Original Article

Terminalia arjuna Bark Extract Reduces High Fat Diet Induced Cardiac Damage in Wistar Rats by Altering Biochemical and Histological Parameters

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ABSTRACT

Objectives: The aim of the present study was to examinethe protective effect of bark extract of TA (*Terminalia arjuna*) on HFD (high fat diet) induced biochemical and histopathological changes in Wistar rats. **Materials and Methods:** Group 1 – control rats received water *ad libitum*, Group 2 – HFD group received corn oil orally at a dose of 10mL/kg, Group 3 (HFD+ Atorvastatin (ATV) 10mg/kg), Group 4 (HFD+TA125 mg/kg), and Group 5 (HFD+TA250 mg/kg) were treated as indicated 6 days a week for 5 consecutive weeks. Serum lipid profile and LCAT enzyme activity were evaluated followed by antioxidant parameters and histopathological assessment of heart tissue. **Results:** HFD group showed elevated levels of lipid profile and alterations in antioxidant parameters, LCAT enzyme activity and histology of heart as compared with control group. Administration of standard drug Atorvastatin (10mg/kg), aqueous bark extract of *T. arjuna* (125 and 250 mg/kg) to HFD group protected the biochemical alterations, LCAT enzyme activity and pathological damage caused to heart tissue. **Conclusion:** The study findings reveal the protective role of *T. arjuna* on HFD induced biochemical and cardiovascular changes implicating antioxidant and therapeutic potential.

Keywords: *Terminalia arjuna*, Histopathology, Atorvastatin, Lipid profile, LCAT enzyme, Rats.

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INTRODUCTION

Cardiovascular illnesses, such as coronary artery disease, stroke, and myocardial infarction are major causes of morbidity and mortality worldwide. Hyperlipidemia is the most common cause of atherosclerosis. Drastic raise in serum triglycerides, total cholesterol, and low-density lipoprotein (LDL) levels are indications of onset of atherosclerosis. However, increased high density lipoprotein has been shown to protect atherosclerosis.[1]In atherosclerosis, inflammation accompanies atherogenesis in an early phase ("fatty streak") to develop lesion ("fibrous plaque") and for progression to complications ("thin-cap fibroatheroma" and ulcerated plaque).[2] Reactive oxygen species can oxidise LDL which leads to cholesterol aggregation in phagocytes and the formation of foam cells, leading to atherosclerosis.[3]

Despite advances in atherosclerosis pathobiology, complexity of disease, cholesterol screening, and the availability of potent and well-tolerated cholesterol-lowering medications, cardiovascular disease remains a primary cause of morbidity and mortality. Controlled diet in the treatment of lipid diseases is important.[4]

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ApoB LPs, a low-density lipoprotein (LDL) drive the atherogenic process.[5] Theapo BLPs initiates and sustains inflammatory response that ultimately leads to clinical ischemia.[6] Exposure to high-cholesterol diets, oxidative stress and mitochondrial dysfunction are important factors for the follow-up of individuals with cardiovascular risk.[7] A study demonstrated that providing high cholesterol dietfor one month induced cardiac lipid dysregulation, characterized by cholesterol agglomeration and elevated A-/H-FABPs in heart, in addition to enhance dsystemic oxidative and inflammatory stresses.[8] High fat diet leads to atherosclerosis resulting in endothelium damage by foam cells.[9] Consistent evidence from numerous and multiple different types of clinical and genetic studies unequivocally establishes that LDL causes ASCVD.[10]

The wealth of knowledge on medicinal plants shows great potential for discovery of new drugs to treat diseases, including diabetes and hyperlipidemia.[11] The medicinal herb, *Terminalia arjuna* Wight and Arn. is found in India, Myanmar, and Sri Lanka, Mauritius.[12-13] *T. arjuna* is known by several names.[13-14] It is a deciduous and evergreen tree that reaches a height of 18 – 24 metres.

Several benefits of *T. arjuna* have been reported viz., aphrodisiac, expectorant, tonic, styptic, anti-dysenteric, purgative, and laxative. *T. arjuna* bark is reported to be useful treating for urinary discharge, strangury, leukoderma, anaemia, hyperhidrosis, asthma, and malignancies. The use of bark powder as an astringent and diuretic is documented in Charaka's texts. The investigations revealed the diuretic potential of hydroalcoholic extract of *T. arjuna* bark and as a prophylactic agent to attenuate acute hypobaric hypoxia induced cerebral vascular leakage through ANP mediated modulation of renin-angiotensin-aldosterone system.[15]

Dietary supplementation of flavonoid-rich nutrients to animals has been shown to protect atherogenic modification, inhibition of cholesterol and oxysterol accumulation in macrophages, reduction in foam cell formation and prevention of atherosclerotic lesions.[16] It can serve as effective hypolipidemidic agent. *T. arjuna* bark contain several micronutrients, phenols, flavonoids, tannin, saponin, alkaloids, glycosides, phytosterols and carbohydrate.[17] The antioxidant activity is largely due to flavonoids.[18] Although evidence favours the traditional use of *T. arjuna* bark extract for cardioprotection, we lack animal studies to confirm the cardio protective role of TA in high fat diet model. Therefore, the aim of the present research was to study the protective effect of bark extract of *T. arjuna* on high fat diet induced biochemical changes and cardiac damage in Wistar rats.

