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Original Research

Evaluation Of Cardioprotective Potential Of Aqueous Extract Of *Tribulus Terrestris* Against Doxorubicin-Induced Cardiotoxicity In Wistar Albino Rats: An Experimental Study

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Abstract

Background: Cardiotoxicity is one of the most terrifying side effects of anticancer agents like Doxorubicin. *Tribulus terrestris* has antihypertensive property and improves cardiac function. Till now only few scientific studies have been performed to evaluate such claims but with inconclusive results. Therefore, the present study was designed to evaluate the cardioprotective activity of *Tribulus terrestris* in experimentally induced cardiotoxicity in albino rats.

Methods: Following approval from institutional animal ethics committee of L.L.R.M. Medical College registered under CPCSEA, India, this study was undertaken in Department of Pharmacology. Thirty Wistar albino rats were randomized into five groups of six rats each. Group I was given normal saline (1 ml/kg) per oral, group II received pellet diet and normal saline for 21 days and then treated with Doxorubicin in a single dose of 20mg/kg intraperitoneally on 21st day. Group III received Carvedilol in doses of 30 mg/kg/day p.o. for 21 days followed by administration of Doxorubicin on 21st day, group IV and V were treated with aqueous extract of *Tribulus terrestris* given in two graded doses (200 mg/kg and 400 mg/kg), per orally respectively for 21 days followed by administration of Doxorubicin 20mg/kg i.p. on 21st day. The rats were observed for 48 hours and then sacrificed under ketamine (75mg/kg) and xylazine (10mg/kg) anesthesia given intraperitoneally. Blood samples (volume ≈ 5ml) were collected from abdominal aorta for performing biochemical tests i.e. Creatinine kinase MB fraction (CK-MB), Lactate dehydrogenase (LDH), Serum glutamate oxaloacetate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT). The animals were sacrificed and heart was dissected out for histopathological study. The data obtained was organized and analyzed by suitable statistical methods i.e. ANOVA followed by Post Hoc test.

Results: CK-MB, LDH, SGOT and SGPT levels were found to be significantly raised ($p<0.001$) in Doxorubicin treated group. *Tribulus terrestris* pre-treated groups exhibited a significant limitation ($p<0.001$) in levels of CK-MB, LDH, SGOT and SGPT in a dose dependent manner following Doxorubicin administration which were comparable to the group treated with the standard cardioprotective drug Carvedilol. Histopathological changes further corroborated cardioprotective potential of *Tribulus terrestris*.

Conclusion: The present study concluded that aqueous extract of *Tribulus terrestris* exhibits a cardioprotective effect against doxorubicin induced cardiotoxicity in a dose dependent manner.

Keywords: *Tribulus terrestris*, Cardiotoxicity, Doxorubicin, Carvedilol.

INTRODUCTION

Cardiovascular disease (CVD) is considered to be one of the most leading causes of global morbidity followed by fatality, particularly in low- and middle-economic countries including India. The incidence rates are rising very high leading to global burden of illness and affecting the quality of life (QOL) of individuals[1]. Cardiovascular diseases cause 17.1 million fatalities each year and this figure is expected to reach upto 24 million in 2030[2].

Cardiotoxicity is one of the most feared side effects of various drug especially anticancer drugs so that the gain in life expectancy might be in the form of heart failure (HF), myocardial ischemia, arrhythmias, hypertension, thromboembolism etc.[3]

Doxorubicin (adriamycin) has been widely used to treat solid and haematopoietic tumours; however, a major limiting factor for the clinical use of DOX is irreversible cardiac toxicity. Doxorubicin induced cardiomyopathy occurs primarily

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through the generation of reactive oxygen species (ROS), which can induce myocardial lipid peroxidation and myofibre degeneration.[4]

The use of herbal medicines is one of the main therapeutic approaches of complementary and alternative medicine (CAM).[5] Herbal extracts have many properties like antioxidant, anti-allergic, anti-inflammatory, antiviral, anti-proliferative and anti-carcinogenic effects. Natural antioxidants, which are capable of protecting cells from oxidative injury, should be included in potential antioxidant therapy. Therefore, there is a need for identifying alternative, natural and safer sources of antioxidants.[6]

