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Original Article

ETHANOL-INDUCED ALTERATIONS IN CARDIAC ENZYMES–AMELIORATIVE EFFECT OF *THESPESIA POPULNEA* LEAF EXTRACT

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ABSTRACT

Objective: This study covers the estimation of changes in cardiac enzymes such as ATPases and antioxidant enzymes following ethanol-administration in rats, and the possible ameliorative effect of leaf extract of the plant *Thespesia populnea* (TP) on these changes.

Methods: Male adult Wistar rats were divided into 10 groups of six rats each. Vehicle controls received 5% gum acacia. Experimental groups received ethanol (20%, 2g/kg); or TP leaf extract (200 mg/kg and 400 mg/kg respectively); or vitamin E (25 mg/kg); or carvedilol (1 mg/kg) per orally every morning for 6 w, individually as well as in combination with ethanol. Following this, changes in the activities of Na^+ ATPase, Ca^{2+}ATPase, Mg^{2+}ATPase, and antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) were determined in the heart tissue and compared with those in vehicle control.

Results: Ethanol (20%, 2g/kg) treatment caused a reduction from the vehicle control in activities of all the examined enzymes, with minimal reduction in Mg^{2+} ATPase activity (29.26%) and maximal reduction in CAT activity (71.05%). With TP leaf extracts of 200 and 400 mg/kg, vitamin E and carvedilol individually, the vehicle controls showed percent changes in enzyme activities ranging from –8.24% for Mg^{2+} ATPase activity to+109.39% for Na^+ ATPase activity caused by carvedilol. When administered along with ethanol, TP leaf extracts, vitamin E and carvedilol reversed the effect of ethanol to various degrees and brought back the enzyme activities to near vehicle control levels. While recovery with 200 mg *Thespesia* leaf extract was less, ranging from 24.1% for Mg^{2+}ATPase activity to 190.91% for CAT activity, 400 mg *Thespesia* extract effected a greater recovery, with a minimum of 48.19% for Mg^{2+} ATPase activity and a maximum of 222.73% for CAT activity, as compared with ethanol-treated rats as controls. These effects could be interpreted in terms of the adverse effects of ethanol on cardiac function and the ameliorative effects, primarily the antioxidant potential, of TP leaf extracts, vitamin E and carvedilol.

Conclusion: The restoration of enzyme activities with TP leaf extract may promote recovery of cardiac tissue from oxidative damage. Results from the current study indicate that treatment with TP leaf extract reduces ethanol-induced oxidative stress in rat heart and hence may help prevent cardiac damage.

Keywords: *Thespesia populnea,* Ethanol, Vitamin E, Carvedilol, ATPases, Cardiac toxicity

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INTRODUCTION

Widespread alcoholism is a serious problem in the modern society. Chronic excessive ethanol consumption has adverse effects on virtually all organs and tissues in the body [1]. Chronic ethanol ingestion leads to alcoholic cardiac myopathy and impaired cardiac function [2, 3]. Ethanol interacts with the cellular constituents, causing profound changes in their structure, organisation and functions. Alteration in lipid components and ion-channels by ethanol can cause changes in membrane function by altering its fluidity [4]. Ethanol consumption has been demonstrated to increase the rate of generation of hydrogen peroxide (H_2O_2) and hydroxyl radical (. OH) in isolated mitochondria in the presence of metal ion-chelate complexes [5].

Various reports suggest the protective effects of herbs and plants possessing phytochemical principles that could effectively prevent the deleterious effects of ethanol caused primarily due to oxidative damage. The results have been promising [6-8]. In recent years, the importance of antioxidant activities of phytoconstituents has gained attention due to their capacity to act as powerful antioxidants. These constituents presumably can protect the human body from free radicals and reactive oxygen species (ROS) [9]. Phytoactive compounds such as flavonoids, phenolics etc. have been studied mainly for their properties against oxidative damage. Oxidative impairment could lead to various diseases such as cancer, cardiovascular problems, inflammation etc. [10, 11].

