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Hypolipidaemic activity of seeds of *Spermac oce hispida* Linn., in Isoproterenol induced Cardiotoxic rats

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ABSTRACT

Lipids play a vital role in pathogenesis of cardiovascular disease by causing hyperlipidemia which in turn, leads to the development of atherosclerosis. Hyperlipidaemia is found to be one of the greatest risk factor for the development of coronary heart disease. The available hypolipidaemic drugs have been associated with number of side effects and people are interested in natural therapies. Our previous studies proved that *Spermac oce hispida* exhibit cardio-protective effects by several mechanisms. *Spermac oce hispida* possesses significant anti-oxidant and cardioprotective activities. The present study aimed to investigate the hypolipidaemic effects of *Spermac oce hispida* seed extract on isoproterenol (ISO) induced cardiotoxic rats. Two different doses of the Hydro alcoholic extract of plant such as 100 and 200 mg/kg body weight was used to prove the hypolipidaemic activity against 100mg/kg body weight of (ISO).After administration of *S. hispida* shows a significant decrease in the levels of cholesterol, triglycerides, LDL, VLDL and significant increase in the level of HDL in serum and heart tissues. Therefore it effectively suppressed the ISO induced hyperlipidemia in rats, suggesting the potential protective role in Coronary heart disease.

Keywords: Myocardial infarction, isoproterenol, antioxidant and coronary heart disease

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INTRODUCTION

Hyperlipidaemia is the major cause for the development of atherosclerosis. An abnormal cholesterol level, higher concentrations of LDL particles and lower concentrations of functional HDL particles in blood is referred as Hypercholesteremia. This is strongly associated with cardiovascular diseases because these promote atheroma development in arteries (atherosclerosis). This disease process leads to myocardial infarction (heart attack), stroke, and peripheral vascular disease.[1] Since high level of blood LDL, especially higher LDL particle concentrations and smaller LDL particle size, contribute to this process more than the cholesterol content of the HDL particles.[2] Hypercholesterolemia also modifies the composition, structure and stability of cellular membranes.

The lowering of cholesterol and lipid levels by drug or dietary interventions could reduce the risk of Coronary heart diseases. The common lipid-lowering drugs such as fibrates, statins, bile acid sequestrants, etc. will regulate the lipid metabolism by different mechanisms, but they also have many adverse effects.[3] Therefore, the development of lipid lowering drugs from natural sources is the best remedy and is in great demand. Medicinal plants continue to provide valuable therapeutic agents, both in modern medicine and in traditional Indian system of medicine.

Spermacoce hispida Linn has been extensively used in Siddha system of medicine for its rich medicinal values. The plant belongs to the family Rubiaceae. *Spermacoce hispida* Linn. Popularly known as 'Nathai suri' in Tamil or 'Shaggy button weed' in English or 'Madanaghanti' in Hindi and 'Madangranta' in Telugu. It is a procumbent herb with stout tap root and stout branches. *Spermacoce hispida* is used as a folk medicine for inflammation, obesity and hyperlipidemia. The plant is widely distributed in Western Ghats of Kerala and in Marudhamalai forest which is an extension of Western Ghats in Tamil Nadu.[4] The seed extract of the plant is used for treating the internal injuries of nerves and kidney. It is suggested that it removes the sign of old age. The tribals living in the Western Ghats of Kerala are using this herb for improving vitality since it purifies blood. Thus all the parts of the plant have an ethnomedical importance.[5]

The plant *Spermac oce hispida* has been studied in a widespread way for its phytochemical composition and a large number of active ingredients such as, Borreline, β-sitosterol, Ursolic acid and Isorhmnatin. The preliminary phytochemical investigation indicates the presence of Saponins, Tannins, Phenolics, Steroids, Essential Oils, Flavonoids and Terpenoids.[6] β-sitosterol is being studied for its potential to reduce benign prostatic hyperplasia and blood cholesterol levels.[7] Hence, in the present study, the Hydro alcoholic seed extract of *S.Hispida* was investigated for Antihyperlipidaemic activity in ISO induced cardiotoxic rats.

