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ANTI HYPERLIPIDEMIC ACTIVITY OF METHANOL EXTRACT OF *DESMOSTACHYA BIPINNATA* ON TRITON X 100 AND HIGH FAT DIET INDUCED HYPERLIPIDEMIA IN MALE WISTAR RATS

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ABSTRACT

To investigate the anti Hyperlipidemic activity of methanol extract of *Desmostachya bipinnata* on Triton x 100 and High Fat Diet induced Hyperlipidemia in male Wistar rats. In this model of Hyperlipidemia, 30 adult male wistar rats (150-200gms) were evenly divided into 5 groups in both groups. Group-1 and Group-2 served as untreated and model controls respectively, while Group-3, 4 and 5 were the treatments groups which were simultaneously treated with standard, 200 and 400 mg/kg extract respectively along with High Fat Diet and Triton x 100. On last day, blood samples for biochemical parameters, were obtained under inhaled diether anaesthesia. HFD and Triton x 100 treatment caused Hyperlipidemia as evidenced by marked elevation in Cholesterol, Triglycerides, LDL, VLDL and decrease in HDL levels. Co-administration of extract with HFD and Triton x 100 decreased rise Cholesterol, Triglycerides, LDL, VLDL and increase in HDL levels. It was observed that the methanol extract of *Desmostachya bipinnata* conferred Anti- Hyperlipidemia activity by biochemical observation against HFD and Triton-x-100 induced Hyperlipidemia in rats. In the near future could constitute a lead to discovery of a novel drug for treatment of drug induced Hyperlipidemia

Key words: *Desmostachya bipinnata,* Anti- Hyperlipidemia activity, methanol extract

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INTRODUCTION

Hyperlipidemia is a condition when abnormally high levels of lipids i.e. the fatty substances are found in the blood. This condition is also called hypercholesterolemia or hyperlipoproteinemia. Human body is complex machinery and for Maintaining the homeostasis various organ and organ system contributes. Any undesirable change (if occurs) will disturb the balance resulting in diseased state. The same way, the change in the level of lipids (They are naturally occurring molecules, which are main component of cell membrane. They acts as blood transporter and their main function is energy storage) will lead to many complications. Virchow in 19th century who identified cholesterol crystals in atherosclerotic lesion and stated that endothelial cell injury initiates atherogenesis (1).In a modification of this hypothesis, it was proposed that the endothelium normally influences the behaviour of arterial smooth muscle cells by providing a barrier to the passage of

plasma proteins, and that the major effect of hemodynamic or other factors that injure the endothelium is to reduce the effectiveness of the barrier2. But now it is clear that higher lipids level, especially cholesterol and triglycerides leads to hyperlipidemia which ultimate speeds up the process of atherosclerosis and disease associated with it (2) Arteries are normally smooth and unobstructed on the inside, but in case of increased lipid level, a sticky substance called plaque is formed inside the walls of arteries. This leads to reduced blood flow, leading to stiffening and narrowing of the arteries (3). Decisions about treatment of elevated lipids currently focus on the LDL cholesterol level as well as the cardiovascular risk status of the individual. In 1991, the National Cholesterol Education Program (NCEP) of the National Heart Blood and Lung Institute (NHLBI) proposed criteria for "acceptable," "borderline," and "high" levels of LDL and TC in children and adolescents These criteria have been adopted as the basis for policy statements and treatment guidelines by many organizations, including the American Academy of Pediatrics (AAP), the American Heart Association (AHA), and the American Medical Association's (AMA) Guidelines for Adolescent Preventive Services. In adults, LDL is strongly associated with a higher risk, and HDL is associated with a lower risk, of coronary heart disease (CHD). Lowering lipids through dietary or pharmacological therapy has been shown to decrease the incidence of atherosclerotic events. Since lipid levels have been observed to track into adulthood, adolescents with hyperlipidemia are also at greater CHD risk. The extent of abnormal lipids and other cardiovascular risk factors during childhood and adolescence is related to the severity of atherosclerosis seen in autopsies of young adults. Hypercholesterolemia is a metabolic condition that determines the onset of chronic degenerative diseases such as atherosclerosis. The formation of initial lesions appears to originate, more often, from the focal increase in lipoprotein content within regions of the intima, due not only to changes in the permeability of the overlying endothelium, but mainly because they bind to constituents of the cellular matrix, increa¬sing the residence time of lipid-rich particles within the arterial wall. In the extracellular space of the intima, lipoproteins may undergo changes and evidence points to a pathogenic role for such modifications. Hypercholesterolemia, therefore, is an important risk factor for cardiovascular diseases and reduction of plasma cholesterol and endothelial protection become important steps for the control of atherosclerotic di¬sease and its complications such as acute myocardial infarction and systemic hypertension (4-6).

