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Hypolipidemic Activity of *Orthosiphon stamnineus* Benth Bark Extract

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ABSTRACT

The American heart association has identified the primary risk factor associated with atherosclerosis as evaluated levels of total cholesterol and triglyceride in the blood. Atherosclereosis, 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. Therefore therapists consider the treatment of hyperlipidemia to be one of the major approaches towards decelerating the atherogenic process. Allopathic hyperlipidemic drugs are available at large in the market but the side effects and contra- indications of these drugs have marred their popularity. Recently herbal hyperlipidemics have gained importance to fill the lacunae created by the allopathic drugs. The present study has been carried out to evaluate the antihyperlipidemic effect of plant *Orthosiphon stamineus* Benth against cholesterol diet induced hyperlipidemia in wister rats. Hydro-alcoholic extract of bark of *Orthosiphon stamnineus* Benth (HAEOSB) was studied for its *in-vivo* anti-hyperlipidemic potential using cholesterol diet induced hyperlipidemia model in rats. The result of study indicated that HAEOSB possess significant hypolipidemic activity at doses 500 and 750 mg/kg.

Keywords: HAEOSB, Nicotinic acid, cholesterol diet

INTRODUCTION

Plant Material

The plant *Orthosiphon stamnineus* Benth (Labiatae) is commonly known as 'Java Tea' usually occurs in Asam, Burma, Nicobar, Islands and Deccan. The plant is traditionally used as diuretic, anti-diabetes and anti-hypertensive[1-3]. However, no systemic study on anti-hyperlipidemic activity of the bark has been reported in the literature. In present investigation, we have screened hydro-alcoholic extract of bark of *Orthosiphon stamnineus* Benth (HAEOSB) for its anti-hyperlipidemic activity.

MATERIALS AND METHODS

Bark of *Orthosiphon stamnineus* Benth was collected from foothill of Yercaud, Tamilnadu, India. The plant material was identified and authenticated by Dr. A. Marimuthu; Principal, Government Arts College, Attur, Tamilnadu, India.

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Preparation of Extract

The bark were dried under shade and then coarsely powdered with a mechanical grinder. The powder was passed through sieve No. 40 and stored in and airtight container for the extraction. The marc left after Petroleum ether extract was dried and then extracted with ethanol 95% v/v (75-78^{0}C) and distilled water mixture in proportion of 50:50 up to 72 hrs. After completion of extraction, the solvent was removed by distillation. Dark brown color residue (yield-14.85%) was obtained. The residue was then stored in a dessicator. The extract was subjected to phytochemical screening[4-6].

Selection and acclimatization of animals:

Wister rats (150-180gm) of either sex and of approximately the same age, procured from listed suppliers of Venkataswara Enterprises, Bangalore, India were used for the study. They were housed in polypropylene cages and fed with standard rodent pallet diet (Hindustan Lever Limited, Bangalore) and water *ad libitum.* The animals are exposed to alternate cycle of l2hrs of darkness and l2hrs light. Before each test, the animals were fasted for at least 12 hrs and the experimental protocols were subjected to the scrutinization of the Institutional Animal Ethical Committee (P.Col. /14/2006) and were cleared by the same. All experiments were performed in the morning according to current guidelines for care of laboratory animals and the

ethical guidelines for investigations of experimental pain in conscious animals[7]. The standard organistic canula and syringe were used for drug administration in experimental animals. Animals were housed in plastic bottom cages and were allowed free access to standard laboratory feed and water. All the animals have been divided into five groups and placed in separate cages, each consisting of 6 animals. The animals were acclimatized to the laboratory conditions for one week before the onset of experiment.

Induction of Hyperlipidemia:

It is a standard model for evaluating anti- hyperlipidemic activity. Cholesterol Diet was used to induce hyperlipidemia in rats[8]. Cholesterol diet is administrated in rats by oral route. It significantly increases serum total cholesterol, triglyceride, AST and ALT levels.

Selection of Dosage:

The dose of Extract (HAEOSB) was arbitrarily chosen as 500 mg/kg. Dose of nicotinic acid (270 mg/kg) was calculated based on a human dose of 3 gm per day. The drugs were suspended in 4% gum acacia for oral administration.

