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EVALUATION OF ANTIHYPERLIPIDEMIC ACTIVITY OF METHANOLIC EXTARCT OF *GLYCOSMIS PENTAPHYLLA* ON EXPERIMENTALLY INDUCED HYPERLIPIDEMIA IN ALBINO RATS

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ABSTRACT

The present study was carried out on 60 male albino rats, divided into 10 groups 6 in each. Rats were post treated with normal saline, 200, 400mg/kg of MEGP and 10mg/kg of Atorvastatin as standard orally for triton and high cholesterol died induced hyperlipidemia models respectively. After administration of Triton and high diet cholesterol there is significant increase in the levels of TG and TC. The blood is withdrawn retro orbital method to estimate above serum parameters. Triton and high cholesterol treated group showed significance increase (P<0.0001) the levels of TC, TG, LDL & VLDL compared to normal group. Post-treatment with extract *of Glycosmis pentaphylla* at different doses (200mg/kg and 400mg/kg), significantly (P<0.001) prevented the elevation of these parameters when compared to hyperlipidemia rats. Triton and high cholesterol treated group showed significance (0.0001) decrease in HDL and compared to normal groups. Post-treatment with extract *of Glycosmis pentaphylla* at different doses (200mg/kg, 400mg/kg), significantly (P<0.001) prevented the reduction of these parameters when compared to hyperlipidemic rats. The present study suggests that post-treatment with methanolic extract of *Glycosmis pentaphylla* showed dose dependent antihyperlipidemic action against Triton and Cholesterol diet induced hyperlipidemia. The overall antihyperlipidemic action of methanolic extract of *Glycosmis pentaphylla* is probably due to its antioxidant, and free radical scavenging activity.

KEYWORDS: Hyperlipidemia, *Glycosmis pentaphylla,* Atorvastatin, Triton, Total Cholesterol (TC), Triglycerides (TG), LDL & VLDL,

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INTRODUCTION

Human body is complex machinery and for maintaining the homeostasis various organs and organ systems contribute. Any undesirable changes will disturb the balance resulting in the diseased state. The same way, the change in the level of lipids will lead to many complications[1]. But lipids are naturally occurring molecules, which are main component of a cell membrane. They acts as blood transporter and their main function is energy storage[2,3]. Hyperlipidemia is the condition of abnormally elevated levels of any or all lipids and/or lipoproteins in the blood. It has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases, stroke and atherosclerosis among all Hyperlipidemia is the primary cause of death[4]. The American Heart Association has identified the primary risk factor associated with the atherosclerosis is the elevated levels of cholesterol and triglyceride in the blood. Therefore the therapist considers the treatment of hyperlipidemia to be one of the major approaches towards decelerating the atherogenic process. Atherosclerosis referred to as a “silent killer”, is one of the leading causes of death in the developing countries like India. In the general population, the cardiovascular disease risk from increased LDL cholesterol is supported by observations that cholesterol-lowering therapy greatly diminishes the clinical manifestations of atherosclerosis, particularly since the advent of inhibitors of 3-hydroxy-3- methyl glutaryl coenzyme a reductase (i.e., statins) that profoundly lower LDL cholesterol. In contrast to the situation with LDL cholesterol, the relation between HDL cholesterol and atherosclerosis is an inverse one[5-7]. World health organization reports that high blood cholesterol contributes to approximately 56% of cases of cardiovascular diseases worldwide and causes about 4.4 million deaths each year. Hyperlipidemia is a metabolic disorder, specially characterized by alterations occurring in serum lipid and lipoprotein profile due to increased concentrations of Total cholesterol (TC), Low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), and triglycerides (TG) with a concomitant decrease in the concentrations of high density lipoprotein cholesterol (HDL-C) in the blood circulation. Currently, the use of alternative medicines and especially the consumption of phytochemicals have been rapidly increasing worldwide. As herbal medicines are less damaging than synthetic drugs and they have better compatibility thus improving patient tolerance even on long-term use[8]. The leaves of *Glycosmis pentaphylla are found in* almost all districts of Kerala India, Sri Lanka to South East Asia and West Malaysia. The plant is used in indigenous medicine for cough, rheumatism, anaemia and jaundice. The juice of the leaves, which is bitter and used in fever, liver complaints and as vermifuge. A paste of the leaves with ginger is applied for eczema and skin infections. A decoction of the root is given for facial inflammations. The present study was aimed to study the methanolic extract of *Glycosmis pentaphylla* Linn leaves were selected to investigate the antihyperlipidemic activity on experimentally induced hyperlipidemia in rats. The present study was indicated to study the Evaluation of antihyperlipidemic activity of methanolic extract of *Glycosmis pentaphylla* on experimentally induced hyperlipidemia in Albino Rats.

