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Research Article

TO EVALUATE THE CARDIOPROTECTIVE AND ANTI-OXIDANT EFFECT OF METHANOLIC EXTRACT OF THE LEAVES OF *TRIBULUS TERRESTRIS* IN WISTAR RATS

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ABSTRACT

Objectives: The aim of the current study was to evaluate the cardio protective and anti-oxidant effect of methanolic extract of the leaves of *Tribulus terrestris* (TT) in Wistar Rats.

Methods: Extracted leaves of TT were evaluated for cardioprotective and antioxidant activities in *in vivo* model. Thirty rats were divided into 5 groups. Group I and II served as normal control and disease control (doxorubicin 20 mg/kg I.P.), respectively, while Group III was the treated group with standard drug (vitamin-E 10 mg/kg P.O). Group IV and V served as the test groups, which were pre-treated with 250 mg/kg and 500 mg/kg body weight per day of "TT," and the treatment was given for 7 days, On the 5th day, doxorubicin was given I.P 20 mg/kg, then 48 h after I.P of doxorubicin, animals were sacrifice and then blood samples was taken for biochemical estimation, and the rat heart for histopathological examination. Along with it, antioxidant studies were carried out using heart tissue homogenate from each group.

Results: The administration of doxorubicin to control group's rats presented a significant increase in serum total cholesterol (TC), triglycerides (TGs), low-density lipoprotein, creatine kinase-MB, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), and decrease in high-density lipoprotein (HDL). Rats treated with TT showed decreased TCs, TGs, low-density lipoprotein, CKMB, SGOT, SGPT, and increases HDL levels. The histopathological studies also showed that the TT significantly minimized the damage induced by doxorubicin.

Conclusion: It can be concluded that the TT could be a potential candidate for the treatment of cardiotoxicity.

Keywords: Cardiotoxicity, Doxorubicin, Vitamin E, *Tribulus terrestris*, Antioxidant activity.

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INTRODUCTION

The cardiovascular system consists of heart and blood vessels which circulate blood all through the body [1]. A heart is a muscular part in most animals, which pumps blood through the blood vessels of the circulatory system, the human heart is only the size on a fist; however, it is the strongest muscle among the human body. It beats about 70 times a minute, even though it rate can doubled in the course of exercising or at times of excessive feeling [2]. In humans, the heart is placed into the lungs, in the center compartment of the chest [3]. The heart is divided into four chambers: upper left and right atria; then lower left and right ventricles [4,5]. Blood is pumped out beyond the left chambers of the heart; such is transported thru arteries of ever-decreasing size, subsequently accomplishing the capillaries of entire the tissues, such as much the skin and other body organs. Having delivered its oxygen and nutrients and having accumulated waste a product, blood is added returned in accordance with the right chambers of the heart thru a system of ever-enlarging veins. During the circulation thru the liver, waste products are removed [6]. Cardiovascular disorder (CVD) is abnormal functioning of the heart or blood vessels [7]. CVD is not an unaccompanied disease, however, a cluster on diseases and injuries that affect the cardiovascular system. In general, it has an effect on human beings in later life, although, according to a leading cardiologist, by means of around 35 years old, most that will get a form of CVD meanwhile have the beginnings of the ailment [8]. CVD is the largest alone contributor in imitation of the 57 million deaths registered by the World Health Organization in 2002 [9]. It was acknowledged that quantity one cause of death globally is due to cardiovascular illnesses because annually more human beings die from heart ailments than beside any other grounds. Approximately 17.5 million human beings died beyond CVDs in the year 2012, representing 31% of every global death. Of these deaths, 7.4 million were due according to coronary heart disease [10]. Healthy human lifestyles is constantly cardinal for human animal beginning from his birth to the end of lifestyles, numbers of diseases, minor and major, lead into key roles in distributing the healthful human life cardioprotection consists of all mechanism or potential to that amount contributed in accordance with upkeep regarding the heart by way of reducing or too preventing myocardial damage [11]. Myocardia infection makes the will increase contribution on mobility and mortality statics in rising countries due to theirs lifestyles fashion modifications [12]. CVD is a group concerning disorders/diseases over the heart and blood vessels, which includes heart attack and stroke. Cardiovascular ailments include: coronary heart disease, cerebrovascular disease, raised blood pressure, peripheral vein disease, rheumatic heart disease, congenital heart disease, and heart defeat [13]. Today CVD accounts for 30% over death worldwide, which includes nearly 40% in high-income countries and as regards 28% of low- and middle-income countries, myocardial infarction is the obstacle of blood supply to portion of the heart, inflicting heart cells according to die, normally due to occlusion over a coronary artery. It creates an important vital cause over morbidity and mortality within developing counters appropriate to increased excessive prevalence of risk factors and also getting older of theirs populations. There are many chance factors for heart diseases: age, gender, tobacco use, physical inactivity, excessive booze consumption, poorly diet, obesity, genetic predisposition and household history on cardiovascular disease, raised blood pressure, raised blood sugar, advanced blood cholesterol, undiagnosed celiac disease, psychosocial factors, poverty and low academic status, or flatulence pollution [14].