Searching the safe and potent remedies from the herbal origin for the treatment of hyperlipidemia and related cardiovascular disorders has become most fascinating and desired area of research for the pharmacologists. Literature review showed that some of the medicinal plants have been scientifically investigated and reported against various disorders. Ochna obtusata is extensively used in ayurveda for variety of condition. However its antihyperlipidemic activity has not been investigated scientifically so far. Keeping in view of pathophysiological complications of hyperlipidaemia and therapeutic efficacy of herbal medicines, the plant Ochna obtusata has been evaluated for antihyperlipidemic activity.

MATERIALS AND METHODS

Collection and authentication

The plant was collected during the march 2014 from Sri Venkateshwara University Tirupati, India. The plant was authenticated by Dr.Madhava Chetty, Department of Botany and voucher specimen of the plant were preserved at institute herbarium library.

Preparation of extracts

The shade dried and powdered leaves of A. nervosa were extracted with ethanol by using Soxhlet extractor. The extract was filtered and then solvent was evaporated under reduced pressure to a solvent free concentrated mass, which was then stored in air-tight container in a cool and dry condition.

Chemicals

Triton X-100(a non-ionic detergent, iso octyl polyoxy ethylene phenol, formaldehyde polymer) was obtained from Technicho lab chemicals, Coimbatore. Atorvastatin was obtained from Moral labs, Chennai. All other chemicals were of analytical grade and obtained locally.

Experimental Animals

Wistar albino adult male rats weighing 200-250g were obtained from the animal house. The animal were

grouped and housed in polyacrylic cages (38x 23x 10 cm) with not more than five animals per cage and maintained under standard laboratory under standard laboratory conditions (temperature 25+2oC) with dark and light cycle (14/10 hour). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water ad libitum. The mice were acclimatized to laboratory condition for 10 days before commencement of experiment. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) constituted under CPCSEA.

Acute Toxicity Studies

The acute oral toxicity study of the extract was carried out by using wistar rats of either sex weighing between 150-200 g as per revised OECD (Organisation for Economic Cooperation and Development) Guidelines 423. The ethanol extract of whole aerial part from *Desmostachya bipinnata* was administered orally to overnight fasted animals at the dose of 250 mg/kg, 500 mg/kg, 1000 mg/kg and 3000 mg/kg of body weight. After administration of the extracts, the animals were observed continuously for the first two hours, for any toxic manifestation. Thereafter, observations were made at regular intervals for 48 h. Further the animals were under investigation up to a period of 2 week for mortality and general behaviour

Anti hyperlipidemic studies

Induction of Hyperlipidemia by Triton-x-100

Hyperlipidemia was induced in Wistar albino rats by single intraperitoneal injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 h (7).The animals were divided into five groups of six rats each. The first group was given standard pellet diet, water and orally administered with 2% Tween 80. The second group was given a single dose of triton administered at a dose of 100mg/kg, i.p. After 72 hours of triton injection, this group received a daily dose of 2% Tween 80 (p.o) for 7 days. The third group was administered a daily dose of Atorvastatin 10 mg/day Fourth group *Desmostachya bipinnata* 200mg/kg suspended in 2% Tween 80, p.o., for 7 days, after inducing hyperlipidemia. Fifth group was administered with the *Desmostachya bipinnata* 400 mg/kg, p.o. for 7 days (8).

