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Research Article

ANTI-HYPERLIPIDEMIC ACTIVITY OF METHANOLIC EXTRACT OF *SYZYGIUM ALTERNIFOLIUM* BARK AGAINST HIGH-FAT DIET AND DEXAMETHASONE-INDUCED HYPERLIPIDEMIA IN RATS

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ABSTRACT

Objective: The present study is an attempt to investigate its anti-hyperlipidemic activity of methanolic extract of *Syzygium alternifolium* (MESA) using high-fat diet (HFDs) and dexamethasone-induced hyperlipidemia.

Methods: MESA bark was evaluated for anti-hyperlipidemic activity in HFD and dexamethasone-induced hyperlipidemic rats. A comparison was also made between the action of *S. alternifolium* bark extract and a known anti-hyperlipidemic drug atorvastatin (10 mg/kg body weight). The results of the study were expressed as mean ± standard error, and data were analyzed using one-way analysis of variance test followed by Dunnett's t-test for multiple comparisons. Values of $p<0.05$ were considered as significant.

Results: Oral administration of 100, 200 mg/kg body weight of the MESA bark exhibited a significant reduction ($p<0.01$) in serum lipid parameters such as total cholesterol, triglycerides, low-density lipoprotein (LDL), very LDL, and increase in high-density lipoprotein in hyperlipidemic rats of both models as compared to hyperlipidemic control statistically. These extracts were found to possess better anti-hyperlipidemic potential.

Conclusion: Our results demonstrated that MESA bark possessed significant anti-hyperlipidemic activity and hence it could be a potential herbal medicine as an adjuvant with existing therapy for the treatment of hyperlipidemia.

Keywords: *Syzygium alternifolium*, Hyperlipidemia, High-fat diet, Dexamethasone.

INTRODUCTION

In 2002 cardiovascular diseases (CVD's) contributed to approximately a third of entire global deaths, whereas by the year 2020. It is expected that CVD's will become the leading cause of death and disability worldwide [1]. Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis - Associated conditions such as coronary heart diseases (CHD), ischemic cerebrovascular disease, and peripheral vascular diseases [2].

Atherosclerosis referred to as a "silent killer" is one of the leading causes of death in the developed countries and is on the rise in developing countries like India [3]. The American Heart Association has identified the primary risk factor associated with atherosclerosis as elevated levels of cholesterol and triglycerides (TG) in the blood. Therefore, therapists consider the treatment of hyperlipidemia to be one of the major approaches toward decelerating the atherogenic process [4]. Allopathic hypolipidemic drugs are available at large in the market, but the side effects and contraindications of these drugs have masked their popularity. Recently, herbal hypolipidemics have to gain importance to fill the lacunae created by the allopathic drugs [5].

Herbal medicine represents one of the most important fields of traditional medicine. WHO recognized that medicinal plants played an important role in the health care of about 80% of the world population in developing countries and depended largely on traditional medicine [6]. Plant derived products are present in 14 of the 15 therapeutic categories of pharmaceutical preparations that are currently recommended by medicinal practitioners, and they form an important part of the health care system in the western world [7]. It is estimated that about 75% of the 120 biologically active plant-derived compounds, presently in use worldwide, have been derived through follow-up researchers to verify the authenticity of data from folk and ethnomedicinal uses. So, there is a great scope for new drug discoveries based on traditional plant uses [8]. There is a need to establish the pharmacological activities for identifying and comparing the various crude drugs for potency.

Syzygium alternifolium (Wt.) Walp. (Myrtaceae) is an endemic aromatic tree, distributed in Assam and Andhra Pradesh, states of India. Locally, it is known as mogi/movi. The plant parts were used in traditional medicine to cure various diseases *viz.*, tender shoots and fruits for dysentery, seeds for diabetes and stem bark was used to treat gastric ulcers [9]. The anti-hyperlipidemic effects of methanolic extract of *S. alternifolium* (Wt.) Walp (MESA) bark against high-fat diet (HFD) and dexamethasone-induced hyperlipidemia in rats have not been reported so far scientifically. Hence, the present study has been carried out to evaluate the anti-hyperlipidemic effect of MESA against HFD and dexamethasone-induced hyperlipidemia in rats.

METHODS

Plant materials

S. alternifolium (Wt.) Walp plant bark was collected in the month of November 2014 from Sheshachalam hills, Tirupati, Andhra Pradesh, India. The plant was then taxonomically identified and authenticated by the botanist Dr. K. Madhava Chetty, Assistant Professor in S.V. University, Tirupati, India.

