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Anti-Hyperlipidemic Activities of a Novel Polyherbal Formulation in High Fat Diet Rat Model.

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INTRODUCTION

1.1 Hyperlipidemia

Hyperlipidemia is considered one of the major risk factors causing cardiovascular diseases (CVDs). CVDs accounts for one third of total deaths around the world, it is believed that CVDs will turn out to be the main cause of death and disability worldwide by the year 2020 (Ginghina *et al.*, 2011; Jorgensen *et al.*, 2013). Hyperlipidemia is an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters and phospholipids and or plasma lipoproteins including very low-density lipoprotein and low-density lipoprotein, and reduced high-density lipoprotein levels (Mishra *et al.*, 2011; Jeyabalan and Palayan, 2009). Hypercholesterolemia and hyper triglyceridemia are the main cause of atherosclerosis which is strongly related to ischemic heart disease (IHD) (Brouwers*et al.*, 2012). There is a strong relation between IHD and the high mortality rate. Furthermore elevated plasma cholesterol levels cause more than four million deathsin a year (Kumar *et al.*, 2012). Atherosclerosis is a process of arteries hardening due to deposition of cholesterol in the arterial wall which causes narrowing of the arteries. Atherosclerosis and atherosclerosis-associated disorders like coronary, cerebrovascular and peripheral vascular diseases are accelerated by the presence of hyperlipidemia (Wells *et al.*, 2007).

Hyperlipidemia relates to increased oxidative stress causing significant productionof oxygen free radicals, which may lead to oxidative modifications in low-density lipoproteins, which present a significant function in the initiation and progression of atherosclerosis and associated cardiovascular diseases (Mishra *et al.*, 2011).

1.2 Hyperlipidemia classification

Hyperlipidemia in general can be classified to:

Primary

It is also called familial due to a genetic defect, it may be monogenic: a single gene defect or polygenic: multiple gene defects. Primary hyperlipidemia can usually be resolved into one of the abnormal lipoprotein patterns. (Tripathi, 2008).

Secondary

It is acquired because it is caused by another disorder like diabetes, nephritic syndrome, chronic alcoholism, hypothyroidism and with use of drugs like corticosteroids, beta blockers and oral contraceptives. Secondary hyperlipidemia together with significant hypertriglyceridemia can cause pancreatitis (Joseph, 2011). The main cause of hyperlipidemia includes changes in lifestyle habits in which risk factor is mainly poor diet in which fat intakeform saturated fat and cholesterol exceeds 40 percent of the total calories uptake (Joseph, 2011).

1.3 Causes and Risk Factors of Hyperlipidemia

Dietary Causes Dietary Fats and Fatty Acids: Dietary fatty acids are divided intothree major classes (saturated, monounsaturated and polyunsaturated fatty acids). The foods that contribute to saturated fatty acids (e.g. myristic acid, palmitic acid, stearic acid, etc) meats (e.g. beef, pork, processed meat products, poultry), 2) milk and other dairy products (e.g. butter, cheese, ice cream, yoghurt), 3) tropical fats (e.g. Coconut, palm oils) and 4) egg (contain proportionately less saturated fat compared to other animal food sources). Monounsaturated fatty acidsare present as oleic acid in olive oil, avocado, animal fats, etc. Polyunsaturated fatty acids are the omega-3 fatty acids (e.g. linoleic acid) and omega-6 fatty acids (e.g. linolenic acid) (Fauci *et al.*, 2008). Food choices made by individuals can influence intake of the different saturatedfatty acids. Selecting leaner cuts of meat are high in palmitic acid and limiting the amount of lean meat would help in lowering saturated fat intak (Sereday *et al.*, 2004). Milk and other dairy products are high in myristic acid content. Substituting skim milk and non-fat dairy products for whole milk products will result in a reduction of saturated fat such as myristic acid intake.

Dietary Cholesterol: Like other sterols, cholesterol is a sterol i.e. a combination of steroid and alcohol) and lipid (a type of fat). It is found in foods such as eggs and dairy products and is also manufactured in the body, especially the liver. Cholesterol also stabilizes a cell against temperature changes. It is a major part of the membranes of the nervous system, the brain, the spinal cord and the peripheral nerves. In particular, it is incorporated into the myelin sheath that insulates the nerves from the surrounding tissue. Cholesterol is also the forerunner of important hormones such as the female sex hormone, oestradiol and the male sex hormone, testosterone and of vitamin D. Cholesterol is also used to produce the bile which is required to digest the fats in food. Nearly most of the body tissues are capable of making cholesterol, but the liver and intestines make the most. The dietary cholesterol is responsible for both the development of hypercholesterolemia and atherosclerosis has been the focus of many investigators. Many studies in rabbits (and other animal models) and in human diet and epidemiologic investigations indicated the importance of dietary cholesterol on serum cholesterol levels and its associated effects. However, other investigations have come to opposite conclusions

after reviewing numerous human feeding studies (although many continue to support the view that dietary cholesterol is the major hypercholesterolemic and atherogenic nutrient in the diet) (Ruixing *et al.*, 2006).

1.3.1 Other Dietary Factors

Carbohydrates: Dietary recommendations to lower the total fat intake include increasing dietary carbohydrate intake because favorable plasma lipid and lipoprotein levels have been reported for populations and individuals whose habitual diet is rich in carbohydrates. High carbohydrate consumption being associated with a decrease in HDL cholesterol levels. Plasma triglyceride levels are not elevated in these individuals, possibly because obesity is rare (Charney, 1999).

Fiber: Studies have shown that only water-soluble fiber plays a role in lipoprotein metabolism in humans. A meta-analysis of 20 studies found that intake of oat products reduces serum cholesterol levels. The mechanism by which dietary fiber affects plasma lipid levels is unknown. Insoluble fibers in wheat and vegetables do not to reduce cholesterol, but they do have other beneficial effects.

Protein: Soy protein also lowers serum cholesterol levels in animals and in hypercholesterolemic individuals when compared with casein (a dairy protein) andbeef proteins. The mechanism underlying these changes is unknown but it has been stated that soy protein affects cholesterol absorption, bile acid absorption, the insulin-glucagon ratio, serum thyroxine levels and hepatic LDL-receptor activity.

Obesity: For a given level of body mass index (BMI), obesity is associated with hyperlipidemia, insulin resistance and hypertension and independent predictor of coronary artery disease (CAD). A meta-analysis of 70 studies indicated that weight reduction was related to increases in HDL cholesterol levels and significant decreases in total, LDL and VLDL cholesterol and triglyceride levels (Woollett *etal.*, 1992). Although they are not always coincident, obesity is also often accompanied by hyperlipidemia. Both obesity and hyperlipidemia are independently associated with atherosclerosis, non-alcoholic fatty liver disease and insulin resistance (Cortse *et al.*, 1983).

Diabetes and Insulin Resistance: Insulin resistance (type II diabetes) is associated with a number of lipid and lipoprotein abnormalities (Keys *et al.*, 1985). The lipid abnormality is associated with insulin resistance and hyperinsulinemia is hypertriglyceridemia. VLDL and total triglycerides are elevated in individuals with type II diabetes although the exact roles of insulin resistance and hypertriglyceridemia are disputed.

Physical Exercise/Activity: Sedentary lifestyles contribute to the development and maintenance of obesity (Keys *et al.*, 1985). Diet can also change in plasma lipoprotein concentrations that occur with exercise.

Alcohol Intake: Low dose ethanol consumption in healthy volunteers modestly activates hepatic de novo lipogenesis and that the major quantitative fate of ethanolis acetate produced in the liver. The acetate released into the plasma which inhibits lipolysis in peripheral tissues by 53% and whole body lipid oxidation is decreasedby 73%. Alcohol intake is second only to diabetes mellitus as a cause of hyperlipidemia in the population, about 25% of hospitalized alcoholics have fasting blood triacylglycerol concentrations above

normal limits and 17% have concentrations >3 mmol/L. Hypertriglyceridemia is seen mostly in patients with fatty liver and rarelyin cirrhosis patients. Patients with cirrhosis have a lower capacity to produce blood lipids than do subjects without liver injury when challenged with diet and alcohol experimentally.

