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# A Study on Oxidative Stress Induced Cardiotoxicity by Cisplatin and Protective Evaluation by *Tinospora cordifolia* and vitamin C

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**A B S T R A C T**

The ameliorative potential of *Tinospora cordifolia* (TC) and vitamin C (Vit. C) was studied against oxidative stress induced cardiotoxicity by cisplatin. 36 male *Wistar* rats were divided into 6 groups(n = 6). Group 1 was maintained as Normal control. Cisplatin (7.5 mg/kg B.W) was administered intraperitoneally to the groups 2, 5 and 6 on day 1. Groups 3 and 4 were administered for 14 days (p/o) with aqueous leaf extract of TC (400 mg/kg B.W) and vitamin C (100 mg/kg B.W), respectively from day 1. Group 5 and 6 received aqueous leaf extract of TC (400 mg/kg B.W) and vitamin C(100 mg/kg B.W), respectively for 14 days orally starting from the 1st day of cisplatin injection. Body weights, antioxidant parameters and cytokine profile were assessed. The present study revealed a significant ($P < 0.05$) increase in TBARS, protein carbonyls, TNF-*alpha* and IL-10, while significant ($P < 0.05$) decrease in GSH in group 2 compared to group 1. The groups 5 and 6 showed significant improvement in all the parameters in comparison to group 2.In conclusion beneficial effects of TC and vitamin C could be attributed to their antioxidant and immunomodulatory actions.

## Introduction

Cancer is leading cause of death worldwide (Oh *et al.*, 2020), deaths due to cancer constitute 2–3% of the annual deaths recorded worldwide and kill about 3500 million people annually all over the world (Polu *et al.*, 2017). Chemotherapy and Radiotherapy are common means of cancer treatment (Yanez *et al.*, 2003).

Cisplatin referred as Pencillin of cancer drugs and prescribed widely for first and effective treatment of cancer. Cisplatin used as gold standard by researchers against which new medicines are compared (Stephen, 2005). Cisplatin is one of the remarkable successes in 'the war on cancer'. It has activity against a broad spectrum of tumors, including testicular, bladder, cervical, ovarian, and lung cancers and solid tumors resistant to other treatment regimens (El-Awady *et al.,* 2011).The anticancer mechanism of cisplatin includes the formation of highly reactive platinum complexes, which bind with nucleophilic DNA via intrastrand and interstrand crosslinking with guanine nucleotides (Hanigan and Devarajan, 2003), thus preventing DNA repair causing DNA damage and subsequent apoptosis within cancer cells (Aldossary, 2019). Life is harsh for cancer survivors (Katta *et al.,* 2018). Despite definitive curative effect of cisplatin, major side effects of the cisplatin treatment include nephrotoxicity, cardiotoxicity, neurotoxicity, ototoxicity etc., thus limiting its clinical use. Several studies revealed cisplatin associated cardiotoxicity (Chowdhury *et al.,* 2016). Cardiotoxicity is one of the most feared adverse effect of cisplatin chemotherapy (Al-Shimaa, 2017). Mechanism of toxicity due to cisplatin is related to its ability to generate ROS, such as superoxide anion and hydroxyl radical with the reduction of various antioxidants (Hussein *et al.,* 2012).Oxidative stress plays a key role in cisplatin induced cardiotoxicity characterized by ROS production, lipid peroxidation and inhibition of antioxidant enzymes such as GSH, SOD and CAT. Excess of ROS target mitochondria resulting in mitochondrial dysfunction and subsequently induce caspase-dependent apoptosis (Zhao, 2019).

Plants have been one of the outstanding sources of medicines since the beginning of human civilization. There is flourishing demand for plant-based food supplements, pharmaceutical and health products (Joshi and Kaur, 2016). *Tinospora cordifolia*, commonly known as Amrita and Guduchi belongs to the family of Menispermaceae, is a succulent, glabrous woody climbing shrub native to India and thrives well in the tropical region. It is regarded as an essential herbal plant of Indian system of medicine and has been used in the treatment of fever, dysentery, diabetes, urinary problems, skin diseases, leprosy and many more diseases (Sharma *et al.,* 2019).Different classes of compounds like alkaloids, diterpenoid lactones, sesquiterpenoids, glycosides, phenolics, steroids, aliphatic compounds and polysaccharides are identified as bioactive components of *Tinospora cordifolia* (Bonvicini *et al.,* 2014).These compounds are found in various parts of the plant but highly concentrated in the leaves, stem and roots of the plant (Kumar *et al.,* 2017). *T.cordifolia*has anti-inflammatory, anti-spasmodic, anti-oxidant, anti-allergic, anti-malarial, immunomodulatory. (Saha and Ghosh, 2012).The pharmacological activities could be attributed to the tannins, glucosides, polyphenols, catechins, epigallactocatechins etc., reported to have free radical scavenging activity(Singh *et al.,* 2003). Most of the phytochemical components were found in aqueous extract due to high solubility of active compounds of *Tinospora cordifolia* leaf in this solvent (Madhavi *et al.,* 2017).