Table 1: Percentage yield of the leaves of *Tribulus terrestris* extract

Plant extract in	Percentage yield	Colour	Nature
Methanol	5.4	Dark green	Sticky

Tribulus terrestris (TT) belongs to the family *Zygophyllaceae*. Commonly, the plant is known by puncture vine, caltrop, and yellow vine. It can be found in Southern Asia, Southern Europe, throughout Africa and Australia. The genus Tribulus comprises about 20 species in the world. Theplant revealed the presence of alkaloids, flavonoids, glycosides, saponins, and tannins.TT is used in folk medicines as a tonic, aphrodisiac, palliative, astringent, stomachic, antihypertensive, diuretic, lithotriptic, and urinary disinfectant. It has been used for centuries in Ayurveda to treat impotence, venereal diseases, and sexual debility. In traditional Chinese medicine, the fruits were used for treatment of eye trouble, edema, abdominal distension, emission, morbid leukorrhea, and also preliminary evidence shows may improve blood sugar control and cholesterol in diabetes patient. Conventionally, people have used this plant for a variety of potential effects, TT is widely used as a general health supplement, as well as in supplements that claim to increase testosterone levels in Unani medicine [15]. Some reactive oxygen species and free radical have been widely accepted as being harmful to human health by triggering many diseases. Natural oxidant from plant, either as crude solvent extract as an individually isolated compound can decrease the risk, considerable safe, and effective [16].

MATERIALS AND METHODS

Collection of plant materials

The leaves of the plant TT were collected from Tirupathi, Andhra Pradesh, India in June. These plant species were authenticated by Dr. MudhaChishti, Department of Botany, Sri Padmavati Women's University, Tirupati, Andhra Pradesh. The collected plant material (leaves) was washed thoroughly with water to remove the adhering soil, mud, and debris. All old insect damage or fungus-infected leaves, and flowers were removed. The plant material was dried in the shade at room temperature to a constant mass. The plant material was coarsely powdered using a blender. The powder was stored in an airtight container and was protected from light.

Preparation of extract

The course ground powder of TT was transferred into the extraction glass and the plant material was loaded into the main chamber of the Soxhlet extractor. Then this part of the extractor is connected to the round bottom flask containing the extraction solvent. The grind coarse powder was packed in the tightly in the Soxhlet extractor and methanol solvent was used for the extraction of the TT leaves powder. In this extraction process, 250 ml solvent was used and was carried out for about 6 h. The extract was again re-extracted under the same conditions to ensure complete extraction. The methanol was filled into the solvent vessel and extracted at a temperature of 75°C for 6 h. The solvent was drained into a beaker by opening the spigot on the Soxhlet extractor. The solvent was removed from the extractor and dried. The extract was then stored in dry airtight bottles for the pharmacological studies. The portion of the extract which is non-soluble remains in the thimble and it was discarded [17].

Experimental animals

Wistar rats weighing between 150 and 200 g were maintained in standard laboratory conditions at room temperature (25±2°C) with 12 h light/dark cycle. The animals were given pellet chow and water ad libitum except during experimentation. The study protocols were duly approved by the Institutional Animal Ethics Committee at Karnataka College of Pharmacy, Bengaluru. Studies were performed in accordance with the CPCSEA guidelines. Reg No: 1564/PO/RE/S/11CPCSEA.

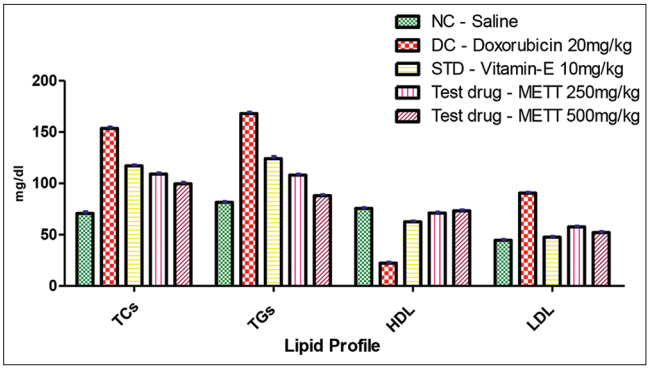

Fig. 1: Effect of test items on lipid profile. Values were expressed as mean±SEM for six animals in each group. Data were analyzed using two-way ANOVA followed by Bonferroni post-tests

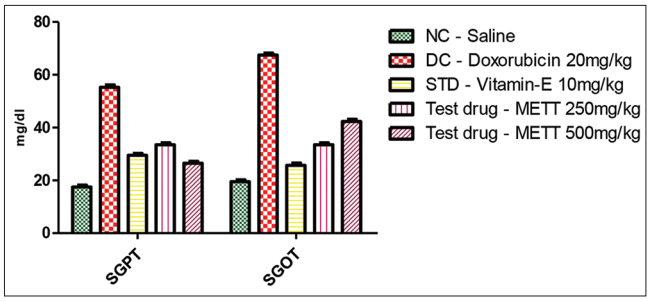

Fig. 2: Effect of test items on serum glutamic-pyruvic transaminase and serum glutamic-oxaloacetic transaminase. Values were expressed as mean±standard error of the mean for six animals in each group; data were analyzed using two-way ANOVA followed by Bonferroni post-tests