In the quest to study plants that might provide alleviation from the development of cardiotoxicity, identifying natural products possessing the potential to provide cardioprotection has been one of the most explored areas. *Thespesia populnea* (TP) of the Malvaceae family is a large tree found in tropical regions and coastal forests of India. The plant is used in the treatment of skin ailments such as scabies, psoriasis, wounds and ulcers [12] and inhibits tumor formation [13]. The leaves, flowers and fruits of TP are used in *Ayurveda* for the control of diabetes [14, 15]. Studies on rats showed that its bark and flowers possess astringent, hepatoprotective and antioxidant activities [17, 18]. Phytochemical study of the leaf extract indicates the presence of lupeol, lupenone, β-sistosterol and, acacetin, kaempferol, quercetin, ferulic acid, vanillic acid, syringic and melilotic acids [19].

Pharmacological and biochemical studies have been carried out on animal tissues to examine the protective effects of TP [14, 16-18]. Na^+/K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase are integral membrane proteins present in all mammalian cells and are involved in the transport of Na^+, K^+, and Ca^{2+} ions across the plasma membrane. They are necessary for maintaining the electrochemical gradient, which is important in the processes of electrical excitation, contraction of the muscle cells and transport of other ions [20]. It has been demonstrated that ethanol administration alters these enzymes and thus produces organ damage [21, 22]. Superoxide dismutase (SOD) is responsible for the catalytic dismutation of the potentially toxic superoxide anion radical to H_2O_2. It is an effective defence of the cells against the endogenous and exogenous generation of ROS [23]. Catalase (CAT) is present in peroxisomes and catalyzes the decomposition of H_2O_2 to yield oxygen (O_2) and

water (H_2O) [24]. The equilibrium between these enzymes is an important process for the effective removal of oxygen stress in intracellular organelles [25].

In view of the importance of ATPase and antioxidant enzymes in biological systems and the possible damage that could be inflicted by ethanol on these enzymes and consequently on heart function, the present study has been taken up to establish the alterations in ATPase and antioxidant enzyme activities due to chronic ethanol administration in rats and to examine the possibility of counteracting ethanol effects by employing TP leaf extract.

MATERIALS AND METHODS

Procurement and maintenance of experimental animals

Wistar strain, adult male albino rats (n = 60) weighing 200±20 g were obtained from Sun Pharma Advanced Research Company Pvt Ltd (SPARC Pvt Ltd). The rats were housed in clean polypropylene cages, maintained in a temperature-controlled room (25±2 °C) with a photoperiod of 12 h light and 12 h dark cycle. The rats were provided with a standard pellet diet (VRK Nutritional Solutions, Laboratory Animal Diets, Pune, India) and water *ad libitum* throughout the experimental period. The protocol for this study was approved by the Institutional Animal Ethics Committee (Regd. No. 1029/PO/ERe/S/07/CPCSEA) in its proposal number BIP/IAEC/ 2015/07 dated 3rd July 2015.

Chemicals

All chemicals used in the study were AnalaR grade and were obtained from the following scientific companies: Sigma (St. Louis, MO, USA); Fisher Scientific (Pittsburgh, PA, USA); Merck (Mumbai, India); Ranbaxy (New Delhi, India); Qualigens (Mumbai, India); SD Fine Chemical Limited (Gujarat, India); Chemdyes Corporation (Gujarat, India). Vitamin E and carvedilol were used as reference standards.

Selection of the plant material

Thespesia populnea (TP) belonging to Malvaceae family was selected for the present study owing to its acknowledged medicinal properties [26, 27]. Fresh leaves of TP were collected. The plant material was taxonomically identified and authenticated (Voucher specimen No.: BSI/AZRC/I.1202/Tech./2012-13 (P1. Id.)/719) by the Botanical Survey of India, Jodhpur, Rajasthan, India. The leaves were thoroughly cleaned, and the good ones were handpicked and shade-dried. Sufficient quantity of leaves was powdered in an electric grinder, sieved using a 24 mesh sieve to obtain fine leaf powder, which was used for extraction. The leaf powder was defatted with petroleum ether and then air-dried.

Following this, the powder was soaked in water and allowed for percolation [28] for 24h, and the solvent was filtered using a moist muslin cloth. The extract was recovered, water was added to the leaf powder and the extraction was continued. This process was repeated three to four times till a colourless extract was obtained. The extract was distilled and concentrated under reduced pressure in a Buchi Rotovapour (R-114) to yield a dark-colored residue and then dried in a vacuum desiccator to remove any remaining water [29, 30]. The required quantity of aqueous extract was suspended in 5% gum acacia at required concentration doses, calculated according to the body weight, and used in all experiments [31].

