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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2021; 10(3): 235-242

www.thepharmajournal.com
Received: 21-01-2021
Accepted: 24-02-2021

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Cardio protective actions of *Tinospora cordifolia* and vitamin C against experimental toxicity due to Cisplatin

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Abstract
The present study aimed to evaluate the protective effects of aqueous leaf extract of *Tinospora cordifolia* and vitamin C against cisplatin induced cardiotoxicity. A total of 36 Male *Wistar* rats were divided into six groups (n = 6). Group 1(Normal Control) was administered Normal saline. Group 2 was administered single dose of cisplatin @ 7.5 mg/kg body weight intraperitoneally on day 1. Groups 3 and 4 were administered for 14 days (p/o) with aqueous leaf extract of *Tinospora cordifolia* @ 400 mg/kg body weight and vitamin C @100 mg/kg bodyweight, respectively from day 1. Group 5 and 6 received aqueous leaf extract of *Tinospora cordifolia* @ 400 mg/kg body weight and vitamin C @ 100 mg/kg body weight, respectively for 14 days (p/o) along with cisplatin @ 7.5 mg/kg body weight intraperitoneally on day 1. After 14 days of experimental procedure, blood was collected and serum was separated for estimation of cardiac biomarkers; rats were euthanized and heart tissue samples were collected for histopathological examination. The biochemical assays showed a significant ($P < 0.05$) increase in serum LDH, CPK and troponins in group 2 when compared with group 1, and there was significant ($P < 0.05$) improvement in the treatment groups 5 and 6. Histopathological studies revealed degenerative changes, loss of architecture and necrosis in heart tissue of group 2 treated with cisplatin alone. These changes were reversed in groups 5 and 6. In conclusion, *Tinospora cordifolia* and vitamin C were found to possess protective action against cisplatin induced cardiotoxicity.

Keywords: Cardiotoxicity, Cisplatin, Heart, *Tinospora cordifolia* and Vitamin C

Introduction
Despite many advancements in drug development and molecular targeted therapy, traditional chemotherapy continues to be the major treatment option. Cisplatin is one of the most important anticancer drugs known for its definitive curative effect [1]. Cisplatin is an important therapeutic tool to combat different tumor types including solid tumors, hematological malignancies, bladder, neck, head, gastric, testicular, esophageal, ovarian and pulmonary cancers, lymphomas and osteosarcoma [2].
Cisplatin acts by its interference with purine bases on DNA resulting in the formation of DNA adducts, thus preventing DNA repair, causing DNA damage and subsequently induces apoptosis within the cancer cells [3]. Cisplatin therapy is associated with adverse effects such as nephrotoxicity, hepatotoxicity, gastrointestinal toxicity, cardiotoxicity, neurotoxicity etc. [4]. Cardiotoxicity is one of the most serious dose-limiting toxicity of cisplatin chemotherapy [5]. Oxidative stress has been recognized as a major factor of cisplatin induced cardiotoxicity [6]. In the past few decades there have been several reports of cisplatin associated cardiotoxicity manifested as both acute reactions and delayed effects [7]. Cisplatin-treated cancer survivors recorded increased risk of cardiovascular events, which impair their quality of life [8]. Thus, Cisplatin-induced cardiovascular toxicity has become an important concern [9].
The clinical usefulness of cisplatin is limited because of its cardiotoxic side effects and alleviating such toxic effect by creating new agents remains a major goal [6]. *Tinospora cordifolia* regarded as treasure from nature as it is salutary in many ways to human being [10]. *Tinospora cordifolia* commonly known as Guduchi belong to the family of Menispermaceae [11]. It is considered as an essential herbal plant of Indian system of medicine and has been used in the treatment of fever, dysentery, urinary problems, skin diseases, leprosy, diabetes and many more diseases [12]. *T.cordifolia* medical applications include usages as anti-oxidant, cardiovascular protective, anti-inflammatory etc., [13]. The pharmacological activities could be

attributed to the flavonoids, tannins, polyphenols, glucosides, catechins, epigallocatechins etc., reported to have free radical scavenging potential [14].

Vitamin C is an active reducing agent involved in various biological effects and plays a crucial role in the metabolism and detoxification of many endogenous and exogenous compounds [15]. Antioxidants such as vitamin E, vitamin C, carotenoids and flavonoids have been reported to show protective effects in cisplatin induced toxicity [16]. Several studies have demonstrated the antioxidant and protective effect of the vitamin C on the DNA damage induced by environmental xenobiotic, chemotherapeutic agents and ionizing radiations [17]. Thus, in light of the above facts, the objective of the current study is to evaluate whether administration of *Tinospora cordifolia* and vitamin C could protect against cisplatin-induced cardiotoxicity.

Material and methods

All chemicals were of analytical grade and they are obtained from Qualigens Pvt. Ltd., Mumbai and SRL Pvt. Ltd., Mumbai, India.

Plant material

Tinospora cordifolia plant leaves were collected from Hyderabad, India. The plant species were authenticated by Scientist, Agricultural College, Hyderabad, India.