MATERIALS AND METHODS

Animals

Wistar male rats (180–280 g) were selected for this research. The wistar rats were allowed to adapt by acclimatization for a week in the laboratory environment in poly propylene cages (3 rats per cage) with sterile paddy husk. The rats were provided with pellet diet (Biogen, Bangalore) and filtered water *ad libitum* and monitored at the Centre for Laboratory and Animal Research (CLAR), Saveetha Medical College with standard protocols. The bedding husk material was changed daily to provide the hygienic environment.

Ethics

Animals were housed and well maintained in accordance with the guidelines issued by the "Committee for the purpose of Control and Supervision of Experiments on Animals", India. The study Protocol was permitted by Institutional Animal Ethics Committee of Saveetha Medical College (SU/CLAR/RD/005/2019 Dated August 09, 2019).

Experimental Groups

Corn oil was orally given at a dose of 10mL/kg 6 times a week for 5 consecutive weeks as a high fat diet to induce cardiac toxicity. Low dose (125mg/kg) and high dose (250mg/kg) of aqueous bark extract of *T. arjuna* was orally administered 6 times a week for five consecutive weeks. As therapeutic intervention. The therapeutic doses were given 30 min before administration of corn oil. Thirty male Wistar albino rats were utilized for this work. Animals were divided into 5 groups (6 rats each). The Group 1 rats served ascontrol (Instead of corn oil water administered orally). The Group 2 rats were high-fat induced. The groups from 3 to 5 received high fat diet and treated with standard drug atorvastatin (10mg/kg), low dose aqueous bark extract of *T. arjuna* (125mg/kg) and high dose aqueous bark extract of *T. arjuna* (250 mg/kg) respectively.

Sample Preparations

Blood was collected in vacutainer tubes without anticoagulants from each animal by retroorbital puncture following isoflurane anaesthesia. It was left for 20 min for clotting and then centrifuged at a speed of 3000 rpm at 4°C for serum separation purpose. The separated serum was stored at −80°C for further use. The animals were sacrificed by cervical dislocation and chest cavity was opened by vertical incision. The heart was removed. The heart was rinsed gently in cold saline, blotted with Whatman filter and stored in sterile plastic

vials at -80°C for further biochemical analyses. For tissue morphological studies, the cardiac tissues were kept in 10% formalin solution and then processed quickly for sectioning.

Terminalia arjuna – Stem bark aqueous extract

Terminalia arjuna bark powder was procured from Herbal Care and Cure Centre, Chennai. One Kilogram TA bark powder was soaked in two litres of boiled hot water. The mixture was boiled for 30 min in a conical flask and kept for 24 hr. It was then transferred into 250 mL conical flask and mixed thoroughly by shaking continuously at a speed of 200rpm for a day at 37°C. Muslin cloth was utilized to filter the extracts. The filtered extract was dried under minimum pressure at 40°C in a centrifugal type evaporator and refrigerated at 4°C for further use. The yield was approximately 18%. The HPTLC plates were prepared in the optimized solvent system, and then it is desiccated in air. It is scanned at 254 nm using CAMAG TLC scanner 3. The standard stock solution was processed in HPLC grade methanol for receiving the sample. The prepared extract was dissolved in methanol and then sonicated for 10 min and the final volume of the solution was made up to 5 mL. The required concentration of the samples was processed from the stock solution by proper dilution.

Biochemical Analysis

Total plasma cholesterol, triglycerides (TG), LDL cholesterol, HDL cholesterol, Lecithin –cholesterolacyl transferase (LCAT) and atherogenic index (AI) were calculated to evaluate the development of dyslipidemia in the model. The amount of Total cholesterol (TC) presentin plasma was calculated by enzymatic method.[19] The absorbance was measured at 510 nm. High-density lipoprotein cholesterol (HDL) was calculated by diagnostic kit based on the enzymatic method.[19] HDL was estimated at 500 nm as described earlier. The values are expressed as mg/dL. Triglycerides were estimated using the diagnostic kit based on the enzymatic method.[19] The absorbance rate of triglycerides was measured at 510 nm. LDL were calculated using the formula derived by Friedewal *et al.*[20]

$$LDL\text{-}C = (TC) - (HDL\text{-}C) - (TG/5)$$

All Cholesterol values are expressed as mg/dL. LCAT was determined by ELISA method according to the manufacturer's instructions using commercially available kit (Daiichi Pure Chemicals, Tokyo, Japan). The LCAT activity was read at 510 nm using microplate reader (MINDRAY model, India).