Contraceptives and Other Pharmacologic Agents: Premenopausal women, using oral contraceptives containing a relatively low dose of estrogen combined with a medium or high dose of progestin had a 24 % higher median concentration of LDL cholesterol than who are not using hormones. Glucocorticoids and estrogens elevate triglycerides and raise levels of HDL cholesterol (Hegsted *et al.,* 1985). Antihypertensives have variable effects on lipids and lipoproteins. Although short- term use of thiazide raises cholesterol, triglycerides and LDL cholesterol, long-term usage is not associated with significant alterations in lipid levels (Bananome andGrundy, 1988)

1.4 Treatment of hyperlipidemia

In 1987 the National Institute of Health (NIH) established the National Cholesterol Education Program (NCEP) to be directed by the Adult Treatment Panel (ATP) forthe purpose of issuing information for health professionals and the general public concerning testing, evaluating, monitoring and treating hyperlipidemia. An important criterion of ATP guidelines is the development of treatment goals for hyperlipidemia based on patient's risk of CHD.

ATP recommends two methods of treatment:

1) Therapeutic lifestyle changes; 2) Drug therapy.

Therapeutic lifestyle changes

Diet modification, regular physical activity, smoking cessation, and weight reduction should be tried as initial treatment, especially in mild cases of hyperlipidemia and in persons without CHD or CHD risk equivalent and<2 risk factors. It should be kept in mind that when dieting, cholesterol intake is reduced. At the same time, production of cholesterol, especially by the liver, increases. It is recommended that the intake should be restricted to 25%-35% of energy intake and that saturated fatty acids make up less than 7% of energy intake and that cholesterol intake should be less than 200 mg daily. The intake of plant sterol esters and soluble fibre is advisable. A healthy diet can result in 10% to 15% reduction of cholesterol blood level.

Drug therapy

High LDL, the presence of risk factors, and documentation of CHD should qualify initiating drug therapy along with TLC. During the early stages of the hyperlipidemia, blood monocytes and platelets attach to a vessel wall at the sites of endothelial damage. The release of the mediators such as platelet derived groth factors leads to a proliferation of smooth cells in the intimal and medial lining of the vessel, collagen synthesis, cholesterol uptake and the beginning of thehyperlipidemic plaque results. Plaque ruptures are resulting in the acute syndromesof unstable angina, myocardial infarction and sudden cardiac death (Scott, 1991).

Diagnosis of hyperlipidemia

Hyperlipidemia typically shows no symptoms and can only be detected by a bloodtest. Screening for

hyperlipidemia is done with a blood test called a lipid profile. According to National Cholesterol Education Program (NECP) screening (National cholesterol education program, 1994) should start at age 20, and if the report isnormal, it should be repeated at least every five years. Normal levels for a lipid profile (AAFP, 2013) are listed below table 1.1.

Table 1.1: Normal levels for a lipid profile

Lipids	Desirable value	Borderline	High Risk
Cholesterol	Less than 200 mg/dl	200-239 mg/dl	240 mg/dl
Triglycerides	Less than 140 mg/dl	150-199 mg/dl	200-499 mg/dl
HDL cholesterol	60 mg/dl	40-50 mg/dl	Less than 40 mg/dl
LDL cholesterol	60-130 mg/dl	130-159 mg/dl	160-189 mg/dl
Cholesterol/HDL ratio	4.0	5.0	6.0

Pharmacological treatment

Numbers of hypolipidemic drugs are available in the market for the treatment of hyperlipidemia. The existing hypolipidemic drugs are listed in table 6. In 1975, the results of the Coronary Drug Project indicated that the drugs are relatively ineffective for preventing myocardial infarction in patients with pre-established CHD. This project examined the effects of estrogens, D-thyroxin, clofibrate and nicotinic acid. The high-dose estrogens were discontinued in 1970 because of an increased number of fatal cardiovascular events without any indication of benefit. The low-dose estrogens were discontinued in 1975 because of suggestion of an excess incidence of mortality from cancer. Dthyroxin was discontinued in 1971 because of increased mortality in this group (The coronary drug project, 1957).

Ayurvedic treatment

Ayurvedic medicine is one of the world's oldest medical systems. Ayurvedic therapeutics is based on the "laws" of nature. Its approach to health-care is based on understanding the interrelationship of body, mind and spirit. The aim of ayurveda medicine is to integrate and balance these elements to prevent illness and promote wellness through diet, nutrition, herbs, yoga, meditation and daily seasonal routines (Tarabilda, 1998). Ayurvedic medicine has been used for thousands of years for treatment of various metabolic disorders. However, few studies have been conducted to evaluate the effectiveness of Ayurveda herbal medicine formulae on hyperlipidemia. Higher quality studies, such as randomised clinical trials, are lacking (Singh *et al.*, 2007)

Home medications

Besides, pharmacological and ayurvedic treatment, some home remedies are also beneficial in the treatment of hyperlipidemia. Some home ingredients which help in lowering lipid and cholesterol level in the body are listed in table 1.2.

Table 1.2: Home remedies for dipping high cholesterol levels

Ingredients	Role
Nuts	Almonds lower LDL by 4.4%, Walnuts lower LDL by 16%.
Oatmeal	Drops LDL by 12-24%.
Orange juice	Reduce blood cholesterol level.
Coriander seeds	Lower cholesterol and triglycerides levels.
Fish oil	Lower triglycerides levels.
Honey	Lower cholesterol level.
Soyabeans	Reduce the production of new cholesterol.
Indian Gooseberry	Reduces excess cholesterol build-up.
Brown Rice	Lower cholesterol level.
Turmeric	Lowers LDL cholesterol levels.
Brinjal	Lowers LDL cholesterol levels.
Coconut oil	Increases HDL and improves the LDL/HDL ratio.
Fenugreek seeds	Lowers cholesterol level by 14%.
Beans	Lowers LDL level
Avocados	Lowers cholesterol level and boost up HDL level.
Olive oil	Lowers LDL-C levels.
Apples	Lowers cholesterol level.
Broccoli	Lowers blood cholesterol level.
Chocolate	Maintain HDL-C and reduces LDL-C levels.

Plants having hypolipidemic activity

Medicinal plants have always been considered as a healthy source of life for all people due to its rich therapeutic properties and being 100% natural (Edeoga *et al.*, 2005). Medicinal plants are widely used by the majority of populations to cure various diseases and illness and have a high impact on the world's economy (Bauman, 2000-2003). Over the past decade, herbal medicine has become a topic of global importance, making an impact on both world health and international trade. Continuous usage of herbal medicine by a large proportion in the developing countries is largely due to the high cost of Western Pharmaceuticals and Healthcare (Cunningliam, 1988). Medicinal plant based drug industries is progressing very fastin India. The medicinal plants play a major role in hypolipidemic activity(Muramatsu and Fukuyo, 1986). The advantages of herbal medicines are effectiveness, safety, affordability and acceptability. Some plants having hypolipidemic property are listed in table 1.3.

Table 1.3: Plants having hypolipidemic activity

Plant	Botanical name	Part used	Family
Inca wheat	*Amaranthus caudatus*	Leaves	Amaranthaceae
Palash	*Butea monosperma*	Leaves	Fabaceae
Amaltas	*Cassia fistula*	Legume	Fabaceae
Guggul	*Commiphora mukul*	Gum resin	Burseraceae
Kesraj	*Eclipta alba*	Flower	Asteraceae
Kalajam	*Eugenia Jambolana*	kernels	Myrtaceae
Pipal	*Ficus racemosa*	Bark	Moraceae
Mulethi	*Glycyrrhiza glabra*	Root	Leguminoceae
Bottle gourd	*Lagenaria siceraria*	Fruit	Cucurbitaceae
Musli	*Cholophytum borivilianum*	Root	Liliaceae
Drumstick tree	*Moringa oleifera*	Leaves, root, seed	Moringaceae
Snake jasmine	*Rhinacanthus nasutus*	Whole plant	Acanthaceae

1.5 Plant Profile:

1.5.1 *Ficus racemosa*

Botanical Name: Ficus racemosa

Common Names: Cluster fig, Gular, Udumbara, Indian fig tree

Family: Moraceae

Description: Ficus racemosa is a large, deciduous tree that can grow up to 20 meters tall. It is commonly found in various parts of India, Southeast Asia, and Australia. The tree is known for its distinctive fruits, which grow in clusters directly on the trunk and branches.

Leaves: The leaves are ovate to elliptic, with a smooth margin and a leathery texture. They are alternately arranged on the branches.

Flowers: The flowers of Ficus racemosa are tiny and unisexual, enclosed within a syconium (a fleshy structure that contains the flowers).

Fruits: The fruits are globose, green when unripe, and turn red or dark purple when ripe. They grow in large clusters directly from the trunk or major branches.

Habitat: Ficus racemosa typically grows in tropical and subtropical regions. It prefers moist, well-drained soils and is often found along riverbanks and in lowland forests.