MATERIALS AND METHODS

Plant material

Glycosmis pentaphylla Linn leaves were collected and authenticated by Dr. K. Madhava Chetty, Dept. of Botany, Sri Venkateshwara University, Tirupathi, Andhra Pradesh, India.

Preparation of extract

Fresh leaves were collected, shade-dried and then dried in a hot air oven at 25ºC and mechanically powered. Transferred it to a round bottomed flask and then went with soxhlet extraction using 90% methanol for 24 hours. Extract was dried by placing it on a big petriplate on electric water bath (70ºC0) and then kept in an oven at 30ºC for 2 hour. The percentage yield of extract was obtained 36.5% in our experimental condition[9].

Phytochemical screening

Preliminary Phytochemical screening of the extract was subjected for the presence of Phytosterols, glycosides, carbohydrates, Flavonoids, Alkaloids, Tannins, Proteins, Saponins[9,10].

Animals

Wistar albino rats of either sex (150–200 g) were used. The animals were housed at room temperature (22-28 ºC) for 12 hr dark and 12 hr light cycle and given standard laboratory feed and water *ad-libitum*. The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee of Smt. Sarojini Ramulamma College of pharmacy bearing the number is 51/01/C/CPCSEA/2013/002.

Drugs and Chemicals

Atorvastatin used from Cadila Pharmaceuticals Ltd. India, the other chemicals and analytical reagents grade obtained from Sicra labs (Prasanthi nagar,Hyd,,A.P,India)

Toxicity studies

Rats were fasted overnight prior to drug administration. Each animal received a single dose of plant extract (2000 mg/kg, p.o.). Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours) and daily thereafter, for a total of 14 days. All observations were systematically recorded with individual records being maintained for each animal. Observations included changes in skin, mortality and general behavioural pattern. Attention was given for observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. No death was observed till the end of study[11,12].

Selection of Dose of the Extract

LD_{50} was done as per OECD guidelines for fixing the dose for biological evaluation. The LD_{50} of the extract as per OECD guidelines falls under class four values with no signs of acute toxicity of 2000 mg/kg. The biological evaluation was carried out at doses of 200 and 400 mg/kg body weight.

Methodology

The present animal study experiment was conducted in high fat diet model and triton induced model.

High fat diet model

The standard diet was prepared by blending the standard animal diet. The hypercholesterol diet was prepared by mixing the standard powdered diet with 1.5% cholesterol and 0.2% cholic acid. The animals were fed a high cholesterol diet for a period of 10 days. To confirm the induction of hyperlipidemia, the blood sample was collected by retro orbital vein. The TC concentration of the blood samples was then determined using standard diagnostic kit. The rats were than divided into 5 groups of 6 animals in each group based upon their cholesterol concentration levels, after which the rats are subjected for the treatment for a period of 10 days the treatment is given orally at a dose range of 200mg and 400mg/kg. At the end of treatment period, the animals were used for the study of various biochemical parameters. Blood was collected by orbital plexus of rat under ether anaesthesia[13] and centrifuged by using the centrifuge at 2000 rpm for 30 min to get serum and estimate the physical and biochemical parameters.

Triton induced model

Hyperlipidaemia was induced in Wistar albino male rats by single intraperitoneal injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline after overnight fasting for 18 hours .The rats were divided into five groups of six rats in each group and were treated with single dose/day (p.o.) of standard drug or extracts .The first group was given Normal saline 0.9% w/v or Distilled water (Served as normal control). The second group was given a single dose of triton at a dose 100 mg/kg, i.p. After 72 hours of triton injection, this group received a daily dose of 5 % CMC (p.o.), for 7 days (Served as Triton control). The third group with Standard Atorvastatin 10 mg/kg p.o. for 14 days (served as standard). Fourth and fifth group was administered a daily dose of Plant extract for seven days, after inducing hyperlipidemia. On 8th day of treatment, the blood was collected by retro orbital sinus puncture, under mild ether anaesthesia in plane tubes. Serum obtained

by immediate centrifugation of blood samples using semi ultra-cooling centrifuge at 3000 rpm for 5 minutes at room temperature and was directly used for estimating serum lipid profiles (serum TC, TG, LDL-C and HDLC). All samples were stored at 4°C until analysis[14-17].

Evaluation of Biochemical Parameters

Total cholesterol (TC), Triglyceride (TG), High Density Lipoprotein (HDL), was measured by using ERBA Diagnostic Kits. Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) were measured by using Friedewald's formula.

