

Full Length Research Paper

Therapeutic efficacy of *Achyranthes aspera* saponin extract in high fat diet induced hyperlipidaemia in male wistar rats

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Accepted 30 September, 2011

Achyranthes aspera Linn belonging to the family *Amaranthaceae* is used in the treatment of lipid disorders in the Indian system of medicine. The present study was undertaken to evaluate the hypolipidemic activity of saponin extract of *A. aspera* (SAA) at 1200 mg/kg body weight in male wistar rats fed on high fat (HF) diet for 8 weeks. Significant reduction ($p < 0.05$) in food efficiency ratio (FER), body weight gain, visceral organ weight indices, serum total cholesterol (TC), triglycerides (TG), very low density lipoproteins (VLDL-C), low density lipoproteins (LDL-C), atherogenic index (AI), hepatic TC and TG levels was observed in SAA treated rats when compared to HF diet alone fed rats. Significant elevation ($p < 0.05$) in levels of serum high density lipoproteins (HDL-C), fecal TC and TG was observed in SAA treated group when compared to HF diet alone fed group. These results suggest that SAA has both hypolipidemic and weight reducing effects on HF diet fed rats. This may be mediated through reduced absorption and elevated excretion of lipids by saponins of *A. aspera* extract.

key words: *Achyranthes aspera*, high fat diet, hyperlipidaemia, orlistat, saponins.

INTRODUCTION

Increased fat consumption has been associated with the risk of hyperlipidemia via alteration of total cholesterol (TC) and triglycerides (TG) levels in plasma and tissues (Estadella et al., 2004). On the other hand, elevated levels of plasma low density lipoproteins (LDL-C) and TG, accompanied by reduced high density lipoproteins (HDL-C) levels, is associated with an increased risk of cardiovascular diseases (CVDs). Consumption of diet with more fat accelerates the development of obesity and heart problems (Bowen and Borthakur, 2004).

In developing countries, the incidence of CVD is increasing alarmingly. India is on the verge of cardiovascular epidemics (Okrañec et al., 2004). The circulatory system disorders are going to be the greatest killers in India by the end of year 2015 (Kaul et al., 1998).

It is well established that increased levels of blood cholesterol especially LDL-C is an important risk factor for cardiovascular complications since it favours lipid deposition in tissues including blood vessels. Lipid lowering trials have clearly established that reduction of TC or LDL-C is associated with decreased risk of atherosclerosis and coronary heart disease (Brown et al., 1998; Grundy et al., 2004). Furthermore, epidemiological studies have also shown an inverse correlation between high HDL-C level and the risk of CVD (Wierzbicki, 2005).

Currently, available hypolipidemic drugs include statins and fibrates. The former corrects the altered blood lipid profile by inhibiting the biosynthesis of cholesterol and the later acts by enhancing the clearance of TG rich lipoproteins (Robert et al., 2006). However, consumption of these synthetic drugs leads to hyperuricemia, diarrhea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function (Kumar et al., 2008). In view of these adverse effects, the search for natural products with lipid-lowering potential and with minimal or no side

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effect is recommended. In recent times, research interest has focused on various herbs that possess hypolipidemic property that may be useful in reducing the risk of CVD (Craig, 1999). Because of their perceived effectiveness, minimal side effects and relative low cost, herbal drugs are prescribed widely even when their biologically active compounds are unknown (Valiathian, 1998).

Achyranthes aspera Linn (*Amaranthaceae*), popularly known as apamarga, is a commonly available plant in India and had claims in the treatment of hyperlipidaemia in ayurveda, an Indian system of medicine (Anil and Mahesh, 2009). Saponins from different plant sources were proved to have hypolipidemic activity (Bao et al., 2005; Ji et al., 2005; Rachh et al, 2010; Fang et al, 2007) and the presence of saponins was reported in *A.aspera* (Michl et al., 2000). Therefore, the present study was aimed to investigate the hypolipidemic activity of saponin extract of *A.aspera* (SAA) in high fat (HF) diet fed male Wistar rats.

