

**Evaluation of Cardioprotective Effect of *Thespesia populnea* with
Special Reference to Antioxidant Activity**

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a. Title of the thesis and abstract

Thesis title: Evaluation of Cardioprotective Effect of *Thespesia populnea* with Special Reference to Antioxidant Activity.

Abstract

Objective: To evaluate the cardioprotective effect of the plant *Thespesia populnea* on adriamycin- and ethanol-induced cardiotoxicity.

Methods

Aqueous extract of *Thespesia populnea* (TP) was subjected to preliminary phytochemical screening as per standard procedures, followed by HPTLC analysis. The *in vitro* antioxidant activity of the extract was also examined. Adriamycin-cardiotoxicity was induced by injecting adriamycin to male, albino Wistar rats weighing 180-220g, in the dose of 2.5mg/kg i.p. (15mg/kg cumulative dose) every alternate day during the 3rd and 4th week of the 4 week-treatment schedule. Ethanol-cardiotoxicity was developed by orally administering ethanol (20%, 2g) to the rats daily for 6 weeks. Rats were administered aqueous extract of TP leaf, orally in the doses of 200mg/kg and 400 mg/kg body weight respectively, daily for 4 weeks in adriamycin cardiotoxicity and 6 weeks in ethanol cardiotoxicity. Vitamin E (25 mg/kg, p.o.) and carvedilol (1mg/kg, p.o.) were used as reference standards and were also administered similar to TP treatment.

At the end of the treatment schedule, physical parameters such as food intake and body weight changes, cardiac parameters such as heart weight, left ventricle wall thickness and cardiac ejection fraction, and biochemical parameters such as serum cardiac biomarkers (c-reactive protein (CRP); creatine kinase MB (CK-MB), creatine kinase (CK), lactate dehydrogenase (LDH), alanine aminotransferase (AlAT), aspartate aminotransferase (AAT) activities; lipid profile comprising total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and high density lipoprotein (HDL); cardiac oxidative stress parameters such as lipid peroxidation, superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione reductase (GR), glutathione peroxidase (GPX), glutathione S-transferase (GST) were assessed in adriamycin- and ethanol-induced cardiotoxicity models. Cardiac ejection fraction was determined using langendorff technique, and ECG was determined using student physiograph. Histopathological studies were carried out at the end of the study for both the models.

Results

Phytochemical screening of the TP leaf extract revealed the presence of flavonoids, phenolic acids, glycosides, saponins, tannins and carbohydrates. A dose-dependent increase in the *in vitro* free radical scavenging activity of the extracts was observed. Aqueous extract of TP produced a significant ($P<0.001$) improvement in body weight and food intake in adriamycin- and ethanol-treated rats, accompanied by a significant ($P<0.01$) increase in left ventricle wall thickness and cardiac ejection fraction. A significant ($P<0.001$) lowering of levels of CRP; and CK, CK-MB, LDH, AlAT and AAT activities that were elevated by adriamycin was observed upon the administration of TP extract. In the animals subjected to adriamycin stress and ethanol stress respectively, aqueous extract of TP produced statistically significant ($P<0.001$) antioxidative effects by lowering lipid peroxidation and elevating the reduced glutathione (GSH) level; superoxide dismutase (SOD), catalase, glutathione reductase (GR), glutathione peroxidase (GPX), glutathione S-transferase (GST) and cardiac ATPase (Na^+ , Ca^{2+} and Mg^{2+} ATPases) activity. Changes in ECG showed a reduction in the prolongation of QT interval, QRS and RR interval. Histopathological changes showed extensive myofibril loss and separation of muscle fibres indicative of dilated cardiomyopathy and severe damage to tissue architecture in adriamycin-treated rats; and breaks in the muscle fibres accompanied by extensive vacuolization, and necrosis of muscle fibres with areas of congestion in ethanol-treated rats. Treatment with TP extract in both the models of cardiotoxicity caused minimal myofibrillar vacuolization with absence of necrosis, mild areas of congestion and restoration of normal cardiac architecture manifested as intact myofibrils. Recovery of cardiac tissue from damage accompanied by an improvement in the cardiac function was observed in the rats in both the models of cardiotoxicity.

