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Original Article

Triptonide Protects against Doxorubicin-induced Cardiotoxicity in Rats by Regulating Oxidative Stress and Cardiac Biomarkers

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ABSTRACT

Background: Doxorubicin is an anthracycline anti-cancer drug and one of the most widely used chemotherapeutic medications to treat both solid and hematological tumors. However, due to the major adverse effect of cardiotoxicity, the clinical use of doxorubicin was highly restricted. Objectives: The current research was undertaken to explore the salutary properties of the triptonide on the doxorubicin-induced cardiotoxicity in rats. **Materials and Methods:** Rats were given 2.5 mg/kg of doxorubicin to produce cardiotoxicity, which was then treated with 25 mg/kg of triptonide. A set of rats was treated with 50 mg/kg of triptonide alone. Plethysmography on the tail-cuff was used to measure the blood pressure indicators. Using assay kits, the concentrations of oxidative and antioxidative biomarkers and cardiac function markers were measured. Using established techniques, the antioxidant enzyme activity was assessed. The histopathological study was performed on the heart tissues to analyze the doxorubicin-induced histological changes. **Results:** The heart weight was improved by triptonide treatment in the doxorubicin-induced rats. Triptonide effectively reduced the blood pressure indicators in the doxorubicin-induced rats. In the doxorubicin-induced rats, triptonide significantly decreased the LDH, CK, and AST activities and the status of myoglobin, H-FABP, GP-BB, and CK-MB. The triptonide therapy decreased the levels of INF-γ, MCP-1, and TGF-β in the serum of doxorubicin-induced rats. The findings of the histopathological examination showed that triptonide had therapeutic benefits. **Conclusion:** In summary, the results of this study supported the hypothesis that triptonide could ameliorate the biochemical and histological changes in the rats' hearts that were caused by doxorubicin.

Keywords: Creatine kinase, Cardiac damage, Myoglobulin, Doxorubucin, Triptonide.

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INTRODUCTION

Acute Myocardial Infarction (AMI), which has a high morbidity rate, is the leading cause of mortality worldwide.[1] According to data from the World Health Organization (WHO), AMI accounts for 30% of all annual fatalities worldwide and is predicted to account for more than 23 million deaths annually by 2030. AMI is a condition where the unstable ischemia syndrome contributes to myocardial necrosis.[2,3] Myocardial Infarction (MI) occurs when blood supply to the heart is suddenly obstructed, leading to ischemia and necrosis of the affected myocardial tissues. Reperfusion may lead to damage and necrosis of myocardial tissues; hence, quick reperfusion of the affected heart muscle is an ultimate goal of MI treatment.[4]

Doxorubicin, an anthracycline antineoplastic medication, is regarded as one of the most potent oncology drugs ever developed.[5] Since it is well known, extremely effective, and has had remarkable achievements in treating both solid and hematological tumors, doxorubicin has been a cornerstone of anti-cancer therapy.[6] However, due to the major adverse effect of cumulative dose-related cardiotoxicity, which causes arrhythmia and cardiomyopathy, the clinical use of doxorubicin is restricted. The precise pathophysiology of doxorubicin-induced cardiotoxicity is yet unknown; however, it may result from a number of different mechanisms. One of the possible contributing factors, along with the release of Nitric Oxide (NO), decreased Adenosine Triphosphate (ATP) generation, mitochondrial dysfunction, oxidative stress, and inflammation.[7]

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The mechanisms of cardiotoxicity brought on by doxorubicin are thought to involve multifactorial pathways. It has been suggested that inflammation and oxidative stress may have a pivotal role.[8] One of the main causes of doxorubicin cardiotoxicity is increased oxidative stress, which results in constant ROS through a variety of contributory mechanisms. These ROS have the potential to promote lipid peroxidation, which could lead to oxidative injury to myocyte mitochondria and cell membranes.[9] When antioxidant mechanisms aren't working as well, ROS damage could occur. The buildup of doxorubicin in cardiac mitochondria results in redox cycling of doxorubicin, which leads to overproduction of ROS, which causes mitochondrial cardiomyocytes to malfunction because myocardial tissues lack appropriate antioxidant systems.[10]

