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Evaluation of anti-hyperlipidemic activity of Euphorbia Hirta bark against triton ® WR 1339 induced hyperlipidemia

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***Abstract*---**Hyperlipidemia is the major risk factor causing many cardiovascular disorders like myocardial infarction and stroke, atherosclerosis. The world has complimented many herbal substances of therapeutic importance. There are many reports about the use of herbal medicine for reduction of lipid profile and reducing the cardiovascular risk in clinical subjects and experimental pharmacology. The plant *Euphorbia hirta* bark was evaluated for anti-hyperlipidemic activity by Triton (Superinone, Triton WR-1339), induced hyperlipidemia in rats. This study was carried out on the action of *Euphorbia hirta*barkethanolic extracts, a known anti-hyperlipidemic drug, Simvostatin (10 mg/kg body wt.), was taken as a

standard drug. The statistical parameters of mean± S.E were employed for the calculation of one way analysis of variance test (ANOVA) and Dunnet's test for many comparison's P < 0.05 were considered as significant. The studies were shown. There was a significant (P<0.01) reduction in serum levels in lipid profile experiments. It has reported a significant lower total cholesterol, triglycerides, low density lipoprotein (LDL), very low density lipopreotein (VLDL) and an increase in high density lipoprotein (HDL).The study confirmed that ethanolic extract of *Euphorbia hirta* bark having significant anti-hyperlipidemic action proving a potential herbal medicine as an alternative to currenttreatment of hyperlipidemia.

***Keywords*---**euphorbia hirtabark, Triton ®, hyperlipidemia, simvastatin.

Introduction

The traditional medicine system is a very rich heritage of India, which has great potential in mitigating the disease of the universe. For more than 2000 varieties of species grown in wide areas which is been included in the Ayurveda, Unani, Siddha and traditional medicines, As herbal medicines generally have fewer side effects compared to the modern medicines, their dosage level are tolerant which has gained in great demand in developed and developing countries. Herbal medicines may be a choice in primary health practices due to broad biological and medicinal properties, and are cost effective compared to other modern medicines [1]. In non-insulin dependent and insulin dependent diabetes mellitus, it also facilitates this lipid malfunctioning in the body and causes many. There are reports that this type of complication in which both hyperlipidemia and lipid disorders may cause cardiovascular complications [2]

Chronic hyperlipidemia may also initiate atherosclerosis, which is another risk factor for the cardiovascular system [3] The drugs which are meant for reduction of hyperlipidemia has been reported many pharmacological action [4] The treatment and curative approaches for hyperlipidemia is by either one of the methods such as decreased production of endogenous lipid production and decreased absorption of lipids from the diet. Increased lipid levels result from better absorption in the gut or more endogenous synthesis and therefore reducing hyperlipidemia reduces different approaches are to be thought scientifically by promoting to prevent endogenous synthesis or to decrease lipid absorption. Both factors can be evaluated in normal animals without artificial diets. A study mentioned that overweight and obese persons have major risk factors for cardio vascular disease in all the age groups. The medical confirmation of obesity is based on Body mass Index (BMI). The range is available from 25 -36, but above 30 BMI is considered as Obese. BMI is defined as their weight in kg divided by the square of their height in meters. The BMI is a risk factor which is already known is due to the imbalance of lipid levels leading to deposition of extra the lipid in body and blood vessels, leading obesity and obesity and increased BMI, and an alteration in lipid metabolism which are caused by multiple factors. These

unregulated lipids are deposited in adipose tissues and vascular systems, and this condition also causes atherosclerosis .e narrowing of arteries due to deposition of cholesterol is a risk factor for cardiovascular diseases [5].

Lipid management is the only approach to mitigating the major risk factors, I.e. hyperlipidemia and atherosclerosis for reducing cardiovascular diseases. This approach will reduce the risk of cardiac death, stroke, myocardial infraction, revascularization and peripheral artery disease by 25-80% of the adults. The categories of statins are the preferred choice for treating hyperlipidemia. These drugs lower the cholesterol formation and interference of biosynthesis of cholesterol. So far there is no single medicine available for anti-hyperlipidemic agents that has the least side effects and effects at present. This created an interesting opportunity to search for more efficient, safer and cost effective anti-hyperlipidemic agents. Folklore and medicinal plants have proven diverse biological activities and pharmacology. Based on review of literature, it has been found that not much has been investigated scientifically so far on this plant; the plant which was selected for the study has been explored for many phytochemicals present in the plant [6] the phytochemical analysis revealed the presence contains glycosides, carbohydrates, sterols, flavonoids and Saponins flavonoids, triterpenoids, alkanes, amino acids, and alkaloids *Euphorbia hirta* bark has illustrative biological studies also reported possesses antibacterial, anthelmintic, anti-asthmatic, sedative, antispasmodic, antifertility, antifungal, and antimalarial properties.[7]