Collection of blood

On the 8thday, blood was collected by retro orbital sinus puncture, under mild ether anaesthesia. The collected samples were centrifuged for 10 minutes. Then serum samples were collected and used for various biochemical experiments. The animals were then sacrificed and the liver collected (9).

Biochemical analysis

The serum and liver extract were assayed for total cholesterol, triglycerides, phospholipids, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) using standard protocol methods (10).

RESULTS AND DISCUSSION

Phytochemical screening of the extract shows the presence of chemical constituents like Alkaloids, steroids, fixed oils, cardio tonic aglycones, flavonoids ,saponins ,carbohydrates, proteins, resins. The presence of the steroids reduces the absorption of cholesterol and decreases the cholesterol concentration. Secondary metabolite like the flavonoids, saponins, reduces the cholesterol levels. Saponins will act as anti hyperlipidaemics by binding with the cholesterol and is readily absorbed by the bile acids causing the reduction in extra hepatic circulation and increases the metabolism of cholesterol to sterols through the fecal excretion. Saponins will as reported to increase the lipoprotein lipase activity and helps in the faster removal of free fatty acids from circulation causes decrease in fatal cholesterol.

According to the OECD guide linesno.423 toxicity studies were performed on the mice upto the dose levels of 2000 mg/kg, no death of the Rats observed .So the LD50was found to be 2000mg/kg. ED50 was 1/10 th of LD50 value. So, ED50 = 2000/10 = 200 mg/kg and 2000/5= 400 mg/kg.

Elevated cholesterol levels will promotes the atherosclerosis. High cholesterol levels are associated with the increased incidence of coronary heart diseases. Reduction in the cholesterol and the HDL concentration significantly reduces the cholesterol levels. Atorvastatin is a member of the drug class of statins, it is the first specific inhibitor used for lowering cholesterol (hypo-lipidemic agent) in those with hyper-cholesterolemia and so preventing cardio vascular disease.It is a naturally occuring drug found in food such as oyster mushrooms and red yeast rice.It

reduces the levels of "bad" cholesterol (LDL) and Triglycerides in the blood, while increasing levels of "good" cholesterol (HDL). It is an inhibitor of 3-hydroxy-3 methyl glutaryl-CoA reductase (HMG-CoA reductase), an enzyme that catalyses the conversion of HMG-CoA to mevalonate.Mevalonate is a required building block for cholesterol biosynthesis and Atorvastatin interferes with its production by acting as a reversible competitive inhibitor foe HMG-CoA,which binds to the HMG-CoA reductase. It works by slowing the production of cholesterol in the body. Buildup of cholesterol and fats along the walls of the blood vessels (A process known as Atherosclerosis) decreases blood flow and therefore, the oxygen supply to the heart, brain and other parts of the body. Lowering blood levels of cholesterol and fats may help to decrease the risk of heart disease, Angina (chest pain), strokes and Heart attacks. In addition to taking a cholesterol-lowering medication, making certain changes in our daily habits can also lower the blood cholesterol levels.

Effect of different extracts of *Desmostachya bipinnata* on serum lipid profile and Atherogenic Index, % protection

The serum level of triglycerides and cholesterol and it can be seen that the HFD group and Triton-x-100 show significant hyperlipidemia when compared with the normal control group. The extract treated groups and the standard treated group significantly decreased the serum levels of cholesterol and triglycerides when compared with the HFD control group and Triton-x-100 ($p<0.05$). The effect of ethanol extract on serum lipid levels was as better that of the standard treated group, showing the hypolipidemic potential of the plant. An increase of HDL-cholesterol level was also observed. Decrease in glucose levels are observed in methanolic extract compared to HFD control group($p<0.001$). Both 200 and 400 mg/kg body wt. of *Desmostachya bipinnata* treated animals and 10 mg/kg body wt of Atorvastain treated animals in both models showed decrease in the atherogenic index and increased percentage of protection (Fig-1-5).

