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EVALUATION OF ANTIOXIDANT AND ANTIHYPERLIPIDEMIC POTENTIAL OF *SIDA CORDIFOLIA LINN.* IN EXPERIMENTAL ANIMALS

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ABSTRACT

The present study was aimed to evaluate antioxidant and antihyperlipidemic activity of an aqueous extract of root of *Sida cordifolia Linn.* (SCAE) against Triton WR-1339 and High fat diet (HFD) induced hyperlipidemia in experimental animal. Effect of simultaneous administration of SCAE in different doses (200 & 400 mg/kg) by oral route was estimated in Triton WR-1339 and HFD induced hyperlipidemic animals by estimating serum lipid levels of cholesterol (TC), Triglycerides (TG), Low density lipoproteins (LDL), High density lipoprotein (HDL) and Very low density lipoprotein (VLDL) and atherogenic index. Whereas antioxidant activity was carried out by estimating serum levels oxidative marker Superoxide dismutase (SOD) and Catalase (CAT). From the study, it was revealed that the aqueous extract of *Sida cordifolia p*ossesses significant hyperlipidemic activity in acute as well as chronic hyperlipidemic models in the company of promising antioxidant activity. So, it was concluded that aqueous extract of *Sida cordifolia p*ossesses potential antioxidant and antihyperlipidemic activity in experimental animals.

Keywords: *Sida cordifolia Linn.* , Hyperlipidemia, Antioxidant, High fat diet, Atherogenic index

INTRODUCTION

Hyperlipidemia, (mainly increased level of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein (LDL) cholesterol along with decrease in high-density lipoprotein (HDL) cholesterol) is the predictor of coronary artery disease (CAD). Hyperlipidemia is an important risk factor in the initiation and progression of atherosclerotic impasse [1, 2].

Therefore, prime consideration in the therapy for hyperlipidemia and arteriosclerosis is to attenuate the elevated blood serum/plasma levels of lipids. Oxidation is one of the destructive processes, wherein it breaks down and damages various molecules. Oxygen via its transportation produces reactive oxygen species (ROS) such as super oxide, hydroxyl radicals, and hydrogen peroxide. They provoke uncontrolled reactions [3]. Free radicals attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA [4, 5].

The body possesses several defence systems comprising enzymes and radical scavengers [3]. Some of them constitute the repair systems for biomolecules that are damaged by the attack of free radicals [5]. Antioxidants are compounds that act as inhibitors of the oxidation process and are found to inhibit oxidant chain reactions at small concentrations and thereby eliminate the threat of pathological processes [3]. Phenolic compounds present in medicinal plants have been reported to

possess powerful antioxidant activity [4]. Flavanoids are a major class of Phenolic compounds present in medicinal plants and are found to have potential role in prevention of various diseases through their antioxidant activity [6].

Sida cordifolia, popularly known as "Malva Branca", grows as a bush of up to 2 m. It is used in the folk medicine for several purposes: antirheumatic, antipyretic [7], laxative, diuretic, antiinflammatory, analgesic [8, 9], hypoglycaemic [8], anti asthmatic, in the treatment of nasal congestion and as aphrodisiac [10, 11]. Further studies showed antiviral [12], antimicrobial [13] and anti fungal [14] activities.

A preliminary phytochemical screening of *Sida cordifolia* demonstrated the presence of alkaloids, steroids, flavonoids and saponins. Chemical studies of the leaves of this plant revealed the presence of ephedrine, pseudoephedrine (vasoconscrictor), vasicinone [15], vasicine and vasicinol (bronchodilators) [16].

In view of the above findings, *Sida cordifolia Linn.* was selected to evaluate its antioxidant and antihyperlipidemic potential in experimental animals. Since antioxidant rich herbs possess significant activity against various disease condition characterized by induced oxidative stress like atherosclerosis, hyperglycemia, nephrotoxicity etc.