Materials and Methods

Collection and identification of Plant material

The plant was identified and authenticated by Prof (Dr) Noel Pinto, St. Agnes College, Mangaluru, and Karnataka, India. And a herbarium of the plant will be kept in our institution. *Euphorbia hirta* bark was collected from local areas of Mangalore, Karnataka and KasaragodDist, Kerala. The sample deposition library has preserved the voucher specifying NGSM/PL/021 for future reference. *Euphorbia hirta* bark was collected during the mansion season. The bark was dried in a shade. Later the bark was made into fine particles. The powder was passed through 40 # mesh particle size and stored in an airtight container at room climate at 25 °C.

Chemicals

Triton -WR 1339Sigma Aldrich,.Simvastatin(Dr. Reddy's Laboratories, Hyderabad), Diagnostic kits for estimation were purchased from Merck Diagnostics India Ltd. Anesthetic ether (Ozone International, Mumbai), and all other chemicals were of analytical grade.

Plant *Euphorbia hirta* bark extraction

About 3 kg of the coarsely powdered *Euphorbia hirta* bark plant material of mesh size 40# were extracted using ethanol as an extracting substance, a simple maceration was employed for extraction at normal room temperature for 07

daysoccasional shaking and stirring in a conical flask. The extract was allowed to concentrate to dryness at room temperature to avoid any decomposition during the process. All the extract was stored under refrigeration to determine active constituents a preliminary phytochemical analysis was employed [8]

Experimental Animals

The pharmacological screening to determine the desired activity of the objective animal studies was designed as per the standard animal experimentation protocol in accordance with CPCSEA guidelines as well as GLP and other relevant safety profile measures The adult experimental animal's rats of belonging to *wister* strain (150-200g) was collected from animal house of NGSM Institute of Pharmaceutical sciences, acclimatized as per the standard protocol 12 h light and 12 h dark cyclesAnimal feed pellets provided by m/s Goldmohar rat feed, Mumbai, India, were considered as a standard basal diet for the experimental animals.All the animals was provided with food andwater *adlibitum*. The protocol was approved by CPCSEA for conducting animal experimentation, at NGSM Institute of Pharmaceutical sciences (Ethical committee IAEC reg. no.: 627/02/a/CPCSEA).

Preparation of dose for dried extracts

Euphorbia hirta bark Ethanolic extracts was made as suitable formulation using distilled water using Tween-80 as suspending agent dose is mentioned as weights only [9]

Preparation of standard drugs

HMG co A Reeducates inhibiters (statins) competitively inhibit the conversion of 3 hydroxy 3 methyl glutaryl co enzyme A (HMG-CoA) to mevalonate (rate limiting step in CH synthesis) by the enzyme HMG co A reductase. Therapeutic dose reduce CH synthesis. Thesi results in increase in LDL receptor expression on liver cells, uptake of IDL and LDL. Simvastatin is one of the products currently used to treat hyperglycemia and atherosclerosis in clinical subjects. Simvastatin is a competitive inhibiter of the enzyme HMG CoA that reducataseinhibiters or "statins". The HMG Co A catalyzes the early and rate limiting step in cholesterol synthesis bio synthesis. Simvastatin 10 mg/kg was used as the reference standard drug for comparing the hyperlipidemia activity.

Acute oral toxicity studies

Pharmacological screening using animals as well as in clinical subjects is very detrimental to the dose of drug used. In order to safely guard and prevent the toxic and harmful effects, it's compulsory to conduct a toxicity test and satisfy the norms of OECD 423 guidelines and CPCSEA. There were no signs of toxicity observed in subjects up to 3000 mg/kg/b.w [10].