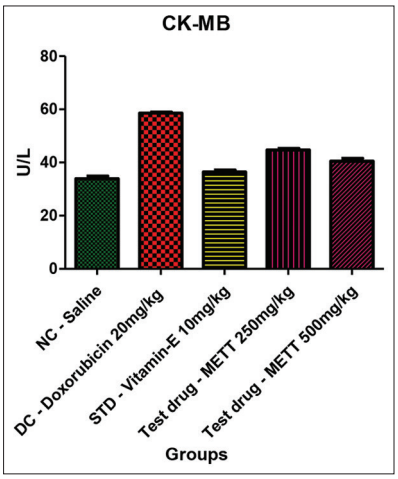

Fig. 3: Effect of test items on creatine kinase-MB. Values were expressed as mean±standard error of the mean for six animals in each group. Data wre analyzed using one-way ANOVA followed by Tukey's test. p<0.001* with all the groups except NC - Saline versus STD - Vitamin-E 10 mg/kg i.e. p>0.05ns. STD - Vitamin-E versus test drug METT 500 mg/kg i.e. p<0.05* and Test drug - METT 250 mg/kg versus test drug - METT 500 mg/kg, i.e., p<0.01**.**

Acute toxicity study

An acute toxicity study was conducted for the methanolic leaves extract of TT as per OECD guidelines 425 using Swiss albino mice. Each animal was administered methanolic extracts by the oral route. The animals

Table 2: Two-way ANOVA comparison and followed by Bonferroni posttests for doxorubicin, Vitamin E, and *Tribulus terrestris* on lipid profile

Row factor	Difference	t	p	Summary
NC - Saline versus DC - doxorubicin 20 mg/kg				
TCs	82.67	54.03	<0.001	***
TGs	86.50	56.53	<0.001	***
HDL	−53.33	34.85	<0.001	***
LDL	46.00	30.06	<0.001	***
NC - Saline versus STD - Vitamin-E 10 mg/kg				
TCs	46.17	30.18	<0.001	***
TGs	42.50	27.78	<0.001	***
HDL	−13.00	8.496	<0.001	***
LDL	3.000	1.961	>0.05	NS
NC - Saline versus test drug - METT 250 mg/kg				
TCs	38.17	24.95	<0.001	***
TGs	26.50	17.32	<0.001	***
HDL	−4.500	2.941	<0.05	*
LDL	13.00	8.496	<0.001	***
NC - Saline versus test drug - METT 500 mg/kg				
TCs	28.67	18.74	<0.001	***
TGs	6.500	4.248	<0.001	***
HDL	−2.200	1.438	>0.05	NS
LDL	7.500	4.902	<0.001	***
DC - Doxorubicin 20 mg/kg versus STD - Vitamin-E 10 mg/kg				
TCs	−36.50	23.86	<0.001	***
TGs	−44.00	28.76	<0.001	***
HDL	40.33	26.36	<0.001	***
LDL	−43.00	28.10	<0.001	***
DC - Doxorubicin 20 mg/kg versus test drug - METT 250 mg/kg				
TCs	−44.50	29.08	<0.001	***
TGs	−60.00	39.21	<0.001	***
HDL	48.83	31.91	<0.001	***
LDL	−33.00	21.57	<0.001	***
DC - Doxorubicin 20 mg/kg versus test drug - METT 500 mg/kg				
TCs	−54.00	35.29	<0.001	***
TGs	−80.00	52.29	<0.001	***
HDL	51.13	33.42	<0.001	***
LDL	−38.50	25.16	<0.001	***
STD - Vitamin-E 10 mg/kg versus test drug - METT 250 mg/kg				
TCs	−8.000	5.229	<0.001	***
TGs	−16.00	10.46	<0.001	***
HDL	8.500	5.555	<0.001	***
LDL	10.00	6.536	<0.001	***
STD - Vitamin-E 10 mg/kg versus test drug - METT 500 mg/kg				
TCs	−17.50	11.44	<0.001	***
TGs	−36.00	23.53	<0.001	***
HDL	10.80	7.058	<0.001	***
LDL	4.500	2.941	<0.05	*
Test drug - METT 250 mg/kg versus Test drug - METT 500 mg/kg				
TCs	−9.500	6.209	<0.001	***
TGs	−20.00	13.07	<0.001	***
HDL	2.300	1.503	>0.05	NS
LDL	−5.500	3.595	<0.01	**

Values were expressed as mean±SEM for six animals in each group, data were analyzed using two-way ANOVA followed by Bonferroni posttests. TCs: Total cholesterol, TGs: Triglycerides, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, SEM: Standard error mean, NS: Not significant

were observed for any changes continuously for the first 2 h and up to 24 h for mortality.