MATERIALS AND METHOD

Animals

Adult male albino wistar rats weighing 150-250g were maintained in Tamil University, Thanjavur, Tamil Nadu, India. The animals were housed in polypropylene cages. They were fed with standard diet and water *ad libitum* and housed under standard environmental conditions. They were segregated into different groups as follows:

Experimental design

Group1: The rats of group 1 serve as control and they did not receive any treatment

Group 2: Rats were administered with Isoproterenol (100mg/kg b.wt) dissolved in 0.9% saline subcutaneously twice at the interval of 24 hours.

Group 3: Rats were administered with 100mg/kg body wt. of *Spermac oce hispida*seed extract for 45 days. ISO was injected subcutaneously on 45th day.

Group 4: Rats were administered with 200mg/kg body wt. of *Spermac oce hispida*seed extract for 45 days. ISO was injected subcutaneously on 45th day

Group 5: Rats were administered with 100mg/kg body wt. of *Spermac oce hispida*seed extract alone for 45 days.

Group 6: Rats were administered with 200mg/kg body wt. of *Spermac oce hispida*seed extract alone for 45 days.

Group 7: Rats were administered with Vitamin E at 100 mg/ kg b.wt. for 45 days. ISO was injected subcutaneously on 45th day.

On 45th day one hour after the administration of test sample/ standard, ISO (100 mg/ kg) dissolved in normal saline was injected subcutaneously to all rats, other than the Group I, V and VI at an interval of 24 hours for two days to induce experimental cardiotoxicity/myocardial infarction.[8] On the 47th day, all the rats were sacrificed by cervical dislocation after an overnight fasting.

Blood was collected from the retro-orbital sinus without anti-coagulant for isolation of serum. The blood was centrifuged and the serum was used for the biochemical assay. The heart was excised immediately and washed off from blood with ice cold physiological saline. Then, the tissue was blotted in between filter papers to absorb moisture and weighed in a balance.

10 % organ homogenate was prepared in 0.1 M Tris-HCl buffer (pH 7.4) solution. The homogenate was centrifuged at 3000 rpm for 15 minutes and the supernatant was used for the various biochemical parameters. Biochemical estimations were carried out in serum and heart.

The total cholesterol was estimated by the method of Zak *et al.*, (1953).[9] Serum triglyceride level was estimated using standard kit Glycerol-3-phosphate oxidase-4-aminophenazone

method. Tissue triglyceride was estimated by the method of Rice, (1970).[10] Serum HDL-cholesterol was estimated as that of serum cholesterol estimation after precipitating the LDL and VLDL using standard HDL-cholesterol precipitant from Randox Kit. The LDL cholesterol was calculated using the formula LDL= Total cholesterol – [HDL cholesterol + (triglycerides/5)]

Statistical Analysis

The results were statistically evaluated by one way Analysis of Variance (ANOVA). They were further evaluated by Duncan Multiple Range test (DMRT) and the results were expressed as Mean ± Standard deviation (SD) for six rats in each group. A value of $P<0.05$ was considered statistically significant. All the statistical analysis was computed using SPSS software version 12.0.

RESULTS AND DISCUSSION

The results of Lipid profile in serum and heart were shown in tables. Table 1 and 2 shows the levels of Cholesterol and Triglycerides in normal and ISO induced cardiotoxic rats. The cholesterol and Triglyceride is found to be significantly increased in serum and heart homogenate when compared to control rats. But these levels were found to be significantly reduced in hyperlipidaemic rats treated with hydroalcoholic extract of*S.hispida.* Table 3 shows the effect of HAE on serum LDL, HDL and VLDL in normal and ISO induced cardiotoxic rats. The levels of LDL and VLDL were significantly higher in ISO induced rats when compared to those in control rats, while the HDL levels were significantly decreased when compared to control rats. After the treatment with hydroalcoholic seed extract of *S. hispida* at the doses 200 mg/kg in ISO induced cardiotoxic rats a significant reduction in LDL, VLDL and significant increase in HDL were observed when compared to control rats were shown in the table3.

