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Evaluation of anti-atherosclerotic potential of *Tinospora cordifolia* in high-fat diet-induced atherosclerosis with associated changes in histological and biochemical parameters on Wistar rats

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Abstract

Background Atherosclerosis is a chronic inflammatory disease. Atherosclerosis starts with fatty streak which is an accumulation of lipid-laden foam cells in the intimal layer of the artery. Atherosclerosis is a major cause of vascular disease globally. Hydro-alcoholic extract of *Tinospora cordifolia* stem possesses hypolipidemic and thrombolytic activity that might be effective in Atherosclerosis. Hence the present study is to determine the anti-atherosclerotic effect of ethyl acetate fraction of stem of *Tinospora cordifolia* (EATC) in the high-fat diet-induced atherosclerotic model on Wistar rats. Atherosclerosis was induced in male Wistar rats by high-fat diet (cholesterol, cholic acid, Propylthiouracil and salad oil) for 60 days. After completion of induction oral administration of EATC (100, 200 mg/kg) and Atorvastatin (10 mg/ kg) were given to animals for next 20 days. The success of the model was determined by histological and biochemical parameters at 80 days.

Results EATC was able to significant decreased the raised serum level of total cholesterol, triglyceride, Low Density Lipoprotein, V Low Density Lipoprotein and significant increased the serum High Density Lipoprotein level as compared to the diseased control group. EATC exhibited less damage to the endothelial lining of the aorta and showed significant protective effect by lowering the deposition of cholesterol thereby increasing the lumen size compared to the diseased control group.

Conclusion The present study reveals that *Tinospora cordifolia* could be a potential source of drug in treatment of Atherosclerosis.

Keywords Atherosclerosis, Atorvastatin, High-fat diet, *Tinospora cordifolia*

Background

Atherosclerosis is the condition during which an artery wall thickens due to the results of a build-up of fatty substances such as cholesterol and triglycerides. Over time, fatty deposits made of lipids and another cellular substance also increase at the injury site and harden, narrowing the arteries.

The organs and tissues connected to the blocked arteries then don't receive adequate blood to function

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properly. Ultimately, a segment of the fatty deposits may break off and enter in bloodstream. Furthermore, the smooth lining of the plaque may rupture, discharge cholesterol and other substances into the bloodstream. This may cause a blood clot, which can block the blood flow to a specific part of the body, such as occurs when blocked blood flow to the heart causes a heart attack. This blood clot can also go to other parts of the body, restrict flow to another organ. The hyperlipidemia and thrombosis are the leading cause of atherosclerosis [1].

Hydroxymethyl glutaryl coenzyme A reductase (HMG-CoA) inhibitors (commonly known as statins) have been one among the foremost widely prescribed groups of medicine for atherosclerosis globally.

Recently, concern has been addressed regarding the over-prescription of statin drugs also because of the potential for dire adverse effects from statin therapy. This has resulted in multiple numbers of patients ceasing statin therapy among questions about the potential risk of long-term statin use [2]; the potential side effect associated with statins is Myalgia [3], Rhabdomyolysis [4], hepatotoxicity [5], increase the risk of diabetes mellitus, peripheral neuropathy [6], mood symptoms, and irritability [7].

As the limitation with currently available treatment, there is a large scope for research using herbs and herbal product based on anti-atherosclerotic properties. *Tinospora cordifolia* is also known as Guduchi. It belongs to the family of Menispermaceae and is found in Myanmar, Sri Lanka, India, and China. The plant is commonly used as traditional ayurvedic medicine and has different remedial properties such as [8] it's stem possess antihyperlipidemic [9], thrombolytic [10], antidiabetic [11], antihepatotoxic [12] and antipyretic actions [13]. Leaves of *T.cordifolia* have shown antidiabetic activity [14] while root possesses antiulcer and antistress activity [15]. *Tinospora cordifolia* has been mentioned as it is best in "Shonitavibandhprashmana" (one that removes obstruction in blood) in Agrya Prakarana by Acharya Charaka. Kushta has been mentioned by Sushruta during description of Rakta mokshana (blood-letting) [10]. These pharmacological actions of the plant are due to presence of phytochemical constituents like flavonoids, tannins, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds. These phytochemicals are present in a various part of the plant, including root, stem, and whole plant [16].