Preparation of dose

A dose of 1/10th and 1/20th of 5000 mg/kg were considered to be a high dose and low dose prepared by dissolving in miliQ water. The doses were prepared as per the OECD guideline no. 425.

Animals were divided into 5 groups each group consisting of 6 rats.

- Group-1: Normal control (normal saline for 7 days)
- Group-2: Doxorubicin (20 mg/kg IP) only on 5th day.
- Group-3: Vitamin-E (10 mg/kg, P.O.) for 7 days + doxorubicin (20 mg/kg IP) 48 h before scarification).
- Group-4: TT extract, low dose (250 mg/kg, P.O. for 7 days) + doxorubicin (20 mg/kg IP) on the 5th day (48 h before scarification).
- Group-5: TT extract, high dose (500 mg/kg, P.O. for 7 days) + doxorubicin (20 mg/kg IP) on the 5th day (48 h before scarification).

Symptoms and mortality in each group were recorded and compared with those rats who received doxorubicin alone. Forty-eight hours after doxorubicin administration, the rats were sacrificed and autopsied. The heart was dissected and histology of the heart was studied, and blood was collected for serum estimation. The serum was separated immediately by cold centrifugation and was used for determination of the myocardial infarction marker enzymes lactate dehydrogenase, creatine kinase-MB (CK-MB), serum glutamic-pyruvic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), along with serum total cholesterol (TC), triglycerides (TGs), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). The enzymes, lipids, and uric acid were estimated using commercial diagnostic kits.

Table 3: Two-way ANOVA comparison and followed by Bonferroni post-tests for Doxorubicin, Vitamin E, and *Tribulus terrestris* on *serum glutamic-pyruvic transaminase* and *serum glutamic-oxaloacetic transaminase*

Row factor	Difference	t	p	Summary
NC - Saline versus DC - Doxorubicin 20 mg/kg				
SGPT	37.70	33.08	<0.001	***
SGOT	48.00	42.12	<0.001	***
NC - Saline versus STD - Vitamin-E 10 mg/kg				
SGPT	12.00	10.53	<0.001	***
SGOT	6.200	5.440	<0.001	***
NC - Saline versus test drug - METT 250 mg/kg				
SGPT	16.00	14.04	<0.001	***
SGOT	14.00	12.28	<0.001	***
NC - Saline versus test drug - METT 500 mg/kg				
SGPT	9.000	7.897	<0.001	***
SGOT	22.80	20.00	<0.001	***
DC - Doxorubicin 20 mg/kg versus STD - Vitamin-E 10 mg/kg				
SGPT	−25.70	22.55	<0.001	***
SGOT	−41.80	36.68	<0.001	***
DC - Doxorubicin 20 mg/kg versus test drug - METT 250 mg/kg				
SGPT	−21.70	19.04	<0.001	***
SGOT	−34.00	29.83	<0.001	***
DC - Doxorubicin 20 mg/kg versus test drug - METT 500 mg/kg				
SGPT	−28.70	25.18	<0.001	***
SGOT	−25.20	22.11	<0.001	***
STD - Vitamin-E 10 mg/kg versus test drug - METT 250 mg/kg				
SGPT	4.000	3.510	<0.01	**
SGOT	7.800	6.844	<0.001	***
STD - Vitamin-E 10 mg/kg versus test drug - METT 500 mg/kg				
SGPT	−3.000	2.632	<0.05	*
SGOT	16.60	14.56	<0.001	***
Test drug - METT 250 mg/kg versus test drug - METT 500 mg/kg				
SGPT	−7.000	6.142	<0.001	***
SGOT	8.800	7.721	<0.001	***

SGOT: *Serum glutamic-oxaloacetic transaminase, SGPT: Serum glutamic-pyruvic transaminase*

Table 4: The phytochemical constituent test of crude extract of *Tribulus terrestris*

Serial number	Constituents	Tests	Results
1	Tests for alkaloids	Mayer'stest	+
		Wagner'stest	+
		Hager'stest	+
2	Tests for carbohydrates	Molisch'stest	−
		Benedict'stest	−
		Fehling'stest	−
3	Tests for flavonoids	Zinc-HCl reduction test	+
		Lead-acetate test	+
		Shinodatest	+
4	Tests for glycosides cardiac glycosides	Baljit'stest	+
		Legal'stest	+
		Anthraquinones glycosides	
		Borntrager'stest	+
		Saponin glycosides	
		Hemolytic test	+
5	Tests for proteins	Ninhydrin test	−
		Million'stest	−
6	Test for saponin	Foamtest	+
7	Tests for steroids and triterpenes	Liebermann–Burchard test	+
		Salkoswski test	+
		Gelatine test	+
		Lead acetate test	+

Where: (+) Present and (−) Absent

Collection of blood samples

At the end of the study, blood was collected from the rat by cardiac puncture under mild ether anesthesia. Collected blood samples were allowed to clot for 10 min at room temperature and they were centrifuged at 3000 rpm for 10 min. The serum obtained was used for the study of biochemical parameters such as SGOT, SGPT, CKMB, HDL, LDL, TC and TG [18].

