the minimal output necessary for the body to remain alive. It depends essentially on the lean mass (which encompasses mostly the muscles) and represents 70% of the total energy output.

Physical activity:

This of course varies greatly, from the sedentary person to the hard labourer. It accounts on average for 20% of the total energy output. Because of the excess weight an obese person carries around, even when exerting only moderate physical activity he will be using up more energy than someone with normal weight.

It is therefore easy to understand why physical activity alone will not enable an obese person to achieve maximum weight loss. However, if the main element of a correctly balanced diet is an overall decrease in food intake, sport will play a significant role as it will assist in maximising its effects. Sport has moreover been shown to be beneficial in the long term as a means to prevent a relapse.

Thermogenesis:

This phenomenon results from the metabolism of food - absorption and storage - after meals. It represents 10% of the total energy output. A certain amount of the calories absorbed during a meal are therefore immediately 'burnt off' by digestion. This explains why jumping a meal does not fit into sensible dieting recommendations.

Taking into account the above facts, and coupling these the notion that moderate but constant overeating can lead to a state of obesity, it is easy to understand the difficulties to obtain good therapeutic results with diets. In particular, it is known that only to maintain their lean body mass - in particular with regard to the muscular component - obese people are dependent on hypercalorific food rations.

There is a true inequality for weight-gain in normal subjects, which can

explain a feeling of unfairness. The famous experiences of Sims, carried on with inmates, are very relevant: some of them had a high-calorie diet (more than 10000 calories per day, whereas the normal intake is an average of 2200 calories). Only a minority of them could gain some weight (more than 6 kg), precisely the ones that had a family background of obesity, which is an acknowledged cause of obesity.

Factors that encourage obesity

The above statements clearly show how obesity in itself is a complex phenomenon. Without getting too specific, the main factors that induce obesity can be roughly divided into the following main groups.(6)

Factors related to Food

These intervene at three levels.

Excess intake (quantity):

Food intake must vary according to a person's physical activity. A sedentary person will use up much less energy than a labourer. Too much food and not enough physical activity will inevitably lead to a person putting on excess weight.

Imbalance in intake (quality):

Without going into too much detail, we know that fat (lipids) plays an important part in creating an imbalance. Excess intake of so-called fast-acting sugars (sweetened drinks, chocolate, etc.) is also detrimental. In effect, these sugars are stored as fat and are then difficult to get rid off.

Eating disorders:

These include the absence of regular meal times, a problem characteristic of modern industrialised countries; and an overall poor lifestyle incompatible with regular meals. In a few cases there can be an alteration of the central nervous system

regulating the appetite, but this is only true for a small minority of people.

Factors related to energy output

This relates to the state of being sedentary in the large sense of the term, and also to the change from an active to a sedentary lifestyle.

Genetic factors

There is certainly a genetic predispostition (linked to numerous genes, not just one) which makes an individual, or members of a same family more susceptible to becoming obese within a given environment. We should not, however, conclude that obesity is a genetic illness, except for very rare and specific cases such as the Prader-Willi syndrome which affects adolescents and combines obesity and mental deficiency.

Psychologic factors

As described in the chapter on the psychological aspects of obesity, there is no such thing as a typical psychological profile for an obese person. In other words, there is no specific trait of character or anatomy of the psychism that predisposes towards obesity. On the other hand, certain psychological states such as depression or stress can influence weight gain. Matters are further complicated by the fact that weight gain in turn can lead to a number of abnormal psychological traits which could wrongly be interpreted as the cause of obesity.

Social and cultural factors

It is wrong to infer that poverty predisposes towards obesity, however an inverse relationship between income and obesity can be observed in all western countries. This can be explained by different eating habits (more fat in the diet for the poorer classes of the population) and unequal rights to healthcare. Hardly one century ago obesity was considered as a sign of wealth and prosperity. Today the

tendency is towards the opposite, where the rich can be seen ' taking care of their body '. It is in fact the life styles that have been adopted today which exert a bad influence on eating habits : global reduction in physical activity due to improved means of transport which lead to people walking less, a reduction of sport activities in schools, an increase in fast-food restaurants and vendors of high calorific sweet drinks and foods, the disappearance of regular eating times, snacks eaten in front of the television, and more recently videos and an increased use of computers for recreation means.

TYPES OF OBESITY

1. Inactivity Obesity

It is no secret that a lack of physical activity can cause you to become overweight. In this type of obesity, once-strong parts of the body quickly gain fat and become unhealthy.

2. Food Obesity

If you overeat, and particularly if you overindulge in unhealthy foods, you may suffer from food obesity. Excessive sugar intake can also cause food obesity, which may lead to accumulation of fat around the middle part of the body.

3. Anxiety Obesity

Anxiety or depression can often lead to overeating and accumulation of fat in the body, since the body must constantly survive in fight-or-flight mode. To treat this type of obesity, you must control your anxiety. Common treatments include medication, a decrease in caffeine intake, and herbs.

4. Venous Obesity

Venous circulation is one obesity cause that is genetic in nature, rather than habitual in nature. if anyone in your family suffers from venous circulation, you run the risk of the same condition. This type of obesity is particularly common in pregnancy. Exercise is the best solution for this problem.

5. Atherogenic Obesity

People whose fat tends to accumulate in the stomach area often suffer from atherogenic obesity. This is a particularly dangerous condition since it can affect your other organs and lead to breathing problems. It is extremely important to avoid drinking alcohol if you have atherogenic obesity.

6. Gluten Obesity

You are likely no stranger to the many health problems that gluten can cause. In fact, gluten can actually cause obesity. This type of weight gain is most common in women. It is often spotted during periods of hormonal change, like puberty, pregnancy, and menopause.

Hyperlipidaemia

Lipid is another word for fat. Lipids are easily stored in the body and serve as a source of energy. Cholesterol and triglycerides are lipids. When the concentration of triglycerides or cholesterol in your blood is too high, it is called hyperlipidaemia. Having a lipid level that is too high increases your risk of heart attacks and strokes.[3]

A health Bloor (1943) has proposed the following classification of lipids based on their chemical composition.

A. Simple lipids or Homolipids.

These are esters of fatty acid with farious alcohols.

1. Fats and oils (triglycerides, triacylglycerols).

These are esters of fatty acids with a trihydroxy alcohol,glycerol. A fat is solid at ordinary room temperature wheras an oil is liquid.

2. Waxes

These are esters of fatty acids with high molecular weight monohydroxy alcohols.

B. Compound lipids or Heterolipids.

These are esters of fatty acids with alcohol and possess additional group(s) also.

1. Phospholipids (phosphatids),

These are compounds containing, in addition to fatty acids and glycerol, a phosphoric acid, nitrogen bases and other substituents.

2. Glycolipids (cerebrosides).

These are the compounds of fatty acids with carbohydrates and contain nitrogen but no phosphoric acid. The glycolipids also include certain structurally-related compounds comprising the groups, gangliosides, sulfolipids and sulfatids.

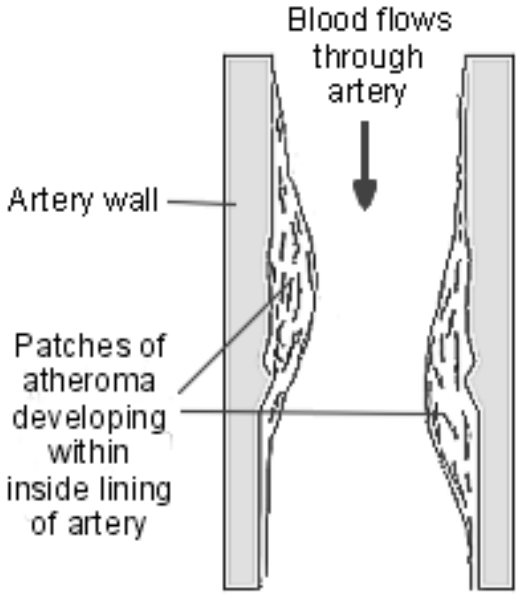
C. Derived lipids.

These are the substances derived from simple and compound lipids by hydrolysis. These include fatty acids, alcohols, mono- and diglycerides,steroids, terpenes and carotenoids.y diet and medicines can help lower your lipid levels.

Glycerides and cholesterol esters, because of their uncharged nature, are also called neutral lipids.However, Conn and Stumpf (1976) have traditionally classified lipids into following 6 classes :

1. Acyl glycerols
2. Waxes
3. Phospholipids
4. Sphingolipids
5. Glycolipids
6. Terpenoid lipids including carotenoids and steroids

Atheroma and cardiovascular diseases



Section of an artery

Patches of atheroma are like small fatty lumps that develop within the inside lining of blood vessels (arteries). Atheroma is also known as atherosclerosis and hardening of the arteries. Patches of atheroma are often called plaques of atheroma.

Over months or years, patches of atheroma can become larger and thicker. So in time, a patch of atheroma can make an artery narrower. This can reduce the blood flow through the artery. For example, narrowing of the heart (coronary) arteries with atheroma is the cause of angina.

Sometimes, a blood clot (thrombosis) forms over a patch of atheroma and completely blocks the blood flow. Depending on the artery affected, this can cause a heart attack, a stroke, or other serious problems.

Cardiovascular diseases are diseases of the heart (cardiac muscle) or blood vessels. However, in practice, when doctors use the term cardiovascular disease they usually mean diseases of the heart or blood vessels that are caused by atheroma.

In summary, cardiovascular diseases caused by atheroma include angina,

heart attack, stroke, transient ischaemic attack (TIA) and peripheral arterial disease. In the UK, cardiovascular diseases are a major cause of poor health and the biggest cause of death.

Blood levels of cholesterol

Cholesterol blood levels are very important but must be considered in an overall assessment of your risk of cardiovascular disease .The following blood cholesterol levels are generally regarded as desirable:

- Total cholesterol (TChol) - 5.0 mmol/L or less. However, about 2 in 3 adults in the UK have a TChol level of 5.0 mmol/L or above.
- LDL cholesterol: 3.0 mmol/L or less.
- HDL cholesterol: 1.2 mmol/L or more.
- TChol/HDL ratio: 4.5 or less. That is, your TChol divided by your HDL cholesterol. This reflects the fact that for any given TChol level, the more HDL, the better.

As a rule, the higher the LDL cholesterol level, the greater the risk to health. A blood test only measuring total cholesterol may be misleading. A high total cholesterol may be caused by a high HDL cholesterol level and is therefore healthy. It is very important to know the separate LDL cholesterol and HDL cholesterol levels.

Your level of LDL cholesterol has to be viewed as part of your overall cardiovascular health risk. The cardiovascular health risk from any given level of LDL cholesterol can vary, depending on the level of your HDL cholesterol and on any other health risk factors that you may have. Therefore, a cardiovascular risk assessment considers all your risk factors together.

Triglycerides

Triglycerides are the main form of fat stored in the body. When you think

of fat on your hips or stomach, you're thinking of triglycerides.

Triglycerides are the end product of digesting and breaking down the bulky fats that are present in our food. Any food we eat that isn't used for energy immediately - carbohydrates, fat, or protein - is also converted into triglycerides. They are bundled into globules and transported through the blood by lipoproteins, like cholesterol. The triglycerides are taken up by fat (adipose) cells, to be used for energy if food isn't available later.

Normal levels of lipids

Fat chemicals (lipids) are measured in millimoles per litre which is a measure of how concentrated they are in 1 litre of blood. It is usually written as mmol/L. The following levels are generally considered desirable. However, a doctor or nurse will advise on the level for you to aim for. They will consider any other diseases or risk factors that you may have

- Total cholesterol (TChol) - 5.0 mmol/L or less.
- LDL cholesterol after an overnight fast: 3.0 mmol/L or less.
- HDL cholesterol: 1.2 mmol/L or more.
- TC/HDL ratio: 4.5 or less. That is, your TChol divided by your HDL cholesterol. This reflects the fact that, for any given TChol level, the more HDL, the better.
- Triglycerides (TGs): 1.7 mmol/L or less after an overnight fast.

  Generally, the higher the LDL cholesterol level, the greater the risk to health. However, your level of cholesterol has to be viewed as part of your overall cardiovascular health risk. The cardiovascular health risk from any given level of cholesterol can vary, depending on the level of your HDL cholesterol and on other health risk factors that you may have.

Causes of hyperlipidaemia

Hyperlipidaemia is often found when people are overweight or have an unhealthy diet. It can also be the result of drinking too much alcohol. It can be something that you may have inherited through your family genes (known as primary) and approximately 1 person in 500 will have this cause. It may be because of another medical condition that you may have, such as diabetes, when it is known as secondary. Other causes include:

- An underactive thyroid (hypothyroidism).
- Obstructive jaundice.
- Cushing's syndrome.
- Anorexia nervosa.
- Nephrotic syndrome.
- Chronic kidney disease.

Some prescribed medicines can affect your cholesterol level, including:

- Thiazide diuretics (used to control blood pressure).
- Glucocorticoids (steroids).
- Cyclosporine (used after organ transplants).
- Antiretroviral therapy (used to treat HIV infection).
- Beta-blockers (used to control heart rate).
- The combined oral contraceptive pill.
- Atypical antipsychotics (used in some mental health problems).
- Retinoic acid derivatives (used in some skin conditions).

Symptoms of hyperlipidaemia

Hyperlipidaemia is often found during routine screening when your doctor is trying to assess your risk of having heart attacks or strokes. This may be as part of an annual health check if you are over 40 years of age, or if you have a close

relative who had these problems at a young age.

Usually, the diagnosis is made after a fasting blood test. Fasting means at least 12 hours when you have not eaten. You are allowed to drink water.
There are also changes that may be visible on your body if you have the inherited form of hyperlipidaemia.(3)

- **Premature arcus senilis** - this is a white or grey ring that is visible when your doctor looks at the front of your eyes.
- **Tendon xanthomata** - these are hard nodules that you may find in the tendons of the knuckles and the Achilles (at the back of your ankle).
- **Xanthelasmas** - fatty deposits in the eyelids.

Lowering lipid levels

Changing from an unhealthy diet to a healthy diet can reduce a cholesterol level. However, dietary changes alone rarely lower a cholesterol level enough to change a person's risk of cardiovascular disease from a high-risk category to a lower-risk category. However, any extra reduction in cholesterol due to diet will help.

A healthy diet has other benefits too apart from reducing the level of cholesterol.
Briefly, a healthy diet means:

- At least five portions, or ideally 7-9 portions, of *a variety of* fruit and vegetables per day.
- A third of most meals should be starch-based foods (such as cereals, wholegrain bread, potatoes, rice, pasta), plus fruit and vegetables.
- Not much fatty food such as fatty meats, cheeses, full-cream milk, fried

food, butter, etc. Use low-fat, mono-unsaturated or polyunsaturated spreads.

- Include 2-3 portions of fish per week, at least one of which should be oily (but, if you are pregnant, you should not have more than two portions of oily fish a week).
- Limit salt to no more than 6 g a day (and less for children).
- If you eat red meat, it is best to eat lean red meat, or eat poultry such as chicken.
- If you do fry, choose a vegetable oil such as sunflower, rapeseed or olive.

Foods that contain plant sterols or stanols can reduce total blood cholesterol level and LDL cholesterol by about 10%. There does not seem to be much evidence, however, that this has an effect on preventing cardiovascular disease. The National Institute for Health and Care Excellence (NICE) therefore does not recommend that these products should be used routinely until more information is available.

Antihyperlipidemic Drugs

Lipid disorders[3,7]

Disorders of lipid metabolism are manifest by elevation of the plasma concentrations of the various lipid and lipoprotein fractions (total and LDL cholesterol, VLDL, triglycerides, chylomicrons) and they result in cardiovascular disease and atherosclerosis.

Type of hyperlipidemia

1. Primary hyperlipidemia
2. Secondary hyperlipidemia

CLASSIFICATION- based on the pattern of lipoprotein on electrophoresis or ltracentrifugation.

Familial Chylomicronemia (I):increased Chylomicrons due to deficiency of lipoprotein lipase or its cofactor.

Familial Hypercholesterolemia (IIA): levels of LDL tend to increase with normal VLDL.

Familial Combined (mixed)Hyperlipidemia (IIB): elevated levels of VLDL, LDL.

Familial Dysbetalipoproteinemia (III): Increased IDL resulting increased TG and cholesterol levels.

Familial Hypertriglyceridemia (VI): Increase VLDL production with normal or decreased LDL.

Familial mixed hypertriglyceridemia (V): Serum VLDL and chylomicrons are increased

Secondary hyperlipidemias results from:

Liver disease, Biliary disease, Obesity, Hypothyroidism, Diabetes, Diet, Alcohol excess, Renal disease (nephrotic syndrome), Drugs (HIV protease inhibitors, thiazide diuretics, oral contraceptive steroids) The most severe hyperlipidaemias usually occur in patients with concurrent conditions, e.g. diabetes Mellitus with one of the primary hyperlipidemias.

Drug therapy: the primary goal of therapy is to:

- Decrease levels of LDL
- Increase in HDL

Anti-hyperlipidemic drugs are mainly classified into 5 types [7]

- HMG CoA REDUCTASE INHIBITORS: E.g. Atorvastatin, Fluvastatin, Lovastatin, Pravastatin, Simvastatin.
- FIBRATES: E.g. Fenofibrate, Gemfibrozil, Clofibrate
- Anion –exchange resins(BILE ACID SEQUESTRANTS): E.g. Colesevelam, Colestipol, Cholestyramine

- Nicotinic acid: E.g. NIACIN.
- CHOLESTEROL ABSORPTION INHIBITORS: E.g. Ezetimibe.
- OTHER DRUGS E.g. Alpha-tocopherol acetate (vitamin E), Omega-3 marine triglycerides (Maxepa), Orlistat

HMG-CoA Reductase Inhibitors (HMGs or statins) Pravastatin , Simvastatin , Atorvastatin , Fluvastatin , Lovastatin. (They are most potent LDL reducers)

Mechanism of action of statins

- Block the rate-limiting enzyme for endogenous cholesterol synthesis, hydroxy-methylglutaryl Coenzyme A (HMG CoA) reductase.
- Increased synthesis of LDL-receptors (upregulation) in the liver
- Increased clearance of LDL from the circulation

Note: Plasma total cholesterol and LDL-cholesterol fall to attain a maximum effect 1 month after therapy.