Conclusion

From the results of the present study, it could be concluded that TP extract exhibits cardioprotective effect by virtue of its antioxidant potential and its ability to restore the cardiac biomarkers and lipid profile back to near normal levels, with an accompanying membrane stabilizing effect. The electrocardiographic changes and the histopathological manifestations induced by adriamycin and ethanol respectively were effectively countered by TP extract, thus recovering the cardiac tissue from damage.

Keywords: Oxidative stress, Cardiotoxicity, *Thespesia populnea*, Vitamin E, Carvedilol, Antioxidative effects.

b. Brief description on the state of the art of the research topic

Cardiotoxicity due to various chemical and therapeutic agents is a serious problem affecting people who are exposed to such agents. A variety of synthetic medicines employed in the treatment of different diseases can also generate free radicals in the body, which may cause oxidative stress and other undesirable effects. Oxidative stress causes the production of reactive oxygen species (ROS), which enhance the peroxidation of lipids, protein and DNA, leading to cell injury.

Many human disorders such as stroke, congestive heart failure, hypertension and coronary artery disease, ischemia, reperfusion injuries, auto-immune diseases, etc. have been linked to the generation of free radicals. This situation could also arise consequent on exposure to acute or chronic alcohol. Such conditions call for research on ameliorative measures for effective control of disease states such as those stated above.

Plants are rich source of antioxidants including several phyto-constituents such as flavonoids, phenolic compounds, tannins, glycosides, saponins etc., which are capable of terminating free radical reactions, thus preventing the human body from oxidative damage. For the present study, the plant *Thespesia populnea* (TP) belonging to Malvaceae family was selected owing to its acknowledged medicinal properties.^{1,2} Phytochemical analysis of TP leaves claims the presence of lupeol, lupenone, β -sitosterol, acacetin, quercetin, vanillic acid, syringic, melilotic and ferulic acids.^{3,4,5}

A detailed survey of literature shows that flavonoids/phenolic acid principles in plants/herbs are responsible for their antioxidant activity. So it is hypothesized that due to the presence of the above active principles in TP, its antioxidative effect oxidative stress in cardiac injury could be explored.

c. Definition of the Problem

The study aims to explore the cardioprotective effect of TP against adriamycin and ethanol induced cardiotoxicity in rats. Since the cardiac damage produced by these agents is routed through free radical generation, the role of antioxidant activity of TP in ameliorating these toxic effects is also evaluated and compared with standard drugs like vitamin E and Carvedilol.

Adriamycin is a widely used anti-cancer drug for the treatment of acute myeloblastic leukemia, soft tissue and bone sarcomas, breast carcinoma, etc. Like many other synthetic

drugs, adriamycin can induce the development of cardiac cardiomyopathy and dysfunction, leading to congestive heart failure and death^{6,7} and the basic underlying mechanism for such effects is understood to be oxidative stress.⁸

Ethanol is a widely abused agent and is implicated in the development of multifarious health problems. The response of the body to chronic or acute administration of ethanol has been shown to result in the generation of oxygen-derived free radicals that cause alterations in the cardiac muscle.⁹ The metabolism of alcohol produces ROS that are detrimental to the cellular antioxidant defense system,¹⁰ causing cell injury.¹¹

The generation of free radicals is suggested to be the underlying cause of various health problems/disorders including cardiotoxicity. Therefore search has been on to find the different ameliorative measures to overcome the burden of oxidative stress-induced health disorders.

d. Objective and scope of work

Objectives

- The objective of the project was to investigate the cardio-protective activity of TP leaf extracts against adriamycin- and ethanol-induced cardio-toxicity.

