ANTIHYPERLIPIDEMIC ACTIVITY OF *STELLARIA MEDIA* LEAVES ON INDUCED HYPERLIPIDEMIC SPRAGUE DAWLEY RATS

A Dissertation submitted to

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

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In partial fulfilment of the award of the degree of

MASTER OF PHARMACY

IN

Branch-IV - PHARMACOLOGY

Submitted by

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TAMILNADU.

MAY – 2019

CERTIFICATES

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"ANTIHYPERLIPIDEMIC ACTIVITY OF *STELLARIA MEDIA* LEAVES ON INDUCED HYPERLIPIDEMIC SPRAGUE DAWLEY RATS"** submitted by the student bearing **Reg. No: 261725205** to "**The Tamil Nadu Dr.M.G.R.Medical University – Chennai**", in partial fulfilment for the award of Degree of **Master of Pharmacy** in **Pharmacology** was evaluated by us during the examination held on.....

Internal Examiner **External Examiner**

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DECLARATION

"ANTIHYPERLIPIDEMIC ACTIVITY OF *STELLARIA MEDIA* LEAVES ON INDUCED HYPERLIPIDEMIC SPRAGUE DAWLEY RATS" submitted to "**The Tamil Nadu Dr.M.G.R Medical University - Chennai",** for the partial fulfillment of the degree of **Master of Pharmacy** in **Pharmacology,** is a bonafide research work has been carried by during the academic year 2018-2019, under the guidance and supervision of **Mr. V.Venkateswaran, M.Pharm., Assistant Professor,** Department of Pharmacology, J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

I further declare that this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma, associate ship and fellowship or any other similar title. The information furnished in this dissertation is genuine to the best of my knowledge.

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Dedicated to

Almighty, Parents,

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My Family

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Chapter - I

Introduction

1.0 INTRODUCTION

1.1.Hyperlipidemia

Hyperlipidemia is manifested as hypercholesterolemia and hypertriglyceridemia. Hypercholesterolemia is the most common hyperlipidemia. The lipids that are involved in hypercholesterolemia are cholesterol, an essential component of cell membrane and a precursor of steroid hormone synthesis and triglycerides are important energy source, they are transported in blood as lipoproteins. The consequence of hyperlipidemia is to cause atherosclerosis, thus the risk of coronary heart diseases and strokes. The risk of heart diseases in future also depends on many other factors that influence the health of a person's level of cholesterol, blood vessels and blood circulation.

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases. Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death. Hyperlipidemia is characterized by elevated serum total cholesterol, low density lipoprotein, very low density lipoprotein and decreased high density lipoprotein levels. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease. Among these hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease. The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular disease or cerebrovascular disease. It is actively involved in the screening of herbal formulations and synthetic drugs for its anti-hyperlipidemic activity.

The term “dyslipidemia” now a days is increasingly being used to describe abnormal changes in lipid profile, replacing the old term hyperlipidemia. Hyperlipidemia means abnormal increase in fat levels of blood. These fats include cholesterol and triglycerides. These are important for our body to function, but when their levels are high they, can cause heart disorders.

1.2. Hepatoprotective associated Hypolipemic Mono-Herbal Medicines

Restorative plants are critical wellsprings of hepatoprotective medications. Very nearly 160 phytoconstituents from 101 plants have been guaranteed by Pharmacopeia Foundation to have hepatoprotective action. Home grown medications are most generally utilized than allopathic medications as hepatoprotectives in light of the fact that these are normally cheap, better social adequacy, enhanced similarity with human body and insignificant symptoms. Different classes of phytoconstituents like flavonoids, triterpenes, lignans, steroids, glycosides, polyphenols, saponins, coumarins and unpredictable oils and so forth gangs hepatoprotective action.

Basically diabetes is characterized by hyperglycemia, a condition of lack of insulin and development of complications in nephrons of kidney, peripheral nerves and retinal damages. Considerable effect on heart has also be the problem of developing further complications leading to atherosclerotic threats to brain, myocardium and lower extremities. Hyperglycemia causes various kind of injury to vascular system viz, increased pace of high glucose flux, intracellular production of advanced glycation products, activation of protein kinase and abnormal hexoseamine pathway. The increased mitochondrial reactive oxygen species (ROS)

would lead to microvascular changes heart and other vital functions of organs and their complex pathways. The damage breaks out by ROS production both mitochondrial and non-mitochondrial results in, tumor formation, age-related degeneration, inflammatory conditions and diabetes mellitus and obesity. Better understanding of ROS production and its intervention strategies leading to solution to this problem with newer technologies. In this context major factor for onset of diabetes has been evidenced due to ROS generation. Further various animal studies confirm that embryos are more vulnerable to the oxidative stress especially in type 2 diabetes. Maternal abnormalities were developed and observed to be more prominent in heart and reduction in pregnancy of the animals has been notified .The existing methods of treating diabetes do not combat diabetic complications, so there is an increased need for effective treatment, which is essential to fight with diabetic complications in relation to considerable reduction of ROS by using various technology and herbal drugs.

1.3. Consequences for Insulin Resistance and ROS production

In a condition pertaining to the high plasma levels of glucose and free fatty acids leads to increased production of reactive oxygen species (ROS) and to a least of reactive nitrogen species (RNS). In turn the initiation of various kinases starts occurring; proceed to phosphorylation of the insulin receptor and nitric oxide generation. Both the aforementioned pathways cause the signaling of insulin and suppress it drastically. Cascading reactions lends increased insulin resistance in liver, skeletal muscle and adipose tissues.

Increased free fatty acid level and lipid content are the prime factor for insulin resistant type 2 diabetes. Besides, the production of ROS could be more due to free fatty acids are common and mitochondrially how ROS is produced is still not understood and yet to be explored.

1.4. ROS and associated Hypertension

The considerate onset of hypertension is due to the non-phagocytic NADPH oxidase (Nox1, Nox 2 and Nox 4), apart from other factors for increased diabetic and hypertensive complications such as mitochondrial generation, inflammation, hypertrophy apoptosis, fibrosis, angiogenesis and rarefaction. Miscellaneous occurrence for ROS bounds to xanthine oxidase, cyclooxygenase, lipoxygenase and nitric oxide synthase.

Normal physiological processes affected by ROS are immunity, endocrine functions, and embryogenesis and signal transduction at cellular level. The intervention has given a tool to effectively control the ROS generation by antioxidants or nitric oxide production, to minimize the vascular injury, renal dysfunction and prevent target organ damage in diabetes and hypertension

Lipids are transported in the blood by being incorporated within lipoproteins. Lipoproteins are macromolecular disc like complexes of lipids and specific proteins called apoproteins. These apoproteins are crucial in the regulation of lipoprotein metabolism (they act as enzymes, cofactors or cell receptor ligands). Distinct classes of lipoproteins are found depending on the variation in lipid and apoprotein composition. Chylomicrons and their remnant contain apoprotein ß48 which is formed in the intestine. Apoprotein ß100 is synthesized in the liver and are found in

(VLDL, IDL, LDL and lipoprotein a) Lipoproteins that convey lipids into the artery wall. Plasma cholesterol and triglyceride are clinically important because they are major treatable risk factors for atherosclerosis and cardiovascular diseases. Hypertriglyceridemia also predispose to acute pancreatitis

Hyperlipidemia is an increase (hyper) in the lipids (lipi), which are a group of fats or fat like substances in the blood (demia). Cholesterol and the triglycerides are the two lipids in the blood. Elevation of one or both of these lipids is seen in hyperlipidemia. Serum cholesterol levels above 240 mg/dL and triglyceride levels above 150 mg/dL are associated with atherosclerosis. Atherosclerosis is a disorder in which lipid deposits accumulate on the lining of the blood vessels, eventually producing degenerative changes and obstruction of blood flow. Atherosclerosis is considered to be a major contributor in the development of heart disease. Triglycerides and cholesterides are insoluble in water and must be bound to a lipid-containing protein (lipoprotein) for transportation throughout the body. Although several lipoproteins are found in the blood, this chapter will focus on the low-density lipoproteins (LDL), the high-density lipoproteins (HDL), and cholesterol. Low-density lipoproteins (LDL) transport cholesterol to the peripheral cells. When the cells have all of the cholesterol they need, the excess cholesterol is discarded into the blood. This can result in an excess of cholesterol, which can penetrate the walls of the arteries, resulting in atherosclerotic plaque formation. Elevation of the LDL increases the risk for heart disease. High-density lipoproteins (HDL) take cholesterol from the peripheral cells and bring it to the liver, where it is metabolized and excreted. The higher the HDL, the lower the risk for development of atherosclerosis. Therefore, it is desirable to see an increase in the HDL (the “good” lipoprotein)

because of the protective nature of its properties against the development of atherosclerosis and a decrease in the LDL. A laboratory examination of blood lipids, called a lipoprotein profile, provides valuable information on the important cholesterol levels, such as: • Total cholesterol • LDL (the harmful lipoprotein) • HDL (the protective lipoprotein) •. HDL cholesterol protects against heart disease, so the higher the numbers the better. An HDL level less than 40 mg/dL is low and considered a major risk factor for heart disease. Triglyceride levels that are borderline (150–190 mg/dL) or high (above 190 mg/dL) may need treatment in some individuals. Atherosclerosis is a disease characterized by deposits of fatty plaques on the inner walls of arteries. These deposits result in a narrowing of the lumen of the artery and a decrease in blood supply to the area served by the artery. When these fatty deposits occur in the coronary arteries, the patient experiences coronary artery disease. Lowering blood cholesterol levels can arrest or reverse atherosclerosis in the vessels and can significantly decrease the incidence of heart disease. Hyperlipidemia, particularly elevated serum cholesterol and LDL levels, is a risk factor in the development of atherosclerotic heart disease. Other risk factors, besides cholesterol levels, play a role in the development of hyperlipidemia. Additional risk factors include family history of early heart disease, cigarette smoking, high blood pressure, Age, Low HDL levels, obesity and Diabetes.