Preparation of plant extract

The aqueous extract prepared according to the procedure adopted by Madhavi *et al* [18]. Collected leaf material washed under running tap water to remove microbes and dust and then air dried under shade at room temperature for 15 days. The leaf material was crushed well into fine powder, packed into airtight polythene bags for further use and stored at room temperature. The aqueous extract is prepared by soaking 5 gm of dried powder in 100 ml of water and shaken well. The solution left at room temperature for 72 hours and then filtered with the help of Whatman No.1 filter paper. The filtrate was kept at low temperature (4 °C) for further use.

Animals and Experimental Design

This study was conducted using 36 male *Wistar* rats aged about 3 months with an average body weight of 180 ± 10 g were obtained from Vyas labs, Hyderabad, which were divided into six equal groups (n=6) with different treatments. Animals were housed in polypropylene cages, in a clean ventilated room under controlled environmental conditions (20–22ºC) and 12 hour dark and light cycles, sterilized dried clean, autoclaved rice husk used as bedding material and was changed on alternate days. Animals were fed on standard balanced diet and water was provided *ad libitum* throughout the experimental period. Animals were left for 7 days to acclimate to experimental conditions. The experimental protocol was approved by the Institutional Animal Ethical Committee (No.4/22/C.V.Sc.,Hyd.IAEC-Rats/29.02.2020) and were in accordance with the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental design

Group - I	Normal saline orally for 14 days.
Group - II	Cisplatin (7.5 mg/kg body weight, ip) injection on day 1.
Group- III	Aqueous leaf extract of *Tinospora cordifolia* @ 400 mg/kg body weight was orally administered for 14 days starting from the day 1.
Group- IV	Vitamin C @ 100 mg/kg body weight was orally administered for 14 days starting from the day 1.
Group - V	Cisplatin (7.5 mg/kg, ip) injection on day 1 + Aqueous leaf extract of *Tinospora cordifolia* @ 400 mg/kg body weight was orally administered for 14 days starting from the day 1.
Group- VI	Cisplatin (7.5 mg/kg, ip) injection on day 1 + Vitamin C @ 100 mg/kg body weight was orally administered for 14 days starting from the day 1.

Blood collection

Blood collection was carried out at the end of the experiment for sero-biochemical analysis. Feed withdrawn 12 h before the blood collection and blood collected from retro-orbital plexus into serum vacutainers and centrifuged at 3000 RPM for 15 min and serum was separated and stored at -80^{0}C till analysis. The serum samples were analyzed for the activity of LDH and CPK, and concentration of serum troponins.

Biochemical analysis

Marker enzyme assays

The marker enzymes, LDH and CPK were assayed in the serum by using the standard kits supplied from ERBA diagnostics Ltd., Surat, India.

Troponins estimated as serum salt soluble proteins by Lowry's method of protein estimation [19].

Histopathological studies

Tissue pieces of heart were collected from the rats that were sacrificed at the end and fixed in 10% neutral buffered formalin (NBF) for histopathology. The small representative pieces of fixed tissues were cut and subjected to overnight washing under running tap water. The tissues were then dehydrated in ascending grades of alcohol, cleared in xylol and embedded in paraffin at 55-56 °C. The paraffin blocks were cut into thin sections of 5-micron thickness by microtome. The cut sections were lifted on grease free glass slides which were precoated with Mayer's egg albumin and kept in incubator overnight at 37 °C for drying. The slides were stained with routine Haematoxylin and Eosin (H & E) stain [20] [21] and the stained sections were mounted with DPX mountant and kept ready for microscopic examination.

Statistical Analysis

All the values were expressed as mean ± SE (n=6). Statistical analysis was performed by using SPSS version 21 software using one-way analysis of variance (ANOVA) followed by Duncan's test as post hoc analysis. The value of $P<0.05$ was considered to be statistically significant.

Results

Serum biochemical parameters

The activity of serum LDH (U/L) in cisplatin treated group (group 2) was significantly ($P<0.05$) increased when compared to Normal control (group 1) on 14th day. Treatment groups administered *Tinospora cordifolia* (group 5) and vitamin C (group 6) revealed a significant ($P<0.05$) improvement in the LDH levels in comparison to cisplatin treated group (group 2) (Table 1 and Fig. 1).