Natural antioxidants protect the cells from oxidative injury (Ibrahim *et al.,* 2019). Vitamin C is an important water-soluble vitamin known for antioxidant, immuno-modulatory and anticarcinogenic actions (Tomofuji *et al.,* 2009). Ghavamiand and Sardari, (2020) documented that vitamin C was efficient in reducing the systemic toxicity of cisplatin in mice model. Ray and Roy (2006) stated that vitamin C increased the therapeutic index of the drug by reducing cisplatin-induced lipid-peroxidation mediated

through free radical mechanisms. Antioxidants such as vitamin C, vitamin E, flavonoids and carotenoids have been reported to show protective effects in cisplatin induced toxicity (Koyuncu *et al.,* 2017). In the light of these observations, an experimental study was framed to evaluate the protective effect of *Tinospora cordifolia* and vitamin C against oxidative stress induced cardiotoxicity by cisplatin.

## Materials and Methods

### Chemicals

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

### Plant material and Preparation of plant extract

The fresh leaves of *Tinospora cordifolia* plant were collected from Hyderabad, India. The plant species was authenticated by Scientist, Agricultural College, Hyderabad, India. Collected leaf material was washed under running tap water to eradicate dust and microbes and then shade dried at room temperature for 15 days. The leaf material was crushed well into fine powder in an electronic grinder and packed into airtight polythene bags for further use and stored at room temperature. Then, 5 gm of dried powder is soaked in 100 ml of water and shaken well. The solution was further kept at room temperature for 72 hours and then filtered with the help of Whatman No.1 filter paper (Madhavi *et al.,* 2017*)*. The filtrate was kept at low temperature (4 °C) for further use.

### Animals and Experimental Design

Thirty six male *Wistar* rats aged about 3 months with an average bodyweight of 180 ± 10 g were obtained from Vyas labs, Hyderabad, which were divided into six equal groups (n=6) with different treatments. The animals were housed in poly-propylene cages and maintained with 12 h dark/light cycle at college animal house under hygienic conditions having ambient temperature (20–22$^0$C) for acclimatization (1 week) prior to beginning of experiment. Animals were fed on standard balanced diet and water was supplied *ad libitum* throughout the experimental period. All the experimental procedures and protocols used in this study were reviewed and 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 CPCSEA.

After experimental procedure, blood was collected. Feed was withdrawn 12 h prior to the blood collection and blood was 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 cytokine profile (TNF-*alpha* and IL-10).On the 14$^{th}$ day, after blood collection, rats were euthanized by carbon dioxide exposure and heart tissues were collected, homogenized and stored at -80$^0$C for further estimation of GSH, TBARS and protein carbonyls.

### Body weights

Body weights were recorded on 0$^{th}$, 7$^{th}$ and 14$^{th}$ day.

### Antioxidant profile

### Estimation of glutathione (GSH)

GSH was estimated on the reaction of reduced glutathione (GSH) with 5-5’ dithiobis-2-nitrobenzoic acid (DTNB) to give a compound that absorbs light at 412 nm(Moron *et al.*,1979).100 µl of 25% trichloroacetic acid

was added to 400 µl of homogenate and then centrifuged, supernatant was collected and used. To 2.0 ml of 0.6 mM DTNB in 0.2 M sodium phosphate (pH 8), 0.1ml of sample was added and 0.9 ml of 0.2 M phosphate buffer and the absorbance was read at 412 nm against a reagent blank. The standards (0.05-5 mg/ml) were also treated in the same manner.