Dose fixation for TP leaf extract

Dose-dependent studies were done to select the effective dose of TP leaf extract to counter ethanol effects. TP doses (100, 200, 300, 400 and 500 mg/kg body wt.) were tested for their effectiveness by *in vivo* studies in rats for 28 d, with 4 rats per dosage group, and ATPase activities were examined in the heart tissue. It was found that the effect was dose-dependent and doses from 200 to 400 mg/kg of TP effectively up-regulated the enzyme activities. Hence, a lower dose of 200 mg/kg and a higher dose of 400 mg/kg were chosen for determining the efficacy of TP to counter ethanol effects.

Treatment protocol

The rats were divided into 10 groups of six animals each, and the treatment was given daily via orogastric tube for 6 w.

Group I**:** Received only 5% gum acacia (5 ml/kg per day p. o.) for 6 w and served as vehicle control (VC).

Group II**:** Received only ethanol (20%, 2g/kg, p. o.) for 6 w.

Group III**:** Received only aqueous leaf extract (200 mg/kg) for 6 w.

Group IV**:** Received only aqueous leaf extract (400 mg/kg) for 6 w.

Group V**:** Received aqueous leaf extract (200 mg/kg) plus 20% ethanol, 2g/kg, p. o. for 6 w.

Group VI**:** Received aqueous leaf extract (400 mg/kg) plus 20% ethanol, 2g/kg, p. o. for 6 w.

Group VII**:** Received only vitamin E (25 mg/kg, p. o.) for 6 w. This group served as drug control or reference control.

Group VIII**:** Received vitamin E plus 20% ethanol, 2g/kg, p. o. for 6 w.

Group IX**:** Received only carvedilol (1 mg/kg, p. o.) for 6 w. This served as drug control or reference control.

Group X**:** Received carvedilol plus 20% ethanol, 2g/kg, p. o. for 6 w**.**

Induction of ethanol-cardio toxicity and isolation of tissue

Ethanol-cardiotoxicity was induced following Husain and Somani [32]**.** Ethanol 20% (2 g/kg, PO) was given via orogastric tube daily for 6 w to the group. In the groups receiving TP extract (200 mg/kg and 400 mg/kg, respectively), vitamin E (25 mg/kg, p. o.) and carvedilol (1 mg/kg, p. o.) respectively, ethanol was given additionally via orogastric tube daily for 6 w. Individual control groups were maintained for all the groups under treatment. At the end of the dosing schedules for all the experimental groups, the heart was excised under euthanasia in chilled Tris buffer (10 mmol pH 7.4) and used to prepare homogenates for enzyme assays.

Assay of ATPase activities

ATPase activities were assayed by the method of Fritz and Hamrick [33] as reported by Desaiah and Ho [34]. Na^+/K^+ and Mg^{2+}ATPase activities were estimated in the mitochondrial fraction. The reaction mixture in a volume of 3.0 ml contained 3 mmol ATP, 3 mmol magnesium chloride ($MgCl_2$), 100 mmol sodium chloride ($NaCl_2$), 20 mmol potassium chloride (KCl), 135 mmol imidazole-hydrochloric acid buffer (pH 7.5), and 0.3 ml of mitochondrial suspension as the enzyme source. The reaction mixture was incubated at 37 °C for 30 min. The reaction was stopped by the addition of 0.1 ml of 50% trichloroacetic acid (TCA). The samples were then assayed for inorganic phosphate using the method of Lowry and Lopez [35] as modified by Phillips and Hayes [36]**.**

The colour was read at 620 nm in a spectrophotometer. Mg^{2+}ATPase activity was measured in the presence of 1 mmol ouabain, a specific inhibitor of Na^+/K^+ ATPase [37]**.** Ouabain-sensitive Na^+/K^+ ATPase activity was obtained from the difference between total ATPase activity and Mg^{2+}ATPase activity. The enzyme activity was expressed as µmol of inorganic phosphate formed/mg protein/h. Ca^{2+}ATPase activity was determined by measuring the inorganic phosphate liberated during the hydrolysis of ATP. The activity was estimated in the mitochondrial fraction. The reaction mixture in a volume of 3.0 ml contained 135 mmol imidazole-hydrochloric acid buffer (pH 7.5), 5 mmol $MgCl_2$, 0.05 mmol $CaCl_2$, 4 mmol ATP, and 0.3 ml of mitochondrial suspension as the enzyme source. The reaction mixture was incubated at 37 °C for 30 min and stopped by the addition of 0.1 ml of 50% TCA.