The purpose of the current study was to investigate whether the administration of an aqueous extract of *Sida cordifolia Linn.*, has any protective effect against triton and high fat diet (HFD) induced hyperlipidemia in experimental animals.

MATERIALS AND METHODS

Preparation of extract: The authenticated root was shade dried and powdered coarsely. Extraction was done according to standard procedures using analytical grade solvents. The powdered drug was extracted by the process of maceration. The extracts obtained were concentrated under reduced pressure and the percentage yield of aqueous extract was calculated.

Animals: Healthy Wistar albino rats of either sex weighing between 150-200 g were taken for the study. They were housed under controlled conditions of temperature (23±2 °c), humidity (55±5%) and 12h light and 12h dark cycles. The animals were fed with standard pellet diet and water *ad libitum*. The research protocol was approved by Institutional Animal Ethics Committee (IAEC) of M. P. Patel college of Pharmacy, Kapadwanj, Gujarat, India on 11th February 2011 with reference no JET/MCP/IAEC/2011/3/2.

Acute toxicity studies: Acute toxicity study for aqueous extracts of *Sida cordifolia Linn.* was conducted as per OECD guidelines 423 using Albino wistar rats. Each animal was administered the aqueous solution of the extract by oral route. The animals were observed for any changes continuously for the first 2h and up to 14th Day for mortality [16].

Antihyperlipidemic activity:

1. Triton induced hyperlipidemia: The method of Tamasi et al [17] was used for the evaluation of antihyperlipidemic activity [18]. Albino wistar rats weighing 150-200g were divided into seven groups each group containing six animals. Animals were fasted for 16 hours prior to the experiment with water *ad libitum*. Group I served as normal control whereas Group II served as hyperlipidemic untreated animals. Group III was treated with standard antihyperlipidemic drugs whereas Group IV to VII was treated with different dose of test extract.

On the first day of experiment, all the animals received triton wr-1339 at 100 mg/kg body weight by intraperitoneal route except animals of Group I (normal control). At the end of the experimental study, blood sample were withdrawn under light ether anesthesia by retro orbital puncture for the assessment of antihyperlipidemic activity.

2. High fat diet (HFD) induced hyperlipidemia [19]

Diet composition:

Healthy albino wistar rats weighing 150-200g were divided into different groups each groups contain six animals. Treatment was given for 42 days as per treatment protocol.

	Normal Diet (%)	High Fat Diet (%)
Wheat flour	45.0	45.0
Sucrose	20.0	20.0
Casein	20.0	20.0
Coconut oil	10.0	10.0
Salt mixture	4.0	4.0
Vitamin mixture	1.0	1.0
Cholesterol	--	1.0
Cholic acid	--	0.1

Treatment protocol:

Group I – Normal control group animals received normal diet

Group II- Hyperlipidemic group animals received high fat diet for 42 days
Group III- Standard drug treated animals received Simvastatin – 10 mg/kg, p.o. with HFD
Group IV to VII- Animals received HFD and different dose of test extract
At the end of the experimental study, blood samples were withdrawn under light ether anaesthesia by retro orbital puncture for assessment of antihyperlipidemic activity.

3. **Assessment of antihyperlipidemic activity:** For the assessment of antihyperlipidemic activity, following parameters were recorded.

Biochemical parameters: The main biochemical parameters recommended by the national cholesterol education program (NCEP) guidelines (2002) for lipid screening i.e. total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), high density lipoprotein cholesterol (HDL-C) and triglycerides were measured [20].

Cardiac risk factors: The cardiac risk factor ratio recommended by the NCEP guidelines 2002 was estimated by calculating TC: HDL-C ratio (Atherogenic index) [20].

4. **Antioxidant activity:** In vivo antioxidant activity was carried out by estimating biochemical markers like superoxide dismutase (SOD) and catalase (CAT) concentration in serum of oxidative stress condition induced by hyperlipidemia in experimental animals [21,22].