## Estimation of Thiobarbituric acid reacting substances (TBARS)

Malondialdehyde (MDA), the product of lipid peroxidation, estimated by reaction with thiobarbituric acid as per the procedure described by Balasubramanian *et al.*,(1988). 1 g of tissue sample with 10ml of 0.2 M Tris HCl buffer (pH 7.2) was taken in a tissue homogenizer to get a 10% homogenate. 500 µl of supernatant from the homogenate, 1 ml of 10% trichloroacetic acid (TCA) and 1ml of 0.67% thiobarbituric acid were taken in a tightly stoppered tube. The tube was further heated to boiling temperature for 45 min. After cooling the tube, the contents were centrifuged (3500 rpm, 10 min). The supernatant absorbance was read against blank at 532nm. The concentration of test samples were obtained using molar extinction coefficient of MDA.

## Estimation of protein carbonyls

Protein carbonyls were estimated based on the reaction of amino carbonyls with 2, 4-dinitrophenyl hydrazine to form hydrazones, which can be detected spectrophotometrically at 372 nm (Levine *et al.*,1990). 1 ml of homogenate supernatant containing 1 mg protein was added to 4 ml of 10 mM DNPH in 2.5 M HCl. Samples were vortexed and incubated at room temperature for 1 h in dark.

Protein was then precipitated by adding 5 ml of 20 % trichloroacetic acid and centrifuged at 3000 rpm for 10 min, the supernatant was discarded to collect protein precipitate, which was washed thrice with 4 ml of ethanol: ethyl acetate (1:1) solution. The final protein precipitate was re-dissolved in 2 ml of 6 M guanidine HCl in 20 mM of potassium phosphate and kept at 37 0C for 10 min and was further centrifuged to remove the insoluble substances and the absorbance was read at 372 nm against 2.5 M HCl blank.

A known concentration of bovine serum albumin dissolved in 6 M guanidine HCl in 20 mM of potassium phosphate was used as standard.

## Cytokine profile

The assay employs a quantitative sandwich enzyme immunoassay technique to measure TNF – *alpha* and IL-10. The ELISA kit was procured from Krishgen Bio systems, Mumbai.

## Statistical Analysis

Data were subjected to statistical analysis by applying one-way analysis of variance (ANOVA) using the statistical package for social sciences (SPSS; version 21). followed by Duncan's test as post hoc analysis. The value of $P<0.05$ was considered to be statistically significant.

## Results and Discussion

In the present study, antioxidant profile, cytokine profile and were analyzed on $14^{th}$ day. The results obtained in the present experiment are presented as below. The mean body weight (g) in group 2 was significantly ($P<0.05$) decreased on $7^{th}$ and $14^{th}$ day, when compared to group 1, while treatment groups 5 and 6 showed significant ($P<0.05$) improvement in their body weights during the experimental period.(Table 1; Fig. 1).

The concentration of GSH (µ mol/mg protein) in heart revealed a significant (P<0.05) decrease in toxic control group 2 when compared to group 1, whereas treatment groups 5 and 6 revealed a significant (P<0.05) improvement as compared to toxic control group 2 (Table 1; Fig. 2).

The concentration of TBARS (n moles of MDA released/mg protein) (Table 1; Fig. 3) and protein carbonyls (n moles of carbonyl/mg protein) (Table 1; Fig. 4), in heart revealed a significant (P<0.05) increase in toxic control group 2 when compared to group 1, whereas treatment groups 5 and 6 showed a significant (P<0.05) decrease as compared to toxic control group 2.

The concentration of serum TNF-α (pg/ml)(Table 1; Fig. 5) and IL-10 (pg/ml) (Table 1; Fig. 6)revealed a significant (P<0.05) increase in toxic control group 2 when compared to group 1, whereas treatment groups 5 and 6 showed a significant (P<0.05) decrease as compared to toxic control group 2.

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 (Vertically).