Figure 1.1: Leaves and fruits of *Ficus racemosa*

Uses:

Traditional Medicine: Various parts of the plant (leaves, fruits, bark, and latex) are used in traditional medicine for treating a range of ailments, including diabetes, diarrhea, ulcers, and respiratory disorders.

Culinary: The fruits are edible and are used in various culinary preparations in some cultures.

Chemical Constituents

The stem bark of *Ficus racemosa* Linn contains tannin, wax, saponin gluanol acetate, β-sitosterol (A), leucocyanidin- 3 – O – β – D - glucopyrancoside, leucopelargonidin – 3 – O – β – D - glucopyranoside, leuc12 opelargonidin – 3 – O– α– L - rhamnopyranoside, lupeol (C), ceryl behenate, lupeol acetate, α-amyrin acetate(B), leucoanthocyanidin and leucoanthocyanin from trunk bark lupeol, β- sitosterol and stigmasterol were isolated (Husain *et al.,* 1992). Fruit contains glauanol, hentriacontane, β sitosterol, glauanolacetate, glucose, tiglic acid (E), esters of taraxasterol, lupeol acetate (D), friedelin (F), higherhydrocarbons and other phytosterol (Suresh *et al.*, 1979). A new tetracyclic triterpene glauanol acetate whichis characterized as 13α, 14β, 17βH, 20 α H-lanosta-8, 22-diene-3β acetate and racemosic acid were isolated from the leaves. An unusual thermo stable aspartic protease was isolated from latex of the plant (Devaraj *et al.*, 2008).

1.5.2 *Linum usitatissimum*

Botanical Name: Linum usitatissimum

Common Names: Flax, Common Flax, Linseed.

Family: Linaceae

Botanical Description:

- **Growth Habit:** Annual herb.
- **Height:** Typically 0.3 to 1.2 meters.
- **Stem:** Erect, slender, and branching.
- **Leaves:** Simple, alternate, narrow lanceolate, 2-4 cm long.
- **Flowers:** Small, blue, five-petaled, blooming in summer.

- **Fruit:** Dry capsule (boll), containing several seeds.

Origin and Distribution: Native to the region extending from the eastern Mediterranean to India. Widely cultivated in temperate and tropical regions worldwide. Cultivation prefers well-drained soils and moderate climates. Requires full sun exposure. Grows best in cool climates and is usually planted in the spring.

Chemical Compositions:

It is a leading source of n-fatty acid, α-Linolenic acid (ALA) (52 % of the total fattyacid), and of phenolic compounds commonly known as Lignans (> 500 μg/g, as is bases), in addition to containing hydrocolloidal gum, also referred to as Mucilage (about 8 % of seed weight), and a good quality of protein and fiber. It also contain 30-40 % of fixed oil and a small quantities of cyanogenetic glycosides (0.05-0.001%) mainly linustatin, neolinustatin and linamarin; lignans; phenyl propane derivatives including linusitamarin (Evans, 2002). It also contain amygdalin, resin, wax sugar and ash 3-5 p.c. Ash contain sulphate, chlorides of potassium, calcium and magnesium, β- Carotene forms 22 to 30 % of the total carotenoids (Kapoor, 2005).

Health Benefits:

Heart Health: Omega-3 fatty acids and lignans contribute to cardiovascular health.

- **Digestive Health**: High fiber content aids in digestion and helps prevent constipation.
- **Anti-inflammatory**: Lignans and omega-3 fatty acids have anti-inflammatory properties.
- **Antioxidant**: Lignans and phenolic acids act as antioxidants, protecting cells from damage.
- **Hormonal Balance**: Lignans can have beneficial effects on hormonal balance, especially in postmenopausal women.

Figure 1.2: Seeds of *Linum usitatissimum* L

1.5.3 Zingiber officinale (Ginger)

Botanical Name: Zingiber officinale.

Common Names: Ginger, Ginger root.

Family: Zingiberaceae.

Botanical description

Ginger is herbaceous rhizomatous perennial, reaching up to 90 cm in height under cultivation. Rhizomes are aromatic, thick lobed, pale yellowish, bearing simple alternate distichous narrow oblong lanceolate leaves. The herb develops several lateral shoots in clumps, which begin to dry when the plant matures.

Leaves are longand 2-3 cm broad with sheathing bases, the blade gradually tapering to a point. Inflorescence solitary, lateral radical pedunculate oblongcylindrical spikes.

Medicinal uses

Ayurveda recommends *Zingiber officinale* (ginger) to manage various disease conditions in spite of mentioning modern perspectives on antiviral, radioprotective, anti-inflammatory, and anticancer and antioxidant effects. Even though, traditional Ayurveda classics provide strong literature base to administer ginger in various diseases mentioning complication or associated symptoms of many disorders. Recent advances in phytochemistry and ethnomedicinal studies elaborate uses of ginger in viral infections, carcinogenic conditions and physiological needs.

Figure 1.3: Rhizome of *Zingiber officinale*

Chemical Constituents

Ginger is known for its rich array of bioactive compounds, which contribute to its medicinal and culinary uses. Some key chemical constituents include:

Essential Oils

- **Gingerol:** The major pungent compound in fresh ginger. Gingerol has anti-inflammatory and antioxidant properties.
- **Shogaol:** Formed from gingerol when ginger is dried or cooked. It is more pungent and possesses strong anti-inflammatory and anti-cancer properties.
- **Zingerone:** Formed from gingerol during cooking, contributing to ginger's aroma and flavor.
- **Paradols:** Found in small quantities, contributing to ginger’s flavor and potential health benefits.

Non-Volatile Compounds

- **Diarylheptanoids:** These include gingerdiols, gingerdiones, and hexahydrocurcumin. They exhibit anti-inflammatory, antioxidant, and anticancer activities.
- **Flavonoids:** Such as quercetin and kaempferol, which have antioxidant properties.
- **Amino Acids:** Essential for protein synthesis and overall health.
- **Vitamins and Minerals:** Including vitamins B6 and C, potassium, manganese, magnesium, and copper.

1. REVIEW OF LITERATURE

Hussien and Aziz, (2021) worked on antibacterial activity of *Linum Usitatissimum* L. The results of Microbiological tests were displayed some difference between the effect of *Linum usitatissimum* L. on the declining of the bacterial species growth. Aqueous extract of *Linum usitatissimum* L. showed inhibition zone againstbacteria ranged from 16 to 25 mm, while alcoholic extract achieved inhibitionzone against bacteria ranged from 17.5 to 27 mm. In present study we indicate that extracts derived from Flaxseed might be the active source of antibacterial compounds andthe hopeful alternate to antibiotic therapy.

Selvi *et al.*, (2019) assessed the various phytochemical compositions, Gas chromatogram and Mass spectrometry (GC-MS) analysis, and antibacterial potentialof different extracts of *L. usitatissimum*. The crude extracts of *L. usitatissimum* revealed the presence of several biologically active phytochemicals with the highest quantity of steroids, terpenoids, flavonoids, tannins, cardiac glycosides, protein, and amino acids. The antibacterial potency was explored against pathogenic bacteria, and the highest inhibitory activity of ethanol and methanol extracts was obtained against *Staphylococcus aureus*. The GC-MS analysis provides different peaks determining the presence of 10 phytochemical compounds with different therapeutic activities. The phytoactive principles were described with their molecular formula, retention time, molecular weight, and peak area (%).

Mao *et al.*, (2019) reported Ginger (*Zingiber officinale* Roscoe) is a common and widely used spice. It is rich in various chemical constituents, including phenoliccompounds, terpenes, polysaccharides, lipids, organic acids, and raw fibers. The health benefits of ginger are mainly attributed to its phenolic compounds, such as gingerols and shogaols. Accumulated investigations have demonstrated that ginger possessesmultiple biological activities, including antioxidant, anti- inflammatory, antimicrobial, anticancer,neuroprotective, cardiovascular protective, respiratory protective, antiobesity, antidiabetic, antinausea,and antiemetic activities.