The inorganic phosphate formed was estimated by the method of Lowry and Lopez [35] as modified by Phillips and Hayes [36]**.** The colour was read at 620 nm in a spectrophotometer**.** Mg^{2+}ATPase activity was measured in the presence of 0.5 mmol ethylene glycol tetra acetic acid (EGTA), and this value was subtracted from total ATPase activity to obtain Ca^{2+} ATPase activity. The enzyme activity was expressed as µmol of inorganic phosphate formed/mg protein/h.

Assay of antioxidant enzymes

Superoxide dismutase (SOD)

SOD activity was determined according to the method of Misra and Fridovich [38] at room temperature. The heart tissue was homogenised in ice-cold 50 mmol phosphate buffer (pH 7.0) containing 0.1 mmol ethylene diamine tetra acetic acid (EDTA) to give 5% homogenate (w/v). The homogenates were centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant was separated and used for enzyme assay. 100 µl of tissue extract was added to 880 µl carbonate buffer (0.05 M, pH 10.2, containing 0.1 mmol EDTA). 20 µl of 30 mmol epinephrine (in 0.05% acetic acid) was added to the mixture and the optical density was measured at 480 nm for 4 min in a Hitachi U-2000 Spectrophotometer. The enzyme activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 unit.

Catalase (CAT)

Catalase activity was measured by a slightly modified version of Aebi [39] at room temperature. The heart tissue was homogenised in ice cold 50 mmol phosphate buffer (pH 7.0) containing 0.1 mmol EDTA to give a 5% homogenate (w/v). The homogenates were centrifuged at 10,000 rpm for 10 min at 4 °C. The resulting supernatant was used as enzyme source. 10 µl of 100% EtOH was added to 100 µl of tissue extract and then placed in an ice bath for 30 min. Following this, the tubes were kept at room temperature and 10 µl of Triton X-100 RS were added. In a cuvette containing 200 µl of phosphate buffer and 50 µl of tissue extract, 250 µl of 0.066 M H_2O_2 (in phosphate buffer) were added, and the decrease in optical density was measured at 240 nm for 60 s in a UV spectrophotometer. The molar extinction coefficient of 43.6 M cm^{-1} was used to determine CAT activity. One unit of activity is equal to the mol of H_2O_2 degraded/mg protein/min.

Statistical analysis

The assay of enzyme activities was carried out with six separate replicates from each group. The values were expressed as mean±standard deviation (SD) from six animals. The significance of differences between the control and treated animals for different parameters was determined by using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons posthoc tests using Graphpad Prism 5 computer package software. P values of at least<0.05 were considered as statistically significant.

RESULTS

The levels of Na^+/K^+ATPase, Ca^{2+} ATPase, Mg^{2+} ATPase, SOD and CAT activities were recorded in the cardiac tissue in vehicle control and following the administration of ethanol, TP leaf extracts (200 mg/kg and 400 mg/kg respectively), vitamin E (25 mg/kg) and carvedilol (1 mg/kg) separately and after pre-treatment with TP leaf extracts, vitamin E and carvedilol before the administration of ethanol. The results are presented in Tables 1 and 2 and fig. 1 and 2. The changes in enzyme activities were expressed with reference to the vehicle control.

Na^+/K^+ ATPase

The vehicle control group recorded the activity of Na^+/K^+ATPase at around 1.49 µmol. TP extract of 200 mg/kg did not cause any significant change in the control activity, whereas increases of 12.98%, 42.95% and 109.39% in the enzyme activity were recorded in groups that received TP leaf extract in the dose of 400 mg/kg, vitamin E and carvedilol respectively. On the other hand, the rats that received ethanol showed a significant decrease of 46.98% in the enzyme activity. Pre-treatment with TP extract (200 mg/kg or 400 mg/kg) or vitamin E or carvedilol before the administration of ethanol could revert this decrease by 102.53%, 140.52%, 120.25%, and 146.83% respectively when compared to ethanol-administered group as the control, and brought back the activity to around the vehicle control (table 1; fig. 1).