As per the previous studies *T.cordifolia* showed antihyperlipidemic and thrombolytic activity which is important aspect in the treatment of atherosclerosis [9] hence the current study designed to determine antiatherosclerotic potential *T.cordifolia.*

Methods

Collection of plant material and preparation of plant extracts

T. cordifolia stem was obtained from the local area of Pune District; Maharashtra, India. The stems were collected in winter season (November), stems were shade dried and pulverized with the help of grinder. The powdered stem was then preserved in an airtight container. The dried powder material (600 gm) was soaked in 1500 ml of 90% ethanol for ten days and was shaken occasionally. The whole mixture was filtered by a piece of clean, white cotton followed by Whatman filter paper. The filtrate was dried using a vacuum rotary evaporator at an optimum temperature of 40 °C to prevent loss of important plant constituents and to obtain the crude ethanolic extract of *T.cordifolia* (yield-20 gm). The concentrated aqueous ethanol extract was partitioned by the ethyl acetate fraction (Kupchan method) [8].

Drugs and chemicals

T. cordifolia stem was purchased from Manikarnika aushdhalaya Pune, MS (India). Chemicals for high-fat diet (cholesterol, cholic acid, and vitamin D3) were purchased from Uttam Chemicals Pune, MS (India). All other chemicals were purchased from local sources and were of analytical grade.

Experimental animal

Healthy male Wistar rats (120–170 g) were procured from D-Global building, Sai Park, Shewalwadi road, Uruli Devachi Pune, MS (India). Animals were housed in a group of 6 per cage in standard polypropylene cages (32.5 × 21 × 14) cm lined with raw husk. The animal house was maintained on 12 light/dark cycle approximately 22 ± 2 °C, relative humidity 60–70%. All animals were provided with a standard laboratory diet (Nutrivet Lifescience, Maharashtra, India) and water ad libitum. All experimental procedures were carried out by the guidelines prescribed by CPCSEA and study was approved by the IAEC. (Approval No. MCP/IAEC/003/2019).

Induction of atherosclerosis

High-fat diet with vitamin D3 induces atherosclerosis in rats. Rats were fed esophageal injection with vitamin D3 (700,000 IU/kg) in the first four days followed by feeding with high lipid emulsion (4% of cholesterol, 1% cholic acid, 0.5% PTU and salad oil) [17]. The success of the model was determined by gross anatomy and histology at 80 days after the beginning of the diet. The standard group received Atorvastatin and the test groups rats were received EATC (100 mg/kg & 200 mg/kg p.o.) in the last 20 days. The normal control group received distilled water and the disease control group as well as diseased

group both received high-fat diet with regular chow to all groups.

Animal grouping and dosing methods

Grouping was done by simple allocation of the animal.

The rats were divided into five groups, six animals in each group.

Group I: Normal control rats receiving vehicle i.e. distilled water.

Group II: Disease control rats administered with a high-fat diet for 80 days.

Group III: Standard group (HFD 60 days followed by 20 days 10 mg/kg standard (Atorvastatin).

Group IV: Test group 1 (HFD 60 days followed by 20 days 100 mg/kg EATC).

Group V: Test group 2 (HFD 60 days followed by 20 days 200 mg/kg EATC).

Biochemical analysis

The blood samples were withdrawn on the 60th and 80th day from the retro-orbital venous plexus of rats without any coagulant for the separation of serum. After collecting the blood into microcentrifuge tubes, it was kept aside for 1 h at room temperature and then serum was isolated by centrifugation at 2000 rpm for 15 min and stored until analyzed for various biochemical parameters. The biochemical analysis was carried out by auto-analyser with ERBA kit which is present in our institute's laboratory.

Histopathology

Aorta and liver were isolated and fixed in 10% neutral buffer formalin and then dehydrated by successively passing through a gradient of a mixture of ethyl alcohol and water. The samples were rinsed by xylene and embedded in paraffin. Tissue sections (5 µm thickness) were cut stained with hematoxylin and eosin dye (H and E) and histological examination were done using light microscopy.

Statistical analysis

All the data were expressed as mean ± standard error of mean using one-way analysis of variance followed by the turkey comparison test using computerized GraphPad prism (version 5.0, trial version, GraphPad software, USA) software at a level of significance of $p < 0.05$.

Results

Effect of EATC on Body weight in high-fat diet-induced atherosclerotic model in rats

Body weight

It was observed that a significant increase in body weight in rats fed with high-fat diet for 60 days. Atorvastatin and EATC (100 mg/kg and 200 mg/kg) treated groups had significantly showed decreased in body weight when compared to diseased control group after 20 days of treatment (Table 1).