Balasubramanian *et al.*, (2019) antibacterial activity of leaf and fruit parts of *Ficus racemosa* plant. Phytochemical analysis revealed alkaloids, flavonoids, phenols that contributed for higher antioxidant and antibacterial activity. The fruit exhibited significant inhibition when compared with leaf. Bacteria from wounds of diabetic patients were isolated and identified as *Staphylococcus* spp., *Pseudomonas* spp., *Klebsiella* spp. and *Escherichia coli*. MIC for all the three (Ethanol, toluene and ethyl acetate) extracts was performed against wound isolates. Among all wound bacteria tested *Staphylococcus* spp. showed lowest MIC (0.07 mg/ml) with toluene extract of fruit. The MIC for *E. coli* and *Pseudomonas* spp. was found to be 0.15 mg/ml and for *Klebsiella* spp. (0.31 mg/ml).The MIC 0.625 mg/ml was obtained for toluene extracts of leaf against *Staphylococcus* spp. and *Klebsiella* spp. Antibacterial study revealed that the extracts of leaf and fruit exhibited good inhibition activity against wound isolates. Study of well diffusion assay of *Ficus racemosa* extract revealed that 75 and 100 µg/ml concentration was found to have significant control over wound pathogens. Highest inhibition was obtained for *Staphylococcus* spp. and *Klebsiella* spp. with fruit extract with a zone of inhibition of 26 ± 0.10 and 24 ± 0.13 mm, respectively at 100 µg/ml. Toluene extract of fruit had higher antioxidant

activity with IC_{50} of 0.75 µg/ml followed by ethanol extract 1.42 µg/ml and correlated with antibacterial activity.

Han *et al.*, (2018) Flaxseed (*Linum usitatissimum* L.) is important source of oil and protein for industrial, pharmaceutical, and nutritional applications. In order to estimate the effects of lyophilized aqueous extract of flaxseed shell (AEF) and evaporated ethanolic extract of flaxseed shell (EEF), we studied their DPPH, ABTS, DMPD and O2•- scavenging effects. Total antioxidant activity by ferric thiocyanate method, Fe3+, Cu2+ and [Fe3+-(TPTZ)2]3+ reducing ability, and Fe2+ chelating activity. Also, α-tocopherol, BHA, trolox, and BHT were used as positive controls.The results clearly AEF and EEF demonstrated effective antioxidant activity. The quantity of p-hydroxybenzoic, vanillin, p-coumaric acid, ascorbic acid, ferulic acid, and ellagic acid were investigated by LC-MS/MS.

Rafieian-kopaei *et al.*, (2017) analgesic and anti-inflammatoryeffects of *Linum usitatissimum* L were evaluated. Xylene test was used for anti-inflammatory evaluation in which 48 mice were randomly designated into 6 groups of 8 eachincluding: control, dexamethasone as positive control (15 mg/kg), and experimental groups (42, 85, 170, and 340 mg/kg, respectively). For analgesic evaluation, 192 mice were randomly designated into 4 sets of 6 groups of 8 mice, including control, morphine as positive control, morphine plus naloxone, experimental groups (200 and 500 mg/kg extract), and extract along with naloxone group, which received 500 mg/kg. The analgesic activities were evaluated at 5, 15, 30, and 60 minutes, respectively, in each set. Both doses showed analgesic activity, the 200 mg/kg possessed higher effects ($P < .05$). Naloxone reduced a section of its effect ($P<.001$). The 170 mg/kg dose of the extract showed anti-inflammatory activity ($P<.05$).

Shah *et al.*, (2016) reported traditional systems of medicine, different parts (leaves,stem, root, fruit, seeds, latex and even whole plant) of *Ficus Racemosa* Linn (commonly known in all over India as udumbara, gular have been recommended for the treatment of diarrhea, diabetes, hypertension, gastric ulcer, wound healing etc. *Ficus Racemosa* Linn. Showed a wide range of pharmacological actions like hypoglycemic, hypolipidemic, renal anti-carcinogenic, anti-diuretic, anti-tussive,hepatoprotective, radioprotective, anti-ulcer, anti-inflammatory, anti-diarrhoel andanti-fungal. β-sitosterol, glauanol acetate, the active constituent present in *Ficus Racemosa* L., has been found to be largely responsible for the therapeutic potentialsof gular.

Vijay and Devanna, (2016) investigated its antihyperlipidemic activity by *in vivo* animal model. Hyperlipidemia model can be induced by administered with dexamethasone in rats with significant increase in serum cholesterol and triglyceride (TG) levels along with increase in the atherogenic index. The ethanolic extract of leaves of *L. aspera* Linn. (200 and 400 mg/kg) treatment has shown significant inhibition against dexamethasone-induced hyperlipidemia in rats by maintaining the serum levels of cholesterol, TGs and near to the normal levels.

Rafael Henrique Oliveira Lopes *et al.*, (2016) investigated the antioxidant and hypolipidemic activity of hydroethanolic extract of *Curatella americana* L. leaves (ExC). The antioxidant activity of ExC was assessed by 2,2-diphenyl-1- picrylhydrazyl free radical (DPPH) scavenging capacity and protection against hemolysis induced by 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), followed by quantification of malondialdehyde (MDA). Wistar rats with hyperlipidemia induced by high-fructose diet (60%) were treated for

60 days with water, simvastatin (30 mg·Kg−1), ciprofibrate (2 mg·Kg−1), and ExC (200 mg·Kg−1). ExC revealed IC50 6.0 ±0.5 of µg·mL−1, an intermediary valueamong positive controls used in the assay of DPPH scavenging capacity. At all concentrations (50 to 125 µg·mL−1) and times (60 to 240 min) evaluated, ExC protected erythrocytes against AAPH-induced hemolysis, which was confirmed by lower MDA levels. In vivo tests showed a reduction of 34 and 45%, respectively, in serum concentration of cholesterol and triglycerides in hyperlipidemic rats treated with ExC, a similar effect compared to the reference drugs, simvastatin and ciprofibrate, respectively.

Syed Safiullah Ghori *et al.*, (2015) designed to perform preliminary phytochemical screening, acute oral toxicity and to evaluate antihyperglycemic activity of wholeplant of *Glycosmis pentaphylla* ethanolic extract. *Glycosmis pentaphylla*, whole plant was extracted using ethanol as solvent by soxhlet apparatus. The extract was subjected to preliminary phytochemical screening. Acute oral toxicity studies were performed to determine test dose. The evaluation of antihyperlipidemic activity was done using Triton X 100 and High Fat Diet induced hyperlipidemia models in Wistar albino rats.Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, glycosides, saponins, tannins, flavonoids, proteins, and amino acids. Doses up to 2000mg/kg were found to be safe after acute toxicity tests. Cholesterol, triglycerides, HDL, LDL, VLDL, SGOT, SGPT, Total protein and glucose were measured. The results suggested that EGP (ethanolic extract Glycosmis pentaphylla) possess antihyperlipidemic activity against hyperlipidemia induced by Triton X 100 and also High Fat Diet induced experimental models.

Sikarwar and Patil, (2014) investigated the possible antihyperlipidemic effect of *Pongamia pinnata* (Leguminosae) leaf extract in triton (400 mg/kg b.w.) induced and atherogenic diet induced hyperlipidemic rats. Petroleum ether, chloroform, ethanol and aqueous extracts of leaves were evaluated for antihyperlipidemic. Antihyperlipidemic drug simvastatin (10mg/kg body wt.) was used as a positive control. The results of the study were expressed as mean± S.E.M. and data was analyzed by using one way analysis of variance test (ANOVA) followed by Dunnett's t-test for multiple comparisons. In diet induced model, chloroform extract showed significant serum lipid lowering parameters like total cholesterol, triglycerides, low density lipoprotein (LDL), very low density lipopreotein (VLDL) and increase in high density lipoprotein (HDL) in hyperlipidemic rats of both models as compared to hyperlipidemic control statistically. In triton induced model, oral administration of (500 mg/kg body wt.) of the chloroform extract and alcoholic extract were able to reduce serum lipid level significantly as compared tohyperlipidemic control.

Brahma Srinivasa Rao *et al.*, (2013) the anti-hyperlipidemic effect of methanolic extract of whole plant of *Rhinacanthus nasutus* ((RNM) was tested in Triton and fat diet induced hyperlipidemic rat models. Here, Acute hyperlipidemia was induced by administration of single dose of Triton X 100 (400 mg/kg,i.p) and Chronic hyperlipidemia was induced by feeding fat diet for 21 days to rats. Treatment with RNM (200 and 400 mg/kg, p.o) significantly reduced the hyperlipidemia i.e., decreased levels of serum Total Cholesterol,Triglycerides,Low Density Lipoprotein Cholesterol (LDL-C),Very Low Density Lipoprotein Cholesterol (VLDL-C), and increase of serum High Density Lipoprotein Cholesterol (HDL-C) when

compared to vehicle control and standard drug Atorvastatin (10 mg/kg). The results demonstrated that methanolic extract of whole plant of *Rhinacanthus nasutus* possessed significant *antihyperlipidemic* activity.