Ca^{2+} ATPase

In vehicle control group, the Ca^{2+}ATPase activity was found to be around 1.58 µmoles. TP leaf extract in the doses of 200 mg, 400 mg, vitamin E and carvedilol caused significant increases in the enzyme activity by 7.59%, 34.18%, 18.99%, and 1.9% respectively from the vehicle control. In contrast, administration of ethanol caused a significant decrease in the enzyme activity by 50%. Pre-treatment with TP extract in both the doses, vitamin E and carvedilol before the administration of ethanol caused recovery of the enzyme activity from the level in ethanol-administered group by 107.59%, 130.38%, 91.14%, and 79.75% respectively, and reverted the activities to the vehicle control level (table 1; fig. 1).

Mg^{2+} ATPase

The vehicle control recorded the activity at around 3.52 µmoles. The Mg^{2+}ATPase activity was lowered by 29.26% in ethanol-treated rats as compared to the vehicle control group. Administration of the TP leaf extract at 200 and 400 mg/kg respectively and vitamin E to the rats individually caused slight increases of 3.98%, 7.39%, and 6.53% respectively in the enzyme activity, while a non-significant decrease of 8.24% was recorded with the administration of carvedilol. Pre-treatment with TP extract at 200 or 400 mg, vitamin E or carvedilol prior to ethanol administration reverted the enzyme activity from the level in ethanol-treated group by 24.1%, 48.19%, 30.12, and 26.51% respectively, and brought back the activity to the vehicle control level (table 1; fig. 1).

Table 1: Changes in Na^+/K^+ATPase, Mg^{2+}ATPase and Ca^{2+} ATPase activities (expressed as µmol of inorganic phosphate formed/mg protein/h) in the presence of ethanol (20%, 2g/kg, p. o.), TP leaf extract (200 and 400 mg/kg), vitamin E (25 mg/kg, p. o.) and carvedilol (1 mg/kg, p. o.) separately and in combination

Experimental group	Na^+/K^+ ATPase	Ca^{2+} ATPase	Mg^{2+} ATPase
Vehicle control (5% Gum acacia)	1.49±0.17	1.58±0.15	3.52±0.28
Ethanol (20%)	0.79±0.12***	0.79±0.18***	2.49±0.24***
TP leaf extract (200 mg)	1.50±0.18 NS	1.70±0.16 NS	3.66±0.19 NS
TP leaf extract (400 mg)	1.67±0.19 NS	2.12±0.12***	3.78±0.26 NS
Standard (vitamin E)	2.13±0.29***	1.88±0.14**	3.75±0.32 NS
Carvedilol	3.12±0.26***	1.61±0.10 NS	3.23±0.22 NS
TP leaf extract (200 mg)+ethanol	1.60±0.12 NS	1.64±0.09 NS	3.09±0.33 NS
TP leaf extract (400 mg)+ethanol	1.90±0.14 **	1.82±0.14 NS	3.69±0.31 NS
Vitamin E+ethanol	1.74±0.20*	1.51±0.18 NS	3. 24±0.27 NS
Carvedilol+ethanol	1.95±0.22*	1.42±0.07 NS	3.15±0.16 NS

Note: Each value is mean±standard deviation (SD) of 6 independent observations. * P<0.05; ** P<0.01; *** P<0.001; NS: Not significant

Superoxide dismutase (SOD)

The vehicle control recorded the activity at around 16.45 units. SOD activity decreased by 54.16% in the ethanol-administered group compared to the vehicle control. Administration of the TP leaf extract at 200 or 400 mg/kg, vitamin E and carvedilol to the rats individually caused increases of 5.11%, 15.14%, 9.91%, and 13.01% respectively. Pre-treatment with TP extract at 200 or 400 mg, vitamin E or carvedilol prior to adding ethanol reverted the enzyme activity from the level in ethanol-treated group by 59.55%, 82.76%, 91.91%, and 82.89% respectively, and brought back the activity to the level of vehicle control (table 2, fig. 2).

Catalase (CAT)

In vehicle control, the CAT activity was found to be about 0.76 µmoles. The activity decreased by 71.05% in the ethanol-treated group compared to the vehicle control. TP extract (200 or 400 mg/kg), vitamin E, and carvedilol caused increases of 7.89%, 14.47%, 3.95%, and 6.58% respectively in the enzyme activity from the vehicle control. Pre-treatment with TP extract (200 or 400 mg/kg) or vitamin E or carvedilol prior to ethanol administration reverted this decrease by 190.91%, 222.73%, 231.82%, and 204.54% respectively when compared to ethanol-administered group as the control, and brought back the activity to around the vehicle control (table 2, fig. 2).

