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Research Paper

Catecholamine-induced myocardial fibrosis and oxidative stress is attenuated by *Terminalia arjuna* (Roxb.)

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Abstract

Objectives Myocardial fibrosis and oxidative stress accompany a number of cardiac disorders such as hypertrophic cardiomyopathy, hypertensive heart disease and cardiac failure. Stem bark of *Terminalia arjuna* has been advocated for cardiac ailments. The present study evaluated the effects of *T. arjuna* bark extract on myocardial fibrosis and oxidative stress induced by chronic beta-adrenoceptor stimulation.

Methods Aqueous extract of *T. arjuna* bark was evaluated at 63, 125 and 250 mg/kg given orally for antifibrotic and antioxidant effects in rats given the selective β-adrenoceptor agonist isoprenaline (5 mg/kg s.c.) for 28 days. Captopril (50 mg/kg per day, given orally), an inhibitor of angiotensin-converting enzyme used as a standard cardioprotective drug, was used as a positive control.

Key findings Isoprenaline caused fibrosis, increased oxidative stress and cardiac hypertrophy (increased heart weight : body weight ratio and cardiomyocyte diameter). The *T. arjuna* bark extract and captopril significantly prevented the isoprenaline-induced increase in oxidative stress and decline in endogenous antioxidant level. Both also prevented fibrosis but not the increase in heart weight : body weight ratio.

Conclusions *T. arjuna* protects against myocardial changes induced by chronic beta-adrenoceptor stimulation.

Keywords collagen; echocardiography; isoprenaline; left ventricular hypertrophy; *Terminalia arjuna*

Introduction

Deleterious effects of catecholamines on the heart due to increased sympathetic activity play a key role in the aetiopathology of left ventricular hypertrophy (LVH), which is an important risk factor for the development of cardiac failure and sudden cardiac death.[1,2] Sustained effects of catecholamines on the heart cause myocyte hypertrophy, which has been shown to promote oxidative stress in cardiac myocytes.[2,3] Oxidative stress possibly adversely modulates collagen biosynthesis, triggering cardiac fibrosis, LVH and subsequent heart failure.[3,4] In fact, fibrosis is considered as a hallmark that differentiates between pathological and physiological hypertrophy.[5] Downregulation of endogenous antioxidants has been implicated in increasing both oxidative stress and hypertrophic response of the heart.[6] Statins have also been shown to reduce LVH by preventing oxidative stress.[7] All these lines of evidence imply a central role of oxidative stress in the development of LVH.

Terminalia arjuna (Roxb.) is a deciduous tree of the family Combritaceae and has been reported to be beneficial in many cardiac ailments in ancient Indian medical literature, *Ayurveda*.[8,9] In preclinical studies, the bark of the plant was shown to be beneficial against oxidative stress induced by myocardial injury.[10] Prolonged treatment with *T. arjuna* has been shown to augment endogenous antioxidants in the heart, and this has been proposed as the mechanism of protection against ischaemia–reperfusion-induced myocardial injury.[11,12] Arjunolic acid, a triterpene present in *T. arjuna* bark, protected against acute isoprenaline-induced myocardial changes.[13] Treatment with bark extract has been reported to prevent the myocardial damage induced by doxorubicin administration.[14] Clinical studies have shown that *T. arjuna* bark powder has potent hypocholesterolaemic

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effects in addition to antioxidant properties, which are comparable to those of vitamin E. There is also encouraging evidence of beneficial effects in patients with refractory heart failure or coronary artery disease.[15–17]

The present study was designed to test the hypothesis that the antioxidant properties of *T. arjuna* could be beneficial in preventing myocardial fibrosis and oxidative stress in a rat model of cardiac hypertrophy.

Materials and Methods

Preparation of aqueous extract of *T. arjuna*

Stem bark of *T. arjuna*, obtained from the southern part of India (Madurai district, Tamilnadu) during the months of September/October, was authenticated by the Department of Pharmacognosy, Pharmaceutical Technology, Jadavpur University, Kolkata, India (vide voucher specimen no. 53). The dried pulverised powder of the bark was extracted with double-distilled water (DDW) by hot continuous percolation using Soxhlet apparatus for 72 h. The extract was then filtered and lyophilised. A 25% yield was obtained following extraction. The aqueous extract of *T. arjuna* bark was evaluated qualitatively for the presence of glycosides, flavonoids, polyphenols, saponins and terpenoids.[18]

Quantitative evaluation of the aqueous extract of *T. arjuna* by TLC

The presence of arjunolic acid and terminoic acid in the extract was determined by TLC using toluene : ethyl acetate : formic acid (7 : 3 : 0.5 v/v) as the solvent system. Standard solutions of arjunolic acid and terminoic acid (both 1 mg/ml) were prepared. Lyophilised aqueous extract of *T. arjuna* (250 mg) was extracted four times with 10 ml *n*-butanol and the final volume made up to 50 ml. Samples of this solution and a standard solution (10 μl of each) were applied to a precoated silica gel $60F_{254}$ plate (E. Merck, Darmstadt, Germany) of uniform thickness (0.2 mm). The plate was developed in the solvent system up to a distance of 8 cm and observed under 254 nm, UV 366 nm. The R_f values of the bands resolved were noted.