Sultana *et al.*, (2013) methanolic extract was prepared from the leaf and stem bark of *F. racemosa*. Their total phenolics and free radical scavenging capacity was determined using folin-ciocalteu reagent (FCR) assay and DPPD-radical scavenging assay respectively. Stem bark and leaves of *F. racemosa* contains 242.97 and 235.45 mg of GAE / gm of dried extract of phenolics respectively. Current studies also show that F. racemosa barks contain a high antioxidant activity. IC50 value of bark extract was found to be 19µg/ml which is even better that of the standard BHT.

Mashhadi *et al.*, (2013) reviewed on ginger effects as an anti-inflammatory andanti-oxidative. The anticancer potential of ginger is well documented and its functional ingredients like gingerols, shogaol, and paradols are the valuableingredients which can prevent various cancers.

Sikarwar and Patil, (2012) investigated the possible antihyperlipidemic effect of *Salacia chinensis* root extract in triton (400mg/kg b.w.)-induced and atherogenic diet-induced hyperlipidemic rats. Oral administration of 500 mg/kg body wt. of the chloroform extract and alcoholic extract of *Salacia chinensis* root exhibited a significant reduction ($P<0.01$) in serum lipid parameters like total cholesterol, triglycerides, low density lipoprotein (LDL), very low density lipopreotein (VLDL) and increase in high density lipoprotein (HDL) in hyperlipidemic rats of both models as compared to hyperlipidemic control statistically. These extracts were found to possess better antihyperlipidemic potential as compared to pet ether and aqueous extract.

Goyal, (2012) worked on antimicrobial activity of ethanolic root extract were evaluated against four bacteria and four fungi at different concentration by using disc diffusion method. The test extract was found to be bacteriostatic and fungistaticin action thus can be used as a source of antibiotic substances for drug development that can be used in control of this bacterial and fungal infection.

Girija and Lakshman, (2011) investigated the anti-hyperlipidemic activity of methanol extracts of leaves of three plants of Amaranthus. It was found that all the three plants at 400mg/kg dose showed significant anti-hyperlipidemic effect ($P<0.01$), whereas 300mg/kg dose is less significant in the entire parameters used for evaluation of anti hyperlipidemic effect ($P<0.05$).Methanol extracts of *Amaranthus caudatus, Amaranthus spinosus, Amaranthus viridis* showed significant anti- hyperlipidemic effect and this study provides the scientific proof for their traditional claims

Lakshmi *et al.*, (2011) ethanol extract of unripe pods and leaves of *Bauhinia purpurea* was evaluated for antihyperlipidemic activity in cholesterol high fat diet (CHFD) induced hyperlipidemia. The groups of rats selected for the study were treated with atorvastatin, ethanol extract of unripe pods and ethanol extract of leaves daily for the whole period. Changes in body weight and the analysis of serum lipids were carried out at the end of the study. There was a marked decrease in bodyweight, total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels. Also there was a significant increase in high density lipoprotein levels after the treatment with *Bauhinia purpurea* extracts. Ethanol extract of leaves

showed a marked effect over body weight reduction andalso had a significant effect on the lipoprotein profile. There is a lowered atherogenic index, TC: HDL-c and LDL: HDL-c ratios in the extract treated groups.

Girija and Lakshman, (2011) investigated the anti-hyperlipidemic activity of methanol extracts of leaves of three plants of *Amaranthus*. It was found that all the three plants at 400 mg/kg dose showed significant anti-hyperlipidemic effect ($P<0.01$), whereas 300 mg/kg dose is less significant in the entire parameters usedfor evaluation of anti hyperlipidemic effect ($P<0.05$). Methanol extracts of *Amaranthus caudatus, Amaranthus spinosus, Amaranthus viridis* showed significant anti-hyperlipidemic effect and this study provides the scientific proof for their traditional claims.

Boopathy Raja *et al.*, (2010) fruit extract of *Helicteres isora* was used to evaluate the anti-hyperlipidemic activity in streptozotocin induced diabetic rats. The serum and liver lipid levels were abnormal in streptozotocin induced diabetic rats than inthe control rats. Total cholesterol, triglycerides, phospholipids, LDL and VLDL were elevated and the HDL level was significantly decreased in diabetic rats. After treated with *Helicteres isora* fruit extract (HiFE), the lipid levels of diabetic rats were restored to near normal level.

2. AIM & OBJECTIVE

Aim

The aim of this research is to evaluate the anti-hyperlipidemic activity of a polyherbal extract containing *Ficus racemosa*, *Linum usitatissimum*, and *Zingiber officinale* in an animal model, and to compare its efficacy and safety with that of a standard lipid-lowering drug.

Objectives

- The primary objective is to assess the lipid-lowering effects of a polyherbal extract composed of *Ficus racemosa*, *Linum usitatissimum*, and *Zingiber officinale*, and to compare its efficacy with that of a standard pharmaceutical agent, such as Orlistat.
- To develop polyherbal preparation according to the composition of plant extracts and their dose.
- To determine therapeutic doses of polyherbal preparation as peracute oral toxicity study.
- To evaluate the anti-hyperlipidemic activity of polyherbal preparation in experimental rats using the HFD model for induction of hyperlipidemia and atorvastatin as standard drug applying various parameters.

PLAN OF WORK

1. Literature Review.
2. Collection and Identification of Plant.
3. Selection of solvent for extraction.
4. Extraction of plant material.
5. Preliminary phytochemical screening of extract.
6. Formulation of Poly-herbal formulation.
7. Antihyperlipidemic activity of plant extract.
   - Acute oral toxicity studies.
   - Diet-induced hyperlipidemic model.
   - Biochemical Evaluation of Serum.
   - Statistical Analysis.
8. Result and discussion.
9. Summary and Conclusion.
10. Compilation and Submission of thesis.

5. MATERIALS AND METHOD

5.1 Collection of plant material

Fruits of *Ficus racemosa,* seeds of *Linum usitatissimum,* rhizome of *Zingiberofficinale* were collected from local area of Bhopal (M.P.) in the month of February,2021.

5.1.1 Cleaning

After procurement of plant material, they were cleaned properly. The cleaning process involved the following steps. Very first the decayed or deterioratedplant material was removed. This was followed by washing with tap water and distilled water. The washed plant material was wrapped in blotting paper in order toremove extra water.

5.1.2 Drying

Drying of fresh plant parts were carried out in sun but under the shade.

5.2 Extraction procedure

Following procedure was adopted for the preparation of extracts from the shade dried and powdered herbs (Mukherjee, 2007):

5.2.2 Defatting of plant material

45.6 gram of shade dried of *Ficus racemosa,* 50.1 gram of *Linum usitatissimum* and 48.4 gram of *Zingiber officinale* was coarsely powdered and subjected to extraction with petroleum ether using soxhlation method. The extraction was continued till the defatting of the material had taken place.

5.2.3 Extraction by soxhlation process

Defatted dried powdered of *Ficus racemosa, Linum usitatissimum, Zingiberofficinale* were exhaustively

extracted with hydroalcoholic solvent (ethanol: water:70:30) using soxhlation method by soxhlet apparatus (Kokate, 1994). The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts.

5.2.4 Determination of Percentage yield

The percentage yield of yield of each extract was calculated by using formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}} \times 100$$

5.3 Phytochemical screening:

Phytochemical examinations were carried out for all the extracts as per the standardmethods.

1. **Detection of alkaloids**: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence ofalkaloids.

b) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. **Detection of carbohydrates:** Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. **Detection of glycosides:** Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence ofcardiac glycosides.

5. **Detection of saponins**

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

6. **Detection of phenols**

a) Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

7. **Detection of flavonoids**

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourlesson addition of dilute acid, indicates the presence of

flavonoids.

b) Lead acetate Test: Extracts were treated with few drops of lead acetate solution.Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Detection of proteins and aminoacids

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of diterpenes

a) Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

5.4 Polyherbal Formulation of Plant Extract

Equal portion from each plant extracts (*Ficus racemosa, Linum usitatissimum, Zingiber officinale)* was mixed in the ratio of 1:1:1 (1000mg of each extract) and subjected for animal activity.

5.5 Animals:-

Wistar rats (180–250 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments.

5.6 Acute toxicity studies

Acute oral toxicity was conducted according to the method of Organizationfor Economic Co-operation and Development (OECD) (OECD Guideline 423, 2001). Animals were kept fasting providing only water, polyherbal formulation *(Ficus racemosa, Linum usitatissimum, Zingiber officinale)* (200mg/kg/day) was administered orally for 4 days of different groups of rats (n=6) and the animals werekept under observation for mortality as well as any behavioral changes for evaluation of a possible antihyperlipidemic effect.

