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Counteraction of adriamycin-induced alterations in cardiac enzymes by *Thespesia populnea* leaf extract

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ABSTRACT

This study examined the ameliorative effect of *Thespesia populnea* leaf extract on the changes in ATPase and antioxidant enzyme activities in rat heart induced by the anthracycline adriamycin. Adriamycin is a potent broad-spectrum antitumor agent, but has adverse effects on the heart, resulting in cardiomyopathy and heart failure. Anthracyclines are suggested to render the cardiac tissue susceptible to free radical damage, and different parts of *T. populnea* have been shown to possess antioxidant activity. Thus, whether the action of adriamycin, if any, on the enzyme activities can be countered by *T. populnea* would be interesting to study. In the present investigation, male adult Wistar rats were divided into 10 groups of six rats each. Adriamycin (15mg/kg i.p. cumulative dose), *T. populnea* leaf extract (200 mg/kg and 400 mg/kg respectively), vitamin E (25mg/kg p.o.) and carvedilol (1mg/kg p.o.) were administered separately and in combination with adriamycin to the rats, and changes in ATPase, superoxide dismutase and catalase activities were determined in the heart tissue. Administration of adriamycin decreased the ATPase and antioxidant enzyme activities. *Thespesia* leaf extracts, vitamin E and carvedilol elevated the enzyme activities individually, and also reversed the effect of adriamycin to different degrees when administered along with it. The findings suggest that *T. populnea* leaf extracts recover the ATPase and antioxidant enzyme activities in the heart of rats from adriamycin stress. This attribute of the plant may be due to its ability to scavenge free radicals and lower the oxidative stress. The results provide preliminary pharmacological support for the use of *T. populnea* in preventing alterations in membrane homeostasis and conferring cardio-protection.

INTRODUCTION

Adriamycin (Doxorubicin) is an important antitumor anthracycline antibiotic drug used in the treatment of cancer. It possesses a potent broad-spectrum antitumor activity, and is commonly used in various cancer treatments (Green *et al*., 2006, Verma *et al*., 2008). In spite of being one of the most preferred drugs for treating several cancers, the clinical use of adriamycin is limited by its potential adverse effects on the heart such as irreversible dilated cardiomyopathy and heart failure (Liu Xi *et al*., 2002). The induction of free radical production is the best described major mechanism through which adriamycin injures the myocardium (Xu*et al*., 2001). Earlier reports suggested the use of different chemical agents to prevent adriamycin-induced cytotoxicity (Fadillioglu *et al*., 2003), and some of them have shown promising results. These chemicals include natural products or phyto-constituents, for example the most commonly used and investigated compounds such as vitamin E, C, A, carotenoids, coenzyme Q, flavonoids, polyphenols, herbal antioxidants, selenium, and virgin olive oil (Quiles *et al*., 2002). Many chemical compounds and herbal formulations have been studied for their antioxidant activity in various tissues by using *in vivo* models such as isoproterenol-induced myocardial infarction (Sathish *et al*., 2003). These compounds have been studied mainly for their properties against oxidative damage leading to various degenerative diseases, such as cardiovascular diseases, inflammation, cancer, etc., and have been suggested to be associated with diverse functions, including nutrient uptake, protein synthesis, enzyme activity, photosynthesis, allelopathy, etc. (Einhellig *et al*., 1986).

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Identifying natural products possessing the potential to provide cardio-protection has therefore been the most explored area for investigating remedies for target organ damage. *Thespesia populnea* (TP) of the Malvaceae family is a large tree found in tropical regions and coastal forests of India. The leaves, flowers, and fruits are useful in treating cutaneous infections such as scabies and psoriasis. The decoction of the leaf is commonly used for the treatment of skin and liver diseases. Oil of the leaf mixed with vegetable oil is useful in urethritis, gonorrhoea, as an astringent, and for hepato-protective and antioxidant activities (Ilavarasan *et al*., 2003a, b). The phytochemical study of leaf extract indicates the presence of lupeol, lupenone, β-sistosterol and, acacetin, kaempferol, quercetin, ferulic acid, vanillic acid, syringic and melilotic acids (Rastogi *et al*., 1979). Several studies focussing on the antioxidant properties of such phytochemicals show that their beneficial effects result via various mechanisms and hence can lead to favourable outcomes in disease states.