In general, the higher the LDL level and the more risk factors involved, the greater the risk for heart disease. The main goal of treatment in patients with hyperlipidemia is to lower the LDL to a level that will reduce the risk of heart disease. The primary care provider may initially seek to control the cholesterol level by encouraging therapeutic life changes (TLC). This includes a cholesterol-lowering

diet (TLC diet), physical activity, quitting smoking , and weight management. The TLC diet is a low-saturated fat and low cholesterol-eating plan that includes less than 200 mg of dietary cholesterol per day. In addition, 30 minutes of physical activity each day is recommended in the TLC. Walking a brisk pace for 30 minutes a day 5 to 7 days a week can help raise the HDL and lower LDL. Added benefits of a healthy diet and exercise program include a reduction of body weight. If TLC does not result in bringing blood lipids to therapeutic levels, the primary health care provider may add one of the antihyperlipidemic drugs to the treatment plan. The TLC is continued along with the drug regimen. In addition to control of the dietary intake of fat, particularly saturated fatty acids, antihyperlipidemic drug therapy is used to lower serum levels of cholesterol and triglycerides. The primary health care provider may use one drug or, in some instances, more than one antihyperlipidemic drug for those with poor response to therapy with a single drug. Three types of antihyperlipidemic drugs are currently in use, as well as one miscellaneous antihyperlipidemic drug. The various types of drugs used to treat hyperlipidemia are: Bile acid sequestrants, HMG-CoA reductase inhibitors, Fibric acid derivatives and Niacin. The target LDL level for treatment is less that 130 mg/ dL. If the response to drug treatment is adequate, lipid levels are monitored every 4 months. If the response is inadequate, another drug or a combination of two drugs is used. Antihyperlipidemic drugs decrease cholesterol and triglyceride levels in several ways. Although the end result is a lower lipid blood level, each has a slightly different action. Health is defined as soundless of physical, mental or moral condition, especially freedom from bodily pain or disease, but true health is more than that. It includes the joy of living, the power and ability to lead a satisfying and purposeful life.

1.5. Familial (primary)

Familial hyperlipidemias are classified according to the Fredrickson classification which is based on the pattern of lipoproteins on electrophoresis or ultracentrifugation. It was later adopted by the World Health Organization (WHO).

1.6. Hyperlipoproteinemia type I

Type I hyperlipoproteinemia is a form of hyperlipoproteinemia associated with deficiencies of lipoprotein lipase.

1.7. Hyperlipoproteinemia type II

Hyperlipoproteinemia type II, by far the most common form, is further classified into type IIa and type IIb, depending mainly on whether there is elevation in the triglyceride level in addition to LDL cholesterol.

1.8. Type IIa

This may be sporadic, polygenic, or truly familial as a result of a mutation either in the LDL receptor gene on chromosome 19 or the ApoB gene. The familial form is characterized by tendon xanthoma, xanthelasma and premature cardiovascular disease. The incidence of this disease is about 1 in 500 for heterozygotes, and 1 in 1,000,000 for homozygotes.

1.9. Type IIb

The high VLDL levels are due to overproduction of substrates, including triglycerides, acetyl CoA, and an increase in B-100 synthesis. They may also be caused by the decreased clearance of LDL. Prevalence in the population is 10%.

- Familial combined hyperlipoproteinemia (FCH)
- Secondary combined hyperlipoproteinemia

1.10. Hyperlipoproteinemia type III

This form is due to high chylomicrons and IDL (intermediate density lipoprotein). Also known as broad beta disease or dysbetalipoproteinemia, the most common cause for this form is the presence of ApoE E2/E2 genotype. It is due to cholesterol-rich VLDL (β-VLDL). Prevalence is 0.02% of the population.

1.11. Hyperlipoproteinemia type IV

This form is due to high triglycerides. It is also known as *hypertriglyceridemia*. According to the NCEP-ATPIII definition of high triglycerides (>200 mg/dl), prevalence is about 16% of adult population.

1.12. Hyperlipoproteinemia type V

This type is very similar to type I, but with high VLDL in addition to chylomicrons. It is also associated with glucose intolerance and hyperuricemia

1.13. Unclassified familial forms

Non-classified forms are extremely rare:

- Hypo-alpha lipoproteinemia
- Hypo-beta lipoproteinemia

1.14. Acquired (secondary)

Acquired hyperlipidemias may mimic primary forms of hyperlipidemia and can have similar consequences.[9] They may result in increased risk of premature atherosclerosis or, when associated with marked hypertriglyceridemia, may lead to pancreatitis and other complications of the chylomicronemia syndrome. The most common causes of acquired hyperlipidemia are:

- Diabetes Mellitus
- Use of drugs such as diuretics, beta blockers, and estrogens

Other conditions leading to acquired hyperlipidemia include:

- Hypothyroidism
- Renal Failure
- Nephrotic Syndrome
- Alcohol Usage
- Some rare endocrine disorders and metabolic disorders

Treatment of the underlying condition, when possible, or discontinuation of the offending drugs usually leads to an improvement in the hyperlipidemia. Specific lipid-lowering therapy may be required in certain circumstances.

Another acquired cause of hyperlipidemia, although not always included in this category, is postprandial hyperlipidemia, a normal increase following ingestion of food.

For treatment of type II, dietary modification is the initial approach but many patients require treatment with statins to reduce cardiovascular risk. If the triglyceride level is markedly raised, fibrates may be preferable due to their beneficial effects. Combination treatment of statins and fibrates, while highly effective, causes a markedly increased risk of myopathy and rhabdomyolysis and is therefore only done under close supervision. Other agents commonly added to statins are ezetimibe, niacin and bile acid sequestrants. Dietary supplementation with fish oil is also used to reduce elevated triglycerides, with the greatest effect occurring in patients with the greatest severity. There is some evidence for benefit of plant sterol-containing products and ω_3-fatty acids

Recently, there has been increasing interest in the use of medicinal plants. The use of medicinal plants in modern medicine suffers from the fact that though hundreds of plants are used in the world to prevent or to cure diseases. Recently search for appropriate antihyperlipidemic agent has been focused on plants used in traditional medicine because of leads provided by natural products that may be better treatment than currently used drugs.

1.14.1. Definition:

- Abnormally high level of any lipoprotein species.
- Hyperlipoproteinemia (hyperlipidemia)
- Hyperlipemia (hypertriglycridemia).

1.14.2. Causes:

- Primary hyperlipidemia → genetic factors.
- Secondary hyperlipidemia → Disease.

- Liver & biliary disease
- Hypothyroidism ↓ (metabolism)
- Obesity – diabetes
- Drug – oral contraceptives
- Alcohol

1.14.3. Clinical consequences:

- Atherosclerosis
- Coronary heart disease
- Acute pancreatitis

1.14.4. Lipoprotein classification:

- 5 classes & lipoprotein

1- Chylomicron

Largest lipoprotein

Transport dietary TGs

2- VLDL

Carry endogenous TGs synthesized in liver to peripheral tissue

3- IDL- intermediate density lipoprrptein. Remnant of VLDL

4- LDL- low density lipoprotein. Bad , the oxidized form of LDL is v. dangerous → ischemic heart disease.

5- HLD- high density lipoprrptein. Good Look at figure of types of hyperlipidemias + the formation of different lipids (lippencot)

1.14.5. Classification of antihyperlipidemia :

1- Fibric acid derivatives (fibrates)

2- Bile acid binding resin

3- HMG-coA reductase inhibitors

4- Niacin

5- Ezetimibe

1.14.6. Fibrates

- Clofibrate
- Fenofibrate
- Gemfibrozil
- Besafibrate
- Cilorofibrate

1.14.7. Mechanism of action:

- Are ligands for nuclear transcription receptors called peroxisome proliferator activated receptor – alpha (PPAR-a)
- Increase lipoprotein lipase activity
- Increase lipolysis of TGs (VLDL + chylomicrons)

1.14.8. Pharmacological action:

- Increase VLDL clearance.
- Reduce hepatic VLDL production.
- Mainly reduce VLDL, Moderately reduce LDL.

1.14.9. Pharmacokinetics:

- Finofibrate is a prodrug (ester).
- Well absorbed orally.
- Bound to plasma proteins.
- Cross the placenta
- Metabolized in liver (enterohepatic circulation).
- Renal excretion as glucouronides.
- Plasma 1\2 life is 20hr for fenofibrate , 1.5hr for gemfibrozil.
- Absorbtion is enhanced in the presence of food.
- Fenofibrate is more effective than gemfibrozil.

1.14.10. Uses: hyper TAG

- Type 3 (dysbetalipoproteinemia)
- Type 4 (hypertriglyceridemia) ↑VLDL.
- Type 5 (elevated VLDL+chylomicrons)

1.14.11. Adverse effect:

- GIT: gastrointestinal upset.
- Myopathy: more common in alcholics.
- Inflammation of the muscles with ↑creatine level.
- Lithiases: increase in gallstone incidence and gallbladder diseases.

1.14.12..Drug interaction:

- Increase oral anticoagulant (warfarin) activity.

- Fibrates ↑ coagulation, so dose must be reduced when given with anticoagulant.
- Aminotransferase elevation: drug must be stopped.
- Arrhythmias.

1.14.13. Contraindications:

- Hepatic\renal disease.
- Billiary tract disease.
- Combination with statins.

1.15. Bile-Acid binding (resin)

- Colestipol(colestid).
- Cholestyramine (Questran, Questran light).
- Colesevelam (welcohol).