To reduce the risk of developing cardiotoxicity from doxorubicin, early diagnosis of a heart injury is advantageous to the patient. Many cardiac biomarkers, including Creatine Kinase (CK) as the initial biomarker to evaluate the heart injury, have been utilized to predict a cardiotoxic event.[11] Additionally, LDH has previously been employed as an indicator for cardiac enzymes, and the rise in LDH level represents a heart injury. CK-MB, which is more sensitive than LDH in terms of acute myocardial injury, is becoming a critical cardiac injury biomarker. Compared to skeletal muscle, cardiac muscle has higher levels of CK-MB.[12] Symptoms of doxorubicin-induced cardiac toxicity include high levels of the enzymes LDH and CK, as well as changes in the shape and function of the heart that can lead to cardiomyopathy and heart failure.[13]

Therefore, it is necessary to investigate medicines that can stop the oxidative cardiac damage caused by doxorubicin in cancer patients. Triptonide is a major bioactive compound present in the *Tripterygium wilfordii* plant. Triptonide acts as a new and effective antitumor agent against lymphoma, prostate cancer, and nasopharyngeal cancer.[14-19] Triptonide also possesses immunosuppressive and anti-inflammatory effects.[17] Triptonide has also been shown to block lung tumorigenicity,[18] limit gastric cancer development and metastasis,[19] target numerous senescence-promoting pathways in leukemia cells,[20] and suppress pancreatic cancer cell proliferation.[21] Apart from its excellent anti-cancer and other biological activities, the cardioprotective roles of triptonide against drug-induced cardiotoxicity remain to be explored. Therefore, the current investigation was conducted in order to determine whether the triptonide has any beneficial effects on the damage that doxorubicin causes to cardiac tissues of the rats.

MATERIALS AND METHODS

Chemicals

The triptonide, doxorubicin, and other chemicals were purchased from Sigma-Aldrich, USA. Thermofisher, MyBioSource, and Biocompare, USA, have provided the ELISA assay kits to evaluate the biochemical markers.

Experimental animals

The Wistar rats, which were 10 to 12 weeks old, were caged in clean conditions with a 12-hr light/dark cycle, a temperature of 21 to 24°C, and a relative humidity of 50% to 60%. They were also given access to regular pellet food and water. Prior to the start of the research, all animals were acclimated to the lab environment for seven days. This work was approved by ethical guidelines (No. SXBH2021-076) Shanxi Bethune Hospital.

Experimental protocol and sample collections

All the rats were separated into four groups, each comprising six rats (n=6). Rats in group II were given alternate days of 2.5 mg/kg doxorubicin for 14 days to induce cardiotoxicity, while group I was served as a control. 25 mg/kg of triptonide was administered orally to group III for 3 days before the doxorubicin administration and for the duration of the study period. The group IV rats were fed triptonide alone (50 mg/kg). Rats were ultimately sacrificed after being put under anesthesia, and a blood sample was then collected to prepare the serum by centrifuging at 5000 g for 20 min. The prepared serum was stored at -20°C for future research. For histological evaluations, a portion of the collected heart tissues was deposited at -20°C.

Determination of blood pressure markers

The status of blood pressure indicators in both the control and treated rats was assessed. Tail-cuff plethysmography and a pressure meter were used to measure the Heart Rate (HR), Systolic Arterial Pressure (SAP), Mean Arterial Pressure (MAP), and Diastolic Arterial Pressure (DAP) of the experimental rats.

Quantification of antioxidant markers

By using previously established techniques, the level of antioxidants and oxidative stress in the homogenate of cardiac tissues was determined. By using the Ohkawa *et al.* approach,[22] the status of TBARS was determined in the heart tissues. Superoxide Dismutase (SOD) activity was detected in accordance with Marklund and Marklund's method.[23] The Catalase (CAT) enzyme activity was measured using the Sinha technique.[24] Glutathione (GSH) status was measured using the Ellman method.[25]

Determination of serum cardiac biomarkers

Lactate Dehydrogenase (LDH), CK, and AST status in the serum of control and treatment rats were examined using the corresponding assay kits in agreement with the recommended guidelines of the manufacturer (Biocompare, USA).