Decrease in food intake and weight loss are the most serious and common health problems in patients with cisplatin chemotherapy (Lin *et al.*, 2018).Weight loss observed in the cisplatin treated group may be because of reduced appetite and enhanced catabolic rate seen as obvious side effects of chemotherapy. It could also be due to CP induced dysfunction of the gastrointestinal system (Hassan *et al.*, 2010).Cisplatin shows dose- dependent gastric stasis and stomach distension associated with food retention and responsible for reduced feed intake (Cabezos *et al.*, 2008). Factors such as nausea and vomiting, diarrhoea, constipation, altered taste and depression are responsible for cisplatin chemotherapy associated weight loss(Yoshizawa *et al.*, 2019). In the present study, the body weights of rats in toxic control group 2 were significantly reduced when compared to non-toxic control groups (1, 3 and 4) at respective time intervals i.e., on $7^{th}$ and $14^{th}$ day. The decrease in body weights in cisplatin group is attributed to oxidative stress and ROS generation, which leads to altered metabolism (Kumar *et al.*, 2017). The results are in concurrence with the findings of Zhao *et al.*, (2018) and Afsar *et al.*, (2019), who reported that cisplatin treatment resulted in significant reduction in body weights in mice and rats, respectively. Weight gain observed in the treated animals (groups 5 and 6) may be due to restoration of antioxidant defenses as evident from the findings of this study. Significant increase in the average weekly body weight with *Tinospora cordifolia* against various toxicitieswas also reported by Ambasta *et al.*, (2017) in mice against arsenic induced toxicity and Nageswari *et al.*, (2018) in rats against zidovudine induced immunotoxicity.

In our present study, there is significant decrease in GSH and increase in TBARS and protein carbonyls in heart tissue of rats of the toxic control group 2 when compared to the non-toxic groups 1, 3 and 4.MDA plays a vital role in the toxicity of several xenobiotics (Koyuncu *et al.*, 2017). MDA, the end product of lipid peroxidation, is the most important indicator of the oxidative damage. In oxidative damage, there is an increase in the amount of MDA and decrease in the amount of endogenous antioxidant molecule GSH (Nooriand Mahboob, 2010). Increase in the MDA level attributes to cisplatin-induced generation of oxygen free radicals, which evoke extensive tissue damage, reacting with membrane lipids, proteins and nucleic acids

(Satoh *et al.,* 2003;Conklin and Nicolson, 2008; Topal *et al.,* 2018), resulting in oxidative stress which plays an important role in cisplatin cardiotoxicity (Afsar *et al.,* 2019).GSH, an omnipresent intracellular peptide, has various functions such as maintaining cellular antioxidant and oxidant balance, detoxification, maintenance of thiol and modulation of cell proliferation (Koyuncu *et al.,* 2017).The decline in GSH level in cisplatin-treated rats resulted in an enhanced lipid peroxidation (Karthikeyan *et al.,* 2007). ROS production by cisplatin inhibits GSH by interfering with its disulfide bond, Cardiac GSH depletion during oxidative stress results in accumulation of GSSG and results in impaired cell function. Superoxide anion radicalis responsible for endothelial dysfunctioning in cardiovascular diseases (Chowdhury *et al.,* 2016). Depletion of GSH and related antioxidants by cisplatin shifts the cellular redox status, results in the accumulation of endogenous reactive oxygen species within the cells (Arany and Safirstein, 2003).Wang *et al.,* (2009) confirmed that myocardial damage is responsible for the increased levels of MDA and decreased activities of GSH, SOD, CAT and GSH-Px. Thus, the findings are in concurrence with the studies depicting that oxidative damage plays an important role in cisplatin induced cardiotoxicity.

Cardiac tissue has very low level of antioxidant enzymes such as superoxide dismutase and catalase, thus heart is the most susceptible organ to oxidative stress(Al-Majed *et al.,* 2006). The observed decrease in heart GSH levels, increase in MDA levels and decreased activities of SOD are suggestive of the oxidative stress caused by cisplatin treatment (El-Awady *et al.,* 2011). The results are in concurrence with the findings of Kiran *et al.,* (2015), who reported that up-regulated levels of MDA and down-regulated activity of antioxidant defenses like GSH result in cisplatin-induced cardiotoxicity.

Proteins are damaged by ROS directly and are the targets of secondary modifications by aldehyde products of lipid peroxidation or ascorbate autooxidation, further resulting in carbonyl modification of protein (PCO). The assessment of PCO is a widely used marker for oxidative protein modification and it is sensitive and early marker of oxidative stress to tissues as compared to lipid peroxidation (Ilaiyarajaand Khanum, 2011).

