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EVALUATION OF HYPOLIPIDEMIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *MIMUSOPS ELENGI* LEAVES AGAINST TRITON WR-1339 INDUCED HYPERLIPIDEMIA IN ALBINO RATS

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ABSTRACT

Experimental evidences are suggesting that the plasma Hypercholesteromic state is a main contributor of the devolopement of cardiovascular diseases like atherosclerosis. The plant Mimusops elengi is used in traditional medical practices of India to treat many diseases. The hydroalcoholic extract of the plant when tested for antihyperlipidemic potential, exhibited a dose dependent activity in albino rats when compared to standard drugs. *Mimusops elengi* leaf extract has not shown any side effects during acute toxicity studies. Hyperlipidemia was developed by I/P injection of triton WR-1339 200mg/kg. The animals are divided into normal, Triton treated group, Triton+Rosuvastatin, Triton+Herb extract (200mg/kg and 400mg/kg). Mimusops elengi leaf extract found to produce hypolipidemic potential by significant reduction in cholesterol, triglycerides, LDL, VLDL and also increases the HDL levels at reduced doses. The plant also showed antioxidant potential by increasing SOD, catalase, GSH and decreasing lipidperoxidation.

Key words: *Mimusops elengi*, Hypolipidemic activity, Oxidative Stress.

INTRODUCTION

The benificial effect of lowering Hypercholesterolemia in the prevention of coronary heart diseases is well established (Simons LA, 2002). In many developing countries, most Hyperlipidemic individuals use medicinal plants as folk medicine to treat hyperlipidemia (Hicham Harnati *et al*., 2007). The use of traditional medicine of medicinal plants in most developing countries, as a normative basis for the maintenance of good health has been widely observed. Moreover, in these societies herbel remedies have become more popular in the treatment of minor ailments, and also on account of the increasing costs of personal health maintenance (Edgar J Dasilva, 1999). *Mimusops elengi* Lin [Syn:Pogadam Family : Sapotaceae]. It is a medium sized evergreen tree found in tropical regions. It is used in traditional medicine for many of its uses.

Earlier studies on this plant showed its protective activity on lipidperoxidation and antoxidant enzymes in experimental diabetic rats (Shaik J *et al*., 2011). It is evaluated for its anthelmintic potential (Diener W *et al*., 1995). The Present Study was undertaken to elucidate the hypolipidemic and antoxidant potential of *Mimusops elengi* leaves in albino rats.

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MATERIALS AND METHODS

Plant Materials

The plant material was collected in and around Tirupati and Tirumala hills. The Plant material was

athentified by Dept of Dravya guna, S.V. Ayurvedic College, Tirupati.

Preparation of plant extract

Shade dried and coarsely powdered plant (350 gm) was subjected to cold maceration process using hydroalcohol (70:30) as solvent. The extraction was continued for 5 days at room temperature with occasional shaking, filtered, collected and concentrated at 40^0C on a heating mantle until a softy mass obtained. It was air dried to remove all the traces of solvent.

Animals

Healthy albino rats of wister strain of either sex weighing about 150-200g used for the study were purchased from Raghavendra enterprises: Bengaluru. The animals were caged individually and kept in air conditioned room at a temperature of 22 $\pm2^0$C with 50% ±10% relative humidity with 12hr light and dark cycle. Animals were maintained at standard rat pellet diet and drinking water ad libitum. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC No: 1016/A/06/CPSCEA/014/2013) and all the experiments were carried out according to guidelines of committee for the purpose of control and supervision of experiments on animals, India.

Acute toxicity Studies

Acute oral toxicity was performed as per OECD-423 gudelines. Four groups of rats each containing 2 animals were fed with four different doses (50mg / kg, 300mg/kg,1000mg/kg and 2000mg/kg P.O) of HAME and continuously observed for 24 hours to detect behavioural, neurological and autonomic parameters. No toxicity signs were produced with the four doses (Mishra G *et al*., 1968).

Triton model of Hyperlipidemia

Triton WR-1339 (Tyloxapol, sigma-Aldrich, USA) was dissolved in normal saline (PH 7.4) and administerd intraperitoneally to the rats (200mg/kg B.W) in order to develop an acute hyperlipidemia in them.

Experimental design for Anti hyperlipidemic activity

The experiment was conducted for 8days. Rats were divided into 5 groups each consisting of six animals. The first group received only the vehicle, i.e., normal saline, Second group was administered vehicle up to 7 days + Triton WR-1339 (200mg/kg I.P) dissolved in normal saline on 8^{th} day. Third group was given Rosuvastatin (1mg/kg) dissolved in normal saline for 7days + Triton WR-1339 (200mg/kg ip) on 8^{th} day. Fourth group was given HAME (200mg/kg B.W) P.O for 7days + Triton WR-1339 (200mg/kg I.P) on 8^{th} day. Fifth group was given HAME (400mg/kg B.W) Triton WR-1339 (200mg/kg I.P) in normal saline on 8th day.