Measurement of cardiac biomarkers

Myoglobin (Myo) levels in the serum of the treated rats were measured using an assay kit using the manufacturer's protocols (MyBioSource, USA). Following the manufacturer's instructions,

the H-FABP, GP-BB, and CK-MB contents in the serum of the control and treatment rats were examined (Biocompare, USA).

Measurement of inflammatory markers

Using the appropriate assay kits and following the recommended protocols of the manufacturer (Raybiotech, USA), the status of Interferon-γ (INF-γ) and Monocyte Chemoattractant Protein-1 (MCP-1) in the serum of control and experimental rats were determined.

Histopathological analysis

The removed cardiac tissue was treated with 10% formalin, followed by the addition of ethanol to dehydrate it. Then, using a microtome, tissue blocks were created by paraffin embedding and cut at a thickness of 5 µm. To find the histological changes, the sliced tissues were stained with hematoxylin and eosin (H&E) and examined under a microscope at a magnification of 40×.

Statistical analysis

The significance level for the obtained data from biochemical assays were fixed as $p<0.05$ using the one-way ANOVA and Dunnett's tests and final data are provided as the mean±SD of triplicates. These tests were performed using the Prism GraphPad-8 software.

RESULTS

Effect of triptonide on the heart weight in the doxorubicin-induced cardiotoxic rats

When compared to controls, the rats showed a progressive decrease in heart weight due to the doxorubicin-induced cardiotoxicity. The heart weight of the doxorubicin-induced cardiotoxic rats, on the other hand, showed an impressive recovery after treatment with 25 mg/kg of triptonide (Figure 1). The heart weight of the rats did not significantly change when they were given 50 mg/kg of triptanide alone, making it more comparable to the control.

Effect of triptonide on the blood pressure indicators in the doxorubicin-induced cardiotoxic rats

According to Figure 2, when doxorubicin-induced cardiotoxic rats were compared to the control group, blood pressure indicators such as HR, SAP, DAP, and MAP were significantly reduced. These alterations in the blood pressure markers were significantly controlled by the triptonide therapy. In the doxorubicin-induced cardiotoxic rats, the administration of 25 mg/kg of triptonide significantly improved the HR, SAP, DAP, and MAP. When rats were only given 50 mg/kg of triptonide, there were no changes in their blood pressure indicators level (Figure 2).

Effect of triptonide on the antioxidants in the heart tissues of doxorubicin-induced cardiotoxic rats

Comparing the doxorubicin-induced cardiotoxic rats to the control group, Figure 3 shows that the TBARS level was significantly elevated while the antioxidant levels were significantly decreased. Intriguingly, in the doxorubicin-induced rats, the 25 mg/kg of triptonide therapy showed a significant reduction in the TBARS and an improvement in antioxidants, including SOD, CAT, and GSH. The rats treated with 50 mg/kg of triptonide alone, which closely resembled the control rats, did not exhibit any significant alterations in these markers (Figure 3).

Effect of triptonide on the levels of serum cardiac biomarkers in the doxorubicin-induced cardiotoxic rats

The serum levels of cardiac biomarkers such as AST, CK, and LDH were examined, and the findings are represented in Figure 4. The doxorubicin-induced rats showed a striking rise in the serum status of AST, CK, and LDH. On the other hand, rats treated with triptonide (25 mg/kg) showed gradually lower levels of AST, CK, and LDH (Figure 4). The AST, CK, and LDH levels of the rats treated with triptonide (50 mg/kg) alone did not significantly differ from those of the control.

Effect of triptonide on the cardiac biomarker levels in the serum of doxorubicin-induced cardiotoxic rats

By analyzing the serum levels of cardiac biomarkers, the cardiotoxic effects of doxorubicin on the rats were evaluated. In contrast to the control group, the doxorubicin-induced rats had noticeably higher serum levels of Myo, H-FABP, GP-BB,