Preparation of extract

The bark was dried under shade then coarsely powdered with a mechanical grinder. The powder was passed through a sieve and stored in an airtight container for the extraction, and Extracted with methanol 70% (75-78°C) up to 72 hrs. After completion of extraction, the solvent was removed by distillation. Dark brown residue was obtained. The residue was concentrated and then stored in desiccators [10].

Preliminary phytochemical screening

The extracts were subjected to phytochemical investigation for plant secondary metabolites such as alkaloids, tannins, flavonoids, glycosides,

saponins, carbohydrates, phenolic compounds, fixed oils, terpenoids, and steroids by utilizing standard methods [11].

Experimental animals
Wistar rats (150-180 g) of either sex approximately the same age, procured from listed suppliers of Albino labs, Hyderabad, India were used for the study. They were housed in polypropylene cages and fed with standard rodent pellet diet and water *ad libitum.* The animals were exposed to an alternate cycle of 12 hrs of darkness and 12 hrs of light. All the experimental works with the animals were carried out after obtaining approval from the Institutional Animal Ethics Committee (Reg. No. 1175/ac/08/CPCSEA).

Acute toxicity studies
An acute oral toxicity study was performed as per Organization for Economic Co-operation and Development 423 guidelines (acute toxic class method). Wistar rats (n=6) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose level of 5 mg/kg body weight (b.w.) by intragastric tube and observed for 14 days. If mortality was observed in 2-3 animals, then the same dose was observed in one animal, and then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 50, 300, and 2000 mg/kg b.w. [12].

Experimental design

Anti-hyperlipidemic activity

Anti-hyperlipidemic activity animals were divided into five groups with six animals per group.

Group 1: Normal control.
Group 2: Hyperlipidemic control (Vehicle 1 ml/100g/day p.o).
Group 3: Hyperlipidemic treated with MESA bark (100 mg/kg, b.w./day p.o).
Group 4: Hyperlipidemic treated with MESA bark (200 mg/kg, b.w./day p.o).
Group 5: Hyperlipidemic treated with atorvastatin (10 mg/kg, b.w./day p.o).

The animals were administered with corresponding treatment for 1 month.

Induction of hyperlipidemia

High cholesterol diet was prepared by mixing cholesterol 2%, sodium cholate 1%, and coconut oil 2%, with powdered standard animal food [2,13]. The diet which was prepared as pellets was placed in the cage carefully and was administered for 20 days.

Dexamethasone-induced hyperlipidemia in rats
To induce hyperlipidemia by using dexamethasone, (a glucocorticoid excess is known to evoke plasma lipid elevation) dexamethasone (10 mg/kg/day, s.c) was administered to rats for 8 days to induce hyperlipidemia. The animals were divided into five groups each group contains six rats. After the induction of hyperlipidemia, MESA was given to the rats for 8 days in the dose of 100 mg/kg and 200 mg/kg, on 9th day blood was collected from retro-orbital plexus, after overnight fasting for the study of biochemical parameters.

Group1: Normal control.
Group 2: Dexamethasone control (Vehicle 1 ml/100 g/day p.o).
Group 3: Dexamethasone (10 mg/kg/day, s.c) + MESA bark (100 mg/kg, b.w./day p.o).
Group 4: Dexamethasone (10 mg/kg/day, s.c) + MESA bark (200 mg/kg, b.w./day p.o).
Group 5: Dexamethasone (10 mg/kg/day, s.c) + Atorvastatin (10 mg/kg, b.w./day p.o).

Biochemical assays for lipids
Total cholesterol estimation was done by using the span total cholesterol diagnostic kit. Serum TGs and high-density lipoproteins (HDL) were estimated by span TGs diagnostic kit. Cholesterol, TGs, and HDL profile were estimated using standard monograph. Low-density lipoproteins (LDL) cholesterol was calculated using formula:

LDL = Total cholesterol−HDL−TGs/5

Very LDLs (VLDL) was calculated using the formula:

VLDL=TGs/5.

The liver was fixed in 10% formalin for histopathological studies.

Measurement of coronary disease risk factor
Atherogenic index (AI), which is a measure of the atherogenic potential of an agent, was calculated using the following formula, and the results were tabulated.

AI = LDL-cholesterol/HDL-cholesterol

% Protection = AI of control - AI of treated group × 100 AI of control

***In vivo* antioxidant studies**
Antioxidant studies are performed using determination of superoxide scavenging activity, lipid peroxidation (LPO) assay, glutathione (GSH) assay, and catalase activity [14]. The results were shown in Tables 4 and 5.