Table 1 Effect of HAE on Cholesterol in normal and ISO induced cardiotoxic rats

Groups	Cholesterol	
	Heart (mg/100 gm of tissue)	Serum (mg/dl)
Normal	246.1 ± 30.2 [a]	65.2 ± 8.3 [a]
ISO	405.4 ± 80.1 [b]	93.1 ± 8.1 [b]
100 mg/kg b.wt. HAE + ISO	282.5 ± 91.2 [a]	79.8 ± 35.4 [ab]
200 mg/kg b.wt. HAE + ISO	257.1 ± 73.5 [a]	68.2 ± 5.9 [ab]
100 mg/kg b.wt. HAE	261.0 ± 101.2 [a]	62.6 ± 11.9 [a]
200 mg/kg b.wt. HAE	255.0 ± 92.2 [a]	64.5 ± 13.1 [a]
Vitamin E + ISO	270.4 ± 94.5 [a]	78.1± 9.2 [a]

Values are Mean ± SD (n=6). Significant difference was observed between different groups using One Way ANOVA followed by DMRT. Values with different letters like a,b,ab,c of same column are differ significantly ($P<0.05$).

Table 2 Effect of HAE on Triglycerides in normal and ISO induced cardiotoxic rats

Groups	Triglyceride	
	Heart (mg/100 gm of tissue)	Serum (mg/dl)
Normal	321.1 ± 74.1 [a]	59.1 ± 9.2 [a]
ISO	462.1 ± 135.1 [b]	105.1 ± 16.5 [c]
100 mg/kg b.wt. HAE + ISO	385.1 ± 86.9 [ab]	89.5 ± 14.1 [bc]
200 mg/kg b.wt. HAE + ISO	309.4 ± 41. [a]	82.4 ± 11.9 [ab]
100 mg/kg b.wt. HAE	314.7 ± 65.3 [a]	59.5 ± 8.4 [a]
200 mg/kg b.wt. HAE	301.4 ± 52.1 [a]	51.9 ± 7.5 [a]
Vitamin E + ISO	304.9 ± 48.8 [a]	78.6 ± 20.3 [ab]

Values are Mean ± SD (n=6). Significant difference was observed between different groups using One Way ANOVA followed by DMRT. Values with different letters like a,b,ab,c of same column are differ significantly (P<0.05).

Table 3: Effect of HAE on serum LDL, HDL and VLDL in normal and ISO induced cardiotoxic rats

Groups	LDL	HDL	VLDL
Normal	58.52± 5.3 [a]	22.56± 5.3[a]	12.1± 7.3 [a]
ISO	115.21± 7.6[b]	13.25± 4.7[b]	21.0 ± 9.3[c]
100 mg/kg b.wt. HAE + ISO	72.55± 5.9[c]	18.95± 7.2[c]	17.9 ± 12.3 [bc]
200 mg/kg b.wt. HAE + ISO	64.48± 3.2[a]	20.19± 8.1[a]	15.48 ± 12.8 [ab]
100 mg/kg b.wt. HAE	56.35± 3.5[a]	21.31± 7.4[a]	12.0 ± 9.5 [a]
200 mg/kg b.wt. HAE	57.68± 4.22[a]	22.16± 6.7[a]	10.51 ± 8.6 [a]
Vitamin E + ISO	59.12± 1.28[a]	21.15± 5.4[a]	14.5 ± 18.2 [ab]

Values are Mean ± SD (n=6). Significant difference was observed between different groups using One Way ANOVA followed by DMRT. Values with different letters like a,b,ab,c of same column are differ significantly (P<0.05).