Antioxidant assay

All groups were subjected to determination of the heart enzymatic antioxidants, reduced glutathione (GSH), glutathione-Peroxidase (GPx), catalase enzyme (CAT), super oxide dismutase (SOD) was carried out by the method done by Razygraev,[21] and Boriskin,[22] respectively. The determination of GSH activity,[21] was evaluated by measuring the absorbance at 412 nm. Absorbance values were associated with a standard curve generated from known standard GSH, and the values obtained were expressed in U/mg protein. The unit of catalase activity,[22] was expressed as the quantity of enzyme needed to decompose 1*m*ol H_2O_2 per min. The reaction was ended by adding 4% ammonium molybdate and the absorbance measured at 410 nm. The determination of the SOD activity,[19] depends upon the production of superoxide radicals by xanthine oxidase and xanthine, that react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to make a red formazon dye. The absorbance was measured at 505 nm and the SOD effect was then measured and expressed in U/mg protein. GPx activity was assayed using the Cayman's Glutathione Peroxidase Assay Kit (Cayman Chemical Company, USA). The determination of GPx activity was evaluated in a spectrophotometer by measuring the absorbance at 340 nm. The measure of GPx activity in the tested samples is the difference in the absorbance change (ΔA_{340}/min) measured for the sample containing the enzyme, and in the rate of decrease in absorbance (ΔA_{340}/min) for the control sample. It is noted that the rate of declination of A_{340} is directly proportional to the GPx activity in the sample, and the values obtained were expressed in U/mg protein.

Histopathology

Heart tissue slices were kept in 10% formal dehyde and fixedin paraffin wax blocks. Sections of 5 µm thickness were stained with Sirius red and then investigated under light microscope for exploring the pathological changes.

Statistical Analysis

All values are expressed as mean ± SE. One way ANOVA was used to test the significance of biochemical data from different groups with Bonforani "*t*" test for multiple comparison. Significance was set at $P<0.05$. Sigma plot 14.5 (Systat software Inc, USA) was utilised for statistical analysis and for plotting the graphs.

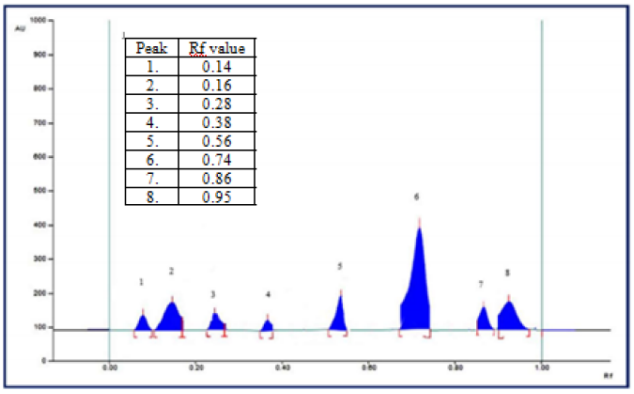

Figure 1: HPTLC Profile of bark extract of *Terminalia Arjuna*.

Table 1: HPTLC profile of *T. arjuna* extract.

Peak	R_f value	Maximum Peak Height	Peak area (%)	Assigned Substances
1.	0.14	140.8	3.75	Unknown Substances
2.	0.16	184.3	24.91	Flavonoids
3.	0.28	147.3	5.76	Triterpenoidess
4.	0.38	110.5	2.24	Unknown Substances
5.	0.56	179.4	11.06	Glycosides
6.	0.74	371.3	76.15	Phenolic Compounds
7.	0.86	151.3	6.23	Tannins
8.	0.95	160.6	28.65	Saponins

Figure 1 represents the HPTLC profile in which R_f value represents the substance shown in Table 1.

RESULTS

The Phenoliccompounds, flavonoids, glycosides, saponin, tannin present in *T. arjuna* were determined by HPTLC methods.

Effect of bark extract on Total Cholesterol

The TC levels (mean±SEM) of control, HFD, HFD+ATV, HFD+TA125, and HFD+TA250 were 121.75 ± 3.6, 187.85 ±4.6, 127.28 ± 1.7, 152.71 ± 5.9, and 130.71 ± 4.0 mg/dl, respectively. The TC levels of HFD group increased by 1.54 folds compared with control group. However, in HFD + TA-treated groups, the increase in the TC level was less than the HFD group (Figure 2). In comparison with HFD group, there was 1.47, 1.23, and 1.44 fold decrease in TC levels in HFD+ATV, HFD +TA125, and HFD+TA250 groups respectively.