1.15.1. Chemistry:

- Polymeric cationic exchange resins.

1.15.2. Pharmacokinetics:

- Insoluble in water.
- Not absorbed systemically, not metabolized.
- Excreted unchanged in feces.

1.15.3. Mechanism of action:

- Bind bile acid in the intestine , so prevent their reabsorbtion.

- Increased bile acid clearance causes increased conversion of cholesterol to bile acids.
- Increase the uptake of plasma LDL by the liver (up regulation of LDL receptors).
- Resins have NO effect on patients with homozygous familial hyperlipiemia.

1.15. 4. Clinical uses:

- Type 2a (familial hypercholesterolemia).
- Type2b (combined hyperlipidemia).
- VLDL may increase so niacin for example is given to reverse this.
- Pruritis: is incomplete biliary obstruction (cholestasis and bile salt accumulation).
- Sever digitalis toxicity

1.15.5. Adverse effect:

1. Constipation, bloating

2. reduced absorption of drugs (warfarin, digoxin, thiazides ,statins, iron salts) .

3. Interfere with absorption of fat soluble vitamins.

4. Increase serum triglyceride (VLDL)

1.16. HMG-CoA reductase inhibitors

1.16.1. Chemistry:

- Structural analogs of HMG-CoA

(3_hydroxy_3_methyl glutaryl_coenzyme A)

1.16.2. Drugs:

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

1.16. 3. Mechanism of action:

- Reversible competitive inhibition of HMG-CoA reductase .
- Inhibit denovo synthesis of cholesterol.
- Up regulation LDL high affinity receptors.
- Small decrease in plasma triglycerides , slight increase in HDL cholesterol.

1.16.4. Pharmacokinetics:

- Prodrugs (lovastatin & simvastatin –GIT)
- Active drugs:

  Atorvastatin – Pravastatin – Fluvastatin –Rosuvastatin.

- All are given orally at night.
- All have high first pass effect.
- Excreted mainly in bile.
- 5-20 % is excreted in the urine.
- T½ = 1-3 hrs except Atrovastatin (14 hrs), Rosuvastatin (19 hrs).
- Food enhances absorption except Provastatin.

1.16.5. Clinical uses:

- Treatment of elevated LDL plasma levels: monotherapy or with bile-acid binding resins or ezetimibe or niacin.

1.16.6. Adverse effect:

- Aminotransferase elevation.
- Myopathy (increase in creatine kinase, muscle pain) → medication should be stopped.
- Consequence of myopathy: myoglob inuria & acute renal failure ARF (Rhabdomyolysis).
- Increase warfarin blood level.

1.16.7. Contraindications:

- Female → pregnant or lactating.
- Children.
- Severe liver disease.
- Severe kidney disease.

1.16.8. Drug Interactions:

- Simvastatin – lovastatin – atorvastatin one metabolize by CYT P450 3A4.
- Flovastatin – Rosuvastatin one metabolize by CYT P450 2Ca.
- Provastatin by sulfation
- Erythromycin – ketoconazole – Cimatidine – cyclosporine.
- Rifampicine – phenytoin - phenobarbitone

1.17. Niacin:

- water soluble vitamin (B3)

1.17.1. Pharmacokinetics:

- Given orally (2-3 gm/day in divided dose up to 6 gm)
- Excreted with urine.
- Given with meals (because it irritates gastrointestinal mucosa).

1.17.2. Mechanism Of Action:

- Inhibits lipolysis in adipose tissue (due to intracellular lipoprotein lipase inhibition).
- ↓ TG synthesis in the liver.
- VLDL secretion.
- Decrease VLDL and LDL plasma levels.
- Increase HDL cholestrol in plasma.

1.17.3. Clinical Uses:

- Familial hyperlipidemia.
- Heterogenous familial hypercholestrolemia (+ resin /statins)
- Most effective in increasing HDL cholesterol.

1.17.4. Adverse effects:

- Cutanuous flushing + warm sensation
- GIT disturbance
- Hyperuricemia + gout (allopurinol)

- Impaired glucose tolerance
- Aminotransferase elevation
- Arrhythmia
- Hypotension

1.17.5. Contraindications:

- Sever peptic ulcers
- Diabetic (if insulin resistance is increased)

1.18. Cholesterol absorption inhibitors ((Ezetimibe))

- Inhibits intestinal absorption of dietary and biliary cholesterol in small intestine
- Decrease hepatic cholesterol stores
- Increase clearance of cholesterol from the blood
- Reduce LDL level
- Metabolized in liver (phase 2 , active glucouronide)
- Long plasma half life 22hrs. (enterohepatic circulation)
- No effects on fat soluble vit.
- Excreted mainly in feces (80 %)
- Daily dose of 10 mg/kg
- Synergestic action with statins
- Plasma level: (see table of effects on LDL ,HDL,TG)
- Increased (fibrates)
- Reduced (cholestyramin)
- Not affected by digoxin or warfarin

1.18.1. Drug combination

1.18.2. Ezetinibe + statins

- Treatment for hypercholesterolemia

1.18.3. Niacin + statins

- Treatment for familial combined hyperlipidemia

1.18.4. Niacin + rosins

- Treatment of familial combined hyperlipidemia
- Familial hypercholesterolemia

1.18.5. Resins + statins

- Treatment for familial hypercholesterolemia

1.18.6. Resins + fibrates

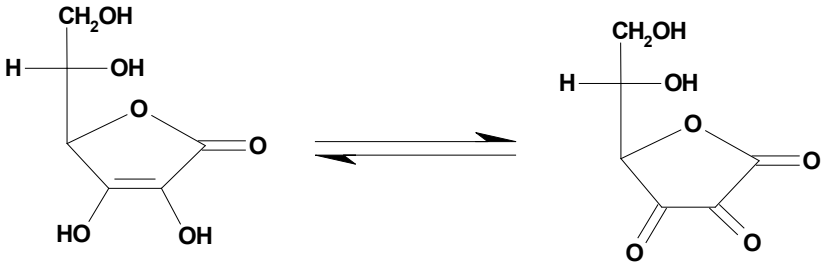
- Treatment for familial combined hyperlipidemia
- Increased risk of choliothiasis.

Natural Antioxidants

Hydrophillic Antioxidants

Ascorbic Acid – Vitamin C

AA is easy oxidised to dehydroascorbic acid (DAA)



Ascorbic acid **Dehydroascorbic acid**

Therefore, AA can protect other compounds against oxidation

DAA is unstable, easy hydrolysed (opening of lactone ring), products already have not a vitamin activity

1.19. Function in organism

1.19.1. Anti-scorbutic factor

- co-enzyme of prolylhydroxylase;
- it catalyze hydroxylation of proline and formation of net structure of collagen from globular (water soluble) proto-collagen, which is formed in liver;
- this reaction ensure good function of ligament tissue
- minimal intake for scorbut prevention – about 10 – 30 mg per day

1.19. 2. Anti-oxidant

- Inactivation of reactive oxygen compounds, e.g. free radicals
- Reduction of some oxidation products
- Therefore, certain protective effect against tumor and atherogenesis

Recommended daily intake

- Minimum: 10 – 30 mg (see above)
- General recommendations: about 70 mg per day
- Human with higher necessity (smokers; people in the cities with strong automobile transport; brainworkers; managers; sportsmen): to 200 and more mg per day

1.19.3. Sources of ascorbic acid

Fruits – apple, kiwi, mango, citrus fruits, black currant

Vegetables – cabbage, capsicum, lettuce, potatoes

Fortification of fruits products (juices, jam ...) by synthetic ascorbic acid

1.19. 4. Possibility of lipid protection

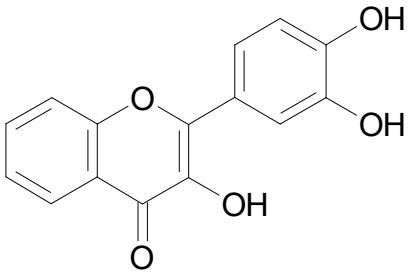
Ascorbic acid must be chemically transformed to lipophillic compound – after reaction (esterification) with fatty acid – depside bond

Ascorbyl palmitate is mainly used as lipid antioxidant

1.19.5. Bioflavonoids

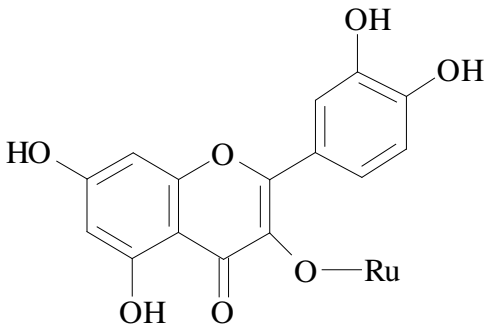
Something called as vitamin P – phenols, flavonoids, terpenes etc.

Structure of most active compounds



Lower, but significant amounts it is possible to found in some vegetables, lower amount in fruits

Rutin and its aglycone quercetin are most significant flavonoids from vegetables



Some herbs contain very high concentrations of antioxidative bioflavonoids

Rosemary; sage; oregano; saturea; thyme; mentha ....