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. Anthracyclines such as adriamycin are reported to alter these enzymes and disturb the cardiac function (Babula *et al*., 2013). Superoxide dismutase (SOD) is responsible for the catalytic dismutation of the potentially toxic superoxide anion radical to H_2O_2. It is an effective defense of the cells against endogenous and exogenous generation of reactive oxygen species. Catalase (CAT) is present in peroxisomes and catalyzes the decomposition of H_2O_2 to yield O_2 and water (Ichikawa *et al*., 1994). The equilibrium between these enzymes is an important process for the effective removal of oxygen stress in intracellular organelles (Sharma *et al*., 2001). Whether the alterations produced by adriamycin on membrane-bound enzyme activities or energy-dependent transport systems or antioxidant systems can be countered by *Thespesia populnea* extract has not been established till date. Therefore, this study was undertaken to examine the effect of adriamycin treatment on the heart with reference to ATPase and antioxidant enzyme activities as indices, and to examine the ameliorative effect of *Thespesia populnea* leaf extract on adriamycin effects.

MATERIAL 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 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.

Selection of plant material

The plant *Thespesia populnea* (TP) belonging to Malvaceae family was selected for the present study owing to its acknowledged medicinal properties (Elakkiya *et al.,* 2011, Sahitya Chetan *et al*., 2012). Fresh leaves of *T. populnea* were collected. The plant material was taxonomically identified and authenticated by Botanical Survey of India, Jodhpur. The leaves were thoroughly cleaned, and 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 that 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 (Agrawal *et al*., 2007) for 24h, and the solvent was filtered using a moist muslin cloth.

The extract was recovered and water was added to the leaf powder and the extraction was continued. This process was repeated three to four times until a colorless 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. The percentage yield of the aqueous extract obtained was 13.6%. The total flavonoid content and total phenolic content of the extract was determined (Makkar *et al*., 1993; Zhishen *et al*., 1999). The total flavonoid and phenolic contents of aqueous extract of *T. populnea* was found to be 164µg quercetin equivalent/ mg of extract and 560 µg gallic acid equivalent/ mg of extract respectively.

The required quantity of aqueous extract (AQ-E) was suspended in 5% gum acacia at required concentration doses, calculated according to the body weight, and used in all experiments (Ghosh, 1984).

Dose fixation study for TP leaf extract

Earlier reports on *T. populnea* suggested different dosages of extracts for different parts for different experimental designs/protocols (Ilavarasan *et al*., 2003a, b, Haja Sherief *et al*., 2011, Shah *et al*., 2011). Since this is the first study reporting the effectiveness of TP on adriamycin-induced toxicity in rats, we have attempted to determine the effective dose of TP for adriamycin-induced toxicity. Dose-dependent studies were done to select the effective dose to counter adriamycin-induced stress. TP doses (100, 200, 300, 400 and 500 mg/kg body wt.) were tested for their effectiveness by *in vivo* studies in Wistar rats for 28 days, 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 200mg/kg and a higher dose of 400mg/kg were chosen for determining the efficacy of TP against adriamycin-induced cardiac stress.

Treatment protocol

The rats were divided into 10 groups of six animals each, and the treatment was given daily via orogastric tube for 28 days.

Group I received 5% gum acacia only (5 ml/kg per day p.o.) for 28 days and served as vehicle control (VC).

Group II received adriamycin (ADR) (15mg/kg during 3rd and 4th weeks in 6 equally divided doses of 2.5mg/kg i.p.)

Group III were given only aqueous leaf extract (200mg/kg) for 28 days.