Hypercholesterolemia and hypertriglyceridemia are the two important factors that have been very clearly indicated to be responsible for the development of atherosclerotic lesion of coronary artery leading to myocardial infarction. A lot of experimental and epidemiological evidence suggest the direct relationship between atherosclerosis and evaluated levels of plasma lipids. An increased risk of atherosclerosis is always abbeyed with high blood concentration of total lipid, LDL, VLDL Total cholesterol with low concentration of HDL.[11]

Isoproterenol is a well-known cardiotoxic agent due to its ability to destruct myocardial cells. It induces myocardial necrosis by a multiple step mechanism. It accomplishes severe stress in the myocardium, resulting in infarct-like necrosis of the heart muscle with an increase in the level of myocardial lipids.[12] ISO-treated cardiotoxicity is related with increased levels of circulatory lipids. Hypercholesterolemia and hypertriglyceridemia are the risk factor for the development of Myocardial infarction. Moreover, ISO promotes lipolysis in the myocardium.

[13] It mainly elevates the LDL cholesterol level in the blood, which in turn leads to the build-up of harmful deposits in the arteries, thus favouring coronary heart disease.[14]

High density lipoprotein is the main substrate for lecithin: cholesterol acyl transferase for cholesterol esterification and incorporation.[15] In the present work, ISO administration has resulted in increased cholesterol, triglycerides (TGL) and LDL level and decreased HDL level as compared to the normal animals ($P<0.05$). HAE pretreatment is observed to reverse the lipid profile to normal levels significantly ($P<0.05$).

Increased cholesterol levels in the heart of isoproterenol intoxicated rats might be due to increased uptake of low density lipoprotein-cholesterol from the blood by the tissues.[16] The decreased level of cholesterol during HAE pretreatment might be due to any one of the following reasons such as decrease in cholesterol biosynthesis or increased removal of cholesterol from the circulation or decrease in absorption of dietary cholesterol or increase in the excretion of cholesterol via bile and feces. Increased conversion of cholesterol to bile acids would lead to reduction in cholesterol levels and hence raise the expression of low density lipoprotein receptors on liver cells, leading to an increased removal of cholesterol from the circulation.[17] The hypocholesterolemic effect of HAE (200 mg/kg b.wt.) might be due to HMG CoA reductase inhibiting activity.

Fibrate acts as an agonist for PPAR– α, which is responsible for decreasing TGL and increasing HDL level.[18] Fibrates lower blood triglyceride levels by reducing the liver's production of and by speeding up the removal of triglycerides from the blood. The decreased TGL and increased HDL against ISO administered rats observed on HAE treatment, reveals that HAE might exhibit an activity similar to that of fibrate. This could be due to lipid lowering property of the drug. LDL particles are sometimes referred to as bad cholesterol because they can transport their content of lipid molecules into artery walls, attract macrophages, and thus drive atherosclerosis. In contrast, HDL particles are often called good cholesterol or healthy cholesterol because they can remove lipid molecules from macrophages in the wall of arteries.[19] The increased LDL in ISO administered rats might be due to increased inhibition of apo lipoprotein B synthesis. Effect of HAE on decreasing LDL-cholesterol could be due to decrease of VLDL-cholesterol synthesis and secretion from the liver leading to a long term decrease in LDL concentration.

The result from our study clearly indicates that the diseased rats exhibit both the hyperlipidemic condition and an increase in the levels of lipid peroxides which paves way for the formation of ox-LDL. The action of antioxidants is commonly linked to the inhibition of lipoprotein oxidation.[20] The presence of various enzymatic and nonenzymatic antioxidants in HAE might have reduced the risk of LDL oxidation and thereby, protect the heart from ox-

LDL induced damage. Various phytoconstituents such as Borreline, Ursolic acid, Isorhmnatin, flavonoids and vitamin C present in *S.Hispida* might be responsible for the observed hypolipidemic activity.

CONCLUSION

In conclusion, the present study demonstrated that the hydroalcoholic seed extract of *S. hispida* exhibited maximum efficacy in lowering the elevated lipid levels. The observed cholesterol-reducing action indicates that the seed possesses some potential medicinal value and could be suggested for the heart and obese patients.

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