MATERIALS AND METHODS

Plant material

A. aspera was collected from Tirumala hill region and the identification was confirmed with taxonomist, Department of Botany Sri Venkateswara University, Tirupati, AP, India. Voucher specimen was deposited for future reference.

Extraction of saponins

Saponins were extracted as per the method outlined by (Sivaramakrishna et al., 2005). The powdered whole plant material (500 g) was extracted with 70% ethanol by soxhlet method and was concentrated under vacuum. The dark colored residue was refluxed with n-butanol for 2 h and soluble constituents were separated by filtration. The n-butanol layer was sequentially washed with distilled water, alkali (2% KOH) and distilled water. Then, it was evaporated and dried under vacuum to obtain a dark green color powder (16 g). Charcoal treatment was given to the powder and the filtrate was further dried under vacuum to give saponin rich extract (8.1 g). The extract was tested for saponins by Libermann- Burchard test.

Acute toxicity study

The acute toxicity of 70% saponin rich ethanol extract of *A.aspera* (SAA) was evaluated in rats using the up and down procedure. Male rats weighing 150±10g received SSA starting at 120 mg/kg body weight orally by gavage. The animals were observed for toxic symptoms continuously for the first 4 h after dosing. The number of survivors was noted after 24 h and the animals were then maintained and observed daily for next 13 days for any further toxicity.

Experimental animals

Male wistar rats weighing 150±10 g were purchased from Sri Venkateswara Animal Agency at Bangalore, India. They were housed individually in polypropylene cages under hygienic and standard environmental conditions (25±2°C, 60-70% humidity, 12 h

light/dark cycle). The rats were fed on standard rodent pellet chow and acclimatized to the lab conditions for 2 weeks before commencement of the experiment. After acclimatization, the control groups were maintained on standard chow obtained from Hindustan Liver limited, Bombay and the experimental groups were fed on HF diet (carbohydrates 39%, fat 21.5%, protein 34.5% and vitamin and mineral mixture 5% AIN 93) obtained from NIN, Hyderabad, India. The treatment groups were orally administered with SAA at 120 mg/kg body weight or orlistat (OL) at 25 mg/kg body weight for 8 weeks once a day (between 8:00 AM to 10:00 AM).

Experimental design

The rats were randomly divided into five groups (n=6/group). Normal standard diet control (NDC), normal standard diet+saponin rich ethanol extract of *A. aspera* (ND+SAA), high fat diet control (HFDC), high fat diet+saponin rich ethanol extract of *A. aspera* (HFD+SAA) and high fat diet+ orlistat (HFD+OL). Body weights and food and water consumption in all groups were measured every day. Faecal samples were collected during the last 3 days of the experiment, weighed, and freeze-dried. Faeces were ground to produce a homogeneous powder and stored at -20°C until analysis. At the end of the feeding period, under sodium pentobarbital (60 mg/kg, i.p) anesthesia following 4-5 h of food deprivation blood was drawn from the heart into heparinized tubes and centrifuged at 2500 g for 15 min at 4°C. All serum samples were frozen at -70°C, and these samples were used to measure the biochemical parameters. After collecting the blood samples, the liver, kidney, perirenal fat and peritoneal fat pads were immediately excised and weighed.

Lipid profile and atherogenic index (AI)

Serum lipid parameters such as TC, TG and HDL-C levels were measured by enzymatic colorimetric methods using kits purchased from Kamineni life sciences, Pvt, Hyderabad, India. Very low density lipoproteins (VLDL-C) and LDL-C levels were calculated as per Friedewalds et al. (1972) formula. Hepatic and fecal lipids were extracted in chloroform: methanol (2:1) mixture and dried according to Folch et al. (1957). Dried lipid extract was dissolved in 1% triton X 100 as per the methodology (Thounaojam et al., 2009) and TC, TG were analyzed using above mentioned kits. AI was calculated as per Kayamori and Igarashi (1994) formula.