Group IV were given only aqueous leaf extract (400mg/kg) for 28 days.

Group V received aqueous leaf extract (200 mg/kg) for 4 weeks and then received adriamycin during the 3rd and 4th weeks (TP200 + ADR).

Group VI received aqueous leaf extract (400 mg/kg) for 4 weeks and then received adriamycin during the 3rd and 4th weeks (TP400 + ADR).

Group VII received vitamin E (25 mg/kg, p.o.) for 4 weeks. This group served as drug control or reference control (VIT E).

Group VIII received vitamin E, for 4 weeks, which was followed by adriamycin during the 3rd and 4th weeks (VIT E + ADR).

Group IX received carvedilol (1mg/kg, p.o.) for 28 days. This served as drug control or reference control (CV).

Group X received carvedilol, for 4 weeks, which was followed by adriamycin during the 3rd and 4th weeks (CV + ADR).

Induction of adriamycin cardiotoxicity and isolation of tissue

Adriamycin cardiotoxicity was induced following Timao Li *et al*. (2000). Adriamycin was injected in the dose of 2.5mg/kg (15mg/kg cumulative dose) in the adriamycin group (disease control) and in the groups receiving *T. populnea* extract (200mg/kg, 400 mg/kg respectively), vitamin E (25 mg/kg, p.o.) and carvedilol (1mg/kg, p.o.) every alternate day during the 3rd and 4th weeks of the 28-day treatment protocol. 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 (10mM pH 7.4) and used to prepare homogenates for enzyme assays.

Assay of ATPase activities

ATPase activities were assayed by the method of Fritz *et al*. (1966) as reported by Desaiah *et al*. (1979). Na^+/K^+ and Mg^{2+}ATPase activities were estimated in mitochondrial fraction. The reaction mixture in a final volume of 3.0 ml contained 3mM ATP, 3mM $MgCl_2$, 100mM $NaCl_2$, 20 mM KCl, 135 mM imidazole-hydrochloric acid buffer (pH 7.5) and 0.3ml of mitochondrial suspension as enzyme source. The reaction mixture was incubated at 37°C for 30 minutes. The reaction was stopped by the addition of 0.1 ml of 50% TCA. The samples were then assayed for inorganic phosphate using the method of Lowry *et al*. (1946) as modified by Phillips *et al*. (1977). The colour was read at 620nm in a spectrophotometer. Mg^{2+}ATPase activity was measured in the presence of 1 mM ouabain, a specific inhibitor of Na+/K+ATPase (McIlwain, 1963). Ouabain-sensitive Na^+/K^+ATPase activity was obtained by the difference between total ATPase activity and Mg^{2+}ATPase activity. The enzyme activity was expressed as µmoles 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 final volume of 3.0 ml contained 135 mM imidazole-hydrochloric acid buffer (pH 7.5), 5 mM $MgCl_2$, 0.05mM $CaCl_2$, 4mM ATP, and 0.3ml of mitochondrial suspension as enzyme source. The reaction mixture was incubated at 37°C for 30 minutes and stopped by the addition of 0.1 ml of 50% TCA. The inorganic phosphate formed was estimated by the method of Lowry *et al*. (1946) as modified by Phillips *et al*. (1977). The colour was read at 620 nm in a spectrophotometer. Mg^{2+}ATPase activity was measured in the presence of 0.5 mM EGTA, and this value was subtracted from total ATPase activity to obtain Ca^2+ATPase activity. The enzyme activity was expressed as µmoles of inorganic phosphate formed/mg protein/h.