Triton ® -induced hyper lipid emic model

In adherence to the standard protocol, the animals selected for the study were kept fasting for 24 h. Hyperlipidemia was induced in experimental rats using Triton WR 1339 in a standard method. The chemical composition of Triton (Superinone, Triton WR-1339), a mixture of polymer of p-isooctylpolyoxyethylenephenol and formaldehyde, a nonionic surfactant. The Experimental animal weighing 150- 180 g Rats were selected for the study, each group consisting 06 animals. The distribution of animals was done randomly with both sexes of animals. The experimental animals were divided into various groups; Group01 the animals to be observed in normal condition were kept in this group. On first day for the induction of desired hyperlidemia condition the animals in group numbers 02 to 05 was injected Triton WR1339 at a dose of 300 mg/kg intra-peritoneal. The normal group was administered saline onlyOn the second day onwards, rats employed in the groups 3,4, and 5 were fed *Euphorbia hirta* bark extracts orally dispersed in distilled water by gastric intubation at doses of 100, 200 and 400 mg/kg b.w. respectively.For Group 2 animalsThe formulation of standard HMG co A Reeducates inhibiters (statins) was administered Simvastatina dose of 10 mg/kg b.w (p.o.) mixed in 0.5%CMC for the observation of therapeutic effect for comparison with test and control groups, whereas 0.5% CMC alone was administered to the rats in served as the hyperlipidemic control (Group 1)In accordance to the protocol On day 03, blood sample was withdrawn from the retro-orbital plexus of all animals, and the serum was separated and stored at -20 °C until further use Table-1. [11]

Table-1: Experimental design

Group	Dosing schedule
I.I	Served as Normal control received standard pellet diet.
I.II	Standard drug Simvastatin(10mg/kg/day p.o.)and Triton® WR1339 was administered 300 mg/kg intra peritoneal route.
I.III	Test 1:*Euphorbiahirta* bark (100mg/kg/day p.o.)and Triton® WR1339 was administered 300 mg/kg intra peritoneal route.
I.IV	Test 2:*Euphorbiahirta* bark (200mg/kg/day p.o.)and Triton® WR1339 was administered 300 mg/kg intra peritoneal route.
I.V	Test 3:*Euphorbiahirta* bark (400mg/kg/day p.o.)and Triton® WR1339 was administered 300 mg/kg intra peritoneal route.

Collection of blood sample form experimental animal

For estimation of the lipid profile of the experimental subject, the blood samples were taken by retro-orbital sinus puncture method under mild ether anesthesia. Samples were centrifuged at 3000 RPM for 10 min using centrifugal apparatus.

Bio-chemical analysis

The bio chemical assay was done for analyzing the lipid parameters, by using the serum sample. the lipid profile assay for total cholesterol, triglycerides, phospholipids, high-density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) the method of CHOD-PAP for Serum total

cholesterol, triglyceride the method of GPO-PAP. Low density very low density cholesterol were calculated by using Friedewald formula and VLDL: TG/5 respectively.[12,13]

Statistical analysis

Readings of mean± S.E.one way analysis of variance test (ANOVA) followed by Dunnett'st-test for multiple comparisons were considered for statistical analysis and P< 0.05considered as significant. [14]

Results and Discussion

Preparation of Extract

Plant extracts was subjected for Phytochemical analysis of *Euphorbia hirta* bark extracts showed the positive report oftriterpenoids, tannins, steroids, saponins, Glycosides, carbohydrates, sterols, flavonoids, triterpenoids, alkanes, amino acids, and alkaloids.

Acute toxicity study

The toxicity study is essential for selection of doses and the study was executed in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals [CPCSEA] guidelines with good laboratory practices. According to OECD- 423 guidelines adopted on 17th December 2001.A female albino rat was selected, the dose of 2000 mg/kg/b.w. of *Euphorbia hirta* bark extract was administered for screening for toxicity. The observation was carried out at regular intervals for every 30 minutes of initial dosing. Upon observations for 24 h many parameter was observed toxicity parameters nervous system associated reflexes on behavioral pharmacology, autonomic systems pharmacology, all the vital organs function, physiology of respiratory, circulatory, and behavior pattern were also found to be normal. It's deemed that the LD50 is 2000 mg/kg/b.w, but for therapeutic applications its 1/10th value is chosen as 200 mg/kg/b.w takes as the median dose and another two doses, 100 mg/kg/bw as a lower dose and 400 mg/kg/b.w higher dose.

The Antihyperlipidemic Activity

The desired pharmacology activity of Anti hyperlipidemic Activity of *Euphorbia hirta* bark extract at the dose of 100, 200, &400 mg/kg b.w has showed a significant results there is decrease in lipid contents of LDL at 400 mg/kg b.w. of *Euphorbia hirta* bark extract where as a HDL-C was increased remarkably [Table-2]

Table-2 Effect of *Euphorbia hirta* extract in Triton® induced hyperlipidemia model

Treatment group	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
I Norma	85.44±2.63	64.78±3.53	47.7±1.7	23.60±1.28	12.78±1.01
II	186.8±4.5	194.4±3.07	24.03±1.80	141.27±4.8	63.60±2.52