Statistical analysis: The data were expressed as mean ± SEM. Statistical differences between means were determined by one – way ANOVA followed by Tukey's post hoc test using graph pad instant software, San Digeo, U.S.A. Values of $P < 0.05$ were considered as significant.

RESULTS

***Acute toxicity studies*:** Acute oral toxicity study was carried out to find out safety of test extract (SCAE) under the study according to OECD Test guideline 425 (modified). Animals were observed for 14 days (post administration) with special attention for first 4 hours after administration.

It was observed that all animals were only slightly sedated within first hour of administration, and were normal and active within two hours of post treatment. No other sign of toxicity was observed during total duration of observation, and all the animals survived 14 days post administration of test drugs.

From the above observation, it was concluded that test extract (SCAE) was safe up to 2000 mg/kg and LD_{50} was greater than 2000 mg/kg. D_1 (200 mg/kg) and D_2 (400 mg/kg) were selected, which represents $1/10^{th}$ and $1/20^{th}$ of 2000 mg/kg to evaluate antioxidant and antihyperlipidemic activity.

Effect of SCAE on serum lipid profile in Triton-induced hyperlipidemic rats: Administration of triton resulted in significant increase ($P < 0.001$) in serum levels of cholesterol, triglycerides, LDL and VLDL. A significant reversal in serum levels of cholesterol, triglycerides, LDL and VLDL was observed in animals treated with SCAE and simvastatin. A standard antihyperlipidemic drug (simvastatin) produced maximum cholesterol lowering effect compared to SCAE 200 and SCAE 400 whereas maximum triglycerides, LDL and VLDL inhibitory effect was procured by SCAE 400 treated animals which was greater than that of simvastatin also SCAE 400 mg/kg produced significant increase ($P < 0.001$) in serum HDL levels, which was greater than that of the simvastatin. The groups treated with SCAE and simvastatin demonstrated significant decrease in atherogenic index compared to triton induced hyperlipidemic animals ($P < 0.001$) (Table 1).

Effect of SCAE on serum lipid profile in HFD-induced hyperlipidemic rats: HFD treated animals demonstrated significantly elevated serum levels of cholesterol, triglycerides, LDL and VLDL ($P < 0.001$) while HDL level was significantly decreased ($P < 0.01$) compared to normal control group. However, SCAE 400 group recorded significant decrease in cholesterol, triglycerides, LDL ($P < 0.001$) and VLDL ($P < 0.05$) while no significant change in HDL level was observed compared to HL control. Also there was significant reduction ($P < 0.001$) observed in atherogenic index in SCAE and simvastatin treated animals compared to HFD induced hyperlipidemic animals. Overall, effect produced by SCAE in dose of 400 mg/kg on serum lipid profile was comparable to standard antihyperlipidemic drug simvastatin (Table 2).

Antioxidant activity: Table 3 and 4 shows the activities of enzymatic antioxidant markers superoxide dismutase (SOD) and catalase (CAT) in normal and experimental animals of each group. SOD and CAT activities were significantly decreased ($P < 0.001$) in both triton as well as HFD treated animals compared to vehicle treated animals. In triton

induced hyperlipidemic rats SCAE in dose of 400 mg/kg have shown significant increase ($P < 0.05$) in both oxidative markers whereas in HFD induced hyperlipidemic rats can increase only CAT activities significantly ($P < 0.05$) whereas SCAE in dose of 200 mg/kg was not able to increase activities significantly.

DISCUSSION

The potentially reactive derivatives of oxygen ascribed as reactive oxygen species (ROS) such as superoxide radical, hydroxyl radical and hydrogen peroxide are continuously generated inside the human body as a consequence of exposure to exogenous chemicals and a number of endogenous metabolic process involving redox enzymes and bioenergetics electron transfer [6].

Owing to the ROS overproduction and inadequate antioxidant defense, there is upsurge of ROS and this culminates in oxidative stress. It is quite interesting to note that plants have good antioxidant ability and are safer than the synthetic antioxidants. The antioxidant activity can be contributed to various mechanisms like prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxidise, reductive capacity and radical scavenging activity [6].

