Antihyperlipidemic potential of *Albizia amara* (Roxb) Boiv. bark against Triton X-100 induced hyperlipidemic condition in rats

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ABSTRACT

Background: The plant *Albizia amara* (Roxb.) Boiv. bark was used in traditional medical practices of India to treat cardiovascular diseases. Hyperlipidemia is the greatest risk factor of coronary heart disease. **Objective:** The objective of this study was to screen the potential of *A. amara* against the condition of hyperlipidemia in rats. **Materials and Methods:** The antihyperlipidemic activity of *A. amara* ethanolic extract (AAEE) was studied on Triton X-100 induced model of hyperlipidemia in rats. Hyperlipidemia in experimental rats was evidenced by an enhancement in the levels of serum cholesterol, triglycerides (TGs), low density lipoprotein (LDL), very LDL (VLDL) and decrease in high density lipoprotein (HDL). **Results:** AAEE showed significant antihyperlipidemic effect by lowering the serum levels of biochemical parameters such as a significant reduction in the level of serum cholesterol, TG (104.1 ± 3.39), LDL (48.2 ± 2.19), VLDL (20.81 ± 0.67) and increase in HDL (47.25 ± 2.05) level with an increase in a dose of AAEE (41.39 ± 1.24) < (47.25 ± 2.05), which was similar to the standard drug atorvastatin. The results of serum glutamate oxaloacetate transaminase and serum glutamate pyruvate transaminase also revealed that the plant extract was found to be safe on liver. Histopathological evaluation also revealed the positive effect of the plant extract. Preliminary phytochemical analysis revealed the presence of phytoconstituents such as saponins, glycosides and tannins. The preliminary chemical constituents stood as a strong evidence for the study. **Conclusion:** Summing up the evidences of the pragmatic study, we can conclude that the extract of *A. amara* (Roxb.) Boiv. Bark aids in declining the condition of hyperlipidemia in rats.

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INTRODUCTION

Hyperlipidemia is a metabolic disorder specifically characterized by alterations occurring in serum lipid and lipoprotein profile due to increased concentrations of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), Very LDL-C (VLDL-C) and triglycerides (TGs) with a concomitant decrease in the concentrations of high density lipoprotein cholesterol (HDL-C) in the blood circulation.[1] Hyperlipidemia characterized by hypercholesterolemia is the most prevalent indicator for susceptibility to cardiovascular diseases.[2] Although several factors, such as a diet high in saturated fats and cholesterol, age, family history, hypertension and lifestyle play a significant role in causing heart failure, the high levels of cholesterol particularly TC, TG, and LDL-C is mainly responsible for the onset of coronary heart diseases (CHDs). A 20% reduction of blood cholesterol level can decrease about 31% of CHD incidence, and 33% of its mortality rate.[3] Cardiovascular diseases are leading cause of death in both industrialized and developing nations. Disorders of oxidative stress are the prime factors for initiation and progression of these diseases.[4] A herbal approach for hypercholesterolemia is a wise choice with comparatively less adverse effects and is relatively inexpensive. They are also effective in reducing the lipid levels in the system.[5] The consumption

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of synthetic drugs may lead to complications such as hyperuricemia, diarrhea, nausea, myositis, gastric irritation, flushing, dry skin, and abnormal liver function. It has been reported that traditional systems boost the immune potential to act against various diseases.[6] This study mainly focuses on reducing the risk of developing ischemic heart disease or the occurrence of further cardiovascular disease or cerebrovascular disease in patients with hyperlipidemia.[7]

The solution of Triton X-100 has successfully been used to induce hyperlipidemia in rats in previous studies, and it was chosen as the hyperlipidemic model due to its convenience, reproducibility and availability.[8]

Albizia amara (Mimoseace) known as "oil cake tree" which is an endemic plant in dry areas of Tamil Nadu, Andhra and Karnataka in India is reported. *A. amara* is a small to moderate-sized, much-branched deciduous tree with smooth, dark green, scaly bark. It resembles the *Acacias,* but lacks thorns. Its root system is shallow and spreading. The leaves are pinnately compound, with 15-24 pairs of small, linear leaflets, on 6-15 pairs of pinnate. The yellow, fragrant and globose flowers are in clusters. They develop when the tree is almost leaf-less. Flowers pedicelled, yellow, fragrant, in 12-20 globose heads. Fruits are oblong pods, about 10-28 × 2-5 cm, light brown, puberulous, thin, and 6-8 seeded; seeds flattened, 8-13 × 7-8 mm. The seeds of *A. amara* were used as an astringent, treating piles, diarrhea, gonorrhea, leprosy, leucoderma, erysipelas, and abscesses. The leaves and flowers have been applied to boils, eruptions, and swellings, also regarded as an emetic and as a remedy for coughs, ulcer, dandruff, and malaria.[9,10]