Derivatisation was done with anisaldehyde sulfuric acid reagent followed by heating for 10 min at 100°C and the R_f values of the bands noted.

Animals

The study was approved by the Institute Animal Ethics Committee (approval no. 289/IAEC/05) and all animal care and experimental protocols were in compliance with the US National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publication #85-23, 1985).

Laboratory-bred male Wistar rats (150–200 g, 10–12 weeks old) were maintained under standard laboratory conditions of 25 ± 2°C, relative humidity 50 ± 15% and normal 12 h light–dark cycle. Commercial pellet diet (Ashirwad, Chandigarh, India) and water were provided *ad libitum*. Rats were randomly distributed into groups of six rats for the experiment.

Chemicals

All chemicals were of analytical grade or higher and were obtained from Sigma Chemicals (St Louis, MO, USA), except L-4-hydroxyproline, which was from Fluka (Steinheim, Germany) and solvents, which were from Merck (Mumbai, India). DDW was used for all biochemical assays.

Experimental protocol

Appropriately weighed amounts of aqueous extract of *T. arjuna* bark were dissolved in an appropriate volume of DDW and administered by oral gavage at 63, 125 and 250 mg/kg once daily for 28 days. These doses were selected on the basis of earlier studies in our laboratory of the cardioprotective effect of *T. arjuna* bark; the appropriate dose for the aqueous extract was calculated according to the yield.[11] These three groups of rats were also given isoprenaline (5 mg/kg, s.c.) daily for 28 days to produce LVH. One group received only isoprenaline daily. A positive control group was given captopril (50 mg/kg orally by gavage) once daily for 28 days along with isoprenaline. The control group was given normal saline (1 ml/kg) s.c. once a day for 28 days.

Body weight and food and water intake were measured at intervals of 7 days. At the end of the 28-day experimental protocol, animals from all the groups were anaesthetised (ketamine HCl 50 mg/kg and xylazine 10 mg/kg, i.p.) and echocardiography was performed as described below. Rats were then euthanised with an overdose of ether anesthesia and the heart was harvested rapidly, washed in ice-cold saline, blotted dry with paper towel and weighed. The right ventricular tissue and atrial appendages were separated for biochemical estimations and stored in liquid nitrogen. Heart samples for light microscopic studies were stored in 10% buffered formalin (pH 7.2).

Echocardiography

The chest hair was shaved and rats were examined in the supine position, with the transducer probe placed gently in the left parasternal position. Heart function was evaluated by two-dimensional M-mode echocardiography with a 10–11.5 MHz neonatal cardiac probe transducer with a high frame rate and shallow focus (10–25 mm) from a short-axis view at the level of the papillary muscles of the left ventricle (LV) using a fully digitised Wipro GE system (Vivid 7 dimension, Oslo, Norway). LV posterior wall thickness and interventricular septal thickness were recorded in diastole; the LV internal dimension was recorded in both diastole (LVDd) and systole (LVDs) from a short-axis view at the level of the papillary muscle from the trailing edge of the septum to the leading edge of the posterior wall.[19] The images, each composing of 5–9 consecutive heart cycles, were digitally transferred online to a computer and analysed by an analyst who was blinded to the treatment groups. Three representative cycles were analysed and averaged. Left ventricular endocardial fractional shortening (FS) and relative wall thickness (RWT) were calculated using standard methods.[20]

Biochemical parameters

Myocardial thiobarbituric acid reactive substances

Myocardial thiobarbituric acid reactive substances (TBARS) were measured according to the method of Ohkawa *et al*.[21] A sample of the left ventricle was homogenised at 4°C in

50 mmol/l potassium phosphate buffer (pH 7.4; 1 : 10) and then 0.2 ml of the homogenate was transferred into a test tube, followed by addition of 0.2 ml 8.1% sodium dodecyl sulfate, 1.5 ml 30% acetic acid (pH 0.5), 1.5 ml 0.8% thiobarbituric acid and the volume made up to 4.0 ml with DDW. Test tubes were heated at 95°C for 60 min. and then cooled. DDW (1.0 ml) and *n*-butanol : pyridine (5.0 ml of a 15 : 1 (v/v) mixture) were added to the test tubes and centrifuged at 4000*g* for 10 min. The absorbance of colour developed in the organic layer was measured at 532 nm. Commercially available 1,1,3,3-tetraethoxypropane was used as a standard for malondialdehyde. Data are given as nmol/g tissue (wet weight).