However, there are still many herbs, which have been claimed to possess therapeutic potential against cardiovascular disorders, but have not been scientifically proven. One such plant is *Tribulus terrestris* which is claimed to possess cardioprotective action. *Tribulus terrestris* (TT) is an annual herb of the Zygophyllaceae family, commonly known as Gokshur or Gokharu or puncture vine, has been used for a long time in both the Indian and Chinese systems of medicine for treatment of various kinds of diseases including heart diseases.[7] The important phytoconstituents of this plant include flavonoids, alkaloids, saponins, lignins, amides and glycosides.[8] It has multitherapeutic potential like diuretic, aphrodisiac, cardiotonic, appetiser, anthelminthic, carminative, expectorant and laxative.[9] It is also effective against cough, asthma, anaemia, and inflammation, and in the treatment of male urinary problems and impotence.[10] *Tribulus terrestris* has antihypertensive property and improves cardiac function.[11]

Till now only few scientific studies have been performed to evaluate such claims but with inconclusive results. Therefore, the present study was designed to evaluate the cardioprotective activity of *Tribulus terrestris* in experimentally induced cardiotoxicity in albino rats.

MATERIALS AND METHODS

Study design

This prospective randomized experimental study was conducted in the Department of Pharmacology, L.L.R.M. Medical College, Meerut from October 2021 to September 2022.

The study was commenced after obtaining approval (Approval no. IAEC/2021/01 dated 27/8/21) from the Institutional Animal Ethical Committee of Lala Lajpat Rai Medical College, Meerut, India registered under CPCSEA India (Registration No. 819/Po/Re/S/04/CPCSEA).

Experimental Animals:

Healthy albino Wistar female rats weighing 150-200g were procured from the rat rearing unit of the Central Animal House of the institute. The selected animals were grouped and housed in polypropylene cages in CPCSEA approved animal house of LLRM Medical College and maintained under standard laboratory conditions of alternating periods of light and darkness of 12hr each and under controlled conditions of temperature (25 ± 2^0C) and relative humidity (45 to 55%) with free access to standard rat pellet diet and tap water *ad libitum*. After one week of acclimatization, the animals were considered suitable for study. Pregnant female rats were not included in the study.

The doses of *Tribulus terrestris* extracts used in the study were calculated on the basis of previously documented LD_{50} on rats as per OECD guidelines (OECD-423).

Method of Preparation of Extract:

The plant material *Tribulus terrestris* was obtained from local market. Then, aerial parts of the plant were dried in shade and its extract was obtained through the Soxhlet apparatus. 2.5g of the dried plant material of *Tribulus terrestris* was refluxed with 100ml of water in a round bottom flask fitted with condenser on a heating mantle at set temperature 100^0 C for 5-6 hrs. The solution was filtered through Whatman no. 1 filter paper. The residue was refluxed again with fresh water adopting similar condition and filtered as above. After filtering, the resulting extract was concentrated through rotavapor and dried in freeze drier. The approximate percentage yield of the extract was 31.1% w/w i.e., 0.7g. From the solid extract collected, 40mg/ml stock solution was made for each.[12]

Study Outline:

Evaluation of Cardioprotective Activity:

The animals were randomly divided into four groups of six animals each. The groups were described as:

Group I: Control group was given 0.9 % NaCl solution in a single oral dose of 1ml/kg b.w. for 21 days.

Group II: In addition to pellet diet and tap water *ad libitum* the animals of this group were treated with doxorubicin in a single dose of 20 mg/kg intraperitoneally on 21st day.

Group III: This group was treated with Carvedilol (standard cardioprotective drug) 30 mg/kg b.w. per orally for 21 days followed by administration of Doxorubicin 20 mg/kg i.p. as in group -II.

Group IV: This group was treated with aqueous extract of *Tribulus terrestris* (200mg/kg b.w.) as a single oral dose every morning for 21 days followed by administration of doxorubicin 20mg/kg i.p. on 21st day.

Group V: This group was treated with aqueous extract of *Tribulus terrestris* (400mg/kg b.w.) as a single oral dose every morning for 21 days followed by administration of doxorubicin 20mg/kg i.p. on 21st day.

ISSN:0975-3583,0976-2833 VOL14,ISSUE05,2023

Animals of all the groups were fasted for 48 hours (during which tap water remained freely available) following administration of Doxorubicin. Then they were sacrificed using Ketamine (75mg/kg) and Xylazine (10 mg/kg) anesthesia, given intraperitoneally. Blood sample (5ml) was collected from abdominal aorta for performing blood test i.e. CK, LDH, SGOT, SGPT. After blood collection the animals were sacrificed and heart was dissected out for histopathological study. The data thus obtained was appropriately organized and analyzed by suitable statistical methods i.e. ANOVA followed by Post Hoc test.