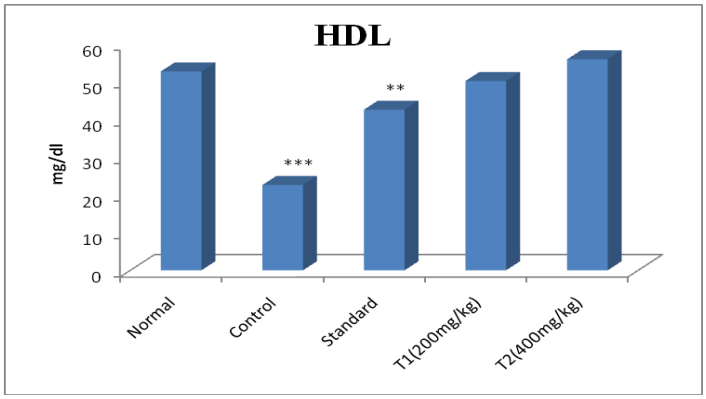

Fig-1 Histogram showing the effect of *Desmostachya bipinnata* on HDL of animals

N = 6; Significance: *** $P<0.001$, ** $P<0.01$, * $P<0.05$ from control

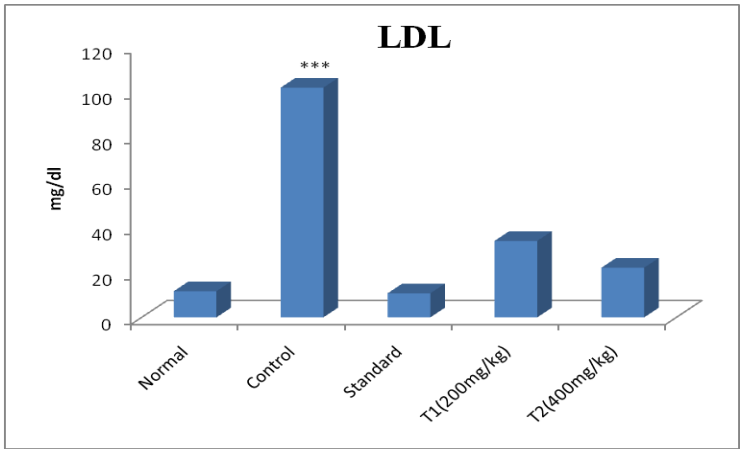

Fig-2 Histogram showing the effect of *Desmostachya bipinnata* on LDL of animals

N = 6; Significance: *** $P<0.001$, ** $P<0.01$, * $P<0.05$ from control

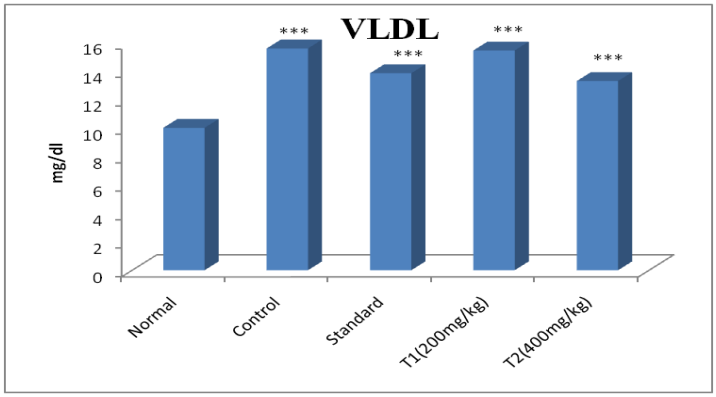

Fig-3 Histogram showing the effect of Desmostachya bipinnata on VLDL of animals

N = 6; Significance: *** $P<0.001$, ** $P<0.01$, * $P<0.05$ from control

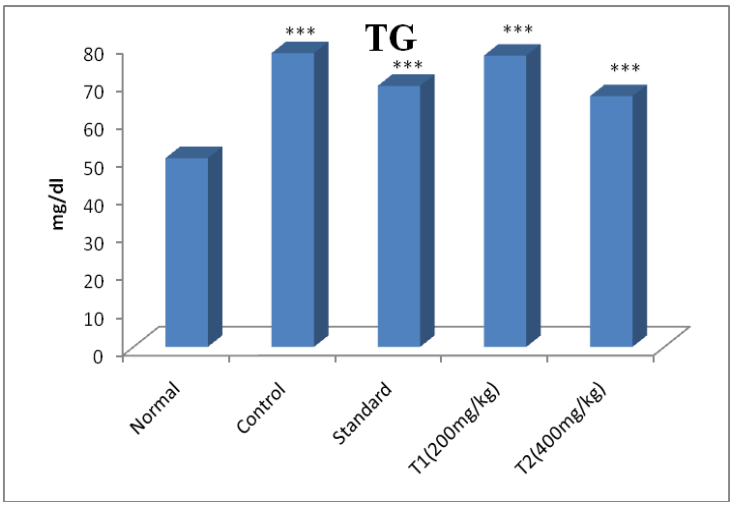

Fig-4 Histogram showing the effect of *Desmostachya bipinnata* Triglycerides of animals

N = 6; Significance: *** *P*<0.001, ** *P*<0.01, * *P*<0.05 from control