Myocardial reduced glutathione

Myocardial levels of reduced glutathione (GSH) were measured by the method of Ellman.[22] The homogenate, as prepared above, was mixed with 10% trichloroacetic acid in a 1 : 1 ratio and then centrifuged for 10 min at 1200*g*. The supernatant was then mixed with 2 ml 0.3 mol/l phosphate buffer (pH 8.4), 0.4 ml DDW and 0.5 ml 5,5-dithiobis (2-nitrobenzoic acid). The reaction mixture was incubated for 10 min and the absorbance measured at 412 nm. The level of GSH was determined from a standard curve constructed using commercially available GSH. Data are given as μg/g wet weight of tissue.

Myocardial catalase

Myocardial catalase activity was quantified according to the method of Aebi.[23] A sample of left ventricle was homogenised at 4°C (1 : 10) in 50 mmol/l potassium phosphate buffer (pH 7.4) and centrifuged at 3000*g* for 10 min. Supernatant (50 μl) was added to a 3 ml cuvette containing 1.95 ml 50 mmol/l phosphate buffer (pH 7.0). Then, 1.0 ml hydrogen peroxide (30 mmol/l) was added and the change in absorbance at 240 nm measured at 15 and 30 s. Data are given as units/mg protein compared with the standard.

Myocardial superoxide dismutase

Myocardial superoxide dismutase (SOD) was measured by the method of Kakkar *et al.*[24] A sample of left ventricle was homogenised in 0.25 mol/l Tris sucrose buffer and centrifuged at 10 000*g* for 15 min at 4°C. The supernatant was fractionated by 50% ammonium sulfate and dialysed overnight. Aliquots of the supernatant (100 μl) were added to sodium pyrophosphate buffer (pH 8.3) followed by addition of 0.1 ml phenazine methosulfate (186 μmol/l), 0.3 ml nitroblue tetrazolium (300 mmol/l) and 0.2 ml NADH (780 μmol/l). The reaction mixture was incubated for 90 s at 30°C and the reaction stopped by adding 1.0 ml glacial acetic acid. *n*-Butanol (4.0 ml) was then added and the samples centrifuged at 3000*g* for 10 min. The absorbance of the organic layer was measured at 560 nm. Data are expressed as units/mg protein. The protein concentration was measured by the Bradford method.[25]

Myocardial collagen content

The myocardial collagen content was measured in terms of L-4-hydroxyproline content, measured as reported previously.[26] Approximately 100 mg of myocardial tissue was weighed and dried at 60°C to constant weight. The dried tissue was powdered and incubated at 105°C in 6 M HCl (6 ml acid per 100 mg tissue) for 18 h in a tightly closed container. The mixture was then filtered through Whatman III filter paper and vacuum dried. The dried tissue extract was reconstituted with DDW and the pH adjusted to 6.0 using NaOH solution. Isopropanol was mixed with reconstituted tissue lysate (4 ml per 100 mg tissue) and aliquots taken for estimation. Samples were taken in duplicate and mixed with acetate–citrate–isopropanol buffer and 100 μl oxidant solution. After 5 min incubation, 1.3 ml Ehrlisch's reagent was added and incubated for 30 min at 60°C. The mixture was cooled and absorbance measured at 558 nm, using acetate–citrate–isopropanol buffer as the blank. The myocardial collagen content was calculated from the standard curve prepared using commercially available L-4-hydroxyproline.

Light microscopy

Heart tissue was fixed in 10% buffered formalin (pH 7.2), routinely processed and embedded in paraffin. Paraffinised sections (5 μm) were cut and stained with haematoxylin and eosin (H&E) for study of myocardial necrosis and picrosirius red for study of myocardial fibrosis.

Morphometry

Morphometry was used for quantitative assessment of cardiac cell hypertrophy using NIH image analysis software (Image J, Wayne Rasband, Washington DC, USA). Myocyte size in H&E-stained sections was determined as reported previously.[27] The sections were screened, fields selected and captured under a Nikon E600 microscope fitted with a camera (Olympus DP-71) along with the in-built scale at 20 × magnification. Each image was retrieved and analysed using Image J software. Transverse sections of myocytes with a distinct cell membrane and visible nucleus were selected for morphometric analysis. The myocyte fibre diameter (μm) was measured perpendicularly across the myocyte at the level of the nucleus. Ten random fields were assessed for each heart section and a total of at least 100 myocytes per heart were measured and mean values taken. The analysis was carried out by two individuals blinded to the study groups.