Effect of EATC on serum lipid level in high-fat diet-induced atherosclerotic model in rats

Serum total cholesterol (TC) (Table 2), Triglycerides (TG) (Table 3), Low-density lipoprotein (LDL) (Table 4), and Very low-density lipoprotein (VLDL) level (Table 5), were found to be increased and High-density lipoprotein (HDL) level (Table 6) and Total protein (TP) were decreased in rats fed with the high-fat diet for 60 days. Atherogenic index ratio (AI ratio) (Table 7) was significantly decreased by EATC (100 mg/kg), EATC (200 mg/kg) and atorvastatin (10 mg/kg) treated groups. A significant reduction in serum TC, TG, LDL, VLDL, level, and increase the HDL and Total protein level (Table 8) were observed in EATC (100 mg/kg), EATC (200 mg/kg) treated groups and standard atorvastatin (10 mg/kg) when compared to diseased control groups. Atherogenic index ratio (AI ratio) was significantly decreased by EATC (100 mg/kg), EATC (200 mg/kg) and atorvastatin (10 mg/kg) treated groups (Tables 2).

Evaluation of transaminase enzyme level in serum

It has been observed that EATC (100 mg/kg, 200 mg/kg) and, Atorvastatin (10 mg/kg) groups were showed a significant decreased in serum SGOT, SGPT, ALP compared to disease control groups (Table 9).

Table 1 Effect of EATC on Body weight in high-fat diet-induced atherosclerotic model in rats

Animal group	Body weight (g)		
	0th day	60th day	80th day
Normal control (NC)	115.333 ± 1.542	119.167 ± 1.167	123.833 ± 0.872
Disease control (DC)	127.833 ± 1.990	150.333 ± 2.246	172.333 ± 3.106
STD (AVT-10 mg/kg)	141.000 ± 1.238	168.000 ± 2.145	151.667 ± 1.563^{a*b*}
EATC (100 mg/kg)	145.000 ± 1.592	176.333 ± 1.382	166.157 ± 1.400$^{a*b\#c\#}$
EATC (200 mg/kg)	139.833 ± 1.352	171.833 ± 0.910	150.833 ± 0.910^{a*b*}

As depicted in Table values are expressed as mean ± SEM; n = 6; Data analyzed by One-way ANOVA test followed by Tukey's multiple test for comparison

NC, normal control; *DC*, disease control; *EATC*, ethyl acetate fraction of stem of *Tinospora cordifolia*

[a] As compared with normal control group, [b]as compared with disease control group, [c]as compared with standard (Atorvastatin) group, Level of significance †$P < 0.05$; #$P < 0.01$; *$P < 0.001$

Table 2 Effect of EATC on TC level in high-fat diet-induced atherosclerotic model in rats

Exp. group	TC (mg/dL) 60 days	TC(mg/dL) 80 days
Normal control (NC)	58.333 ± 4.897	58.5 ± 5.025
Disease control (DC)	133.667 ± 7.658	141.8 ± 6.332
STD (AVT-10 mg/kg)	128.000 ± 7.099	$78.8 \pm 5.128^{a\dagger b*}$
EATC (100 mg/kg)	145.833 ± 2.195	$114.1 \pm 1.887^{a*b\#c*}$
EATC (200 mg/kg)	141.833 ± 4.483	$94.1 \pm 3.487^{a*b*}$

As depicted in Table Values are expressed as mean ± SEM; n = 6; Data analyzed by One-way ANOVA test followed by Tukey's multiple test for comparison

NC, normal control; *DC*, disease control; *EATC*, ethyl acetate fraction of stem of *Tinospora cordifolia*

[a] As compared with normal control group, [b]as compared with disease control group, [c]as compared with standard (Atorvastatin) group, Level of significance †$P < 0.05$; #$P < 0.01$; *$P < 0.001$

Table 3 Effect of EATC on TG level in high-fat diet-induced atherosclerotic model in rats

Exp. group	TG (mg/dL) 60 days	TG(mg/dL) 80 days
Normal control (NC)	87.167 ± 4.875	88 ± 4.604
Disease control (DC)	154.500 ± 3.836	165.5 ± 4.425
STD (AVT-10 mg/kg)	156.500 ± 3.658	$101.8 \pm 4.167^{b*}$
EATC (100 mg/kg)	158.667 ± 5.731	$118.3 \pm 5.371^{a\#b*}$
EATC (200 mg/kg)	155.667 ± 5.661	$106.5 \pm 5.864^{b*}$