Statistical analysis

All values are expressed as mean ± standard deviation (SD). Data was analyzed by one-way analysis of variance (ANOVA), and then differences among means were analyzed using the Turkeys multiple comparisons post hoc-test. Differences were considered significant at p<0.05.

RESULTS

Toxicity study

Over the study duration of 14 days, there were no deaths recorded in the male and female animals up to 1.2 g/kg body weight of the 70% ethanolic extract of *A. aspera* orally. During the observation period, animals did not produce any variations in the general appearance. The acute toxicity study does not show any toxic symptoms,

Table 1. Effect of SAA and OL on body weight gain and FER in HF diet fed rats.

Parameter	NDC	ND+SAA	HFDC	HFD+SAA	HFD+OL
Initial weights (g)	148.83±8.81	148.5±6.74	149±9.42	148.5± 5.71	149.83±6.58
Final weights (g)	242.5±5.68 ^a	234.83±4.57 ^a	316.0±5.29 ^b	242±4.91 ^a	245±9.46 ^a
Body weight gain (g/8 weeks)	11.70±1.41 ^a	10.79±1.16 ^a	20.87±1.93 ^b	11.79±1.40 ^a	11.95±1.84 ^a
Percentage gain in body weight	66.29	58.24	108.72	71.38	78.19
FER	0.77±0.079 ^a	0.84±0.08 ^a	1.65±0.21 ^b	1.11±0.13 ^c	1.24±0.17 ^c

Values represent mean ± S.D of 6 rats. Values that share the same superscript do not differ significantly from each other ($p < 0.05$). NDC, Normal standard diet control; ND+SAA, normal standard diet + saponin rich ethanol extract of *A. aspera*; HFDC, high fat diet control; HFD + SAA, high fat diet+saponin rich ethanol extract of *A. aspera*; HFD + OL, high fat diet+ orlistat.

Table 2. Effect of SAA and OL on visceral organ weight indices (g/100g body weight) in HF diet fed rats.

Organ indices	NDC	ND+ SAA	HFDC	HFD+SAA	HFD+OL
Liver	4.00 ± 0.238 ^a	3.94 ± 0.115 ^a	5.02 ± 0.409 ^b	4.00 ± 0.489 ^a	4.155 ± .141 ^a
Kidney	0.96 ± 0.091 ^a	0.87 ± 0.068 ^a	1.10 ± 0.104 ^a	0.93 ± 0.043 ^a	0.95 ± 0.046 ^a
Peritoneal fat	1.48 ± 0.239 ^a	1.43 ± 0.278 ^a	5.58 ± 0.327 ^b	1.47 ± 0.144 ^a	1.67 ± 0.138 ^a
Perirenal fat	1.41 ± 0.211 ^a	1.33 ± 0.214 ^a	2.91 ± 0.302 ^b	1.39 ± 0.191 ^a	1.43 ± 0.129 ^a

Values represent mean ± S.D of 6 rats. Values that share the same superscript do not differ significantly from each other ($p < 0.05$). NDC, Normal standard diet control; ND+SAA, normal standard diet + saponin rich ethanol extract of *A. aspera*; HFDC, high fat diet control; HFD + SAA, high fat diet+saponin rich ethanol extract of *A. aspera*; HFD + OL, high fat diet+ orlistat.

changes in behavior, or mortality at 1.2 g/kg body weight dose. One tenth of this dose was used for the present study. All animals survived until the scheduled euthanasia and no gross pathological alteration was found in the internal organs.

Body weights and food efficiency ratio (FER)

Intake of HF diet for 8 weeks led to significant increase in body weight gain in HFDC rats when compared to NDC rats. In SAA or OL treated group, there was a significant reduction in body weight gain when compared to HFDC rats. Consumption of HF diet resulted in significant increase in FER when compared with the NDC rats. However, there was significant decrease in FER in SAA or OL treated group when compared to HFDC group ($p < 0.05$, Table 1).

Visceral organ weight indices

There was a significant increase in liver, kidney, peritoneal and perirenal adipose weight indices in HFDC rats when compared to NDC group. But under the influence of SAA or OL, significant decrease was observed when compared with HFDC group ($p < 0.05$, Table 2).