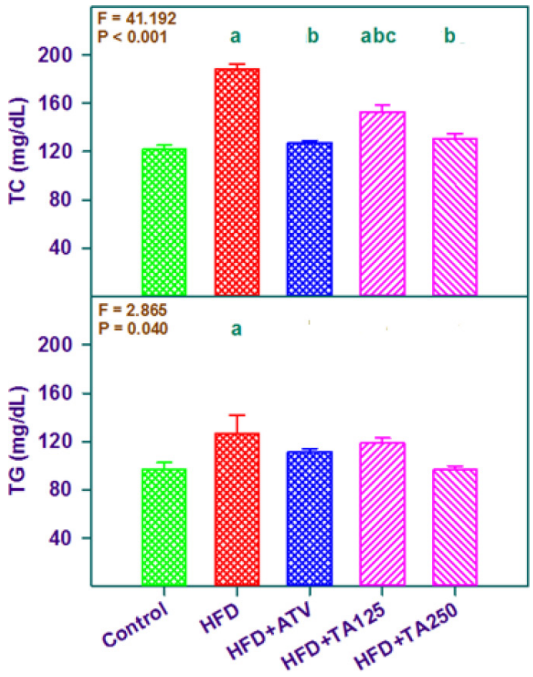

Figure 2: Assessment of Lipid Profile (TC, TG) in control, HFD and drug treatment groups.

Effect of bark extract on Triglycerides

The TG (mean ± SEM) levels of control, HFD, HFD+ATV, HFD+TA125, and HFD +TA250 were 96.61 ± 6.0, 126.4 ±15.7, 111.6 ± 2.5, 118.47 ± 4.3, and 96.45 ± 3.3 mg/dl, respectively. The TG levels of HFD group increased by 1.30 fold as compared with control group. However, in HFD+TA-treated groups, the increase in the TG level was less than that seen in HFD group (Figure 2). In comparison with HFD group, there was 1.13, 1.06, and 1.31 fold decrease in TG levels in HFD+ATV, HFD+TA125, and HFD+TA250 groups respectively.

Effect of bark extract on LDL

The LDL (mean ± SEM) levels of control, HFD, HFD+ATV, HFD+TA125, and HFD+TA250 were 124.32 ± 4.6, 144.94 ± 9.5, 130.71 ± 7.4, 121.48 ± 1.7, and 110.71 ± 2.3mg/dl, respectively., the LDL levels of HFD group increased by 1.17 fold compared with control group. However, in HFD+TA-treated groups, the increase in the LDL levels was lesser than the HFD group (Figure 3). In comparison with HFD group, there was 1.10 fold, 1.19 fold and 1.31 fold decrease in LDL levels in HFD+ATV, HFD+TA125 and HFD+TA250 groups respectively.

Effect of bark extract on HDL

The HDL (mean ± SEM) levels of control, HFD, HFD+ATV, HFD+TA125, and HFD+TA250 were

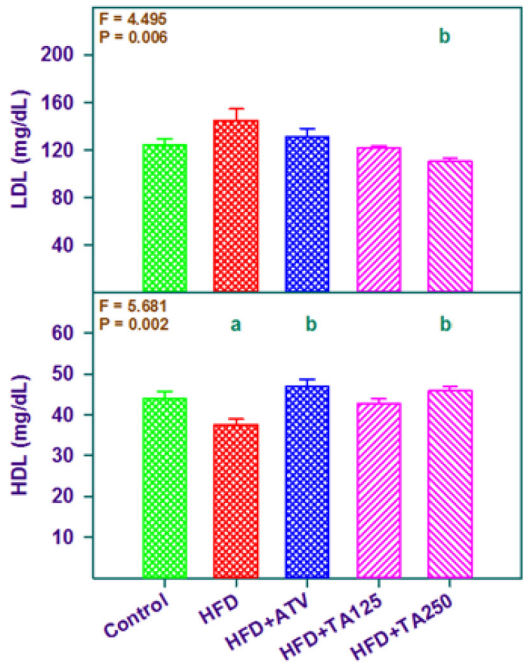

Figure 3: Assessment of Lipid Profile (LDL, HDL) in control, HFD and drug treatment groups.

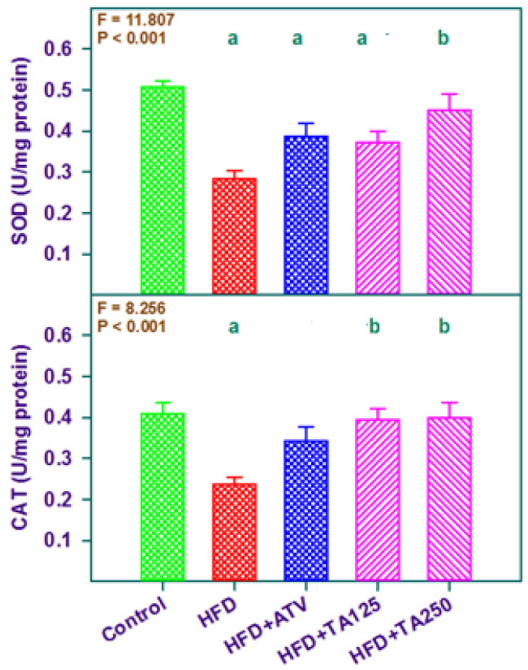

Figure 4: Evaluation of antioxidant Parameters (SOD, CAT) in control, HFD and drug treatment groups.

