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Potential of *Sida rhomboidea.Roxb* Leaf Extract in Controlling Hypertriglyceridemia in Experimental Models

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ABSTRACT

The present study was undertaken to evaluate the antihypertriglyceridemic activity of *Sida rhomboidea.roxb* leaf extract (FESR) (200 and 400 mg/kg bodyweight) in Triton WR 1339 and oral lipid emulsion induced hypertriglyceridemia in rats. Plasma cholesterol (TC), Triglyceride (TG), HDL and Triglyceride secretion rate (TGSR) were assessed in control and Triton WR 1339 rats. TG profile in lipid emulsion treated rats was evaluated at an interval of 0, 3, 6, 9 and 12 hour post emulsion and area under curve (AUC) was calculated. FESR (200 & 400 mg/kg) treatment to Triton WR 1339 treated rats recorded significant decrement in plasma TC ($p<0.05$), TG ($p<0.05$) while HDL ($p<0.05$) was increased at 6th hour and 24th hour post Triton injection. Lowered levels of TGSR were recorded in Triton + FESR treated groups at 6th hour. In the second experiment oral lipid emulsion induced hypertriglyceridemia was significantly suppressed by FESR (200 & 400 mg/kg), indicated by lowered AUC values compared to its control (Lipid emulsion only). Results clearly substantiate the antihypertriglyceridemic potential of *S. rhomboidea. Roxb* leaf extract mediated via decreased intestinal absorption and increased catabolism of TG. The present study is of merit in providing pharmacological evidence for use of SR leaf extract as a folklore medicine for controlling obesity amongst north-eastern population of Indian subcontinent.

Keywords: *Sida rhomboidea. Roxb,* Hypertriglyceridemia, Hypercholesterolemia, Triton WR 1339.

INTRODUCTION

Hyperlipidemia & hypercholesterolemia are the key risk factors for Cardiovascular Disorders (CVD) (1) which has been reported as the most common cause of death in developed as well as developing nations (2, 3). Hypertriglyceridemia in combination with abnormally low concentrations of HDL cholesterol (High Density Lipoprotein Cholesterol) is one of the most common, atherogenic profile of lipid metabolism of high prevalence seen in Indian population (4). Since, synthetic drugs have been shown to have side effects, clinical importance of the herbal drugs has received considerable attention in recent years (5) as medicinal products of herbal origin have been reported to have hypolipidemic and hypocholesteremic properties (6, 7). WHO has in fact recommended use of indigenous plants as an alternative remedy especially in developing countries (8). *Sida rhomboidea.Roxb* (Syn. S. rhombifolia linn, fam.Malvaceae) is a shrubby weed found throughout India (9). In ayurveda it is known as "mahabala" (10). Studies have reported that the aerial parts have n-alkanes, long chain alcohols, sterol (11) ephedrine, sterculic acid,linoleic acid, phenyl ethylamines, cellulose and lignin (12). The extracts of the plant have been studied for various biological activities like anti-inflammatory, antipyretic, antibacterial, hepatoprotective and antinociceptive (13-16). It is also beneficial in fever, heart diseases and urinary disorders (9). A decoction

prepared from aerial parts of the plant is use as a folklore medicine against obesity, hypertension and diabetes in parts of North Eastern India. No pharmacological study relating the role of *S.rhomboidea. Roxb* in controlling hypertriglyceridemia has been reported so far. Present study focuses on possible anti hypertriglyceridemic activity of SR aqueous leaf extract in experimental models.

MATERIALS AND METHODS

Plant material and Extraction

SR leaves were collected from Imphal district India in month of June and shade dried. The plant was identified by Dr.Hemchand Singh, Taxonomist, Department of Botany, D.M.College of Science and a sample (voucher specimen No.216) was deposited at the herbarium of the Department of Botany. Leaves of SR were shade dried and powdered. Hundred gm of the leaf powder was boiled in distilled water at 100°C for 3 hours. Resulting filtrate was concentrated by heating till it formed a semisolid paste which was then freeze dried. The yield was 24% W/W. Different doses of freeze dried extract (FESR) were prepared by dissolving known quantity of FESR in 0.5% carboxymethyl cellulose.