Blood samples were withdrawn from the retroorbitalvenous plexus of rats for the separation of serum at the end of treatment schedule. After collecting the blood in the micro-centrifuge tubes, kept aside for 20min at room temp and then serum was isolated by centrifugation at 2500 rpm for 15min and stored until analysed for biochemical estimation of lipid parameters.

Instruments

Absorbance was recorded in analytical UV-visible spectrophotometers.

Biochemical studies

The lipid profile such as total serum cholesterol, triglycerides, HDL cholesterol, LDL, VLDL, atherogenic index, LDL/HDL ratio were studied, Liver homogenate was tested for SOD, CAT, GSH & Lipid peroxidation. Histopathological study of liver was carried out. On 8^{th} day, at the end of treatment schedule rats were sacrificed, liver was fixed in 10% formalin then embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin. Examined under light microscope and photographs were taken.

Statistical analysis

All the data was expressed as mean ± SEM. Statistical significance between more than two groups were tested using two way ANOVA Followed by Bonferroni multiple comparison test using computer based fitting program (Prism graph pad). Statistical significance was set accordingly.

Table 1. Effect of HAME leaves on Serum Total Cholesterol, Triglyceride and HDL-Cholesterol levels (mg/dl)

S. No	Group	Total Cholesterol(mg/dl)	Triglyceride(mg/dl)	HDL-Cholesterol(mg/dl)
1	Normal	116.25± 6.16	97.76±17.03	36.65±0.97
2	Triton control	156.77±2.93*	164.13±3.24*	25.60±1.19*
3	Triton + Rosuvastatin	132.61±3.55**	111.57±12.26**	31.49±1.71**
4	Triton + HAME(200mg/kg)	134.54±2.14**	112.99±9.67**	28.67±0.48**
5	Triton+HAME	132.73±1.38**	112.86±5.1**	34.22±2.61**

	(400mg/kg)			

All the Values were expressed as mean ± SEM. N = 6
*=p < 0.001, when compared to the normal
**= p < 0.001, when compared to the control

Table 2. Effect of HAME leaves on Serum LDL-cholesterol and VLDL- cholesterol (mg/dl)

S. No	Group	LDL-Cholesterol(mg/dl)	VLDL- Cholesterol (mg/dl)
1	Normal	63.76±10.22	19.17±3.50
2	Triton control	100.04±1.72*	32.82±0.64*
3	Triton + Rosuvastatin	79.72±5.64**	22.31±2.45**
4	Triton + HAME(200mg/kg)	87.78±1.24**	22.59±1.93**
5	Triton+HAME (400mg/kg)	77.60±5.57**	20.12±0.87**

All the Values were expressed as mean ± SEM. N = 6
*=p < 0.001, when compared to the normal
**= p < 0.001, when compared to the control

Table 3. Effect of HAME leaves on Serum LDL/HDL Ratio and Atherogenic index

S. No	Group	LDL/HDL Ratio (mg/dl)	Atherogenic Index
1	Normal	1.73±0.29	2.30±0.20
2	Triton control	3.91±0.13*	5.23±0.19*
3	Triton + Rosuvastatin	2.53±0.16**	3.20±0.10**
4	Triton + HAME(200mg/kg)	3.06±0.08**	3.75±0.12**
5	Triton+HAME (400mg/kg)	2.30±0.29**	3.22±0.32**

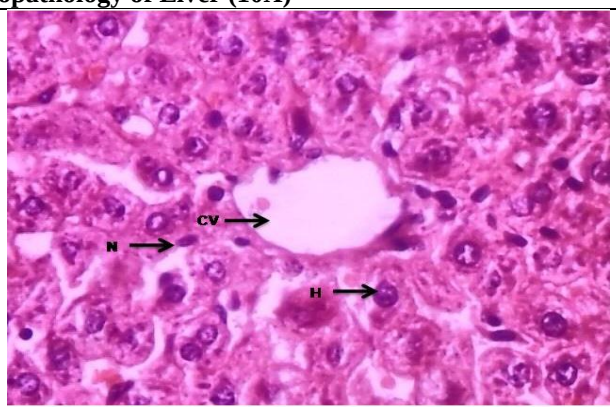
All the Values were expressed as mean ± SEM. N = 6
*=p < 0.001, when compared to the normal
**= p < 0.001, when compared to the control