Statistical analysis

Statistical analysis was done using SPSS 11.5 for Windows. Data are expressed as means ± SD. One-way analysis of variance followed by Bonferroni's post-hoc analysis for multiple comparisons was used to test for significance, set at $P < 0.05$.

Results

Characterisation of aqueous extract of *T. arjuna*

R_f values in the present solvent system were −0.44 for arjunolic acid and −0.28 for terminoic acid. The aqueous extract of *T. arjuna* was found to contain 0.39% (w/w) arjunolic acid and 0.35% (w/w) terminoic acid (Figure 1).

Changes in hypertrophic parameters

Changes in heart weight : body weight ratio (HW : BW) and cardiomyocyte diameter are shown in Table 1. There was a significant ($P < 0.001$) increase in HW : BW and myocyte diameter in the isoprenaline group compared with the control

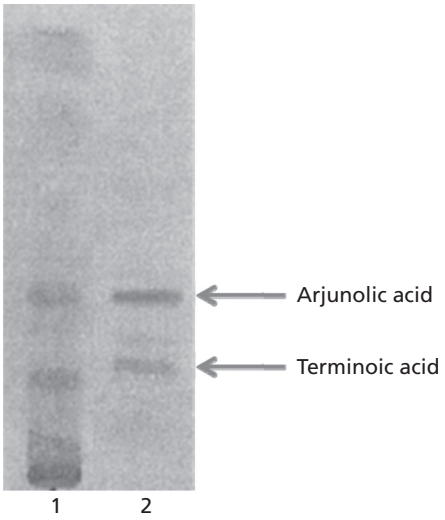

Figure 1 Thin-layer chromatogram of aqueous extract of *Terminalia arjuna* (lane 1) and standard terpenoids (arjunolic acid and terminoic acid) present in the bark (lane 2)

group. Treatment with *T. arjuna* or captopril did not prevent the increase in HW : BW.

Echocardiographic parameters

Echocardiographic parameters are summarised in Table 1. A significant increase in relative wall thickness ($P < 0.01$) was observed in the isoprenaline group but there were no significant changes in LVDd, LVDs or FS. Ventricular wall thickness was similar in rats treated with *T. arjuna* and captopril to the isoprenaline group. There were no significant differences in LVDd, LVDs and FS among the groups studied.

Myocardial lipid peroxidation

Myocardial TBARS levels are shown in Table 2. There was significant increase in myocardial TBARS level in the isoprenaline group compared with the control group ($P < 0.001$). The increase in myocardial TBARS level was prevented by treatment with *T. arjuna* extract at all three doses ($P < 0.01$ at 63 and 125 mg/kg; $P < 0.001$ at 250 mg/kg). Treatment with captopril also significantly prevented the increase in myocardial TBARS level ($P < 0.001$).

Myocardial endogenous antioxidants

Myocardial reduced glutathione

There was a significant decrease in myocardial GSH level in the isoprenaline group compared with the control group ($P < 0.001$; Table 2). The decrease in myocardial GSH level was prevented by treatment with *T. arjuna* extract 125 and 250 mg/kg (both $P < 0.01$). Captopril also significantly prevented the decrease in myocardial GSH level ($P < 0.01$).

Myocardial catalase activity

There was a significant decrease in myocardial catalase activity in the isoprenaline group compared with the control group ($P < 0.001$; Table 2). This decrease in catalase activity was prevented by treatment with *T. arjuna* extract ($P < 0.01$ at 63 mg/kg; $P < 0.001$ at 125 and 250 mg/kg) and captopril ($P < 0.001$).

Myocardial superoxide dismutase activity

Myocardial SOD activity was significantly decreased in the isoprenaline group compared with the control group ($P < 0.001$; Table 2). The decrease in SOD activity was decreased by treatment with *T. arjuna* extract at 125 ($P < 0.05$) and 250 mg/kg ($P < 0.01$) and captopril ($P < 0.05$).

Myocardial collagen content

There was significant increase in myocardial collagen content in the isoprenaline group compared with the control group ($P < 0.001$; Table 2). This increase in collagen content was prevented by treatment with the *T. arjuna* extract at 63 ($P < 0.05$) and 250 mg/kg ($P < 0.01$) and captopril ($P < 0.01$).