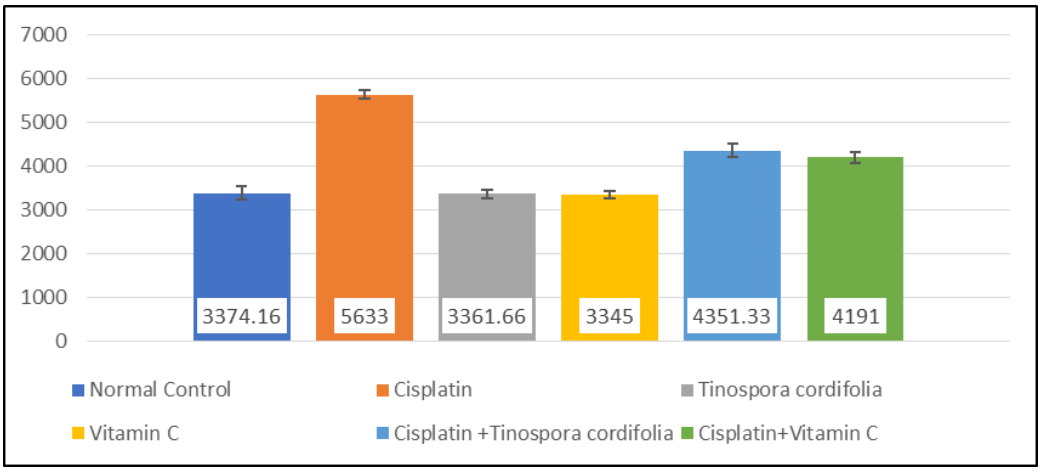

Fig 1: Serum LDH activity (U/L) in different groups of rats

The activity of serum CPK (U/L) in group 2 was significantly ($p<0.05$) higher in cisplatin treated group compared to Normal control group (group 1) on 14th day. Administration of *Tinospora cordifolia* (group 5) and vit C (group 6) showed significantly ($p<0.05$) lower values when compared to group 2 (Table 1 and Fig. 2).

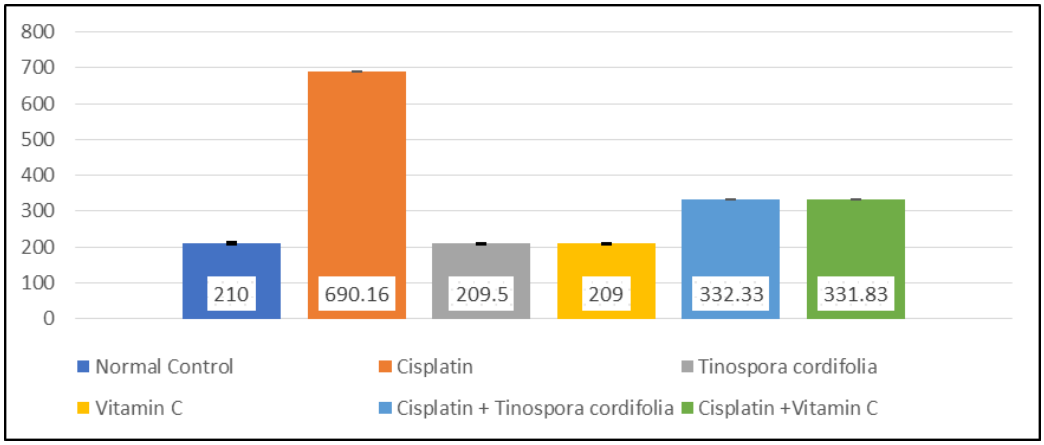

Fig 2: Serum CPK activity (U/L) in different groups of rats

The concentration of serum Troponin (ng/ml) in cisplatin treated group (group 2) was significantly ($p<0.05$) higher compared to group 1 on 14th day, whereas treatment groups administered *Tinospora cordifolia* (group 5) and vitamin C (group 6) exhibited significantly ($p<0.05$) lower values in comparison to group 2 (Table 1 and Fig. 3).