Control				7	
III Standard	96.60±5.6	154.2±4.70	38.60±2.31	28.4±3.07	35.362±2.46
IV 100 mg/kg Extract	161.2±3.19***	180.9±2.62*	30.78±1.35*	128.9±2.92***	43.36±1.92***
V 200 mg/kg Extract	119.6±3.28***	169.8±2.48***	33.0.9±2.63**	70.85±2.92***	41.24±3.34***
VI 400mg/kg Extract	100.8±2.35***	161.9±2.56***	36.17±1.82***	52.34.±3.34**	39.02±1.86***

The values are expressed as Mean ± SEM, n=6 rats in one group. *P<0.05, **P<0.01, **P<0.001, when compared with control group.

Discussion

The preliminary phytochemical screening of ethanolic extract of *Euphorbia hirta*revealed the presence of alkaloids, flavonoids, glycosides, steroids, tannins, saponin, proteins and carbohydrates.Suppressing the action of lipases is the main mechanism of action of Triton which is surfactant it block the uptake of lipoproteins from circulation by extra hepatic tissues, resulting in increased blood lipid concentration. [15,16] Inhibition of cholesterol biosynthesis and increases the level of lipolytic enzymes to evacuate the lipid from blood stream The screening for antihyperlipdemic model Triton ® -induced hyper lipid emic is a well-established for natural substance and synthetic drug testing procedures, the results tabulated were shown a significantantihyperlidemic action at dose 400 mglkgl.w orally Lowered both plasma TG and TC levels.

The anti-hyperlipidemic activity of the *Euphorbia hirta* was found to be evident in both the excretory and synthesis phase of triton ® induced hyperlipidemia in rats. Triton acts as the surfactant which decreases the action of lipase and blocks the uptake of the lipoproteins from the circulation to the extra hepatic tissues, which in turn results in an increase in the level of the circulatory lipids in the blood.

The bio active substance Flavonoids augments the activity of lecithin acyl transferase (LCAT), which regulates the blood lipid level. LCAT plays a key role in the co-operation of free cholesterol into HDL (this may increase the HDL) and transfers it back to the VLDL and the LDL, which are later taken back by the cells of the liver. It can be suggested that the use of plant extract as a remedy for hyperlipidemia may be possible. Further studies can be carried out to determine individual active constituents/ molecules causing this action. The phyto constituent flavonoids have properties of decreasing the LDL and increasing HDL in experimental subjects. Movement of cholesterol from the certain tissues to the liver for catabolism and excretion, decreased levels of TG, TC and LDL and increased levels of HDL were witnessed from the study, the combined action a well as synergistic effects of bioactive molecules such as flavoinoids, saponns, steroids in the ethanolic extract of *Euphorbia hirta* was responsible [17]