Assay of antioxidant enzymes

Superoxide Dismutase (SOD)

SOD activity was determined according to the method of Misra *et al*. (1972) at room temperature. The heart tissue was homogenized in ice-cold 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (w/v). The homogenates were centrifuged at 10,000 rpm for 10 min at 0°C in cold centrifuge. 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 0.1mM EDTA); and 20 µl of 30 mM epinephrine (in 0.05% acetic acid) was added to the mixture and the optical density was measured at 480 nm for 4 min on 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 (1984) at room temperature. The heart tissue was homogenized in ice-cold 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give a 5% homogenate (w/v). The homogenates were centrifuged at 10,000 rpm for 10 min at 0°C in cold centrifuge. 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. After 30 min 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 moles 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 ± SEM from six animals. The significance of differences between the control and treated animals for different parameters was determined by using one-way ANOV followed by Tukey's multiple comparison Post-hoc test 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, Mg^{2+}ATPase, Ca^{2+}ATPase, SOD and CAT activities were estimated in the cardiac tissue in vehicle controls and following the administration of adriamycin, TP leaf extracts (200 mg/kg and 400mg/kg respectively), vitamin E and carvedilol separately and after pre-treatment with *T. populnea* leaf extracts, vitamin E (25mg/kg) and carvedilol(1mg/kg) before the administration of adriamycin. The results are presented in Tables 1& 2 and Figures 1 & 2.

Na^+/K^+ATPase

The vehicle control group recorded the activity of Na^+/K^+ATPase at around 1.49 µmoles. The activity was significantly lowered in adriamycin-treated rats as compared to the vehicle control group. While TP extract of 200 mg/kg did not cause any perceptible change in the control activity, a significant increase in the enzyme activity was recorded in TP400, VIT E, CV groups (receiving *T. populnea* leaf extract in the doses of 400 mg/kg, vitamin E and carvedilol respectively). Pre-treatment with *Thespesia populnea* extract in the dose of 200mg/kg caused partial restoration of the ATPase activity towards the vehicle control, while pre-treatment with TP 400mg/kg before the administration of adriamycin increased the enzyme activity and restored it to normal. Similarly, the rats treated with vitamin E + adriamycin and carvedilol + adriamycin showed significant increases in the Na^+/K^+ATPase activity and restored it fully to the level of vehicle control. Pre-treatment with vitamin E was found to be slightly more effective compared to treatments with TP and carvedilol (Table 1; Fig. 1).