As depicted in Table Values are expressed as mean ± SEM; n = 6; Data analyzed by One-way ANOVA test followed by Tukey's multiple test for comparison

NC, normal control; *DC*, disease control; *EATC*, ethyl acetate fraction of stem of *Tinospora cordifolia*

[a] As compared with normal control group, [b]as compared with disease control group, [c]as compared with standard (Atorvastatin) group, Level of significance †$P < 0.05$; #$P < 0.01$; *$P < 0.001$

Table 4 Effect of EATC on LDL level in high-fat diet-induced atherosclerotic model in rats

Exp. group	LDL (mg/dL) 60 days	LDL(mg/dL) 80 days
Normal control (NC)	23.333 ± 3.252	23.333 ± 3.528
Disease control (DC)	135.167 ± 8.252	151.833 ± 6.467
STD (AVT-10 mg/kg)	133.667 ± 5.011	$45.000 \pm 4.597^{b*}$
EATC (100 mg/kg)	150.667 ± 5.777	$93.000 \pm 4.107^{a*b*c*}$
EATC (200 mg/kg)	149.667 ± 2.155	$76.500 \pm 7.442^{a*b*c\#}$

As depicted in Table values are expressed as mean ± SEM; n = 6; Data analyzed by One-way ANOVA test followed by Tukey's multiple test for comparison

NC, normal control; *DC*, disease control; *EATC*, ethyl acetate fraction of stem of *Tinospora cordifolia*

[a] As compared with normal control group, [b]as compared with disease control group, [c]as compared with standard (Atorvastatin) group, Level of significance †$P < 0.05$; #$P < 0.01$; *$P < 0.001$

Table 5 Effect of EATC on VLDL level in high-fat diet-induced atherosclerotic model in rats

Exp. group	VLDL (mg/dL) 60 days	VLDL(mg/dL) 80 days
Normal control (NC)	17.433 ± 0.975	17.600 ± 0.921
Disease control (DC)	30.900 ± 0.767	33.100 ± 0.885
STD (AVT-10 mg/kg)	31.300 ± 0.732	$20.967 \pm 0.833^{b*}$
EATC (100 mg/kg)	32.733 ± 1.530	$23.667 \pm 1.074^{a\#b*}$
EATC (200 mg/kg)	31.133 ± 1.132	$21.300 \pm 1.173^{b*}$

As depicted in Table values are expressed as mean ± SEM; n = 6; Data analyzed by One-way ANOVA test followed by Tukey's multiple test for comparison

NC, normal control; *DC*, disease control; *EATC*, ethyl acetate fraction of stem of *Tinospora cordifolia*

[a] As compared with normal control group, [b]as compared with disease control group, [c]as compared with standard (Atorvastatin) group, Level of significance †$P < 0.05$; #$P < 0.01$; *$P < 0.001$

Table 6 Effect of EATC on HDL level in high-fat diet-induced atherosclerotic model in rats

Exp. group	HDL (mg/dL) 60 days	HDL(mg/dL) 80 days
Normal control (NC)	37.500 ± 3.030	37.8 ± 3.240
Disease control (DC)	22.333 ± 1.358	16.6 ± 1.961
STD (AVT-10 mg/kg)	19.833 ± 1.046	$35.6 \pm 2.028^{b*}$
EATC (100 mg/kg)	18.833 ± 1.167	$29.3 \pm 1.909^{b\#}$
EATC (200 mg/kg)	16.667 ± 1.145	$31.33 \pm 1.585^{b*}$

As depicted in Table values are expressed as mean ± SEM; n = 6; Data analyzed by One-way ANOVA test followed by Tukey's multiple test for comparison

NC, normal control; *DC*, disease control; *EATC*, ethyl acetate fraction of stem of *Tinospora cordifolia*

[a] As compared with normal control group, [b]as compared with disease control group, [c]as compared with standard (Atorvastatin) group, Level of significance †$P < 0.05$; #$P < 0.01$; *$P < 0.001$

Table 7 Effect of EATC on Atherogenic index level in high-fat diet-induced atherosclerotic model in rats

Exp. group	AI (mg/dL) 60 days	AI(mg/dL) 80 days
Normal control (NC)	0.728 ± 0.212	0.555 ± 0.088
Disease control (DC)	5.095 ± 0.533	8.483 ± 1.231
STD (AVT-10 mg/kg)	5.547 ± 0.532	$1.240 \pm 0.198^{b*}$
EATC (100 mg/kg)	6.873 ± 0.543	$3.028 \pm 0.269^{a\dagger b*}$
EATC (200 mg/kg)	7.780 ± 0.605	$2.047 \pm 0.247^{b*}$