Histopathology studies

A portion of the heart of animals in all groups was stored in a container for 12 h in 10% formalin (10 mL of formaldehyde in 90 mL of normal saline) solution and subjected to histopathological studies. The rats were sacrificed by light ether anesthesia. The heart was then quickly removed and preserved in 10% formalin solution for histopathological examinations [19].

Estimation of heart anti-oxidants

Estimation of Superoxide dismutase (SOD)

0.8 ml of 0.1 M sodium carbonated buffer (pH = 10.2), 0.1 ml of supernatant, 0.1 ml of epinephrine were added to the quartz cuvette. The absorbance at 295 nm by using a spectrophotometer was measured. The absorbance change for 0 min and 1 min was recorded [20]. Formula to calculate SOD:

$$\% \text{ inhibition of pyrogallol auto oxidation} = \frac{\Delta A\text{test}}{\Delta A\text{control}} \times 100$$

$$\text{SOD activity (u/mg)} = \frac{\% \text{ inhibition of pyrogallol}}{50}$$

Estimation of catalase

1.95 ml phosphate buffer (50 MM, pH 7.0), 1.0 ml H_2O_2 (0.17 MM), and 0.05 ml homogenate (10%, w/v) in a total volume of 3.0 ml was added. The absorbance at 240 nm using a spectrophotometer was measured. The absorbance change for 0 min and 1 min was recorded [21,22]. Formula to calculate catalase activity:

$$2.3\ \Delta t \times \left(\frac{\text{Einitial}}{\text{Efinal}} \times 1.63 \times 10^{-3} \right)$$