HISTOPATHOLOGICAL EXAMINATION

The heart was excised from the animal and washed with normal saline. Whole of the heart was placed in 10% neutral formalin for 12-24 hours. It was then dehydrated and cleared with ethanol and xylene, respectively, followed by embedding in paraffin wax from which blocks were prepared. Sections of 5µm thickness were prepared from the blocks using microtome.[13] These were processed in alcohol-xylene series and were stained with Harris Haematoxylin and Eosin stain and then subjected to histopathological examination.[14]

STATISTICAL ANALYSIS

Mean ± SE was calculated for each group to observe the general trend of the group. The statistical analysis was carried out using one way analysis variation (ANOVA) followed by Post-Hoc Test. P- values were estimated by referring to appropriate tables.[15]

OBSERVATIONS AND RESULTS

BIOCHEMICAL PARAMETERS

Effect of aqueous extract of *Tribulus terrestris* (TT) on serum CK-MB, LDH, SGOT and SGPT.

1.CK-MB

The mean CK-MB level in normal saline treated group was 20.67±0.33IU/L. It was found to be significantly increased ($p<0.001$) to 287.67±4.09 IU/L following Doxorubicin administration (20mg/kg,i.p.). Pretreatment with standard drug Carvedilol (30mg/kg p.o.), significantly ($p<0.001$) limited the rise in CK-MB levels to 60.33±2.52 after administration of Doxorubicin (20mg/kg,i.p) (Tab 1, Fig 1).

Tribulus terrestris extract exhibited dose dependent limitation of CK-MB rise following Doxorubicin administration. When *Tribulus terrestris* extract was administered in lower dose of 200 mg/kg per orally for 21 days, a significant limitation ($p<0.01$) of CK-MB rise (179.67±1.45 IU/L) was observed when compared to Doxorubicin treated group (20mg/kg,i.p) but it did not match the efficacy of Cavedilol treated group. However, in dose of 400 mg/kg per orally for 21 days the *Tribulus terrestris* extract had better efficacy, in limiting the CK-MB rise to 89±1.73 IU/L following administration of Doxorubicin (20mg/kg,i.p), which was found to be statistically significant ($p<0.001$) (Tab 1, Fig 1).

2. LDH

The mean LDH level in normal saline treated group was 265±8.14 IU/L. It was found to be significantly increased ($p<0.001$) to 1177.67±7.96 IU/L following administration of Doxorubicin (20mg/kg,i.p). (Tab 1, Fig 1)

Tribulus terrestris extract demonstrated dose dependent limitation of LDH rise after Doxorubicin administration. Although the dose of 200 mg/kg per orally for 21 days showed a significant limitation ($p<0.01$) of LDH rise (801±8.38IU/L) when compared to Doxorubicin treated group (20mg/kg,i.p.) but I did not match the efficacy of Carvedilol treated group. However, in dose of 400 mg/kg per orally for 21 days the *Tribulus terrestris* extract had much better efficacy as expected, in limiting the LDH rise to 584.33±6.12 IU/L following administration of Doxorubicin (20mg/kg,i.p), which was found to be statistically significant ($p<0.001$) (Tab 1, Fig 1).

3. SGOT:

The mean SGOT level in normal saline treated group was 30.67±1.20 IU/L. It was found to be significantly increased ($p<0.001$) to 220.33±1.45 IU/L following administration of Doxorubicin (20mg/kg,i.p) (Tab 1, Fig 1).

Tribulus terrestris extract resulted in dose dependent limitation of rise in SGOT levels after Doxorubicin administration. Although the dose of 200 mg/kg per orally for 21 days demonstrated a significant limitation ($p<0.01$) of SGOT rise (189.67±4.80 IU/L) when compared to Doxorubicin treated group. On increasing the dose of *Tribulus terrestris* the performance of drug against cardiotoxicity induced by doxorubicin was enhanced. For the dose of 400 mg/kg *Tribulus terrestris* showed further decrease in the elevation of SGOT level to 111±3.79 IU/L, which was highly significant ($p<0.001$) (Tab 1, Fig 1).

ISSN:0975-3583,0976-2833 VOL14,ISSUE05,2023

4. SGPT

The mean SGPT level in normal saline treated group was 35.33±0.33 IU/L. It was found to be significantly increased ($p<0.001$) to 246.67±3.52 IU/L following administration of Doxorubicin (20mg/kg,i.p) (Tab 1, Fig 1).