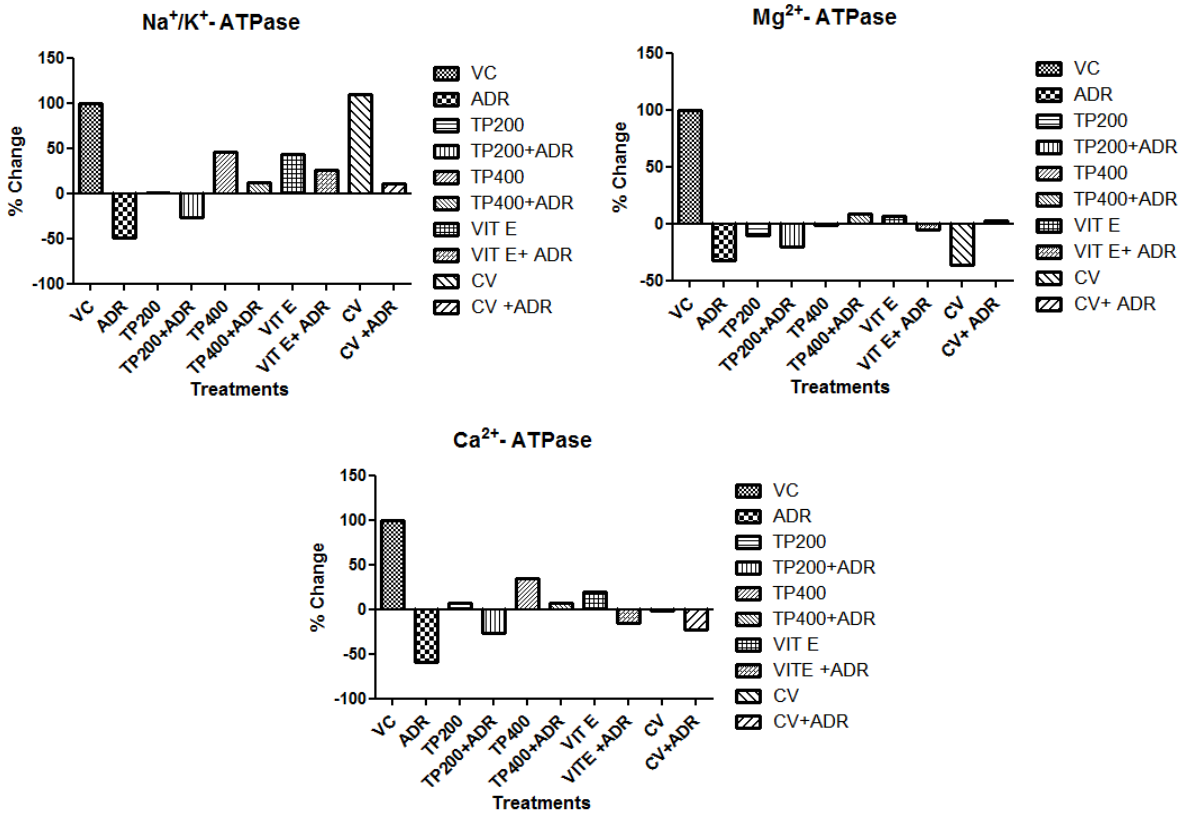

Fig. 1: Effect of *T. populnea* leaf extract on percent changes in Na^+/K^+ATPase, Mg^{2+}ATPase and Ca^{2+}ATPase enzyme activities in rat heart in the presence of Adriamycin, *Thespesia* leaf extract (200 & 400 mg), Vitamin E (25mg/kg p.o.) and Carvedilol (1mg/kg p.o.) separately and in combination.

Table 1: Changes in Na^+/K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase activities (expressed as µmoles of inorganic phosphate formed/mg protein/h) in the presence of Adriamycin, *Thespesia* leaf extract (200 & 400 mg), Vitamin E (25mg/kg p.o.) and Carvedilol (1mg/kg p.o.) separately and in combination.

Experimental Group	Indices	Na^+/K^+ ATPase	Mg^{2+} ATPase	Ca^{2+} ATPase
Vehicle Control (5% Gum acacia)	Mean ± SD	1.49 ± 0.27	3.52 ± 0.28	1.58 ± 0.15
Disease Control (Adriamycin, 15 mg/kg cumulative dose)	Mean ± SD 't' test	0.80 ± 0.07 P<0.001	2.38 ± 0.35 P<0.001	0.64 ± 0.25 P<0.001
Thespesia Leaf Extract (200 mg/kg)	Mean ± SD 't' test	1.50 ± 0.29 NS	3.14 ± 0.19 NS	1.70 ± 0.16 NS
Thespesia Leaf Extract (400 mg/kg)	Mean ± SD't' test	2.18 ± 0.42 P<0.001	3.49 ± 0.36 NS	2.12 ± 0.12 P<0.001
Thespesia Leaf Extract (200 mg/kg) + Adriamycin	Mean ± SD't' test	1.09 ± 0.12 P<0.01	2.81 ± 0.28 P<0.01	1.16 ± 0.33 P<0.01
Thespesia Leaf Extract (400 mg/kg) + Adriamycin	Mean ± SD't' test	1.67 ± 0.29 NS	3.81 ± 0.44 NS	1.70 ± 0.19 NS
Standard(Vitamin E, 25 mg/kg, p.o.)	Mean ± SD't' test	2.13 ± 0.29 P<0.001	3.75 ± 0.32 NS	1.88 ± 0.14 P<0.01
Vitamin E (25 mg/kg, p.o.) + Adriamycin	Mean ± SD't' test	1.87 ± 0.23 P<0.01	3.32 ± 0.36 NS	1.33 ± 0.08 P<0.05
(Carvedilol, 1mg/kg, p.o.)	Mean ± SD't' test	3.12 ± 0.26 P<0.001	2.23 ± 0.22 P<0.001	1.56 ± 0.10 NS
Carvedilol(1mg/kg, p.o.) + Adriamycin	Mean ± SD't' test	1.66 ± 0.30 NS	3.60 ± 0.27 NS	1.21 ± 0.13 P<0.01

Note: Each value is mean ± Standard Deviation of 6 independent observations. The values are significant at least at P<0.05. NS: Not significant.