Hyperlipidemia is one of the important risk factor involved in the development of cardiovascular diseases. Atherosclerosis and congestive heart diseases (CHD) are strongly associated with abnormal lipid metabolism and plasma lipoproteins. Triton WR-1339 treated rats are considered to be a useful acute hyperlipidemic model associated with inactive lipoprotein lipase.

Triton WR-1339 acts as a surfactant to block the uptake of lipoprotein from the circulation by extra hepatic tissue resulting in an increased level of circulatory lipoproteins [23]. Triton WR-1339 induced hyperlipidemic rats treated with SCAE produced reversal of increase in serum cholesterol, triglycerides, LDL and VLDL whereas increased serum HDL levels was found.

The main causative factor for atherothrombotic disease is the disturbances occurring in lipid metabolism. Despite the presence of different hypolipidaemic drugs in the market, their therapeutic application is usually associated with severe side effects. Hence, efforts are being made to find safer and more efficient anti-hyperlipidaemic drugs. In that respect, medicinal plants have been considered as promising resources for the discovery of new drugs for hyperlipidemia [24].

Hence SCAE was evaluated in high fat diet induced hyperlipidaemia. In this chronic model serum TC, TG, VLDL-C and LDL-C levels were increased which are the causative agents to promote atherosclerosis and other cardiovascular diseases. LDL carries cholesterol from liver to the peripheral cells and smooth muscle cells of the arteries. A rise in LDL-C may cause deposition of cholesterol in arteries and aorta and hence it is direct risk factor for CHD [25].

In the present study, acute and chronic models of hyperlipidemia were used to evaluate antihyperlipidemic activity whereas superoxide dismutase and catalase, oxidative markers were measured to evaluate antioxidant activity. SCAE shown significant antioxidant activity which can be contributed the presence of flavonoids and other phytoconstituent.

CONCLUSION

The present study revealed that aqueous extracts of *Sida cordifolia Linn.* reversed the hyperlipidemia induced by triton and HFD in experimental animals demonstrating promising antihyperlipidemic activity. Whereas by significantly affecting oxidative markers like SOD and catalase, extract have shown promising antioxidant activity. *Sida cordifolia Linn.* might have exhibited antihyperlipidemic activity by virtue of its antioxidant potential.

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Table 1: Effect of SCAE on serum lipid profile in triton-induced hyperlipidemic rats

Groups	TG (mg/dl)	TC (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)	Atherogenic index
Normal Control	57.83 ± 1.25	64.00 ± 2.02	35.83 ± 1.17	14.33 ± 1.12	26.17 ± 2.40	2.57 ± .27
HL Control (P_1)	158.33 ± 3.71 ^{c}	115.17 ± 2.45 ^{c}	66.00 ± 1.93 ^{c}	32.00 ± 1.65 ^{c}	19.67 ± 1.20 ^{ns}	5.94 ± 0.30 ^{c}
Simvastatin (P_2)	70.00 ± 2.49 ^{c}	89.33 ± 2.44 ^{c}	48.83 ± 1.83 ^{c}	19.33 ± 1.76 ^{c}	24.50 ± 1.31 ^{ns}	3.71 ± 0.27 ^{c}
SCAE 200 (P_2)	90.17 ± 2.24 ^{c}	84.83 ± 3.52 ^{c}	49.50 ± 1.61 ^{c}	26.00 ± 1.97 ^{ns}	22.67 ± 1.58 ^{ns}	3.83 ± 0.32 ^{c}
SCAE 400 (P_2)	74.17 ± 1.87 ^{c}	73.33 ± 2.50 ^{c}	38.67 ± 2.08 ^{c}	19.33 ± 1.65 ^{c}	26.83 ± 1.49 ^{a}	2.78 ± 0.19 ^{c}