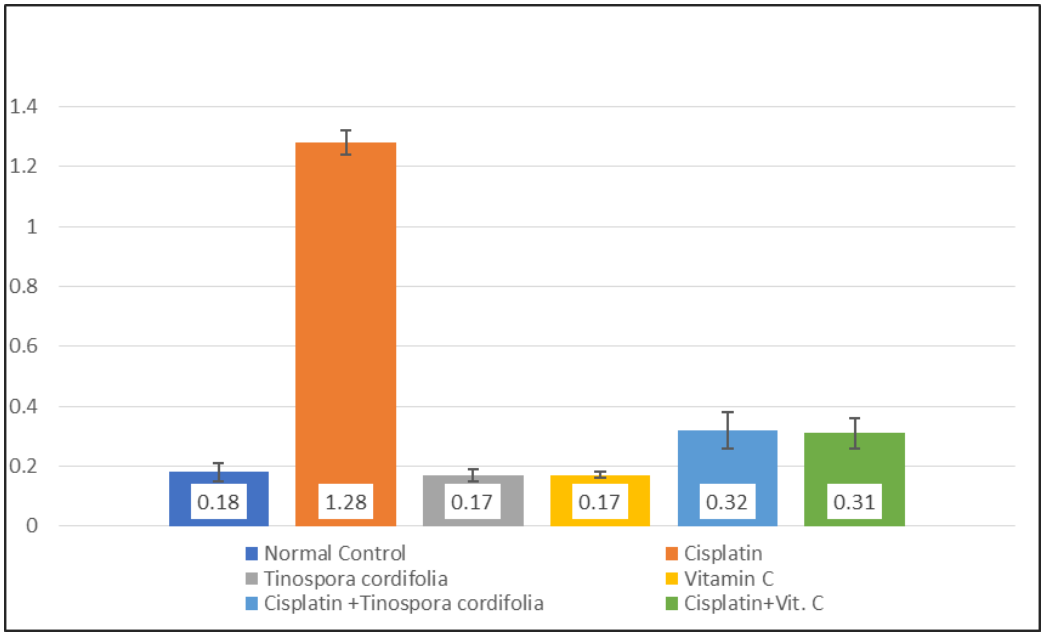

Fig 3: Serum troponins as salt soluble proteins (ng/ml) in different groups

Table 1: LDH (U/L), CPK (U/L) and Serum troponins (ng/ml) of different groups of rats

Group	LDH	CPK	Serum troponins
Normal Control	3374.16 ± 156.28 [c]	210.00 ± 2.12 [c]	0.18 ± 0.03 [c]
Cisplatin	5633.00 ± 108.66 [a]	690.16 ± 0.30 [a]	1.28 ± 0.04 [a]
Tinospora cordifolia	3361.66 ± 103.96 [c]	209.50 ± 1.38 [c]	0.17±0.02 [c]
Vitamin C	3345.00 ± 85.06 [c]	209.00 ± 0.93 [c]	0.17 ± 0.01 [c]
Cisplatin + *Tinospora cordifolia*	4351.33 ± 150.57 [b]	332.33± 0.84 [b]	0.32 ± 0.06 [b]
Cisplatin + vitamin C	4191.00 ± 125.45 [b]	331.83 ± 0.47 [b]	0.31 ± 0.05 [b]

Values are Mean + SE (n =6) One way ANOVA with Duncan's post hoc test (SPSS). Means with different alphabets as superscripts differ significantly (P < 0.05) among the groups

Histopathology

Light microscopic examination of the heart tissue of rats in normal control group 1 showed regularly arranged cardiac myofibers with several cardiomyocytes consisting of centrally located nuclei without significant changes (Fig. 4). In the groups 3 (Fig 13), 4 (Fig 14), heart tissue histomorphology revealed normal architecture of the cardiac muscle fibers. However, the cardiac histological architecture in tissues of rats treated with cisplatin alone (group 2) revealed severe myocardial degenerative changes (Fig. 5) characterized by disarrayed cardiac muscle fibers (Fig. 6) associated with deeply stained pyknotic nuclei (Fig.7), congestion of blood vessels, interstitial edema (Fig. 8), vacuolar degeneration (Fig. 9), hemorrhage (Fig. 10), infiltration of mononuclear cells (Fig. 11) and moderate myocardial fiber necrosis (Fig. 12). In rats treated with *Tinospora cordifolia* justified the protective action, revealing mild congestion and mild myocardial degenerative changes (Fig. 15), mild hemorrhages and mild disruption of cardiac muscle fibers (Fig. 16) compared to cisplatin treated group 2. Vitamin C treated group revealed very mild cardiac tissue injury and myocardial level of organization was clear (Fig. 17) compared to cisplatin treated group 2.

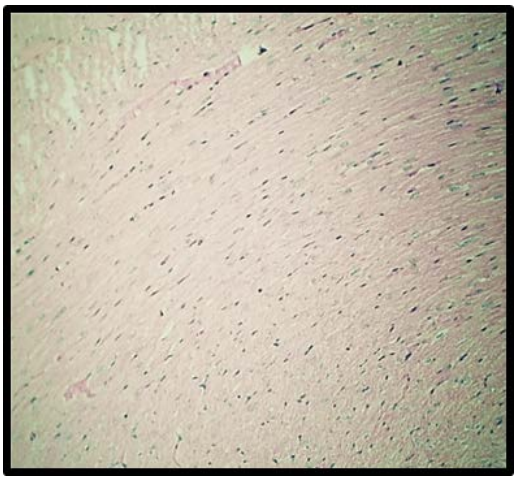

Fig 4: Photomicrograph of heart tissue showing normal appearance of myocardium with regularly arranged cardiomyofibres with cardiomyocytes consisting of single central nuclei (Group 1)

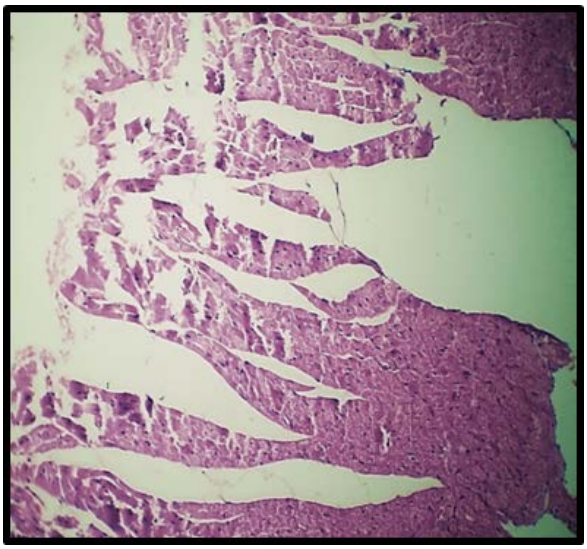

Fig 5: Photomicrograph of heart tissue showing marked disruption of cardiac muscle fibres associated with deeply stained nuclei (Group 2) H&E X100

