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Assessment of Lipid Lowering Effect of *Sida rhomboidea.Roxb* Methanolic Extract in Experimentally Induced Hyperlipidemia

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ABSTRACT

This inventory scrutinizes the effect of methanolic extract of *Sida rhomboidea.Roxb* (SR) on high fat diet-induced hyperlipidemia in male *Charles foster* rats. The changes in body weight, food and water intake, feed efficiency ratio and whole weight of liver and epididymal fat pad were recorded in both control and experimental groups. In addition to the above parameters, the changes in plasma lipid profile, hepatic lipid profile, fecal total lipids (TL), total cholesterol (TC), triglycerides (TG), cholic acid (CA) and deoxycholic acid (DCA) and activity levels of plasma SGOT and SGPT were also assessed in both control and experimental groups. Plasma TC, TG, low density lipoproteins (LDL), very low density lipoproteins (VLDL) and free fatty acids (FFA) levels were decreased along with significantly increased plasma HDL levels. Hepatic TL, TC, TG were significantly decreased in HL+SR400 group, whereas fecal TL, TC, TG, CA and DCA contents were higher after feeding SR to hyperlipidemic rats, probably due to reduced intestinal absorption and effective elimination of TC, TG and bile acids through feaces. The results obtained with methanolic extract of SR are in accordance with our previous study using crude extract. Hence, this study further adds to the existing information that the polar fraction of SR is equally potent as the crude extract. This report provides an ideal platform for the isolation, purification and characterization of known bioactive/novel compounds present in the polar fraction of SR.

Key words: Hypercholestremia, hypertriglyceridemia, *Sida rhomboidea.*Roxb

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INTRODUCTION

Hypertriglyceridemia and hypercholesterolemia are established causative factors for the development of atherosclerosis and cardiovascular diseases.[1] A decrement of 20% in plasma TC can reduce not only 31% risk of coronary artery disease, but also the mortality rate by approximately 33%.[2] Several synthetic hypocholesteromic agents such as statins, fibrates, resins and nicotinic acid are capable of efficiently reducing plasma TC levels,[3,4] but low-density lipoprotein (LDL) does not undergo any significant alteration.[5] Also, synthetic hypolipidemic agents have one or more side effects and are unable to increase HDL levels.[6] Recent studies have shown that many compounds of herbal origin are able to reduce plasma TG and TC levels and elevate HDL.[7-9] These attribute in reducing the risk of cardiovascular disease (CVD).

Sida rhomboidea.Roxb. (SR, family Malvaceae) is known as "Mahabala" in ayurveda and is found throughout India.[10] It is used in many parts of North Eastern India as a home remedy against obesity and diabetes. Phytochemical analysis

of the aerial parts have shown presence of n-alkanes, long-chain alcohols, sterols, ephedrine, sterculic acid, linoleic acid, phenyl ethylamine, cellulose and lignin.[11,12] It has been shown to have antiinflammatory, hepatoprotective, antibacterial effects and antinociceptive properties.[13-16] It is also considered beneficial in controlling fever, cardiovascular disease and urinary disorders.[17]

Previous studies from our laboratory have documented that aqueous leaf extract of SR has lipid lowering[18] and anti hypertriglyceridemic properties[19] , whereas methanolic extract of SR has shown to have antioxidant and free radical scavenging properties (Unpublished observations). Hence, this inventory is an effort to assess the effect of SR on the lipid profile of experimentally induced hyperlipidemia in male *Charles foster* rats.

MATERIALS AND METHODS

Plant collection and identification

*Sida rhombodiea.*Roxb (SR) leaves were collected from Imphal district of India in the month of June. The plant was identified at the Department of Botany, D. M. College of Science, Manipur, Imphal and a sample (voucher specimen No.216) was deposited at the herbarium of the Department of Botany.

Preparation of methanolic extract

The leaves of the plant were washed and rinsed with tap water and shade dried. The dried leaves were subjected to extraction using methanol in soxhlet apparatus and resultant filtrate was concentrated under reduced pressure by rotary evaporator (Buchi, Germany). A semisolid paste obtained by this process was stored at 0°C. The extractive value of the SR was 21% w/w, which was further dissolved in 0.5% carboxy methyl cellulose (CMC).