Histopathological studies
A portion of liver tissue (liver slices) of normal control, dexamethasone control, cholesterol control, and co-treated groups of rats with atorvastatin and MESA (100 mg/kg) and MESA (200 mg/kg) were stored in containers for 12 hrs in 10% formalin solution and subjected to histopathological studies [15]. Observed microscopically for histopathological changes, i.e., normal liver, damaged and recovered liver was studied and compared. The results were shown in Fig. 1.

Statistical analysis
All the values were expressed as mean ± standard error of mean. The datas were statistically analyzed by one-way analysis of variance followed by Dunnett's t-test, and values $p<0.05$ was considered to be significant [16].

RESULTS

Acute toxicity studies
Administration of *S. alternifolium* bark extracts in the doses of 50, 300, and 2000 mg/kg resulted in no mortalities or evidence of adverse effects implying that *S. alternifolium* is non-toxic. Throughout 14 days of the treatment, no changes in behavioral pattern, clinical signs and body weight of mice in both control and treatment groups were observed. This shows that *S. alternifolium* was safe up to a dose of 2000 mg/kg.

Preliminary phytochemical screening
Preliminary phytochemical screening was done and found the presence of steroids, terpenoids, flavonoids, alkaloids, phenolic compounds, saponins, tanins and carbohydrates. The results were given below in Table 1.

Anti-hyperlipidemic activity
When MESA was evaluated for its anti-hyperlipidemic activity against HFD and dexamethasone-induced hyperlipidemia model, it showed a statistically significant activity in dose of 100 mg/kg and 200 mg/kg by oral administration. After 21 days, HFD and 8 days treatment with dexamethasone, a significant rise in lipid and lipoprotein levels were observed in serum in dexamethasone-induced group, when compared to the normal group. The results were depicted in Tables 2 and 3.

In vivo antioxidant studies

In vivo antioxidant studies showed that superoxide dismutase (SOD), GSH, and catalase levels have increased and LPO levels decreased in the groups treated with extracts. The results were shown in Tables 4 and 5.

Table 1: Phytochemical investigation of *Syzygium alternifolium* (wt.) Walp bark methanolic extract

S. No.	Constituents	Report
1	Carbohydrates	+
2	Steroids	+
3	Alkaloids	+
4	Saponins and flavonoids	++
5	Tannins and phenolic compounds	++

+: Presence of constituents

Histopathology

The histopathological study showed recovery of the damaged liver cells in the drug treated group. The reputed cells of the intoxicated liver were reformed. The degree of vascularization was also reduced as compare to the hyperlipidemic group. Multiple foci of inflammation and necrosis noticed in centrilobular region of liver, Furthermore, infiltration of inflammatory cells noticed in the inflammatory region of liver. In HFD model mild to moderate sinusoidal space dilatation along with hemorrhages noticed in the sinusoidal space of liver and multiple foci of inflammation along with infiltration of inflammatory cells particularly lymphocytes noticed in the centrilobular region of liver. The results were shown in Fig. 1.

DISCUSSION

The present studies were performed to assess the anti-hyperlipidemic activity and to prove its claim in folklore practice against various

Table 2: Effect of MESA bark on HFD induced hyperlipidemia

Groups	Treatment	TC mg/dl	TG mg/dl	LDL mg/dl	VLDL mg/dl	HDL mg/dl	AI mg/dl
I	Control	102.3±1.9	79.8±1.6	37.7±2.3	15.8±0.3	46.83±2.1	1.7±0.06
II	Cholesterol control	219.6±11.1^{a}	185.5±6.9^{a}	162.9±11^{a}	30.3±1.1^{a}	26.33±0.6^{a}	5.9±0.3^{a}
III	MESA 100 mg/kg	131.6±2.7^{a,**,A}	129±1.6^{a,**,B}	63.3±2.8^{b,**,A}	25.8±0.3^{a,**,A}	41.33±0.3^{b,**,A}	1.5±0.1^{a,**,B}
IV	MESA 200 mg/kg	116.6±1.7^{a,**,B}	109±1.54^{a,*,B}	48.7±1.6^{a,**,B}	21.6±0.2^{a,*,B}	43.8±0.9^{b,**,B}	1.1±0.5^{a,**,B}
V	Atorvastatin 10 mg/kg	110.3±3.4^{b,**}	104.1±5.3^{b,**}	46.5±4.0^{b,**}	20.83±1.0^{b,**}	47.5±0.4^{b,**}	0.9±0.1^{b,**}