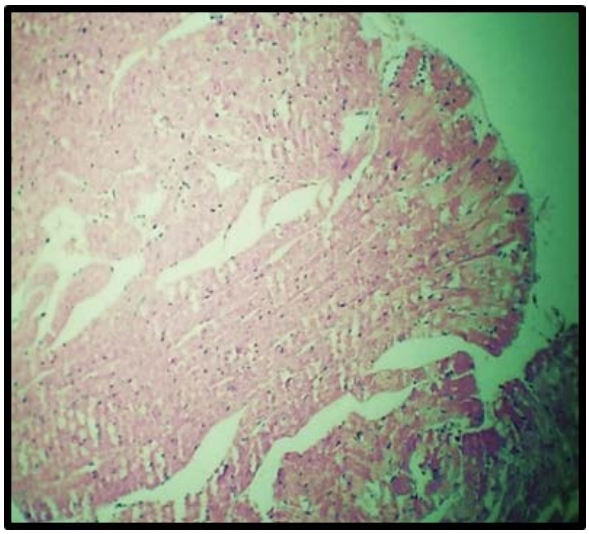

Fig 6: Photomicrograph of heart tissue showing moderate to marked myocardial degenerative changes with disarrayed cardiac muscle fibers (Group 2) H&E X100

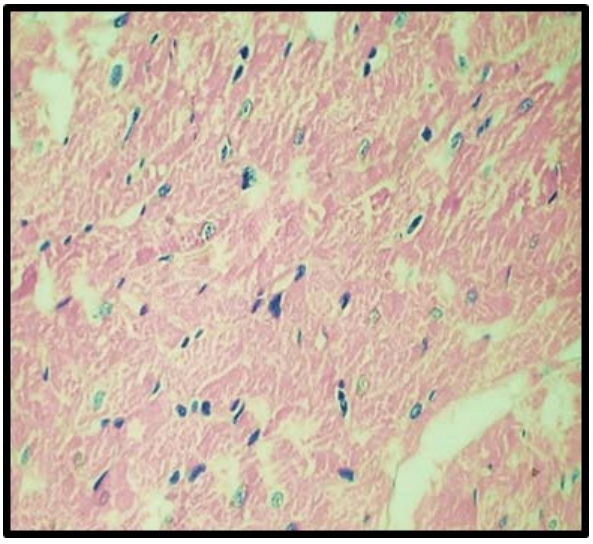

Fig 7: Photomicrograph of heart tissue showing degenerated cardiac muscle fibers associated with pyknotic (arrow) and condensed nuclei (Group 2) H&E X400

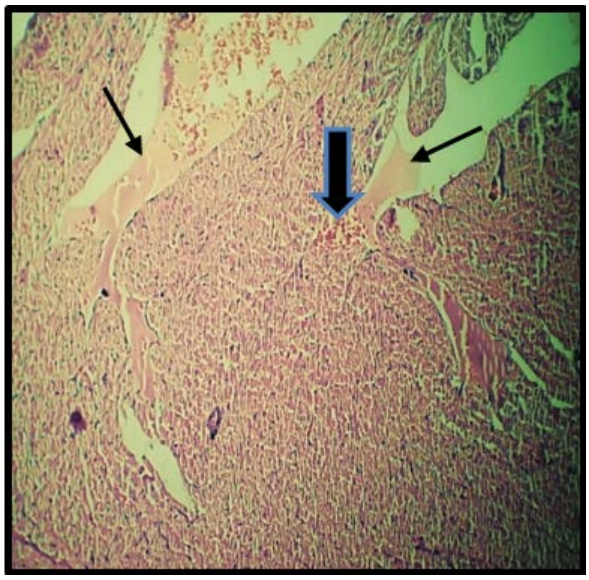

Fig 8: Photomicrograph of heart tissue showing moderate interfibrillar edema (thin arrow) and hemorrhages (thick arrow) (Group 2) H&E X100

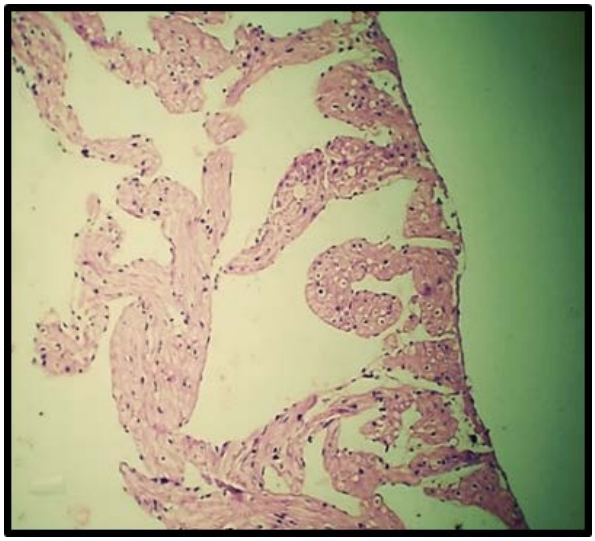

Fig 9: Photomicrograph of heart tissue showing disarrayed cardiac muscle fibers with vacuole (arrow) (Group 2) H&E X 100