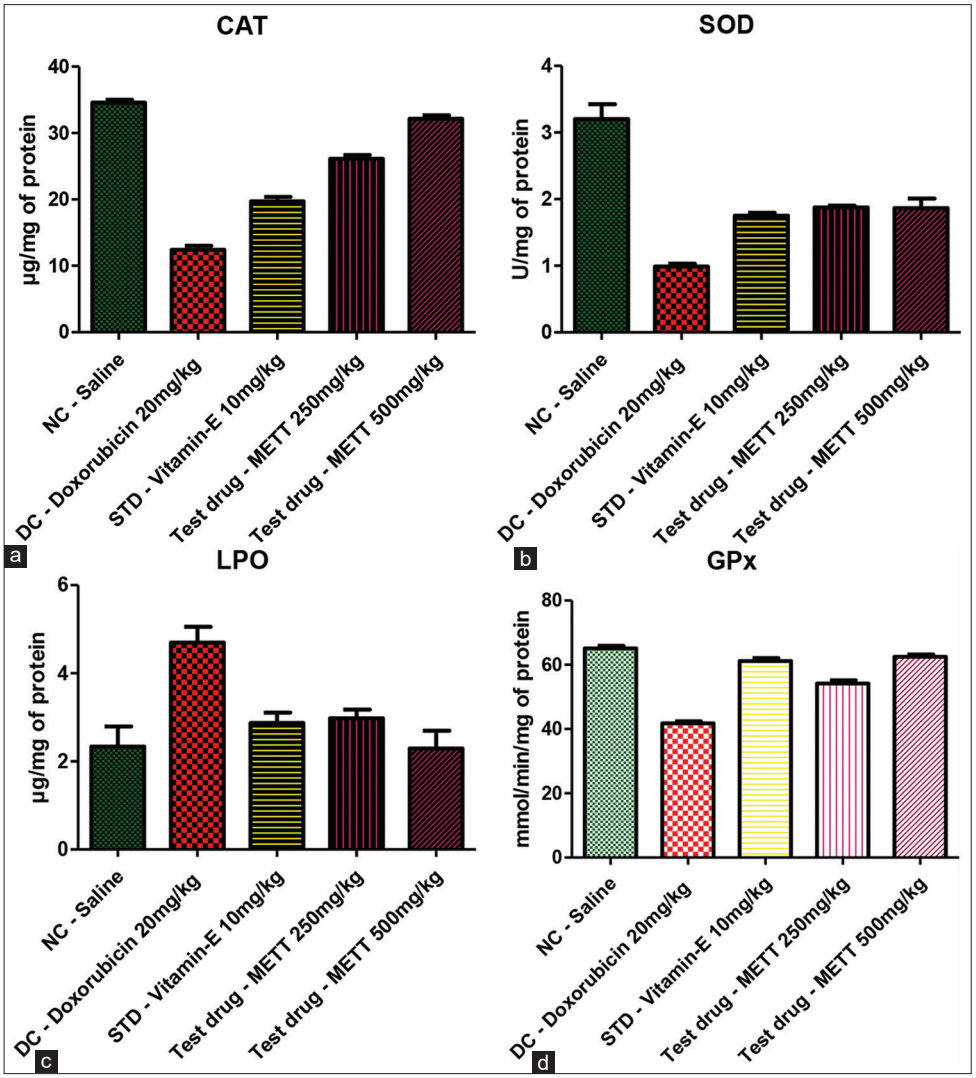

Fig. 4: (a) Effect of test items on catalase. Values were expressed as mean±SEM for six animals in each group; Data were analysed using one-way ANOVA followed by Tukey's test. p<0.001* compared with all the groups except NC - Saline versus test drug - METT 500 mg/kg i.e. p<0.05*. (b) Effect of test items on SOD. Values were expressed as mean±SEM for six animals in each group; Data were analyzed using one-way ANOVA followed by Tukey's test. p<0.001*** compared with all the groups except DC - Doxorubicin 20 mg/kg versus STD - Vitamin-E 10 mg/kg i.e. p<0.01** and STD - Vitamin-E 10 mg/kg versus Test drug - METT 250 mg/kg, 500 mg/kg, and Test drug - METT 250 mg/kg versus test drug - METT 500 mg/kg i.e. p>0.05 ns. (c) Effect of test items on LPO. Values were expressed as mean±SEM for six animals in each group; Data were analysed using one-way ANOVA followed by Tukey's test. p>0.05 ns with all the groups except NC - Saline versus DC - Doxorubicin 20 mg/kg, DC - Doxorubicin 20 mg/kg versus test drug - METT 500 mg/kg i.e. p<0.01***, DC - Doxorubicin 20 mg/kg versus STD - Vitamin-E 10 mg/kg i.e. p<0.01**, DC - Doxorubicin 20 mg/kg versus test drug - METT 250 mg/kg i.e. p<0.05*. (d) Effect of test items on GPx. Values were expressed as mean ± SEM for six animals in each group; Data were analyzed using one-way ANOVA followed by Tukey's test. p<0.001*** with the all the groups except NC - Saline versus STD - Vitamin-E 10 mg/kg i.e. p<0.05* and NC - Saline versus test drug - METT 500 mg/kg, STD - Vitamin-E 10 mg/kg versus test drug - METT 500 mg/kg i.e. p>0.05ns.**

Estimation of lipid peroxidation

To 1.0 ml of the sample, 2.0 ml of TCA- TBA-HC1 reagent was added and mixed thoroughly. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1,000 g for 10 min. The absorbance was determined at 535nm against a blank that contains all the reagents except the sample. The results were expressed as nmoles of MDA formed/min/mg protein using an extinction coefficient of the chromophore 1.56 × 105 $M^{-1}cm^{-1}$ [23-26].

Formula to calculate lipid peroxidation:

$$OD532\times100\times\left(\frac{1.56\times TV}{dwt}\right)\times1000$$

Estimation of glutathione peroxidase

To 0.2 ml of tris buffer, 0.2 ml of EDTA, 0.1 ml of sodium azide, and 0.5 ml of tissue homogenate were added. To this mixture, 0.2 ml of glutathione and 0.1 ml of hydrogen peroxide were added. The contents were mixed well and incubated at 37°C for 10 min along with a tube containing all the reagents except the sample. After 10 min, the reaction was arrested with the addition of 0.5 ml of 10 % TCA, centrifuged and the supernatant were assayed for glutathione by Ellman's method. To 2.0 ml of the supernatant, 3.0 ml of disodium hydrogen phosphate solution and 1.0 ml of DTNB reagent were added. The colors developed were read at 412 nm. Standards in the range of 200–1000 jag were taken and treated in a similar manner. The activity was expressed in terms of fig of glutathione consumed/min/mg protein [27-30].

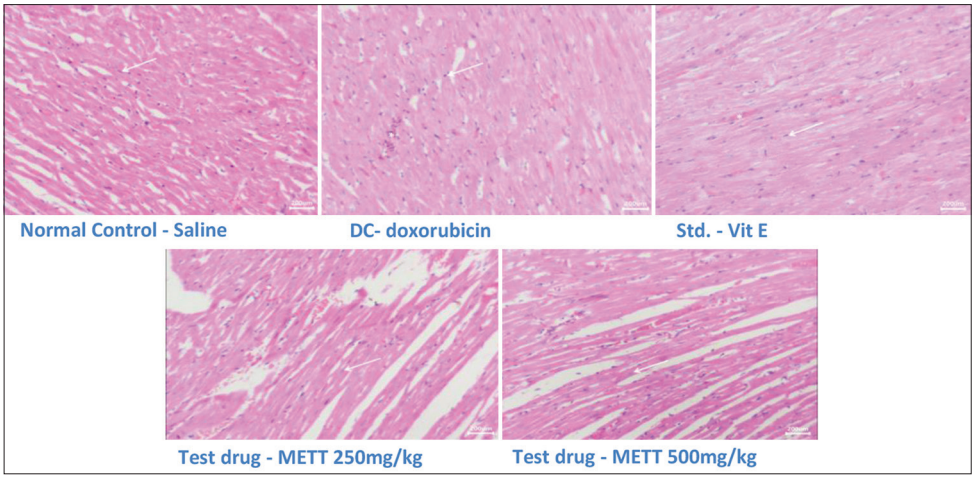

Fig. 5: Histopathological assessment of Heart tissue

Formula to calculate Glutathione peroxidase:

$$GPX=\frac{2(mRate\text{-}mRate\ B)\times VRM\times DF}{6.22\times VS}\times 1.63\times 10^{-3}$$

Statistical analysis

The results are expressed as mean±S.E.M from n=6 rats in each group. The significance of difference among the groups was assessed using ANOVA followed by Tukey's test/Bonferroni post-tests compared between normal control (Untreated) versus all groups. $p<0.05$ was considered statistically significant.