Table 2: Changes in superoxide dismutase (SOD) (expressed as units of superoxide anion reduced/mg protein/min) and catalase (CAT) (expressed as µmol of hydrogen peroxide (H_2O_2) degraded/mg protein/min) activities in the presence of ethanol (20%, 2g/kg, p. o.), TP leaf extract (200 and 400 mg/kg), vitamin E (25 mg/kg, p. o.) and carvedilol (1 mg/kg, p. o.) separately and in combination

Experimental group	SOD	Catalase
Vehicle control (5% Gum acacia)	16.45±0.59	0.76±0.07
Ethanol (20%)	7.54±0.45***	0.22±0.06***
Thespesia leaf extract (200 mg)	17.29±0.53 NS	0.82±0.03 NS
Thespesia leaf extract (400 mg)	18.94±0.55*	0.87±0.05*
Standard (vitamin E)	18.09±0.26 NS	0.79±0.07 NS
Carvedilol	18.59±0.55*	0.81±0.08 NS
Thespesia leaf extract (200 mg)+ethanol	12.03±0.48***	0.64±0.08*
Thespesia Leaf extract (400 mg)+ethanol	13.78±0.56***	0.71±0.14 NS
Vitamin E+ethanol	14.47±0.51*	0.73±0.15 NS
Carvedilol+ethanol	13.79±0.55***	0.67±0.11 NS

Note: Each value is mean±standard deviation (SD) of 6 independent observations. * P<0.05; *** P<0.001; NS: Not significant

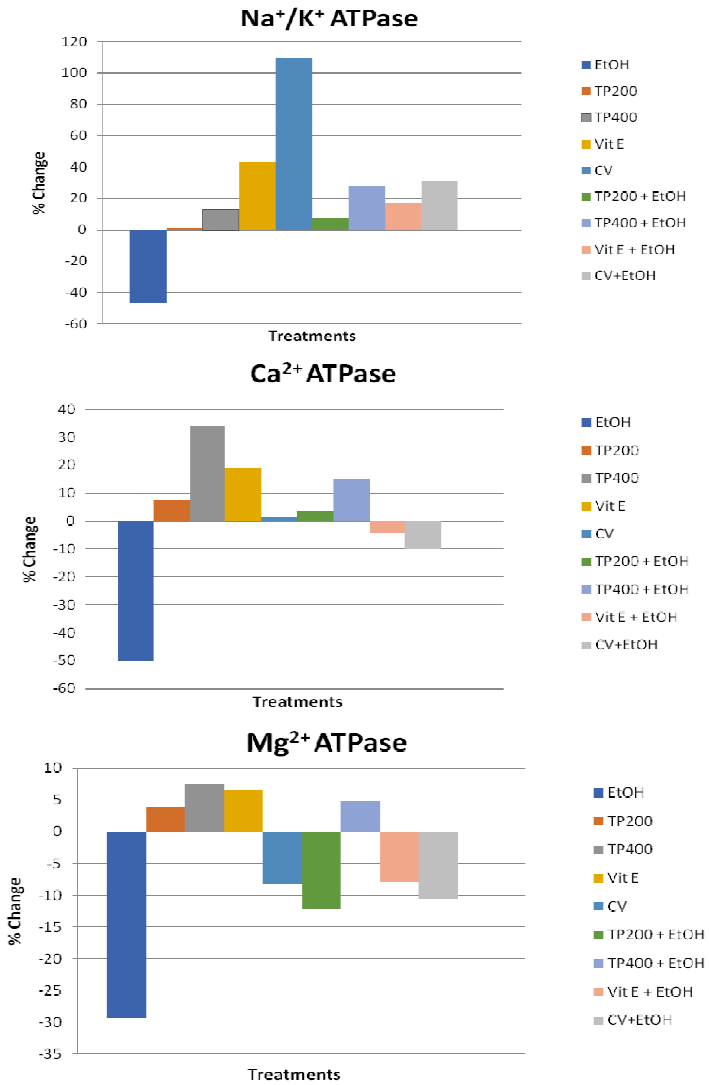

Fig. 1: Percent changes in Na^+/K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase enzyme activities in rat heart tissue following the administration of ethanol, TP leaf extract (200 and 400 mg/kg), vitamin E and carvedilol individually as well as in combination with ethanol. The percent changes between means each of 6 independent observations were calculated by comparing with vehicle controls. Note: EtOH–ethanol; TP 200 and TP 400–TP leaf extracts; Vit E–vitamin E; CV–carvedilol; TP 200+EtOH and TP 400+EtOH–TP leaf extracts+ethanol; Vit E+EtOH–vitamin E+ethanol; CV+EtOH–carvedilol+ethanol