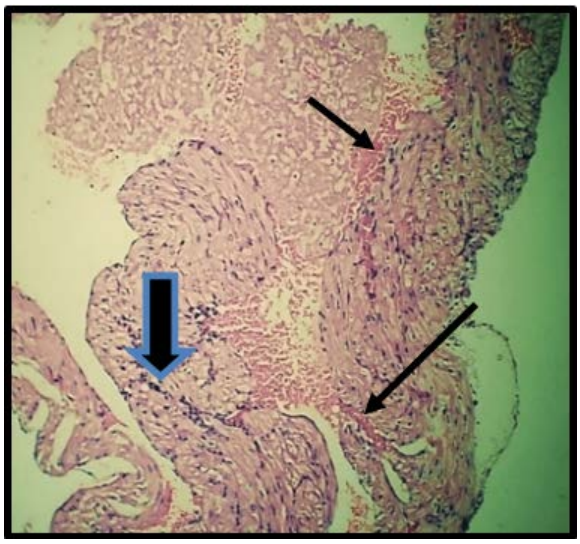

Fig 10: Photomicrograph of heart tissue showing marked hemorrhages (thin arrow) and marked round cell infiltration (thick arrow) (Group 2) H&E X100

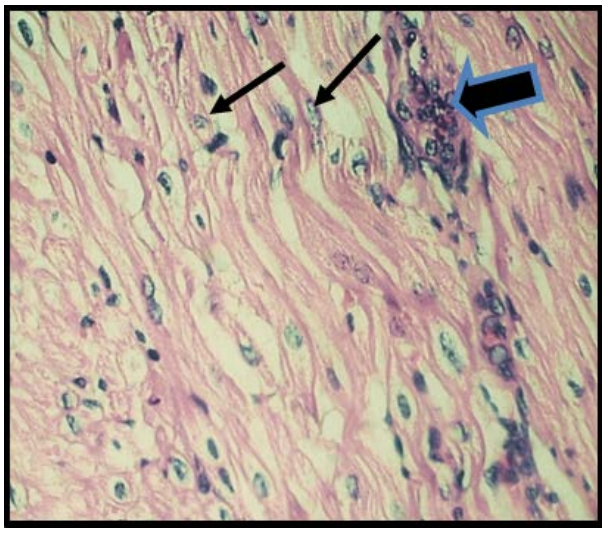

Fig 11: Photomicrograph of heart tissue showing moderate degenerative changes in cardiomyocytes nucleus (thin arrow) and infiltration of mononuclear cells (thick arrow) (Group 2) H&E X400

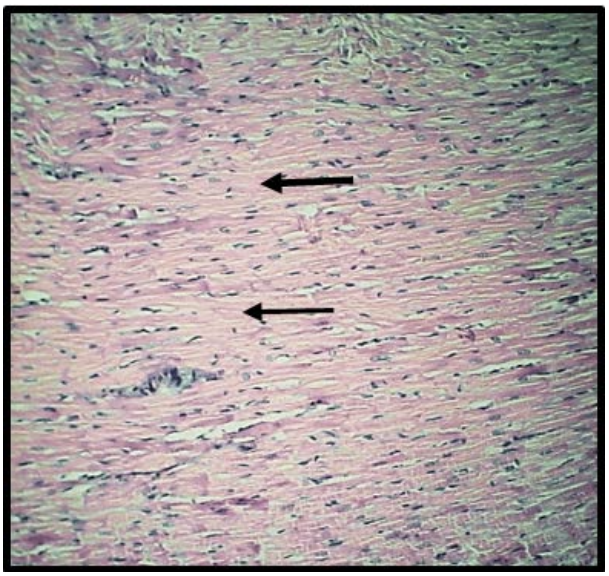

Fig 12: Photomicrograph of heart tissue showing moderate myocardial fiber necrosis (arrow) (Group 2) H&E X100