Treatment Protocol:

Rats were made hyperlipidemic by the oral administration (P.O) of Cholesterol (400 mg/kg) along with cholic acid (50 mg/kg) in coconut oil for 20 days, once daily. The rats with elevated cholesterol level were divided into 4 groups of 8 animals each and given drug/ vehicle treatment for 10 days. During these 10 days all groups also received Cholesterol in same dose as earlier. In each group 8 animals were taken. Animals were kept fasted throughout the experimental period, but were provided water *ad libitum.*

Group – I: **Hypercholesterolemic Control (HC- C)**
-Animals received vehicle (Acacia 4%) 5 ml/kg P.O
Group – II: **Standard group (HC- NIC)**
-Animals received Nicotinic acid 270 mg/kg; P.O
Group- III: **Test Group – I (HC- HAEOSB- I)**
-Animals received HAEOSB (500 mg/kg; P.O)
Group- IV: **Test Group – II (HC- HAEOSB- II)**
-Animals received HAEOSB (750 mg/kg; P.O)

Blood samples for biochemical estimation were withdrawn from rats, after overnight fast, by decapitation after inducing anesthesia. Serum cholesterol and triglyceride levels were determined on day 1 (before treatment) & on day 10 (after treatment). Four groups of normal rats, each with 6 animals weighing 150- 180 gm were selected & given 10-day drug/vehicle treatment as above. However no cholesterol was administered to them, neither as pre-treatment nor during treatment.

The groups are

Group – V: **Normal control (N- C)**
-Animals received vehicle (Acacia 4%) 5 ml/kg P.O
Group – VI: **Standard group (N - NIC)**
-Animals received Nicotinic acid 270 mg/kg; P.O
Group- VII: **Test Group – I (N- HAEOSB- I)**
-Animals received HAEOSB (500 mg/kg; P.O)
Group-VIII: **Test Group – II (N- HAEOSB- II)**
-Animals received HAEOSB (750 mg/kg; P.O)

In each group 8 animals were taken. Animals were kept fasted throughout the experimental period, but were provided water *ad libitum.* Their serum total cholesterol and triglyceride levels were determined on day 1 and day 10 as for hypercholesterolemic rats. The lipid profiles of cholesterol Diet induced Hyperlipidemia model was summarized in Table No.1.

Biochemical estimation

After the experimental period, animals in different groups were sacrificed. The rats were killed by decapitation after inducing anesthesia (pentobarbitone sodium, 60mg/kg) and blood samples were collected into dry clean tubes. After centrifugation for 10 min, the serum samples were taken fro biochemical assay. Serum levels of total cholesterol[9] ,triglyceride[10], AST[8] and ALT[8] were determined by standard biochemical kit obtained from Merck, Germany.

Preparation of tissue homogenate

For estimation of lipids from the tissues, sample of liver were rinsed in ice-cold saline and blotted carefully. Each liver sample placed in ice-cold glass homogenizer containing phosphate buffer (at pH 7.4) [11]. The wet weight of the added sample was determined. After homogenization and centrifugation the supernatant was collected for determination of total cholesterol and triglyceride by standard biochemical kit obtained from Merck, Germany.

Histopathological examination

A small portion of the liver tissues from all the groups was excised immediately after sacrifice. Tissues were fixed in 10% formalin in phosphate buffer (pH 7.0) for 24 hr at room temperature for histopathology. Tissues were embedded in paraffin was and sections were cut 3-5 μm slices and were stained with haematoxylin and eosin (H&E) and observed under light microscope[12].

Statistical analysis

The data were statistically analyzed by Student's t-test and all values were expressed as mean ± SEM. The data were also analyzed by one way ANOVA followed by Dunnet's t-test[13.14].

RESULTS

Serum and tissue lipid profile

There were significant elevation noted in the levels of total cholesterol, triglyceride in the serum, liver tissue of HC diet group in compared to normal diet group (Table 1,2). In the present study HAEOSB significantly prevented the high cholesterol diet induced rise in the levels of total cholesterol, triglyceride in serum and liver tissue of Group IV (HC+HAEOSB) rats compared to group I rats (HC

Table . 1: Effect of HAEOSB On Lipid Profiles in serum against Cholesterol Diet Induced Hyperlipidemia Model

Group	Groups	Total cholesterol (mg/dl)		Triglyceride (mg/dl)		Transaminases (U/L)	
		Pre Treatment	Post Treatment	Pre Treatment	Post Treatment	AST	ALT
I	HC- C	91.16±0.91	84.33±1.04	132.10±1.34	165±1.82	193.33±2.11	178± 1.15
II	HC- NIC	85±2.85	67.33±2.49**	165.43±4.12	111.50±1.35*	335.33±4.4*	284± 1.79*
III	HC- HAEOSB I	92.66±2.63	73.83±2.07**	170.30±3.92	125±1.56*	244.33±3.5*	232± 2.98*
IV	HC- HAEOSB II	98.83±0.83	70.33±1.80**	165.50±3.51	119±2.02*	238± 2.6**	220± 4.75**
V	N- C	62.66±4.40	70.16±2.22	113.33±1.71	109.10±2.11	-	-
VI	N- NIC	62.50±2.22	53.33±1.38	138.17±2.17	100±5.01	-	-
VII	N- HAEOSB I	68±2.06	76.16±2.02	142±2.63	108.67±2.80	-	-
VIII	N- HAEOSB II	68.16±1.43	79.50±1.68	150±3.26	111.50±2.96	-	-