Leafs of strawberries, raspberries, blackberries

Example of very active compounds: Rosmarinic acid, Carnosic acid

Flavonoids are able in foods and also in organism to reduce of dehydroascorbic acid back to ascorbic acid

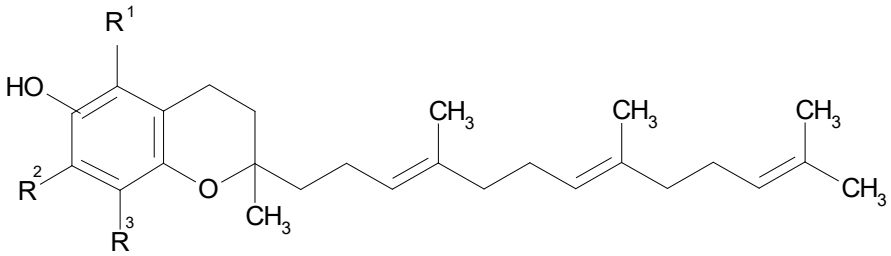
1.19. 6. Ubiquinones

- Coenzymes Q_n, CoQ_n
- Group of 12 compounds of plant or animal origin
- Structure and physiology effects are similar to vitamin E
- Ubiquinones are important for course of redox reactions in aerobe metabolism
- Well-known is Coenzyme Q_{10}, which is often used in cosmetics

1.20. Amino Acids and Peptides with Free Thiol Group

- For example cysteine or glutathione
- Compounds are able to easy oxidation:
- 2 R-SH $\rightarrow$ R-S-S-R
- Similar as flavonoids, compounds are able to reduce of oxidation form of ascorbic acid
- Lipophilic Antioxidants
- Tocopherols – Vitamin E
- 8 compounds with the vitamin E activity – 4 tocopherols, 4 tocotrienols

1.20.1. Structure

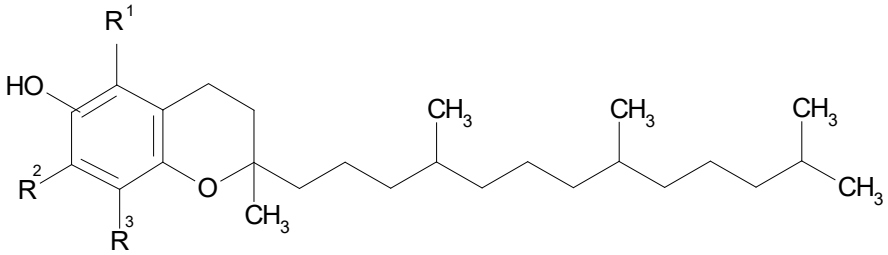


$R^1 = R^2 = R^3 = CH_3$ α - tokotrienol

$R^1 = R^3 = CH_3$, $R^2 = H$ β - tokotrienol

$R^1 = H$, $R^2 = R^3 = CH_3$ γ - tokotrienol

$R^1 = R^2 = H$, $R^3 = CH_3$ δ – tokotrienol



$R^1 = R^2 = R^3 = CH_3$ α - tokoferol

$R^1 = R^3 = CH_3$, $R^2 = H$ β - tokoferol

$R^1 = H$, $R^2 = R^3 = CH_3$ γ - tokoferol

$R^1 = R^2 = H$, $R^3 = CH_3$ δ – tokoferol

Tocopherols are stronger antioxidants than tocotrienols (about 100 : 1)

1.20.2. Function

in organism - antioxidant

– inactivation of free radicals and singlet oxygen

in foods – antioxidant

- Reduction of hydroperoxides to hydroxy derivatives
- Tocopherols are oxidized to quinones
- Quinones can be reduce back to tocopherols by other antioxidants (ascorbic acid, glutathion and mainly glutathion peroxidase)

1.20.3. Recommended daily intake

- 10 – 20 mg; higher necessity with higher intake of polyenoic fatty acids

1.20.4. Sources

- Tocotrienols: mainly germinated wheat
- Tocopherols: mainly plant oils – natural content 10 – 100 mg / kg; α-tocopherol is often added to oils as α-tocopheryl acetate

1.20.5. Insufficiency

- Various symptoms connect with the influence of free radicals
- Strong insufficiency – liver necrosis (extinction of liver cells) or metabolic troubles of muscles and nerves (myopathy, encephalomalacy)

1.20.6. Carotenoids

- Retinoids (c. with vitamin A activity; e.g. ß-carotene): weak or none antioxidant activity
- Other carotenoids with strong antioxidant activity:
    - some xanthophyls, e.g. canthaxanthin or lutein

1.21. Antioxidants and Free radicals

Antioxidants are intimately involved in the prevention of cellular damage -- the common pathway for cancer, aging, and a variety of diseases. Athletes have a keen interest because of health concerns and the prospect of enhanced performance and/or recovery from exercise. The purpose of this article is to serve as a beginners guide to what antioxidants are and to briefly review their role in exercise and general health.

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction, like dominoes. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane. Cells may function poorly or die if this occurs. To prevent free radical damage the body has a defense system of *antioxidants.*

Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Although there are several enzyme systems within the body that scavenge free radicals, the principle

micronutrient (vitamin) antioxidants are vitamin E, beta-carotene, and vitamin C. Additionally, selenium, a trace metal that is required for proper function of one of the body's antioxidant enzyme systems, is sometimes included in this category. The body cannot manufacture these micronutrients so they must be supplied in the diet.

Vitamin E: A fat/soluble vitamin present in nuts, seeds, vegetable and fish oils, whole grains (esp. wheat germ), fortified cereals, and apricots. Current recommended daily allowance (RDA) is 15 IU per day for men and 12 IU per day for women.

Vitamin C: Ascorbic acid is a water/soluble vitamin present in citrus fruits and juices, green peppers, cabbage, spinach, broccoli, kale, cantaloupe, kiwi, and strawberries. The RDA is 60 mg per day. Intake above 2000 mg may be associated with adverse side effects in some individuals.

Beta-carotene is a precursor to vitamin A (retinol) and is present in liver, egg yolk, milk, butter, spinach, carrots, squash, broccoli, yams, tomato, cantaloupe, peaches, and grains.

Epidemiologic observations show lower cancer rates in people whose diets are rich in fruits and vegetables. This has lead to the theory that these diets contain substances, possibly antioxidants, which protect against the development of cancer. There is currently intense scientific investigation into this topic.

Antioxidants are also thought to have a role in slowing the aging process and preventing heart disease and strokes, but the data is still inconclusive. Therefore from a public health perspective it is premature to make recommendations regarding antioxidant supplements and disease prevention. Perhaps the best advice, which

comes from several authorities in cancer prevention, is to eat 5 servings of fruit or vegetables per day[12-15].

Although there is little doubt that antioxidants are a necessary component for good health, no one knows if supplements should be taken and, if so, how much. Antioxidants supplements were once thought to be harmless but increasingly we are becoming aware of interactions and potential toxicity. It is interesting to note that, in the normal concentrations found in the body, vitamin C and beta-carotene are antioxidants; but at higher concentrations they are pro-oxidants and, thus, harmful. Also, very little is known about the long-term consequences of megadoses of antioxidants. The body's finely tuned mechanisms are carefully balanced to withstand a variety of insults. Taking chemicals without a complete understanding of all of their effects may disrupt this balance.

1.22. Recommendations

Follow a balanced training program that emphasizes regular exercise and eat 5 servings of fruit or vegetables per day. This will ensure that you are developing your inherent antioxidant systems and that your diet is providing the necessary components.

2.0 LITERATURE REVIEW

BenbrookCM, et al., (2013) studied the effects of Nigella sativa L. on C- reactive protein, lipid profile, Oxidized low-density lipoprotein (Ox-LDL), antioxidant capacity, apolipoprotein A (Apo A) and apolipoprotein B (Apo B) and fatty streak formation in hypercholesterolaemic male rabbits. Fifteen rabbits were divided into three groups of five rabbits each and fed with normal diet, hypercholesterolaemic diet (1% cholesterol) and hypercholesterolaemic diet supplemented with 5% Nigella sativa L.. They received rabbit laboratory chow diet for a period of 8 weeks.

Balbir, et al., (2012) studied the hypolipidemic and antiatherogenic effect of aqueous extract of leaves of Ficus glumosa (Moraceae) in Wistar albino rats with hypercholesterolemia (HC). In their study, 60 Wistar male rats were divided into 6 groups of 10 rats each. For induction of hypercholesterolemia (HC), 1% of cholesterol was added in the feed of rats. The plant extract was administered to animals at the increasing doses of 225, 300 and 375mg/kg for four weeks.

Elkhateeb A et al., (2014) investigated the effects of formulation variables on development of curcumin nano cubosomal formulations as potential oral delivery systems. Curcumin cubosomes were prepared by homogenization method. A 32 full factorial design was employed to evaluate individual and combined effects of formulation variables, namely polyoxmar 407, glycerol monooleate, and entrapment efficiency. Prepared curcumin nano cubosomes were evaluated for entrapment efficiency percentage (EE %), particle size analysis, zeta potential, microscopic examination. Anti ulcer activity model was followed by pyloric ligation. Anti ulcer activity was evaluated by ulcer index, gastric volume, gastric pH, total acidity and

free acidity. The formulation CF6 having high percentage of entrapment efficiency and when compared with the other formulations. The optimized CF6 formulation should have particle size of about 43 nm and zeta potential of about -17 kv. Formation of cubosomes was confirmed by transmission electron microscopy and optical microscopy. The antiulcer activity of curcumin cubosomes was more than pure curcumin. The developed curcumin loaded cubosomal has given stable, nano-sized vesicles and shown to improve curcumin anti ulcer activity in oral drug delivery.

Srikant rao K, et al., (2012) A significant percentage inhibition in the paw oedema by carrageenan was reported in the studies conducted on the anti-inflammatory effect of aqueous extract of leaves of Holoptelea integrifolia in rats in dose dependent manner. Indomethacin was used as the standard anti-inflammatory drug.

Katavic PL, et al., (2006) evaluated the hepatoprotective and anti-inflammatory activities of the seeds of Plantago major in rats. The carrageenan hind paw oedema model was employed for inflammatory studies and the hepatoprotective activity was observed when the extract was able to significantly reduce the serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels when compared to the CCl4 group.