With aqueous extract of *Tribulus terrestris* there is a dose dependent limitation of SGPT rise following Doxorubicin administration. At the dose of 200 mg/kg per orally for 21 days displayed a highly significant limitation ($p<0.05$) of SGPT rise (197.67±3.93 IU/L) when compared to Doxorubicin treated group (20mg/kg,i.p.). However, on increasing the dose of *Tribulus terrestris* the protective effect of the drug is increased. At the dose of 400mg/kg for 21 days the aqueous extract of *Tribulus terrestris* showed much better efficacy in limiting the SGPT rise to 158.33±2.03 IU/L following administration of Doxorubicin (20mg/kg,i.p.), which was found to be statistically significant ($p<0.001$) (Tab 1, Fig 1).

Table 1: Effect of Carvedilol and *Tribulus terrestris* in their respective doses on Doxorubicin induced changes in various biochemical parameters. (Mean± SE) (n=6)

S. No.	Treatment (p.o mg/kg)	CKMB (IU/L) (Mean±SE)	LDH (IU/L) (Mean±SE)	SGOT (IU/L) (Mean±SE)	SGPT (IU/L) (Mean±SE)
I.	Normal Saline (1ml/100g)	20.67±0.33	265±8.14	30.67±1.20	35.33±2.03
II.	Doxorubicin (20 i.p.)	287.67±4.09^	1177.67±7.96^	220.33±1.45^	246.67±3.53^
III.	Carvedilol 30	60.33±1.45$^{\Omega}$	496.33±6.17$^{\Omega}$	75.67±2.60$^{\Omega}$	114±4.04$^{\Omega}$
VI.	*Tribulus terrestris* 200	179.67±1.45*	801±8.38*	189.67±4.81*	197.67±3.93*
VII.	*Tribulus terrestris* 400	89±1.73$^{\Omega}$	584.33±6.12$^{\Omega}$	111±3.79$^{\Omega}$	158.33±2.03$^{\Omega}$

* $p< 0.01$ as compared to DOX treated group.

$^{\Omega}$ $p < 0.001$ as compared to DOX treated group.

^ $p < 0.001$ as compared to normal saline treated group.

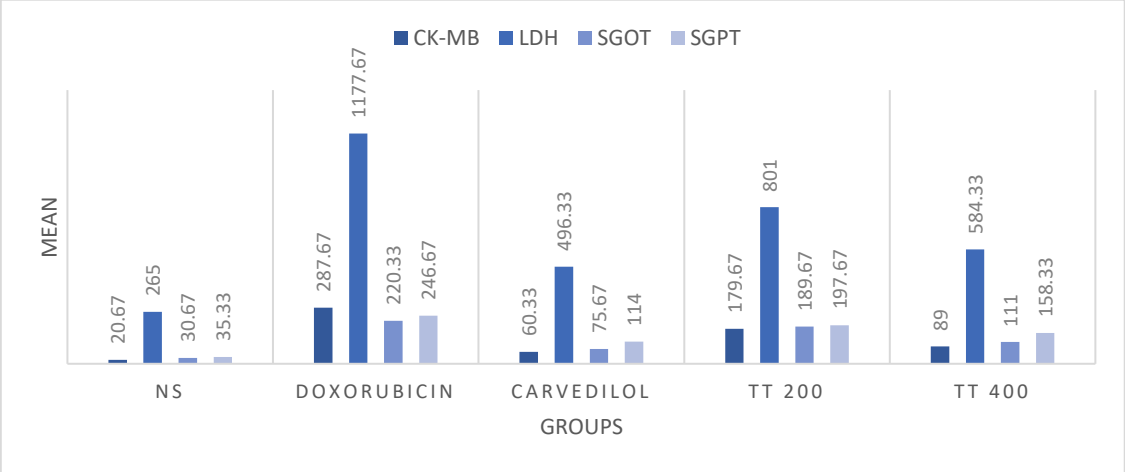

Figure 1: Effect of Carvedilol and *Tribulus terrestris* (TT) in their respective doses on Doxorubicin induced changes in various biochemical parameters with normal saline (NS) group taken as control (Mean± SE) (n=6)

HISTOPATHOLOGICAL CHANGES

The histopathological sections of heart of rats of saline treated group showed normal striated muscles, clearly visible striations and well aligned oval flattened nuclei (Fig. 2). Doxorubicin (DOX) administration resulted in intramuscular haemorrhage, oedema, degeneration of muscle fibres, vacuolation and infiltration with inflammatory cells (Fig. 3).