RESULTS

Percentage yield of the extract obtained was recorded

% yield of the extract was obtained, and methanol extraction revealed a 5.4% yield (Table 1).

Biochemical analysis

A. Effect of doxorubicin, Vitamin E, and TT on lipid profile

In comparison to the disease control drug, doxorubicin, treated groups' TC, TGs, HDL, and LDL levels significantly improved. The statistical analyses were performed using a prism graph (Fig. 1), and the results were analyzed using a two-way ANOVA comparison. Bonferroni posttests with p values of 0.001***, 0.01**, 0.05*, and >0.05ns were performed in comparison to the disease control (Table 2).

B. Effect of doxorubicin, Vitamin E, and TT on SGPT and SGOT

When compared to Doxorubicin, the disease control, treated groups' SGOT and SGPT levels showed a considerable improvement. Prism charts were used to determine the significant assessments, which were then analysed using two-way ANOVA comparison and compared to the disease control using Bonferroni posttests at p<0.001***, p<0.01**, and p<0.05* (Fig. 2 and Table 3).

C. Effect of doxorubicin, Vitamin E, and TT on CK-MB

CK-MB is a cardiac marker used to help diagnose an acute myocardial infarction, myocardial ischemia, or myocarditis. Treated groups were shown to have made a significant improvement in their CK-MB compared to disease control. It calculates the blood concentration of CK-MB, a bound mixture of two forms of the phosphocreatine Kinase enzyme. The statistical analyses were performed using a prism graph (Fig. 3), and the results were compared to the disease control using Bonferroni posttests with p-values of 0.001***, 0.01**, 0.05*, and >0.05ns.

D. Effect of doxorubicin, Vitamin E, And TT extract on Anti-oxidant enzymes

A variety of physiological and pathological states are heavily influenced by oxidative stress. Reactive oxygen species generation and intracellular defense systems carefully control the intracellular oxidative equilibrium. Increased oxidative stress may affect proteins, lipids, and DNA, which may cause cellular inflammation and programmed cell death. The evolution of numerous cardiovascular disorders, including atherosclerosis, heart failure, cardiac arrhythmia, and ischemia-reperfusion injury, is shown to be significantly influenced by oxidative stress, according to evidence. Cardiovascular illnesses linked to oxidative stress can be treated in a variety of ways. In terms of their CK-MB, treated groups showed a considerable improvement when compared to the disease-control drug, doxorubicin. Prism graph was used to determine the significant assessments, and two-way ANOVA comparison was used toanalyse the Statistics and followed by Bonferroni posttests p<0.001***, p<0.01**, p<0.05*, p>0.05ns and compared to the disease control.

Histopathological analysis (heart tissue)

Fig. 5 depicts the evaluations of heart tissue, and the explanation is given below.

Effect of normal saline (10 ml/kg)

Heart showing the normal architecture of cardiac muscle fibers with cross striations. The cytoplasm of skeletal muscle fibers staining pink in color (asterisk) and oval-shaped nucleus staining blue (arrow). Hematoxylin and Eosin stain, Scale bar=200µm.

Effect of doxorubicin (20 mg/kg)

Heart showing the marked distortion of the normal architecture of cardiac myocytes and wide separation of muscle fibers evident by empty spaces. Some of the myocytes show peripheral and condensed nuclei (arrow) along with congestion of blood vessels with red blood cells indicated by dark red color of blood vessels. There was focal infiltration of inflammatory cells mainly mononuclear cells (asterisk). Haematoxylin and Eosin stain, Scale bar=200 µm.

Effect of Vitamin E (10 mg/kg)

Heart showing the return to the normal architecture of cardiac myocytes with cytoplasm staining pink in color with cross striations, oval-shaped nuclei staining blue (arrow). There was congestion of blood capillaries filled with red blood cells (asterisk) indicating the regeneration of cardiac myocytes. Hematoxylin and Eosin stain, Scale bar=200 µm.

Effect of METT (250 mg/kg)

Heart showing the return to the normal architecture of cardiac myocytes with cytoplasm staining pink with cross striations, oval-shaped nuclei staining blue (arrow). There was congestion of blood capillaries filled with red blood cells (asterisk) indicating the regeneration of cardiac myocytes. However, mild degenerative changes in the cardiac muscle fibers were observed. Hematoxylin and eosin stain, scale bar=200 μm.

Effect of METT (500 mg/kg)

Heart showing the normal architecture of cardiac myocytes with cytoplasm staining pink in color with cross striations and oval-shaped nuclei staining blue in color (arrow). There was congestion of blood capillaries filled with red blood cells (asterisk) indicating the regeneration of cardiac myocytes. Hematoxylin and eosin stain, scale bar=200 μm.