Experimental animals

Male *Charles foster* albino rats (220-250 g) were housed and maintained in clean polypropylene cages under controlled room temperature (22 ± 2ºC) and were fed with commercially available rat chow (SLD (Normal diet); M/s Pranav Agro Ltd., Vadodara, India) or high fat diet (HFD)[20] and provided with water *ad libitum*. Experiments on animals were performed in accordance with guidelines of the institutional animal ethical committee (Approval no. 827/ac/04/CPCSEA).

Diet composition

	Normal diet (%)	High fat diet (%)
Wheat flour	45.0	43.9
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

Experimental design

Group-I Control (CN): Animals were fed with SLD and received 0.5% CMC (via gastric intubation) for 42 days.

Group-II Hyperlipidemic (HL): Animals were fed with HFD and 0.5% CMC (via gastric intubation) for 42 days.

Group-III (HL+SR400): Animals were fed with HFD and received methanolic extract of SR (400 mg/kg BW) orally via gastric incubation for 42 days.

Group IV (HL+SS): Animals were fed with HFD and received Simvastatin (10 mg/kg BW) orally via gastric incubation for 42 days.

At the end of the experimental period, overnight fasted animals (for 12 h) were given mild ether anesthesia and blood was collected by retro orbital sinus puncture in EDTA-coated vials. Plasma was obtained by cold centrifugation (4°C) of the vials for 10 min at 3000 rpm. Later animals were sacrificed by decapitation and liver, pancreas, brain and epididymal fat pad were excised and stored at -80°C for further estimations.

Lipid profile

Plasma TC, TG and HDL were analyzed using commercially available kits (Eve's diagnostic, Vadodara, India). VLDL and LDL were calculated by Friedewald's formula.[21] Hepatic and fecal lipids were extracted using chloroform: methanol (2:1) mixture and total lipids were estimated gravimetrically. Dried lipid extract was dissolved in 1% triton X 100[22] and TC and TG were analyzed using the above mentioned kits.

Fecal cholic acid and deoxycholic acids (CA and DCA)

Fecal samples from each group were collected on every third day between days 31 and 42. Dried fecal samples were

eluted with absolute alcohol, filtered and CA and DCA were estimated by the method of Mosback *et al*.[23]

Liver function assessment

Activity levels of serum glutamate oxaloacetate transferase (SGOT, EC 2.6.1.1) and serum glutamate pyruvate transferase (SGPT, EC 2.6.1.2) were assayed in plasma of control and experimental rats using kits (Eve's diagnostic, Vadodara, India).

Statistical analysis

Statistical evaluation of the data was done by one way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. The results are expressed as mean ± SEM using Graph Pad Prism version 3.0 for Windows, Graph Pad Software, San Diego, CA, U.S.A.

RESULTS

Body weight and food intake

HL group recorded higher body weight gain and feed efficiency ratio compared to CN group (51% and 66.73%). However, there was no significant change in food intake of HL *vs* CN group but was decreased in HL + SR400 (22.38%). HL + SR400 group recorded 36.71% decrement in body weight gain coupled with 46.38% decrement in feed efficiency ratio. Although water intake was decreased by about 18.46% in HL + SR400 group, it was unchanged in other groups. HL + SS group recorded no significant change in food and water intake, but recorded decrement in weight gain and feed efficiency ratio (22.06% and 9.54%) as compared to HL group [Table 1].

Liver and epididymal fat pad weight

The weight of epididymal fat pad recorded an increment of 37.65% in HL control group, while there was a decrement of 56.79% in HL+SR group compared to the HL group. The weight of liver increased by 26.54% in HL control group compared to the CN group, whereas it was decreased by 18.48% in HL + SR group compared to HL control group. HL+SS group was also found to exert favorable effect, but was not as effective as SR400. HL+SS group recorded 27.63% decrement in epididymal fat pad weight, but there was no significant change in liver weight as compared to HL group [Table 1].