Degree of protection with ***Tribulus terrestris*** against Doxorubicin induced cardiotoxicity was evident clearly on histopathological examination of cardiac tissue.

Rats treated with *Tribulus terrestris* (200mg/kg/day p.o + DOX 20mg/kg i.p single dose) for 21 days demonstrated gentle widening of the muscle fibres with focal loss of myofibres, inflammation and absence of myonecrosis (Fig.4). Rats treated with *Tribulus terrestris* (400mg/kg/day p.o +DOX 20mg/kg i.p single dose) for 21 days showed small areas of early necrotic changes and almost normal cardiac architecture (Fig. 5) which was similar to that seen in Carvedilol treated groups (parallel arrangement of muscle fibres with peripherally located normal nuclei) (Fig. 4).

ISSN:0975-3583,0976-2833 VOL14,ISSUE05,2023

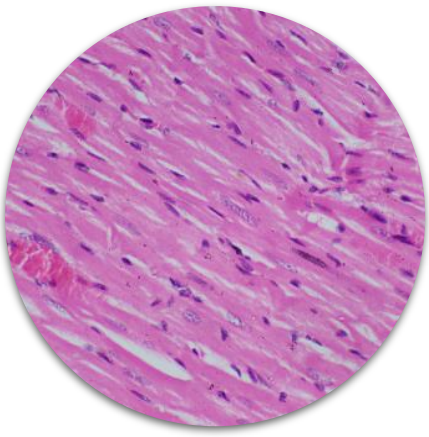

Fig 2-Light micrographs (400X) of section from heart tissue stained with haematoxylin and eosin showing normal histological architecture of heart in rats treated with normal saline (1ml/100g/day p.o).

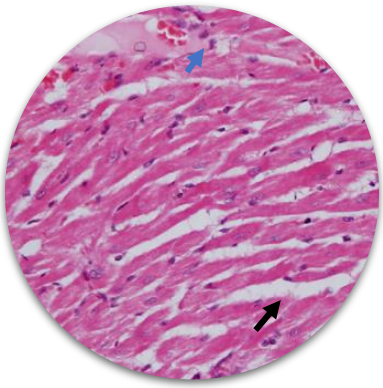

Fig 3-Light micrographs (400X) of section from heart tissue stained with haematoxylin and eosin showing intense inflammatory infiltration (blue arrow), interstitial oedema and degeneration of muscle fibres (black arrow) in rats treated with Doxorubicin (20mg/kg i.p single dose).

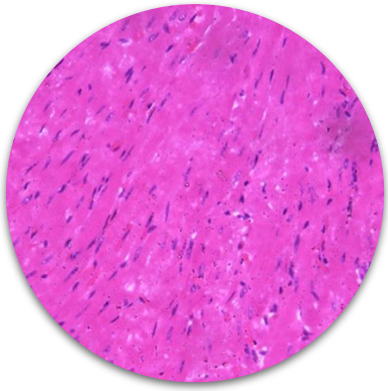

Fig 4- Light micrographs (40X) of section from heart tissue stained with haematoxylin and eosin showing parallel arrangement of muscle fibres with peripherally located normal nuclei seen in rats treated with Carvedilol (30mg/kg/day p.o + Doxorubicin 20mg/kg i.p single dose) for 21 days.

ISSN:0975-3583,0976-2833 VOL14,ISSUE05,2023

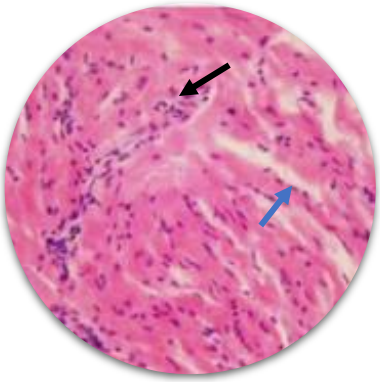

Fig 5- Light micrographs (40X) of section from heart tissue stained with haematoxylin and eosin showing gentle widening of the muscle fibers with focal loss of myofibers (blue arrow), inflammation (black arrow), absence of myonecrosis in rats treated with *Tribulus terrestris* (200mg/kg/day p.o +Doxorubicin 20mg/kg i.p single dose) for 21 days.