Values are expressed as Mean ± SEM, n = 6, *P* Values: ^{a} <0.05; ^{b} <0.01, ^{c} <0.001; ns Non significant, P_1: Compared to Normal control; P_2: Compared to Hyperlipidemic rats

Table 2: Effect of SCAE on serum lipid profile in HFD-induced hyperlipidemic rats

Groups	TG (mg/dl)	TC (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)	Atherogenic index
Normal Control	55.62 ± 2.90	45.31 ± 2.57	37.52 ± 2.07	17.48 ± 1.44	28.08 ± 1.73	1.63 ± 0.10
HL Control (P_1)	91.77 ± 2.70 ^{c}	138.95 ± 2.22 ^{c}	89.93 ± 2.57 ^{c}	32.78 ± 2.20 ^{c}	18.67 ± 1.83 ^{b}	7.79 ± 1.77 ^{c}
Simvastatin (P_2)	66.54 ± 2.04 ^{c}	58.13 ± 2.20 ^{c}	42.43 ± 1.40 ^{c}	20.79 ± 0.98 ^{c}	22.97 ± 1.89 ^{ns}	2.66 ± 0.36 ^{c}
SCAE 200 (P_2)	72.64 ± 1.89 ^{c}	82.99 ± 1.73 ^{c}	60.28 ± 1.45 ^{c}	26.57 ± 1.93 ^{ns}	23.86 ± 1.35 ^{ns}	3.55 ± 0.25 ^{c}
SCAE 400 (P_2)	63.24 ± 2.27 ^{c}	57.58 ± 2.35 ^{c}	44.37 ± 1.54 ^{c}	18.87 ± 0.94 ^{a}	25.66 ± 2.08 ^{ns}	2.29 ± 0.11 ^{c}

Values are expressed as Mean ± SEM, n = 6, *P* Values: ^{a} <0.05; ^{b} <0.01, ^{c} <0.001; ns Non significant, P_1: Compared to Normal control; P_2: Compared to Hyperlipidemic rats

Table 3: Effect of SCAE on serum oxidative enzymes level in triton-induced hyperlipidemic rats

Groups	SOD (U/ml)	CAT (U/ml)
Normal Control	5.02 ± 0.24	18.16 ± 1.43
HL Control (P_1)	3.19 ± 0.16 ^{c}	8.82 ± 0.68 ^{c}
Simvastatin (P_2)	4.88 ± 0.25 ^{b}	15.88 ± 1.33 ^{b}
SCAE 200 (P_2)	3.61 ± 0.25 ^{ns}	12.74 ± 1.10 ^{ns}
SCAE 400 (P_2)	4.60 ± 0.41 ^{a}	17.38 ± 0.91 ^{a}

Values are expressed as Mean ± SEM, n = 6, *P* Values: ^{a} <0.05; ^{b} <0.01, ^{c} <0.001; ns Non significant, P_1: Compared to Normal control; P_2: Compared to Hyperlipidemic rats

Table 4: Effect of SCAE on serum oxidative enzymes level in HFD-induced hyperlipidemic rats

Groups	SOD (U/ml)	CAT (U/ml)
Normal Control	5.10 ± 0.32	20.29 ± 1.56
HL Control (P_1)	4.32 ± 0.50 ^{ns}	9.48 ± 0.75 ^{c}
Simvastatin (P_2)	4.82 ± 0.40 ^{ns}	19.39 ± 1.48 ^{c}
SCAE 200 (P_2)	3.74 ± 0.35 ^{ns}	14.71 ± 1.15 ^{ns}
SCAE 400 (P_2)	4.69 ± 0.67 ^{ns}	19.40 ± 1.50 ^{c}

Values are expressed as Mean ± SEM, n = 6, *P* Values: ^{a} <0.05; ^{b} <0.01, ^{c} <0.001; ns Non significant, P_1: Compared to Normal control; P_2: Compared to Hyperlipidemic rats

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