**P<0.05, ** P<0.01 vs. before treatment*

The data were statistically analyzed by Paired t-test and all values were expressed as mean ± SEM. The data were also analyzed by one way ANOVA followed by Dunnet's t-test and values p<0.05 were considered significant.

Table. 2: Effect of HAEOSB on Lipid Profile in Liver Tissue against Cholesterol Diet Induced Hyperlipidemia Model

Group	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	Transaminase (U/L)	
			ALT	AST
N – C	4.10±0.342	5.21±0.323	-	-
HC – C	7.60±0.501	15.21±1.436	19.45±1.432	17.19±1.234
HC – NIC	5.43±0.260**	6.91±0.501**	32.53±1.781	28.97±2.091
HC – HAEOSB I	6.16±0.531*	8.20±0.451**	25.43±1.52*	21.32±1.332*
HC – HAEOSB II	5.50±0.523*	7.54±0.545**	21.84±1.530**	20.91±0.98**

**P<0.05, ** P<0.01 Vs control*

The data were statistically analyzed by Student's t-test and all values were expressed as mean ± SEM. The data were also analyzed by one way ANOVA followed by Dunnet's t-test and values p<0.05 were considered significant.

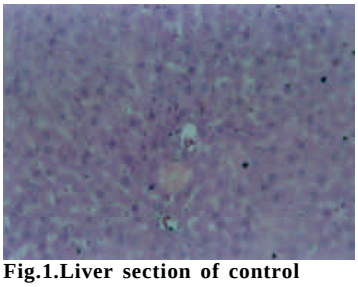

Fig.1.Liver section of control (H&E, x 400)

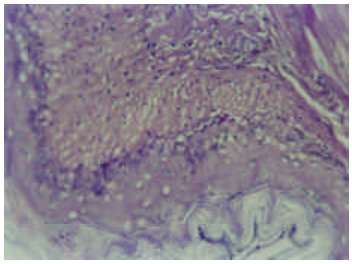

Fig.2.Liver section of High cholesterol (HC) diet treated rats showing marked vascular congestion fatty deposition and foamy degeneration of hepatocytes (H&E, x 400)

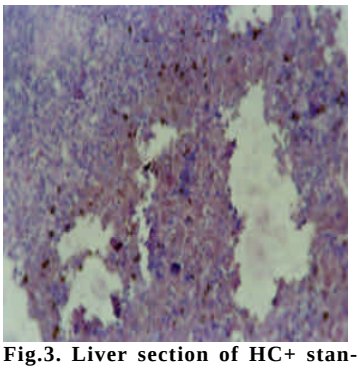

Fig.3. Liver section of HC+ standard drug showing normal hepatocytes and central vein but some degree of swelling (H&E, x 400)

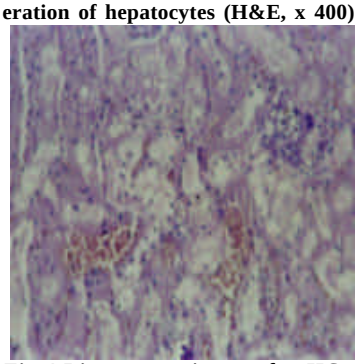

Fig.4.Liver section of HC+ HAEOSB (500 mg/kg) showing recovered normal hepatocytes (H&E, x 400)

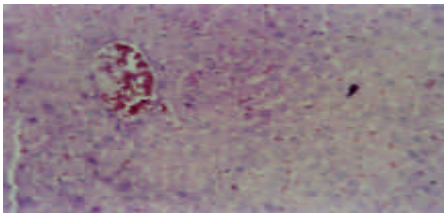

Fig.5.Liver section of HC+ HAEOSB (750mg/kg) showing recovered normal hepatocytes (H&E, x 400)

diet). Serum and tissue transaminases (ALT & AST) levels were also significantly increased in the nicotinic acid group compared to cholesterol diet induced group (Table No. 1,2). This increase in the transaminase levels in liver tissue indicates the propensity to cause hepatotoxicity. This manifested as an elevation of AST & ALT in liver tissue and serum of patients receiving nicotinic acid. This is one of the major limitation of nicotinic acid. However, HAEOSB (500 mg/kg) and HAEOSB (750 mg/kg) did not shown any significant elevation of ALT & AST levels in the serum as well as liver tissues, compared to the nicotinic group. The results are depicted in Table No. 1 and 2.