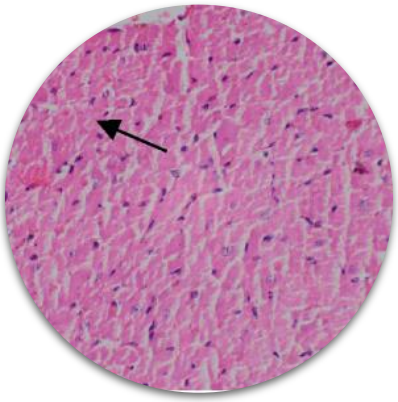

Fig 6- Light micrographs (40X) of section from heart tissue stained with haematoxylin and eosin showing almost normal cardiac muscle morphology with small area of early necrotic changes (arrow) in rats treated with *Tribulus terrestris* (400mg/kg/day p.o + Doxorubicin 20mg/kg i.p single dose) for 21 days

DISCUSSION

Myocardial ischemia and the reperfusion that follows can cause injuries similar to or worse than pure ischemia, this phenomenon is called myocardial ischemia/reperfusion (I/R) injury. I/R injury generates oxygen derived free radicals, which cause lipid peroxidation resulting in additional tissue damage, including cardiomyocyte apoptosis.[16] Generation of reactive oxygen species (ROS) is one of the mechanisms for Doxorubicin induced myocardial toxicity. Additionally, Doxorubicin causes oxidative alteration of the mitochondrial permeability transition pores. This effect is responsible for decrease in mitochondrial calcium loading capacity.[17]

Doxorubicin (DOX) is one of the most effective anticancer drugs to treat various forms of cancers. However, its therapeutic utility is severely limited by cardiotoxicity associated with it. Although multiple mechanisms are involved in doxorubicin-induced cardiotoxicity, generation of ROS which causes lipid peroxidation and depletion of antioxidant enzymes is considered to be a major mechanism involved.[18] The rat model of doxorubicin induced cardiotoxicity has been widely used as a standard method to evaluate cardioprotective drugs and to study myocardial consequences of ischemic disorder.[19] Doxorubicin in a single dose of 20mg/kg intraperitoneally has been used to induce cardiotoxicity in experimental studies also.[20]

The therapeutic actions including cardioprotective effect of most medicinal plants are related to their antioxidant properties which, in turn, could be ascribed to the antioxidant phytochemicals present in them.[21] Several antioxidant vitamins have exhibited protective role against doxorubicin induced cardiotoxicity. Vitamin E decreased cardiac lipid peroxidation and delayed the lethality of a single dose of doxorubicin at 24 hr.[22]

Tribulus terrestris contains phytoconstituents like flavonoids, alkaloids, saponins, lignins, amides and glycosides which have been found to exhibit cardioprotective potential.[23] The study done on *Tribulus terrestris* revealed its high antioxidant activity which plays an essential role in its anti-ischemic activity in an *in-vitro* model.[24] Since mitochondria are the primary resource for ROS production, the effect of ischemia on mitochondria was studied (*in vitro*) and demonstrated that TT fruit (methanolic extract) safeguards mitochondria from ischemic insult via preventing surplus ROS.[25]

ISSN:0975-3583,0976-2833 VOL14,ISSUE05,2023

Results of the present study indicate that administration of DOX (20mg/kg i.p) elevated the serum levels of CKMB, LDH, SGOT and SGPT signifying myocardial damage similar to the studies done by El-Agamy et al. (2016)[26]; Zhang et al. (2017)[27], and this rise of serum markers was found to be statistically significant in the present study also. It was interesting to note that CK-MB levels were significantly low in the *Tribulus terrestris* pretreated rats (Tab.1, Fig.1). *Tribulus terrestris* in the dose of 400 mg/kg conferred more cardioprotection than *Tribulus terrestris* in the dose of 200 mg/kg. In the present study, a significant rise in the LDH levels was observed in rats treated with DOX after 48 hr of the treatment. *Tribulus terrestris* pretreatment caused significant reduction in the elevated levels of LDH indicating amelioration in the severity of cardiotoxicity (Tab 1 Fig 1).

Ravichandra *et al*., (2014) reported rise in levels of SGOT and SGPT following myocardial infarction.[28] DOX treatment elevated these enzyme levels in serum to a significant extent and pretreatment with *Tribulus terrestris* doses significantly reduced the elevated levels of SGOT and SGPT. The results of the serum markers correlated with the histopathological observations in the myocardial tissue of animals treated with either DOX or normal saline (Tab1, Fig1).