Scope of research

This research work encompassed the evaluation of the ability of TP in conferring cardiac protective effects, by determining its antioxidant potential in two models of cardiotoxicity namely, (i) cardiac toxicity induced by a therapeutic drug such as adriamycin and (ii) chemically induced cardiac toxicity due to ethanol.

The effects of TP leaf extract on various aspects involved in cardiac injury such as cardiac changes, changes in serum biomarkers, lipid profile, ATPase and antioxidant enzymes, and electrocardiographic changes, supported by histopathological data were studied in the present work. Since the project aims to explore the usefulness of naturally occurring antioxidants from plant sources, the likely impact it would have is the reduction in oxidative stress and reduction/ reversal from cardio-toxicity to normal cardiac functional status. The project will further pave way for exploring antioxidant principles from different plant sources and studying their effects for treating free radical-induced cardiac damage and other organ toxicities.

e. Original contribution by the thesis.

In the light of absence of any information on the effects by TP extract on the possible alterations/changes that adriamycin and ethanol could bring about while inflicting cardiotoxicity, the present work tries to examine the effects of TP extract on various physical, biochemical, *in vitro* and *in vivo* parameters that have not been explored till date. The study further attempts to explain how cardioprotection might be conferred by the plant.

f. Methodology of Research, Results / Comparisons

1. Methods

1.1 Selection of plant material and preparation of crude extract

Fresh leaves of TP were collected. The plant material was taxonomically identified and authenticated. The leaves were dried and defatted with petroleum ether. The aqueous extract was then prepared.¹² The percentage yield of the aqueous extract obtained was 13.6%. The total flavonoid content and total phenolic content of the extract was determined.^{13,14} 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.¹⁵

1.2 HPTLC fingerprinting of the extract:

HPTLC analysis of the extract was carried out with TLC Silica Gel 60 F254 (Merck) precoated aluminium plates as the stationary phase, and toluene: ethyl acetate: formic Acid (5:4:1, v/v/v) as the mobile phase. 20 µl freshly prepared sample was used for the procedure.

1.3 *Invitro* radical scavenging activity of *Thespesia populnea* extract:

The *in vitro* free radical scavenging activity of TP leaf extract was determined using hydroxyl radical scavenging assay, superoxide anion scavenging assay, DPPH radical scavenging assay, ABTS radical scavenging assay, reducing power assay, and scavenging of hydrogen peroxide (H₂O₂).^{16,17,18,19,20}

1.4 Procurement and maintenance of experimental animals

Wistar strain, adult male, albino rats (n = 60) weighing 200 ± 20 g were housed and maintained as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. All the experimental procedures that were carried out in the

study were approved by the Institutional Animal Ethics Committee (Regd. No. 1029/PO/ERe/S/07/CPCSEA) in its proposal number BIP/IAEC/2015/07.

1.5 Treatment protocol

The effects of TP leaf extracts, vitamin E and carvedilol were studied in two animal models of cardiotoxicity, namely **adriamycin model** and **ethanol model**.

1.5.1 Experimental design for adriamycin cardiotoxicity model²¹:

The rats were divided into 10 groups of six animals each, and the following treatments were given daily via orogastric tube for 4 weeks.

Group I received 5% gum acacia alone (5 ml/kg per day p.o.) daily for 4 weeks. It served as the vehicle control.

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

Groups III and IV were given only TP leaf extract, 200mg/kg (TP200) and 400mg/kg (TP400) respectively, orally, daily for 4 weeks.

Groups V and VI received TP leaf extract (200 mg/kg and 400mg/kg) respectively, daily for 4 weeks (28 days) followed by adriamycin on alternate days during the 3rd and 4th weeks (TP200 + adriamycin and TP400 + adriamycin respectively).

Groups VII and IX received vitamin E (25 mg/kg, p.o.) and carvedilol (1mg/kg, p.o.) respectively, daily for 4 weeks. Both these groups served as drug controls or reference controls.