LDL and VLDL were calculated according to Friedewald Formula:

VLDL cholesterol (mg/dl) = TG ÷ 5

LDL cholesterol (mg/dl) = TC – (HDL + VLDL).

Statistical Analysis

Results were presented statistically by using Graph pad prism version 5.0. All results were expressed as Mean±SEM, analyzed by one way analysis of variance followed by "Dennett's test".

RESULTS

Effect of MEGP on Body weight and Atherogenic Index

Triton and high cholesterol treated group showed significant increase (P<0.0001) the levels of body weight and Atherogenic Index compared to normal group. Post-treatment with extract of *Glycosmis pentaphylla* at different doses (200mg/kg, 400mg/kg), significantly (P<0.001) prevented the elevation of these parameter when compared to hyperlipidemia rats.

Table 1
Effect of MEGP on body weight and atherogenic index in HFD induced Hyperlipidemia in rats.

Group No	Treatment	Body weight	Atherogenic index
I Normal Control	Normal saline	163.9±1.10	2.51±0.02
II Toxic Control	High Fat Diet	210.2±1.53	4.14±0.008
III Standard	Atorvastatin 10 mg/kg and High Fat Diet	171.6±0.98***	2.73±0.05***
IV Test 1	MEGP 200mg/kg	200.1±0.92*	3.24±0.01**
V Test 2	MEGP 400mg/kg	172.2±1.77**	2.93±0.06***

Values are mean ± SEM, n=6. P value *** p< 0.001, **p< 0.01 and *p< 0.05 vs. Toxic control.

Table 2
Effect of MEGP on body weight and atherogenic index in Triton-X-100 induced Hyperlipidemia in rats.

Group No	Treatment	Body weight	Atherogenic index
I Normal Control	Normal saline	159.1±1.16	2.56±0.22
II Toxic Control	Triton-X-100 (100 mg/kg)	205.4±1.43	4.18±0.008
III Standard	Atorvastatin 10 mg/kg and Triton-X-100 (100 mg/kg)	166.9±1.14***	2.72±0.04***
IV Test 1	MEGP 200mg/kg	196.5±0.72**	3.28±0.01*
V Test 2	MEGP 400mg/kg	168.72±1.72***	2.85±0.03***

Values are mean ± SEM, n=6, value ***p< 0.001, **p< 0.01 and *p< 0.05 vs. Toxic control.

Effect of MEGP on serum lipid profile in HFD and Triton induced Hyperlipidemia in rats

Triton and high cholesterol treated group showed significance increase (P<0.0001) the levels of TC, TG, LDL & VLDL compared to normal group. Post-treatment with extract of *Glycosmis pentaphylla* at different doses (200mg/kg, 400mg/kg), significantly (P<0.001) prevented the elevation of these parameters when compared to hyperlipidemia rats.

Effect on TC, TG, LDL & VLDL

Post-treatment with 200mg/kg of extract reduced the serum TC, TG, LDL & VLDL significantly (P<0.001), but at doses 400mg/kg it reduces significantly (P<0.0001) in HFD and in Triton model at dose 200 and 400mg/kg it reduces serum total cholesterol significantly (P<0.0001) and standard group Atorvastatin showed significant reduction in total cholesterol in HFD and in Triton model when compared to hyperlipidemic rats.

Effect on HDL

Post-treatment with 200mg/kg of and 400mg/kg it increases significantly serum HDL (P<0.0001) in HFD and in Triton model at dose 200 and 400mg/kg it increases serum HDL significantly (P<0.0001) and standard group Atorvastatin showed significant increase in HDL in HFD and in Triton model when compared to hyperlipidemic rats.

Table 3

Effect of MEGP on serum lipid profile in HFD induced Hyperlipidem

Group No	Treatment	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
I Normal Control	Normal saline	126.7±2.12	87.87±1.65	43.33±0.82	65.81±2.56	17.57±0.33
II Toxic Control	HFD	220.2±3.82	128.2±2.09	28.27±1.32	166.3±4.44	25.64±0.041
III STD	Atorvastatin 10 mg/kg and HFD	178.8±2.66***	104.5±1.8***	41.26±1.85***	116.4±2.13**	20.90±0.35***
IV Test 1	MEGP 200mg/kg	186.7±1.99*	107.0±2.80**	49.84±2.09**	129.0±2.23*	25.38±0.57**
V Test 2	MEGP 4 00mg/kg	180±2.66***	105.1±3.20***	46.56±1.40***	118.1±2.12**	22.82±0.64***

Values are mean ± SEM, n=6, value ***p< 0.001, **p< 0.01 and *p< 0.05 vs. Toxic control.