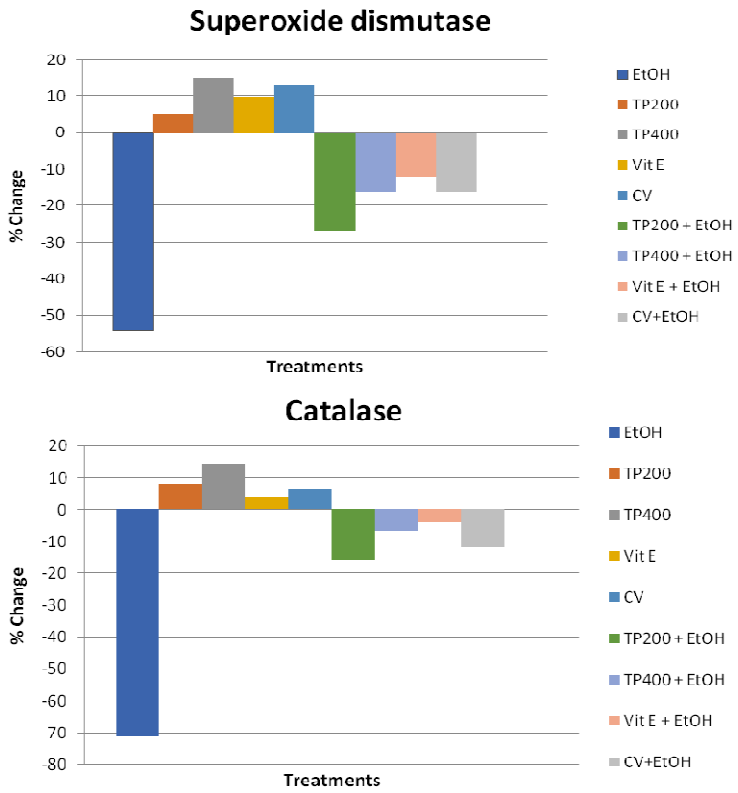

Fig. 2: Percent changes in superoxide dismutase and catalase enzyme activities in rat heart tissue following the administration of ethanol, TP leaf extract (200 and 400 mg/kg), vitamin E and carvedilol individually as well as in combination with ethanol. The percent changes between means each of 6 independent observations were calculated by comparing with vehicle controls. Note is same as for fig. 1

DISCUSSION

Acute and chronic administration of ethanol in several human and animal models has demonstrated cardiotoxic effect [40], with the major ethanol metabolite acetaldehyde contributing to cardiac dysfunction, hypertrophy and heart failure by either its direct toxicity or promoting elevated levels of catecholamines and ROS [41]. ATPases are membrane-bound enzymes involved in energy-mediated translocation of Na^+, $Ca^{2+}$and Mg^{2+}ions [42]. Studies have shown a reduction in the activity of these enzymes upon damage to the myocardium [43, 44]. Calcium overload in the myocardial cells during ischemia activates the Ca^{2+}ATPase, depleting high energy phosphate stores and thereby indirectly inhibiting Na^+ and K^+ transport and inactivating Na^+/K^+ ATPase [44]. Ethanol interacts with the cellular constituents causing profound changes in their structure, organisation and functions. Alteration in lipid components and ion-channels by ethanol can cause changes in membrane function by altering its fluidity [4] and thereby inhibition of Na^+/K^+ATPase and Ca^{2+}ATPase activities [45, 46].

As stated above, inhibition of ATPase activities in the present study is attributable to the interaction of ethanol with the cellular constituents [47]. Administration of TP extract at the dose of 200 mg/kg had only a partial effect on elevating the ATPase activities when followed by ethanol administration, while significant improvement was observed with 400 mg/kg (table 1; fig. 1). Thus, TP extract in the dose of 400 mg/kg has greater efficacy in reverting the inhibitory effect of ethanol on ATPase enzymes. This observation points to a dose-dependent action of TP leaf extract on ethanol effect and is suggestive of TP leaf extract conferring a membrane stabilising protective effect.