Table 4. Effect of HAME on *Invivo* Anti-oxidant parameters

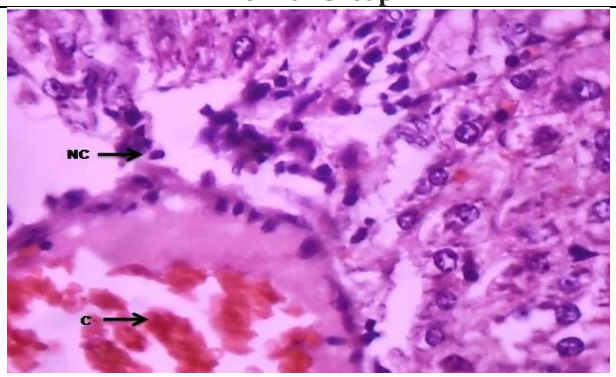
S. No	Group (n=6)	*Invivo* Anti-oxidant parameters			
		SOD (Units/mg)	Catalase (µm of H_20_2 /min/mg)	GSH (µg of GSH/mg of protein	LPO (nm of MDA/mg of protein)
1	Normal	4.23±2.66	2.17± 11.79	4.99 ±3.38	1.60±0.07
2	Triton Control	1.80 ±0.77a	1.18 ±3.05a	1.82±9.29a	3.22± 0.08*
3	Triton + Rosuvastatin	3.40± 0.78b	2.01±9.59b	3.93 ±4.23b	1.30± 0.03**
4	Triton + HAME (200mg/kg)	3.74± 0.52b	2.09 ±10.13b	3.48 ±7.36b	1.20 ±0.01**
5	Triton + HAME (400mg/kg)	3.80±0.37b	2.11±3.21b	3.33±0.78b	1.18±0.01**

All the Values were expressed as mean ± SEM. N = 6.
a = p < 0.05, when compared to the normal
*p < 0.0001, when compared to the normal
b= p< 0.05, when compared to the control
** p < 0.0001, when compared to the control

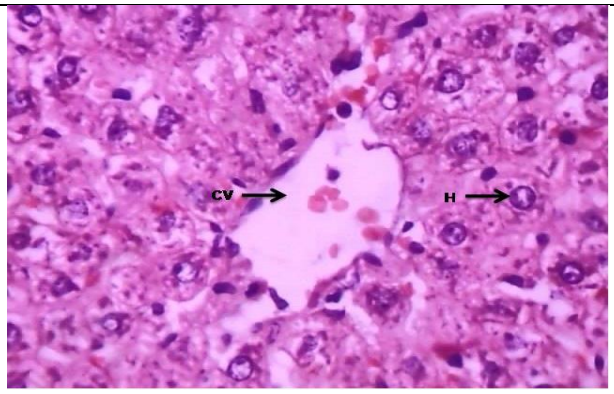
Fig. 1. Effect of HAME on histopathology of Liver (10X)



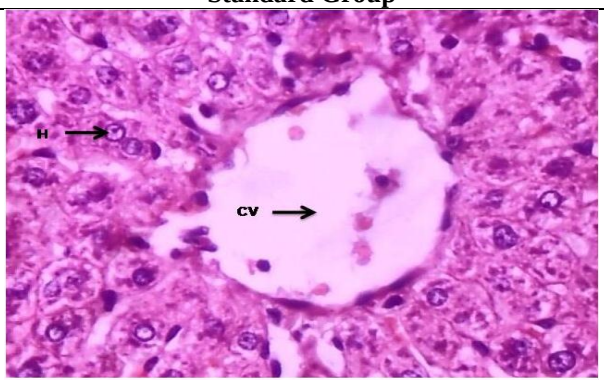
Normal Group



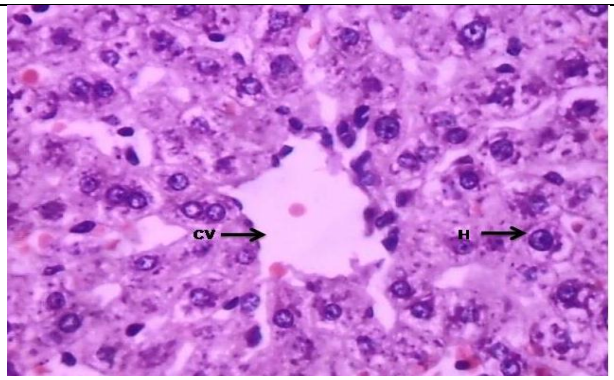
Disease Control



Standard Group



Test 200mg/kg



Test 400 mg/kg

RESULTS

Preliminary Phytochemical screening

Phytochemical screening of HAME revealed the presence of alkaloids, steroids, phenols, flavonoids, saponins, tannins, anthraquinones, carbohydrates, proteins, gums, mucilages, terpenes and glycosides (Campillo JE *et al*., 1994).