Therapeutic uses:

These drugs are effective in lowering plasma cholesterol levels in all types of hyperlipidemias However, patients who are homozygous for familial hypercholesterolemia lack LDL receptors and, therefore, benefit much less from treatment with these drugs. These drugs are often given in combination with other antihyperlipidemic drugs

Pharmacokinetics of statins :

Pravastatin and fluvastatin are almost completely absorbed after oral administration.

- Oral doses of lovastatin and simvastatin are from 30 to 50 percent absorbed.
- Pravastatin and fluvastatin are active, whereas lovastatin and simvastatin must be hydrolyzed to their acid forms.

- Excretion takes place through the bile and feces
- Some urinary elimination also occurs.
- Their half-lives range from 1.5 to 2 hours.

Note: Because of a circadian rhythm to LDL-receptor synthesis, statins are a little more effective if given in the evening rather than in the morning.

Adverse effects

1. Transient, and minor abnormality of liver function tests
2. Myopathy and rhabdomyolysis (disintegration or dissolution of muscle and elevation of muscle enzymes (creatine phosphokinase, CPK),the risk is greater in:
3. In patients with renal insufficiency
4. In patients taking drugs such as cyclosporine, itraconazole, erythromycin, gemfibrozil, or niacin. Plasma creatine kinase levels should be determined regularly.

Drug interactions:

The HMG CoA reductase inhibitors may also increase warfarin levels. Thus, it is important to evaluate INR

Contraindications:

These drugs are contraindicated during pregnancy and in nursing mothers. They should not be used in children or teenagers.

FIBRIC ACID DERIVATIVES (FIBRATES) Bezafibrate Ciprofibrate Fenofibrate Gemfibrozil

Mechanism of action

Agonists at PPAR (peroxisome proliferator-activated receptor) → expression of genes responsible for increased activity of plasma lipoprotein lipase

enzyme → hydrolysis of VLDL and chylomicrons→ ↓serum TGs.↑ clearance of LDL by liver & ↑ HDL.

Therapeutic uses

Hypertriglyceridemia (the most effective in reduction TGs) - combined hyperlipidemia (type III) if statins are contraindicated

Pharmacokinetic

- Well absorbed from the gastrointestinal tract
- Extensively bound to plasma proteins
- Excreted mainly by the kidney as unchanged drug or metabolites.

Contraindications

1. Where hepatic or renal function is severely impaired (but gemfibrozil has been used in uraemic and nephrotic patients without aggravating deterioration in kidney function)
2. Pregnant or lactating women

Adverse effects

1. Gastrointestinal effects
2. Lithiasis: Because these drugs increase biliary cholesterol excretion, there is a predisposition to the formation of gallstones.
3. Myopathy and rhabdomyolysis the risk is greater in:
   - Patients with poor renal Function
   - In patients taking a statin.
4. Fibrates enhance the effect of co-administered oral Anticoagulants.

Anion – exchange resins (BILE ACID SEQUESTRANTS): Cholestyramine,

Colestipol Colesevelam

Mechanism of action:

- Anion exchange resins bind bile acids in the intestine forming complex →loss of bile acids in the stools →↑ conversion of cholesterol into bile acids in the liver.
- Decreased concentration of intrahepatic cholesterol → compensatory increase in LDL receptors →↑ hepatic uptake of circulating LDL → ↓ serum LDL cholesterol levels.

Therapeutic uses:

1. In treatment of type IIA and IIB hyperlipidemias (along with statins when response to statins is inadequate or they are contraindicated).
2. Useful for Pruritus in biliary obstruction (↑ bile acids).

Pharmacokinetics:

Orally given but neither absorbed nor metabolically altered by intestine, totally excreted in feces.

Adverse effects:

1. Gastrointestinal effects: constipation (most common), nausea, and flatulence, anorexia, diarrhea, these effects are dose-related.
2. Impaired absorptions: At high doses, cholestyramine and colestipol impair the absorption of the fat-soluble vitamins (A, D, E, and K).

Note: Colesevelam has fewer gastrointestinal side effects and not impaired absorption of the fat-soluble vitamins (A, D, E, and K).

Drug interactions:

Tetracycline, warfarin, digoxin, thiazide diuretics, phenobarbitone and thyroid hormones should be taken 1 h-2h before or 4 h- 6 h after colestyramine to avoid impairment of their absorption (Because the drug binds anions)

Niacin (nicotinic acid)

Mechanism of action:

- It is a potent inhibitor of lipolysis in adipose tissues → ↓ mobilization of FFAs (major precursor of TGs) to the liver →↓ VLDL (after few hours).
- Since LDL is derived from VLDL so ↓ VLDL →↓ LDL (after few hours).
- ↑ HDL levels
- ↓ Endothelial dysfunction →↓ thrombosis.

Therapeutic uses:

Niacin lowers plasma levels of both cholesterol and triacylglycerol. Therefore, it is particularly useful in the treatment of familial hyperlipidemias. Niacin is also used to treat other severe hypercholesterolemias, often in combination with other antihyperlipidemic agents. In addition, it is the most potent antihyperlipidemic agent for raising plasma HDL levels, which is the most common indication for its clinical use.

Pharmacokinetics:

Niacin is administered orally. It is converted in the body to nicotinamide, which is incorporated into the cofactor nicotinamide-adenine dinucleotide (NAD+). Niacin, its nicotinamide derivative, and other metabolites are excreted in the urine. [Note: Nicotinamide alone does not decrease plasma lipid levels.]

Adverse effects:

1. Cutaneous flush most common side effects accompanied by an

uncomfortable feeling of warmth and pruritus. Administration of aspirin prior to taking niacin decreases the flush, which is prostaglandin mediated. The sustained-release formulation of niacin, which is taken once daily at bedtime reduces bothersome initial adverse effects.

2. Nausea and abdominal pain.
3. Hyperuricemia and gout (Niacin inhibits tubular secretion of uric acid)
4. Impaired glucose tolerance
5. Hepatotoxicity

Cholesterol absorption inhibitors

Ezetimibe

- Selectively inhibits intestinal absorption of dietary and biliary cholesterol in the small intestine → ↓ in the delivery of intestinal cholesterol to the liver → ↓ of hepatic cholesterol stores → ↑ clearance of cholesterol from the blood.
- Ezetimibe lowers LDL cholesterol and triacylglycerols
- Increases HDL cholesterol.

Pharmacokinetic

- Metabolized in the small intestine and liver via glucuronide conjugation (a Phase II reaction), with subsequent biliary and renal excretion.
- Both ezetimibe and ezetimibe-glucuronide are slowly eliminated from plasma, with a half-life of approximately 22 hours.
- Ezetimibe has no clinically meaningful effect on the plasma concentrations of the fat-soluble vitamins A, D, and E. Patients with moderate to severe hepatic insufficiency should not be treated with ezetimibe.

  [Note:] A formulation of ezetimibe and simvastatin has been shown to lower LDL levels more effectively than the statin alone.

Combination drug therapy

Bile acid resins can be safely combined with statins or nicotinic acid (↓ LDL, VLDL cholesterol levels respectively).

- Ezetimibe + statins → synergistic effects.
- Fibrates and statins are CI → myopathy.
- Nicotinic acid and statins (must be cautiously used) → myopathy

OTHER DRUGS:

Alpha-tocopherol acetate (vitamin E)

Has no effect on lipid levels but is a powerful antioxidant. Considerable evidence points to oxidation of LDL as an essential step in the development of atheroma, and therefore interest has centred on the role of either endogenous or therapeutic vitamin E in prevention of atheroma.

Omega-3 marine triglycerides (Maxepa) contain

The triglyceride precursors of two polyunsaturated fatty acids derived from oily fish. They have no place in treating hypercholesterolaemia. Some patients with moderate to severe hypertriglyceridaemia may respond to oral use, although LDLcholesterol may rise.

Orlistat, a weight-reducing agent

It is pancreatic lipase inhibitor, lowers the Glycaemia of diabetes mellitus to a degree that accords with the weight loss, and improves Hyperlipidmia .There is a risk of steatorrhoea and malabsorption of Fat-soluble vitamins A, D and E.

FDA approved new anti-hyperlipidemic drugs

Food and drug administration (FDA), U.S. approved some new drugs for the treatment of hyperlipidemia associated diseases. They are as follows:

I. Livalo (Pitavastatin)

II. Juxtapid (Lomitapide)

III. Kynamro (Mipomersen)

IV. Vascepa (Icosapent ethyl)

Medicinal plants used in the treatment of hyperlipidemia[7]

Numbers of plant species were reported to possess anti-hyperlipidemic activity like *Abelmoschus esculentus, Achyranthus aspera, Allium sativa, Bauhinia variegata, Curcuma longa* and the other plants and plant constituents which were reported to posses anti-hyperlipidemic activity are listed in Table-2 .

Table 2: List of medicinal plants having Hypolipidemic activity

Plant name	Part used
Abelmoschus esculentus	Whole plant
Achyranthus aspera	Roots
Aegle marmelos	Leaf
Allium cepa	Fresh bulbs
Allium sativum	Fresh fruits
Alstonia scholaris	Leaves
Amaranthus viridis	Leaves
Andrographis serpyllifolia	Roots
Anethum graveolens	Essential oil
Asparagus racemosus	Roots
Bauhinia Variegata	Roots & Stems
Cassia fistula	Legume
Catharanthus roseus	Leaves
Curcuma longa	Rhizome
Cymbopogon citrates	Leaves
Eclipta prostate	Leaves
Garcinia combogia	Peel of matured fruits
Glyccyrrhiza glabra	Roots
Hibiscus rosa sinesis	Root

Morus alba	Leaves
Nelumbo nucifera	Leaves
Ocimum basilicum	Whole plant
Pongamia pinnata	Pods, flowers
Ruta graveolens	Leaves, Whole plant
Terminalia chebula	Pericarp
Zingiber officinale	Rhizome

Table 3: List of the Phytoconstituents having Hypolipidemic activity

Biological source	Phytoconstituents
Allium cepa	S-methyl cysteine sulfoxide
Atrium lappa L.	Inulin
Avena sativa L.	Mono and oligosachcharide
Bupleurum chinense DC	Triterpine
Citrus lemon L.	Pectin, citrus flavonoids
Cyamposis tetragonolobus(L)	Gums
Emblica officinalis	Fixed oil and volatile oil
Ginkgo biloba L.	Polyphenolic compounds, Flavonoids
Medicago sativa L.	Saponins
Plantago lanceolata L.	Metamucil

Need for Natural Hypolipidemic agents

The use of statins in the treatment of Hyperlipidemia causes concern in both patients and physicians about the safety associated with such medications. Muscle toxicity or myopathy, is a common adverse effect of this class of drugs. Myopathy progressing to rhabdomyolysis and renal failure is the most serious side effect associated with all statins either in monotherapy or in combination therapy and appears to be doserelated. As statins therapy is for a long term basis, there may be a risk of chronic toxic effects like carcinogenic, teratogenic and mutagenic over a life time of use. Till date, there are very less natural medications available in the

market to treat hyperlipidemia. Therefore it is a need of the day to search for natural medicaments because of their fewer side effects and less expensive as compared with synthetic drugs. So, numerous studies are needed to explore the anti-hyperlipidemic activity of herbs. This may, at least in part, help future studies to screen the newer anti-hyperlipidemic molecules.[7]

PLANT PROFILE

- ***Anisomeles malabarica*** [8]

Plant name	:	*Anisomeles malabarica*(L.)R.Br
Synonym	:	*Nepeta malabarica* L.
Family	:	Lamiaceae

Vernacular names:

Anisomeles malabarica are locally known as Malabar catmint in English,

Hindi	:	Kalpanath, Codhara, Gopoli
Irula	:	Kannu thumbai
Kannada	:	Karitumbi, Gandubirana Gida
Konkani	:	Kaktumbo
Malayalam	:	Karintumpa, Karithumba, Karimthumba, Perumtumpa
Marathi	:	Gojibha
Oriya	:	Vaikuntha
Sanskrit	:	Mahadronah, Vaikunthah
Tamil	:	Aruvaachadachi, peyimarutti
Telugu	:	Mogabheri or Moga- biran.

- **TAXONOMIC CLASIFICATION:**

Division	:	Spermatophyta
Sub-division	:	Angiospermae
Class	:	Dicotyledone
Sub-class	:	Gamopetalae
Series	:	Bicarpellate
Order	:	Lamiales

Family	:	Labiatae or Lamiaceae
Genus	:	Anisomeles
Species	:	Malabarica R.Br.
Botanical name:		Anisomeles malabarica(L) R. Br.

Biological source:

Young leaves

Distribution:

Anisomeles malabarica are of Asiatic origin was limited in its distribution and is found only in south India, Srilanka and Australia.[8]

Parts used:

All parts of the plant

Anisomeles malabarica plant

Description:

Malabar Catmint is a shrubby herb, 0.5-1.5 m tall. Stems are tetragonous, densely villous or woolly. Leaves are ovate to oblong, 3-8 cm x 1.5-3 cm, densely woolly beneath, sparsely hirsute above, hairs 4-celled, petiole 0.5-2.5 cm long, softly woolly. Inflorescence is a single terminal spike, calyx 8.5 mm x 6 mm, longest teeth 3-4 mm long, in fruit 8-10 mm long, teeth hairy inside. Flower up to 1.8 cm long, lower lip about 12 mm x 4 mm, lilac or pale blue, filaments almost at same level, about 8 mm long, style about 1.3 cm long. Nutlets are cylindrical, 1.3 mm x 0.9 mm.

Chemical constituents:

Plant (roots, stems, leaves and flowers) yield essential oil, anisomelic acid, ovatodiolide, diterpenes - malabaric acid, anisomelyl acetate, anisomellol, anisomelolide, crisilineol, betulinic acid and Beta-sitosterol.[8,9]

Medicinal uses:

The whole plant, especially the leaves and the roots are used as astringent, carminative, febrifuge and tonic.

❖ **TRADITIONAL AND ETHANOBOTANICAL USES[9]**

Ethnobotanically, the leaves of *A.malabarica* are used against convulsions, dyspepsia, intermittent fever, colic, boils, tetanus. *Anisomeles malabarica* is useful in halitosis, epilepsy, hysteria, amentia, anorexia, dyspepsia, colic, flatulence, intestinal worms, fever arising from teething in children, intermittent fever, gout, swelling, diarrhoea and rheumatism.The plant also shows properties like antiperotic, diaphoretic, emmenagogue etc. Recently the plant was investigated for its herbicidal activity. Stem paste of *A.malabarica* mixed with coconut oil is applied topically over the wounds, and the Leaves are used to treat Eczema.The plant is also used in curing various ailments like anticancer, allergenic, anthelmintic, antiallergic, antianaphylactic, antibacterial, anticarcinomic, antiedemic, antihistaminic, antiinflammatory, antileukemic, antinociceptive, antiplasmodial, antiseptic and antiperotic properties. *A.malabarica* is being investigated for its pharmacognostical and various biological activities.

REPORTED ACTIVITIES

The reported activities of *Anisomeles malabarica* includes Anti-allergic, Anti-anaphylactic, Anti-bacterial, Anti-cancer, Anti-carcinogenic, Anti-inflammatory, Anti-epileptic potential, Antifertility, Anti-pyretic activity and antispasmodic activity.[8,9]

REVIEW OF LITERATURE

LITERATURE RELATED TO ANTI HYPERLIPIDEMIC ACTIVITY

Huang *et al.*, (2017) reported the dyslipidemic activity of *Malus - toringoides* leaves which is traditionally used as a medicine for treating or preventing cardiovascular disease in Tibet. In addition to the effect of this medicinal plant on thrombosis, they tested its effect on dyslipidemia in a hypolipidemic rat model. Serum levels of total cholesterol (TC), triglycerides (TG), low- and high-density lipoprotein cholesterol (LDL-C and HDL-C, respectively), as well as the antioxidant capacity of glutathione peroxidase (GSHP-X), superoxide dismutase (SOD), and malondialdehyde (MDA) were measured at the end of the study. MT significantly reduced serum TC, TG, and LDL-C and increased the HDL-C content in MT-treated rats compared with the model group. These changes were dose dependent. MT treatment also significantly elevated the activity of SOD and GSHP-X, and decreased the serum levels of MDA compared with untreated hyperlipidemic rats, thereby increasing serum antioxidant capacity. In addition, MT reduced liver steatosis in hyperlipidemic rats. Overall, MT exerts considerable hypolipidemic and antioxidant properties.(10)

Surya *et al* .,(2016) evaluated the antihyperlipidemic activity of *Ficus dalhousiae* stem bark methanolic extract (250 and 500 mg/kg b. wt.) in Triton WR-1339 and high fat diet-induced hyperlipidemic rats. *F. dalhousiae* extract significantly($P < 0.005$) alter the serum TC, TG, LDL-C and HDL-C levels to near normal in Triton WR-1339 and high fat diet-induced hyperlipidemic rats. The liver total cholesterol and triglycerides were also significantly reduced after treatment with 250 and 500 mg/kg of *F. dalhousiae*. The result of their study indicated that *F. dalhousiae* has a significant potential to use as a natural antihyperlipidemic agent.(11)