Groups VIII and X received vitamin E and carvedilol respectively, daily for all the 4 weeks, and received adriamycin on alternate days during the 3rd and 4th weeks (vitamin E + adriamycin and carvedilol + adriamycin respectively).

1.5.2 Experimental design for ethanol- cardiotoxicity model²²

The rats were divided into 10 groups of six animals each, and the following treatments were given daily via orogastric tube for 6 weeks.

Group I received 5% gum acacia alone (5 ml/kg per day p.o.) daily for 6 weeks. It served as vehicle control.

Group II received ethanol (20% Ethanol, 2g/kg, p.o.) daily for 6 weeks.

Groups III and IV were given only TP leaf extract, 200mg/kg (TP200) and 400mg/kg (TP400) respectively orally, daily for 6 weeks.

Groups V and VI received only TP leaf extract (200 mg/kg and 400mg/kg respectively) plus 20% ethanol, 2g/kg, p.o. (TP200 + ethanol and TP400 + ethanol respectively) daily for 6 weeks.

Groups VII and IX received vitamin E (25 mg/kg, p.o.) and carvedilol (1mg/kg, p.o.) respectively, daily for 6 weeks. Both these groups served as drug controls or reference controls.

Groups VIII and X received vitamin E plus 20% ethanol, 2g/kg, p.o. (vitamin E + ethanol) and carvedilol plus 20% ethanol, 2g/kg, p.o. (carvedilol + ethanol) respectively, daily for 6 weeks.

1.6 Isolation of tissue

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. For the estimation of serum biochemical parameters, blood samples were collected at the end of the experimental period and dispensed into clean plain glass test tubes. They were allowed to stand for 30 min at room temperature to clot. Serum for the assays was then separated from the clot by centrifugation at 5000 rpm for 10 min and used for the estimation of the parameters.

1.7 Statistical Analysis:

Data were expressed as mean \pm standard deviation (SD). Statistical analysis was done using one-way or two way analysis of variance (ANOVA) followed by Tukey's Multiple comparisons test or Bonferroni's post hoc test using Graph Pad Prism version 5.30 for Windows, Graph Pad Software, San Diego, California, USA. P values of <0.05 were considered as significant.

Results

2. Analysis of plant extracts

2.1 Phytochemical screening of plant extracts

The aqueous extract of TP leaf was subjected to phytochemical screening, and it was observed that the extract consisted of flavonoids, phenolic acids, glycosides, saponins, tannins and carbohydrates. The total flavonoid content and phenolic acid content was determined for the leaf extract. The total flavonoid and phenolic contents of aqueous extract

of TP were found to be 164µg quercetin equivalent/ mg of extract and 560 µg gallic acid equivalent/ mg of extract respectively.

2.2 HPTLC fingerprinting analysis

Since flavonoids were the major phytoconstituents that may be responsible for the protective effects of TP, HPTLC analysis of the extract was carried out to determine the presence of the flavonoid quercetin in the extract. Densitograms obtained from HPTLC analysis of aqueous extract of TP leaves showed the presence of quercetin corresponding with the R_f value 0.38, of standard flavonoid quercetin.

2.3 *In vitro* free radical scavenging activity:

The TP extract in the doses of 50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml and 250µg/ml exhibited a dose-dependent increase in free radical scavenging activity in comparison to standard antioxidants ascorbic acid and butylated hydroxytoluene, thus confirming the antioxidant property of the extract *in vitro*.

3. Studies on adriamycin-induced cardiotoxicity

3.1 Effect of TP leaf extract treatment on food intake and body weight changes

At the end of treatment period, the rats in adriamycin group exhibited significant decrease in food intake ($P < 0.001$) at the end of 4 weeks as compared to vehicle control group. Treatment with TP leaf extract (TP200 & TP400), vitamin E and carvedilol respectively was observed to step up the food intake significantly ($P < 0.001$) when administered in combination with adriamycin, in comparison to the animals receiving adriamycin alone.