DISCUSSION

Numerous research works have proven its uses beyond the ethno-medicinal uses in experimental animals. Alkaloids and flavonoids, glycosides are responsible for their pharmacological activities [31].

Thus, the present study was carried out to evaluate cardioprotective and anti-oxidant activity of methanolic extract of leaves of TT in doxorubicin-induced cardiotoxic in the rat. On preliminary phytochemical screening of the plant, it was revealed that the plant is rich in antioxidant properties due to the presence of chemical constituents like steroidal saponins, flavonoids, flavonol glycosides, alkaloids, and tannins (Table 4). As a result of which, the leaves of the plant TT may possess cardioprotective activity.

Acute toxicity studies were conducted, single-dose administration of 5000 mg/kg body weight not showed mortality. Hence, upon calculation of ED50 the dose of methanolic extract of leaves of TT was recommended for prophylactic and therapeutic effects in reducing cardiovascular diseases, and that has been reviewed [32].

Due to its structure, doxorubicin interferes with nucleoside metabolism and can be incorporated into RNA and DNA, leading to cytotoxicity and cell death [33]. Doxorubicin has diverse adverse effects such as cardiotoxicity, nephrotoxicity, and hepatotoxicity which restrict its wide and extensive clinical usage. It causes marked organ toxicity coupled with increased oxidative stress and apoptosis [34]. Life-cardiac side effects of doxorubicin are still speculative and based on cardiac symptoms. There is no evidence of a single mechanism responsible for doxorubicin-induced cardiotoxicity, and the underlying mechanisms might be multifactorial. Several investigators postulated its direct toxic effects on cardiomyocytes and vascular endothelial cells [35-36]. The leaves of the plant TT contain antioxidant properties, which enable it to reduce lipid peroxidation by generating an extra electron, as a result of which it stabilizes the unstable reactive oxygen species and ultimately prevents its generation. Thus, pre-treatment of rats with METT showed a significant reduction in elevated such as SGOT, SGPT, CKMB, HDL, LDL, TC and TG. In addition, treatment with METT preserved the physiological weight gain of the animals over 10 days, as the body weight on day 11 was significantly more compared with their baseline body weight. In addition to biochemical changes, a decrease in body weight may be due to reduced ingestion of food and increase in waste and toxicity in the blood may be due to marked necrosis of heart tissue. The present study revealed that the high dose of methanolic extract of leaves of TT possesses cardioprotective action which might be due to the presence of anti-oxidant properties of the extract. fixed as $1/10^{th}$ and $1/20^{th}$ of 5000 mg/kg as high dose and low dose, respectively. Myocardial infarction remains a leading cause of death worldwide and prompt treatment for a heart attack is indispensable to save life. In the traditional Indian medicinal system, a major role has been played by the plants, especially, in the aspect of cardioprotection. Several herbs and herbal products have been the potential therapeutic effects of vitamin E in AMI can be comprised of biological actions such as antioxidant and anti-inflammatory effects, as well as a synergism with other antioxidant molecules. Indeed, Vitamin E, mainly α-tocopherol, is the major peroxyl radical scavenger in biological lipid phases such as membranes or LDL [37]. The antioxidant action has been ascribed to its ability to act chemically as a lipid-based free radical chain-breaking molecule, thereby inhibiting lipid peroxidation through its own conversion into an oxidized product, α-tocopheroxyl radical. α-Tocopherol can be restored by the reduction of the αtocopheroxyl radical with redox-active reagents such as vitamin C or ubiquinol [38].

CONCLUSION

The experimental studies carried out on the extract of leaves of TT possess dose-dependent cardioprotective and antioxidant activity. The higher dose 500 mg/kg showed significant protection compared to lower dose 250 mg/kg. Administration of doxorubicin in control rats showed significant increase serum TC, TGs, low-density lipoprotein, CKMB, SGOT, SGPT, and decrease in high-density lipoprotein. Rats treated with TT (250 mg/kg and 500 mg/kg) showed decreased TC, TG, LDL, CKMB, SGOT, and SGPT and increases HDL levels. The histopathological studies also showed that the plant extract significantly minimized the damage induced by doxorubicin. A significant decrease in GPx, SOD, LPO, and Catalase was seen in the 5-fluorouracil-treated group when compared with the control group and a dose-dependent increase in GPx, SOD, LPO, and Catalase was observed on animals treated with METT along with doxorubicin. TT 500 mg/kg prevented the alterations in marker enzymes of myocardial infarction, and oxidative stress and showed normal myofibrillar structures and revealed a marked protection by the extract against myocardial necrotic damage. TT elevated HDL level and decreased LDL cholesterol level. The higher dose showed significant Cardioprotective activity compared to lower dose 250mg/kg. The potential chemical constituents are present on leaves of TT had a potential to treat cardiotoxicity.

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AUTHORS' CONTRIBUTIONS

All the authors contributed to the preparation of the final manuscript.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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