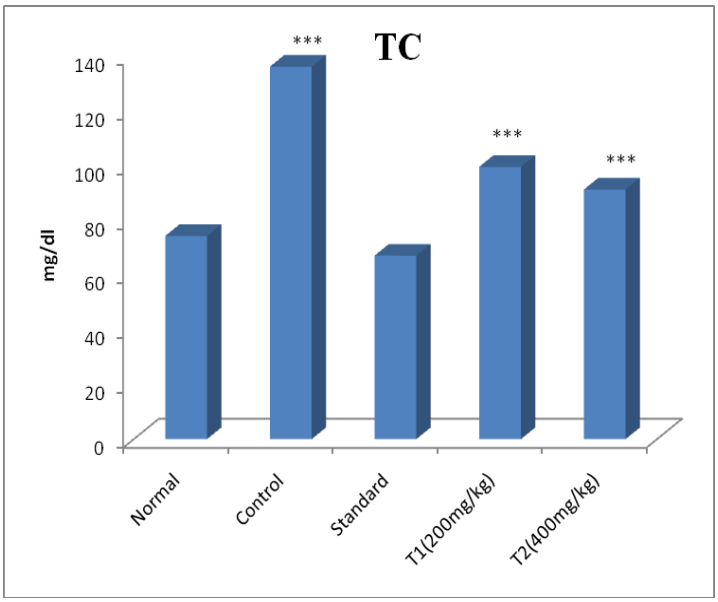

Fig-5 Histogram showing the effect of *Desmostachya bipinnata* on Total Cholesterol of animals

N = 6; Significance: *** *P*<0.001, ** *P*<0.01, * *P*<0.05 from control

Effect of different extracts of *Desmostachya bipinnata* on Total protein profile

The serum level of total protein and it can be seen that the Triton-x-100 group shows significant decrease in total protein levels when compared with the normal control group. The extract treated groups and the standard treated group significantly increased the serum levels of total protein when compared with the Triton-x-100 control group (p<0.001). The effect of methanol extract on levels was better as that of the standard treated group, showing the hypolipidemic potential of the plant (Fig-6).