Light microscopy

The histopathological analysis showed myocardial damage predominantly in the subendocardial region. Figure 2a shows H&E-stained sections of myocardial tissue from the control group, showing normal histomorphology. Tissue from the isoprenaline group showed myocardial necrosis, loss of myocytes and inflammatory cell infiltration (Figure 2b). Myocardial tissue from rats treated with *T. arjuna* extract at 63 mg/kg show areas of necrosis, and infiltration of inflammatory cells is clearly visible (Figure 2c). Less necrosis and fewer inflammatory cells are present in samples from rats treated with 125 mg/kg *T. arjuna* extract (Figure 2d). Tissue from rats treated with 250 mg/kg or captopril shows relatively smaller

Table 1 Changes in left ventricular structure and function at the end of the study, measured by M-mode echocardiography. Myocardial hypertrophy was measured as heart weight : body weight ratio and microscopic evaluation of cardiomyocyte diameter.

	LVDd (mm)	LVDs (mm)	FS (%)	RWT	HW : BW (mg/g)	Cell diameter (μm)
Control	5.55 ± 0.22	2.93 ± 0.24	46.98 ± 3.94	0.50 ± 0.04	2.78 ± 0.33	21.66 ± 3.14
Isoprenaline group	5.78 ± 0.26	2.92 ± 0.16	49.47 ± 3.80	0.62 ± 0.06††	5.24 ± 0.48†††	34.02 ± 4.94†††
Isoprenaline + TA 63 mg/kg	5.72 ± 0.26	2.88 ± 0.23	49.58 ± 2.98	0.59 ± 0.04†	4.80 ± 0.49†††	34.83 ± 7.13††
Isoprenaline + TA 125 mg/kg	5.85 ± 0.48	2.85 ± 0.30	51.14 ± 5.44	0.58 ± 0.07	5.70 ± 0.68†††	31.21 ± 4.29†††
Isoprenaline + TA 250 mg/kg	5.87 ± 0.27	2.88 ± 0.27	50.85 ± 3.21	0.58 ± 0.04	4.94 ± 0.42†††	34.42 ± 4.88†††
Isoprenaline + captopril	5.45 ± 0.31	2.73 ± 0.24	49.67 ± 6.91	0.63 ± 0.04†††	4.87 ± 0.55†††	32.30 ± 4.92†††

FS, fractional shortening; HW : BW, heart weight : body weight ratio; LVDd, left ventricular dimension in diastole; LVDs, left ventricular dimension in systole; RWT, relative wall thickness; TA, *Terminalia arjuna* bark extract. Values are means ± SD ($n = 6$). $^{\dagger}P < 0.05$; $^{\dagger\dagger}P < 0.01$; $^{\dagger\dagger\dagger}P < 0.001$ vs control group.

Table 2 Biochemical parameters of oxidative stress in myocardial tissue

	TBARS (nmol/g tissue)	GSH (μg/g tissue)	SOD (units/mg protein)	Catalase (units/mg protein)	Collagen (μg/g tissue)
Control	18.4 ± 1.5	334.2 ± 35.6	5.8 ± 0.3	48.3 ± 4.0	275.0 ± 24.5
Isoprenaline	25.5 ± 1.7†††	239.8 ± 15.6†††	3.6 ± 0.4†††	11.8 ± 2.2†††	398.9 ± 27.3†††
Isoprenaline + TA 63 mg/kg	23.7 ± 2.3†††,**	256.2 ± 38.7†††	4.6 ± 0.4†	20.0 ± 2.8†††,**	322.5 ± 25.8*
Isoprenaline + TA 125 mg/kg	22.9 ± 2.4††,***	289.6 ± 18.6*	4.8 ± 0.5*	22.1 ± 3.8†††,***	336.0 ± 31.8
Isoprenaline + TA 250 mg/kg	18.2 ± 1.0***	306.4 ± 13.8**	5.2 ± 0.7**	29.4 ± 4.5†††,***	302.4 ± 53.3**
Isoprenaline + captopril	18.2 ± 1.1***	300.3 ± 19.7**	4.6 ± 0.6††,*	26.7 ± 4.5†††,***	315.9 ± 41.0**

GSH, glutathione; SOD, superoxide dismutase; TA, *Terminalia arjuna* extract; TBARS, thiobarbituric acid reactive substances. Values are means ± SD (n = 6). TBARS, GSH and collagen are per g tissue wet weight. Myocardial fibrosis is expressed in terms of collagen content in the heart estimated in terms of L-hydroxyproline. $^{\dagger}P < 0.05$; $^{\dagger\dagger}P < 0.01$; $^{\dagger\dagger\dagger}P < 0.001$ vs control; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs isoprenaline.