Values are expressed as mean±SEM, (n=6). All the groups were compared with control group and standard group. All the data were statistically analyzed by one-way ANOVA followed by Dunnett's test, and significant values are expressed as ^{a}p<0.001, ^{b}p<0.05, **p<0.01, *p<0.05, ^{A}: p<0.01, ^{B}: p<0.05. ns: Non-significant. TC: Total cholesterol, TG: Triglyceride, LDL: Low-density lipoprotein, HDL: High-density lipoproteins, AI: Atherogenic index, MESA: Methanolic extract of *Syzygium alternifolium*, HFD: High-fat diet, SEM: Standard error of mean

Table 3: Effect of MESA in dexamethasone-induced hyperlipidemia

Groups	Treatment	TC mg/dl	TG mg/dl	LDL mg/dl	VLDL mg/dl	HDL mg/dl	AI mg/dl
I	Control	103.83±2.8	77.5±2.078	42.6±2.8	15.5±0.4	47.83±2.4	0.89±0.06
II	Cholesterol control	212.5±10.2^{a}	173.1±12.9^{a}	150.1±12^{a}	34.6±2.5^{a}	27.5±0.8^{a}	5.49±0.48^{a}
III	MESA 100 mg/kg	127.3±2.4^{b,**,B}	124.3±3.4^{a,**,B}	59.3±2.04^{a,**,B}	24.8±0.6^{a,**,B}	43.1±0.9^{a,**,A}	1.37±0.04^{a,**,B}
IV	MESA 200 mg/kg	113.8±1.3^{a,**,A}	117.5±3.4^{a,*,B}	42.8±1.7^{a,**,B}	23.5±0.6^{a,*,B}	47.5±0.5^{a,**,A}	0.9±0.04^{a,**,B}
V	Atorvastatin 10 mg/kg	103±1.06^{b,**}	99.6±2.17^{b,**}	33.3±1.07^{b,**}	19.9±0.4^{b,**}	49.6±0.7^{b,**}	0.66±0.02^{b,**}

Values are expressed as mean±SEM, (n=6). All the groups were compared with control group and standard group. All the data were statistically analyzed by one-way ANOVA followed by Dunnett's test and Significant values are expressed as ^{a}p<0.001, ^{b}p<0.05, **p<0.01, *p<0.05, standard (A=p<0.01, B=p<0.05),. ns: Non-significant, MESA: Methanolic extract of *Syzygium alternifolium*, TC: Total cholesterol, TG: Triglyceride, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, HDL: High-density lipoproteins, AI: Atherogenic index

Table 4: *In vivo* antioxidant studies in HFD induced hyperlipidemia

Groups	SOD (U/mg of protein)	GSH (µM/min/mg of protein)	Catalase (µM/mg of protein)	LPO (nM MDA/g of protein)
I	9.4±0.1	9.35±0.08	10.48±0.2	6.15±0.09
II	2.1±0.1^{a}	1.2±0.05^{a}	1.16±0.04^{a}	14.97±0.08^{a}
III	4.86±0.1^{a,**,A}	4.45±0.13^{a,**,A}	3.45±0.07^{a,**,A}	11.53±0.09^{a,**,A}
IV	6.26±0.21^{a,*,A}	6.3±0.12^{a,**,B}	5.71±0.06^{a,**,A}	9.76±0.03^{a,**,A}
V	7.8±0.08^{b,**}	7.8±0.05^{b,**}	8.9±0.13^{b,**}	7.31±0.04^{b,**}

Values are expressed as mean±SEM, (n=6). All the groups were compared with control group and standard group. All the data were statistically analyzed by one-way ANOVA followed by Dunnett's test andSignificant values are expressed as ^{a}p<0.001, ^{b}p<0.05, **p<0.01, *p<0.05, standard (A=p<0.01, B=p<0.05). ns: Non-significant, SOD: Superoxide dismutase, GSH: Glutathione, LPO: Lipid peroxidation, HFD: High-fat diet, SEM: Standard error of mean

Table 5: *In vivo* antioxidant studies in dexamethasone-induced hyperlipidemia

Groups	SOD (U/mg of protein)	GSH (µM/min/mg of protein)	Catalase (µM/mg of protein)	LPO (nM MDA/g of protein)
I	9.6±0.1	10±0.05	12.68±0.19	5.3±0.05
II	1.68±0.23^{a}	1.78±0.04^{a}	1.18±0.04^{a}	15.93±0.05^{a}
III	5.6±0.21^{a,**,A}	4.45±0.13^{a,**,A}	5.15±0.05^{a,**,A}	12.3±0.18^{a,**,A}
IV	6.4±0.13^{a,*,A}	6.3±0.18^{a,*,A}	7.18±0.07^{a,**,A}	10.8±0.04^{a,**,A}
V	7.7±0.11^{b,**}	8.51±0.08^{b,**}	10.25±0.095^{b,**}	7.26±0.06^{b,**}