Oxidative modification of proteins leads to loss of protein function and shows detrimental effects on cell and tissues (Dalle-Donne *et al.,* 2003). The results are in accordance with the findings of Rjiba *et al.,* (2012), who reported that cisplatin induces significant increase in protein carbonyl generation and decreases the levels of GSH in heart of rats. Hassan *et al.,* (2010) stated that carbonyls and MDA contents are considered respectively as final products for estimation of protein oxidation and lipid peroxidation, respectively and reported that cisplatin treated rats showed tremendous increment in MDA content and carbonyl content. Our findings revealed that *T. cordifolia* and vitamin C administration increased GSH levels, suppressed the increase in MDA concentration (an index of lipid peroxidation) and suppressed the increase in protein carbonyls (an index of protein degradation). These findings suggest that protective action of *Tinospora cordifolia* is in harmony with the previous reports (Stanley and Menon 2001; Goel *et al.,* 2002). *T.cordifolia* extract inhibits the lipid peroxidation and superoxide and hydroxide radicals *in vitro* due to its antioxidant and free radical scavenging properties (Mathewand Kuttan, 1997).

**Table.1** Experimental design

| | |
|---|---|
| Group 1 | Normal saline orally for 14 days |
| Group 2 | Cisplatin (7.5 mg/kg body weight, ip) injection on day 1. |
| Group 3 | Aqueous leaf extract of *Tinospora cordifolia* @ 400 mg/kg body weight was orally administered for 14 days starting from the day 1. |
| Group 4 | Vitamin C @ 100 mg/kg body weight was orally administered for 14 days starting from the day 1. |
| Group 5 | 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 6 | 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. |

**Table.2** Average body weights (g), GSH (µ mol /mg protein),TBARS (nm MDA released/mg protein), Protein carbonyls (nmoles of carbonyl /mg protein), TNF-*alpha* (pg/ml) and IL-10 (pg/ml) in different groups of rats.

| **Groups** / **Parameter** | **Normal Control** | **Cisplatin** | **TC** | **Vit. C** | **Cisplatin + TC** | **Cisplatin + Vit.C** |
|---|---|---|---|---|---|---|
| **Day 7 body weight** | 220.66 ± 1.02$^{a}$ | 135.50 ± 0.42$^{c}$ | 220.50 ± 0.76$^{a}$ | 221.50 ± 1.72$^{a}$ | 160.66 ± 1.58$^{b}$ | 161.16 ± 1.01$^{b}$ |
| **Day 14 body weight** | 246.50 ± 2.89$^{a}$ | 133.16 ± 0.79$^{c}$ | 247.50 ± 2.37$^{a}$ | 248.66 ± 3.81$^{a}$ | 194.00 ± 0.93$^{b}$ | 195.50 ± 1.33$^{b}$ |
| **GSH** | 9.63 ± 0.22 $^{a}$ | 4.98 ± 0.27 $^{c}$ | 9.68 ± 0.23 $^{a}$ | 9.75 ± 0.18 $^{a}$ | 7.03 ± 0.22 $^{b}$ | 7.49 ± 0.24 $^{b}$ |
| **TBARS** | 55.50 ± 1.74$^{c}$ | 77.83 ± 1.70$^{a}$ | 54.66 ± 1.45$^{c}$ | 53.16 ± 1.44$^{c}$ | 61.66 ± 1.22$^{b}$ | 60.33 ± 1.38$^{b}$ |
| **Protein carbonyls** | 1.75 ± 0.08 $^{c}$ | 7.36 ± 0.42 $^{a}$ | 1.58 ± 0.05 $^{c}$ | 1.49 ± 0.03 $^{c}$ | 3.36 ± 0.23 $^{b}$ | 3.03 ± 0.18 $^{b}$ |
| **TNF-*alpha*** | 106.83 ± 4.24$^{c}$ | 381.83 ± 7.43$^{a}$ | 105.50 ± 2.21$^{c}$ | 105.83 ± 4.54$^{c}$ | 230.00 ± 4.95$^{b}$ | 227.33 ± 2.06$^{b}$ |
| **IL-10** | 54.50 ± 1.50$^{c}$ | 374.50 ± 1.08$^{a}$ | 53.83 ± 1.30$^{c}$ | 52.16 ± 0.83$^{c}$ | 294.16 ± 3.68$^{b}$ | 291.50 ± 4.77$^{b}$ |