As depicted in Table values are expressed as mean ± SEM; n = 6; Data analyzed by One-way ANOVA test followed by Tukey's multiple test for comparison

NC, normal control; *DC*, disease control; *EATC*, ethyl acetate fraction of stem of *Tinospora cordifolia*

[a] As compared with normal control group, [b]as compared with disease control group, [c]as compared with standard (Atorvastatin) group, Level of significance †$P < 0.05$; #$P < 0.01$; *$P < 0.001$

Table 8 Effect of EATC on Total protein level in high-fat diet-induced atherosclerotic model in rats

Exp. group	TP (mg/dL) 60 days	TP(mg/dL) 80 days
Normal control (NC)	6.467 ± 0.582	6.4 ± 0.577
Disease control (DC)	3.417 ± 0.192	2.5 ± 0.076
STD (AVT-10 mg/kg)	3.033 ± 0.126	5.4 ± 0.236[b*]
EATC (100 mg/kg)	3.250 ± 0.092	4.4 ± 0.119[a*b#]
EATC (200 mg/kg)	2.950 ± 0.043	4.6 ± 0.128[a#b*]

As depicted in Table Values are expressed as mean ± SEM; n = 6; Data analyzed by One-way ANOVA test followed by Tukey's multiple test for comparison

NC, normal control; *DC*, disease control; *EATC*, ethyl acetate fraction of stem of *Tinospora cordifolia*

[a] As compared with normal control group, [b]as compared with disease control group, [c]as compared with standard (Atorvastatin) group, Level of significance †$P < 0.05$; #$P < 0.01$; *$P < 0.001$

Table 10 Effect of EATC on blood clotting time in high-fat diet-induced atherosclerotic model in rats

Exp. group	Blood clotting time (sec) on 80th day
Normal control (NC)	130 ± 2.70
Disease control (DC)	77 ± 1.45
STD (AVT-10 mg/kg)	116 ± 6.75[b*]
EATC (100 mg/kg)	98 ± 7.24[b#]
EATC (200 mg/kg)	109 ± 4.57[b*]

As depicted in Table values are expressed as mean ± SEM; n = 6; Data analyzed by One-way ANOVA test followed by Tukey's multiple test for comparison

NC, normal control; *DC*, disease control; *EATC*, ethyl acetate fraction of stem of *Tinospora cordifolia*

[a] As compared with normal control group, [b]as compared with disease control group, [c]as compared with standard (Atorvastatin) group, Level of significance †$P < 0.05$; #$P < 0.01$; *$P < 0.001$…)

Blood clotting time

Atorvastatin (10 mg/kg) and EATC (100 mg/kg and 200 mg/kg) treated groups had significantly increased in blood clotting time when compared to diseased control group after 20 days of treatment (Table 10).

Histopathological study

a) Histopathological study of Aorta

Figure 1 Shows photomicrographs of histopathology sections of rat aorta of different experimental groups. Photomicrographs are taken under 400× magnification. (a) Shows normal histological structure of rat aorta. (b) Experimental rat aorta shows Showed foam cell deposition hemorrhagic streak and fibrous deposition in endothelial layer. (c) Experimental rats treated with Atorvastatin (10 mg/kg) shows reduction in foam cell deposition and fibrous deposition in endothelial layer. (d) Oral administration of 100 mg/kg of EATC for 20 days in experimental rats shows slight changes in foam cell deposition and fibrous deposition in endothelial layer. (e) Treatment with 200 mg/kg of EATC shows improvement in histological structure.

b) Histopathological study of liver:

Figure 2 Shows photomicrographs of histopathology sections of rat liver of different experimental groups. Photomicrographs are taken under 400× magnification. (a) Shows normal histological structure of rat liver showing systematically arranged hepatocytes (b) Experimental rat liver shows microvesicular cytoplasmic vacuolation (c) Experimental rats treated with Atorvastatin (10 mg/kg) for 20 days significantly attenuated hepatocytes. (d) oral administration of 100 mg/kg of EATC for 20 days in experimental rats show slight changes in vacuolization in liver (e) treatment with 200 mg/kg of EATC shows improvement in histological structure.