An accompanied reduction in body weights was observed with adriamycin ($P < 0.001$) at the end of 4 weeks as compared to vehicle control group. TP extract (200mg/kg and 400 mg/kg respectively), vitamin E and carvedilol respectively significantly increased ($P < 0.001$) the body weight at the end of 4 weeks, when administered in combination with adriamycin in comparison to the animals treated with adriamycin alone.

3.2 Effect of TP leaf extract treatment on changes in heart weight, thickness of left ventricle wall and cardiac ejection fraction

Rats treated with adriamycin showed an increase ($P < 0.001$) in heart weight accompanied by a decrease ($P < 0.001$) in the thickness of the left ventricle wall and lowered % cardiac ejection fraction (EF). Treatment with TP extract (200mg/kg, 400 mg/kg), vitamin E and carvedilol

prior to adriamycin administration ameliorated the changes produced by adriamycin ($P<0.01$) with respect to heart weight ($P<0.001$), thickness of the left ventricle wall ($P<0.001$) and % EF respectively.

3.3 Effect of TP treatment on serum biochemical parameters:

Rats receiving adriamycin treatment alone recorded highly significant ($P<0.001$) elevations in the serum enzymes CRP, CK-MB, CK, LDH, AlAT, AAT, and lipid profile, TC, TG, LDL-c, VLDL-c accompanied by a significant ($P<0.001$) lowering of HDL-c. The serum biochemical changes were restored to near normal values upon treatment with TP extract (200mg/kg, 400 mg/kg respectively), vitamin E and carvedilol at the end of 4 weeks of treatment. The changes in all the serum parameters caused by adriamycin were significantly improved, as manifested by a decrease ($P<0.001$) in CRP, CK-MB, CK, LDH, AlAT, AAT activities and lipid profile (TC, TG, LDL-c, VLDL-c) levels along with an increase ($P<0.001$) in HDL-c levels.

3.4 Effect of TP leaf extract treatment on antioxidant enzyme activity in the heart tissue

Adriamycin administration significantly ($P<0.001$) elevated the malondialdehyde levels and lowered ($P<0.001$) the endogenous antioxidants GSH, SOD, CAT, GPX, GR, and GST as compared to vehicle control group. Treatment with TP extract (200mg/kg and 400mg/kg), vitamin E and carvedilol prior to adriamycin-treatment significantly lowered ($P<0.001$) the lipid peroxidation ((MDA content) and elevated ($P<0.001$) the endogenous antioxidants GSH, SOD, CAT, GPX, GR, GST to near control values by the end of 4 weeks of treatment.

3.5 Effect of TP treatment on cardiac ATPases: Na^+/K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase

Administration of adriamycin decreased ($P<0.001$) the ATPase activities, namely Na^+/K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase as compared to vehicle control group. TP leaf extract in the dose of 400mg/kg vitamin E and carvedilol significantly elevated the Na^+/K^+ ATPase ($P<0.001$), Ca^{2+} ATPase ($P<0.001$), Mg^{2+} ATPase ($P<0.001$) respectively, when administered along with adriamycin and also reversed the effect of adriamycin when compared to adriamycin treatment alone. The effect of TP leaf extract in the dose of 200mg/kg plus adriamycin and carvedilol plus adriamycin on Mg^{2+} ATPase activity was observed to be not statistically significant.

3.6 Effect of TP treatment on changes in the ECG (QT interval, QRS complex duration and RR interval)

ECG findings in adriamycin-treated rats, when compared to vehicle control group, showed a significant prolongation ($P<0.001$) of QT interval, QRS complex duration and RR interval, which was suggestive of ventricular arrhythmias, of defective conduction and a decreased ventricular function. Such changes in the ECG were significantly reverted close to the vehicle control values upon treatment with TP leaf extract in the dose of 200mg/kg ($P<0.01$) and 400 mg/kg ($P<0.001$), vitamin E ($P<0.001$) and carvedilol ($P<0.001$) in comparison to adriamycin treatment alone.