Morphological and Histopathological Observation

The liver of the high cholesterol (HC) diet group was significantly enlarged and the color of liver becomes pale. The physical appearance, i.e. color, size and smoothness of the liver of HEAOSB with HC diet group remain unaltered comparing those of the normal control group. Microscopical examination of liver section of control group (Fig. No. 1) showed normal arrangement of hepatocytes with clear broad of central vein at portal layer. Microscopical examination of liver section of cholesterol diet treated group (Fig. No. 2) showed various degrees of pathological changes such as centrilobulor fatty degeneration, cloudy swelling and necrosis of hepatic cells. The histopathological study showed recovery of the damaged liver cells in the drug treated group. The ruptured cells of the intoxicated liver were reformed. The degree of vascularization was also reduced as compare to hyperlipidemic group. (Fig. No. 4, 5). But, situation is somewhat different in case of cholesterol diet group. There is degeneration of hepatocytes, swelling observed in the standard treatment group (Fig. No. 3). It is due to increase in the levels of AST and ALT in the liver tissue. Microscopical examination of liver section of HAEOSB (500 mg/kg & 750 mg/kg) treated group (Fig. No. 4, 5) clearly showed normal hepatic cells and central vein, which are comparable with nicotinic acid, treated group (Fig. No. 3).

DISCUSSION AND CONCLUSION

The phytochemical and pharmacological studies on the bark of the plant *Orthosiphon stamineus* Benth was done. The phytochemical constituents were extracted by successive solvent extraction. Hydro- alcoholic extract of *Orthosiphon stamineus* Benth (HAEOSB) shows the presence of main phytoconstituent Orthosiphonin (A bitter glycoside) and Neo- orthosiphonin A – E (Flavanoid) [15-17]. Hence, it was selected for the pharmacological studies. In the pharmacological studies, hydro- alcoholic extract of *Orthosiphon stamineus* Benth (HAEOSB) shows significant antihyperlipidemic activity in dose dependent manner. The anti-hyperlipidemic activity was evaluated by using cholesterol diet induced hyperlipidemia. It was found that HAEOSB was more effective in higher dose as compared to lower dose as an anti-hyperlipidemic agent against cholesterol diet induced hyperlipidemia model. Present studies reveal that hydro- alcoholic extract of *Orthosiphon stamineus* Benth (HAEOSB) can be used as effective antihyperlipidemic. Further experiments are required to prove the mechanism and advantage of HAEOSB over other drugs.

REFERENCES

1. Kirtikar *et al*. Indian Medicinal Plants 2nd ed., p-2121.
2. Thirumalai Natarajan *et al*. Siddha Materia Medica 4th ed., p- 66.
3. Nadkarni A K *et al*. Indian Materia Medica. 1996:138.
4. Trease and Evans *et al*. Pharmacognosy, 15 thed., New Delhi p. -138.
5. Harboum J B *et al*. Phytochemical Methods- A Guide To Modern Techniques of Plant Analysis. Reprint 1976 Harsted Press, p-4-6.
6. Pulok *et al*. quality control of herbal drugs 1sted. 2002:389-398.
7. Zimmerman M *et al*. ethical guidelines for invesigation of experimental pain in conscious animals 1983:16:109-10.
8. Kutty G N *et al*.synthesis and hypolipidemic activity of a thiazolidinone derivatives, Indian Drugs 2004:41(2): 76-79
9. Parekh A C, *et al*. Anal Chem. 1970; 42: 1423.
10. Horn W T, *et al*. J Lipd Res. 1981; 122: 377.
11. Weiser M., *et al*. J Clin Pharmcol. 1997; 37: 453.
12. Galigher *et al*. Essentials of Practical Microtechnique, 2nd ed. Lea and Febiger, Philadelphia, 1971, p-77.
13. S.K. Kulkarni handbook of experimental pharmacology 3 rd ed. p-172-79.
14. Courtesy; http :// faculty vasser .edu / lowry/ ank3. html.
15. Lyckander IM *et al*. Lipophilic flavonoids from Orthosiphon Spicatus prevent Oxidative inactivation of 15- lipoxygenase. 1996:54(4): 239-46.
16. Malter K E *et al*. Flavonoids; Orthosiphon Spaicatus Planta Medica 1989:55(6); 569-70.
17. Ohashi K *et al*. Indonesian Medicinal Plants XXIII: Chem. Pharm. Bull.2000: 48(3) 433-5.

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