No scientific report is available to date to validate these folkloric uses; hence, we are reporting the antihyperlipidemic activity of ethanolic extract of *A. amara.*

MATERIALS AND METHODS

Collection of plant material

Bark of *A. amara* was collected after authentication by Dr. K. Madhava chetty, Assistant professor, Department of botany, Sri Venkateswara University, Tirupati, AP, India. The bark was collected from the Chittore forest. The voucher number is ALBZ/AM/2013.

Preparation of plant extracts

The collected bark was washed thoroughly with water and dried in the shade. Ethanolic extract was obtained by extracting powder with 95% ethanol by Soxhlet extraction method for 72 h. After completion of the extraction, the solvent was removed by rotary evaporator method. The ethanolic extract was used for further antihyperlipidemic study.

Preliminary phytochemical analysis

The ethanol extract of *A. amara* was subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents; it revealed the presence of glycosides, saponins and tannins.[11]

Animals

The study was carried out after obtaining the Institutional Animal Ethics Committee approval number 769/2010/CPCSEA. Albino Wistar rats were used in this experiment. They were procured from Sainadh enterprises, Hyderabad, India. Animals were housed in poly acrylic cages maintained under standard conditions of 18°C ± 2°C and 12 h light/dark cycle. Animals had free access to standard chow diet and water, *ad libitum*.

Chemicals

Triton X-100 (a nonionic detergent, iso-octyl polyoxy ethylene phenol, formaldehyde polymer) was procured from Merck specialties Private Limited, Mumbai. Atorvastatin was obtained from Moral labs, Chennai. All other chemicals were of analytical grade and obtained locally.

Acute toxicity test

The acute toxicity tests were performed according to the Organization of Economic Cooperation and Development 423 guidelines.[12]

A. amara was found to be safe up to 2000 mg/kg body weight when administered orally. Two doses were selected for the study 250 mg/kg and 500 mg/kg.

Antihyperlipidemic studies

Induction of hyperlipidemia

Hyperlipidemia was induced in Wistar albino rats by single intraperitoneal injection of freshly prepared solution of Triton X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 h. The animals were divided into five groups of six rats each. The first group was given standard pellet diet, water and orally administered with 10% dimethyl sulfoxide (DMSO). The second group was given a single dose of triton administered at a dose of 100 mg/kg, i.p. After 72 h of triton injection, this group received a daily dose of 10% DMSO (p.o) for 7 days. The third group was administered a daily dose of *A. amara* ethanolic extract (AAEE) 250 mg/kg dissolved in 10% DMSO, p.o., for 7 days, after inducing hyperlipidemia. The fourth group was administered a daily dose of AAEE 500 mg/kg dissolved in 10% DMSO, p.o., for 7 days, after inducing hyperlipidemia. The fifth

group was administered with the standard atorvastatin 10 mg/kg, p.o. for 7 days.[13,14]

Collection of blood

On the 8th day, blood was collected by retro orbital sinus puncture, under mild ether anesthesia. The collected samples were centrifuged for 10 min, and then serum samples were collected and used for various biochemical experiments. The animals were then sacrificed, and the livers were collected.[15]

Histopathological study

Histopathological study was performed in all the groups of animals. The liver sections were isolated and preserved in 10% formalin.[16] The liver sections were then evaluated for any architectural changes.

Biochemical analysis

The serum extract was assayed for TG, TC, HDL, LDL, VLDL by using standard kits supplied by, Ang Strom PvT LtD, Vadodhara, India.

The fraction of LDL-C in the serum was calculated by using Friedewald's equation as follows:[17]
LDL-C = Total cholesterol − (HDL-C + VLDL-C)
VLDL-C = Triglyceride/5

Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) activities were also measured.