3.7 Histopathological studies

Histopathological studies exhibited adriamycin's abnormal effects on the myocardium. The manifestations of adriamycin treatment were myofibrillar breaks and detachment of muscle strands, accompanied by degeneration of muscle fibers. By TP extract, vitamin E and carvedilol treatments, these changes were reverted to the control condition entailing preservation of normal cardiac architecture, minimal vacuolization and absence of separation of muscle strands.

4. Studies on ethanol-induced cardiotoxicity

4.1 Effect of TP treatment on food intake and body weight changes induced by ethanol

Rats in ethanol group exhibited a significant decrease ($P<0.001$) in food intake and body weight ($P<0.001$) as compared to vehicle control group. Treatment with TP leaf extract improved the food intake ($P<0.01$) accompanied by an increase in body weight ($P<0.01$) when compared to the animals treated with ethanol alone at the end of 6 weeks of treatment.

4.2 Effect of TP treatment on changes in heart weight, thickness of left ventricle wall and cardiac EF induced by ethanol

Rats receiving ethanol exhibited an increase in heart weight ($P<0.001$) followed by a decrease in the thickness of the left ventricle wall ($P<0.001$) and reduced % EF ($P<0.001$) as compared to vehicle control group. Treatment with TP extract in the dose of 400mg/kg significantly ($P<0.001$) reduced and reverted the alterations in the heart weight, whereas a slightly less significant ($P<0.05$) reduction in heart weight was observed with vitamin E. The effects shown by TP200 and carvedilol on heart weight were not significant. TP in the dose of 200mg/kg, 400mg/kg, vitamin E and carvedilol plus ethanol respectively effected an increase

in the thickness of the left ventricle wall ($P<0.001$) and cardiac ejection fraction ($P<0.001$) at the end of the 6 week-treatment schedule.

4.3 Effect of TP treatment on changes in serum biochemical parameters induced by ethanol

Administration of ethanol to rats caused significant increments ($P<0.001$) in serum biochemical parameters that included CRP, CK-MB, CK, LDH, AlAT, AAT, and lipid profile, TC, TG, LDL-c, VLDL-c accompanied by a significant ($P<0.001$) lowering of HDL-c as compared to vehicle control group. These changes were modified by TP200, TP400 leaf extract, vitamin E and carvedilol treatments respectively in combination with ethanol and the elevations in all the serum parameters CRP, CK-MB, CK, LDH, AlAT, AAT, and lipid profile, TC, TG, LDL-c, VLDL-c were significantly reduced ($P<0.001$) accompanied by a significant elevation ($P<0.001$) in the HDL-c levels effecting recovery to near normalcy at the end of the 6 week-treatment schedule, as compared to the rats receiving ethanol treatment alone.

4.4 Effect of TP leaf extract treatment on antioxidant enzyme activity in the heart tissue

Administration of ethanol to rats for 6 weeks produced significant elevations ($P<0.001$) in malondialdehyde levels and lowered ($P<0.001$) the GSH, SOD, CAT, GPX, GR and GST activity levels as compared to vehicle control group. Treatment with TP extracts (200mg/kg and 400mg/kg), vitamin E and carvedilol prior to ethanol administration for 6 weeks effected significant ($P<0.001$) lowering of lipid peroxidation (MDA) and elevated ($P<0.01$) the endogenous antioxidants GSH, SOD, CAT, GPX, GR, GST to near control values when compared to ethanol treatment alone.