Morice et al., (2009) reported the in vivo analgesic and anti-inflammatory activity of methanolic extract of the roots of Argyreia speciosa using acetic acidwrithing and carrageenan induced hind paw oedema. The methanolic extract of the roots significantly decreased the acetic acid-writhing and inhibition of carrageenan induced hind paw oedema in rats.

Rahman A, et al., (2009) studied the anti-inflammatory activity of aqueous extract of stem bark of Stereospermum kunthianum in rats. The anti-inflammatory activity was confirmed using carrageenan-induced paw oedema, leukocutes migration, granuloma air pouch tests in rats. They concluded that the extract dosed at 400 mg/kg showed maximum activity and was higher than that of indomethacin (10 mg/kg) and the extract also significantly reduced the number of recruited leucocytes and inhibited the peritoneal exudates formation.

Rastogi et al., (2009) conducted experiments in rats and demonstrated the analgesic and anti-inflammatory activity of Amukkarac curanam, a polyherbal siddha formulation. The experimental methods used were tail immersion and acetic acid writhing method for anti-analgesic activity and cotton pellet-induced granuloma formation for anti-inflammatory activity. Pentazocine (10 mg/kg, intraperitoneally) and aspirin (150 mg/kg, orally) were clinically used standard analgesics and for antiinflammatory activity (Indomethacin, 10 mg/kg, orally) was used.

Ray AB, et al., (2009) reported the anti-inflammatory, analgesic and anti-lipid peroxidative properties of the ethanolic extract of leaves of Wattakaka volubilis in rats. The extract showed potent anti-inflammatory activity in carrageenan-induced rat paw oedema and acetic acid-induced writhing in mice when compared to the standard drug indomethacin in dose dependent manner. The extract also exhibited significant inhibition of FeCl2 ascorbic acid stimulated mice liver lipid peroxidation.

Shah Gagan et al., (2010) investigated twelve medicinal plants of northeast India for the presence of biologically active compounds and also carried out phytochemical screening and anti-oxidant activity of all the twelve medicinal plants.

The antioxidant activity was estimated by using 2, 2- diphenyl-picryl-hydrazyl (DPPH) free radical assay. Oroxylum indicum, Ipomoea aquatica and Moringa oleifera exhibited strong antioxidant activity as compared to other plants. Oroxylum indicum showed the highest antioxidant activity. The present study indicated that these plants are of therapeutic potential due to their high free-radical scavenging activity.

Singh RK, et al., (2009) reported the anti-inflammatory, anti-nociceptive, and antipsychiatric effects of 80% ethanolic extracts of rhizomes of Alpinia officinarum on complete Freund's adjuvant (CFA)-induced arthritis in rats. The ethanolic extract showed acute anti-inflammatory activity by reducing the oedema volume in carrageenan-stimulated arthritis and inhibited NO generation in LPS-induced RAW 264.7 cells. In addition, this extract showed chronic anti-rheumatic and analgesic activities by suppressing the swelling volume, by recovering the paw withdrawal latency, and by inhibiting the flexion scores in CFA-induced arthritis.

3.0. PLANT PROFILE

Stellaria media, chickweed, is an annual flowering plant in the carnation family Caryophyllaceae. It is native to Europe, but naturalized in many parts of North America. It is used as a cooling herbal remedy, and grown as a vegetable crop and ground cover for both human consumption and poultry. It is sometimes called common chickweed to distinguish it from other plants called chickweed. Other common names include chickenwort, craches, maruns, winterweed. The plant germinates in autumn or late winter, then forms large mats of foliage.

Scientific name **:** *Stellaria media*

Kingdom **:** Plantae

Clade **:** Angiosperms

Order **:** Caryophyllales

Family **:** Caryophyllaceae

Genus **:** *Stellaria*

Species **:** *S.media*

Higher classification: Chickweeds

Figure No.1: Leaves and flower of *Stellaria media*

The plants are annual and with weak slender stems, they reach a length up to 40 cm. Sparsely hairy, with hairs in a line along the stem. The leaves are oval and opposite, the lower ones with stalks. Flowers are white and small with 5 very deeply lobed petals. The stamens are usually 3 and the styles 3. The flowers are followed quickly by the seed pods. This plant flowers and sets seed at the same time.

Stellaria media is widespread in North America, Europe and mostly in Asia. There are several closely related plants referred to as chickweed, but which lack the culinary properties of plants in the genus *Stellaria.* Plants in the genus *Cerastium* are very similar in appearance to *Stellaria* and are in the same family (Caryophyllaceae). *Stellaria* has fine hairs on only one side of the stem in a single band and on the sepals. Other members of the family Caryophyllaceae which resemble *Stellaria* have hairs uniformly covering the entire stem. It usually has 3 styles, 3-5, occasionally 8 stamens, variously stated as 8 stamens by Keble Martin and (1-)3(-8) by Clapham, Tutin and Warburg.

4.0 AIM & OBJECTIVE

The ethnobotanical information reports about 900 plants that may possess anti-diabetic potential. Wide arrays of plants representing active principles of numerous chemical compounds have demonstrated activity consistent with their possible use in the treatment of diabetics. Among these are anthraquinones, glycosides, polysaccharides, peptidoglyeans, flavonoids, tannins, steroids, glycopeptides, terpenoids and inorganic ions. The introduction of these indigenous herbal compounds in the management of diabetes mellitus will greatly simplify the management and make it less expensive. Herbal plants are of great value in the field of medicine and cure of diseases. Practical experiences and several modern research studies have shown that therapy using plants is better than using chemical by being safer besides having synergistic effect of their active ingredients and presence of certain minerals and salts. There are still large number of medicinal plants in which active constituents have not yet been investigated even though their medicinal effect is established by folklore and traditional system of medicine. Thus, identification of potential anti-diabetic agents using mechanism-based studies holds great promise for elucidating mechanisms and devising more specific and effective treatments for diabetes-related diseases. The Herbal medicine is still the main stay of about 70-80% of the world population, Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. One of the approaches used in drug discovery which the selection of a plant is based on the prior information on the medicinal use of the plant. The primordial objective of the research is to evaluate the antioxidant and antihyperlipidemic effects of the *Stellaria media* Leaf Extract.

5.0 PLAN OF THE WORK

The objective of the present study will be carried out by the following sequence based on bioassay guides principles.

- Collection and authentication of plant *Stellaria media leaves*
- Plant extracts preparation of *Stellaria media leaves*
- Phytochemical qualitative screening of *Stellaria media leaves*
- Acute toxicity study
- Blood cholesterol lowering effect of the plant extract of *Stellaria media leaves* in both acute and sub-acute *in-vivo* hypolipidemic models will be performed.
- Diet induced model
- Triton induced model
- Evaluation of Parameters: 1. HDL 2. LDL 3. Total Cholesterol 4. Invitro Antioxiant

6.0 MATERIALS AND METHODS

6.1. Collection and preparation of plant extracts

The leaves were initially separated from the main tree and rinsed with distilled water and shade dried and then homogenized into fine powder and stored in air tight bottles. Calculated amount of powder in contrasting amount of organic solvents in a conical flask and then kept in a rotary shaker at 190-220 rpm for 24 h. And then it was filtered with the help of muslin cloth and centrifuged. The supernatant was collected and the solvent was evaporated by solvent distillation apparatus to make the final volume of one-fourth of the original volume. It was stored at 40 °C in air tight bottles for further studies[41].

6.2. Preparation of extract

The leaves were separated, washed thoroughly with water and shade dried for 6 days. 1000 g of powdered was subjected to extraction with methanol, ethanol, chloroform, petroleum ether, ethyl acetate (2000ml) in a round bottom flask at room temperature for 15days. After fifteen days decanted and press the mark up to collect the fluidized product which were concentrated using rotary vacuum evaporator under reduced pressure for collected extract.

6.3. Phytochemical Test

Screening and identification of phytochemical constituents observational study was carried out in extracts as well as powder specimens using the standard procedures.

6.3. 1. Maeyer's reagent

Required quantity of mercuric chloride was dissolved in 60 ml of distilled water. 5.0 g of potassium iodide was dissolved in 20 ml of distilled water. Both solutions were mixed and volume was raised to 100 ml with distilled water.

6.3.2. Dragendorff's reagent

Solution A: 1.7 g of basic bismuth nitrate and 20 g of tartaric acid were dissolved in 80 ml of distilled water. Solution B: 16 g of potassium iodide was dissolved in 40 ml of distilled water. Both solutions (A and B) were mixed in1:1 ratio.

6.3. 3. Test for alkaloids

About 0.5 to 0.6 g of the methanolic plant extract was mixed in 8 ml of 1% HCl, warmed and filtered. Two ml of the filtrate mixed both reagents such as Maeyer's and Dragendorff's

6.3. 4. Test for steroids

About 0.5 g of the ethanolic extract fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid.

6.3. 5. Test for terpenoids

An aliquot 0.5 ml of methanolic extract was mixed with 2 ml of $CHCl_3$ in a test tube. 3 ml of concentrated H_2SO_4 was carefully added to the mixture to form a layer.

6.3. 6. Test for flavonoids

To the substance in alcohol, a few magnesium turnings and few drops of concentrated Hydrochloric acid were added and boiled for five minutes.

6.3. 7. Test for tannins

The 0.5 g of powdered sample of each medicinal plant leaves was boiled in 20 ml of distilled water in a test tube and then filtered. The filtration method used here was the normal.

6.3. 8. Test for Phytosterol

Sample was dissolved in Two ml of acetic anhydride, animated to sweltering, cooled and then 1 ml of concentrated sulfuric acid was added along the side of the test tube.