Plasma lipid profile

HL group recorded significantly elevated levels of TC (48.61%), TG (56.08%), FFA (37.70%), LDL (65.35%) and VLDL (56.12%) while HDL level was significantly decreased (37.55%) compared to CN group. However, HL+SR400 group recorded significant decrease in TC (44.99%), TG (45.89%), FFA (15.67%), LDL (45.37%) and VLDL (45.49%), while HDL level was elevated (44.58%) compared to HL group. HL+SS group recorded decreased levels of TC (30.94%), TG (33.57%), LDL (27.62%) and VLDL (33.70%) while there was no significant change in FFA and HDL levels compared to the HL group. LDL/HDL ratio (Atherogenic index) was highest (7.28) in HL group followed by HL+SS (5.14) and HL+SR400 groups (2.2) and, was the least in the CN group (1.97; Table 2).

Hepatic TL, TC and TG levels

Hepatic total lipids (TL), TC and TG contents were higher (42.22, 50.29, and 55.80%) in HL group compared to CN group. HL+SR400 group recorded significant decrement in TL, TC and TG levels (32.15, 35.40 and 43.71%) compared to HL+SR 400 group. HL+SS group recorded decreased levels of TL, TC and TG (23.43, 19.42 and 34.54%) compared to the HL group [Table 3].

Fecal lipids

The TL, TC, TG, CA, and DCA levels eliminated through

Table 1: Effect of SR on food intake, water intake, weight gain, feed efficiency ratio, liver and epididymal fat pad weight

Parameters	CN	HL	HL+SR400	HL+SS
Food intake$	11.20 ± 0.37	11.61 ± 0.66^{NS}	9.02 ± 0.56^{a}	10.37 ± 0.55^{ns}
Water intake@	24.40 ± 0.50	24.28 ± 1.13^{NS}	22.28 ± 1.66^{ns}	23.30 ± 0.50^{ns}
Weight gain¥	25.09 ± 3.09	51.75 ± 4.70^{B}	32.75 ± 5.87^{a}	40.33 ± 3.22^{ns}
Feed efficiency Ratio	2.24 ± 017	4.40 ± 0.27^{C}	3.23 ± 0.21^{b}	3.98 ± 0.18^{ns}
Liver weight†	9.02 ± 0.39	12.28 ± 0.43^{C}	10.01 ± 0.49^{b}	11.01 ± 0.12^{a}
Epididymal fat Pad weight†	6.97 ± 0.41	11.18 ± 0.45^{C}	4.83 ± 0.56^{c}	8.09 ± 0.44^{c}

Values are mean ± SEM (n=6), where, $ = g/day, @ = ml/day and ¥ = g and † = gm/100 gm BW. A = *P*<0.05, B = *P*< 0.01, C = *P*< 0.001 and NS = non significant when, CN vs HL. a = *P*<0.05, b = *P*< 0.01, c = *P*< 0.001 and ns = non significant when, HL vs HL+SR400 and HL+SS

Table 2: Effect of SR on plasma lipids, lipoprotein profile and Atherogenic Index (AI)

Parameters	CN	HL	HL+SR400	HL+SS
TC†	45.78 ± 3.45	89.09 ± 5.87^{C}	49.00 ± 4.99^{c}	62.08 ± 2.02^{b}
TG†	53.09 ± 5.78	120.90 ± 5.42^{C}	65.90 ± 5.51^{c}	80.31 ± 2.18^{c}
FFA†	38.17 ± 1.85	61.27 ± 1.02^{B}	51.67 ± 1.82^{b}	58.02 ± 0.72^{a}
HDL†	21.89 ± 1.98	13.67 ± 1.89^{A}	24.67 ± 2.27^{b}	14.02 ± 2.20^{ns}
LDL†	34.50 ± 1.99	99.6 ± 2.01^{C}	54.41 ± 2.40^{c}	72.09 ± 1.28^{c}
VLDL†	10.61 ± 1.35	24.18 ± 1.78^{C}	13.18 ± 1.60^{b}	16.03 ± 0.80^{b}
AI	1.57	7.28	2.20	5.14