5.7 Induction of hyperlipidemia

Rats with an average body weight were made hyperlipidemic by giving high-fat diet (HFD) for 15 days. The HFD contained Cholesterol (2%), Cholic acid (1%), Dalda (20%), and Coconut oil (6%) as major constituents. Hyperlipidemia was confirmed by measuring the levels of serum lipids and lipoproteins in the rats (Ntchapda F, 2015).

5.8 Experimental designs

Group –I: Normal (vehicle alone)

Group –II: HFD rats treated with vehicle alone

Group -III: HFD rats treated with polyherbal formulation (100mg/kg, p.o.)

Group –IV: HFD rats treated with polyherbal formulation (200mg/kg, p.o.)

Group –V: Hyperlipidemic rats treated with Orlistat (60 mg/kg/day p.o.)

Animals were divided into five groups of 6 animals each. The first group treated normal vehicle alone. The group II received HFD rats treated with vehicle alone (positive control). The groups III, IV and V received 100 mg/kg and 200 mg/kg of polyherbal formulation and Orlistat (60 mg/kg/day p.o.) respectively for 15 days.

5.9 Biochemical Evaluation in Serum

Serum Triglycerides (TG), total cholesterol (TC), and High density lipoprotien-cholesterol (HDL-C), low density lipoprotein (LDL), serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT)were estimated by using commercial kits as per the manufacturer instructions. Blood was collected from Retro-orbital sinus animals and centrifuged. The serum samples were collected in separate containers for biochemical estimations.

Statistical analysis

The results were expressed in mean±standard deviation. Statistical analysiswas carried out by using one way ANOVA (Dunnett test).

6. RESULTS AND DISCUSSION

6.1 Result of percentage yield of extract

The crude extracts so obtained after the soxhlation process, extract were further concentrated on water bath evaporation the solvents completely to obtain the actualyield of extraction. The yield of extract obtained from sample using different solventis depicted in the table 6.1-6.3.

Table 6.1: Result of percentage yield of *Ficus racemosa*

S. No.	Extracts	% Yield (w/w)
1.	Petroleum ether	2.45
2.	Hydro-alcoholic	6.08

Table 6.2: Result of percentage yield of *Linum usitatissimum*

S. No.	Extracts	% Yield (w/w)
1.	Petroleum ether	1.63
2.	Hydro-alcoholic	3.74

Table 6.3: Result of percentage yield of *Zingiber officinale*

S. No.	Extracts	% Yield (w/w)
1.	Petroleum ether	0.97
2.	Hydro-alcoholic	4.74

6.2 Results of phytochemical Testing

A small portion of the dried extract were subjected to the phytochemical test using Kokate (1994) methods to test for alkaloids, glycosides, saponins, flavonoids and phenol separately for extract of sample. Small amount of extract is suitably resuspended into the sterile distilled water to make the concentration of 1 mg per ml.The outcomes of the results are discussed separately in the table 6.4-6.6.

Table 6.4: Result of phytochemical screening of *Ficus racemosa*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids A) Wagner's Test: B) Hager's Test:	 -ve +ve
2.	Glycosides A) Legal's Test:	 -ve
3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	 +ve -ve
4.	Saponins A) Froth Test:	 +ve
5.	Phenolics A) Ferric Chloride Test:	 +ve
6.	Proteins A) Xanthoproteic Test:	 +ve
7.	Carbohydrate A) Fehling's Test:	 -ve
8.	Diterpenes A) Copper acetate Test:	 -ve

Table 6.5: Result of phytochemical screening of *Linum usitatissimum*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids A) Wagner's Test: B) Hager's Test:	 -ve -ve
2.	Glycosides A) Legal's Test:	 -ve
3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	 +ve +ve
4.	Saponins A) Froth Test:	 +ve
5.	Phenolics A) Ferric Chloride Test:	 ve
6.	Proteins A) Xanthoproteic Test:	 +ve
7.	Carbohydrate A) Fehling's Test:	 +ve
8.	Diterpenes A) Copper acetate Test:	 -ve

Table 6.6: Result of phytochemical screening of *Zingiber officinale*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids A) Wagner's Test: B) Hager's Test:	 -ve -ve
2.	Glycosides A) Legal's Test:	 -ve

3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	-ve +ve
4.	Saponins A) Froth Test:	+ve
5.	Phenolics A) Ferric Chloride Test:	+ve
6.	Proteins A) Xanthoproteic Test:	-ve
7.	Carbohydrate A) Fehling's Test:	+ve
8.	Diterpenes A) Copper acetate Test:	-ve

Table 6.7: Study of extracts (AC) using Acute Toxic Class Method (OECD GUIDELINES 423)

S. No.	Treatment	Dose	Weight of the animal (grams)	Weight of the animal (grams)	Signs of toxicity	Onset of toxicity	Reversible or Irreversible	Duration
Day 1				**Symptoms after Test (4th Day)**				
1.	AC	200mg/kg	180	179	No	Nil	Nil	4 Days
2.	AC	200 mg	185	186	No	Nil	Nil	4 Days
3.	AC	200 mg	198	198	No	Nil	Nil	4 Days
Owing to neither toxicity or Death the dose for given again								
4.	AC	200 mg	198	197	No	Nil	Nil	4 Days
5.	AC	200 mg	199	200	No	Nil	Nil	4 Days
6.	AC	200 mg	200	198	No	Nil	Nil	4 Days

As the study revealed, with the starting dose of 200mg/kg of the extract administered to all the animals exhibited no lethality (within 4 days). As no mortality was observed the test was repeated for the same set of experimental animals which again didn't show any mortality. This was considered under category X (unclassified) according to the GHS sytem for classifying the toxic chemicals.

Also it is reported that there was no mortality or any toxicity found at the selected doses until the period of study.

6.3 Results of anti-hyperlipidemic activity

Anti-obesity effect of the poly-herbal formulation on the high fat diet induced rats. The mean body weight as shown in Table 6.7. The activity levels of TC, TG, LDL,HDL, SGOT and SGPT were observed in normal and experimental animals in group. In group II animals, the activity levels of TC, TG, LDL, SGOT and SGPT were significantly elevated when compared to that of normal groups. On the other hand the HDL was significantly depleted in the HFD fed rat. In group III, IVand V animals, the activity levels of TC, TG, LDL, SGOT and SGPT weresignificantly decreased when compared to that of normal groups. Also HDL level was significantly increased in the same groups.

Table 6.8: Mean Body Weight Change

Group	Drug	Dose	Body weight (g)	
			Onset of study	End of study
I	Normal	Normal saline	180.10±5.50	190.00±7.50
II	Control	HFD	185.05±5.50	300.50±7.50*
V	Polyherbal formulation	100 mg/kg p.o.	186.00±5.50	195.00±5.50*
VI	Polyherbal formulation	200 mg/kg p.o.	185.05±5.00	198.00±5.50*
IV	Orlistat	60 mg/kg p.o.	195.00±5.00	187.50±8.50*

Values are expressed as the mean ± SEM of six observations. *** $P<0.005$ vs. control treatment (One-way ANOVA followed by Dunnett's test)

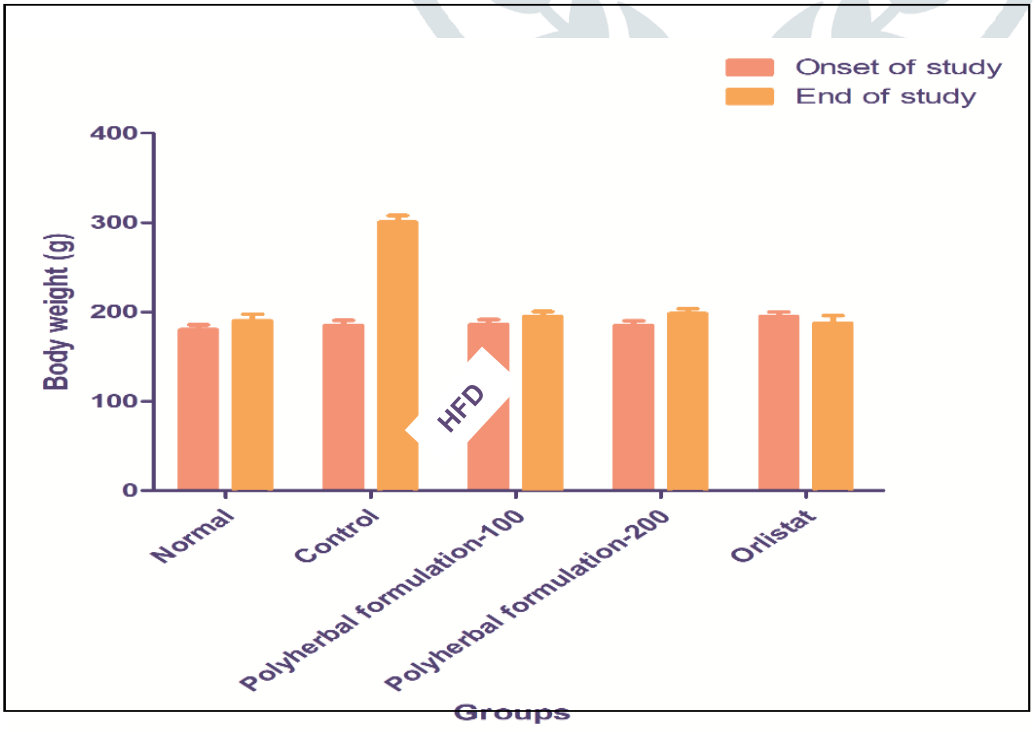

Figure 6.1: Effect of the Polyherbal formulation on body weight in HFDinduced rats