Table 4

Effect of MEGP on serum lipid profile in Triton-X-100 induced Hyperlipidemia in rats

Groups no	Treatment	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
I Normal control	Normal saline	106.2±0.60	84.03±1.34	45.17±1.14	65.91±2.49	16.9±0.27
II Toxic control	Triton-X-100	194.4±1.04	138.7±1.75	25.77±1.12	167±4.5	28.6±0.35
III std	Atorvastatin 10 mg/kg and Triton-X-100	125.9 ±1.12**	100.2±0.67***	41.26±0.82***	116.6±2.13***	18.7±0.42**
IV Test 1	MEGP 200mg	165.1±1.04*	128±1.11*	33.67±0.51**	129±2.30***	25.38±0.55**
V Test 2	MEGP 400mg	131.9±1.31**	104.5±0.91***	39.06±0.76***	118±2.14***	20.9±0.41**

Values are mean ± SEM, n=6, value ***p< 0.001, **p< 0.01 and *p< 0.05 vs. Toxic control.

DISCUSSION

The objective of the present study is to evaluate antihyperlipidemic action of MEGP on triton and high cholesterol diet induced hyperlipidemia in male albino wistar rats. Development of atherosclerotic disease is a complicated process involving accumulation of lipid-containing particles in the walls of coronary arteries & other major arteries within the body. A high-fat diet causes increased cholesterol levels in people, which leads to obesity. High cholesterol diet increased serum cholesterol and LDL-C level significantly A rise in LDL may leads to deposition of cholesterol in arteries and aorta and hence it is a direct risk factor for coronary heart disease. Studies show that both LDL and VLDL have a positive role in atherogenesis. HDL is synthesized mainly in intestine and liver. HDL is considered to be a beneficial lipoprotein as it has an inhibitory effect to the pathogenesis of atherosclerosis. Low level of HDL is associated with high risk of coronary artery disease. The systemic administration of the surfactant triton to rats leads to elevation of serum cholesterol, triglyceride after the 24 hours and there are elevated results were compared with earlier reports. The extract of MEGP tested in the present study significantly prevented hyperlipidemia. Triton and high cholesterol diet induced hyperlipidemia is associated with increased levels of lipids in the serum. Increased levels of total cholesterol, TGs, HDL, LDL and VLDL in the Triton and high cholesterol treated group indicate that Triton and high cholesterol may interfere with metabolism or biosynthesis of lipids. Phytochemical investigation has shown the presence of saponins and phenolic compounds in the Methanolic extract. Observed hypolipidemic activity of all the doses could be attributed to the saponins since saponins have been reported to possess hypolipidemic activity. Phenolic compounds might also contribute to the observed effect as such ingredients from *Anethum graveolens* are reported to reduce TC, TG and TP. The results presented in this study indicated that the Methanolic extract of *Glycosmis pentaphylla* leaves at doses of 200mg, & 400mg, post treatment decreases the Triton and high cholesterol diet induced increased levels of total cholesterol, TGs, HDL, LDL and

VLDL. Among these two doses 400mg, decrease the lipid parameters more significantly than the 200mg/kg, hence the higher dose is more protective than the lower dose. Lowering high cholesterol levels significantly reduce the risk of heart attacks, strokes, and death. Normally hepatocyte stimulates the synthesis of triglycerides and cholesterol during states of increased free fatty acid flux to the liver (e.g., after the fatty meal or in the situation of increased lipolysis) but due to anti-hyperlipidemic drug, there may be inability of hepatocytes to increase cholesterol synthesis and decrease hepatocyte cholesterol concentration by increases the catabolic conversion of cholesterol to bile acids in liver. It is difficult to establish mechanism of action from the present study so further study is much more needed to find out phytoconstituents which are responsible for the observed antihyperlipidemic activity. In Conclusion the effect of MEGP leaves showed dose dependent antihyperlipidemic action against triton and cholesterol diet induced hyperlipidemia. The overall antihyperlipidemic action of methnolic extract of MEGP leaves is probably due to its antioxidant, and free radical scavenging activity. Pretreatment with MEGP leaves at doses 200mg and 400mg/kg significantly restored all the biomarker enzymes in serum induced by triton and high diet cholesterol. It is difficult to establish the exact mechanism of action observed for the antihyperlipidemic action of *Glycosmis pentaphylla*. Further isolation and characterization and purification of active constituents and further experimentation needed to elucidate exact mechanism of action of *Glycosmis pentaphylla* leaves. Hence it could be an alternate therapy in the treatment of oxidative stress induced by antihyperlipidemia.

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