Values are mean ± SEM (n=6), where, † = mg/ dl. A = P<0.05, B = P< 0.01, C = P< 0.001 and NS = non significant when, CN vs HL. a = P<0.05, b = P< 0.01, c = P< 0.001 and ns = non significant when, HL vs HL+SR400 and HL+SS

Table 3: Effect of SR on hepatic and fecal lipid profile

Samples	Parameters	CN	HL	HL+SR400	HL+SS
Liver	TL$	52.93 ± 0.59	91.61 ± 0.85^{C}	62.15 ± 1.56^{c}	70.22 ± 1.44^{c}
	TC$	10.98 ± 044	22.09 ± 0.99^{C}	14.27 ± 1.31^{b}	17.80 ± 0.43^{ns}
	TG$	21.89 ± 0.29	49.53 ± 0.35^{C}	27.88 ± 1.04^{c}	32.42 ± 0.98^{c}
Feaces	TL@	42.18 ± 0.85	45.79 ± 0.60^{A}	58.83 ± 0.97^{c}	51.40 ± 0.22^{c}
	TC@	12.34 ± 0.63	14.91 ± 0.60^{A}	19.07 ± 0.47^{b}	15.18 ± 0.20^{ns}
	TG@	9.84 ± 0.24	10.88 ± 0.25^{A}	22.01 ± 0.82^{c}	16.22 ± 0.18^{c}
	CA‡	29.87 ± 0.88	40.07 ± 3.35^{A}	55.90 ± 2.29^{b}	45.47 ± 1.18^{ns}
	DCA‡	35.40 ± 1.05	35.43 ± 1.80^{NS}	53.52 ± 1.78^{c}	40.40 ± 0.88^{a}

Values are mean ± SEM (n=6), where $ = mg/g tissue and @ = µg/gm, ‡ = mg/g feaces. A = P<0.05, B = P< 0.01, C = P< 0.001 and NS = non significant when, CN vs HL. a = P<0.05, b = P< 0.01, c = P< 0.001 and ns = non significant when, HL vs HL+SR400 and HL+SS

Table 4: Effect of SR on enzymes of hepatic function

Parameters	CN	HL	HL+SR 400	HL+SS
SGOT†	82.68 ± 4.90	123.09 ± 5.13^{C}	71.08 ± 4.08^{c}	102.52 ± 4.13^{a}
SGPT†	35.98 ± 3.09	53.27 ± 2.90^{B}	31.160 ± 3.86^{b}	45.50 ± 2.26^{ns}

Values are mean ± SEM (n=6), where † = unit/L. A = P<0.05, B = P< 0.01, C = P< 0.001 and NS = non significant when, CN *vs* HL. a = P<0.05, b = P< 0.01, c = P< 0.001 and ns = non significant when, HL *vs* HL+SR400 and HL+SS

feaces of HL group were higher by 7.88 17.23, 9.55 and 25.45%, respectively, compared to the CN group. There was no significant change in fecal DCA level in HL group compared to the CN group. The fecal TL (22.16%), TG (50.56%), TC (21.81%), CA (28.31%) and DCA (33.8%) levels eliminated through the feaces were higher in HL+SR400 group compared to HL group. HL+SS group recorded increased levels of TL, TG, and DCA (10.91, 32.92 and 12.30%) while there were no significant changes in TC and CA levels as compared to HL group [Table 3].

Liver function assessment

Activity levels of plasma SGOT and SGPT were significantly elevated (32.82 and 32.45%) in HL control group compared to the CN group and significantly decreased (42.25 and 41.50%) in HL+SR400 group compared to the HL control group. HL+SS group recorded 16.7% decrement in SGOT levels whereas; there was no significant change in SGPT levels as compared to HL group [Table 4].