**Fig.1** Average body weights (g) of rats of different groups of rats

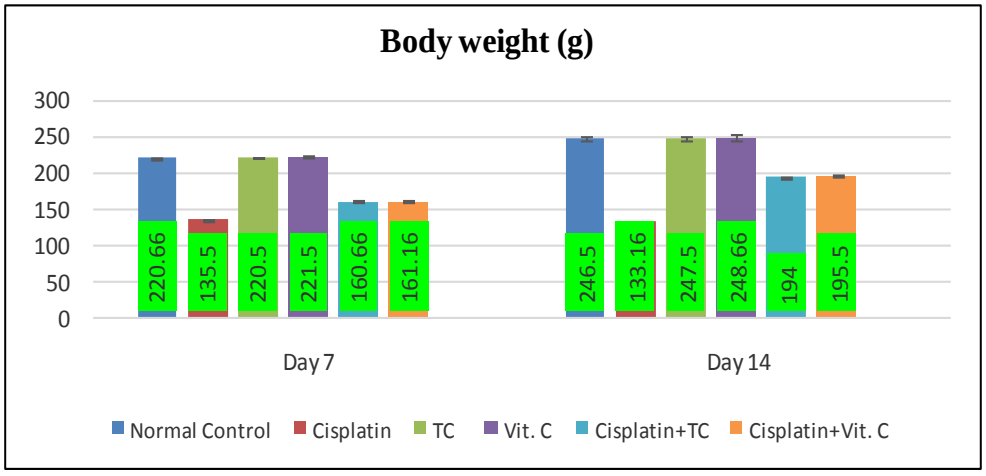



**Fig.2** GSH concentration (µ mol/mg protein) in heart of different groups of rats

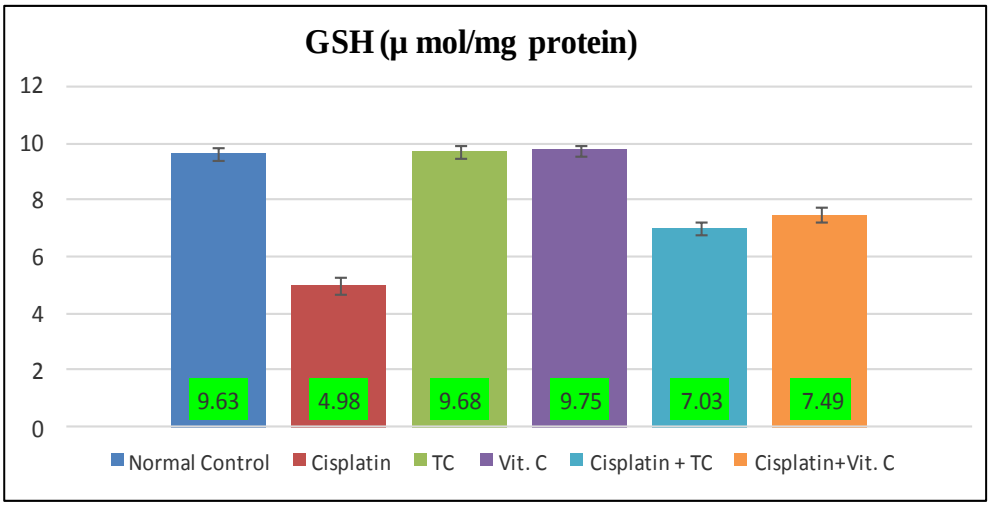


**Fig.3** TBARS concentration (n moles of MDA released/mg protein) in heart of different groups of rats

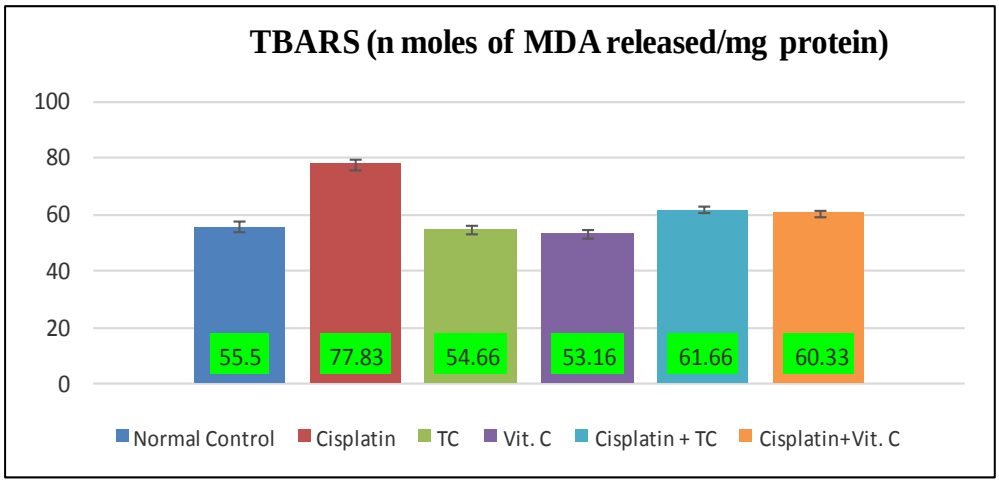