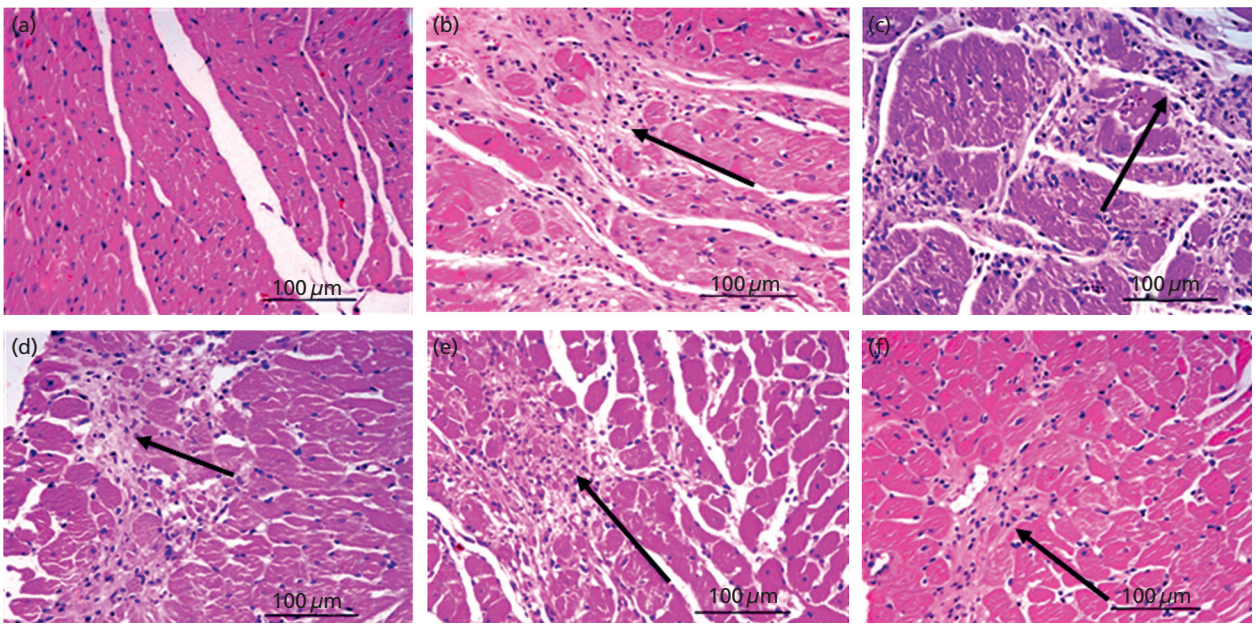

Figure 2 Light microscopic images (×20) of haematoxylin and eosin-stained myocardial sections showing areas of necrosis and inflammatory cells. (a) Control; (b) isoprenaline; (c) isoprenaline + *Terminalia arjuna* extract 63 mg/kg; (d) isoprenaline + *T. arjuna* extract 125 mg/kg; (e) isoprenaline + *T. arjuna* 250 mg/kg; (f) isoprenaline + captopril.

areas of necrosis and/or inflammatory cells (Figure 2e,f). Figure 3 shows sections stained with picrosirius red, showing areas of fibrosis. Samples from the control group did not show any marked areas of fibrosis whereas large fibrotic areas are observed in the isoprenaline groups (Figure 3a,b). Treatment with *T. arjuna* extract partially reduced the fibrosis, observed as relatively lesser replacement fibrosis (Figure 3c–e); however interstitial fibrosis was present. Captopril-treated rats showed similar patterns (Figure 3f).

Discussion

In the present study, chronic administration of isoprenaline led to LVH, characterised by increases in HW : BW and myocardial cell size. This was accompanied by increased myocardial lipid peroxidation and depletion of endogenous antioxidants (GSH, SOD and catalase), subendocardial necrosis coupled with replacement and reactive fibrosis. The current findings corroborate earlier reports in which chronic isoprenaline administration resulted in LVH and increased oxidative stress and fibrosis.[28] Simultaneous treatment with *T. arjuna* or captopril prevented the increase in oxidative stress and fibrosis following chronic isoprenaline administration but the increase in HW : BW and cardiomyocyte diameter were not prevented by either treatment.