4.5 Effect of TP leaf extract treatment on cardiac ATPases: Na^+/K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase

Rats receiving ethanol treatment alone exhibited decreased ($P<0.001$) Na^+/K^+ ATPase, Ca^{2+} ATPase, Mg^{2+} ATPase activities as compared to vehicle control group. TP leaf extracts in the doses of 200mg/kg and 400mg/kg, vitamin E and carvedilol respectively in combination with ethanol elevated the activities of Na^+/K^+ ATPase and Ca^{2+} ATPase and reduced the effect of ethanol to different degrees. TP400 and vitamin E in combination with ethanol elevated ($P<0.05$ and $P<0.01$) Mg^{2+} ATPase activity respectively. The changes effected by TP200 plus

ethanol and carvedilol plus ethanol on the Mg^{2+} ATPase activity were observed to be not significant.

4.6 Effect of TP treatment on changes in the ECG (changes in QT interval, QRS complex duration and RR interval)

Significant changes were observed in the electrocardiographic recordings of ethanol-treated rats as compared to vehicle control group. The changes observed were prolongation of QT interval, QRS complex duration and RR interval ($P<0.001$) suggesting cardiac arrhythmias, defective conduction and a possible decrease in ventricular function. TP in both the doses (200mg and 400mg, respectively), Vitamin E, and carvedilol restored these changes caused by ethanol in the ECG, QT interval ($P<0.001$), QRS complex ($P<0.001$) and RR interval ($P<0.001$) to near control values.

4.7 Effect of TP leaf extract treatment on histopathological alterations due to ethanol

Ethanol treatment produced changes in the histology of the cardiac tissue which confirmed the deleterious effects of the chemical on cardiac tissue. The manifestations that were observed due to ethanol treatment were, myofibrillar vacuolization with separation of myofibrils and loss of cardiac architecture. These changes were reverted to the control situation with preservation of normal cardiac architecture, minimal vacuolization and absence of separation of muscle strands, but with mild areas of congestion. The improvement in the histological manifestations of ethanol confirms the protective effect of TP leaf extract on the cardiac tissue.

g. Achievements with respect to objectives

The following objectives were achieved in this study.

1. Administration of TP leaf extract increased the food intake and body weight, decreased the heart weight, increased the thickness of left ventricle wall and cardiac ejection fraction.
3. Serum biomarkers of significance related to cardiac function that were significantly elevated by treatment with adriamycin and ethanol were brought back to near normal after treatment with TP leaf extract.
4. Biomarkers of cardiac oxidative stress i.e., the endogenous antioxidants were elevated with a simultaneous reduction in lipid peroxidation in the heart tissue with TP leaf extract treatment.

5. Cardiac ATPases that were significantly lowered by adriamycin and ethanol treatments were restored to near normal levels by TP leaf extract .
6. Electrocardiographic changes were improved following treatment with TP leaf extract.
7. Histopathological changes manifested by adriamycin and ethanol were significantly altered and restored to near normal state with TP leaf extract treatment.

h. Conclusion

From the results of the present study, it can be concluded that TP leaf extract ameliorates the physical, serum biochemical and histological changes caused by adriamycin and ethanol respectively in the rat heart. This amelioration may be primarily due to the antioxidant principles present in the extract that might be crucial in conferring cardioprotection.

i. Copies of papers published and a list of all publications arising from the thesis

A) Poster presentation:

Title: *Thespesia populnea* leaf extract counteracts the effects of adriamycin on ATPase activity in rat heart. Indian Pharmaceutical Congress (IPC), Vishakhapatnam. 16-18 December, 2016.

B) Papers published:

1. Sangeetha Lakshmi A. Rajbanshi, Archana N Paranjape, Vasu Apanna. Counteraction of adriamycin-induced alterations in cardiac enzymes by *Thespesia populnea* leaf extract Journal of Applied Pharmaceutical Science, 2017, 7: 38-45.
2. Sangeetha Lakshmi A. Rajbanshi, Archana N Paranjape, Vasu Apanna. Ethanol-induced alterations in cardiac enzymes – ameliorative effect of *Thespesia populnea* leaf extract. International Journal of Pharmacy and Pharmaceutical Sciences, 2017, 9 (2nd revision submitted), Acceptance awaited.

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