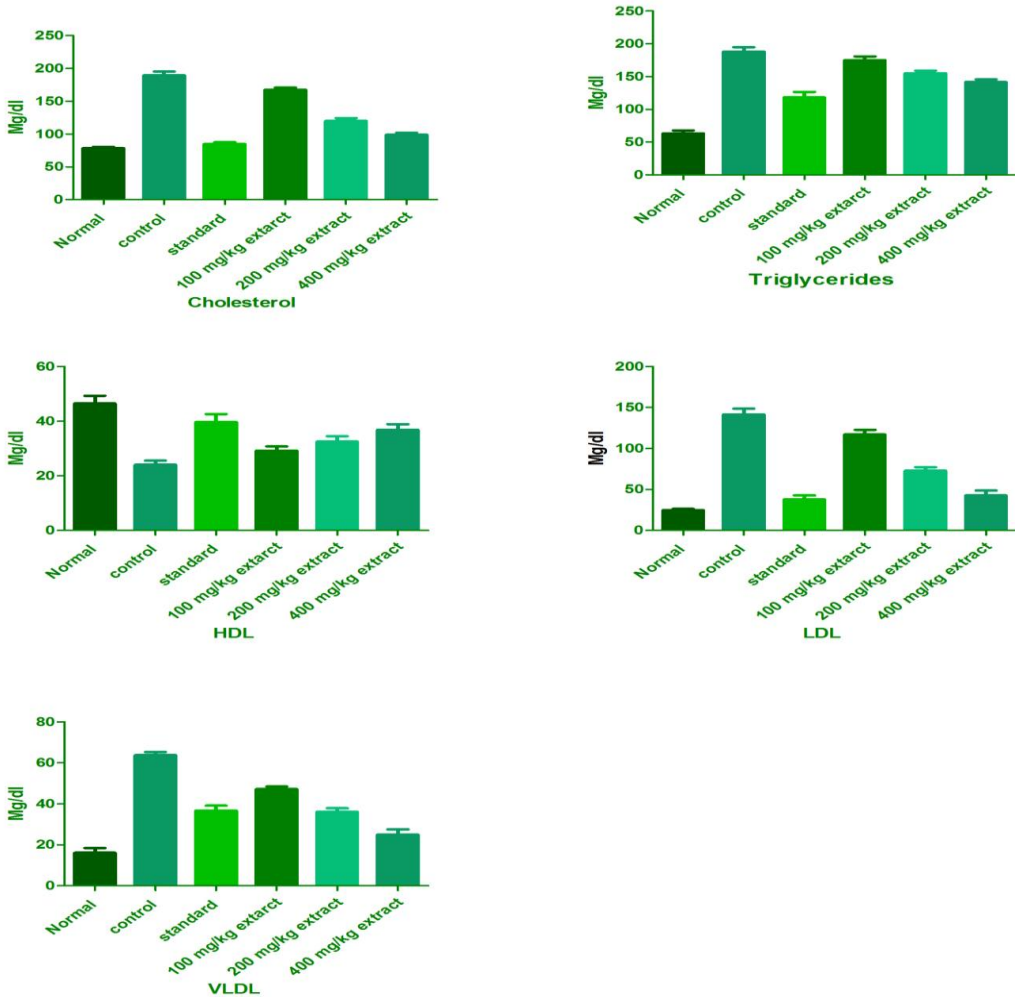

Fig 1: Effect of *Euphorbia hirta* extract in Triton® induced hyperlipidemia model

Conclusion

Antihyperlipidemic activity in the Triton® induced hyperlipidemia model was carried out in the ethanolic extract of *Euphorbia hirta* bark. The hyperlipidemia induced by Triton ® WR-1339, a nonionic surfactant, in experimental animals and compared with standard HMG Co-A enzyme reductase drug Simvastatin 10mg/kg, a potent lipid-lowering agent and studies with ethanolic extracts of at convenient doses 0f 100, 200, and 400 mg/kg/b.w. the extract might be acting by preventing anpreliminary stageof biosynthesis of cholesterol, depleting the plasma cholesterol concentration.

Triton® WR-1339 causing structural modifications in the circulatory lipoproteins and decreases the action of lipid lowering enzymes like; lipases, lipoprotein lipase activity and as a consequence effect is this facilitation and blocking action leading to the uptake of circulating lipids by extra liver tissues, and, in turn, resulting in increased blood lipid concentration [18, 19] More increased level bio active

substances present in ethanolic extract of Euphorbia hirta bark. May decrease the concentrations of triglycerides along with cholesterol. Elevated level of blood cholesterol especially LDL was the major risk factor for cardio vascular risk factor, atherosclerosis, and stroke in experimental animas and HDL as cardio protective protein. [20-22]

1/10th of maximal dose of 2000mg/kg/b.w, limit test was taken as a therapeutic dose determined by the acute oral toxicity of *Euphorbia hirta* bark this dose has significantly decreased the level of cholesterol, triglycerides, phospholipids, VLDL and LDL as compared to hyperlipidemic control. HDL level were increased significantly as compared to control. The anti hyperlipidmeic actions is be due to the increased activity of lecithin: cholesterol acetyl transferase which incorporates free cholesterol, free LDL into HDL and transferred back to VLDL and intermediate density lipoprotein.

There is significant reduction in triglyceride levels which may be due to elevation of endothelial bound lipoprotein lipase action by hydrolyzing triglycerides into free fatty acids or inhibiting lipolysis, which prevents conversion to triglycerides and lipoprotein lipase releasing fatty acids from chylomicrons and very low-density lipoprotein (VLDL) in circulation. Cholesterol synthesis in the liver is activated by triton WR 1339. Moreover, Tritons have an ability to alter very low density [VLDL] lipoproteins, rendering them refractive to the action of lipolytic enzymes of blood and tissues, preventing or delaying their removal from blood [23]. Hence, the hypolipidemic effect of extracts could be due to an increased catabolism of cholesterol into bile acids. . The study has concluded that the plant of *Euphorbia hirta* bark can be utilized for its Antihyperlipidemic activity.

In future, toxicological studies can also be carried out to find out about the toxic and non-toxic nature of the drug substances in the extracts.Similarly the projects can be made on finding out the chemical constituents Isolation and characterization of the responsible for pharmacological activity.

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Conflict of interest: Nil

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