Chronic isoprenaline administration is known to induce LVH and increase myocardial oxidative stress through generation of reactive oxygen species via NAD(P)H oxidase following β-adrenoceptor stimulation.[29] Isoprenaline induces activation of MAP kinase via G-protein-coupled receptors and via activation of various growth factors, which is known to activate genes encoding for cardiac protein synthesis and extracellular matrix remodelling, leading to cardiac hypertrophy.[30,31] Increase in myocardial lipid peroxidation and depletion of endogenous antioxidants such as GSH, SOD and catalase following isoprenaline

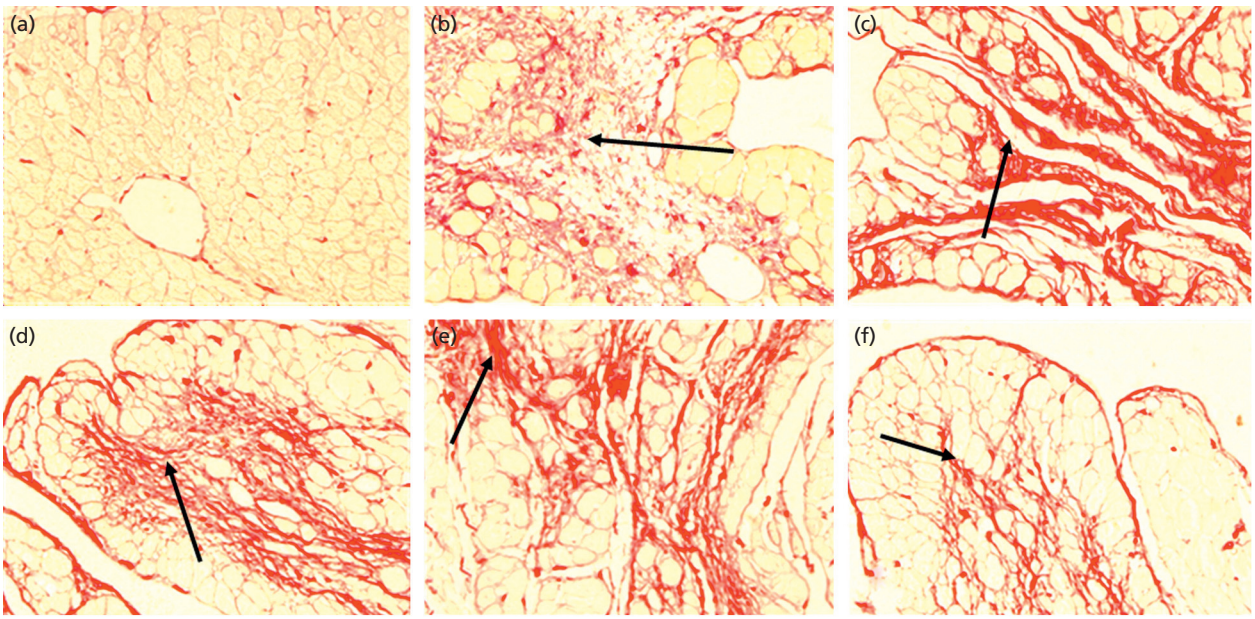

Figure 3 Light microscopic images (×20) of myocardial sections stained with picrosirius red showing areas of fibrosis and tissue damage. (a) Control; (b) isoprenaline; (c) isoprenaline + *Terminalia arjuna* extract 63 mg/kg; (d) isoprenaline + *T. arjuna* extract 125 mg/kg; (e) isoprenaline + *T. arjuna* 250 mg/kg; (f) isoprenaline + captopril.

administration have been reported in animal studies.[32] In recent studies, increased oxidative stress has been incriminated as a major aetiopathological factor in many cardiovascular disorders.[33] Free radicals generated following oxidative stress damage lipid membranes and deplete endogenous tissue antioxidants.

Protection against cardiac oxidative stress by the *T. arjuna* bark has been documented in various clinical and animal studies.[13,15] *T. arjuna* has already been shown to increase levels of endogenous antioxidants in the heart and thereby provides protection against oxidative injury due to either ischaemia–reperfusion[11,12] or high doses of isoprenaline.[10,11] In the present study, treatment with *T. arjuna* prevented the isoprenaline-induced increase in myocardial TBARS, showing maximum efficacy at the 250 mg/kg dose. Moreover, the depletion of endogenous antioxidants, GSH, SOD and catalase, was also prevented. All these myocardial antioxidants (i.e. GSH, SOD and catalase) play important roles in neutralising free radicals and thereby prevent further oxidative stress and tissue damage.