Statistical analysis

Results were presented as mean ± standard error of the mean. The significance of difference among the groups was assessed using one-way analysis of variance followed by Dunnett's test using Graph Pad PRISM Software and $P < 0.05$ was considered as significant.

RESULTS

The preliminary phytochemical screening revealed the presence of phytochemical constituents such as glycosides, saponins and tannins in the ethanolic extracts of *A. amara (Roxb) Boiv* [Table 1].

Acute toxicity studies

Ethanolic extract of *A. amara* did not produce any toxic symptoms or mortality up to the dose level of 2000 mg/kg body weight in rats, and hence the extract was considered to be safe and nontoxic for further pharmacological screening.

Effect of administration of Albizia amara ethanolic extract (250/500 mg/kg, p.o., once daily)/ atorvastatin (10 mg/kg, p.o., once daily) on serum lipid parameter levels in rats induced with Triton X-100

The effects of AAEE on lipid levels in treated rats are shown in Table 2 and Figure 1.

The results showed that the levels of TG, TC, LDL-c and VLDL-c in the HCD treated group were significantly higher ($P < 0.001$), while the level of HDL-c was significantly lower ($P < 0.001$), when compared to normal and other treated groups.

The data on serum lipid profile from groups of rats exposed to high cholesterol diet treated with various doses of AAEE (250 mg/kg), showed a significant decrease ($P < 0.001$) in serum parameters TG, TC, LDL, VLDL and increase in HDL ($P < 0.001$) and AAEE (500 mg/kg) showed significant decrease ($P < 0.001$) in TG, TC, LDL, VLDL and increase in HDL ($P < 0.001$), whereas atorvastatin shows significantly decrease ($P < 0.001$) in TG, TC, LDL, VLDL and increase in HDL ($P < 0.001$) when compared

Table 1: Phytochemical screening of ethanolic extract of *Albizia amara* bark

Chemical constituent	*Albizia amara*
Alkaloid	Negative
Glycoside	Positive
Saponins	Positive
Carbohydrates	Negative
Tannins	Positive
Flavonoids	Negative
Steroids	Negative
Amino acids	Negative

Table 2: Effect of AAEE on serum lipid profile in hyperlipidemic rats

Group	HDL	LDL	VLDL	TG	TC
Normal	41.72±0.53	25.06±0.89	15.37±0.55	76.83±2.77	82.15±1.02
Triton X-100 treated	26.2±0.63^c	92.30±1.16^c	31.96±0.75^c	159.8±3.68^c	150.5±0.91^c
Triton+AAEE 250 mg/kg	41.39±1.24^r	62.99±4.32^r	23.88±0.46^r	119.8±2.30^r	128.3±3.93^q
Triton+AAEE 500 mg/kg	47.25±2.05^r	48.2±2.19^r	20.81±0.67^r	104.1±3.39^r	116.3±3.4^r
Triton+standard atorvastatin	55.62±2.47^r	40.41±1.99^r	18.17±0.49^r	90.86±2.46^r	114.12±3.86^r

Values are expressed as mean±SEM (n=6 animals in each group). *P* values: ^{a}<0.05, ^{b}<0.01, ^{c}<0.001 as compared with normal group. *P*<0.05, ^{q}<0.01, ^{r}<0.001 as compared with triton treated group. SEM: Standard error of mean, AAEE: *Albizia amara* ethanolic extract, HDL: High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein, TG: Triglyceride, TC: Total cholesterol

with that of Triton X-100 treated group. The effect of AAEE as a feed supplement at two doses, that is 250, 500 mg/kg, resulted in a dose-dependent reduction in lipid profiles.

Effects of Albizia amara ethanolic extract on low density lipoprotein/high density lipoprotein and atherogenic index

As depicted from Table 3 and Figure 2, it was found that the triton treated groups markedly elevated levels of LDL/HDL and atherogenic index compared to normal and other treated groups. The supplement of AAEE shows dose dependent attenuated changes.

Effects of Albizia amara ethanolic extract on serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase and Alkaline phosphatases

The effects of AAEE on SGPT, SGOT and ALP levels in treated rats are elucidated in Table 4 and Figure 3.