The myocardial tissue of saline treated rats illustrated clear integrity of the myocardial cell membrane and absence of any inflammatory cell infiltration (Fig. 2). DOX injected rats showed separation of cardiac muscle fibers and infiltration of inflammatory cells (Fig. 3). The reduced inflammatory cell infiltration and normal cardiac muscle fibre architecture in *Tribulus terrestris* treated rats further confirmed their cardioprotective effect (Fig. 5,6). In the present study doxorubicin induced rise in serum markers was significantly decreased by pretreatment with *Tribulus terrestris* (Tab1, Fig1). The results of present study demonstrated that *Tribulus terrestris* provide cardioprotection against doxorubicin induced cardiotoxicity.

In the present study, the aqueous extract of *Tribulus terrestris* has been shown to possess cardioprotective potential. The results could be more effective with use of other extracts as ethanolic, methanolic etc. Further studies of longer duration, on a larger scale are needed for isolation and structure determination of the cardioprotective principles and detailed explanation of mechanism of action of *Tribulus terrestris* as a cardioprotective agent.

CONCLUSION

The study concludes that pretreatment with aqueous extract of *Tribulus terrestris* significantly reduced Doxorubicin induced damage to rat myocardium without any appreciable side effects. Thus, we can conclude that *Tribulus terrestris* have a good cardioprotective action that might be attributed to the antioxidant properties of chemical compounds like polyphenols and flavonoids present in them. Based on these observations, it can be proposed that *Tribulus terrestris* may provide a therapeutic option against drug induced cardiotoxicity.

The present study also carries a scope for further assessment of *Tribulus terrestris* with its hydroalcoholic extract, other dose levels and extended test durations, other biochemical parameters, isolation and structure determination of the cardioprotective principle(s) and a detailed explanation of their mechanism of action in order to conclusively establish *Tribulus terrestris* as a potential cardioprotective drug.

However, an extended study using large number of animals is required so that substantial data can be generated for facilitating further evaluation of these agents through clinical trials.

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Conflict of interest: None declared

Ethical Approval: The study was commenced after getting approval from Institutional Animal Ethical Committee (Approval no. IAEC/2021/01 dated 27/8/21) of Lala Lajpat Rai Memorial Medical College, Meerut, India, registered under CPCSEA India (Registration No. 819/Po/Re/S/04/CPCSEA).

REFERENCES

1. Mounika S, Jayaraman R, Jayashree D, Pravalika KH, Balaji A, Sadiya M, Prathyusha M. A comprehensive review of medicinal plants for cardioprotective potential. Int. J. Adv. Pharm. Biotech. 2021 7(1) 24-29

2. Fuster V, "Global burden of cardiovascular disease: time to implement feasible strategies and to monitor results," Journal of the American College of Cardiology, vol. 65, no. 5, pp. 520–522, 2014.

ISSN:0975-3583,0976-2833 VOL14,ISSUE05,2023

3. Xu MF, Tang PL, Qian ZM, Ashraf M. Effects by doxorubicin on the myocardium are mediated by oxygen free radicals. Life Sci.2001;68:889-901

4. Liu Y, Zhou L, Du B, Liu Y, Xing J, Guo S, et al. Protection against Doxorubicin-Related Cardiotoxicity by Jaceosidin Involves the Sirt1 Signaling Pathway. Hindawi, Oxidative Medicine and Cellular Longevity Volume 2021, Article ID 9984330, 18 pages

5. Liu X, Wu WY, Jiang BH, Yang M, and Guo DA, "Pharmacological tools for the development of traditional Chinese medicine," Trends in Pharmacological Sciences, vol. 34, no. 11, pp. 620–628, 2013

6. Tachjian A, Maria V, and Jahangir A, "Use of herbal products and potential interactions in patients with cardiovascular diseases," Journal of the American College of Cardiology, vol. 55, no. 6, pp. 515–525, 2010.

7. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 13th edn. Pune: Nirali Prakashan Publisher; 2007. p. 370.

8. Shahid MRM, Talpur MM, Pirzada T, Phytopharmacology of Tribulus terrestris, J. Biol. Regul. Homeost. Agents 30 (2016) 785–788.

9. Chhatre S, Nesari T, Somani G, Kanchan D, Sathaye S, Phytopharmacological overview of Tribulus terrestris, Pharmacogn. Rev. 8 (15) (2014) 45–51.

10. Joshi DD, Uniyal R, Different chemo types of Gokhru (Tribulus terrestris): a herb used for improving physique and physical performance, Int. J. Green Pharm. 2 (2008) 158–161.