Table 9 Effect of EATC on SGPT, SGOT, and ALP level in high-fat diet-induced atherosclerotic model in rats

Exp. group	SGOT (U/L)		SGPT (U/L)		ALP (U/L)	
	60 days	80 days	60 days	80 days	60 days	80 days
Normal control (NC)	23.500 ± 3.212	24.16 ± 3.390	120.500 ± 3.538	122.66 ± 3.879	91.167 ± 3.038	91.50 ± 3.191
Disease control (DC)	37.833 ± 3.070	47.00 ± 2.933	157.833 ± 2.7623	173.33 ± 2.848	145.667 ± 2.044	159.33 ± 2.824
STD (AVT-10 mg/kg)	43.667 ± 2.231	22.33 ± 1.978[b*]	157.167 ± 3.341	127.83 ± 5.546[b*]	165.833 ± 1.701	114.33 ± 4.602[a*b*]
EATC (100 mg/kg)	41.000 ± 2.206	29.33 ± 2.836[b#]	166.833 ± 1.641	150.83 ± 1.662[a*b#c#]	154.167 ± 2.496	138.1 ± 2.868[a*b#c*]
EATC (200 mg/kg)	45.667 ± 2.155	25.16 ± 2.774[b*]	155.500 ± 3.658	128.33 ± 3.955[b*]	159.333 ± 2.765	124.33 ± 3.547[a*b*]

As depicted in Table values are expressed as mean ± SEM; n = 6; Data analyzed by One-way ANOVA test followed by Tukey's multiple test for comparison

NC, normal control; *DC*, disease control; *EATC*, ethyl acetate fraction of stem of *Tinospora cordifolia*; *ALP*, Alkaline Phosphatase; *SGOT*, Serum Glutamic-Oxaloacetic Transaminase; *SGPT*, Serum Glutamic-Pyruvic Transaminase

[a] As compared with normal control group, [b]as compared with disease control group, [c]as compared with standard (Atorvastatin) group, Level of significance †$P < 0.05$; #$P < 0.01$; *$P < 0.001$

a) Histopathological study of Aorta :