Liu *et al.*, (2016) investigated the hypolipidemic effects of the aqueous leaf extract of *Carmona microphylla* (Lam.) G. Don. (CAE) *in vitro* and *in vivo*. The lipid-lowering effect of CAE was investigated in oleic acid induced steatosis in HepG2 liver cells, as well as in high-fat diet (HFD)- and triton WR-1339 (TRI)-induced hyperlipidemic mice. The levels of intracellular, serum and/or hepatic total cholesterol (TC); triglyceride (TG); lowdensity lipoprotein-cholesterol (LDL-c); high density lipoprotein-cholesterol (HDL-c); hepatic superoxidedismutase (SOD) activity and malondialdehyde (MDA) were determined by oil-red O staining and appropriate kits. Treatment with CAE inhibited lipid accumulation in HepG2 cells and reduced the elevated levels of serum TC, TG and LDL-c as well as hepatic TC and TG in hyperlipidemic mice induced by HFD. CAE administration also significantly decreased arteriosclerosis index and LDL-c/HDL-c ratio, but improved oxidative status as revealed by increased hepatic SOD activity and decreased MDA level. The lipid ameliorating and antioxidative effects of CAE (600 mg/kg) were comparable to those of the standard lipid-lowering drug, simvastatin (5mg/kg). Their results suggested that *C. microphylla* aqueous extract (CAE) protects against hyperlipidemia induced by HFD in mice and may find therapeutic application in hyperlipidemic patients.(12)

Zhang *et al.*, (2016) investigated the therapeutic effect and potential mechanism of the fruit of *Ficus suspensa* using streptozotocin (STZ)-induced diabetic mice. Crude methanol extract of *F. suspensa* fruit was fractionated with different solvents and the ethyl acetate fraction (EAF) was selected for *in vivo* studies based on the *in vitro* α-amylase and HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl coenzyme A) inhibiting activities. For *in vivo* study, diabetes mellitus was induced in mice with STZ. Diabetic mice were orally administrated with 50, 100 and 200 mg/kg body weight of EAF for 4 weeks. Mouse body weight, blood glucose, glucose tolerance, biochemical parameters and gene expression related to pancreas and liver function were analyzed after EAF

administration. After 4 weeks of EAF intervention, a significant decrease in blood glucose, triglyceride, creatinine total cholesterol, acid phosphatase, alkaline phosphatase, aspartate transaminase, alanine transaminase, and hepatic lipid (triglycerides and cholesterol) content as well as a significant increase in body weight, insulin secretion and glucose tolerance was observed in EAF treated diabetic mice. qRT-PCR analysis revealed that EAF antagonized STZ-induced alteration of the expression of rate-limiting enzymes (glucokinase and phosphorenolpyruvate carboxykinase) in liver and insulin secretion related genes insulin-1, insulin-2 and duodenal homeobox factor-1 in pancreas. The ethyl acetate extract of *Forsythia suspense* (Thunb.) Vahl fruit has potency to develop an anti-hyperglycemic and anti-hyperlipidemic agent for the treatment of diabetes mellitus via modulation of oxidative stress, the hepatic glucose metabolism and pancreatic insulin secretion.(13)

Adeneye and Crooks (2015) investigated the weight losing, antihyperlipidemic and cardioprotective effects of the alkaloid fraction of Hunteria umbellata (H. umbellata) seed. Adult female *Wistar rats* (weight range: 120-150 g) were randomly divided into 4 and 5 treatment groups in the normal and triton-induced hyperlipidemic models, respectively and were daily treated for 14 d before they were humanely sacrificed under inhaled diethyl ether anesthesia. About 5 mL of whole blood was obtained by cardiac puncture from each treated rat, from which serum for lipids assay was subsequently separated. Tissue samples of livers of treated rats were harvested and processed for histopathological analysis. Overall, results of their study showed that repeated oral treatments with 25 and 50mg/kg/day of alkaloid fraction of *H.umbellata* elicited weight losing, antihyperlipidemic and cardioprotective effects in triton WR-1339 induced hyperlipidemic rats that were mediated via *de novo* cholesterol biosynthesis inhibition.(14)

El-Tantawy *et al.*, (2015) investigated the hypolipidemic effect of an

ethanolic extract from the roots and rhizomes of *Panicum repens* L.in rats suffering from high-cholesterol, diet induced hyperlipidemia, and the phytochemicals in the extract were analyzed. The extract was administered p.o.in doses of 250 mg/kg/day together with cholesterol at a dose of 100 mg/kg/day for 7 weeks. The high-cholesterol diet caused a significant increase in total lipids, total cholesterol (TC), total triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and the atherogenic index, whereas the level of high-density lipoprotein cholesterol (HDL-C) was significantly decreased. Administration of the *P. repens* extract ($p < 0.05$) significantly reduced the rise of the serum levels of total lipids, TC, TG, and LDL-C, as well as the atherogenic index, whereas it significantly increased ($p < 0.05$) the level of HDL-C. HPLC analysis of the phenolics and flavonoids in the extract revealed the presence of gallic acid, chlorogenic acid, chicoric acid, primulic acid, rutin, apigenin-7-glucoside, and quercetin. In conclusion, the *P. repens* extract was found to possess hypolipidemic activity in high-fat, diet-induced hyperlipidemic rats.(15)

Rony *et al.,* (2014) studied the hypolipidemic activity of *Phellinus rimosus* studied using tritonWR-1339 and high cholesterol diet (HCD) induced models. The triton induced elevated lipid profile was attenuated by *P. rimosus* or standard drug atorvastatin. Similarly, administration of *P. rimosus* along with HCD significantly decline serum triglyceride, total cholesterol, low-density lipoprotein, with elevating the high-density lipoprotein. Thiobarbituric acid reacting substances in heart and liver significantly decreased; whereas activity of enzymatic antioxidants and level of reduced glutathione were significantly increased. In both models, *P.rimosus* extract showed a significant ameliorative effect on the elevated atherogenic index as well as LDL/HDL-C ratio. The hypolipidemic activity of *P. rimosus* can be ascribed to its inhibitory effect on the liver HMG CoA reductase activity. The results suggest the possible therapeutic potential of this fungus as hypolipidemic agent.(16)

Thirumalai *et al.,* (2014) evaluated the hypolipidemic effect of *Piper*

betel (P. betel) in high fat diet induced hyperlipidemia rat. The methanol leaf extract was tested for hypolipidemic effect in the albino rats at the selected optimum dosage of 250 mg/kg body weight orally. In negative control animals, the activity levels of serum total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and very low density lipoprotein-cholesterol (VLDL) were significantly enhanced when compared to that of normal rat. It could be said that the methanolic leaf extract of *P. betel* exhibited a significant hypolipidemic effect.[17]

Irudayaraj *et al.*, (2013) evaluated the *in vitro* antioxidant and antihyperlipidemic activity of *Toddalia asiatica* (L) Lam. leaves in Triton WR-1339 and high fat diet-induced hyperlipidemic rats. In *in vitro* studies *T. asiatica* leaves ethyl acetate extract showed very good scavenging activity on 2,2-diphenyl-picrylhydrazyl (DPPH) (IC50 605.34 ± 2.62 lg/ml), hydroxyl (IC50 694.37 ± 2.12 lg/ml) and nitric oxide (IC50 897.83 ± 1.48 lg/ml) radicals, as well as high reducing power. In Triton WR-1339 induced hyperlipidemic rats, oral treatment with *T. asiatica* leaves ethyl acetate extract produced a significant ($P< 0.005$) decrease in the levels of serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C), and significant increase in high-density lipoprotein cholesterol (HDL-C) in comparison with hexane and methanol extracts. In high fat diet-fed hyperlipidemic rats, the ethyl acetate extract (200 and 400 mg/kg) significantly altered the plasma and liver lipids levels to near normal.[18]

Thirumalaisamy *et al.*, (2013) evaluated the the effect of the ethyl-acetate fraction of an ethanolic extract from *Streospermum suaveolens* on lipid metabolism in streptozotocin (STZ)-induced diabetic rats. The present study demonstrated that the ethyl-acetate fraction of *Stereospermum suaveolens* exhibits a potent antihyperlipidemic activity in hyperglycemic rats and suggests that the

plant may have therapeutic value in treating the diabetic complication of hyperlipidemia.[19]

Khanna *et al.*,(2002) studied the lipid lowering activity (LLA) of *Phyllanthus niruri* in triton and cholesterol fed hyperlipemic rats. Serum lipids were lowered by *P. niruri* extract orally fed (250 mg/kg b.w.) to the triton WR-1339 induced hyperlipemic rats. Chronic feeding of this drugs (100 mg/kg b.w.) in animals simultaneously fed with cholesterol (25 mg/kg b.w.) for 30 days caused lowering in the lipids and apoprotein levels of VLDL and LDL in experimental animals. The LLA of this drug is mediated through inhibition of hepatic cholesterol biosynthesis, increased faecal bile acids excretion and enhanced plasma lecithin: cholesterol acyltransferase activity.[20]

Veeramani *et al.*, (2012) investigated the antihyperlipidemic effect of crude ethanolic extract of *Melothria maderaspatana* leaf (CEEM) on deoxycorticosterone acetate (DOCA)-salt hypertensive rats. In DOCA-salt hypertensive rats, the level of plasma and tissues of total cholesterol (TC), triglycerides (TG), free fatty acids (FFA) and phospholipids (PL) significantly increased and administration of CEEM significantly reduced these parameters towards normality. Further, the levels of low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) significantly increased while high density lipoprotein cholesterol (HDL-C) decreased in hypertensive rats and administration of CEEM brought these parameters to normality which proved their antihyperlipidemic action. Histopathology of liver, kidney and heart on DOCA-salt induced rats treated with CEEM showed reduced the damages towards normal histology. Their findings provided evidence that CEEM was found to be protecting the liver, kidney and heart against DOCA-salt administration and the protective effect could attribute to its antihyperlipidemic activities.[21]

Umamaheswari *et al* .,(2012) evaluated the *in vitro* anticataract and antioxidant activities of ethanolic seed extract of *Abrus precatorius* Linn.

(Fabaceae) against calcium-induced cataractogenesis using goat lenses. Transparent isolated goat lens were incubated in artificial aqueous humor and divided into seven experimental groups. The extracts at a dose of 100 µg/ml and 200 µg/ml were incubated simultaneously with calcium chloride (10 mM) for a period of 16 h. Vitamin E (100 µg/ml) was used as the standard drug. At the end of the incubation, levels of various biochemical parameters such as protein content, malondialdehyde (MDA), lipid hydroperoxides (LH), Cu2+-induced lipoprotein diene formation, Ca2+ATPase and enzymatic antioxidants like catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione reductase (GSSH) and non-enzymatic antioxidant like reduced glutathione(GSH) were measured in the lens homogenate. Incubation with calcium produced a mature cataract and there was a significant increase in LH and MDA and a decrease in protein content, Ca2+ATPase, Cu2+-induced lipoprotein diene formation and enzymatic and non-enzymatic antioxidants when compared to normal control. Their results indicated that simultaneous incubation of the plant extracts prevented the preoxidative damage caused by calcium, which is evidenced from the improved antioxidant potential. The ethanolic seeds extract of *Abrus precatorius* protected the lens against calcium-induced oxidative damage which might be helpful in delaying the progression of cataract.[22]

Kamesh *et al.*,(2012) explored the effect of alcoholic extract of *Bacopa monniera* (AEBM) on high cholesterol diet-induced rats. AEBM treatment significantly decreased the levels of TC, TG, PL, LDL, VLDL, atherogenic index, LDL/HDL ratio, and TC/HDL ratio but significantly increased the level of HDL when compared to HCD induced rats. Activities on liver antioxidant status (SOD, CAT, GPx, GR, GST) were significantly raised with concomitant reduction in the level of LPO were obtained in AEBM treated rats when compared to HCD rats. Treatment with AEBM significantly lowered the activity of SGOT, LDH and CPK. Histopathology of aorta of cholesterol fed rat showed intimal thickening and foam cell deposition were noted. Their results suggested that AEBM extended

protection against various biochemical changes and aortic pathology in hypercholesterolemic rats. Thus the plant may therefore be useful for therapeutic treatment of clinical conditions associated hypercholesterolemia.(23)

Hariri and Thibault (2010) described the history of using high-fat diet to induce obesity in animals, aims to clarify the consequences of changing the amount and type of dietary fats on weight gain, body composition and adipose tissue cellularity, and explores the contribution of genetics and sex, as well as the biochemical basis and the roles of hormones such as leptin, insulin and ghrelin in animal models of dietary obesity. The major factors that contribute to dietary obesity – hyperphagia, energy density and post-ingestive effects of the dietary fat – are discussed. Other factors that affect dietary obesity including feeding rhythmicity, social factors and stress are highlighted. Finally, they commented on the reversibility of high-fat diet-induced obesity.(24)

Dhulasavant *et al*.,(2010) investigated the hypolipidemic effect of *Cinnamomum tamala* leaves extracts in high cholesterol diet induced hyperlipidemia. Aqueous and ethanolic extracts of leaves of *Cinnamomum tamala* were administered in doses of 400mg/kg /day p.o. each for 10 days. Simultaneous administration of *Cinnamomum tamala* 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.(25)

Ghule *et al* .,(2009) investigated the effect of methanolic extract from *Lagenaria siceraria* in experimentally induced hyperlipidemia in rats. Methanolic extract of *Lagenaria siceraria* fruits (LSFE) (100, 200 and 300 mg/kg; p.o.) was administered to the high fat-diet-induced hyperlipidemic rats for 30 days to

evaluate its antihyperlipidemic activity. Atorvastatin (10 mg/kg; p.o.) was used as a standard drug. At the 30th day, most significant reduction in lipid levels in the LSFE treated rats as compared to the rats fed with high-fat diet at the 0th day were: total cholesterol , low-density lipoprotein cholesterol, triglyceride, very low-density lipoprotein cholesterol ($P < 0.0001$). Conversely, high-density lipoprotein cholesterol levels were significantly ($P < 0.0001$) increased. The increase in weight in rats administered with LSFE was less when compared to rats fed with high-fat diet. Moreover, LSFE also exhibited significant increase in excretion of bile acids. Their results demonstrated that the LSFE has a definite antihyperlipidemic potential. There is also a valid scientific basis for consuming it in the treatment of coronary artery diseases in India.[26]

Kumar *et al.*,(2008) studied the hypolipidemic action of *Anthocephalus indicus* (family, Rubiaceae) fruit extract has been studied in hyperlipidemic rats fed a triton- and cholesterol-rich high-fat diet. In triton WR-1339-induced hyperlipidemic rats, feeding with the fruit extract (500 mg/kg b.w.) exerted a lipid-lowering effect as assessed by reversal of plasma levels of total cholesterol, phospholipids, and triglyceride following reactivation of the post-heparin lipolytic activity. In another model, chronic feeding of this natural product (500 mg/kg, b.w.) to animals simultaneously fed a high-fat diet for 30 days caused lowering of lipid levels in plasma and liver accompanied with stimulation of hepatic lipolytic activity. The hypolipidemic activity of *Anthocephalus indicus* fruit extract was compared with guggulipid, a known lipid-lowering drug, in both models.[27]

Kumari *et al* .,(2006) tested the total alcoholic extract of *Eclipta prostrata* for antihyperlipidemic potential, which exhibited a dose-dependent activity in albino rats when compared to standard drugs. The activity was assessed by studying the lipid profiles of serum, liver and heart of the control and drug-

treated animals. Their results lend support to the traditional use of *Eclipta prostrata* in the treatment of hyperlipidemia.[28]

Galati *et al* ., (1999) evaluated the effects of prolonged administration of a lyophilized stem decoction of *Salvadora persica* in diet-induced rat hypercholesterolemia. The preparation was administered for 15 and 30 days (by gavage at a dose of 500 mg/kg in an aqueous vehicle in a volume of 0.5 ml/100 g b.w.). Cholesterol, HDL, LDL and triglyceride plasma levels were assayed. Their results showed that the *S.persica* decoction significantly lowered cholesterol and LDL plasma levels in rats, proving to be more active at 30days of treatment. The systemic administration of Triton results in a rise in plasma cholesterol and triglyceride levels. The results obtained show that *S.persica* decoction was inactive at 18 hr after treatment, whereas at 27 hr, it was able to reduce cholesterol and LDL plasma levels. In all experiments HDL and triglycerides were unchanged.[29]

LITERATURE RELATED TO PLANT

Sheikh *et al.*,(2016) evaluated the different solvent plant extracts and essential oil of *Anisomeles malabarica* leaves and flowers for *in vitro* anti-inflammatory and anti-oxidant activity using different assay method. The *in vitro* anti-inflammatory activities were analyzed by; Heat Induced Hemolytic and Protein Denaturation method using Diclofenac sodium as a standard. Anti-oxidant potencies were ascertained by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and Iron chelating methods. Ascorbic acid was used as a standard. The percentage inhibition and stabilization values for anti-inflammatory and scavenging and chelation values for anti-oxidant were obtained and tabulated. The result clearly indicates that the methanolic extracts of both flower and leave of the study species shows effective anti-inflammatory and anti-oxidant properties. This scavenging free radical capacity can be used as a good anti-oxidizing agent in various fields of medicine, food industry etc.[30]

Packialakshmi and Periyakkal (2015) tested *Anisomeles malabarica* plant leaves for their FTIR spectroscopic studies revealed different characteristic peak values with various compounds in the leaf. The FTIR method was performed by spectrophotometer system, which was used to detect the characteristic peak values and their functional group of Amines, Ether, Mono substituted, Alkenes and nitro compounds. Leaf extracts of *Anisomeles malabarica* were evaluated for phytochemical screening to identify the active compounds.(31)

Unpaprom *et al.*, (2015) assessed the role of larvicidal activities of methanolic leaf and inflorescence extract of *Anisomeles malabarica* (L.) against malarial vector *Anopheles stephensi* Liston (Insecta: Diptera : Culicidae). The larvicidal activity was assayed against the mosquito species at various concentrations ranging from (20 to 100 ppm) under the laboratory conditions. The LC50 and LC90 value of the methanolic leaf and inflorescence extract of *Anisomeles malabarica (L.)* was determined by Probit analysis. The percentage of mortality of *Anopheles stephensi* after the treatment of *Anisomeles malabarica* on I to IV instar larvae from 20,40,60,80 to100 ppm. The LC50 and LC90 values were represented as follows: LC50 value of I instar was 50.24%, II instar was 54.70%, III instar was 59.03% and IV instar was 64.33%, respectively. LC90 value of I instar 106.99%, II instar was 113.33%, III instar was 118.04% and IV instar was 119.94%, respectively. The present results suggest that the effective *Anisomeles malabarica* leaf and inflorescence crude extracts have potential to be used as an ideal eco-friendly approach for the control of mosquito vectors.(32)