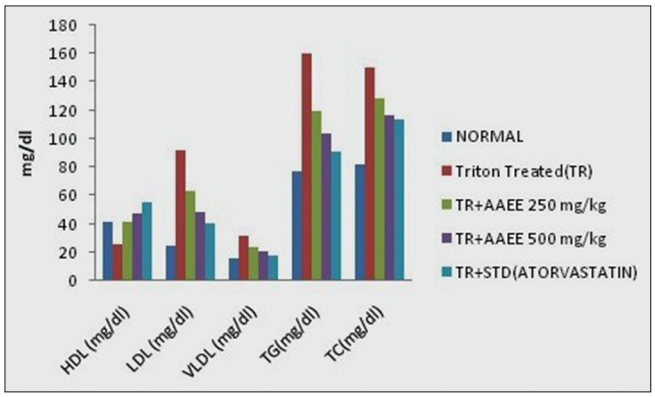

Figure 1: Effect of administration of *Albizia amara* ethanolic extract (250/500 mg/kg, p.o., once daily)/atorvastatin (10 mg/kg, p.o., once daily) on serum lipid parameter levels in rats induced with Triton X-100

The results showed that the levels of SGPT, SGOT and Alkaline phosphatases in the triton treated group were significantly higher ($P < 0.001$) when compared to normal and other treated groups. The AAEE treated groups significantly lowered ($P < 0.001$) the increased SGPT, SGOT, and ALP levels.

Effects of Albizia amara ethanolic extract on liver histopathology of triton induced hyperlipidemic condition

As evident from Figure 4a, the histopathology of liver in rats fed with Triton X-100 showed necrosis of liver cells

Table 3: Effect of AAEE on various biological parameters like LDL-C/HDL-C ratio and atherogenic index in hyperlipidemic rats

Group	LDL/HDL	Atherogenic index
Normal	0.601	0.264
Triton treated	3.528	0.785
Triton+AAEE 250 mg/kg	1.527	0.461
Triton+AAEE 500 mg/kg	1.022	0.344
Triton+standard atorvastatin	0.725	0.213

AAEE: *Albizia amara* ethanolic extract, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol

Table 4: Effect of AAEE on SGOT, SGPT and ALPs in hyperlipidemic rats

Group	SGOT (IU/L)	SGPT (IU/L)	ALPs
Normal	56.06±0.84	52.1±1.89	98.3±1.61
Triton treated (TR)	83.58±1.53^{c}	87.9±2.31^{c}	305.81±4.33^{c}
TR+AAEE 250 mg/kg	47.78±1.97^{r}	61.6±2.12^{r}	192.0±5.48^{r}
TR+AAEE 500 mg/kg	44.34±1.68^{r}	59.3±4.40^{r}	188.3±2.81^{r}
TR+standard atorvastatin	40.25±1.77^{r}	52.0±3.07^{r}	183.1±3.01^{r}

Values are expressed as mean±SEM (n=6 animals in each group). P values: ^{a}<0.05, ^{b}<0.01, ^{c}<0.001 as compared with normal group. P<0.05, ^{q}<0.01, ^{r}<0.001 as compared with triton treated group. AAEE: *Albizia amara* ethanolic extract, SEM: Standard error of mean, SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase, ALP: Alkaline phosphatase, TR: Thyroid hormone receptor