DISCUSSION

This study deals with the effect of SR (400 mg/kg BW) on plasma and hepatic lipid profile and elimination of lipids through feaces in hyperlipidemic rats. There was a significant reduction in plasma and hepatic lipid profiles along with elevation in plasma HDL in HL+SR400-treated rats as compared to HL rats, thus indicating the efficacy of SR extract in preventing the elevation seen in various components of lipid profile under experimentally induced hyperlipidemia. Epidemiological studies have shown that a higher level of HDL in plasma reduces the risk of coronary artery disease (CAD).[24] It is also reported that HDL is potentially capable of inhibiting LDL oxidation, protecting vascular endothelial cells from cytotoxic effects of Ox-LDL and preventing the advent of atherosclerosis.[25] Flavanoids are reported to increase HDL concentration and decrease LDL and VLDL levels in hypercholesteremic rats.[26] Flavanoids and polyphenols found in our SR extract could therefore be considered favorable in increasing HDL and decreasing LDL and VLDL in HL+SR400-treated rats.

HMG Co-A reductase is the key metabolic enzyme for the *de novo* synthesis of cholesterol in liver.[27] Hence, low levels of hepatic TC observed in SR-treated rats could be due to the inhibition of HMG Co-A reductase activity.[18] Further, low levels of cholesterol in liver are known to

elevate LDL-R expression in hepatocytes and consequent withdrawal of LDL from circulation.[28] It can be assumed In this context that lowered LDL levels observed in HL+SR400 group could be attributed to an increased expression of LDL-R triggered by lowered hepatic TC levels. Hepatic cholesterol is catabolised into bile acids or converted to oxysterol that can stimulate cholesterol 7α hydroxylase to form bile acids.[29] Previous reports on anti-hyperlipidemic effect of some natural products suggested higher fecal bile excretion.[30] Hence, it can be speculated that HL+SR400 treatment may convert hepatic cholesterol into bile acids evidences by greater elimination of bile acids through feaces in the experimental group. Phytosterols are known to have cholesterol lowering property because of their greater affinity for micelles than cholesterol. This results in reduced incorporation of cholesterol into micelles[31,32] amounting to increased cholesterol elimination through feaces. Hence, high fecal cholesterol content recorded in HL+SR400 group in the present study could be attributed to a high content of phytosterol in the SR extract. Saponins have been correlated with inhibition of pancreatic lipase leading to reduced intestinal absorption and higher excretion of dietary fats.[33] Hence, high saponin content in our SR can be the possible reason for the higher elimination of TG through feaces of HL+SR400 treated rats. Previous studies have reported increased liver weight and significantly elevated plasma levels of SGPT and SGOT under conditions of hyperlipidemia.[34] Results recorded in this study are in accordance with these reports as HL rats have recorded higher liver weight and impaired liver marked by elevated activity levels of plasma SGPT and SGOT.[35] SR treatment is able to reduce liver weight and plasma SGPT and SGOT levels thus indicating its hepatoprotective activity. A comparison of results obtained in this study with the lipid lowering potential of Simvastatin suggest that, SR is more effective in elevating circulating HDL levels and lowering TG and TC levels and in maintaining hepatic TG metabolism.

The results obtained with methanolic extract of SR are in accordance with our previous study,[18] wherein a crude extract of SR has been reported to have a dose-dependent lipid lowering property by a similar mechanisms as envisaged herein. This study is an addition to the existing information that the methanolic extract consisting of polar fraction of SR is equally potent as the crude extract. It was inferred from our previous study [19] that 400 mg/kg BW of crude extract was the most efficient dose for lowering lipid and cholesterol levels in hyperlipidemic rats. Hence, this study further validates the efficacy of methanolic extract of SR in the same dose. This report also provides an ideal platform for the isolation, purification and characterization of known bioactive/novel compounds present in polar fraction of SR.

CONCLUSION

In continuation with the previous study from our lab, this report adds to the existing wealth of information on the possible therapeutic role of methanolic extract of SR in experimentally induced hyperlipidemia. A detailed investigation has been initiated in our laboratory to identify the active principles from methanolic extract. Hence, it can be concluded that *Sida rhomboidea*.Roxb methanolic extract has an excellent lipid lowering potential and also possess curative properties in conditions of hyperlipidemia and related disorders.

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