Nilofer and Packialakshmi (2014) evaluated the aqueous leaf, flower, stem and boiled leaf extracts of *Anisomeles malabarica* for antibacterial screening by disc diffusion methods along with their phytochemical screening. The *Anisomeles malabarica* extracts are sensitive to five pathogenic organisms such as *Staphylococcus aureus, Staphylococcus epidermis, E.coli, Pseudomonas aeroginosa* and *Bacillus substilis* except *Proteus vulgaris* by disc diffusion

methods. They carried out the phytochemical screening to identify the active compounds in the extracts.[33]

Packialakshmi and Nilofer (2014) reported the antibacterial activity of *Anisomeles malabarica*. Sequential extraction was carried out by using solvent such as ethanol, methanol, petroleum ether and aqueous extract from the leaf and boiled leaf of plant were investigated for preliminary antibacterial property against some pathogenic bacteria. In polar studies the maximum zone of inhibition were found in *Staphylococcus aureus*. In non-polar studies the maximum zone of inhibition were found in *Pseudomonas aeruginosa*. All these findings have confirmed the uses of this plant in a broad spectrum to treat several bacterial infections. Their results showed good antibacterial activity and it could play an important role in herbal formulations for the treatment of infectious diseases.[34]

Vinod *et al.*,(2014) investigated the preliminary bioactive phytochemicals present in the leaves extracts obtained by analytical standard hexane and ethanol solvents. The phytochemicals were analysed such as alkaloids, flavonoids, saponins, tannins and terpenoids from both the leaves extracts. Their study also extends to evaluate the antimicrobial activity of *Anisomeles malabarica* leaves extracts. The in vitro antimicrobial activity was performed by agar well diffusion method against the clinically important multi drug resistant bacterial strains viz., *Staphylococcus aureus* (NCIM 2492), *Bacillus subtillis* (NCIM 2439) and *Klebsiella pneumoniae* (NCIM 2719) with the concentration of extracts ranged from 25 to 75µL. It has shown the concentration dependent antimicrobial activity (MIC). Their study shows the powerful antimicrobial activity against Staphylococcus aureus and *Klebsiella pneumoniae* bacterial strains with maximum inhibitory zone compared with standard antibiotic drug tetracycline.[35]

Ramaraj and Unpaprom (2013) evaluated the larvicidal and pupicidal activities of crude methanol extract of *Anisomeles malabarica*. The extract was assayed for their toxicity against the important vector mosquitoes *Anopheles stephensi*. The plant extract showed larvicidal effects after 24 h of exposure; however, the highest larval and pupal mortality was found in the methanol extract of *Anisomeles malabarica* against the first to fourth instars larvae and pupae. Their result suggested that the plant extract have the potential to be used as an ideal eco-friendly approach for the control of mosquito vector. This paper gives a bird's eye view mainly on the biological activities, pharmacological actions, and plausible medicinal applications of *Anisomeles malabarica*. Different aspects of *Anisomeles malabarica* medicinal values are briefly demonstrated, such as potential anti-allergic, anti-anaphylactic, anti-bacterial, anticancer, anti-carcinogenic, anti-inflammatory, antiepileptic potential, antifertility, anti-pyretic activity and antispasmodic. (36)

Subramanian and Muthukrishnan(2013) investigated the antibacterial properties of various extracts of *Anisomeles malabarica* (L) against both clinical and laboratory isolates of bacteria using the disc diffusion method. Aqueous extracts (10 mm zone diameter of inhibition, MIC 10µg/mL) demonstrated the highest activity, followed by Ethanolic extract (8 mm zone diameter of inhibition, MIC 20µg/mL) methanolic extract demonstrated the least activity against the test bacteria (4 mm zone diameter of inhibition, MIC 40 µg/mL). Generally the antibacterial activities of *Anisomeles malabarica* are comparable to that of standard oflaxcin to a certain extent. Finally, these results suggested that *Anisomeles malabarica* (L) can be used to source antibiotic substances for possible treatment of bacterial infections and for the development of new drugs.(37)

Kavitha *et al* ., (2012) evaluated the *in vitro* antibacterial activity of leaf extracts of *Anisomeles malabarica* against *E.coli, S.aureus, P.mirabilis,*

P.aeruginosa, K.pneumonia. The preliminary phytochemmical analysis of both Ethanolic and Diethyl ether extracts revealed the presence of alkaloids, flavonoids, tannins, safonins, and glycosides. It was found that the ethanolic extract exhibited a maximum antibacterial activity at 200µg/ml and produced 25mm zone of inhibition against *S.aureus* where as Diethyl ether extract produced 30mm zone of inhibition in the same concentration. Their results provide justification for the use of *A. malabarica* to treat various infectious diseases.(38)

Choudhary *et al* .,(2011) isolated and evaluated the anti-epileptic potential of fractions from the ethyl acetate extract (EAE) of *Anisomeles malabarica* leaves.Thus, it may be concluded that the flavonoids fraction of the EA extract of *Anisomeles malabarica* leaves has antiepileptic potential against both MES and PTZ convulsion models. Acute treatment (25 and 50 mg/kg, i.p.) is associated with neurotoxic activity. Whereas, chronic treatment (6.25 and 12.5 mg/kg, i.p., 1 week) also shown significant antiepileptic effect without causing neurotoxic side effects. However, further research is in progress to determine the component(s) of the flavonoids fraction of *Anisomeles malabarica* involved and their mechanism of action in bringing about the desirable anti-epileptic effect.(39)

Vijayalakshmi and Ranganadhan(2011) tested the ethyl acetate extract of leaves from *Anisomeles malabarica* for *in vitro* antioxidant using various free radical scavenging assays. Free radical scavenging assays such as hydroxyl, superoxide anion radicals, 2,2-diphenyl-1-picryl hydrazyl (DPPH) and 2,2'-azinobis-(3-ethyl-enzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays were performed. It was observed that the extract effectively scavenged hydroxyl and superoxide anion radicals. It also scavenges DPPH and ABTS radicals. All the concentrations of leaf extract showed free radical scavenging and antioxidant power and the preventive effects were in a dose-dependent manner. Those various antioxidant activities were compared to standard antioxidants such as ascorbic acid, butylated hydroxytoluene (BHT) and á-tocopherol. Thus, the

results obtained in their present study indicated that *A. malabarica* extract could be considered as a potential source of natural antioxidants.[40]

Singh *et al.*,(2009) investigated the anticonvulsant potential of chloroform, ethyl acetate and methanol extracts of leaves of *Anisomeles malabarica* against pentylenetetrazole (PTZ) and maximal electroshock (MES) induced convulsions. All the three extracts were administered (i.e. 100, 200, 400 mg/kg, p.o.) for 7 days and at the end of the treatment convulsions were induced experimentally. Diazepam and phenytoin (1 mg/kg, i.p. and 25 mg/kg, i.p., respectively) were used as reference anticonvulsant drugs against experimentally induced convulsions. High doses (400mg/kg, p.o.) of chloroform and ethyl acetate extracts both significantly decreased the extent of MES- and PTZ-induced convulsions. On the other hand, ethyl acetate extract at lowest and medium selected doses (i.e. 100 mg/kg, p.o. and 200 mg/kg, p.o., respectively, for 7 days) had also significantly attenuated PTZ-induced convulsions. However, methanol extract at any of the doses used (i.e. 100, 200 and 400 mg/kg, p.o.) did not show any significant effect on PTZ- and MES-induced convulsions. None of the extracts at doses used in the present study have altered locomotor activity and motor coordination. Hence, it may be concluded that chloroform and ethyl acetate extracts of *Anisomeles malabarica* leaves are effective against PTZ- and MES induced-convulsions in rats.[41]

OBJECTIVE OF THE STUDY

Hyperlipidemia is a heterogeneous disorder characterized by an elevation in total cholesterol (TC), triglycerides (TG), very low density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), free fatty acids (FFA), and apolipoprotein B (apo B) levels, as well as reduced high-density lipoprotein cholesterol (HDL-C) levels. These disorders happen as a result of either metabolic disorders or dietary and lifestyle habits. Several studies have revealed that an decrease in HDL-C and increase in TC, LDL-C and TG are associated with an increase in the risk of ischemic heart diseases. Many drugs have been reported to possess hypolipidemic activity like bile acid binding resins, statins, fibrates, niacin and cholesterol absorption inhibitors which are the common treatment for hyperlipidemia. However, severe side effects are associated with the use of these drug for lipid-lowering medications.[3]

Many natural compounds were reported as hypolipidemic agents such as polyphenolics, flavonoids, tannins, alkaloids, phytosterol, unsaturated fatty acids and dietary fibers.[7]

The main objective of this study was to evaluate the *in vitro* cholesterol esterase inhibitory activity and *in vivo* antihyperlipidemic activity of Anisomeles malabarica leaf extract.

PLAN OF WORK

The work involved the following steps,

- Review of literature
- Plant collection and authentication of the leaves of *Anisomeles malabarica,*
- Preparation of cold the extract,
- Phytochemical screening,
- GC-MS analysis,
- Cholesterol esterase inhibitory activity using *in vitro* methods,
- Acute toxicity studies,
- Antihyperlipidemic activity of *Anisomeles malabarica* using *in vivo* methods in rat,
- Determination of serum biochemical parameters,
- Estimation of tissue protein and malondialdehyde in experimental animals.
- Estimation of liver and heart enzymatic antioxidants like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase in experimental animals,
- Estimation of liver and heart non-enzymatic antioxidants like reduced glutathione,
- Histopathological studies of the liver and heart tissues,
- Tabulation, statistical analysis and thesis writing.

MATERIALS AND METHODS

Experimental animals

Albino *wistar* rats of either sex weighing between 125-150 g were used for the study. The animals were housed in polypropylene cages inside a well-ventilated room. The room temperature was maintained at 23±2˚C with a 12 h light / dark cycle. The animals were fed with commercial rat feed pellets and provided with drinking water *ad libitum*. All animal procedures have been approved by ethical committee in accordance with animal experimentation and care.

Drugs and chemicals

Cholesterol Esterase and pNPB (P-nitro phenyl butyrate) were purchased from the Sigma Aldrich, USA. Acetonitrile, taurocholate were purchased from Loba chemicals, Mumbai. Gallic acid were purchased from HiMedia, Mumbai. High Fat Diet (HFD) food for rats were purchased from RSM Hitech Feeds, Namakkal. Atorvastatin is used as a standard drug were purchased from Medopharm, Chennai. Cholesterol, triglycerides, HDL, LDL, AST, ALP, ALT and LDH levels were determined using standard kits obtained from Agappe diagnostics Pvt., Ltd., Kerala.

Instrument used

Digital balance (Sartorius Ltd, USA), UV (Jasco UV-spectometry), pH meter (Elico) and GC-MS.

Plant material

The plant material consists of dried powdered leaves of *Anisomeles malabarica* L. belonging to the family Lamiaceae.

Plant collection and authentication

The leaves were collected near Dharapuram, dry region of Thirupur district, Tamil Nadu, India. The plant was identified and authenticated by Dr. M. Palanisamy, scientist 'D'-In-charge in Botanical Survey of India, T.N.A.U. Campus, Coimbatore, Tamil Nadu bearing the reference number BSI/SRC/5/23/2017/TECH.2814.

Plant extraction

The leaves were separated and dried in shade under room temperature, powdered mechanically and sieved through No.20 mesh sieve. The finely powdered leaves were kept in an airtight container until the time of use.

In the cold maceration process, about 25 gm of the whole or coarsely powdered crude drug is placed in a stoppered container with the 70% ethanol and 30% water and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. On the fourth day the mixture is then strained, the marc is pressed, and the combined liquids are clarified by filtration or decantation after standing.

Phytochemical screening

Chemical tests were carried out for the various fractions of *Anisomeles malabarica* for the presence of phytochemical constituents .[43]

Test for alkaloids

To a little of plant leaves extract a few drops of Mayer's test reagent was added. Formation of precipitate indicates presence of alkaloids.

Test for flavonoids

1ml of extract was taken and few drops of very dilute solution of ferric chloride were added. The color changed to pale green or red brown color which indicates the presence of flavonoids.

Test for saponins

One ml extract and one ml alcohol diluted with 20 ml distilled water and shaken well for 15 minutes. The formation of 1cm layer of foam indicated the presence of saponins.

Test for carbohydrates

Small amount of extract was dissolved separately in 5 ml distilled water and filtered. The filtrate was subjected to molisch's test. Formation of reddish brown ring indicates the presence of carbohydrate.

Test for tannins

To 5ml of extract solution, 1ml of lead acetate solution was added. Flocculent brown precipitate indicates the presence of tannins.

Test for glycosides

A small portion of extract was hydrolyzed with hydrochloric acid for few hours on a water bath and the hydolysate was subjected to legal's test to detect the presence of different glycosides.

Legal's test

To the hydrolysate 1ml of sodium nitroprusside solution was added and then it was made alkaline with sodium hydroxide solution. If the extract produced pink to red color, it indicates the presence of glycosides.

Test for fixed oils and fats

Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hrs. Formation of soaps or partial neutralization of alkali indicates the presence of fixed oils and fats.

Test for phenols (ferric chloride test)

A fraction of the extract was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black color.

Test for gums and mucilage

About 10ml of extract was added to 25ml of absolute alcohol with stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates.

Test for amino acids and proteins (1% Ninhydrin solution in acetone)

2ml of filtrate was treated with 2-5 drops of Ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple color.

Gas Chromatography -Mass Spectroscopy Analysis

Derivatization procedure: [44]

Two procedures were followed. For the crude ethanol ether and ethanol extracts, a small amount of concentrated sample was taken in a separating funnel and shaken by adding water and ethyl acetate in the ratio of 1:4. The upper layer was collected and concentrated in rotary evaporator to about 1.5 ml. Added 100µl N, O-Bis(trimethylsilyl) trifluoroacetamide and trimethyl chlorosilane (BSTFA+TMCS) and 20µl pyridine and heated at 60°C for 30 minutes.

For the layers which are separated from the crude extracts, a small amount of extract was taken and evaporated out totally. To this added acetonitrile and filtered into a conical flask. To the filtrate added 50µl BSTFA+TMCS and heated at 60°C in a water bath for 30 minutes. Filtered using 0.45µ membrane filter to a vial.

GC-MS Analysis:

GC-MS analysis was carried out on a Perkin Elmer Turbo Mass Spectrophotometer (Norwalk, CTO6859, and USA) which includes a Perkin Elmer Auto sampler XLGC. The column used was Perkin Elmer Elite - 5 capillary column measuring 30m × 0.25mm with a film thickness of 0.25mm composed of 95% Dimethyl polysiloxane. The carrier gas used was Helium at a flow rate of 0.5ml/min. 1µl sample injection volume was utilized. The inlet temperature was maintained as 250°C. The oven temperature was programmed initially at 110°C for 4 min, then an increase to 240°C. And then programmed to increase to 280°C at a rate of 20°C ending with a 5 min. Total run time was 90 min. The MS transfer line was maintained at a temperature of 200°C. The source temperature was maintained at 180°C. GCMS was analyzed using electron impact ionization at 70eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library. Measurement of peak areas and data processing were carried out by Turbo-Mass-OCPTVS-Demo SPL software.[44]

***In vitro* Cholesterol esterase inhibitory activity:**

Enzyme inhibition assay is performed in presence of sodium taurocholate with p-nitrophenyl butyrate as chromogenic substrate.[45] Hydrolysis is performed in the presence of high enzyme concentration and products are identified spectrophotometrically. Assay buffer is sodium phosphate buffer (pH-7.0) which is made by adding 100 mM sodium phosphate with 100mM Nacl in deionised water.

Stock solution of cholesterol esterase (19.5 ng/mL) and taurocholate (12mM) were prepared by using (100mM) sodium phosphate buffer of pH-7.0. Stock solution of pNPB (200µM), flavonoid compounds and standard of different concentrations (10-320µg/mL) are prepared by using acetronitrile (6%). A final volume of 1 ml is taken into a cuvette containing 430µL of assay buffer, 500µL of taurocholate solution, 40µL of acetonitrile 10µL of pNPB solution and 10µL of flavonoid solution are added and thoroughly mixed. Incubate for 2 minutes at 25° C, the reaction is initiated by adding 10µL of enzyme solution. The absorbance is measured at 405 nm against a blank.

Percentage inhibition is calculated by using formula,

$$\text{Cholesterol inhibition } \%\text{ I} = \frac{[1 - \text{enzyme activity with inhibitor}]}{\text{Enzyme activity without inhibitor}} x\,100$$

Uninhibited enzyme activity is determined by acetonitrile instead of the inhibitor solution. Control absorbance is measured by adding 100mM sodium phosphate pH 7, instead of enzyme. Gallic acid is used as the reference standard.[45]

Acute toxicity study of AMLE

Acute oral toxicity testing was carried out in accordance with the OECD guideline 420 Acute Oral Toxicity – Fixed Dose Procedure method (OECD, 2002).

Procedure

The acute toxicity study was done by two steps - Sighting study and Main study.

The purpose of the sighting study is to allow selection of the appropriate starting dose for the main study.

Sighting study was conducted using one animal and main study using five animals, in which one animal is taken from the sighting study. Healthy adult female (generally slightly more sensitive than male) Albino *wistar* rats weighing between 220±20%g body weight were procured and kept in cages under ambient temperature (22±3°C) with 12 h light/dark cycle. The animals were randomly selected, marked and kept in their cages for 5 days prior to dosing for acclimatization to laboratory conditions. An animal was fasted over-night but water provided *ad libitum* and received a single dose (2000mg/kg, body weight, *p.o.*) of *Anisomeles malabarica* leaf Extract. After the administration of the extract, food was withheld for further 3-4h.

Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal), and also respiratory rate, circulatory (heart rate and blood pressure), autonomic(salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (drowsiness, gait, tremors and convulsions) changes.

If the animal dosed with 2000mg/kg b.w. during the sighting study survives and remains so without any toxic manifestations then the same dose is selected for the main study. The main study was done in 4 animals other than that taken for the sighting study (a total of 5 animals). The procedure of the main study is similar to that of the sighting study.

Table 5: Design of acute toxicity studies

Study involved	Dose (mg/kg)	No. of animals
	5	1

Sighting study	50	1
	300	1
	2000	1
Main study	2000	5

Selection of dose of the extract

LD_{50} was done as per OECD guidelines for fixing the dose for biological evaluation. The LD_{50} 0f the fractions as per OECD guidelines falls under category 4 values with no signs of acute toxicity at doses of 2000 mg/kg. The biological evaluation of the fractions was carried out at a dose of 200 mg/kg body weight.

High fat diet fed rats of hypercholestremia in rats

Rat was fed with two dietary regimes such as Normal Pellet Diet (NPD) and High fat Diet (HFD). The rat was feeding either NPD or HFD *ad libitum*, respectively, for the period of 50 days.

Composition of High Fat Diet (25 kg)

Maize	:	5 kg
Soya meal	:	5 kg
Coconut cake	:	3 kg
Rice Polish	:	2 kg
Groundnut cake	:	5 kg
Tallow (Animal Fat)	:	5 kg

Experimental Design:

Healthy male or female Albino *wistar* strain weighing (125-150) g were used for the present study. The animals were housed in large spacious cages. Food

and water were given *ad libitum*. The animal house was ventilated with a 12 h light/dark cycle, throughout the experimental period. Rats were allowed to adapt to their environment condition for at least 10 days before the initiation of experiment.(46)

The rats were randomly divided into six groups of six rats each.

Group I:

Control rats fed with normal diet for 50 days.

Group II:

Rats fed with hypercholesterolemic diet (HCD) for 50 days.

Group III:

Rats fed with HCD for 50 days + administrated with low dose of plant extract (100mg/kg, body weight/day orally) for last 35 days.

Group IV:

Rats fed with HCD for 50 days + administrated with High dose of plant extract (200mg/kg, body weight/day orally) for last 35 days.

Group V:

Rats fed with normal diet for 50 days + administrated with plant extract (200 mg/kg, body weight/day orally) for last 35 days.

Group VI:

Rats fed with HCD for 50 days+ administered with standard drug (10 mg kg^{-1} body weight/day orally) for last 35 days.

Measurement of Body weight, Food intake and Water Consumption

In addition, the food intake and water consumption of rats in all the groups were noted down.

Blood Collection:

At the end of the experimental period (50 days), blood was collected by retro-orbital puncture under mild ether anaesthesia. The blood was allowed to clot for approximately 1 hour at room temperature and then centrifuged at 5000 rpm for 10 min to obtain the serum. The levels of serum cholesterol, triglycerides, high density lipoprotein, low density lipoprotein, very low density lipoprotein, aspartate transaminase, alanine transaminase, alkaline phosphatase and lactate dehydrogenase, were determined using a semi-autoanalyser.[47]

Serum parameters

1. Alkaline phosphatase (ALP)
2. Aspartate transaminase (AST)
3. Alanine transaminase (ALT)
4. Lactate dehydrogenase (LDH)
5. High density Lipoprotein (HDL)
6. Low density Lipoprotein(LDL)
7. Very low density lipoprotein.(VLDL)
8. Triglycerides (TG)
9. Total cholesterol (TC)

1. Alkaline phosphatase

Principle

Kinetic determination of ALP according to the following reaction

Para-nitrophenyl phosphate + H_2O $\xrightarrow{ALP}$ p-nitrophenol + Inorganic phosphate

ALP is widely distributed throughout the body, but clinically important one for diagnostic reasons are in bone, liver, placenta and intestine.

Reagent preparation

Mix four volumes of reagent 1 (R1), which contains diethanolamine buffer (pH 10.2) 125 mmol/L, magnesium chloride 0.625 mmol/L with one volume of reagent 2 (R2) contains P-Nitro phenyl phosphate 50 mmol/L. This working reagent is stable for 30 days at 2-8°C.

Procedure

To 20 µl of sample, 1 ml of working reagent was added. Mix and incubate at 37°C for one minute. Measure the change in absorbance per minute during 3 minutes at 405nm.

Aspartate transaminase

Principle

Kinetic determination of aspartate aminotransferase (AST) based upon the following reaction.

L-Aspartate + alpha-ketoglutarate $\xrightarrow{AST}$ Oxaloacetate + L-Glutamate

Oxaloacetate + NADH + H^+ $\xrightarrow{MDH}$ L-Malate + NAD^+

MDH: Malate dehydrogenase

Reagent preparation

Mix four volume of reagent 1 (R1) contains tris buffer (pH 7.8) 88 mmol/L, L-aspartate 260 mmol/L, LDH>1500 U/L, MDH>900 U/L with one volume of reagent 2 (R2) contains alpha-ketoglutarate 12 mmol/L, NADH-0.24 mmol/L. This working reagent is stable for 30 days at 2-8°C.

Procedure

To 100 µl of sample, 1 ml of working reagent was added. Mix and incubate at 37°C for one minute. Measure the change in absorbance per minute during 3 minutes at 340nm.

Alanine transaminase

Principle

Kinetic determination of Alanine transaminase (ALT) according to the following reaction.

$$\text{L-Alanine} + \text{alpha-ketoglutarate} \xrightarrow{\text{MDH}} \text{Pyruvate} + \text{L-Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-Lactate} + NAD^+$$

LDH- Lactate dehydrogenase

Reagent preparation

Mix four volume of reagent 1 (R1) contains tris buffer (pH 7.5) 110 mmol/L, L-alanine 600 mmol/L, LDH>1500 U/L, with one volume of reagent 2 (R2) contains alpha-ketoglutarate 16 mmol/L, NADH-0.24 mmol/L. This working reagent is stable for 30 days at 2-8°C.

Procedure

To 100 µl of sample, 1 ml of working reagent was added. Mix and incubate at 37°C for one minute. Measure the change in absorbance per minute during 3 minutes at 340nm.

Lactate dehydrogenase

Principle

Kinetic determination of lactate dehydrogenase according to the following reaction.

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-Lactate} + NAD^+$$

Reagent preparation

Mix four volume of reagent 1 (R1) contains tris buffer (pH 7.4) 80 mmol/L, pyruvate 1.6 mmol/L, sodium chloride 200 mmol/L with one volume of reagent 2 (R2) contains NADH- 240 mmol/L. This working reagent is stable for 21 days at 2-8°C.

Procedure

To 10 µl of sample, 1 ml of working reagent was added. Mix and incubate at 37°C for one minute. Measure the change in absorbance per minute during 3 minutes at 340nm.

These parameters were determined using standard kits obtained from Agappe Diagnostics, Cochin, India using a Semi Auto-analyser (Mispa Viva, Mumbai, India).

Triglycerides

Principle: Enzymatic determination of triglycerides;

- TGL reacting with water in the presence of lipoprotein lipase to gives Glycerol and Fatty acid.

- Glycerol is reacting with ATP in the presence of glycerol kinase to gives Glycerol -3- phosphate and ADP.
- Glycerol -3- phosphate reacting with 2 molecules of oxygen in the presence of Glycerol -3- phosphate oxidase gives dihydroxyacetophenone and 1 molecule of hydrogen peroxide.
- 2 molecules of hydrogen peroxide reacting with 4- aminoantipyrine and TOPS gives violet coloured complex.

Procedure:

Mix the triglyceride reagent (pipes buffer-5mmol/L, TOPS-5.3MMOL/L, Potassium ferrocyanate-10mmol/L, Magnsium salt- 17mmol, 4- aminoantipyrine-0.8mmol/L, ATP-3.15mmol/L, lipoprotein lipase >1800 U/L, Glycerol-3-phosphate oxidase >3500 U/L, Peroxidase >450) with triglycerides standard concentration- 200mg/dL .Read the initial absorbance at 405 nm, after one minute repeat the absorbance reading after every 1, 2, and 3 minutes.

High Density Lipoprotein

Principle:

The reaction between cholesterol other than HDL and the enzyme for cholesterol assay is suppressed by the electrostatic interaction between polyanions and cationic substances. Hydrogen peroxide is formed by the free cholesterol in HDL by cholesterol oxidase. Oxidative condensation of EMSE and 4-AA is caused by hydrogen peroxide in the presence of peroxidase, and the absorbance of the resulting red- purple quinone is measured to obtain the cholesterol value in HDL. HDL in the presence of polyanions and cationic substances suppress the reaction with enzyme. HDL (cholesterol esters) reacts with one water molecule in the presence of cholesterol esterase gives HDL (free cholesterol) and fatty acids. HDL (free cholesterol) reacts with two molecules of oxygen and one molecule of hydrogen ion in the presence of cholesterol oxidase gives cholestenone and

hydrogen peroxide. Two molecules of hydrogen peroxide react with 4-AA, EMSE, three molecules of hydrogen and one molecule of oxygen in the presence of peroxidase gives violet quinone and five molecules of water.

Procedure:

Mix the reagent-1 (R1: N-ethyl-N-(3-methylphenyl)-N-succinylehyenediame (EMSE) and reagent-2 (R2: cholesterol oxidase 4-aminoantipyrin(4-AA).Absorbance was recorded against reagent blank at 600 nm.

LOW DENSITY LIPOPROTEIN

Principle: This assay method uses a surfactant for selectively solubilizing LDL alone in the cholesterol assay system that employs cholesterol esterase and cholesterol oxidase. It passes the ester cholesterol and free cholesterol contained in the LDL to the cholesterol reaction system to determine LDL cholesterol. The enzyme reactions to other, non-LDL lipoproteins (HDL, VLDL, chilomicrons) are inhibited by the surfactant and by the sugar compounds. These lipoproteins are therefore not passed to the cholesterol reaction system and consequently remain in the reaction liquid as lipoproteins.

Procedure: Mix the reagent-1(R1: HSDA-1mmol/L, good's buffer) and reagent-2 (cholesterol esterase- 2.0U/L, cholesterol oxidase- 1.0mmol/L, 4-Aminoantipyrin-2.5mmol/L and good's buffer). Absorbance was recorded against reagent blank at 600 nm.

VERY LOW DENSITY LIPOPROTEIN

The value of VLDL was calculated by = Triglycerides/5

TOTAL CHOLESTEROL

To 1 ml of reagent solution (10 mM sodium cholate, 0.82 mM 4-aminophenazone, 6 mM phenol, 33 U/L cholesterol hydrolase, 250U/L cholesterol oxidase, 200 U/L horseradish peroxidase in 100 mM Tris buffer, (pH 7 .7) 10 pi serum was added. A reagent blank solution similar to reagent solution without the cholesterol ester hydrolase and cholesterol oxidase was prepared. Incubation was for about 10 min at 37°C. Absorbance was recorded at 500 nm.

Preparation of tissue homogenates

The brain was removed and washed immediately with ice-cold saline to remove blood. A 10% w/v brain homogenate was prepared in ice-cold potassium phosphate buffer (100mM, pH 7.4) followed by centrifugation at 5000g for 10 min. the resulting supernatant was used for the estimation of biochemical parameters.

A. Biochemical parameters

1. Estimation of total protein content
2. Estimation of malondialdehyde (MDA)

B. Determination of enzymatic antioxidants

3. Assay of catalase
4. Estimation of glutathione peroxidase (GPx)
5. Assay of superoxide dismutase (SOD)
6. Estimation of glutathione reductase (GSSH)

C. Determination of non-enzymatic antioxidants

7. Estimation of reduced glutathione (GSH)

A. Biochemical parameters

1. Estimation of total protein content

To 0.1ml of brain homogenate, 4.0 ml of alkaline copper solution was added and allowed to stand for 10 min (Lowry *et al., 1951*). Then, 0.4 ml of

phenol reagent was added very rapidly and mixed quickly and incubated in room temperature for 30 min for colour development. Reading was taken against blank prepared with distilled water at 610 nm in UV-visible spectrophotometer. The protein content was calculated from standard curve prepared with bovine serum albumin and results were expressed as µg/mg brain tissue.[48]

2. Estimation of malondialdehyde (MDA)

One ml of the homogenate was combined with 2 ml of TCA-TBA-HCl reagent (15% trichloro acetic acid (TCA) and 0.375% thiobarbituric acid (TBA) in 0.25 N HCl) and boiled for 15 min. precipitate was removed after cooling by centrifugation at 1000g for 10 min and absorbance of the sample was read at 535 nm against a blank without tissue homogenate. The levels of MDA were calculated using extinction coefficient calculation. The values are expressed as nmoles/min/mg brain tissue.[49]

B. Determination of enzymatic antioxidants

3. Assay of catalase

The reaction mixture contained 2.0 ml of homogenate and 1.0 ml of 30 mM hydrogen peroxide (in 50 mM phosphate buffer, pH 7.0). a system devoid of the substrate (hydrogen peroxide) served as a control. Reaction was started by the addition of the substrate and decrease in absorbance mentioned at 240 nm for 30 seconds at 25°C. the difference in absorbance per unit time was expressed as the activity and three parallel experiments were conducted. One unit is defined as the amount of enzyme required to decompose 1.0 M of hydrogen peroxide per minute at pH 7.0 and 25°C.[50]

4. Estimation of glutathione peroxidase (GPx)

The reaction mixture consists of 0.2 ml of 0.4 M Tris buffer, 0.1 ml of 1.0 mM sodium azide, 0.1 ml of 0.042% hydrogen peroxide, 0.2 ml of 200 mM glutathione and 0.2 ml of brain tissue homogenate supernatant incubated at 37°C for 10 min. The reaction was arrested by the addition 0.1 ml of 10% TCA and the absorbance was taken at 340 nm. Activity was expressed as nmoles/min/mg brain protein.(51)

5. Assay of superoxide dismutase (SOD)

The assay mixture contained 1.2 ml of sodium pyrophosphate buffer (0.052 M, pH 8.3), 0.1 ml of 186 µM Phenazonium methosulphate (PMS), 0.3 ml of 300 µM nitro blue tetrazolium chloride (NBT), 0.2 ml of 780 µM NADH, 1.0 ml of homogenate and distilled water to a final volume of 3.0 ml. reaction was started by the addition of NADH and incubated at 30°C for 1 min. the reaction was stoped by the addition of 1.0 ml of glacial acetic acid and the mixture was stirred vigorously. 4.0 ml of n-butanol was added to the mixture and shaken well. The mixture was allowed to stand for 10 min, centrifuged, the butanol layer was taken out and absorbance was measured at 560 nm against a butanol blank. A system devoid of enzyme served as the control and three parallel experiments were conducted. (52)

6. Estimation of glutathione reductase (GSSH)

The enzyme activity was determined spectrophotometrically by the decrese in absorbance of NADPH at 340 nm. The reaction mixture contained 2.1 ml of 0.25 mM potassium phosphate buffer pH 7.6, 0.1 ml of 0.001 M NADPH, 0.2 ml of 0.0165 M oxidized glutathione and 0.1 ml of bovine serum albumin (10 mg/ml). The reaction was started by the addition of 0.02 ml of brain tissue homogenate with mixing and the decrease in absorbance at 340 nm was measured

for 3 min against a blank. Glutathione reductase activity was expressed as nmoles/min/mg brain protein at 30°C. (53)

C. Determination of non-enzymatic antioxidants

Estimation of reduced glutathione (GSH)

Brain was homogenized in 10% w/v cold 20mM EDTA solution on ice. After deproteinization with 5% TCA, an aliquot of the supernatant was allowed to react with 150 µM DTNB. The product was detected and quantified spectrophotometrically at 416 nm. Pure GSH was used as standard for establishing the calibration curve and three parallel experiments were conducted.(54)

Histopathological studies

The portions of heart and liver were immersed in 10% formalin for 24 h for histopathological examination. The specimens were cleared in xylene and embedded in paraffin. Paraffin bees wax blocks were prepared and cut into 5µM thick sections. The obtained tissue sections were mounted on glass slides and stained with hematoxylin and eosin for histopathological examination using a light microscope.

Statistical analysis

The results would be statistically analyzed by One way-ANOVA followed by Tukey's test using GraphPad Instat software. The values would be expressed as mean ± SEM. $P<0.05$ would be considered statistically significant when compared to standard and negative control.