A number of compounds in *T. arjuna* with established antioxidant properties, such as tannins, saponins, triterpenoids, flavonoids and glycosides, could have been responsible for these antioxidant effects.[10,13] Arjunolic acid has been reported to have beneficial effects against myocardial necrosis caused by isoprenaline.[13] The quantitative analysis of the extract used in the present study confirmed the presence of the terpenoic acids, arjunolic acid and terminoic acid. However, other antioxidants (tannins, saponins, flavonoids) may be present along with arjunolic acid, and the content of arjunolic acid measured in the extract (0.39% w/w) was low compared with doses used earlier by Sumitra *et al.*,[13] so it is not prudent to assign the protective effects observed in the present study to arjunolic acid. It is possible that the crude extract with its different chemical classes of compounds with common pharmacological properties may offer comparable effects to the single chemical entity.

Collagen deposition (ultimately causing fibrosis) is considered to be a hallmark of pathological hypertrophy,[5] whereas absence of collagen deposition in the myocardium is characteristic of physiological hypertrophy.[34] An increase in collagen content and its gradual maturation causes myocardial dysfunction.[35,36] Different forms of fibrosis have been observed following isoprenaline treatment.[37] Myocardial necrosis following isoprenaline treatment is replaced by fibrous tissue, termed replacement fibrosis. Reactive fibrosis is also observed, which could be due to the activation of collagen secretion by cardiac fibroblasts following isoprenaline treatment, leading to perimysial collagen deposition.[38] Recently, increased oxidative stress has been reported to mediate the development of myocardial fibrosis.[39] In an earlier study, treatment with tempol, a membrane-permeable radical scavenger, was shown to prevent the increase in myocardial collagen content.[28] In the present study, the antioxidant effect of *T. arjuna* was associated with prevention of increase in myocardial collagen content. The protection against increased myocardial fibrosis was also evident in light microscopy studies, in which staining with picrosirius red revealed less fibrosis in groups treated with *T. arjuna*.

Activation of myocardial and/or systemic renin–angiotensin systems plays an important role in the development of isoprenaline-induced LVH.[40,41] Angiotensin-converting enzyme (ACE) inhibitors and drugs with antioxidant properties prevent the increase in oxidative stress as well as LVH in different animal models.[7,42] Angiotensin II has also been

implicated in the generation of reactive oxygen species, by a mechanism linked to NAD(P)H oxidase.[29] In the present study, captopril, an ACE inhibitor, prevented myocardial oxidative stress as well as depletion of endogenous antioxidants and had antifibrotic effects. Protection against myocardial fibrosis can be explained by prevention of isoprenaline-induced lipid peroxidation and the ability to reduce myocardial angiotensin II levels; the latter has been reported to be a strong mediator of isoprenaline-induced myocardial fibrosis.[41] The cardioprotective effect of captopril has been also proposed to be due to the presence of a thiol group.[43] To date, no study has reported any inhibition of the renin–angiotensin system by *T. arjuna* bark powder or any of its extracts. Therefore, it is possible that the aqueous extract of *T. arjuna* and captopril acted through different mechanisms to bring about similar effects in oxidative stress and myocardial fibrosis associated with LVH.

Although *T. arjuna* and captopril prevented the increase in myocardial oxidative stress and fibrosis, neither of them prevented the increase in hypertrophic response to isoprenaline. In an earlier study of isoprenaline-induced hypertrophy, two different doses of captopril (50 and 200 mg/kg) were evaluated and the higher dose was found to be effective in reducing the ventricular weight : body weight ratio.[44] However, the ventricular collagen area was increased with the higher dose of captopril compared with the isoprenaline group. The lower dose of captopril did not affect either the ventricular weight : body weight ratio or the collagen area. However, in the present study the same dose prevented the increase in collagen content. In earlier studies, use of the same dose or even lower doses was found to be effective in preventing/reducing cardiac hypertrophy in different animal models.[45,46] Its is not clear whether the effect of captopril observed in the present study is due to the lower dose or a lack of effect on isoprenaline-induced cardiac hypertrophy.[47–49] However, the possibility of higher doses of captopril having antihypertrophic effects cannot be ruled out.

Similar findings have been reported earlier where tempol, a cell-membrane-permeable radical scavenger, failed to prevent LVH, even though it modified the intracellular mediators of pathological hypertrophy, which suggests that different signal pathways are involved in oxidative stress and hypertrophy.[28] As oxidative stress and fibrosis are deleterious elements in the progression of hypertrophy to heart failure, attenuation of these factors might be clinically helpful. Moreover, it is important to note that sparing the hypertrophic response might be useful for maintaining the physiological hypertrophic response.

Conclusions

The present study has shown that *T. arjuna* bark extract is effective in preventing two major deleterious events – oxidative stress and fibrosis – associated with LVH. However, it did not affect the HW : BW. These findings support the earlier hypothesis that isoprenaline-induced hypertrophy is not completely prevented by antioxidants and suggests the role of other pathways independent of oxidative stress.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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