44.03 ± 1.8, 37.65 ±1.3, 46.71 ± 1.7, 42.48 ± 1.2, and 45.92 ± 1.08mg/dL respectively. The HDL levels of HFD group decreased by 1.16 fold as compared with control group. However, in HFD+TA-treated groups, the HDL levels were significantly higher than the HFD group (Figure 3). In comparison with HFD group, there was 1.24,1.13 and 1.21 foldincrease in HDL levels in HFD+ATV, HFD+TA125 and HFD+TA250 groups respectively.

Biochemical Studies

The results showed significant decline in the levels of antioxidant enzymes, viz., SOD, Catalase, GSH and GPX, and LCAT levels in HFD group when compared with control group. However in TA-treated groups, HFD induced changes in the antioxidant enzyme levels as well as LCAT content were significantly reversed ($P < 0.001$).

The SOD (mean ± SEM) levels of control, HFD, HFD+ATV, HFD+TA125, and HFD+TA250 were 0.5 ± 0.01, 0.28 ± 0.01, 0.38 ± 0.03, 0.37 ± 0.02, and 0.45 ± 0.04 U/mg protein, respectively. The SOD levels of HFD group decreased by 1.76 fold as compared with control group. However, in HFD+TA-treated groups, the SOD levels increased which was greater than the HFD group (Figure 4). In comparison with HFD group, there was 1.34 fold, 1.28 fold, 1.55 fold increases of SOD levelsin HFD+ATV, HFD+TA125, and HFD+TA250mg group respectively.

The catalase (mean ± SEM) levels of control, HFD, HFD+ATV, HFD+TA125, and HFD+TA250 groups were 0.40 ± 0.02, 0.23 ± 0.01, 0.34 ± 0.03, 0.39 ± 0.02, and 0.39 ± 0.03 U/mg protein respectively. The catalase levels of HFD group decreased by 1.70 fold as compared with control group. However, in HFD+TA-treated groups, the increase in the catalase level was higher than the HFD group (Figure 4). In comparison with HFD group, there was 1.42, 1.67 and 1.67 fold increase incatalase levels in HFD+ATV, HFD+TA125, and HFD+TA250 groups respectively.

The GSH (mean ± SEM) levels of control, HFD, HFD+ATV, HFD+TA125, and HFD+TA250 groups were 0.51 ± 0.06, 0.26 ± 0.01, 0.43 ± 0.04, 0.39 ± 0.02, and 0.50 ± 0.04 U/mg protein respectively. The GSH levels of HFD group decreased by 1.88 fold as compared with control group. However, in HFD+TA-treated groups, the rise in the GSH level was higher than the HFD group (Figure 5). In comparison with HFD group, there was 1.63, 1.44 and 1.85 fold increase of GSH levels in HFD+ATV, HFD+TA125, and HFD+TA250 groups respectively.

The GPX levels (mean ± SEM) of control, HFD, HFD+ATV, HFD+TA125, and HFD+TA250 groups were 0.43 ± 0.01, 0.26 ± 0.01, 0.38 ± 0.03, 0.34 ± 0.02, and 0.47 ± 0.03 U/mg protein respectively. The GPX levels of HFD group decreased by 1.65 fold as compared with control group. However, in HFD+TA treated groups, the increase in the GPX level was

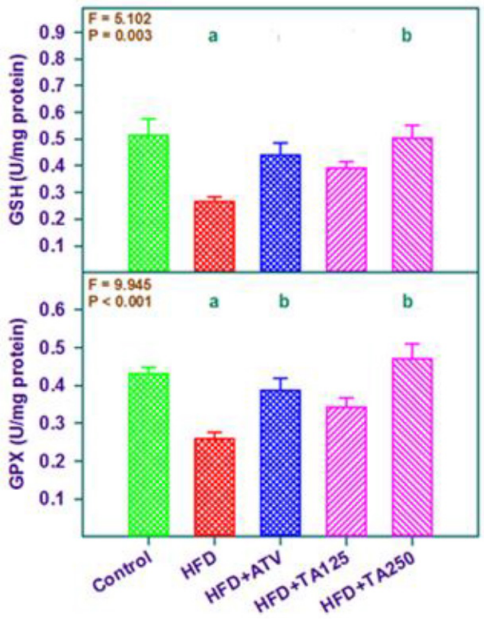

Figure 5: Evaluation of antioxidant Parameter (GSH, GPX) in control, HFD and drug treatment groups.