RESULTS

PHYTOCHEMICAL SCREENING

Phytochemical screening of powdered leaves of *Anisomeles malabarica* showed the presence of alkaloids, flavonoids, saponins, tannins, sterols, oil and fat, phenolic compound, protein and amino acid, gums and mucilages, carbohydrates, and glycosides.(Table-6)

Table 6: Phytochemical screening

S.No	Phytochemical	Interpretation
1	Alkaloid	+
2	Flavonoids	+
3	Saponins	+
4	Tannins	+
5	Sterols	+
6	Oil and Fat	-
7	Phenolic compound	+
8	Protein and Aminoacid	+
9	Gums and mucilage	+
10	Carbohydrates	+
11	Glycosides	+

(+) Present; (-) Absent

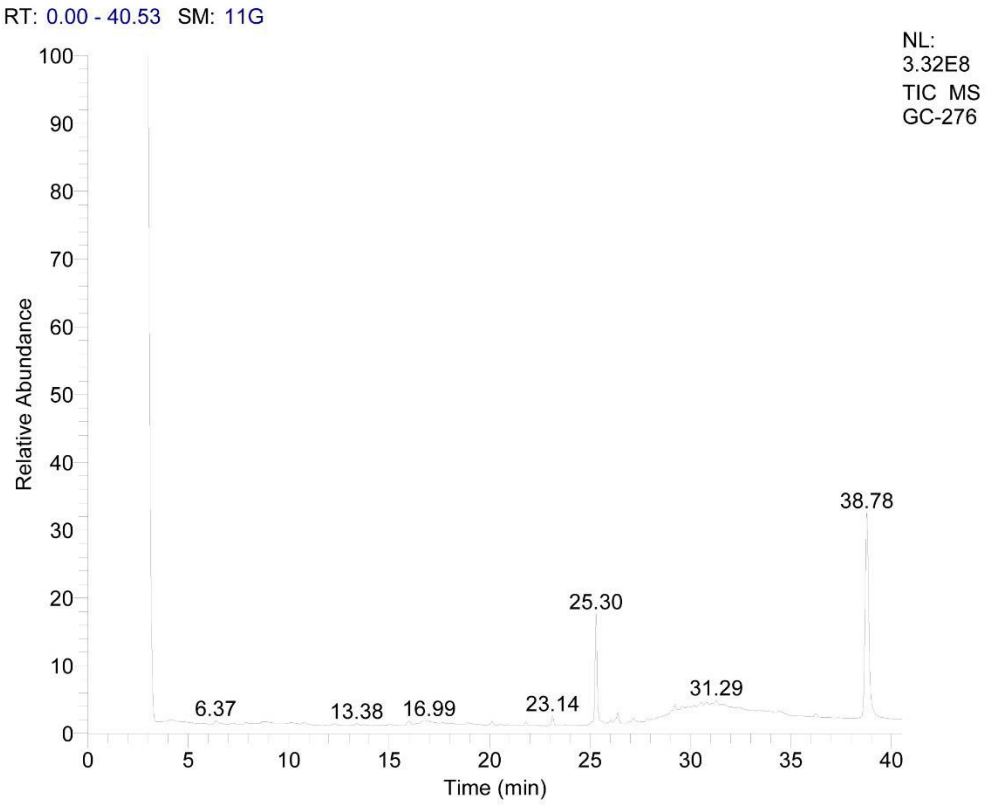
GC-MS Analysis

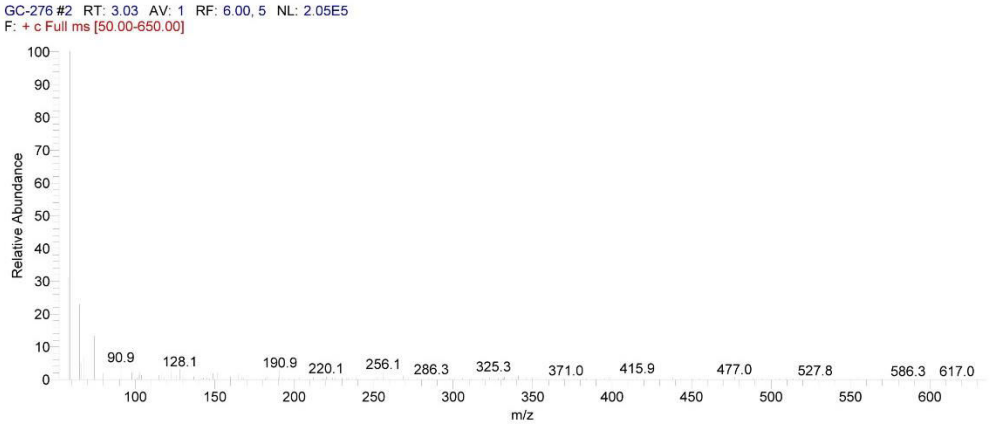
Gas chromatography mass spectroscopy analysis was carried out in a crude leaf ethanolic extract of *Anisomeles malabarica*. The total ion chromatogram (TIC) of ethanol extract of *A.malabarica* showing the GC-MS profile of the compounds were identified. The peaks in the chromatogram were integrated and were compared with the database of spectrum of known components stored in the GC-MS library.

The detailed tabulations of GC-MS analysis of the extracts are given below. Phytochemical analysis by GCMS analysis of the plant extract revealed the presence of different fatty acids, heterocyclic compounds etc.

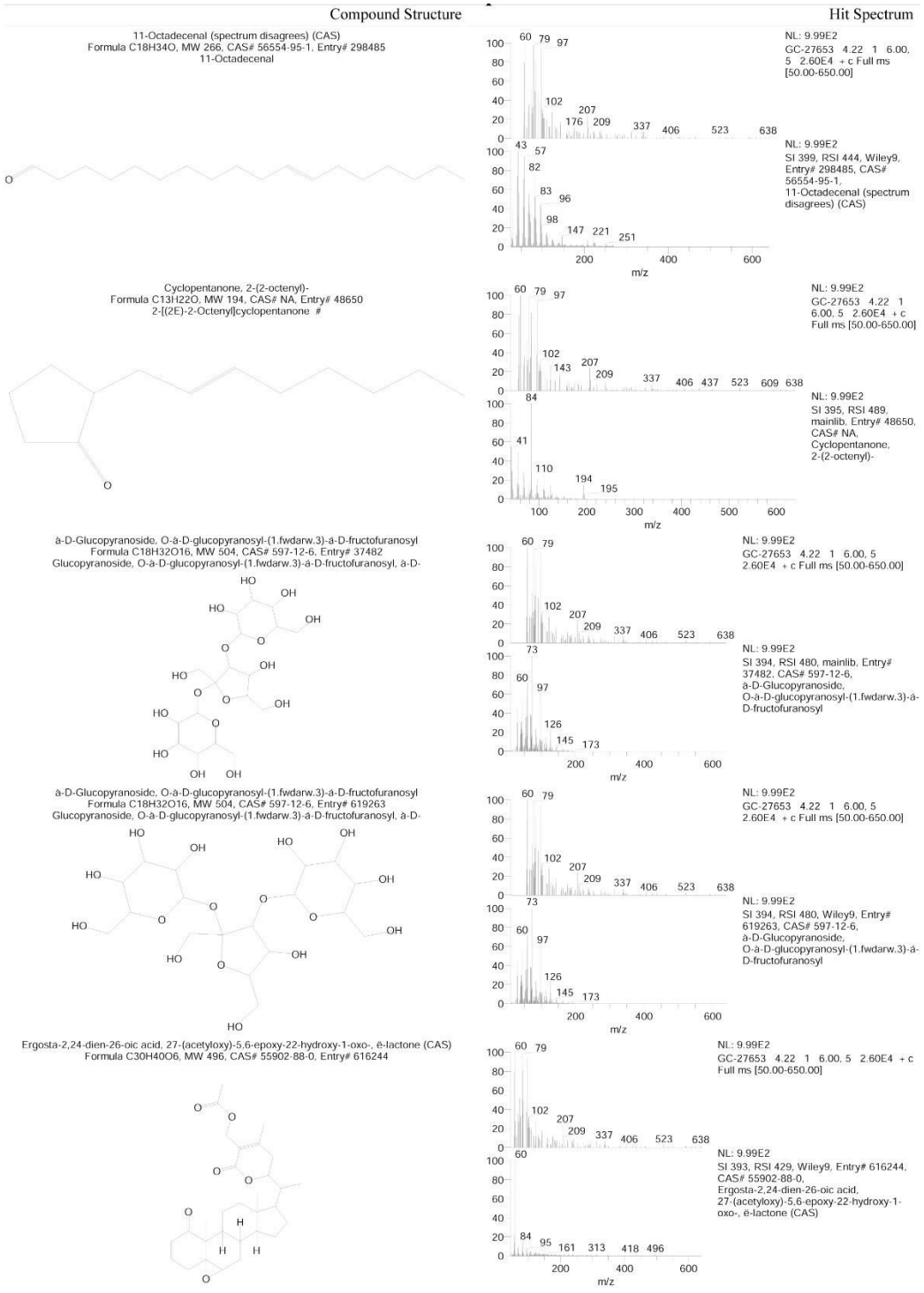
EQUIPMENT : THERMO GC - TRACE ULTRA VER: 5.0,
THERMO MS DSQ II
COLUMN : DB 35 - MS CAPILLARY STANDARD NON - POLAR COLUMN
DIMENSION : 30 Mts, ID : 0.25 mm, FILM : 0.25 µm
CARRIER GAS : He, FLOW : 1.0 ML/Min
TEMP PROG : OVEN TEMP 70 C RAISED TO 260 C AT 6 C /MIN

INJECTION
VOLUME : 1 MICRO LITER





SI	RSI	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %
512	940	Formamide, N-formyl-N-methyl-	19.21	C3H5NO2	87	0.62
509	931	FORMAMIDE, N-FORMYL-N-METHYL-	19.21	C3H5NO2	87	0.62
495	907	ACETOFLUORONITRILE	10.48	C2H2FN	59	0.62
493	823	threo-3,4-Epoxy-4-methyl-2-pentanol	9.67	C6H12O2	116	0.62
487	873	1-Propanamine (CAS)	7.60	C3H9N	59	0.62
477	832	meso-3,4-Hexanediol	5.36	C6H14O2	118	0.62
477	792	9à-HYDROXY-3-METHYL-3-AZABICYCLO(3.3.1)NONANE-9á-D	5.36	C9H16DNO	155	0.62
476	831	meso-3,4-Hexanediol	5.36	C6H14O2	118	0.62
476	792	Tetraethylene glycol monomethyl ether	5.15	C9H20O5	208	0.62
468	730	N-TRIMETHYLAMMONIO-N'-4-METHYLPHENYLSULFONYLFORMAMIDINATE	3.84	C11H17N3O2S	255	0.62



IN VITRO STUDIES

Cholesterol esterase inhibitory activity

The ethanolic leaf extract of *Anisomeles malabarica* was studied for its cholesterol esterase inhibitory activity at various concentrations ranging from 10, 20, 40, 80, 160, and 320 µg/ml. The absorbance of the mixture was measured at 405nm. It was observed that there is a dose dependent increase in the percentage inhibition from the concentration 10µg/ml to 320 µg/ml and the values are shown Table-7. IC_{50} values of the extract was calculated and compared with the standard Gallic acid.

For the extract of AMLE IC_{50} value was calculated and was found to be 46.66±11.66µg/ml.Gallic acid is used as a reference standard and IC_{50} value was found to be 45.66±10.039µg/ml.

Table 7: Cholesterol esterase inhibitory activity of Gallic acid

Concentration (µg/ml)	% Inhibition			Mean ± SEM	IC_{50}(µg/ml)
	I	II	III		
5	3.72	26.69	51.52	27.31±13.802	45.66±10.039
10	6.07	38.78	57.78	34.21±15.101	
20	43.0	23.83	46.77	37.866±7.102	
40	20.02	38.78	56.92	38.573±10.653	
80	28.6	32.46	69.04	43.366±12.885	
160	26.15	62.23	65.53	51.303±12.613	
320	37.51	52.42	69.24	53.056±9.165	

Results are Mean ± SEM of two parallel measurements values

Table 8: Cholesterol esterase inhibitory activity of AMLE

Concentration(µg/ml)	% Inhibition			Mean ± SEM	IC_{50}(µg/ml)
	I	II	III		
5	16.06	40.09	58.14	38.096±12.188	46.66±11.667
10	20.88	50.98	58.76	43.54±11.550	
20	71.29	58.16	24.84	51.43±13.825	
40	34.74	52.21	61.88	49.61±7.942	
80	42.03	49.02	60.34	50.46±5.335	
160	25.9	59.93	64.48	50.10±12.173	
320	37.64	53.56	69.33	53.51±9.148	

Results are Mean ± SEM of two parallel measurements values.

IN VIVO STUDIES

Acute toxicity studies and selection of dose for *in vivo* studies

Acute toxicity was determined as per OECD guidelines (420) using fixed dose procedure for fixing the dose for biological activity. For acute toxicity studies ten female rats weighing 150-200g were taken and they were fasted overnight before the experimental day. Overnight fasted rats were weighed and body weight determined for dose calculation and the test samples (AMLE) were administered orally starting with a lower dose of 5 mg/kg in 0.5 % CMC and the dose was gradually increased in a sequence of 50, 300 and 2000 mg/kg and the signs and symptoms of toxicity and death if any were observed individually at 0, 0.5, 1, 2, 3 and 4h for first 24 h and thereafter daily for 14 days. Food was given to the animals after 4 h of dosing. The animals were observed twice daily for 14 days and body weight changes, food and water consumption, etc.were noted.

In acute toxicity studies, it was found that the animals were safe up to a maximum dose of 2000 mg/kg of body weight. There were no changes in normal behavioral pattern and no signs and symptoms of toxicity and mortality were observed up to the dose of 2000 mg/kg body weight Table-9 & Table-10. Hence, as per the OECD 420 guidelines AMLE can be included in the category 5 or unclassified category of globally harmonized classification system (GHS). Hence, based on these results the AMLE was considered non-toxic and for the biological evaluation the dose of 50 and 100 mg/kg body weight was selected.

Table 9 : OBSERVATIONS DONE FOR THE ACUTE ORALTOXICITY STUDY OF AMLE

Parameters observed		0 h	0.5h	1 h	2 h	4 h	Day 2&3	Day 4&5	Day 6&7	Day 8&9	Day 10&11	Day 12&13	Day 14
Respiratory	Dyspnea	-	-	-	-	-	-	-	-	-	-	-	-
	Apnea	-	-	-	-	-	-	-	-	-	-	-	-
	Nostril discharges	-	-	-	-	-	-	-	-	-	-	-	-
Motor activity	Tremor	-	-	-	-	-	-	-	-	-	-	-	-
	Hyper activity	-	-	-	-	-	-	-	-	-	-	-	-
	Hypo activity	-	-	-	-	-	-	-	-	-	-	-	-
	Ataxia	-	-	-	-	-	-	-	-	-	-	-	-
	Jumping	-	-	-	-	-	-	-	-	-	-	-	-
	Catalepsy	-	-	-	-	-	-	-	-	-	-	-	-
	Locomotor activity	-	-	-	-	-	-	-	-	-	-	-	-
Reflexes	Corneal reflex	+	+	+	+	+	+	+	+	+	+	+	+
	Pinna reflex	+	+	+	+	+	+	+	+	+	+	+	+
	Righting reflex	+	+	+	+	+	+	+	+	+	+	+	+

Table 9 : OBSERVATIONS DONE FOR THE ACUTE ORALTOXICITY STUDY OF AMLE contd……..

Parameters observed		0 h	0.5h	1 h	2 h	4 h	Day 2&3	Day 4&5	Day 6&7	Day 8&9	Day 10&11	Day 12&13	Day 14
Convulsion	Tonic and clonic convulsion	-	-	-	-	-	-	-	-	-	-	-	-
Muscle tone	Hypertonia	-	-	-	-	-	-	-	-	-	-	-	-
	Hypotonia	-	-	-	-	-	-	-	-	-	-	-	-
Ocular sign	Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-
	Miosis	-	-	-	-	-	-	-	-	-	-	-	-
	Mydriasis	-	-	-	-	-	-	-	-	-	-	-	-
	Ptosis	-	-	-	-	-	-	-	-	-	-	-	-
Skin	Edema	-	-	-	-	-	-	-	-	-	-	-	-
	Skin and fur	-	-	-	-	-	-	-	-	-	-	-	-
	Erythema		-	-	-	-	-	-	-	-	-	-	-
Cardiovascular signs	Bradycardia	-	-	-	-	-	-	-	-	-	-	-	-
	Tachycardia	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection	Contraction of erectile tissue of hair	-	-	-	-	-	-	-	-	-	-	-	-
Gastro intestinal signs	Diarrhea	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 10 : LIVE PHASE OBSERVATION

Live phase animals	Observations
1. Body weight every day	Normal
2. Food consumption daily	Normal
3. Water consumption daily	Normal
4. Home cage activity	Normal

Note: Acute toxicity study of AMLE was performed and it was non-toxic up to 2000 mg/kg dose

Table 11:MORTALITY RECORD FOR AMLE IN ACUTE ORAL TOXICITY STUDY

Group	Group 1 (5 mg/kg)	Group 2 (50 mg/kg)	Group 3 (300 mg/kg)	Group 4 (2000 mg/kg)	
No. of animals	1	1	1	1	2
Body weight (g)	150	183	180	200	195
Sex	Female	Female	Female	Female	Female
30 min	Nil	Nil	Nil	Nil	Nil
1 h	Nil	Nil	Nil	Nil	Nil
2 h	Nil	Nil	Nil	Nil	Nil
3 h	Nil	Nil	Nil	Nil	Nil
4 h	Nil	Nil	Nil	Nil	Nil
Day 1	Nil	Nil	Nil	Nil	Nil
Day 2	Nil	Nil	Nil	Nil	Nil
Day 3	Nil	Nil	Nil	Nil	Nil
Day 4	Nil	Nil	Nil	Nil	Nil
Day 5	Nil	Nil	Nil	Nil	Nil
Day 6	Nil	Nil	Nil	Nil	Nil
Day 7	Nil	Nil	Nil	Nil	Nil
Day 8	Nil	Nil	Nil	Nil	Nil
Day 9	Nil	Nil	Nil	Nil	Nil
Day 10	Nil	Nil	Nil	Nil	Nil
Day 11	Nil	Nil	Nil	Nil	Nil
Day 12	Nil	Nil	Nil	Nil	Nil
Day 13	Nil	Nil	Nil	Nil	Nil
Day 14	Nil	Nil	Nil	Nil	Nil
Mortality	**0/1**	**0/1**	**0/1**	**0/2**	

Note: Acute toxicity study of AMLE was performed and it was non-toxic up to 2000 mg/kg dose

Effect of the extract of *A.malabarica* on Body weight

The comparison of body weight between the first day of the study and last day, showed significant increase in the body weight in all groups.And the HCD group is increases when compare to the other groups. Statistical analysis of the results using Student's paired T test showed that the values obtained are significant P values $P<0.05$.