11. Reshma P.L.a, Binu P.b, Anupama Naira, Vineetha R.C.b , Abhilash S.b , Nair R. Harikumaranb, Raghu K.G.: Pretreatment of Tribulus terrestris L. causes anti-ischemic cardioprotection through MAPK mediated anti-apoptotic pathway in rat: Biomedicine & Pharmacotherapy. 111. 2019. 1342-1352.

12. Najafi, Houshang, Firouzifar MR, Shafaat O, Astiyan SC and Hosseini N. "Protective Effects of Tribulus terrestris LN Extract Against acute kidney injury induced by reperfusion injury in rats." IraniaN Journal of kidney diseases (2015); 292-298

13. Talib VH, editor. A handbook of medical laboratory technology. 2nd ed. New Delhi: CBS Publisher & Distributors; 2007.p.155-167.

14. Clayden EC, editor. Practical Section Cutting and Staining- Part 1, London: Churchill Livingstone. 197; p.1071

15. Ghosh MN, editor. Fundamentals of experimental pharmacology.6th ed. Kolkata: Hilton and Company;2015. p.243

16. Zhang S, Li H, Yang SJ; Tribulosin protects rat hearts from ischemia/ reperfusion injury; Acta Pharmacologica Sinica (2010) 31: 671–678

17. Salouege I, Ali RB, Saïd DB, Elkadri N, Kourda N, Lakhal M, Klouz A; Means of evaluation and protection from doxorubicin-induced cardiotoxicity and hepatotoxicity in rats; Journal of Cancer Research and Therapeutics; Volume 10 - Issue 2, 2021, IP: 247.171.0.58

18. Sandamali JAN, Hewawasam RP, Kamani Ayoma Perera Wijewardana Jayatilaka KAPW, Mudduwa LKB; Cinnamomum zeylanicum Blume (Ceylon cinnamon) bark extract attenuates doxorubicin induced cardiotoxicity in Wistar rats; Saudi Pharmaceutical Journal 29 (2021) 820–832

19. Puri A, Maulik SK, Ray R, Bhatnagar V. Electrocardiographic and biochemical evidence for the cardioprotective effect of vitamin E in doxorubicin-induced acute cadiotoxicity in rats. Eur JnPediatr Surg.2005;15:387-391.

20. Haed S, Barshack I, Luboshits G, Wexler D, Deutsch V, Keren G, George J. Erythropoietin improves myocardial performance in doxorubicin-induced Cardiomyopathy. Eur. Heart J.2006;27:1876-1883.

ISSN:0975-3583,0976-2833 VOL14,ISSUE05,2023

21. Bagchi Akinmoladun AC, Ibukun EO, Afor E, Akinrinlola, BL, Onibon TR, Akinboboye AO, Obuotor EM, Farombi EO. Chemical constituents and antioxidant activity of Alstonia boonei. Afr. J. Biotechnol. 2007;6(10):1197-1201.

22. Mimnaugh, E. G., Z. H. Siddik, R. Drew, B. I. Sikic & T. E. Gram: The effects of alpha-tocopherol on the toxicity, disposition, and metabolism of adriamycin in mice. Toxicol. Appl. Pharmacol. 1979, 49, 119–126

23. Shahid MRM, Talpur MM, Pirzada T, Phytopharmacology of Tribulus terrestris, J. Biol. Regul. Homeost. Agents 30 (2016) 785–788.

24. Reshma PL, Lekshmi VS, Sankar V, Raghu KG, Tribulus terrestris (Linn.) attenuates cellular alterations induced by ischemia in H9c2 cells via antioxidant potential, Phytother. Res. 29 (6) (2015) 933–943.

25. Reshma PL, Sainu NS, Mathew AK, Raghu KG, Mitochondrial dysfunction in H9c2 cells during ischemia and amelioration with Tribulus terrestris L, Life Sci. 152 (2016) 220–230.

26. El-Agamy, D.S., Abo-Haded, H.M., Elkablawy, M.A., 2016. Cardioprotective effects of sitagliptin against doxorubicin-induced cardiotoxicity in rats. Exp. Biol. Med. (Maywood, N.J.). 241, 1577–1587.

27. Zhang, Y.Y., Yi, M., Huang, Y.P., 2017. Oxymatrine ameliorates doxorubicin-induced cardiotoxicity in rats. Cell. Physiol. Biochem. 43, 626–635.

28. Ravichandra V, Hanumantharayappa B, Madhava Reddy Papasani Evaluation of cardioprotective activity of galangin against doxorubicin induced Cardiomyopathy. Int. Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(9):86-90.