Table 6.9: Effect of the Polyherbal formulation on serum lipid profile levels inHFD induced rats

Treatment	Dose	Total cholesterol (mg/dL)	Triglycerides (mg/dL)
Normal	Normal saline	105.00 ± 5.00	110.00 ± 5.50
Control	HFD	290.10 ± 5.50	240.0 ± 5.22
Polyherbal formulation	100 mg/kg p.o.	155.00 ± 5.50**	140.50 ± 5.50*
Polyherbal formulation	200 mg/kg p.o.	130.10 ± 5.50***	135.50 ± 5.50*
Orlistat	60 mg/kg p.o.	115.20 ± 5.50***	119.50 ± 5.50*

Values are expressed as the mean ± SEM of six observations. *** P<0.005 vs. controltreatment (One-way ANOVA followed by Dunnett's test)

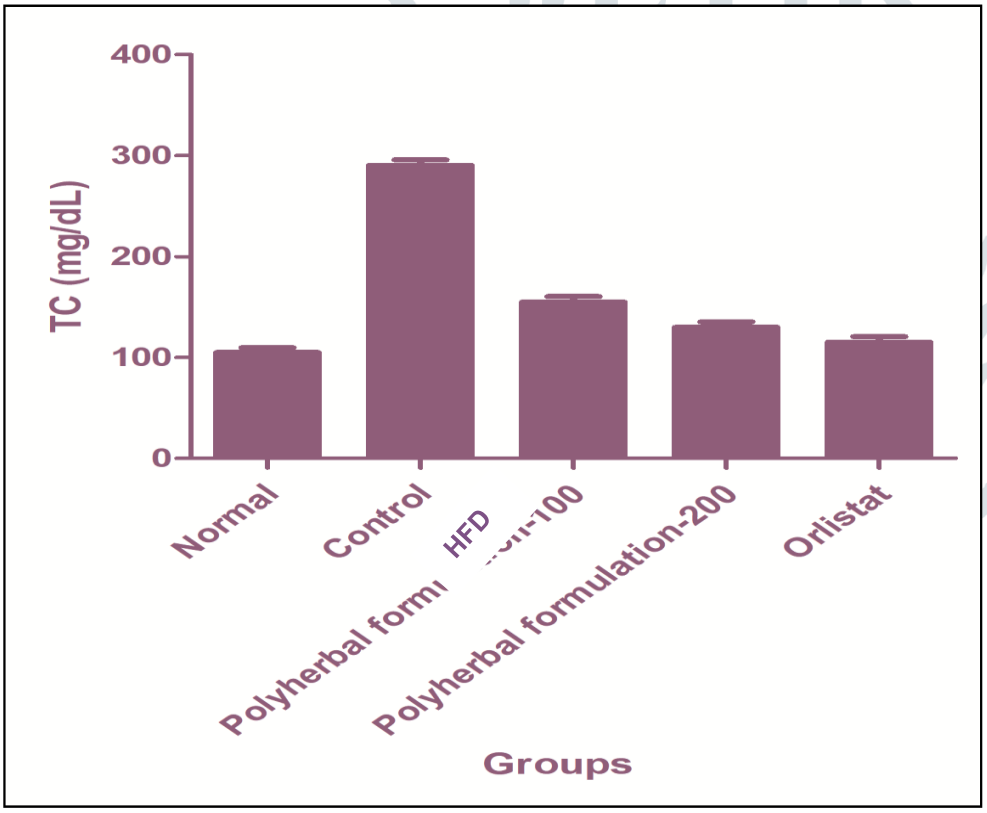

Figure 6.2: Effect of the Polyherbal formulation on serum lipid profile levels-Total cholesterol in HFD induced rats

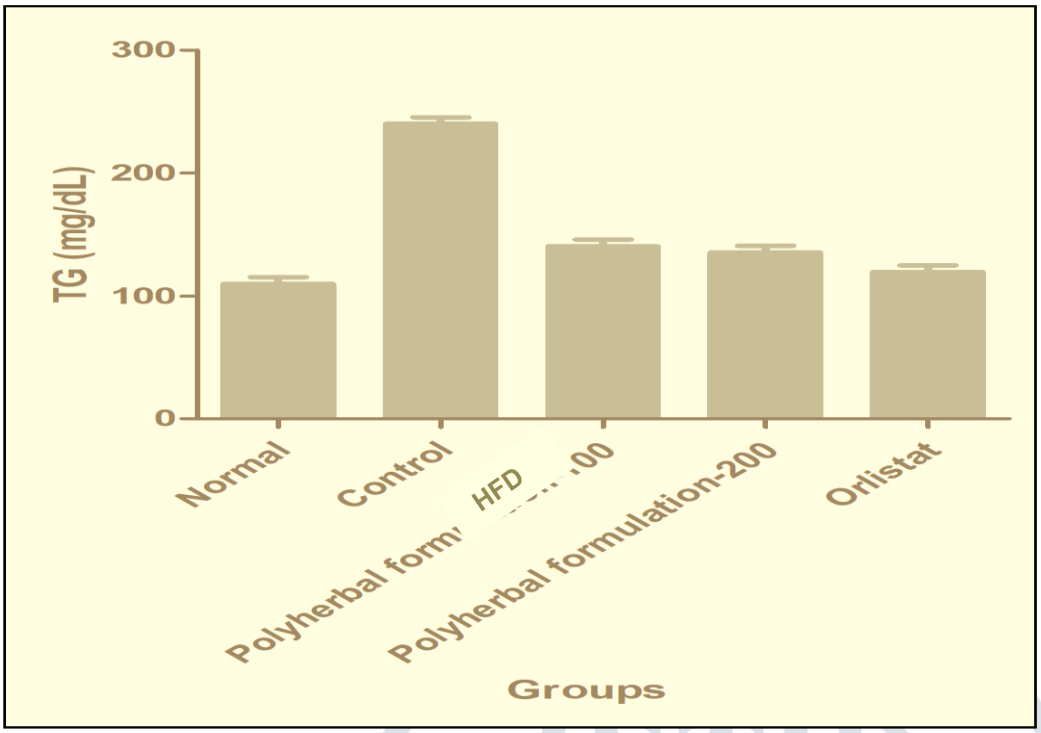

Figure 6.3: Effect of the Polyherbal formulation on serum lipid profile levels-Triglyceride in HFD induced rats

Table 6.10: Effect of the Polyherbal formulation on serum lipid profile levels inHFD induced rats.