Table 12: Effect of AMLE on Body weight

Group Name	Body weight (g)	
	Initial	Final
Normal control	160.83±4.54	200±6.51*
Negative control	199.16±14.17	265±10.40*
AMLE low dose(100 mg/kg)	146±6.96	213.33±3.33*
AMLE high dose(200 mg/kg)	154.16±7.35	228±9.165*
AMLE alone(200 mg/kg)	153.33±3.57	170±6.83*
Standard Atorvastatin(10 mg/kg)	141.6±4.77	222±8.29*

Values are expressed as mean ±SEM (n=6).* denotes P<0.05 When compared to the initial readings (Student's Paired 't' -test)

Average Food Intake

Quantities of food consumed by rats in group I were more when compared to remaining groups, this was because rats were fed with extra HFD for 1 to 25 days during hyerlipidemia induction. The quantities of food intake were the same in all groups, although body weight gain differed significantly between control groups, standard and AMLE treated groups. This difference was probably due to the different doses of AMLE extracts seems to exert a protective effect against overweight in AMLE treated group as compared to HFD control group. The values of average feed intake were tabulated in Table No 13.

Table No 13: Effect of AMLE on Food Intake in HFD Induced Hyperlipidemic Rats.

Groups	Treatment	Average food intake 1-25 days(gm)	Average food intake 25-50 days(gm)
Group I	Normal control	17.28±0.303	15.83±0.215
Group II	Negative control	10.42±0.375	16.64±0.648
Group III	AMLE low dose (100mg/kg)	10.04±0.426	16.62±0.693
Group IV	AMLE high dose (200 mg/kg)	9.99±0.474	16.95±427
Group V	AMLE alone (200 mg/kg)	15.20±0.56	14.50±0.35
Group VI	Standard (10 mg/kg)	10.25±0.250	17.29±0.500

Effect of extract of *A.malabarica* on water consumption

During the treatment, *A.malabarica* extract caused a significant increase in water consumption**.** The amount of water intake were the same in all groups, although there will differed significantly between control groups, standard and AMLE treated groups. This difference was probably due to the different doses of AMLE extracts. The values of average water intake were tabulated in Table No 14.

Table No 14: Effect of AMLE on water consumption in HFD Induced Hyperlipidemic Rats.

Groups	Treatment	Average water intake 1-25 days(ml)	Average water intake 25-50 days(ml)
Group I	Normal control	100.50±3.0	120.10±8.50
Group I	Negative control	106.8±1.41	141.89±1.02
Group III	AMLE low dose (100mg/kg)	187.04±1.23	222.04±1.14
Group IV	AMLE high dose (200 mg/kg)	141.68±2.11	281.82±2.13
Group V	AMLE alone (200 mg/kg)	110.12±5.60	130.58±60
Group VI	Standard (10 mg/kg)	45.95±2.22	74.15±1.33

Serum Biochemical Parameters

The lipid profile was evaluated by estimating triglycerides (TG), total cholesterol (TC), HDL-Cholesterol (HDL-C), LDL-Cholesterol (LDL-C) and VLDL-Cholesterol (VLDL-C) in normal and hyperlipidemic animals. The values were tabulated in Table No.

Triglycerides (TG)

Animals in HFD group exhibited significant ($P<0.001$) increase in triglyceride levels (394.3±2.389mg/dl) compared to normal control group (164.98±6.50mg/dl).

Hyperlipidemic animals treated with 100mg/kg AMLE showed significant ($P<0.05$) reduction in triglycerides levels, animals treated with 200mg/kg AMLE showed significant ($P<0.01$) decrease in triglycerides levels. And animals treated with AMLE alone group shows non-significant ($P>0.05$) in triglycerides level.

Total cholesterol (TC)

Animals in HFD group exhibited significant (P<0.05) increase in total cholesterol levels (193.4±mg/dl) compared to normal control group (122.8±2.18mg/dl). Hyperlipidemic animals treated with 100mg/kg AMLE did not show significant reduction in total cholesterol levels, animals treated with 200mg/kg AMLE showed significant (P<0.01) decrease in total cholesterol levels 180.3±3.21mg/dl and animals treated with AMLE alone group shows non-significant (P>0.05) when compared to the negative control.

High Density Lipoprotein (HDL)

Animals in HFD group exhibited significant (P<0.05) decrease in HDL levels (2.130±0.006mg/dl) compared to normal control group (3.513±0.32mg/dl).

Hyperlipidemic animals treated with 100mg/kg AMLE did not shown significant increase in HDL levels, animals treated with 200mg/kg AMLE showed significant (P<0.05) increase in HDL levels and animals treated with AMLE alone group shows non- significant (P>0.05) when compared to the negative control.

Low Density Lipoprotein (LDL)

Animals in HFD group exhibited significant (P<0.1) increase in LDL levels (101.0±3.33mg/dl) compared to normal control group (54.38±2.94mg/dl).

Hyperlipidemic animals treated with 100mg/kg and 200mg/kg AMLE did not show significant reduction in LDL levels, and animals treated with AMLE alone group shows non- significant(P>0.05) when compared to the negative control.

Very Low Density Lipoprotein (VLDL)

Animals in HFD group exhibited significant($P<0.05$) increase in VLDL levels (68.81±0.92mg/dl) compared to normal control group (28.38±0.74mg/dl).

Hyperlipidemic animals treated with 100mg/kg AMLE showed significant ($P<0.01$) reduction in VLDL levels 64.20±0.59mg/dl, animals treated with 200mg/kg AMLE showed extremely significant decrease in VLDL levels, 34.62±0.90 respectively $P<0.001$.

Standard drug Atorvastatin treated group showed potent antihyperlipidemic activity and showed decrease in triglyceride, total cholesterol, LDLc, VLDLc levels, increase in HDL c levels with extreme significance compared to HFD control group.

Serum Marker Enzymes

The activities of serum marker enzymes like AST, ALT, ALP and LDH were significant ($P<0.05$) increases in the HFD group when compared to the normal control groups. Hyperlipidemic animals treated with 100mg/kg and 200mg/kg AMLE shown significant reduction in LDL levels, and animals treated with AMLE alone group shows non- significant ($P>0.05$) when compared to the negative control.

Table 15:Effect of AMLE on Serum Biochemical Parameters

Groups	Treatment	Serum lipid profile (mg/dl)								
		TC	TG	ALP	LDH	SGOT	SGPT	HDL-C	LDL-C	VLDL-C
Group I	Vehicle control	122.8 ±2.18	164.9 ±6.50	0.26 ±.015	1.33 ±0.18	3.65 ±0.77	3.004 ±0.82	3.51 ±0.32	54.3 ±2.94	28.38 ±0.74
Group II	Negative Control	193.4 ±2.61	394.3 ±2.38###	2.46 ±0.15###	2.13 ±0.006#	16.41 ±4.9#	10.62 ±1.81###	2.13 ±0.006#	101.0 ±3.3#	68.81 ±0.92#
Group II	AMLE low dose (100 mg/kg)	193.4 ±2.61	178.51 ±9.60***	0.64 ±0.03***	0.45 ±0.02***	2.14 ±0.29***	0.025 ±0.00***	0.26 ±0.07**	98.85 ±3.95***	64.20 ±0.59***
Group IV	AMLE high dose (200 mg/kg)	180.3 ±3.21**	144.14 ±9.99**	0.68 ±0.04***	0.48 ±0.19***	3.178 ±1.18**	0.03 ±0.003***	0.35 ±0.07**	60.51 ±2.67***	34.62 ±0.90***
Group V	AMLE alone (200 mg/kg)	119.5 ±1.90^{ns}	208.5 ±24.22^{ns}	0.40 ±0.03^{ns}	1.63 ±0.18^{ns}	8.71 ±1.78^{ns}	2.55 ±1.16^{ns}	2.38 ±0.56^{ns}	50.40 ±1.86^{ns}	25.40 ±0.56^{ns}
Group VI	Standard (10 mg/kg)	129.4 ±2.26***	206.01 ±14.64***	0.52 ±0.10***	0.46 ±0.10***	3.92 ±0.871**	0.03 ±0.004***	3.46 ±0.07*	58.01 ±1.38***	32.90 ±0.55***

Values are expressed in Mean ± SEM (n=6) one way ANOVA followed by Tukey's test. Where, # denotes P<0.05 and ### denotes P<0.001 when compared to vehicle control rats.*denotes P<0.05, **denotes P<0.01 and ***denotes P<0.001 when compared to negative control. ^{ns}denotes P>0.05 when compared to vehicle control group.

Effect of AMLE on Total protein and MDA

There was a significant (P<0.05) decrease in the level of total protein and MDA in Negative control group when compared to the normal control groups. (Table No.16)

When the AMLE treated groups shows significant (P<0.05) in the Total protein and MDA in the tissues (heart and liver) homogenate in the dose 100 and 200 mg/kg when compared to the Negative control group (HFD).AMLE alone treated groups shows non-significant (P>0.05) when compare to the normal control groups.

Effect of AMLE on enzymatic and non-enzymatic antioxidants in control and experimental groups

High Fat Diet animals shows a significant (P<0.05) decrease in the enzymatic antioxidants like catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase and the non-enzymatic antioxidant reduced glutathione in the tissues (heart and liver) homogenate when compared to normal control.(Table No16.)

When the AMLE treated groups shows significant (P<0.05) in the enzymatic antioxidants like catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase and the non-enzymatic antioxidant reduced glutathione in the tissue (heart and liver) homogenate in the dose 100 and 200 mg/kg when compared to the Negative control group (HFD).AMLE alone treated groups shows non-significant (P>0.05) when compare to the normal control groups.

Table 16: Effect of AMLE on the lipid peroxidation and antioxidant enzymes in Liver

GROUPS	Total protein	MDA	SOD	CAT	GSH	GSSH	GP_X
Normal control	14.51 ±0.56	0.41 ±0.098	2.660 ±0.310	2.498 ±0.219	1.813 ±0.408	1.581 ±0.243	3.06 ±0.416
Negative control	7.95 ±1.07###	0.014 ±0.004###	0.028 ±0.004###	0.073 ±0.010###	0.043 ±0.005##	0.023 ±0.003###	0.054 ±0.003###
AMLElow dose(100mg/kg)	2.45 ±1.39*	0.253 ±0.055*	1.54 ±0.2183***	1.388 ±0.202**	1.75 ±0.167**	1.095 ±0.020***	1.504 ±0.403*
AMLEhigh dose(200 mg/kg)	9.02 ±0.71**	0.37 ±0.070**	1.595 ±0.236***	1.404 ±0.230**	1.948 ±0.23**	0.9054 ±0.0206**	1.895 ±0.187**
AMLE alone(200 mg/kg)	12.44 $\pm1.0^{ns}$	0.191 $\pm0.017^{ns}$	1.892 $\pm0.166^{ns}$	1.604 $\pm0.238^{ns}$	1.734 $\pm0.355^{ns}$	1.518 $\pm0.2437^{ns}$	1.979 $\pm0.1941^{ns}$
Standard(10 mg/kg)	7.23 ±1.57*	0.306 ±0.012*	2.24 ±0.021***	1.995 ±0.390***	1.36 ±0.340*	1.06 ±0.0092***	1.55 ±0.244*

Protein = mmoles/min/mg, CAT = mmoles/min/mg wet protein, GPx = µmoles/min/mg protein, SOD = µmoles/min/mg protein, GSSH = µmoles/min/mg protein,GSH = µmoles/min/mg protein, and MDA = µmoles/min/mg protein ;Values are expressed in Mean ± SEM (n=6) one way ANOVA followed by Tukey's test. Where, # denotes P<0.05 and ### denotes P<0.001 when compared to vehicle control rats.*denotes P<0.05, **denotes P<0.01 and ***denotes P<0.001 when compared to negative control. ^{ns}denotes P>0.05 when compared to vehicle control group.

Table 17: Effect of AMLE on lipid peroxidation and antioxidant enzymes in Heart

GROUPS	Total protein	MDA	SOD	CAT	GSH	GSSH	GP_X
Normal control	12.51 ±0.56	0.351 ±0.090	2.77 ±0.393	2.946 ±0.271	2.276 ±0.306	1.88 ±0.278	2.74 ±0.322
Negative control	76.95 ±1.07###	0.034 ±0.005##	0.049 ±0.013###	0.070 ±0.010###	0.031 ±0.0048###	0.0380 ±0.009###	0.090 ±0.03###
AMLE Low dose (100mg/kg)	1.45 ±1.39*	0.330 ±0.07*	1.832 ±0.281***	1.44 ±0.210**	1.962 ±0.259***	1.766 ±0.157**	1.435 ±0.174*
AMLE High dose (200 mg/kg)	5.02 ±0.71**	0.634 ±0.070***	1.72 ±0.20***	1.419 ±0.216**	2.101 ±0.422***	1.642 ±0.4622**	1.611 ±0.32*
AMLE alone (200 mg/kg)	10.44 ±1.0ns	0.247 ±0.017^{ns}	2.055 ±0.1791^{ns}	2.0994 ±0.23^{ns}	1.686 ±0.3006^{ns}	1.3188 ±0.1652^{ns}	1.97 ±0.23^{ns}
Standard (10 mg/kg)	6.23 ±1.57*	0.312 ±0.024*	2.156 ±0.2110***	2.079 ±0.379***	1.7006 ±0.277**	1.678 ±0.331**	1.66 ±0.2610*

Protein = mmoles/min/mg, CAT = mmoles/min/mg wet protein, GPx = µmoles/min/mg protein, SOD = µmoles/min/mg protein, GSSH = µmoles/min/mg protein, GSH = µmoles/min/mg protein, and MDA = µmoles/min/mg protein ;Values are expressed in Mean ± SEM (n=6) one way ANOVA followed by Tukey's test. Where, # denotes P<0.05, ## denotes P<0.01 and ### denotes P<0.001 when compared to vehicle control rats.*denotes P<0.05, **denotes P<0.01 and ***denotes P<0.001 when compared to negative control. ^{ns}denotes P>0.05 when compared to vehicle control group.

Histopathological changes in Heart

Normal control

Section studied from heart shows normal myocardium with myocytes. The blood vessels show normal. There is no evidence of architectural destruction/ edema/ inflammatory infiltrates/ necrosis.(Figure-1)

Negative control

Section studied from heart shows normal myocardium with architecture. Stroma shows focal cytoplasmic vacuolation and also shows scattered lymphocytic infiltration. The blood vessels show normal morphology.(Figure-2)

AMLE low dose (100 mg/kg)

Section studied from heart shows myocardium with myocytes showing cytoplasmic vacuolation (fatty change). The blood vessels show normal morphology. There is no evidence of architectural destruction/ edema/ inflammatory infiltrates/ necrosis. (Figure-3)

AMLE high dose (200 mg/kg)

Section studied from heart shows normal myocardium with myocytes. The blood vessels show congestion. There is no evidence of architectural destruction/ edema/ inflammatory infiltrates/ necrosis. (Figure-4)

AMLE alone (200 mg/kg)

Section studied from heart shows normal myocardium with myocytes. The blood vessels show normal. There is no evidence of architectural destruction/ edema/ inflammatory infiltrates/ necrosis. (Figure-5)

Standard(10 mg/kg)

Section studied from heart shows normal myocardium with myocytes. The blood vessels show congestion. There is no evidence of architectural destruction/ edema/ inflammatory infiltrates/ necrosis.(Figure-6)

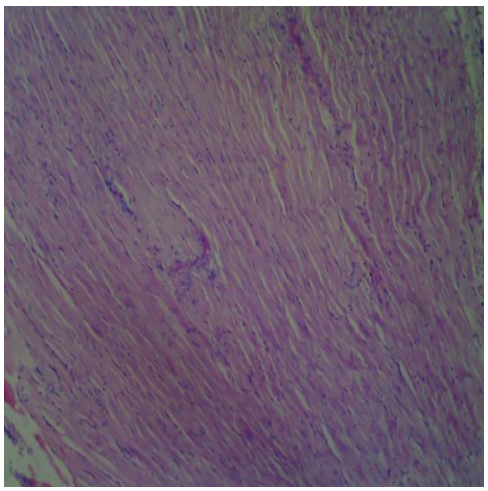


Figure-1 Photomicrograph of heart tissues from normal control group

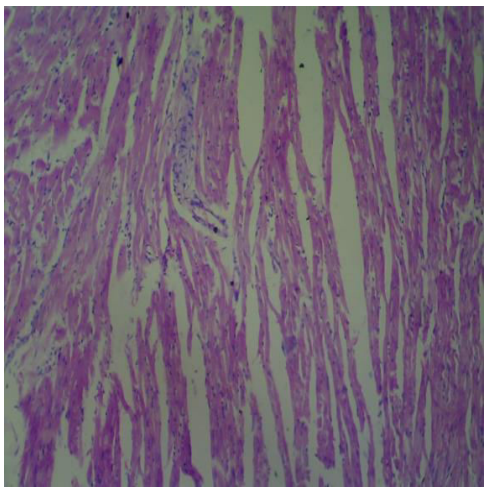


Figure-2 Photomicrograph of- heart tissues from negative control group

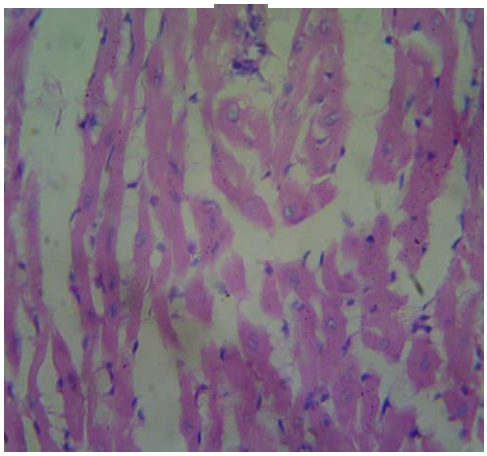


Figure-3 Photomicrograph of heart tissues from AMLE Low dose

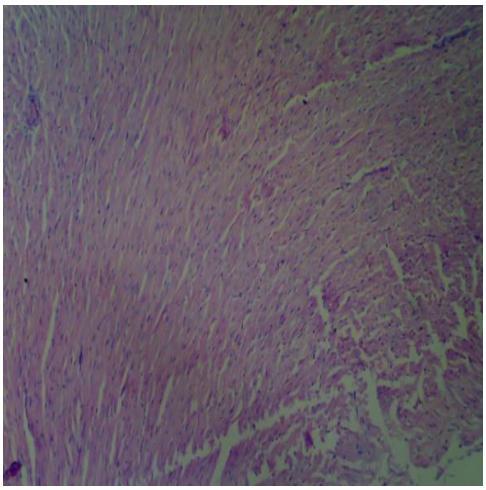


Figure-4 Photomicrograph of heart tissues from

AMLE High dose

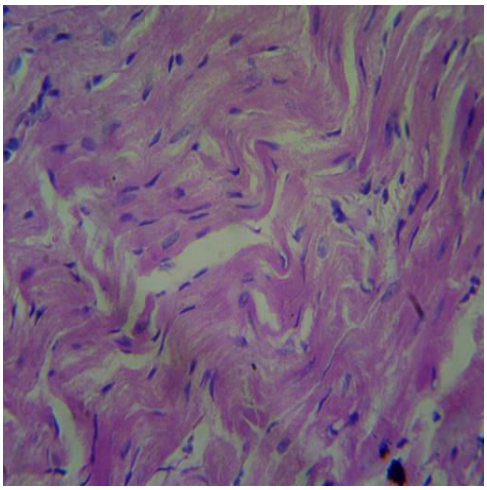


Figure-5 Photomicrograph of heart tissues from

AMLE alone

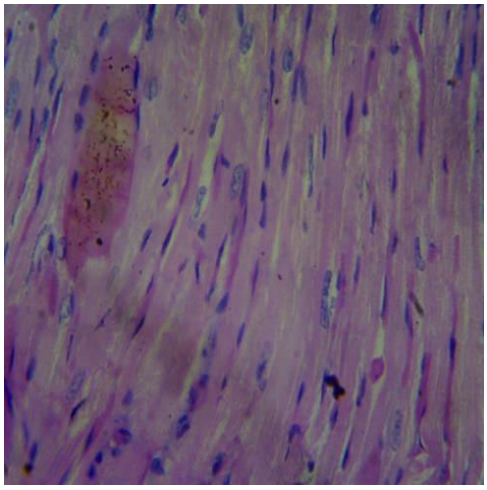


Figure-4 Photomicrograph of heart tissues from

Standard

Histopathological changes in Liver

Normal control

The sections from the liver shows normal lobular architecture. Individual hepatocytes show no significant pathology. Portal traid shows normal morphology. Sinusoids show mild dilatation. Central vein shows normal. (Figure-7)