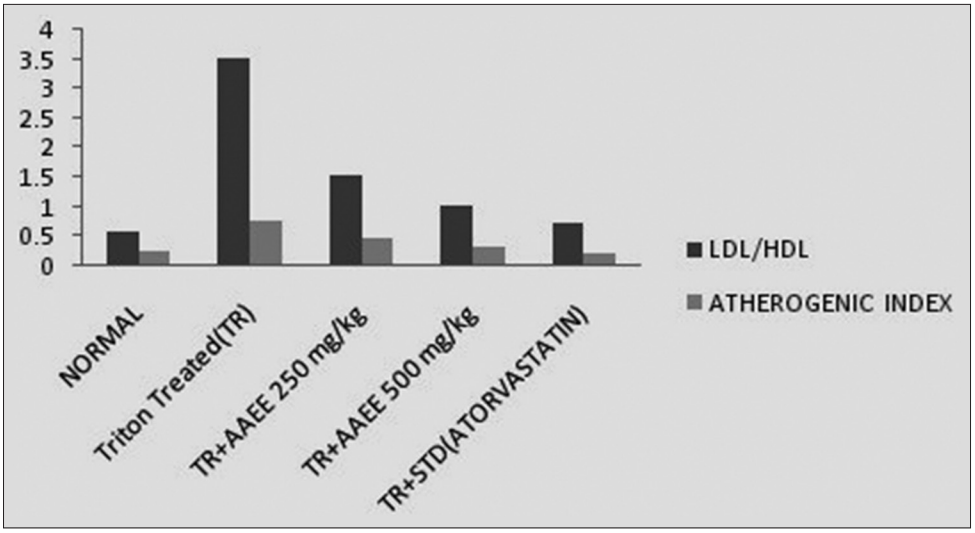

Figure 2: Effects of *Albizia amara* ethanolic extract on low density lipoprotein/high density lipoprotein and atherogenic index

in small groups around central veins and mild periportal inflammation. Hyperlipidemic rats, which simultaneously received AAEE (250 mg/kg) showed that the sinusoids are congested, and no change was observed in hepatocytes. The overall hepatic architecture was maintained [Figure 4b]. Hyperlipidemic rats, which simultaneously received AAEE (500 mg/kg) showed that the hepatic architecture is in normal limits [Figure 4c]. Histopathology of liver of rats treated with Triton X-100 and atorvastatin showed only mild congestion and mild periportal lymphocytic infiltration [Figure 4d]. The overall hepatic architecture was in normal limits. The AAEE showed a significant antihyperlipidemic activity in the animal model and the best activity was shown by AAEE.

DISCUSSION

The main causative factor for cardiovascular diseases is the disturbances occurring in lipid metabolism. Though there are a large class of hypolipidemic drugs used in the treatment, none of the existing ones available are fully effective, absolutely safe and free from adverse events. Hence, efforts are being made to find out safe and effective agents that may be beneficial in correcting the lipid metabolism and preventing cardiac diseases. As the traditional practitioners have used this plant to treat hyperlipidemic conditions and hence, it was considered worthwhile to investigate the claim in experimentally induced hyperlipidemia. Triton X-100 has been widely

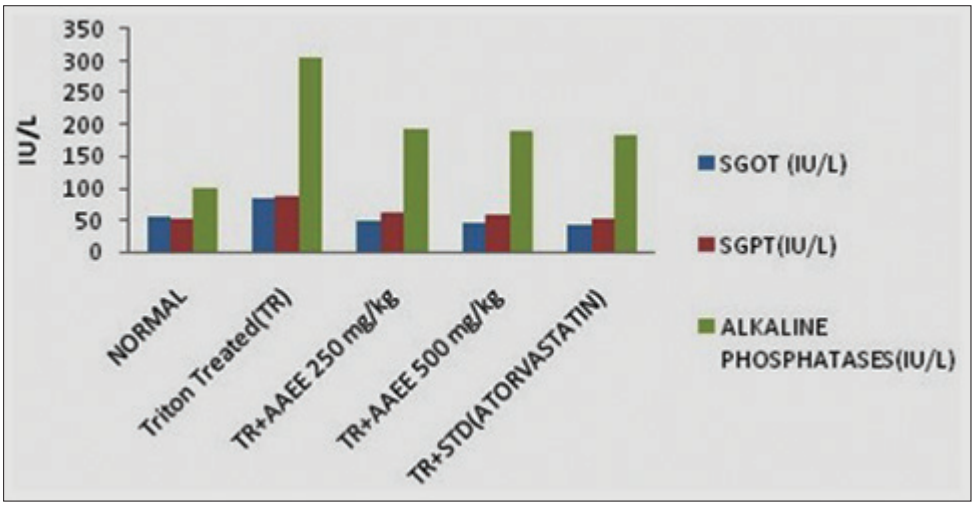

Figure 3: Effects of *Albizia amara* ethanolic extract on serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase and Alkaline phosphatases