Table 2: Changes in superoxide dismutase (SOD) (expressed as units of superoxide anion reduced/mg protein/min) and catalase (CAT) (expressed as micromoles of H_2O_2 degraded/mg protein/min) activities in the presence of Adriamycin, *Thespesia* leaf extract (200 & 400 mg), Vitamin E (25mg/kg p.o.) and Carvedilol (1mg/kg p.o.) separately and in combination.

Experimental Group	Indices	SOD	Catalase
Vehicle Control (5% Gum acacia)	Mean ± SD	16.45 ± 0.89	0.76 ± 0.07
Disease Control (Adriamycin, 15 mg/kg cumulative dose)	Mean ± SD 't' test	6.21 ± 0.83P<0.001	0.15± 0.05P<0.001
Thespesia Leaf Extract (200 mg/kg)	Mean ± SD 't'test	14.09± 0.3P<0.001	0.69 ± 0.03P<0.001
Thespesia Leaf Extract (200 mg/kg) + Adriamycin	Mean ± SD't' test	12.29 ± 1.28P<0.001	0.48± 0.05P<0.001
Thespesia Leaf Extract (400 mg/kg)	Mean ± SD't' test	15.94 ± 0.25P<0.001	0.72 ± 0.08P<0.001
Thespesia Leaf Extract (400 mg/kg) + Adriamycin	Mean ± SD't' test	15.66 ± 1.34P<0.001	0.75 ± 0.06P<0.001
Standard(Vitamin E, 25 mg/kg, p.o.)	Mean ± SD't' test	16.09 ± 0.26P<0.001	0.79 ± 0.07P<0.001
Vitamin E (25 mg/kg, p.o.) + Adriamycin	Mean ± SD't' test	15.9 ± 1.39P<0.001	0.71 ± 0.04P<0.001
(Carvedilol, 1mg/kg, p.o.)	Mean ± SD't' test	14.09 ± 1.25P<0.001	0.72 ± 0.08P<0.001
Carvedilol(1mg/kg, p.o.) + Adriamycin	Mean ± SD't' test	11.61 ± 1.02P<0.001	0.65 ± 0.03P<0.001

Note: Each value is mean ± Standard Deviation of 6 independent observations. The values are significant at least at P<0.05. NS: Not significant.

Mg^{2+}ATPase

The vehicle controls recorded the activity at around 3.52 µmoles. The Mg^{2+}ATPase activity was significantly lowered in adriamycin-treated rats as compared to the vehicle control group. Administration of TP leaf extract at 200 and 400 mg/kg and vitamin E separately to the rats caused slight and non-significant changes in the enzyme activity, while a significant increase in the enzyme activity was observed for the administration of carvedilol. Pre-treatment with TP extract at 200 mg before treatment with adriamycin effected only a partial recovery of the enzyme activity towards the control. Pre-treatment with TP extract at 400 mg, Vitamin E and carvedilol before the administration of adriamycin restored the enzyme activity to the control level (Table 1; Fig. 1).

Ca^{2+} ATPase

In vehicle control group, the Ca^{2+}ATPase activity was found to be around 1.58 µmoles. The activity was found significantly decreased in adriamycin-treated rats when compared to the vehicle control group. TP leaf extract at 200mg/kg and carvedilol administered separately caused non-significant changes in the control activity, while TP extract at 400 mg and vitamin E effected significant increases in the enzyme activity from the control. Pre-treatment with TP extract at 200mg, vitamin E and carvedilol before the administration of adriamycin effected partial recoveries of the enzyme activity towards the control. However, pre-treatments with TP at 400 mg before adriamycin administration restored the enzyme activity completely to the control level (Table 1; Fig. 1).