- Foam Test: Five ml of the test solution taken in a single test tube was traumatized well for 5 mins.
- Olive oil test: - Additional a few drops of olive oil to required quantity in a test tube containg sample.

6.3. 9. Test for glycosides

1.Keller -Killiani test: Additional required quantity of glacial acetic acid + few drops of 5 % ferric chloride solution to a little of dry extract. Further 0.5 ml of concentrated sulfuric acid was added along the side of the test tube carefully.

2. Hydroxyanthraquinone Test To 1 ml of the extract, added a few drops of 10% potassium hydroxide solution.

6.4. Acute toxicity studies

Healthy albino rats of either sex of 2-2½-months-old of body weight 125-150 g were housed in polypropylene cages at 25±2°C with light dark cycle of 12 h in the Animal House of the study center are to be used for the study. It should be acclimatized for seven days. All animals are to be given with standard rat feed and water ad libitum. The experiments were performed after approval of the protocol by the minute of Institutional Animal Ethics Committee IAEC/M.pharm/10/2018 and animal care wastaken as per the guidelines of Committee for the Purpose of Controland Supervision of Experiments on Animals 887/Po/Re/S/2005 CPCSEA, Government of India.

INVITRO ANTIOXIDANT ACTIVITY

6.5. DPPH Assay

0.3mM DPPH solution was prepared by dissolving DPPH (5.91 mg) in 50 ml of methanol. This stock solution was prepared freshly and kept in the dark at ambient temperature when not in used. Extracts were dissolved in 100 ml with methanol, to obtain a solution of 1000µg/ml. From this stocking solutions, various working conc. were produced to get concentration of 20, 40, 60, 80, 100 µg/ml with distilled water. The standard stock solution was prepared by dissolving ascorbic acid (Standard Sample) in suitable solvent (methanol) with a final concentration of 1000 µg/ml and different concentration of 20, 40, 60, 80, 100 µg/ml were prepared by distilled water. 0.3 mM solution of free radical standard in CH_3OH produced and 1 ml of this solution was admixed to three milliliter of test solution in water at various different concentrations and kept for 30 minutes, the absorbance was taken at

517nm. Difference between the absorbance value of sample and control of (DPPH) was obtained and given as percent scavenging of free radical.

6.6. ABTS+ Assay

The standard stock solution was prepared by dissolving ascorbic acid (Standard Sample) in suitable solvent (methanol) with a final concentration of 1000 µg/ml and different concentration of 20, 40, 60, 80, 100 µg/ml were prepared by distilled water. $ABTS^{+}$ radical was freshly prepared by adding 5 ml of 4.9 nM ammonium persulfate solutions to 5ml of 14 mM ABTS solution and kept for 16 hrs in the dark ambience. About 950µl of ABTS radical solution has been added with 50 microliter of sample or referrals solution and the reaction mixture was vortexed for 10 sec and kept for 6 minutes. The absorbance was recorded at 734 nm compared with the manage ABTS. %age suppression was calculated from following formula

6.7. High-Cholesterol Diet Induced Rat Model

Swiss albino rats weighing (200 – 250gm) were used for a standard experimental method as high cholesterol diet consisting of Cholesterol (1%), sodium cholate (0.5%), sucrose (30%), casein(10%), butter (5%) and standard chow diet (53.5%) for 7 days. The animals divided into three groups of control, test and standard drug treated animals. The studies conducted in two stages. In the preliminary stage effective hypolipedemic doses of test and standard drugs are worked out and in the final stage the effect of test and standard drugs are studied. The lipid profile includes total cholesterol LDL, HDL, VLDL and triglycerides were studied. The blood samples were collected after 6, 24 and 48hour of drug administration.

6.8 High-Cholesterol Diet Induced Rat Model

Triton induced rat model

The antihyperlipidemic effects of the above extracts were evaluated in 45 triton induced hyperlipidemic rats starved for 18hours. The rats were divided into 4 Groups of 5 animals each. and then injected with Triton at a dose of 100mg/kg body weight except rats of group 1 which served as a normal vehicle treated group 2 and 3 were treated daily with a dose of 200 and 400mg/kg ethanolic and aqueous extracts respectively immediately after the Triton injection by i.p. administration. Blood samples were collected after 6, 24 and 48 hour of Triton injection evaluates the lipid profiles.

Table No. 1: High-Cholesterol Diet Induced Rat Model

S. No	Group	Drug treatment	Number of animal
1.	Negative control group	Only triton (100mg/kg)	6
2.	Positive control group	Received standard drug atorvastatin 10 mg/kg and HCD for 15 days	6
3.	Test group low dose	Extract low dose(200mg/kg)	6
4.	Test group high dose	Extract high dose(400mg/kg)	6

7.0 RESULTS & DISCUSSION

Extract were a semisolid brownish color extract and the percentage yield was found to be 15.9%.

7.1. Phyochemical Analysis

The phytochemical screening results revealed that the after which it was observed whether the alkaloids were present by the indication of turbidity and/or precipitate formation. Not changed from violet to blue or green was absence of steroids. An interface with a reddish brown coloration was formed in the presence of terpenoids, as positive result. Red coloration identifies the presence of flavonoids (Shinado's test). A colour change was not observed in the test tube, which indicated in the absence of tannins. Un development of darker ring was development at the intersection and the turning of the upper layer to dim green which indicated the test for the presence of phytosterols. Below two observation indicated presence of Saponins formation of stable foam confirmed the test. The formation of a soluble emulsion confirmed the test. The formation of blue colour in acetic acid layer confirmed the test. Absence of red color confirmed the test. Above two observations indicated not present glycosides.

Table No.2: Phytochemical test

S. No.	Phytoconstituents	*Stellaria media* leaves
1.	Alkaloid	+
2.	Terpenoid	+
3.	Saponin	+
4.	Glycoside	-
5.	Resin	-
6.	Phlabotanin	-
7.	Steroids	-
8.	Flavonoid	+
9.	Anthraquinone	+

7.2. Acute toxicity studies

Plant a dose of 2000 mg/kg had no adverse effect on the behavioral responses of the tested mice up to 15 days of observation. Physical observations indicated no signs of changes in the skin, fur, eyes mucous membrane, behavior patterns, tremors, salivation, and diarrhea of the mice. There was no mortality observed and recorded weight loss normal. Based on the above observation fix the doses 200 and 400 mg/kg for anti-diabetic activity.

7.3. Hypolipidemic activity

After the drug treatment HDL level reduced but triglyceride, LDL and Total cholesterol increased where as compared to the normal and control group, indicating drug treated groups have hypolipidemic property. After the drug and extract treated group of animals HDL level was constantly increased dose dependent manner when compare to two dose levels of plant drugs. The reports of levels of triglycerides, LDL and Total cholesterol reduced based on the two dose level usage when compared to the normal and control group, indicating by the illustration has expressed reduction of cholesterol level. After the drug treatment body weight was constantly reduced when compared to triton induced rats.

Administered triton was activated metabolic function on rats to reduce the HDL but levels of triglyceride, LDL and Total cholesterol amplified. Drugs treated group of rats HDL levels were constantly increased when compared with control group. An assortment of triglyceride, LDL and Total cholesterol were abridged compared with control group but positive control have more hypolipidemic action Administered drug produced dose dependent equipotent hypolipidemic action

7.4. Histopathology

Various organs histopathology report revealed that the kidney liver and heart were moderate and mild histological changes observed different dose levels 200 & 400 mg / Kg when compared to various tissues of kidney liver and heart.

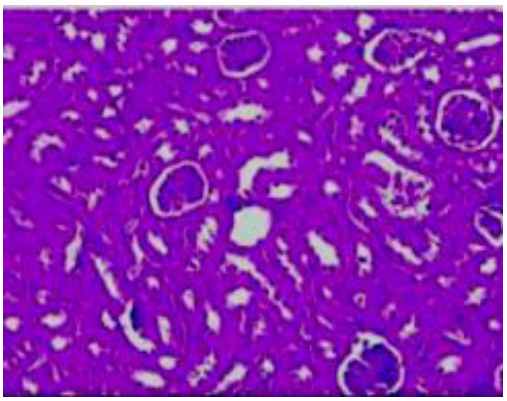


Figure No. 2: Kidney of normal control rat

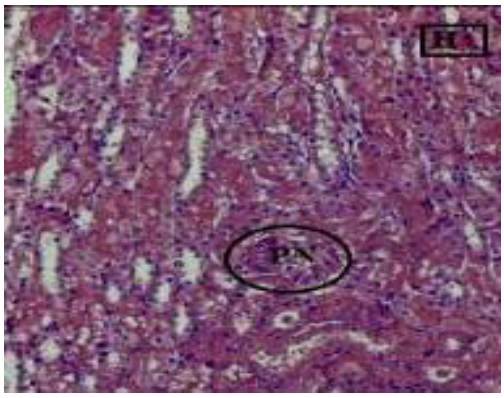


Figure No. 3: Kidney of extract treated with *Stellaria media* leaves

(200mg/Kg)