DISCUSSION & CONCLUSION

Hyperlipidemia is one of the greatest risk factor for number of coronary heart diseases and cerebro vascular diseases (Cignarella A *et al*., 1996). The main causative factor for hyperlipidemia is due to disturbances occurring in lipid metabolism. Though large number of drugs used in the treatment, none of the existing ones available worldwide is fully effective, absolutely safe and free from side effects (Juhan-Vague I and Vague P, 1990). Hence, efforts are being made to find out safe and effective agents that may be beneficial in correcting the lipid metabolism and preventing cardiac diseases. An herbal treatment for hypercholesterolemia has no side effects and is cheap, locally available and are effective in reducing the lipid levels in the system (Kellner A *et al*., 1951).

Among the natural materials, medicinal plants hold promise in the discovery of new drugs. The leaves of *mimusops elengi* showed the presence of saponins, phenolic glycosides, flavonoids, proteins in the Preliminary screening of the drug (Campillo JE *et al*., 1994). The ethanolic extract of the leaves yielded quercitol, hentriacontane, β-Sitosterol and quercetin (Otway S and Robinson DS, 1967). The presence of saponins and flavonoids explains that the plant must have valuable medicinal properties which must be explored to evaluate the plant for hypolipidemic activity.

Triton WR-1339 has been widely used to block clearance of triglyceride rich lipoproteins to induce acute Hyperlipidemia in several animals (Schurr PE *et al*., 1972). This model is widely used for a number of different aims (Sudhakar V *et al*., 2007) and in particular, in rats it has been used for screening natural or chemical hypolipidemic drugs (Valko M *et al*., 2007) because it is convenient in terms of length of treatment period & handling. In this aim, many plants such as vaccinum myrtillus (Moran MS *et al*., 1979) and phyllanthus niruri (Slater TF and Sawyer BC, 1971) have been investigated for their acute hypolipidemic activity in Triton WR-1339 induced hyperlipidemia animals.

Thus, since the proportion of triglycerides in VLDL is several times higher than cholesterol, it is not surprising that the hypolipidemic act as of HAME extract was markedly higher for triglycerides than for cholesterol. This result suggests that the extract is able to restore, at least partially, catabolism of B-lipoproteins. The underlying mechanism of this activity is not elucidated by present study, however as hypothesized by many works with other plants (Fiser RH *et al*., 1974), the restorate as of catabolic metabolism of VLDL could be due to increased stimulation of the lipolytic activity of plasma lipoprotein lipase.

This result suggests that cholesterol lowering activity of the herb extract can be results from the rapid catabolism of LDL cholesterol through its hepatic receptors for final elimination in the form of bile acids as demonstrated by khanna et al. (Khanna AK *et al*., 2002).

Schurr et al. (Schurr PE *et al*., 1972) demonstrated that a parenteral administration of a dose of Triton WR-1339 to adult rats induced Hyperlipidemia. The large increase in cholesterol and triglycerides due to Triton WR-1339 injection results mostly from an increase of VLDL secretion by the liver accompanied by a strong reduction of VLDL and LDL catabolism (Grundy SM and Vega GL, 1987).

HAME and Rosuvastatin showed significant reduction in total cholesterol, Triglycerides, LDL, VLDL, atherogenic index, LDL/HDL ratio.HAME and Rosuvastatin group also increased HDL cholesterol levels but tritonised groups significantly decreased HDL levels. The increased HDL levels may be due to presence of flavonoids, beta carotene and lupeol in the extract.

Flavonoids have antioxidant and free radical scavenging activity as they decrease the oxidative modifications of LDLs.

Saponins acts by binding with cholesterol in intestinal lumen which results in reduction in absorption of cholesterol and steroids also reduce the absorption of cholesterol and increase fecal excretion of cholesterol. Biochemical assay of various lipid profiles revealed that HAME reduced the levels of total lipids, total cholesterol, triglycerides, VLDL, LDL and the activity was observed with 200mg dose.

In addition, increased intracellular generation of reactive oxygen species (ROS) plays a role in chronic inflammatory responses to atherosclerosis (Jana GK *et al*., 2010). A lot of oxygenated compounds such as malondialdehyde (MDA) and conjugated dienes are produced during the attack of free radical to membrane lipoproteins and poly unsaturated fatty acids. The main endogenous antioxidant system include SOD, catalase and glutathione and non enzymatic antioxidants play an important role in alleviating tissue damage due to formation of free radicals. A lot of studies have found that serum MDA levels are higher in animal models of Hyperlipidemia.

In the present study, antioxidant activity of HAME was also studied. Interestingly HAME increased the SOD, catalase and glutathione levels and reduced the lipid peroxidation when compared to Triton control group. The antioxidant activity of HAME may also be responsible for hypolipidemic activity.

On Histopathological examination, Liver tissues from Triton control group showed wide spread ballooning of cells due to deposition of lipids and the treated groups with Rosuvastatin and HAME showed protective effect and decreased lipid accumulation and decreased ballooning of cells (Fig. 1).

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