Superoxide dismutase and Catalase

In the present study adriamycin decreased the activities of superoxide dismutase (SOD) and catalase (CAT) in the heart tissue of rats significantly.

The activities increased in all the treatment groups when compared to the disease control. *T. populnea* extracts in both the doses (200mg/kg and 400 mg/kg), vitamin E and carvedilol individually and in combination with adriamycin elevated both the enzyme activities significantly. However, *T. populnea* in the dose of 200mg/kg and carvedilol plus adriamycin respectively did not restore the enzyme activities to normal (Table 2; Fig. 2).

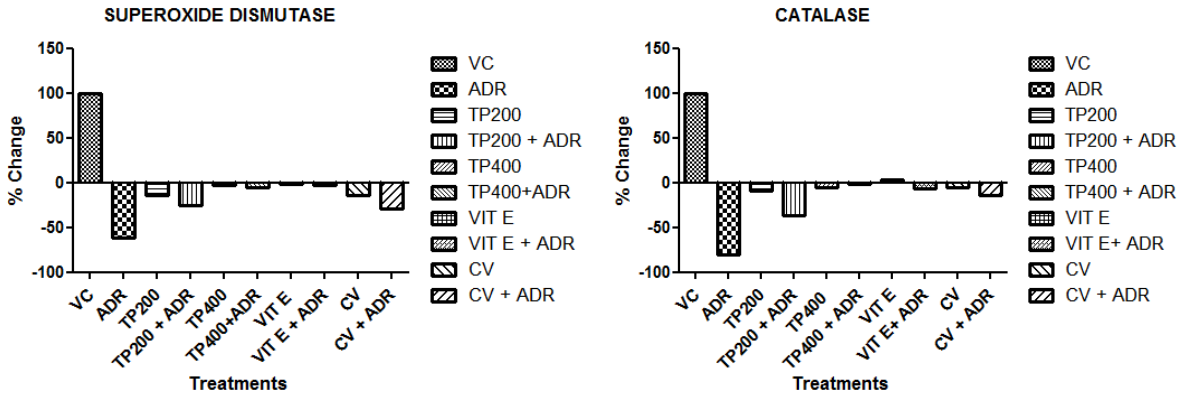

Fig. 2: Effect of *T. populnea* leaf extract on percent changes in superoxide dismutase and catalase enzyme activities in rat heart in the presence of Adriamycin, *Thespesia* leaf extract (200 & 400 mg), Vitamin E (25mg/kg p.o.) and Carvedilol (1mg/kg p.o.) separately and in combination.

DISCUSSION

Adriamycin-induced cardiotoxicity is a complex mechanism, and is commonly believed to be triggered by and linked to oxidative stress caused by the production of reactive oxygen species (ROS) (Oliveira *et al*., 2006; Pereira *et al*., 2011). ATPases are membrane-bound enzymes involved in energy-mediated translocation of Na^+, Ca^{2+} and Mg^{2+} ions. Studies have shown a reduction in the activity of these enzymes upon damage to the myocardium (Upaganlawar *et al*., 2009, Upaganlawar and Balaraman, 2011). 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 (Upaganlawar and Balaraman, 2011). Reduced activity of Na^+/K^+ATPase and Mg^{2+}ATPase has also been attributed to the loss of sulfhydryl (SH) groups (Nicotera *et al*., 1985) and lipid peroxidation (Patel *et al*., 2010).

In the present study the activities of Na^+/K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase were significantly diminished in the heart tissue of adriamycin-treated rats. This may be attributed to the peroxidation of membrane lipids induced by adriamycin, which may lead to reduced activities of membrane-bound ATPases and hence the impairment in cellular homeostasis, which might play a role in subsequent cardiac failure. The above results are in agreement with the earlier reports suggesting deleterious changes in the cardiac tissue brought about by adriamycin (Hanna *et al*., 2014).