Values are expressed as mean±SEM, (n=6). All the groups were compared with control group and standard group. All the data were statistically analyzed by one-way ANOVA followed by Dunnett's test, and Significant values are expressed as ^{a}p<0.001, ^{b}p<0.05, **p<0.01, *p<0.05, standard (A=p<0.01, B=p<0.05). ns: Non-significant, SOD: Superoxide dismutase, GSH: Glutathione, LPO: Lipid peroxidation, HFD: High-fat diet, SEM: Standard error of mean

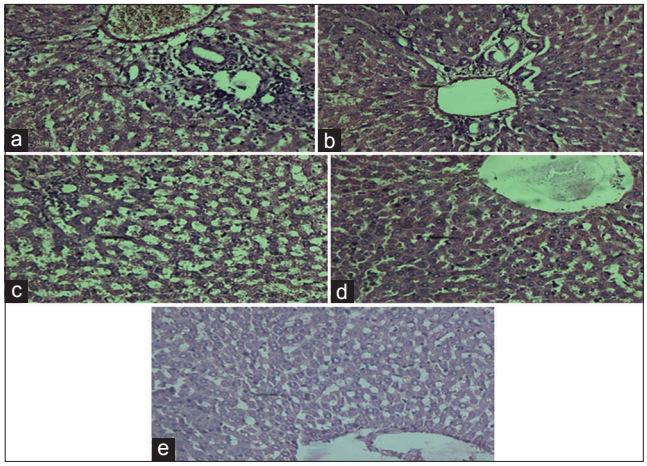

Fig. 1: Histopathology (liver) of high-fat diet (HFD) induced hyperlipidemia model, (a) Normal, (b) HFD induced, (c) methanolic extract of Syzygium alternifolium (MESA) 100 mg/kg, (d) MESA 200 mg/kg, HFD + standard (atorvastatin) 10 mg/kg

disorders. Cholesterol is synthesized in all animal tissue. It's important relates to its role in the stabilization of membrane structures because of its rigid planar structure. Increased amount of cholesterol leads to CVD particularly CHD [17].

The plasma cholesterol was reduced remarkably on treating the HFD rats with methanol extract of *S. alternifolium.* The lipid lowering effects may be due to the presence of plant sterol. Plant sterol reduces the absorption of cholesterol and thus increases the fecal excretion of steroids that results in decrease of body lipids reduction 1% cholesterol produces a 2-3% reduction in CHD risk [18].

The excess of fat diet increased the TG level which is one of the causes of hardening of arteries. HDL is known as the good cholesterol it has reversed the transport function. It carries cholesterol away from the including the coronary categories and drops it off at the liver. HDL is directly anti-androgenic, and it is believed to remove cholesterol from the developing lesions.

LDL is a risk factor and plays a role at several steps of atherosclerosis. A decrease in oxidative stress and protection of LDL from oxidation might, therefore, be a strategy with great promise for prevention of atherosclerosis associated CVD. VLDL production is directly related to the body fat. Severe elevation in the VLDL cholesterol leads to hypercholesterolemia. TGs are mainly stored in the adipose tissue. The plasma lipoproteins are major sources of fatty acid to synthesis triacylglycerol. The excess of fat diet increased the TG level which is one of the causes of hardening of arteries [19].

It has been reported that HFD brings about remarkable modifications in the antioxidant defense mechanisms of rat tissues by increasing the process of LPO, which plays an important role in oxidative stress of biological systems [20]. In the present study, MESA bark showed highly significant reduction in LPO levels compared to HFD group. This effect can be attributed to the phenolic constituents in *S. alternifolium* bark which has the ability to strongly inhibit LPO and high concentration of flavonoids which are potent antioxidants, lead to increase in antioxidant enzymes such as SOD, catalase, and GSH levels (Tables 4 and 5) and finally histopathology study supports the same in Fig. 1.

The preliminary phytochemical studies identified the presence of alkaloids, glycosides, terpenoids, steroids, flavonoids, volatile oils, tannins, proteins, and carbohydrates in MESA.

It is reported that phytosterol is useful in the treatment of hyperlipidemia [21]. Phenolic constituents have the ability to strongly inhibit the LPO process, and flavonoids act as potent antioxidants and free radical scavengers [22]. Phytosterols, triterpenoids, flavonoids, tannins, and phenolic constituents in MESA might be responsible for the hypolipidemic activity. Further experiments are required to prove the mechanism and advantage of *S. alternifolium* (Wt.) Walp (MESA) over other drugs.

CONCLUSION

These results suggested that MESA possess significant anti-hyperlipidemic activity.

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