Negative control:

The sections from the liver shows mild altered architecture. Individual hepatocytes show cytoplasmic vacuolation. Portal traid shows bile duct hyperplasia. Sinusoids show mild dilatation congestion. Central vein shows congestion. (Figure-8)

AMLE low dose (100mg/kg)

The sections from the liver shows altered lobular architecture. Individual hepatocytes show cytoplasmic vacuolation(both micro and macro vesicular steatosis). Portal traid shows bile duct hyperplasia. Sinusoids show mild dilatation. Central vein shows no significant abnormality. (Figure-9)

AMLE High dose (200 mg/kg)

The sections from the liver shows normal lobular architecture. Individual hepatocytes show no significant pathology. Portal traid shows bile duct hyperplasia. Sinusoids show mild dilatation. Central vein shows dilatation and congestion. (Figure-10)

AMLE alone (200 mg/kg)

The sections from the liver shows normal lobular architecture. Individual hepatocytes show no significant pathology. Portal traid shows mild bile duct hyperplasia. Sinusoids show mild dilatation. Central vein shows congestion. (Figure-11)

Standard (10 mg/kg)

The sections from the liver shows normal lobular architecture. Individual hepatocytes show no significant pathology. Portal traid shows mild periportal inflammation and bile duct hyperplasia. Sinusoids show mild dilatation. Central vein shows normal. (Figure-12)

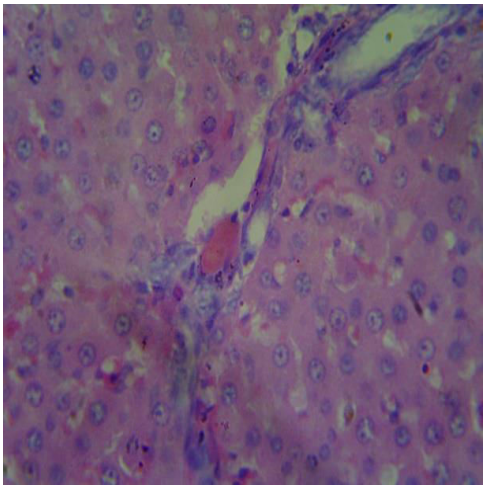


Figure-7 Photomicrograph of liver tissues isolated from normal control group

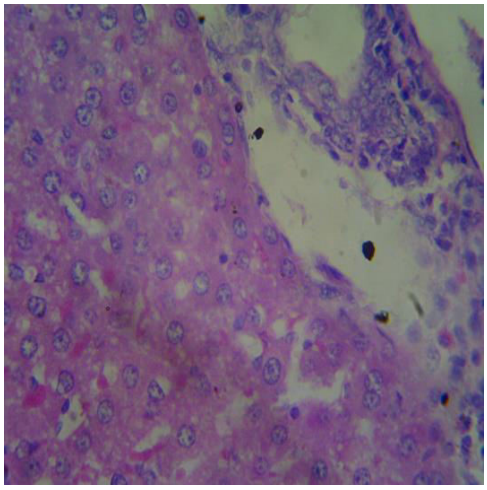


Figure-8 Photomicrograph of liver tissues from negative control group

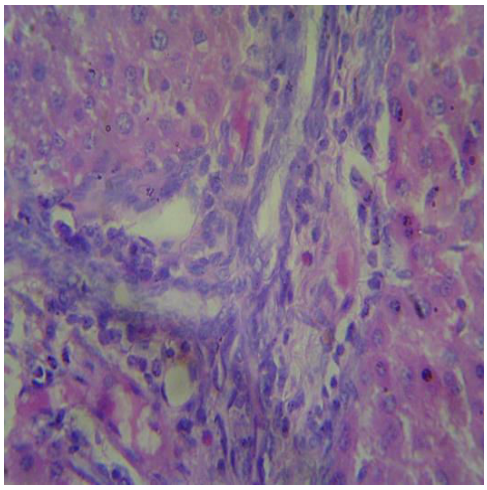


Figure-9 Photomicrograph of liver tissues from AMLE Low dose

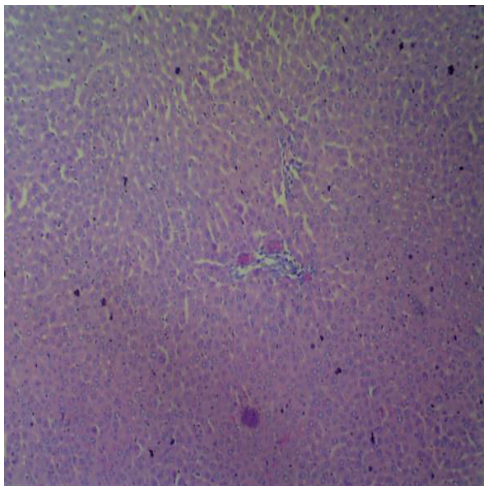


Figure-10 Photomicrograph of liver tissues from

AMLE High dose

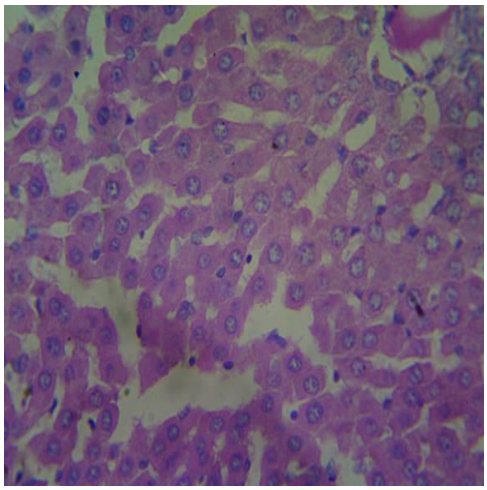


Figure-11 Photomicrograph of liver tissues from

AMLE alone

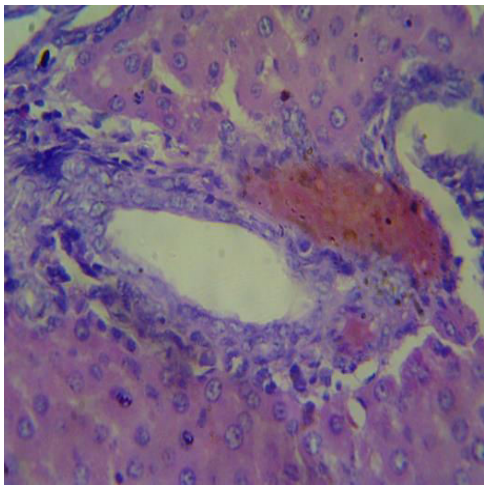


Figure-12 Photomicrograph of liver tissues from

Standard

DISCUSSION AND CONCLUSION

Hypercholesterolemia and the resulting atherosclerosis have been implicated in the pathophysiology of coronary heart diseases and myocardial ischemia. Lowering cholesterol level may decrease the risk of CVD, and therefore enormous efforts have been extended to achieve this aim. The hypocholesterolemic activity of ethanolic leaf extract of *Anisomeles malbarica* (AMLE) against hypercholesterolemia was monitored on the Lipid profile status, antioxidant status, activities of serum cardiac marker enzyme, and histological changes of liver and heart. In this study the high cholesterol diet (HCD) is used which consists of maize, soya meal, coconut cake, rice polish, groundnut cake and animal fat such as tallow which has been used in inducing experimental hypercholesterolemia.(46) In the current study, the HCD fed rats showed increased levels of plasma cholesterol (TC), and triglycerides (TG) levels compared to normal control rats. Treatment with AMLE significantly decreased the levels of serum TC, TG, when compared to HCD induced rats.

Saponins are also reported to precipitate cholesterol from micelles and interfere with enterohepatic circulation of bile acids making it unavailable for intestinal absorption, this forces liver to produce more bile from cholesterol and hence the reduction in serum cholesterol level. Saponins are also reported to lower triglycerides by inhibiting pancreatic lipoprotein lipase. Similarly in our study also, the presence of both flavanoids and saponins in AMLE could have contributed in reducing the levels of lipid status (TC, TG,) elevated levels of serum low density lipoprotein cholesterol (LDL) and very low density lipoprotein cholesterol (VLDL) are often accompanied by premature atherosclerosis and other CVD. A low level of high-density lipoprotein cholesterol (HDL) is also an important risk factor for cardiovascular disease.

The cardioprotective effects of HDL have been attributed to its role in reversing cholesterol transport, its effects on endothelial cells, and its antioxidant activity. Flavanoids can increase HDL-C and also decreases oxidation of LDL-cholesterol. High cholesterol diet increases serum LDL levels and oxidative stress which results in the production of increased oxidized LDL and thereby increases atherosclerotic plaque formation. From our present study it is evident that HCD induced rats showed increased serum LDL and VLDL levels with the concomitant reduction in serum HDL level, when compared to normal rats. Supplementation with AMLE reduced the serum LDL and VLDL levels and increased the serum HDL level which could be due to reduction in plasma total cholesterol and increasing LDL receptor activity by the flavanoids and phytosterol present in the plant extract. Also it could be presumed that the reduction of total cholesterol by AMLE could have been associated with a reduction of its LDL fraction, which is the target of several hypolipidemic drugs. Oxidative stress is believed to contribute to the pathogenesis of hypercholesterolemic atherosclerosis hence, various antioxidant compounds are being evaluated for potential anti-hypercholesterolemic effects.

A high fat diet brings about remarkable modifications in the antioxidant defence mechanisms of rat tissues by the process of lipid peroxidation. Several reports have shown that hypercholesterolemia diminishes the antioxidant defence systems by producing free radicals and thereby elevating the lipid peroxide products, resulting in the production of toxic intermediates. Superoxide dismutase is the first enzyme in antioxidant defense that protects tissues against oxygen free radicals by catalyzing the removal of superoxide radical ($O_2^{\cdot}$-), which damages the membrane and biological structures.Catalase has been shown to be responsible for the detoxification of significant amounts of Hydrogen peroxide.[50] SOD and CAT are the two major scavenging enzymes that remove the toxic free radicals. From our study we observed that there was a reduction in the activity of hepatic SOD

and CAT in HCD induced rats when compared to control rats , this may be due to the enhanced production of Reactive Oxygen Species (ROS) by HCD. This free radical affects the antioxidant activity and hence resulted in the decreased activity of SOD and CAT.[50-52]

Treatment with AMLE restores the HCD induced alteration in the activity of the SOD and CAT to near control due to its free radical scavenging activity. GPx has been shown to be responsible for the detoxification of H_2O_2. Glutathione reductase is responsible for the reduction of oxidised glutathione to glutathione (reduced). The increased oxidant stress in hypercholesterolemic conditions exhausts the GSH pools. Activities of hepatic antioxidant enzymes viz., Glutathione peroxidase (GPx) and Glutathione Reductase (GR) enzymes and Glutathione contents were significantly decreased in HCD induced rats. On oral administration with AMLE, the activities of these antioxidant enzymes in liver were reverted back to normal levels. Earlier it has been reported that AMLE has an antioxidant activity owing to the presence of its saponins, flavanoids and phytosterol.

Lipid peroxidation is regarded as one of the basic mechanisms of cellular damage caused by free radicals. The relationship between LPO and hypercholesterolemia is well recognized, a cholesterol rich diet results in increased LPO by the induction of free radical production. Hypercholesterolemia and lipid peroxidation are believed to be critically involved in development of atherosclerosis. In our study we found that a significant increase in LPO levels were observed in HCD fed groups when compared to the control group. AMLE brought down the level of LPO to near normal. It has been already reported that AMLE has an antioxidant activity owing to the presence of its saponins, flavanoids and phytosterol, thus it decrease the concentration of free radicals, which might terminate the initiation and propagation of LPO. [49]Several reports

showed that high cholesterol level can cause cardiac damage. Elevation in the levels of diagnostic marker enzymes such as Alanine transaminase, Aspartate transaminase, Lactate dehydrogenase in serum of HCD induced rats is due to Peroxide formation induced by hypercholesterolemia in the form of ROS. This ROS production increases cellular membrane permeability, intracellular fluid transfers onto intercellular space, resulting in muscle and cardiac damage which leads to the leakage or release of marker enzymes from cardiac tissue to serum and hence the level of marker enzymes are raised in HCD fed rats. There was a significant elevation in the levels of marker enzymes such as SGOT, SGPT, LDH were observed in HCD induced rats when compared with control rats. Treatment with AMLE significantly reduced the activity of SGOT, SGPT,LDH to near normal levels. Macroscopic observation of liver and heart showed that tenacity of vessel in control group was better than that of hypercholesterolemic rats. Furthermore, surface of intima in rats fed with normal rat chow was smooth and glossy, and there is no thickening of the intima or migration of smooth muscle cells to the intima, whereas surface of intima of HCD induced rats showed a typical plaque characterized by thickening of the intima, migration of smooth muscle cells to the intima, infiltration of macrophages, appearance of foam and lamellar calcification under the endothelium.

From our result we observed that there was an increased foam cell formation which leads to intimal thickening in HCD induced group. These foam cell counts were reduced in AMLE treated group which could be due to its inhibition activity on cholesteryl ester and thereby reduces the specific binding sites of acetyl LDL hence can reduce the foam cell formation and leads to less thickening of intima. The result obtained in this study suggests that the ethanolic extract of *A.malabarica* has beneficial effects in preventing hypercholesterolemia by lowering lipid status, improving antioxidant status as well as protecting the heart and liver morphology. The present experimental data therefore suggest that

ethanolic extract of *A.malabarica* has an atheroprotective potential. Further studies using various other models has to be carried out to confirm these findings.

Sri Ramakrishna Institute of Paramedical Sciences

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COLLEGE OF PHARMACY

Institutional Animal Ethics Committee (IAEC)

CPCSEA Registration No. 1559/PO/E/S/11/CPCSEA dated 06.10.2015

CERTIFICATE

Title	**: Study on *in vitro* cholesterol esterase inhibitory activity and *in vivo* anti-hyperlipidemic activity of *Anisomeles malabarica* leaf extract.**
Nameof the Applicant :	**Mrs. B. Anandhi**
Proposal Number	: **COPSRIPMS/IAEC/PG/P'COLOGY/01/2017-2018**
Approval Date	: **05/05/2017**
Expiry Date	: **04/05/2018**
Number of animals sanctioned with name of the species:	**Female *Wistar* rats – 09 Nos** **Male *Wistar* rats – 32 Nos**
Sex of the animal	:**Male and Female *Wistar* rats**
Methodology	: **Approved**

Recommendations: Nine female *Wistar* rats for acute toxicity studies and 32 male *Wistar* rats for *in vivo* studies were sanctioned.

Dr. T. K. Ravi
Chairman IAEC

CHAIRMAN
IAEC

Sign of Chairman

Date:05/05/2017

Dr. S. Gnanapoongothai
CPCSEA Main nominee

S. Gnanapoongothai 5/5/17

MAIN NOMINEE
CPCSEA

Sign of CPCSEA Main nominee

Date:05/05/2017

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सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2017/Tech. 281h

दिनांक/Date: 6th January 2017

सेवा में / To

Ms. B. Anandhi
II M. Pharm.
Department of Pharmacology
Sri Ramakrishna Institute of Paramedical Sciences
College of Pharmacy
Coimbatore – 641 044

महोदया/Madam,

The plant specimen brought by you for identification is identified as *Anisomeles malabarica* (L.) R. Br. ex Sims (= *Nepeta malabarica* L.) - LAMIACEAE. The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

धन्यवाद/Thanking you,

भवदीय/Yours faithfully,

6/1/17

(डॉ. एम. पलनिसामी/Dr. M. Palanisamy)
वैज्ञानिक 'डी' प्रभारी / Scientist 'D' –In-Charge

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