Animals

Female *Charles foster* albino rats (220-250 g body weight) were housed in polypropylene cages and fed with standard pellet diet (Pranav agro Ltd, Baroda) and water *ad libitum*. The experimental protocol was according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, India and, approved by the animal ethical committee of Department of Zoology, The M.S University of Baroda, Vadodara (Approval No.827/ac/04/CPCSEA).

Triton WR 1339 induced Hypertriglyceridemia in rats

Rats were divided into four experimental groups (n=6) and administered Vehicle, FESR or lovastatin (LVS; 10mg/kg BW) (17) via gastric intubation for 3 consecutive days.

I. Vehicle (0.5% CMC)
II. Triton WR 1339 + Vehicle (0.5% CMC)
III. Triton WR 1339 + FESR (200mg/kg BW)
IV. Triton WR 1339 + FESR (400mg/kg BW)
V. Triton WR 1339 + LVS (10mg/kg BW)

On the fourth day, rats were fasted for 18 hours (water *ad libitum*) and were given single intravenous injection of Triton WR 1339 (200mg/kg bodyweight in 0.9% saline, Sigma USA) (18). Blood was collected at 0, 6 and 24 hour after injection of triton from the orbital sinus in EDTA coated vials. Plasma was obtained by cold centrifugation (4°C) of the vials at 3000 rpm for 10 min. Plasma Triglycerides (TG), Total cholesterol (TC), HDL levels were assessed using analytical kits (Reckon diagnostics ltd) using Autoanalyser (Merck, Micro lab L 300).TG secretion rate (TGSR) was calculated as described earlier (19).

Oral loading of lipid emulsion in rats

Effect of SR extract on absorption of fats was tested by oral fat loading method (20) with modification. Rats were divided into four experimental groups (n=6). Lipid emulsion consisted of 30% corn oil, 5% tween 20 and 65% Carboxy methyl cellulose (w/v) and was administered to control groups (EML), whereas SR extract (200 or 400mg/kgBW) was administered to groups II (EML+SR200) and III (SR400) and, group IV (EML+ORL50) received Orlistat (50 mg/kg BW; Biocon Ltd, Bangalore, India) along with emulsion. Blood samples were collected prior to subjecting animals to lipid emulsion load (0 hr) and post lipid emulsion load at 3, 6, 9 and 12 hours. Plasma TG levels assessed using analytic kits (as mentioned above) and area under curve (AUC; 0-9hr) was calculated for each experimental groups (21).

Statistical analysis

All the values are expressed as mean ± S.E.M using Graph pad prism version 3.0 for windows, Graph pad software, San Diego California USA and data was analyzed statistically by one way ANOVA post Boneferroni's multiple comparison test. $P<0.05$ were considered significant.

RESULTS

Triton WR 1339 induced Hypertriglyceridemia in rats

Triton treated rats (TR) recorded significant elevation in Plasma TC (70%), TG (93%) and lowered HDL (39%) at 6th hr compared to control rats (CN). TR+SR200 and TR+SR400 groups recorded a significant decrease in plasma TC (18%, 35%), TG (42%, 54%) and increase in HDL (26%, 33%) compared to TR rats. These changes were compared to TR+LVS10 group.

There was a reduction in TC (24%), TG (38%) and HDL (5%) in TR group between 6th to 24th hour post triton while, TR+SR200 and TR+SR400 groups recorded 14% & 5% reduction in TC, 20% & 28% reduction in TG respectively and a marginal increase in HDL levels. TR+LVS10 group recorded a significant decrement in TC ($p<0.05$) and TG ($p<0.05$) levels but no change in

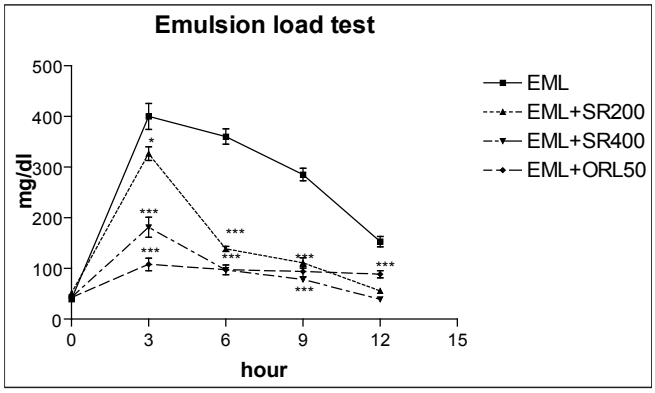

Figure 2. Effect of SR extract on Oral loading of lipid emulsion in rats.*Significant *= P<0.05 when EML Vs EML+SR200, EML+SR400 and EML+ORL50.*

HDL level. TGSR at 6 hours registered a significantly decrement in TR+SR200 (P<0.05), TR+SR400 (P<0.05) and TR+LVS10 group (p<0.05).