TP leaf extract has been reported to exhibit hepatoprotective, anti-inflammatory and antimicrobial effects [26, 27]. This protection may be credited to the presence of antioxidant principles such as flavonoids, phenolic acids and saponins, which have proven antioxidant effects [48]. Thus, restoration of ATPase activity to normal by TP leaf extract in ethanol-treated rats is attributable to the presence of antioxidant phytoconstituents such as ferulic acid, synringic acid and vanillic acid etc. [19]. Therefore, restoration or elevation of the endogenous antioxidant enzyme activities to normal may be attributed to the antioxidant potential of TP protecting the ATPase enzymes from oxidative degradation.

Although several mechanisms have been suggested for the toxicity produced by ethanol, one of the most common mechanisms is by way of generation of free radicals [47]**.** The decrease in SOD activity due to ethanol indicates inefficient scavenging of ROS, which might be implicated to the oxidative inactivation of the enzyme [49]. Ethanol administration also reduces catalase activity in cardiac, liver, kidney and other tissues [50]. Results of the present study are in agreement with the earlier reports, with a reduction in the activity of SOD and CAT.

Vitamin E has been explored for its antioxidant benefits in various conditions of cardiotoxicity, and epidemiological data indicate an inverse association between cardiovascular risk and vitamin E intake from dietary sources and/or supplements [43, 51]. The ameliorative effect of vitamin E in the present study could be attributed to its free radical scavenging ability. Earlier studies also credit the beneficial effect of vitamin E on membrane-bound enzymes to its potential to protect the-SH groups from oxidative damage through the inhibition of peroxidation of membrane lipids [52].

Earlier reports from the literature suggest that the protective role of carvedilol on the heart is due to its antioxidant effect. Recent studies suggest that the drug protects against the oxidation of sarcoplasmic reticulum Ca^{2+} ATPase, inhibits oxidative damage to amino acids, and also reduces oxidative stress in the myocardium in patients with dilated cardiac myopathy [53, 54]. Contrary to these reports on the ATPase elevating effects of carvedilol, the results of the present study showed a slight nonsignificant decrease in activity of Mg^{2+}ATPase upon individual carvedilol treatment. Nevertheless, it was able to reduce the decrease caused by the treatment with alcohol when administered along with it. This effect of carvedilol on the membrane-bound enzymes is probably due to its inherent antioxidant activity as opposed to its beta-receptor blocking action.

This is confirmed by studies indicating the anti-inflammatory and antioxidant properties of carvedilol [55].

The equilibrium between SOD and CAT activities is an important process for the effective removal of oxygen stress in intracellular organelles [56]. The heart tissue has less antioxidant enzyme activity compared to liver and other tissues, making it more vulnerable to peroxidative damage due to oxidative stress [32, 57]. Therefore, the reduced capability of the heart tissue to eliminate superoxide and hydrogen peroxide radicals may thus predispose to oxidative stress in the tissue.

Under normal conditions, scavenging mechanisms operate swiftly to remove excess ROS. Experimental studies on TP have shown that the leaf extract exhibits free radical scavenging properties *in vitro* on hydroxyl radicals, peroxyl radicals, and superoxide free radicals [27]. In the present study SOD and CAT activities were restored to normal by TP leaf extract in rats co-administered with ethanol. These results are suggestive of the superoxide and hydrogen peroxide radical scavenging effects produced by the TP leaf extract in the cardiac tissue. This assumption is supported by the demonstrated *in vitro* radical scavenging potential of the plant [27]. Earlier studies on TP also report the antioxidant activity of the plant in different conditions [17, 58].

CONCLUSION

From the findings of the present study, it can be inferred that TP counters the changes in the activities of ATPases and antioxidant enzymes SOD and CAT brought about by ethanol administration, thereby reverting the abnormal changes towards normal. The use of TP in preventing the alterations in membrane homoeostasis and endogenous antioxidant enzymes could prove to be of benefit owing to the phytochemical protective constituents in the plant. Further studies with TP could provide a better understanding of the effects of the plant at the molecular level and the mechanisms involved in conferring cardioprotection.

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AUTHORS' CONTRIBUTION

This investigation was carried out by the first author Ms. Sangeetha L. A. Rajbanshi under the close supervision of Dr. Archana N. Paranjape (2nd author) who directed the research and reviewed the manuscript and under the advice of the co-supervisor Dr. Vasu Appanna (3rd author) who also reviewed the manuscript.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests.

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