**Fig.4** Protein carbonyl concentration (n moles of carbonyl/mg protein) in heart of different groups of rats

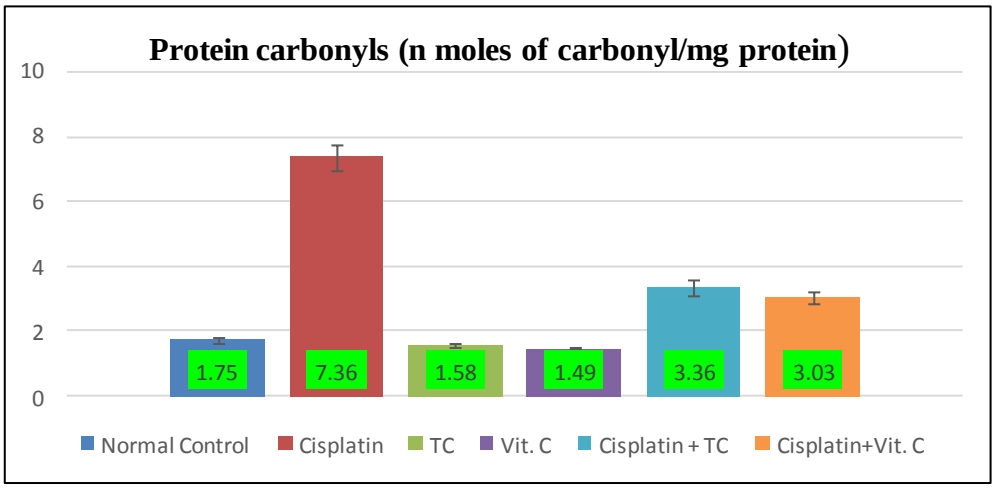


**Fig.5** Serum TNF-α levels (pg/ml)in different groups of rats

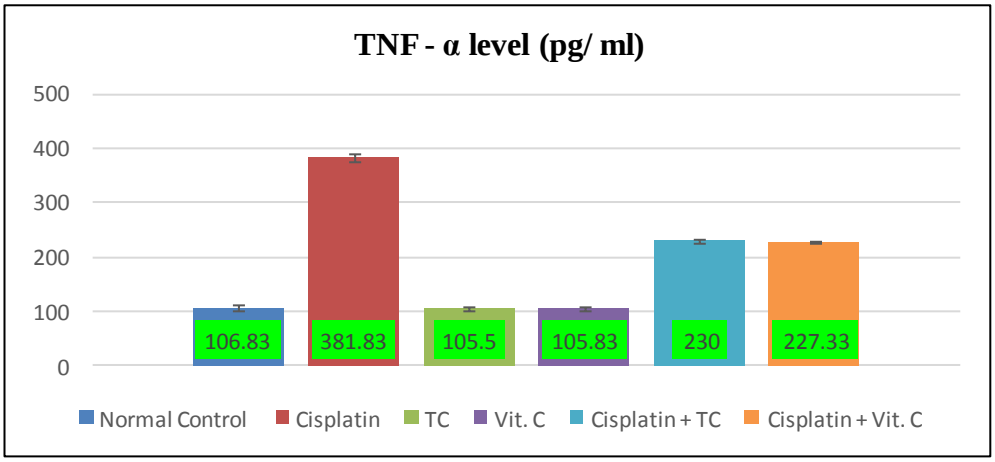
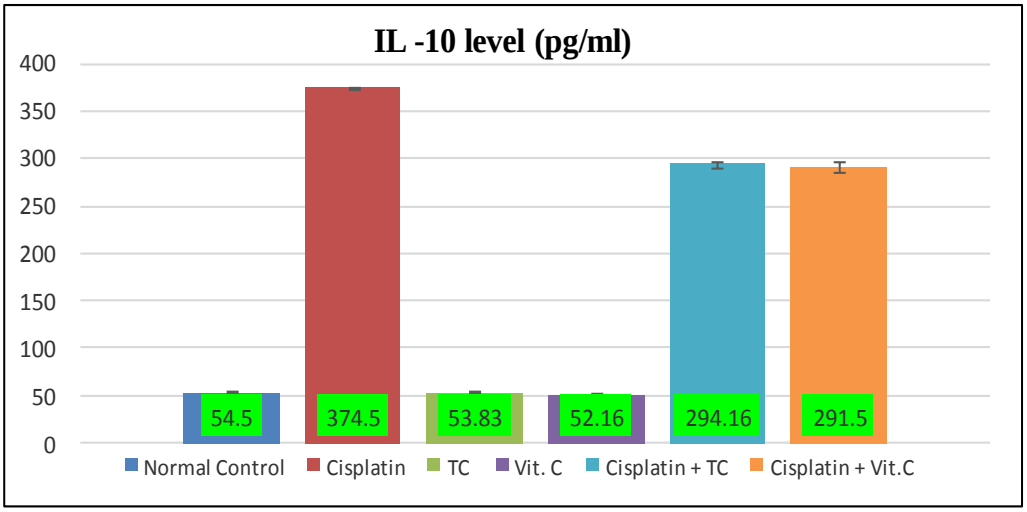