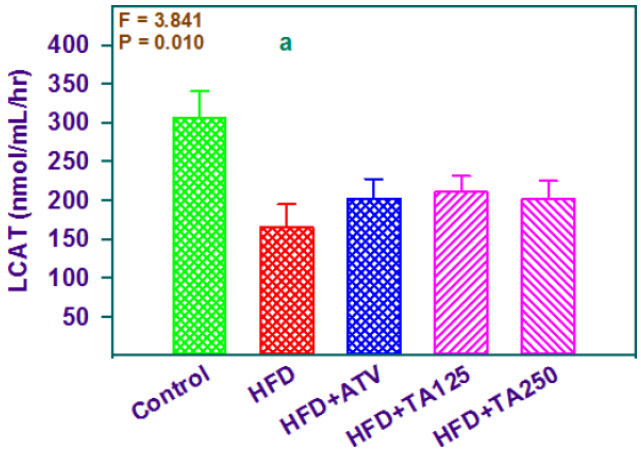

Figure 6: Evaluation of LCAT Enzyme Activity.

greater than the HFD group (Figure 5). In comparison with HFD group, there was 1.5, 1.31 and 1.81 fold increase of GPX levels in HFD+ATV, HFD+TA125 and HFD+TA250 groups respectively.

Effect of bark extract on LCAT

The LCAT levels (mean ± SEM) of control, HFD, HFD+ATV, HFD+TA125 and HFD+TA250 groups were 306.04±107.45, 165.24±89.26, 201.78±77.23, 210.52±62.34 and 201.63±73.80nmol/mL/hr respectively. As compared to control, the LCAT levels of HFD group decreased by 1.85 fold whereas in HFD+TA treated groups, the increase in LCAT was greater than that noticed in HFD group (Figure 6). In comparison with HFD group, there was increase of LCAT levels by

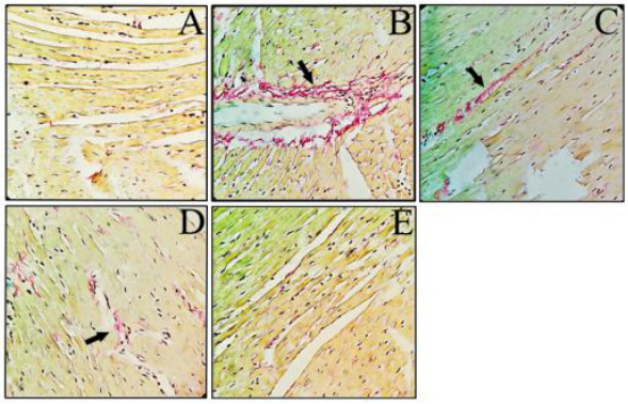

Figure 7: Representative images of heart tissue stained with Sirius red. X40 magnification. Panel-A (control), Panel - B (HFD), Panel -C (HFD + Standard drug Atorvastatin treated) and Panel -D (HFD + TA 125 reated), Panel –E (HFD + TA 250 treated).

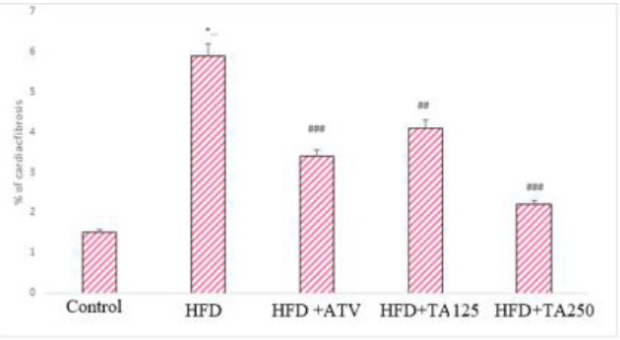

Figure 8: Quantitative analysis of cardiac fibrosis in heart tissue.

1.22, 1.27 and 1.22 fold in HFD+ATV, HFD+125, and HFD+250 groups respectively.

Figure 7 represents sections stained with picrosirius red, representing areas of fibrosis. Samples representing the control group did not confirm any marked areas of fibrosis whereas large fibrotic areas are observed in the high fat fed groups (Figure 7b). Treatment with *T. arjuna* extract partially reduced the fibrosis, observed as relatively lesser areas of fibrosis (Figure 7d–e), (Figure 8); however interstitial fibrosis was present. Atorvastatin-treated rats showed similar patterns (Figure 7c).

All data were reported as mean ± SEM for measurements in triplicate. $^{***}P < 0.001$ statistically significant as compared with control group. $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ statistically significant as compared with HFD group.