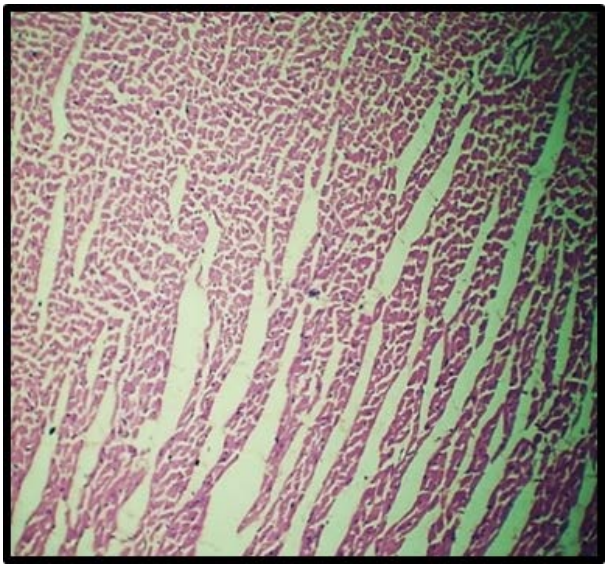

Fig 13: Photomicrograph of heart tissue showing normal morphology of regularly arranged cardiac muscle fibers (Group3) H&E X100

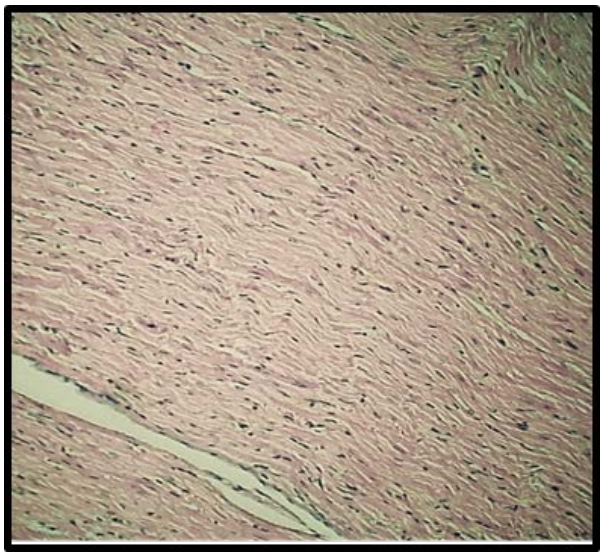

Fig 14: Photomicrograph of heart tissue showing normal appearance of the cardiac muscle fibers (Group 4) H&E X100

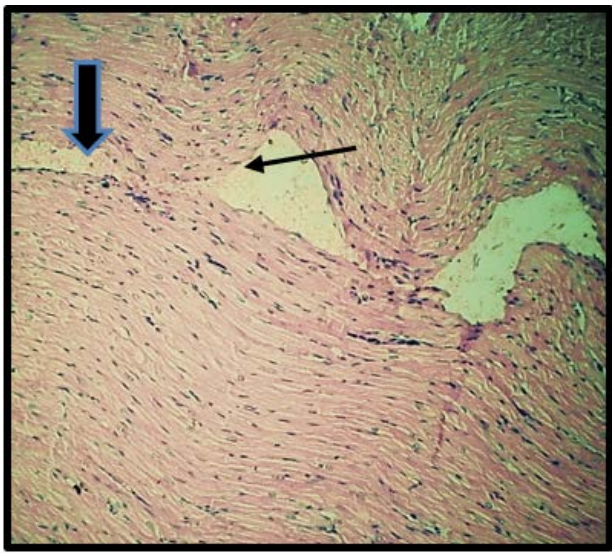

Fig 15: Photomicrograph of heart tissue showing mild congestion (thick arrow) and mild disruption in cardiac muscle fibers (thin arrow) (Group 5) H&E X100

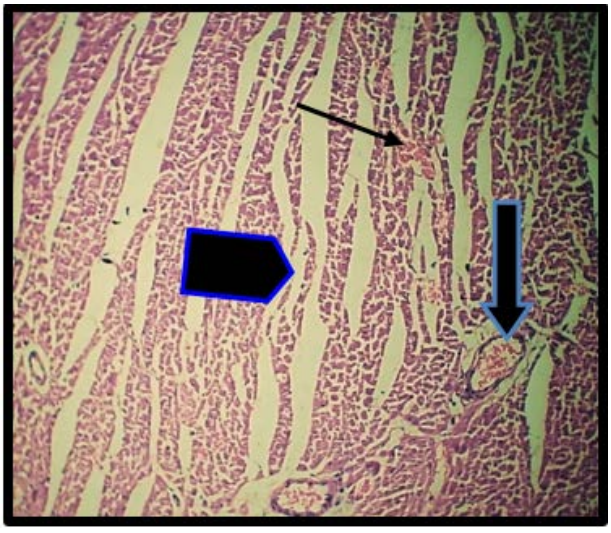

Fig 16: Photomicrograph of heart tissue showing mild irregularity of cardiac muscle fibers with mild congestion (thick arrow), mild hemorrhages (thin arrow) and mild cardiac muscle fiber disruption (arrowhead) (Group 5) H&E X100