Administration of *Thespesia populnea* extract at the dose of 200mg/kg had only partial effect when followed with adriamycin administration, while significant improvement was observed with 400mg/kg (Table 1; Fig. 1). The results further suggest that upon treatment with *T. populnea* extract in the dose of 400mg/kg, the activities of Na^+/K^+ATPase, Mg^{2+}ATPase and Ca^2+ATPase could be restored towards normal levels by reverting the abnormal changes brought about by adriamycin.

This is suggestive of membrane- stabilizing, protective effect conferred by *T. populnea* extract.

The generation of free radicals by adriamycin in the form of doxorubicin semiquinone also has been suggested to play a major role in its cardiotoxic effects (Bachur *et al*., 1979) by increasing oxygen free radical activity (Lee *et al*., 1991). Semiquinones are unstable under aerobic conditions, thereby generating superoxide anion radicals (Sarika Kamble *et al*., 2009). Adriamycin mediates inhibition of cardiac superoxide dismutase (Arai *et al*., 2000) and catalase leading to reduction in the activity of the enzymes. Restoration or elevation of the endogenous antioxidant enzyme activities to normal may be attributed to the antioxidant potential of *Thespesia*, protecting the ATPase enzymes from oxidative degradation (Patel *et al*., 2010). The leaf extract of *Thespesia populnea* has been reported to exhibit hepato-protective, anti-inflammatory and antimicrobial effects due to its antioxidant principles (Elakkiya *et al*., 2011, Sahitya Chetan *et al*., 2012). Hence, the restoration of ATPase activity to normal in adriamycin-treated rats by *Thespesia* is presumably due to the presence of antioxidant phyto-constituents such as ferulic acid, synringic acid and vanillic acid etc. in the leaf extract.

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 (Upaganlawar *et al*., 2009, Jha *et al*., 1998). The ameliorative effect of vitamin E on cardiac ATPases in the present study could be attributed to its free radical scavenging ability. Recent studies on carvedilol suggest that the drug protects against the oxidation of sarcoplasmic reticulum Ca^{2+}ATPase, and also reduces oxidative stress in the myocardium in patients with dilated cardiac myopathy (Dandona *et al*., 2000, Nakamura *et al*., 2002).

Similar to vitamin E, carvedilol elevated the ATPase activities both when administered alone and also by pre-treatment of the rats before administering adriamycin (Table 1; Fig. 1). 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 (Yuan *et al*., 2002). Cardiomyocytes are more susceptible to adriamycin-induced free radical-mediated damage because these cells have relatively low levels of antioxidant enzymes such as SOD and CAT (Doroshow *et al*., 1980, Kalyanaraman *et al.*, 2002), which provide protection by converting hydrogen peroxide into water and oxygen.

Transgenic over-expression of both SOD and catalase has been shown to be cardio-protective (Kang *et al*., 1996, Yen *et al*., 1996). In the present study, SOD activity was significantly inhibited in adriamycin-treated rats, as reported by earlier studies (Shah *et al*., 2009). Experimental studies on *T. populnea* have shown that the leaf extract exhibits free radical scavenging properties *in vitro* on hydroxyl radicals, peroxyl radicals, and superoxide free radicals (Sahitya Chetan *et al*., 2012). In the present study, a decrease in activity levels of cardiac SOD and CAT in adriamycin-treated group was observed, which was effectively reverted to normalcy by *T. populnea* leaf extract (400mg/kg).

In conclusion, the present findings suggest that *T. populnea* ameliorated the activities of ATPases which might contribute to cardio-protection in the rats under adriamycin stress. These ameliorative and restorative properties of the plant may be due to its ability to scavenge free radicals and restoration of the endogenous antioxidant components in the heart. These results provide preliminary pharmacological support to the use of *T. populnea* in preventing alterations in membrane homeostasis which could prove beneficial in conferring alleviation from cardiotoxicity.

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