DISCUSSION

Atherosclerosis is a type of cardiovascular disease caused by plaque build up in the arteries. Statin decrease overall

death rates in patients with a control atherosclerotic cardiovascular disease and atherosclerosis risk of 10% or greater. However people who are at a lower risk may not benefit (10% or less). In cases of true statin intolerance, which is defined as the manifestation of drugs characteristic harmful effects in an individual at recommended doses, non-statin therapy may be investigated.[23] Currently there is no satisfactory therapeutic agents to overcome HFD induced cardiovascular complications. In the present work, the efficacy of TA in HFD induced complications was explored in a rat model. Increased blood lipid profile (TC, TG, LDL) levels with lower HDL levels in HFD group compared with control. Based on previous works treating hyperlipidemia with food or lipid-lowering drugs lower the incidence of cardiovascular disease. Egyptian Herbal drug formulation (HMF) (composed of *T. chebula*, Senae, rhubarb, black cumin, aniseed, fennel and licorice) with L-carnitinehave improved body weight, lipid profile, glucose levels and liver function. Insulin resistance, oxidative stress markers were improved, as were kidney and cardiac marker functions to proveanti-obesity effect.[24-25] Atorvastatin is a statin drug that is used to lower cholesterol levels. The use of atorvastatin may be a therapeutic option for the treatment of vascular diseases caused by inflammation and oxidative stress as evidenced by improved biomarkers and a significant recovery of mitochondrial morphology.[26] But the common side effects of atorvastatin include gastrointestinal problems such as diarrhoea, joint pain, insomnia, urinary infection, nausea and loss of appetite. There fore safe and alternative therapy is required for the management of cardiac diseases and associated problems.

Hyperlipidemia caused by a high-cholesterol diet is related to higher lipid levels in the blood. The findings show that *T. chebula* bark extract has considerable anti-hyerlipidemic Action.[27] The present findings are in agreement with earlier works to support the therapeutic benefits of TA. This attributes tothe heart-health effects rendered by *T. arjuna* containing polyphenols that have the potential to stimulate nitric oxide to benefit endothelial functions.[28] *Terminalia arjuna* therapy for two weeks has led to significant regression of this endothelial abnormality amongst smokers.[29] The presence of a considerable amount of flavonoid components in *T. arjuna* contributes to its antioxidant and antimicrobial effects. As a result the findings support the hypothesis that *T. arjuna* bark is a viable source of natural medicinal comounds.[18] In *T. arjuna*-treated groups, HDL levels were increased due to the effect of plant derived components (phytosterols, flavonoids, saponins and tannins) existing in the bark of *T. arjuna*. Modifications in serum lipid profile levels are indications of dysfunctions of cardiovascular system.[30] *T. arjuna* bark extract significantly lowered cholesterol and LDL cholesterol to normal levels and also lowering triglyceride levels and elevating HDL cholesterol when given orally.[31] Extracts high in flavonoids, tannins, saponins, and phenols appeared to have a variety of effects on lipid metabolism, including suppression of pancreatic amylase activity, decreased lipogenesis activity, and an increase in fatty acid oxidation.[32] The other plant materials of *T. Arjuna* has also shown considerable hypolipidaemic and antioxidant activities in various disease conditions.[33] TA drug treatments (125 and 250 mg/kg) to HFD-induced rats significantly reversed the altered serum enzyme and biochemical parameters to near normal levels.

It was shown that LCAT−/−hamsters display complete loss of HDL (high-density lipoprotein) and elevated triglyceride levels on standard laboratory diet. LCAT−/− hamsters on standard laboratory diet show atherosclerotic plaques showing the relationship between low HDL and atherosclerosis.[34] In this study, LCAT levels were observed to be greater in the drug treated groups (HFD+ATV, HFD+TA125, HFD+TA250) compared to HFD group but the changes were not significant. This fairly indicates that measurement of LCAT activity is useful for predicting atherosclerotic cardiovascular disease (ASCVD) in patients at a high risk for progression of atherosclerosis.[35]

Aqueous extract of *T. arjuna* showed protective action against alcohol induced hepatic and renal injury by ameliorating both the nitrosative and oxidative stress by enhancing the antioxidant status of the system.[36] In alcohol linduced, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) were evaluated in liver and kidney. Antioxidant enzyme activity was found to be significantly reduced. However, rats which had been administered alcohol and given an aqueous we given an aqueous extract of *T. arjuna* (AETA) showed significant reductions in plasma lipid peroxidation levels and both enzymatic and non-enzymatic antioxidant levels are restored to normalcy. Histopathological examinations of the liver and kidneys supported these findings.[37] Antioxidant levels in the cells have increased like GSH, SOD, and CAT help to combat oxidative stress. One of the most efficient therapeutic techniques may be to protect against oxidative stress through this pathway.[38] The findings of this research were as follows: In the drug treated groups, (HFD+ATV, HFD+TA125, HFD+TA250) there was a considerable

rise in SOD, CAT and a reduced GSH and GPx as compared with HFD. In rats consuming atherogenic diets (high in dietary cholesterol) causes oxidative stress as well as the development of inflammatory markers that may lead to cardiovascular diseases.[39]