Treatment	Dose	Low density lipoproteins (mg/dL)	High density lipoproteins (mg/dL)
Normal	Normal saline	80.00 ± 5.00	60.00 ± 5.00
Control	HFD	190.0 ± 5.52	25.00 ± 5.60*
Polyherbal formulation	100 mg/kg p.o.	120.50 ± 5.50**	40.40 ± 5.50*
Polyherbal formulation	200 mg/kg p.o.	105.50 ± 5.50***	45.50 ± 5.60*
Orlistat	60 mg/kg p.o.	95.50 ± 5.50***	50.10 ± 5.50*

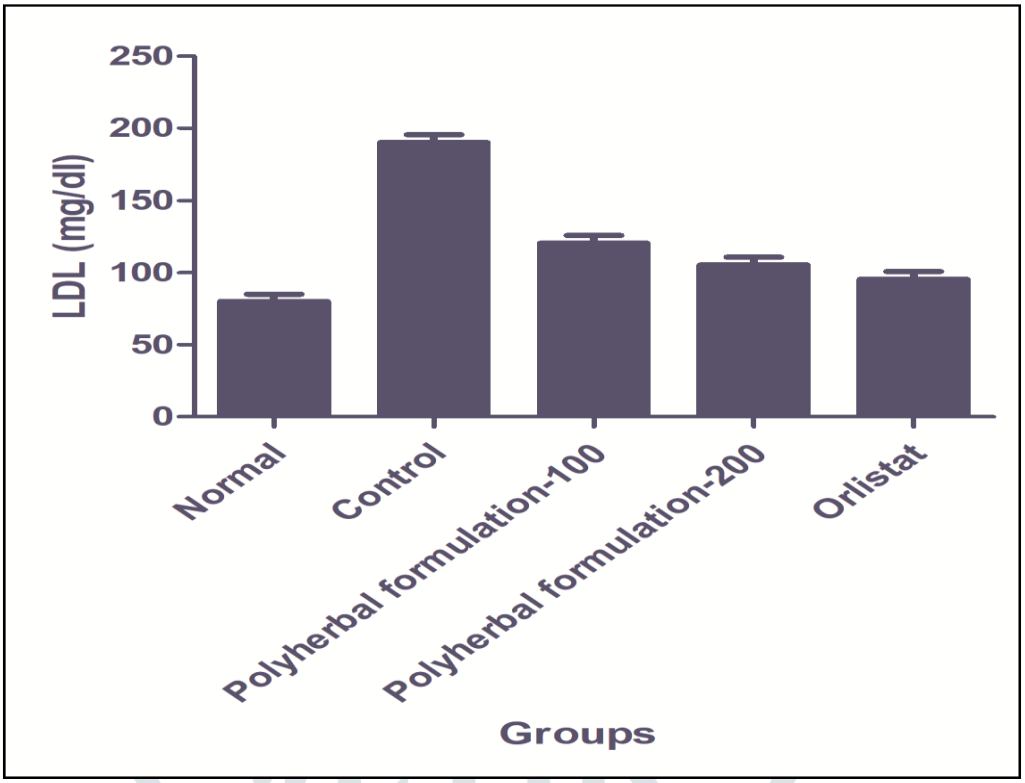

Figure 6.4: Effect of the Polyherbal formulation on serum lipid profile levels-Low density lipoproteins in HFD induced rats

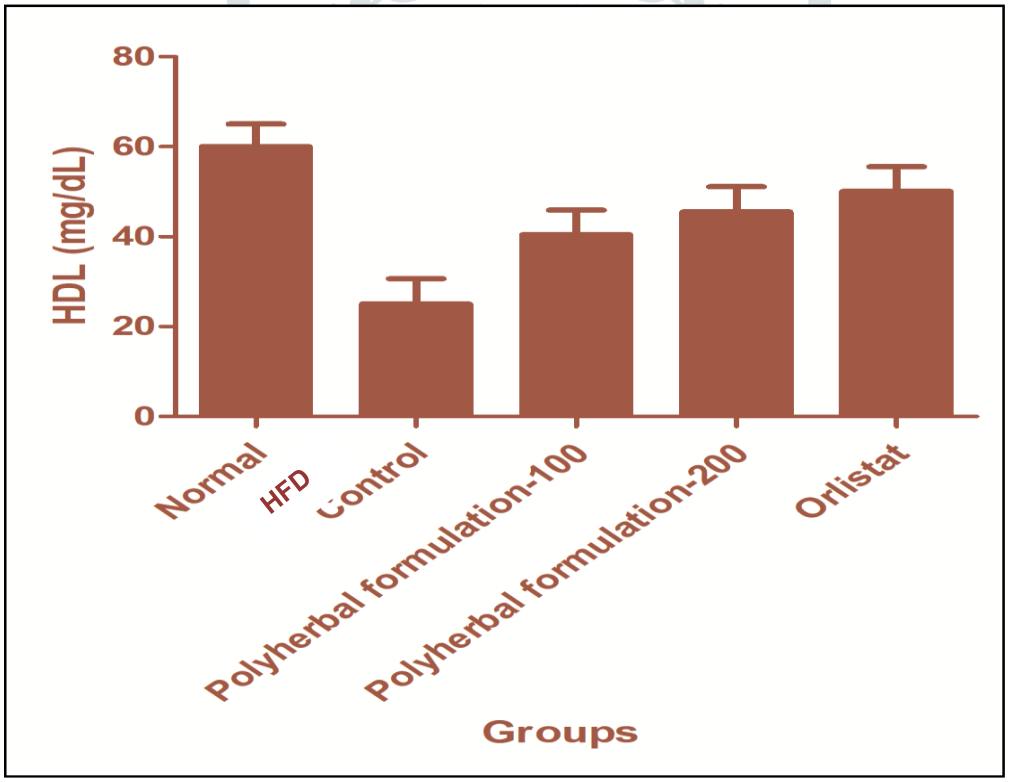

Figure 6.5: Effect of the Polyherbal formulation on serum lipid profile levels-High density lipoproteins in HFD induced rats

7. SUMMARY AND CONCLUSION

In the present study, the effects of Polyherbal formulation on obesity were assessed usinghigh fat diet induced rat model. Cholesterol is synthesized in all animal tissue. It isimportant to relate to its role in the stabilization of membrane structures because of its rigid planar structure. It also as a precursor for the synthesis of steroid hormones.In the present study, feeding rats with diets rich in cholesterol resulted in increasedTC, TG, LDL and decreased HDL levels.

This model was used to study the potential of effect of extract of Polyherbal formulation that contained significant amounts of antioxidants properties. In the current study, HFD significantly increased the TC and TG levels when given to theexperimental animals for 15 days. Oral administration of different doses of Polyherbal formulation produced significant reductions in lipids and significant increase in HDL-C levels to near normal. The 200 mg/kg dose of Polyherbal formulation was the most effective at reducing serum levels of TC and TG after five weeks of treatment when compared with the other two doses. The antihyperlipidemic activity of 100 and 200 mg/kg Polyherbal formulation was comparable to that of orlistat. HDL-C plays an important role in transferring cholesterol and cholesterol esters from tissues and cells to the liver, where they are metabolized to bile acids. Thus, HDL-C achieves an essential function of decreasing cholesterol levels in blood and peripheral tissues and prevent atherosclerosis plaque formation in the aorta. Meanwhile, TG plays a key role in maintaining normal lipid metabolism through regulation of lipoprotein interactions. Increased serum TGlevels were interrelated with an amplified rate of coronary artery disease. On the other hand, the oxidation of LDL-C in the artery walls by oxygen free radicals leads to the production of oxidized LDL-C, which attracts the macrophage scavenger of the immune system. As the study revealed, with the starting dose of 200mg/kg of the extract administered to all the animals exhibited no lethality (within 4 days). As no mortality was observed the test was repeated for the same set of experimental animals which again didn't show any mortality. This was considered under category X (unclassified) according to the GHS system for classifying the toxic chemicals. Also it is reported that there was no mortality or any toxicity found at the selected doses until the period of study. On the other hand the HDL was significantly depleted in the HFD fed rat. In group III, IVand V animals, the activity levels of TC, TG, LDL, SGOT and SGPT were significantly decreased when compared to that of normal groups. Also HDL level was significantly increased in the same groups. Values are expressed as the mean ± SEM of six observations. *** $P<0.005$ vs. controltreatment.

These macrophages accumulate at the arterial wall after ingestion of oxidized LDL-C particle, and their concentration is associated with atheromatous arteriosclerosis plaques. Higher TC, TG, and LDL-C levels and lower HDL-C levels are risk factors for atherosclerosis. These processes may lead to many complications, such as coronary heart disease, ischemic stroke, and occlusive arterial disease of the lower limbs. Administration of Polyherbal formulation to hypercholesterolemic rats resulted in a significant decline in plasma and hepatic total lipids, TC, and LDL-C levels. Furthermore, plasma HDL-C level was found to be increased.

In conclusion, this study demonstrated that Polyherbal formulation exhibited dose- dependent

antihyperlipidemic effect, with the highest dose exerting the most potenteffect in terms of reducing serum and tissue lipids in HFD-fed rats. It can be deduced that the beneficial properties of Polyherbal formulation against hyperlipidemia may be mediated in part through multiple mechanisms and reduction of the endogenous synthesis of TC and TG in the liver. Overall, this study suggeststhat Polyherbal formulation has a potent antihyperlipidemic effect that could be further developed as a lipid-lowering agent.

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