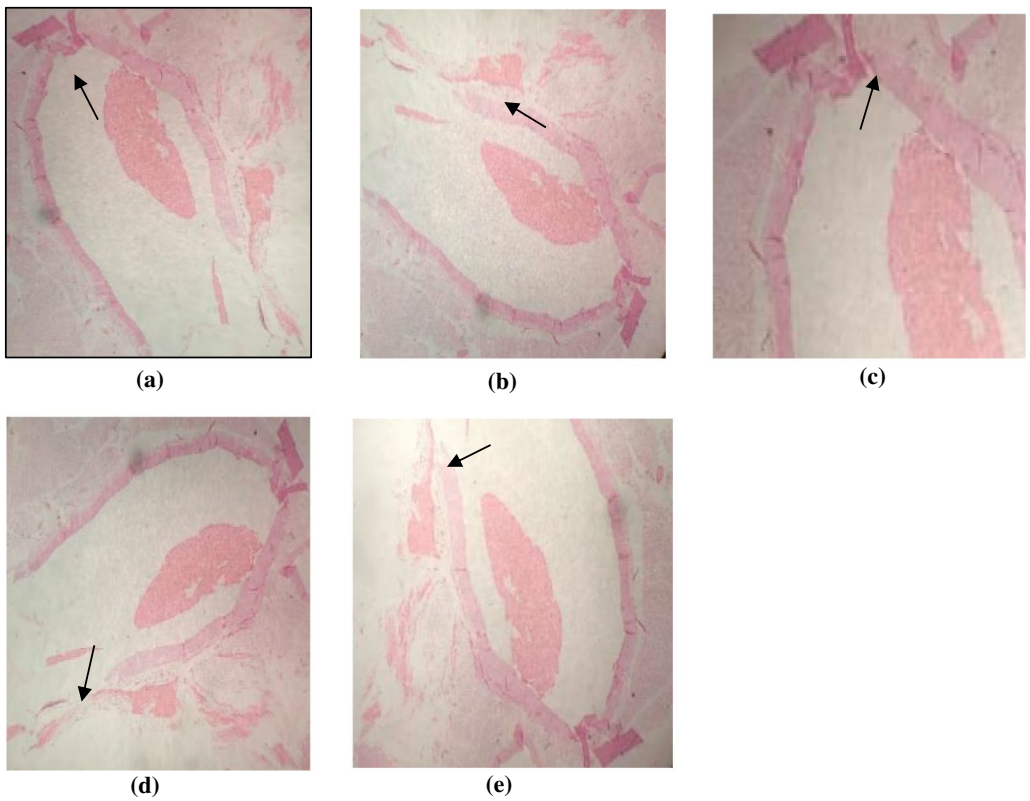

Fig. 1 Effect of EATC on histopathology study of aorta in high-fat diet-induced atherosclerotic model in rats. Figure shows photomicrographs of histopathology sections of rat aorta of different experimental groups. Photomicrographs are taken under 400X magnification. **a** NC—Shows normal histological structure of rat aorta. **b** DC—Experimental rat aorta shows showed foam cell deposition hemorrhagic streak and fibrous deposition deposition in endothelial layer. **c** Atorvastatin (STD)—Atorvastatin shows reduction in foam cell deposition and fibrous deposition in endothelial layer. **d** EATC (100 mg/kg)—Oral administration of 100 mg/kg of EATC for 20 days in experimental rats shows slight changes in foam cell deposition and fibrous. **e** EATC (200 mg/kg)—Treatment with 200 mg/kg of EATC shows improvement in histological structure.

Discussion

As per the evidence hyperlipidemia, thrombosis, and inflammatory reactions contribute to the development of atherosclerosis [18]. The extract of *T.cordifolia* has been proved to have effect in of diabetes mellitus in management of hyperlipidemia and in thrombosis [9]. However, the effects of *T.cordifolia* in the development of atherosclerosis have not been evaluated. Current study was designed to study potential effects of EATC on atherosclerosis in HFD rats. In pathological conditions of atherosclerosis, there is significantly increased in total cholesterol, and phospholipids in plasma which is accompanied by increased serum LDL-C level, with decreased circulating HDL-C. Present study showed that EATC can positively modify lipoprotein profiles. Treatment with EATC showed a marked reduction in TC and LDL-C levels. There was a significant increase in HDL –C levels in EATC treated groups. Recent data suggest that the inhibition of cholesterol absorption may be caused by flavonoids tannin saponins present in EATC could be a mechanism contributing to the positive change in plasma lipoprotein level. Flavonoids are reported to enhance the activity of lecithin cholesterol acyl transferase (LCAT) which plays a key role in the incorporation of free cholesterol into HDL causing increase in HDL-C [19]. Hypertriglyceridemia itself is an independent risk factor and can increased the development of atherosclerosis [20]. The TG build-up caused by dietary cholesterol may be an addition to the decrease of fatty acid beta-oxidation and the preference of cholesterol ester to afflux to LDL during the onset of biosynthesis and secretion of LDL [21]. Most drugs are not promising to decreased TG levels while EATC showed that it not only reduces the lipid profiles but also lowers the plasma level of TG. The significant reduction in the serum TG level observed in EATC treated rats backing the cardioprotective effects which may be due to presence of Tannins which are reported to increase the activity of the endothelium bound lipoprotein lipase activity and hydrolyzes triglycerides [22].

b) Histopathological study of liver:

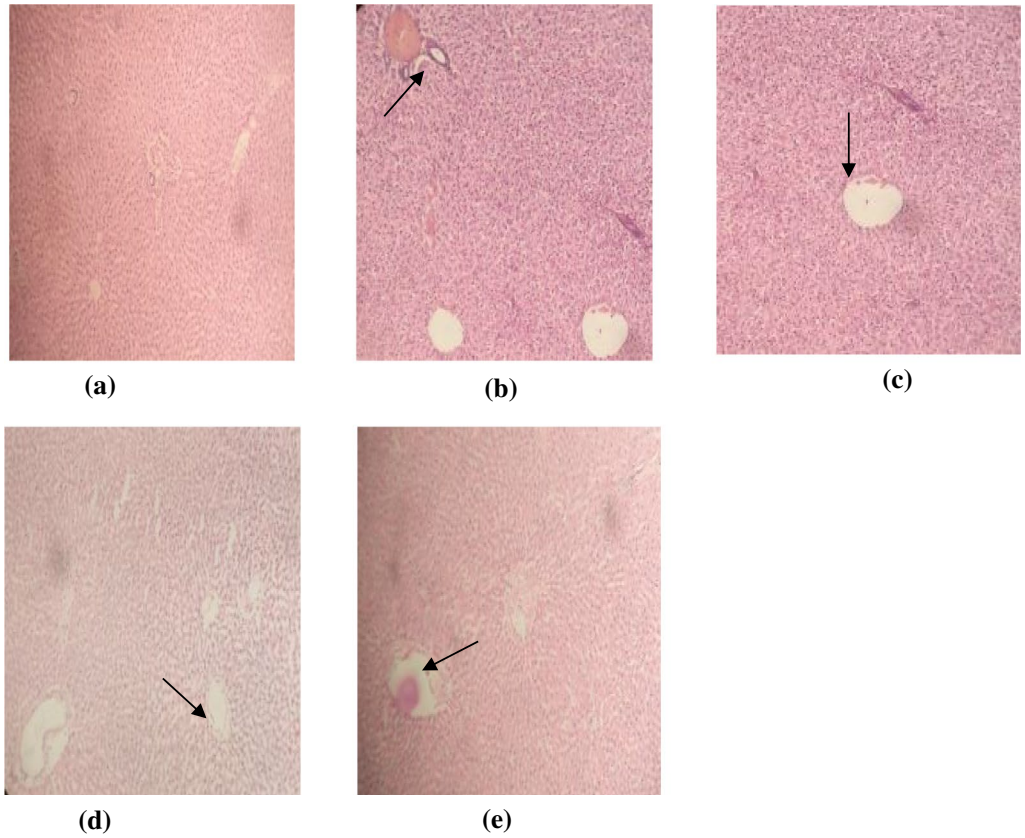

Fig. 2 Effect of EATC on histopathology study of liver in high-fat diet-induced atherosclerotic model in rats. Figure shows photomicrographs of histopathology sections of rat liver of different experimental groups. Photomicrographs are taken under 400X magnification. **a** NC—normal histological structure of rat liver showing systematically arranged hepatocytes. **b** DC—Experimental rat liver shows microvesicular cytoplasmic vacuolation. **c** Atorvastatin (STD)—Experimental rats treated with Atorvastatin (10 mg/kg) for 20 days significantly attenuated hepatocytes. **d** EATC (100 mg/kg)—oral administration of 100 mg/kg of EATC for 20 days in experimental rats show slight changes in vacuolization in liver. **e** EATC (200mg/kg)—treatment with 200 mg/kg of EATC shows improvement in histological structure.

In the induction period, the level of total protein (TP) was decreased in high-fat rats than in a normal group, however it has been observed that the level of TP increased in EATC and, Atorvastatin treated groups, this can be a protective effect in atherosclerosis treatment. In pathological conditions of atherosclerosis inflammatory markers such as SGPT, SGOT and ALP leak from the necrotic heart cells to the blood. These enzymes are not specific for myocardial injury individually; however, evaluation of these enzymes together may be an indicator of myocardial damage [23, 24]. The results of the present study showed that serum SGOT, SGPT, and ALP levels were significantly increased in the diseased control group when compared to the normal control group. There was a significant restoration of these enzymes on administration with EATC which demonstrated its cardio-protective effect.

In atherosclerosis, blood clotting time decreases due to the continuous rise of lipid (LDL, Cholesterol) in the blood which leads to the accumulation of lipid in the endothelium of large vascular arteries. This accumulation makes endothelial injury release macrophage and proinflammatory reagent form plaque blood arteries [25]. The present study showed that the clotting time in EATC (100 mg/kg, 200 mg/kg) and, Atorvastatin (10 mg/kg) treated groups significantly increased in compared to disease control group which inhibited the development of thrombosis in respective groups.

In histopathological studies, the aorta of the diseased control group showed a significant foam cell deposition, hemorrhagic streak and fibrous deposition in the endothelial layer. Hence, it is clear that the HFD fed animals showed a notable increase in lipid composition levels in the aorta. EATC (100 mg/kg, 200 mg/kg) and

Atorvastatin (10 mg/kg) groups significantly decreased the foam cell deposition and fibrous deposition in the endothelial layer with improvement in the histological structure of aorta which confirmed the effectiveness of EATC against hyperlipidemia and atherosclerosis.

Conclusion

The data of present study showed significant increases in serum TC, TG, LDL, VLDL, atherogenic index (TC/HDL and LDL/ HDL ratio), HMG-CoA reeducates activity and a significant decrease in HDL in rats fed with high-fat diet. The present study revealed that EATC able to maintain the lipid and lipoprotein status, which plays an integral role in development of atherosclerosis.

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SN: Concept, Design; KK: Data Collection or Processing, Analysis or Interpretation; SG: Literature Search, Writing and drafting; All authors have read and approved the manuscript.

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Declarations

Ethics approval and consent to participate
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Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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