Triterpenoids of *T. arjuna* are able to prevent myocardial abnormalities and pathological changes induced by cyclosporine.[40] The bark of *T. arjuna* is reported to have co-enzyme Q which is effective to prevent heart disease there by proving cardio-protective potential of *T. arjuna*.[41] Furthermore, whether administered to normal or hyercholesterolemic rabbits. *T. arjuna* bark powder exhibits considerable anti-atherogenic effect.[42]

Gastro protective characteristics, phytoconstituents included in *T. arjuna* extract have been shown to have prostaglandin-inducing properties as well as antioxidant capacity.[43] It was shown that an ethanolic extract of *T. arjuna* had a considerable antioxidant effect, protecting the kidneys and liver from oxidative damage induced by diabetes.[34]

The absence of collagen deposition in the heart is the characteristic pathogenesis of hypertrophy. Myocardial dysfunction is characterized by an increase in collagen content and progressive maturation. Since the adult mammalian heart has limited regenerating potential, a considerable proportion of cardiomyocytes die after a myocardial infarction and are repaired by fibrous tissue.[44] Reactive fibrosis is also seen, which could be due to cardiac fibrolasts stimulating collagen deposition in response to a HFD. The development of cardiac fibrosis has been associated with increases in oxidative stress.[43] Treatment with tempol, a membrane permeable radical scavenger was demonstrated to prevent an increase in myocardial collagen deposition in an earlier findings.[45] The antioxidant activity of *T. arjuna* was associated with significant reductions of myocardial collagen deposition in the current investigation. The protective action against greater myocardial fibrosis was also confirmed through light microscopy experiments, where picrosirius red staning revealed lesser fibrosis in the *T. arjuna* treated groups.

Even with progression of hypertrophy to heart failure, Oxidative stress and fibrosis have a detrimental effect. It is also important to note that delaying the hypertrophic response could enable maintain the physiological hypertrophic response intact. The study found that mast cell activation could induce cardiac fibrosis in hyerlipidemia via tryptase and chymase which was correlated with slight rise in TGF-β/Wnt/β-catenin pathway.[46]

CONCLUSION

The present research findings conclude that administration of aqueous bark extract of *Terminalia arjuna* (250mg/kg) to high fat diet induced rats was effective in reducing the cardiac damage and associated complications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

TA: *Terminalia arjuna*; **ATV:** Atorvastatin; **LDL:** Low density lipoprotein; **ASCVD:** Athersclerotic cardiovascular disease; **SEM:** Standard error Mean; **ANOVA:** Analysis of Variance; **LCAT:** Lecithin cholesterol acyl tranferase; **HFD:** High fat diet; **HPTLC:** High performance thin layer chromatography; **HPLC:** High performance liquid chromatography; **SOD:** Superoxide dismutase; **GSH:** Reduced glutathione; **GPx:** Glutathione peroxidise; **ANP:** Atrial natriuretic peptide; **A-/H-FABPS:** Subtypes of fatty acid binding proteins; **TGF-β/Wnt/β-catenin:** Transforming growth factor superfamily.

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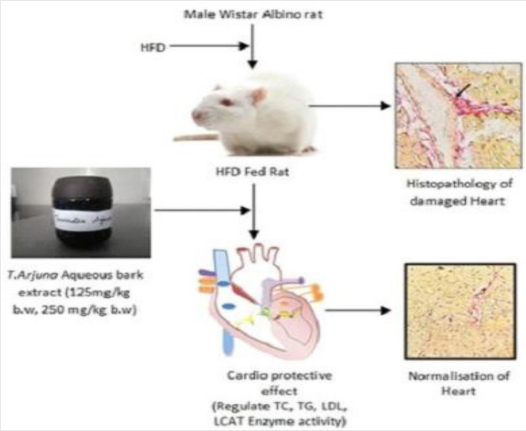
PICTORIAL ABSTRACT



SUMMARY

The present study is focused on amelioration of HFD induced cardiac complications with the administration of *T. arjuna* aqueous bark extract through in vivo studies. HFD induction leads to biochemical and histopathological changes finally causing cardiac complication in rats. This was confirmed through in vivo studies by observing the changes in biochemical and histopathological alterations. Aqueous fraction of bark extract of T. arjuna showed significant reduction in low density lipoprotein and increase in LCAT enzyme activity. Histopathological study also supported beneficial role of T. arjuna bark extract in the normalisation of heart tissue from HFD induced damage. Thus from this study it can be conluded that T. Arjuna bark extract exhibits potential action for amelioration of cardiac complications

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