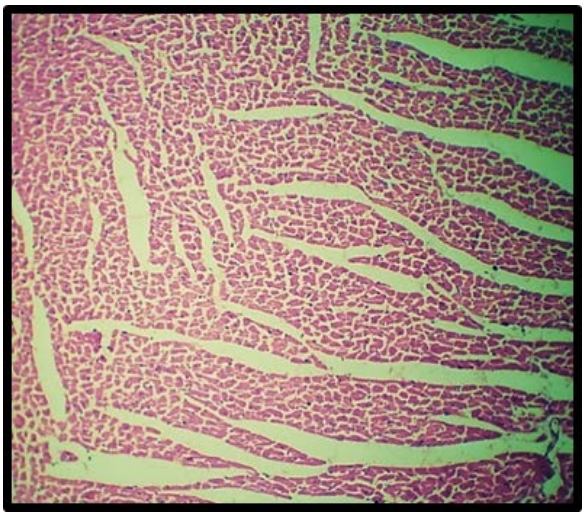

Fig 17: Photomicrograph of heart tissue showing normal myocardial bundle arrangement (Group6) H&E X100

Discussion

Cisplatin causes the disruption of cell membranes, resulting in the release of intracellular proteins such as LDH, CK – MB and cardiac troponins, which are assayed to detect the presence and extent of myocardial injury. Elevated troponins in chemotherapy induced cardiotoxicity reflect myocardial necrosis [22]. Cardiac troponin I is specific and sensitive marker of myocardial damage [23]. Cisplatin is a potential source of production of ROS, such as superoxide anion and hydroxyl radical [24]. ROS overload causes disruption of antioxidant defenses [25]. Cisplatin-induced lipid peroxidation results in elevated levels of serum LDH, troponin-Tn I and CK levels, thus oxidative stress plays an important role in the mediation of cardiac toxicity [26]. Afsar *et al*. [27] reported that the heart injury causing irreversible alterations of cardiac cell membrane structure and function as a result of increased lipid peroxidation, which causes leakage of cardiac enzymes, which increase the CK and cTnI content of serum.

In the present study, the activities of serum LDH and CPK, and the concentration of serum troponins were significantly elevated in the toxic control group 2 when compared to the non-toxic control groups 1, 3 and 4. Cardiotoxicity associated with cisplatin is secondary event following increased lipid peroxidation of cardiac membranes [28]. Myocardial enzymes catalyze the metabolism and regulate the electrical activity of myocardial cells. During acute myocardial infarction, there is a sharp decrease in coronary blood supply and as a result, many cardiac enzymes are released and accumulated in the blood. Myocardial enzymes are often used clinically as relative testing indices in hypoxic-ischemic myocardial damage [2].

Co-administration of cisplatin with *Tinospora cordifolia* and vitamin C (group 5 and 6, respectively) significantly reversed the changes recorded in serum LDH, CPK and troponins when compared to the toxic control group 2. A significant protection in cardiac biomarker levels reflects the Cardioprotective action of *Tinospora cordifolia*, thus maintaining the membrane integrity of myocytes. Cardioprotective activity of *T. cordifolia* is related to its ability to strengthen the myocardial memebrane by its membrane stabilizing action [11]. Cardioprotective action of *T. cordifolia* against ischemia – reperfusion induced myocardial injury is attributed to its free radical scavenging activity [29]. Marked recovery was observed in the markers post

combination treatment by cisplatin with *T. cordifolia* and vitamin C in this study.

These results are further substantiated from histopathology, which revealed normal arrangement of cardiac muscle fibers showing mild congestion and mild inflammatory changes with mild cardiac tissue injury. Normal histology of heart was found in the normal control group 1 with regularly arranged cardiac myofibers, several cardiomyocytes consisting of centrally located nuclei and no abnormal changes in myocardial interstitium. Cisplatin control group revealed severe myocardial degenerative changes characterized by disarrayed cardiac muscle fibers associated with deeply stained pyknotic nuclei. These changes in cardiac muscle fibers may be attributed to reduction in aerobic respiration and reduced ATP production in the damaged cells. Nuclear changes are due to the DNA damage caused by oxidative stress and these nuclear changes are responsible for cellular dysfunction and death [5].

In the cisplatin control group, there was congestion of blood capillaries with cellular infiltration in the intercellular spaces between the cardiac myocytes. These changes may be due to the endothelial injury of blood vessels after cisplatin administration, which may be due to free radical-induced lipid peroxidation [30]. Additionally, interstitial edema, vacuole, haemorrhages, infiltration of mononuclear cells and moderate myocardial fiber necrosis are seen [2, 26]. *T. cordifolia* and vitamin C administration for 14 days significantly improved the cisplatin induced histopathological changes and showed mild myocardial degenerative changes, less inflammatory cell infiltration and mild cardiac tissue injury.

Conclusion

In conclusion, the study revealed that cisplatin induces cardiotoxicity by oxidative stress, thus impairing the serobiochemical parameters and histoarchitecture of heart. Administration of *Tinospora cordifolia* and vitamin C could effectively reverse the pathological changes owing to their antioxidant and cardioprotective actions.

Acknowledgment

The authors are thankful to college of veterinary science, PVNRTVU, Rajendranagar, Hyderabad for providing necessary facilities for research work.

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