Serum lipids and atherogenic index (AI)

Consumption of HF diet substantially increased the levels

of serum TC, TG VLDL-C, LDL-C and substantially decreased the levels of HDL-C when compared to NDC rats. In contrast, SAA or OL treatment had significantly decreased the levels of TC, TG, VLDL-C, LDL-C and significantly increased the levels of HDL-C when compared to HFDC group ($p < 0.05$, Table 3). Intake of HF diet led to significant raise of AI in HFDC group when compared to NDC group. But in SAA or OL treated group, significant reduction in AI was observed when compared to HFDC group ($p < 0.05$, Table 3).

Hepatic and fecal lipids

Significant increase in hepatic TC and TG content was observed in HFDC group when compared to NDC group whereas there was a significant decrease in hepatic TC and TG content in SAA or OL treated group when compared to HFDC group. Fecal lipid analysis revealed that there was a significant elevation in TC and TG in the feces of SAA and OL group when compared to HFDC group ($p < 0.05$, Table 4).

DISCUSSION

The present study revealed that hypolipidemic activity of SAA in HF diet fed rats. The lipid lowering property of SAA is due to the decreased intestinal absorption and increased excretion of cholesterol and TG in feces.

Consumption of HF diet resulted in significant increase

Table 3. Effect of SAA and OL on serum lipid profile and AI in HF diet fed rats.

Parameter	NDC	ND+SAA	HFDC	HFD+SAA	HFD+OL
TC (mg/dl)	75.10±3.89 ^a	72.60±2.03 ^a	165.23±4.49 ^b	90.95±4.53 ^c	99.32±5.36 ^d
TG (mg/dl)	83.02±4.52 ^a	77.88±3.18 ^a	164.89±7.113 ^b	90.70±5.67 ^a	91.43±5.24 ^a
VLDL-C (mg/dl)	16.60±0.90 ^a	15.57±0.63 ^a	32.97±1.42 ^b	18.14±1.13 ^a	18.28±1.04 ^a
LDL-C(mg/dl)	18.50±3.810 ^a	16.09±1.89 ^a	110.56±4.19 ^b	35.30±3.33 ^c	44.05±6.21 ^d
HDL-C (mg/dl)	39.99±2.98 ^a	40.94±1.85 ^a	21.71±1.74 ^b	37.04±2.62 ^a	36.23±2.77 ^a
AI	0.88±0.12 ^a	0.77±0.079 ^a	6.63±0.49 ^b	1.46±0.16 ^c	1.77±0.27 ^c

Values represent mean ± S.D of 6 rats. Values that share the same superscript do not differ significantly from each other (P<0.05). NDC, Normal standard diet control; ND+SAA, normal standard diet + saponin rich ethanol extract of *A. aspera*; HFDC, high fat diet control; HFD + SAA, high fat diet+saponin rich ethanol extract of *A. aspera*; HFD + OL, high fat diet+ orlistat.

Table 4. Effect of SAA and OL on hepatic and fecal lipids in HF diet fed rats.

Parameter	NDC	ND+SAA	HFDC	HFD+SAA	HFD+OL
Hepatic lipids					
TC (mg/g)	14.16±0.64 ^a	12.22±0.72 ^b	29.70±1.17 ^c	17.03±1.25 ^d	18.34±0.78 ^e
TG(mg/g)	24.39±0.72 ^a	20.49±1.28 ^b	53.05±1.63 ^c	25.27±0.83 ^a	28.97±0.93 ^d
Fecal lipids					
TC (mg/g)	8.98±0.46 ^a	10.43±0.70 ^b	8.00±0.68 ^a	17.88±0.58 ^c	17.41±0.56 ^c
TG (mg/g)	9.05±0.63 ^a	11.64±0.67 ^b	10.32±0.73 ^{ab}	19.65±0.74 ^c	18.24±1.40 ^c