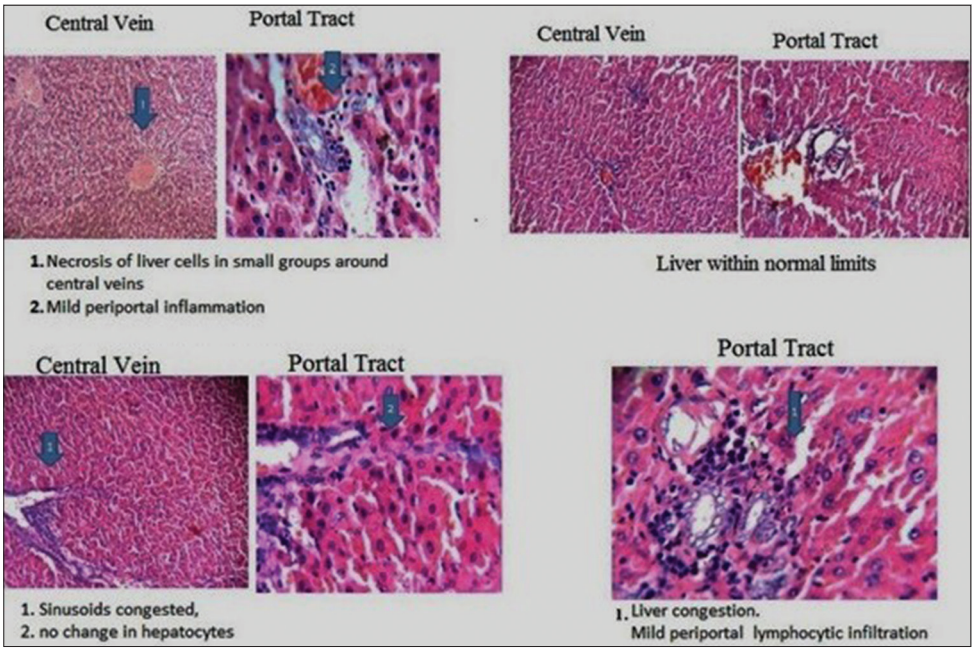

Figure 4: Effects of *Albizia amara* ethanolic extract on liver histopathology of triton induced hyperlipidemic condition

used to block clearance of TGs-rich lipoproteins to induce acute hyperlipidemia in several animals.[18] The large increase in plasma cholesterol and TGs due to Triton X-100 injection results mostly from an increase of VLDL secretion by the liver accompanied by a strong reduction of VLDL and LDL catabolism.[19] In this study, AAEE was selected to screen for it antihyperlipidemic activity in Triton X-100 (100 mg/kg) induced hyperlipidemic rats, which was almost comparable to that of the standard atorvastatin drug used in the treatment. AAEE clearly shows that, at a dose of 250 and 500 mg/kg significantly lowered the TGs and cholesterol levels. The reduction of TC by the *A. amara* extract was associated with a decrease of its LDL fraction, which is the target of several hypolipidemic drugs. This result suggests that cholesterol-lowering activity of the herb extract can be a result from the rapid catabolism of LDL-C through its hepatic receptors for final elimination in the form of bile acids. It is widely accepted that reduction in plasma HDL is a risk factor for developing atherosclerosis. HDL facilitates the translocation of cholesterol from the peripheral tissue, such as arterial walls to liver for catabolism. The increase in HDL may slow down the atherosclerotic process. Increased levels of HDL (cardio protective lipid) may be due to the increase in the activity of lecithin cholesterol acyltransferase, which play a key role in incorporating the free cholesterol into HDL and transferring back to VLDLs or intermediate density lipoproteins, which are taken back by the liver cells. The increased level of HDL-cholesterol and decreased cholesterol level along with its LDL fraction, which is evident from the results could be due to an increased cholesterol excretion and decreased cholesterol absorption through gastro intestinal tract. Several studies have shown that an increase in HDL-C is associated with a decrease in coronary risk. High levels of TC and LDL-C are major coronary risk factors. Saponins have been known to have a lytic action on erythrocyte membranes and this property has been used for their detection. The hemolytic action of saponins is believed to be the result of the affinity of the aglycone moiety for membrane sterols, particularly cholesterol, with which they form insoluble complexes.[20] The result strongly suggests that the hypolipidemic activity of this medicinal plant could be attributed to the presence of the valuable saponin in the extract. The antihyperlipidemic activity of AAEE (250 and 500 mg/kg) against Triton X-100 shows a significant decrease in TC, TG, LDL-C, VLDL ($P < 0.001$) and significant increase in HDL-C ($P < 0.001$) in a dose dependent manner comparing with standard atorvastatin treated group. However, there is a necessity for further research to work for more insight to the possible mechanisms.

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