**Fig.6** Serum IL-10 levels (pg/ml) in different groups of rats




The aqueous extract of *T. cordifolia* leaves revealed the presence of phytosterols, flavonoids, phenols and diterpenes (Madhavi *et al.*, 2017). Phenolic compounds exhibit antioxidant potential, by scavenging the free radicals. Due to the presence of hydroxyl group and redox properties, they act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Polu *et al.*, 2017). Flavonoids act as scavengers of many oxidizing species (hydroxyl radical or peroxy-radicals and superoxide anions) and as quenchers of singlet oxygen (Kaur *et al.*, 2016). In the present study, there is significant increase in serum TNF-α and IL-10 in toxic control group 2 compared to the nontoxic groups 1, 3 and 4. IL-10 is a pleiotropic cytokine produced by macrophages, B-lymphocytes and T-helper 2 (Th2) cells, which can stimulate and suppress the immune response (Othman *et al.*, 2013). Concurrent with the induction of a stress-activated inflammatory response, many agents with anti-inflammatory properties (IL-10) are produced that may prevent tissue injury or help in tissue repair/remodeling subsequent to

injury in different organs and tissues (Deng *et al.,* 2001).Our results are in accordance with the findings of Chowdhury *et al.,* (2016), who reported increased levels of TNF-α (responsible for tissue damage) in the cardiac tissue of animals following cisplatin administration. TNF-α intensifies the cytotoxic effects of cisplatin. Tumor cells exhibit an elevation in constitutive production of several pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1β. They are major mediators of acute inflammation and play decisive roles at different stages of development of tumor, including initiation, promotion, malignant conversion, invasion, and metastasis. Apoptosis and myocyte necrosis can be linked to elevated proinflammatory cytokine levels, such as IL-1β, TNF-α, and interferon-γ. Enhanced levels of TNF-α suggests stimulation of the inflammatory response *in vivo*, which leads to aggravated cisplatin-induced cardiotoxicity (Bahadir *et al.,* 2018). Cytokines, chemokines and cell adhesion molecules together regulate the neutrophil infiltration at the site of cardiac tissue damage. Inflammation related genes for pro-inflammatory cytokines, chemokines and cell adhesion molecules are mostly regulated by the transcription factor NF-κB. Cisplatin mediated ROS generation activates the transcription factor NF-κB, which plays a critical role in cardiac inflammation and injury (Chowdhury *et al.,* 2016).

In the present study, *T.cordifolia* and vitamin C at the doses administered significantly normalized the elevated level of inflammatory cytokines and thus protected the heart from inflammatory insult. Spelman *et al.,* (2006) reviewed the immunomodulatory activity of *T.cordifoliain vitro* and *in vivo,* and found that it plays a prominent role in the modification of modulation of Il -1, Il-6, TNF and INF.Dry leaf extracts of *T.cordifolia*have been reported to exhibit anti-inflammatory activity by inhibiting LPS-induced pro-inflammatory cytokine TNF-α, at both protein and transcript levels (Reddi and Tetali, 2019).

In conclusion, the study revealed that cisplatin induces cardiotoxicity by oxidative stress. Administration of *Tinospora cordifolia* and vitamin C could effectively antagonize the cardiotoxicity by cisplatin owing to their antioxidant and cardioprotective actions.

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