Oral loading of lipid emulsion in rats

A comparison of TG levels at 3 hour post lipid emulsion load recorded 90 % increase in EML rats while, EML+SR200, EML+SR400 and EML+ORL50 groups recorded 84 %, 76% and 61% increase respectively. EML+SR200, EML+SR400 groups registered 83% and 78% decrement in TG levels from 3rd to 12th hour post emulsion compared to 61% decrease in EML group (Fig. 2). AUC values were significantly decreased in EML+SR200 (p<0.05), EML+SR400 (p<0.05) and EML+ORL 50 compared to EML group.

DISCUSSION

Triton induced hypertriglyceridemia has been reported in several animal models (22) and have been used to test potential of Natural / chemical hypolipidemic drugs (23, 24). The large increase in plasma cholesterol and triglycerides due to Triton WR-1339 injection results mostly from an increase of VLDL secretion by the liver accompanied by a strong reduction of VLDL and LDL catabolism (25) due to inhibition of lipoprotein lipase. FESR was able to significantly lower plasma TC and TG in a dose dependent manner. Lipid lowering activity of FESR could be due to an increased stimulation of the lipolytic activity of Plasma lipoprotein lipase (LPL). A similar mechanism of lipid lowering has been reported in other plant (26, 27).

Reduction of plasma HDL cholesterol is a key factor in development of atherosclerosis and ischemic heart diseases (28). Decrement in HDL levels in TR treated groups corroborates previous reports (29) but a significant increment in HDL levels in FESR treated groups clearly indicates its protective action. HDL/TC ratio in Triton induced hyperlipidemia represents the proportion of cholesterol component and may provide valid indices for calculation of cardiovascular risk (29, 30). High HDL/TC ratio after FESR treatment can be consider as an indicator of reduced cardiovascular risk compared to TR group. Such favourable changes in HDL levels however not recorded after LVS treatment. These observations could be considered significant because synthetic cholesterol lowering drugs lowers plasma TC but have no significant positive effect on plasma HDL levels (31).

Table 2. Effect of SR extract and Lovastatin on plasma Total cholesterol (TC), Triglycerides (TG), and HDL at 24th hr post Triton WR 1339.

Groups	Treatment	TC(mg/dl)	TG(mg/dl)	HDL(mg/dl)	HDL/TC
1	Vehicle	39.08±2.89	45.35±8.65	24.67±0.78	0.63
2	TR	98.62±2.17###	433.7±15.70###	14.13±0.82###	0.15
3	TR+SR200	91.50±1.13*	300.8±10.93*	21.77±0.98*	0.21
4	TR+SR400	80.05±1.26***	215.2±16.63***	23.41±0.99**	0.28
5	TR+LVS10	81.99±1.24***	223.7±23.29***	14.19±0.50ns	0.17

*Significant # = P<0.05 when Group 1 Vs Group 2 and Significant *= P<0.05 when Group 2 Vs Group 2, 3, 4 & 5.*

Table 1. Effect of SR extract and Lovastatin on plasma Total cholesterol (TC), Triglycerides (TG), and HDL at 6th hr post Triton WR 1339.

Groups	Treatment	TC(mg/dl)	TG(mg/dl)	HDL(mg/dl)	HDL/TC
1	Vehicle	39.08±2.89	45.35±8.65	24.67±0.78	0.63
2	TR	130.0±2.61###	655.2±11.06###	14.98±1.03##	0.12
3	TR+SR200	106.9±2.37***	377.8±15.05***	20.33±1.14*	0.19
4	TR+SR400	84.3±1.41***	299.6±19.98***	22.38±1.47**	0.27
5	TR+LVS10	91.8±2.10***	308.9±12.31***	16.04±1.23ns	0.17

Significant # = P<0.05 when Group 1 Vs Group 2 and Significant *= P<0.05 when Group 2 Vs Group 2, 3, 4 & 5.