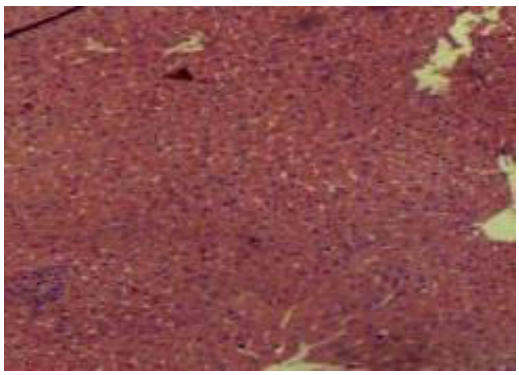


Figure No. 4: Liver of normal control rat

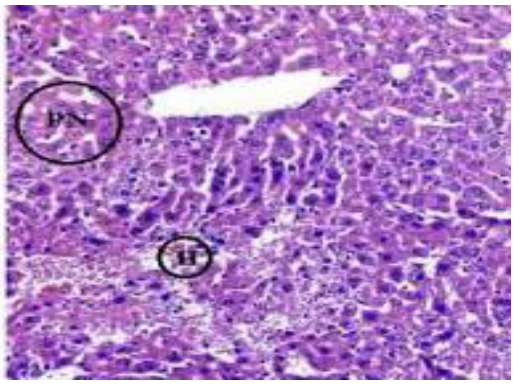


Figure No. 5: Liver of extract treated with *Stellaria media* leaves (200mg/Kg)

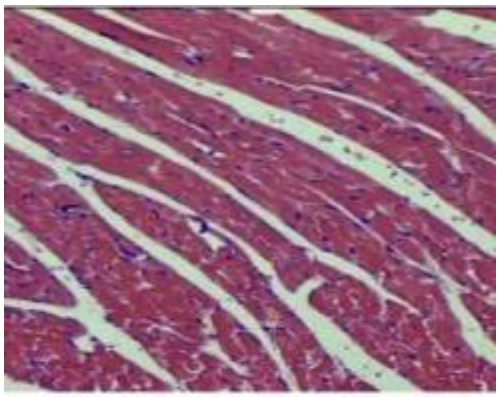


Figure No. 6: Heart of normal control rat

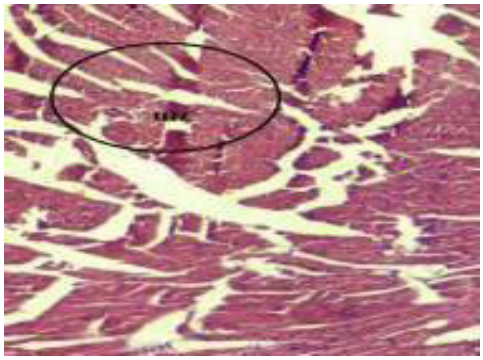


Figure No. 7: Heart of extract treated with *Stellaria media* leaves (200mg/Kg)

Table No. 3: DPPH Assay

Conc. (µg/ml)	Percentage of inhibition (%)		
	Ascorbic acid	**Extract 200mg/kg**	**Extract 400mg/kg**
20	51.33 ± 0.14	33.57 ± 0.82	34.56 ± 0.74
40	63.45 ± 0.96	43.59 ± 0.75	43.27 ± 0.59
60	74.66 ± 0.76	59.28 ± 0.33	58.31 ± 0.46
80	88.56 ± 0.54	63.61 ± 0.67	66.58 ± 0.38
100	99.51 ± 0.36	72.34 ± 0.09	75.31 ± 0.35

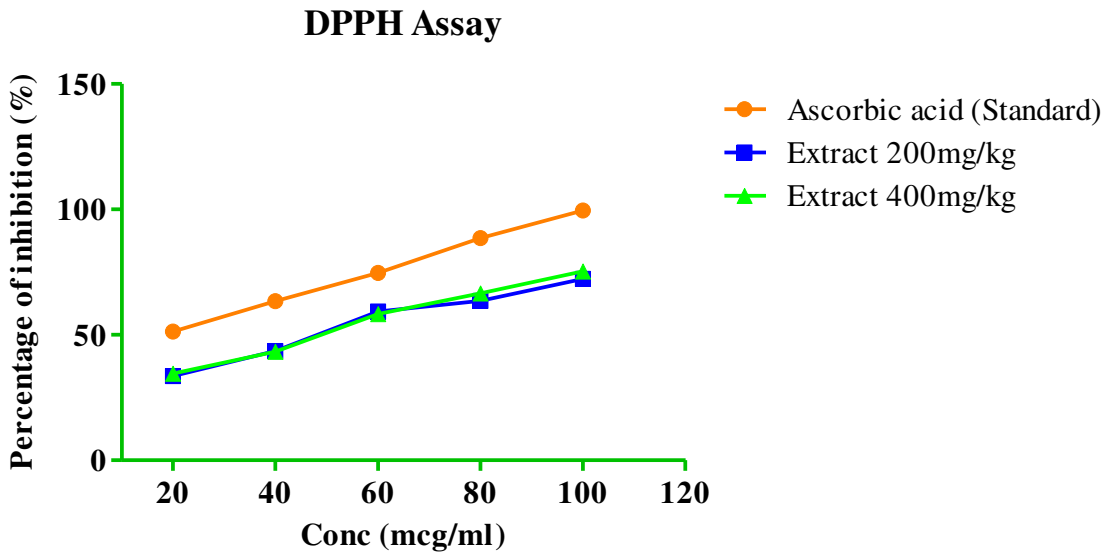


Figure No. 8: DPPH Assay

Table No. 4: ABTS+ Assay of *Stellaria media* leaves

Conc. (µg/ml)	Percentage of inhibition (%)		
	Ascorbic acid	**Extract 200mg/kg**	**Extract 400mg/kg**
20	45.26 ± 0.78	41.78 ± 0.34	44.56 ± 0.45
40	56.21 ± 0.56	53.01 ± 0.18	60.67 ± 0.56
60	70.25 ± 0.57	65.24 ± 0.37	66.03 ± 0.87
80	76.88 ± 0.87	71.26 ± 0.69	72.65 ± 0.91
100	89.53 ± 0.99	87.51 ± 0.58	89.56 ± 0.87

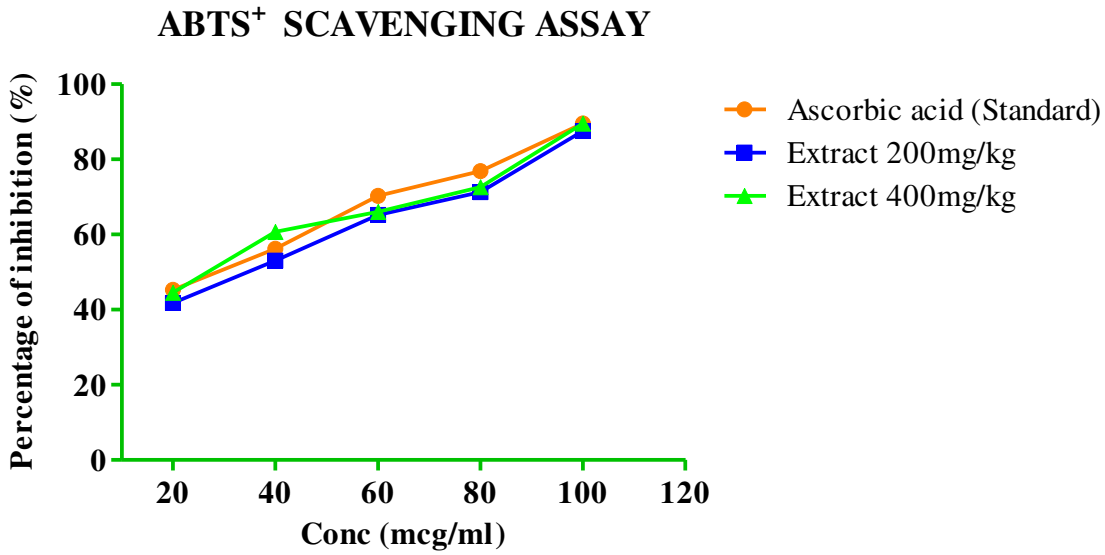


Figure No. 9: ABTS+ Assay of Stellaria media leaves

Table No. 5: High-Cholesterol Diet Induced Rat Model

Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
Control	99.56± 8.56	82.15± 6.16	37.81± 1.58	27.03± 1.23	29.71± 2.91
HCD	193.33 ± 7.33	150.92± 6.53	19.51± 1.36	29.77± 1.30	139.24± 5.47
HCD+ Simvastatin (10mg/kg)	112.33± 9.15*	118.22± 5.28*	30.65± 2.54*	20.61± 1.03*	59.82± 3.97*
HCD+ 200 mg Extract	151.00±8.36*	122.54±3.29*	23.91± 1.58*	27.41± 0.75*	101.68± 9.85*
HCD+ 400mg Extract	124.33± 7.01*	118.73± 5.56*	31.33± 2.19*	22.33± 1.45*	72.62± 3.88*