Values represent mean ± S.D of 6 rats. Values that share the same superscript do not differ significantly from each other (P<0.05). NDC, Normal standard diet control; ND+SAA, normal standard diet + saponin rich ethanol extract of *A. aspera*; HFDC, high fat diet control; HFD + SAA, high fat diet+saponin rich ethanol extract of *A. aspera*; HFD + OL, high fat diet+ orlistat.

in body weight gain, FER and visceral organ weight indices. In case of rats treated with SAA, remarkable reduction in the aforementioned parameters was observed when compared to HFDC rats. Increased FER in HF diet fed rats was due to palatability and energy density which would contribute to hyperphagia through reduced satiation signaling (Savastano and Covasa, 2005). Increase in body weight gain and organ indices was due to hyperphagic effect in HFDC rats. Reduction in body weight gain, FER and visceral organ weight indices in SAA treated rats was due to lowered lipid absorption in intestinal tract by the saponins leading to reduced fat accumulation in the whole body and visceral organs.

These results suggest that SAA has weight reducing property and is in line with Anil and Mahesh (2009) study. CVD is a major complication of hyperlipidemia and cause of death in the world, mainly due to atherosclerosis. Abnormal plasma lipids are risk factors for CVD (NIH, 2000). Evidences from lipid lowering trials have clearly established that reduction of TC or LDL-C is associated with decreased risk of atherosclerosis and coronary heart disease (Brown et al., 1998; Grundy et al., 2004). Earlier studies reported that the alcoholic extract of *A. aspera* lowered lipid profile in triton-induced hyperlipidemic rats. In addition, chronic administration of the same extract to normal rats, lowered serum lipids followed by significant reduction in the levels of hepatic lipids (Khanna et al.,

1992).

In the present study, SAA significantly reduced the levels of serum TC, TG, VLDL-C, and LDL-C and significantly increased the levels of HDL-C when compared to HFDC rats. This desirable effect was due to saponins which could prevent intestinal lipid absorption (Oakenfull and Sidhu, 1990). High TC or LDL-C concentrations are a risk factor for coronary heart disease (Woo et al., 2008). AI is used as a marker to assess the susceptibility of atherogenesis (Kottai et al., 2005). The clinical complications of atherosclerosis could be diminished when lipid concentration is lowered by hypocholesterolemic agents (Yang et al., 2006). In the present study, decreased serum lipids and AI implies that the SAA has protective role against CVDs.

Significant reduction in hepatic lipids and significant elevation in fecal lipids was observed in SAA treated rats when compared to HFDC rats. Saponins were known to precipitate cholesterol from micelles and interfere with enterohepatic circulation of bile acids making them unavailable for intestinal absorption of lipids (Oakenfull and Sidhu, 1990). In addition, they were also reported to inhibit pancreatic lipase in HF diet fed animals, leading to greater fat excretion due to reduced intestinal absorption of dietary fats (Han et al., 2002). In the present study, decreased levels of hepatic lipids were due to reduced intestinal absorption which resulted in elevated excretion

of lipids through feces in SAA treated rats.

Pancreatic lipase inhibition using OL has been widely used in the pharmacotherapy of morbid obesity. However, the effects of OL on the secretion of appetite regulating gastrointestinal hormones and appetite sensations are still debated (Ellrichmann et al., 2008). The present study indicated that the hypolipidemic activity of SAA is more effective and safer than OL in HF diet fed rats.

In conclusion, the SAA is a potent inhibitor of HF diet induced hyperlipidemia, over weight and prevents CVDs by reducing excess accumulation of body fat and altering the lipid profile in blood.

Abbreviations

SAA, Saponin extract of *A. aspera*; **HF**, high fat; **FER**, food efficiency ratio; **TC**, total cholesterol; **TG**, triglycerides; **VLDL-C**, very low density lipoproteins; **LDL-C**, low density lipoproteins; **AI**, atherogenic index; **HDL-C**, high density lipoproteins; **CVDs**, cardiovascular diseases; **OL**, orlistat; **NDC**, normal standard diet control; **ND**, normal standard diet; **HFDC**, high fat diet control; **HFD**, high fat diet.

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