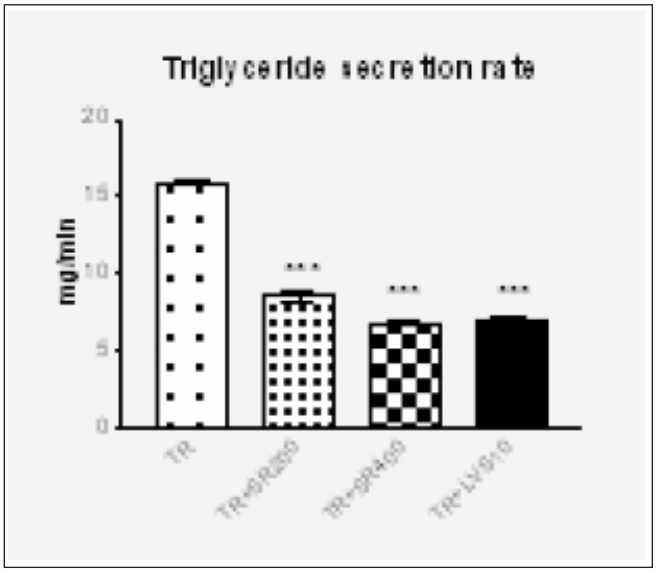

Figure 1. Effect of SR extract on triglyceride secretion rate at 6th hr post Triton WR 1339.*Significant *= P<0.05 when TR Vs TR+SR200, TR+SR400 and TR+LVS10.*

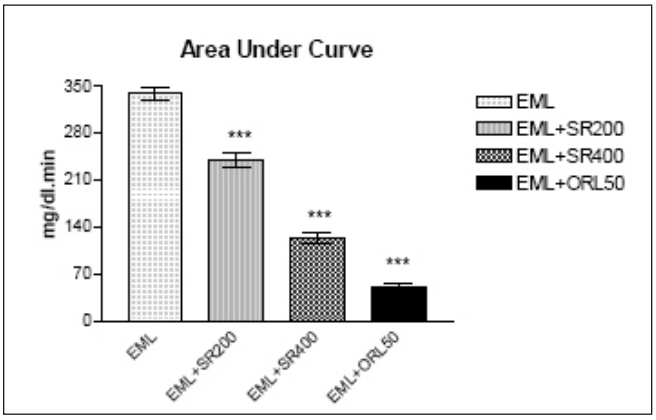

Figure 3. Effect of SR extract Area Under Curve during Oral loading of lipid emulsion in rats.*Significant *= P<0.05 when EML Vs EML+SR200, EML+SR400 and EML+ORL50.*

In the second experiment TG absorption and clearance have been demonstrated after the feeding of lipid emulsion to fasted rats. This experimental model is widely used to study effect of various drugs on intestinal absorption of TG (32). A close scrutiny of TG levels at 3hour stage indicates reduced fat absorption in SR treated rats compared to its control indicated by low AUC values in FESR treated rats. This observation can possibly be due to inhibition of pancreatic lipase leading to poor absorption of fats. This speculation is drawn on the basis of previous reports where plant extracts of Panax japonicus and *Actinidia arguta* have been shown to inhibit pancreatic lipase and reduce intestinal absorption of TG (33, 34) Orlistat is a well known pancreatic lipase inhibitor (35) and a comparison of its AUC values with that of FESR treated groups further strengthens this hypothesis. Antihypertriglyceridemic property of Platycodi Radix and *Acanthopanax senticosus* extracts has been attributed to presence of saponins (36, 37). Antihypertriglyceridemic properties of SR extract may be attributed to the presence of saponins (unpublished observation).Higher elimination of plasma TG (18-22%) in FESR group compared to EML group between 3 to 12 hour post lipid emulsion load can be due to increased activity of Plasma LPL.

CONCLUSION

It can be concluded from the present study that *S.rhomboidea.Roxb* extract prevents experimental hypertriglyceridemia via decreased intestinal absorption and increased catabolism of TG. This study is the first report on role of SR in controlling hypertriglyceridemia thus confirming its folklore use by North Eastern population for obesity.

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