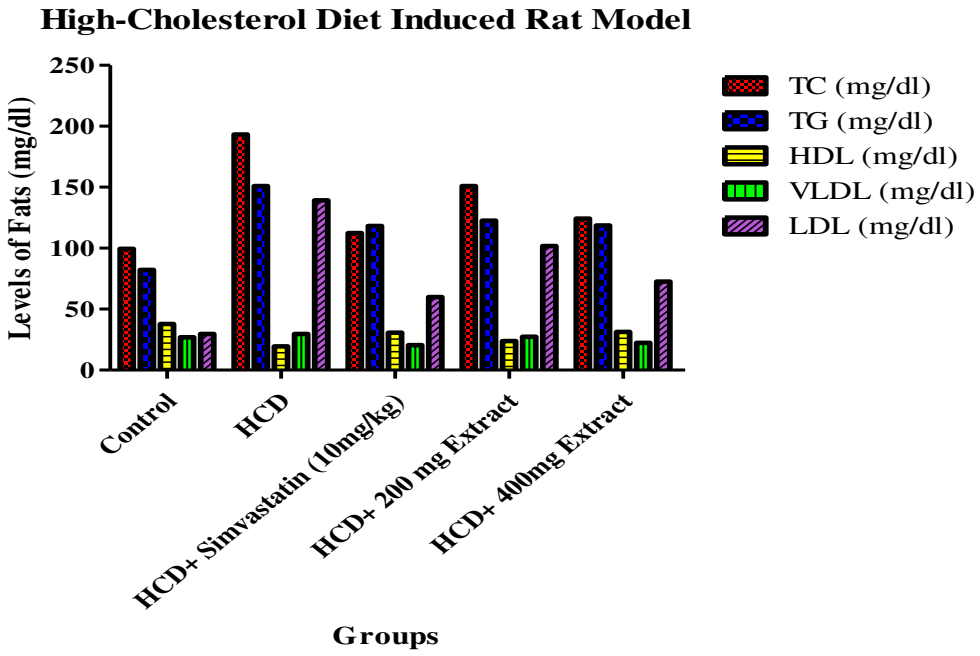


Figure No. 10: High-Cholesterol Diet Induced Rat Model

Table No. 6: High-Cholesterol Diet Induced Rat Model

Days	Mean Body weight (gm)(% change in body weight)				
	Normal	**HCD**	**Standard**	**200 mg/kg**	**400mg/kg**
Initial	148.33± 7.05	138.66± 8.7	146.33± 8.8	144.83± 7.6	144.44± 7.3
5th day	161.33± 6.55	164.66± 7.8	165.33 ± 7.7	166.50± 7.9	167.44± 8.03
10th day	169.10± 8.6	179.66± 8.9	181.66 ± 9.0	181.00 ± 8.9	180.00 ± 8.8
15th day	178.33± 8.05	187.33 ± 8.5	189.53 ± 9.05	188.00± 8.9	185.33 ± 8.3
20th day	183.66 ± 8.0	199.33± 8.9	192.33± 8.7	192.66 ± 8.9	190.33 ± 8.6

Figure No. 11: High-Cholesterol Diet Induced Rat Model

Table No. 7: Triton Induced Hyperlipidemic rat

Parameters	Normal	Triton WR1339	Extract 200mg/kg	Extract 400mg/kg	Simvastatin
TC (mg/dl)	95.33± 1.56	247.33±7.19	166.7± 5.26	72.12± 8.19	181.5 ±17.56
TG (mg/dl)	52.11± 5.34	697.42 ±8.81	634.77± 3.34	174.5#± 2.93	449.23 ± 8.15
HDL (mg/dl)	60.35± 7.21	50.81 ±7.31	28.56 ± 2.12	36.6 ± 9.68	30.59 ± 8.64
LDL (mg/dl)	23.12± 1.38	72.16±2.45	68.31 ± 9.49	8.21**± 9.28	64.12 ± 4.43
VLDL (mg/dl)	13.27± 1.42	137.07 ±6.56	70.16± 08.0	46.13± 6.63	88.32± 8.33

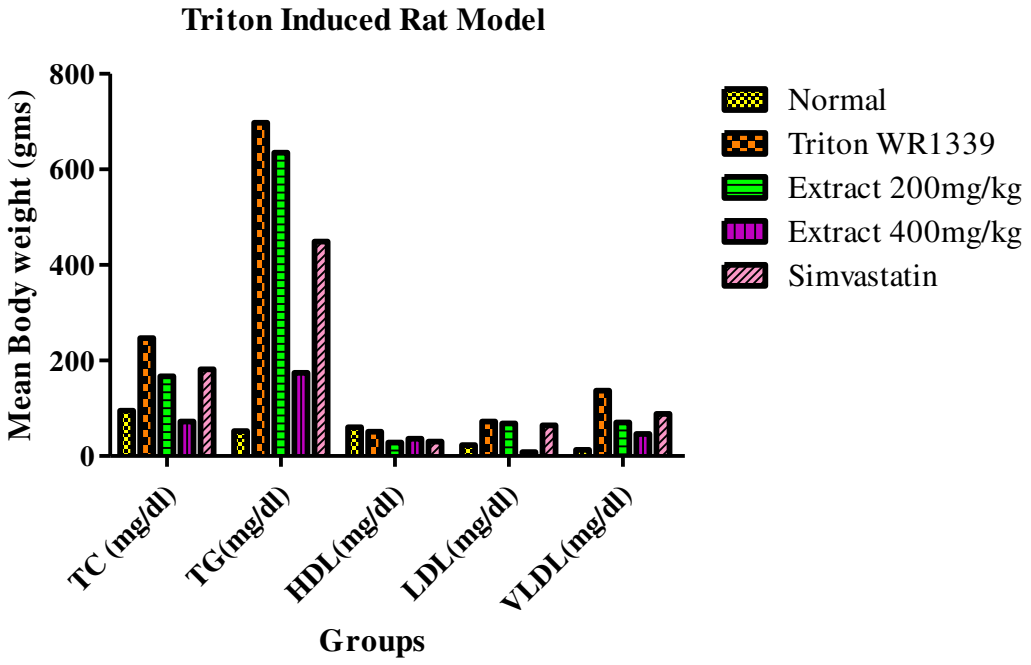


Figure No. 12: Triton Induced Hyperlipidemic rat

Table No. 8: Observation of hematological parameters in rats

Parameter	Hb (gm %)	RBC (million/mm^3)	WBC (10^3 cells/ mm^3)
Normal control	13.12±0.19	4.31±0.45	8.65±0.56
Positve control	9.84±0.56*	3.06±0.23*	12.86±0.7*
Ext (200 mg/kg) HCD	11.15±0.55ns	3.90±0.8ns	10.31±0.67a
Ext(400 mg/kg) HCD	11.97±0.24a	4.03±0.17a	10.02±0.8a
Ext (200 mg/kg) TIR	6.93±0.84aa	4.88±0.37aa	8.84±0.58aaa
Ext(400 mg/kg) TIR	6.66±0.86a	4.36±0.19aa	9.6±0.29aa

HCD- High Cholesterol Diet

TIR- Triton Induced Rats

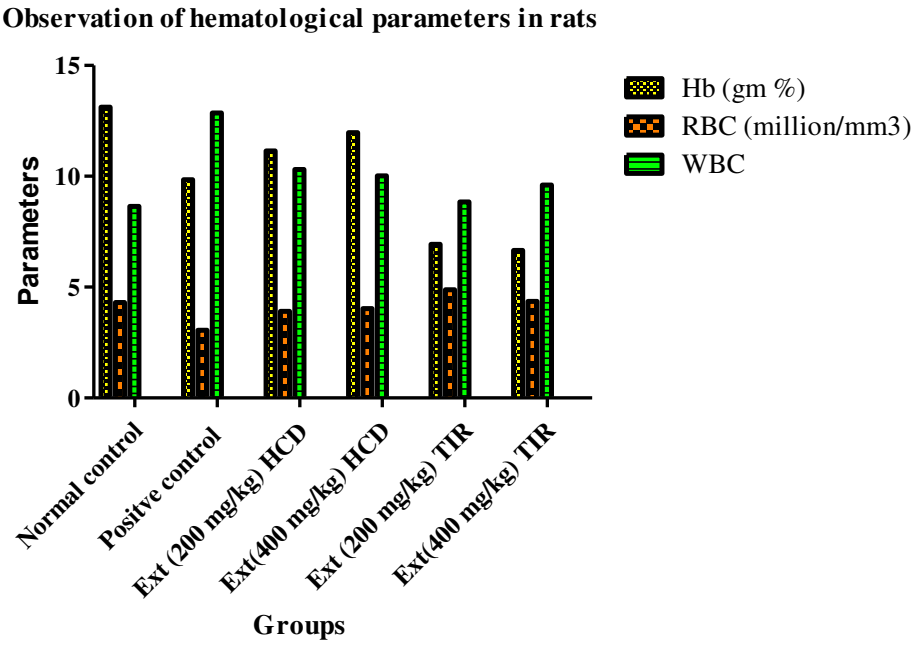


Table No. 13: Observation of hematological parameters in rats

8.0 CONCLUSION

Plant materials are used throughout the developed and developing world as home remedies, in over-the-counter drug products, and as raw material for the pharmaceutical industry, and they represent a substantial proportion of the global drug market. Certain herbs have become popular over the years, but the public, medical practitioners and the media still have a poor understanding of herbal medicine. Evidence is emerging on the dangers of herbs. As in most situations, the truth lies hidden under the media hype, poorly understood science, and exaggerated claims. Lack of experience, information, and education about herbs make consumers, physicians, and other orthodox health care provider's easy victims of market exploitation and herbal myths. There is no rational reason behind the tendency to equate "natural" with "harmlessness." The fact that something is natural does not necessarily make it safe or effective. In addition, a lack of knowledge of phytochemistry leads to misinterpretation and misunderstanding. It is very likely that some herbs will have side effects, interact with other medications, and be toxic. Information on isolated constituents should not be applied directly to the whole herb and studies on in vitro forms should not be confused with oral administration which was established by pharmacological screenings. In current scenario, herbs are the potent sources of medicines used in the treatment of various disease and disorders. Since, plants are used as medicine there is prompt need of evaluation of plant species, therefore, the present work was conceived to evaluate the phytochemical and pharmacological screening of few Indian medicinal plants.

Thus the results of the present investigation clearly indicated that the selected medicinal plants possess good antihyperlipidemic activity in atherogenic diet

induced hyperlipidemic rats and led to the development of new Herbal formulation possessing antihyperlipidemic and antiatherosclerotic activities

Hence it is going to be concluded that the potential benefits of the extracts of *Stellaria media* has been demonstrated well in advance and can be used further to demonstrate the antihyperlipidemic as well as controlling of both triglyceride levels and reducing the risk of factors of cholesterol inducers. The aforementioned results of the research suggest that the *Stellaria media,* found to have the potential antihyperlipidemic action.

The results found are encouraging for further studies on the selected plants and to identify the bioactive compounds.

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