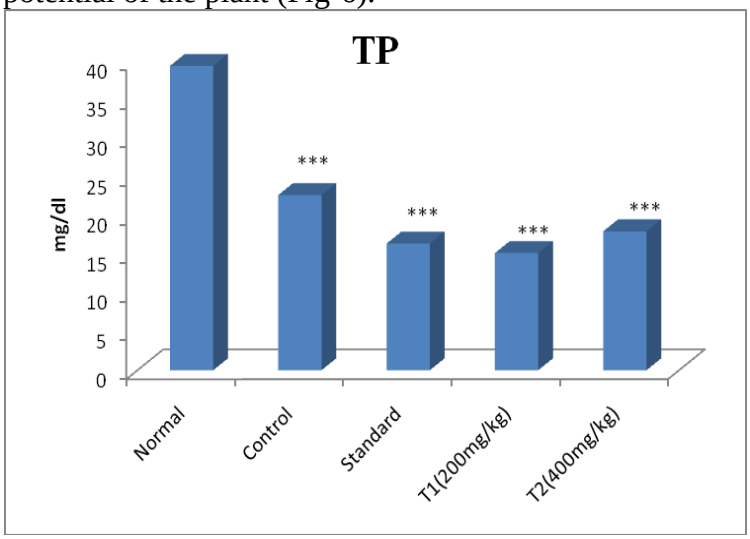

Fig-6 Histogram showing the effect of *Desmostachya bipinnata* on Total protein of animals

N = 6; Significance: *** *P*<0.001, ** *P*<0.01, * *P*<0.05 from control

Effect of different extracts of *Desmostachya bipinnata* on SGOT, SGPT and ALP levels

AST, ALT, SGOT, SGPT, and GGT and Alkaline Phosphatase are abbreviations for proteins called enzymes which help all the chemical activities within cells to take place. Injury to cells releases these enzymes into the blood. They are found in muscles, the liver and heart. Damage from alcohol and a number of diseases are reflected in high values. AST/SGOT, ALT/ SGPT are also liver and muscle enzymes. They may be elevated from liver problems, hepatitis, excess alcohol ingestion, muscle injury and recent heart attack. [100] An atherogenic diet has been reported to induce glomerulosclerosis/nephropathy and mild tubular and hepatic damage experimental rats [101] In case of the effect of methanol extract on enzymes (SGOT, SGPT and ALP), the extract shows significantly lower levels of SGOT, SGPT and ALP in comparison to Triton-x-100 control group (p<0.05). Here the maximum reduction was observed for

standard followed by methanolic extract (Table-1).

Table-1 Effect of different extracts of *Desmostachya bipinnata* on SGOT, SGPT and ALP levels

Triton x-100					
TEST	**NORMAL**	**CONTROL**	**STANDARD**	**T1**	**T2**
ALP	76.85±2.707	180.41±2.23***	113.83±1.54***	135.76±0.81***	82.3±1.573
GPT	34.34±2.286	57.90±2.504***	37.08±2.741	50.79±1.385***	45.83±2.893**
GOT	42.49±4.388	54.80±2.975*	42.78±3.262	41.27±1.520	45.12±3.061

CONCLUSION

Phytochemical screening of the extract shows the presence of chemical constituents like Alkaloids, steroids, fixed oils, cardio tonic aglycones, flavonoids, saponins, carbohydrates, proteins, resins. Acute toxicity tests were performed according to the OECD guide line no.423, LD50 value was found to be 200mg/kg and 400mg/kg. Anti Hyperlipidaemic activity was performed by using the high fat diet and Triton-x-100 induced method. In the present study an increase in plasma HDL-cholesterol with a concomitant percentage decrease from other lipid was observed. It can be concluded from the present data that the levels of total serum cholesterol, triglyceride and MDA which are actually raised in high fat diet, can be lowered significantly with Desmostachya bipinnata Dc. And total proteins which is actually lowered in Triton-x-100 can be raised significantly with *Desmostachya bipinnata* Dc and antioxidant parameters SOD, GSH, Catalase which which are actually lowered in high fat diet can be raised significantly with *Desmostachya bipinnata* Dc. Atherogenic index which actually raised in atherogenic diet and Triton-x-100, can be lowered significantly with Desmostachya bipinnata Dc and a very good % protection was seen with Desmostachya bipinnata and standard drug. From this we can conclude that the extract (*Desmostachya bipinnata* Dc.) showed the anti Hyperlipidaemic activity.

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