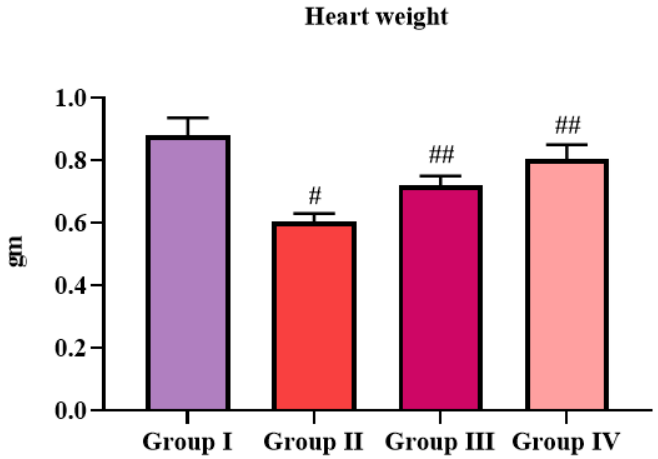

Figure 1: Effect of triptonide on the heart weight in the doxorubicin-induced cardiotoxic rats.

The triptonide treatment considerably increased the heart weight in the doxorubicin-induced rats. Values are given as the mean ± SD of three triplicate assays. The Prism GraphPad-8 software was used to perform the statistical analysis using one-way ANOVA and DMRT assays. Note: "#" represents $p < 0.01$ when compared to control and "##" represents $p < 0.05$ when compared to the doxorubicin-induced cardiotoxic rats.

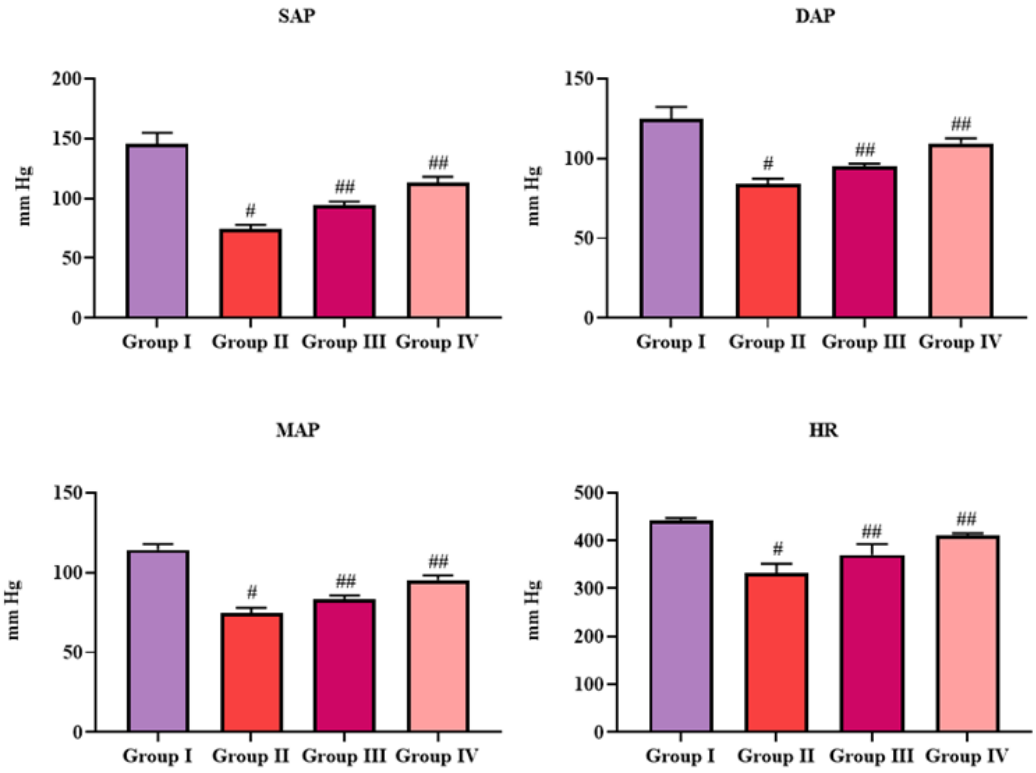

Figure 2: Effect of triptonide on the blood pressure indicators in the doxorubicin-induced cardiotoxic rats.

The levels of blood markers were effectively increased by the triptonide treatment in the doxorubicin-induced rats. Values are given as the mean ± SD of three triplicate assays. The Prism GraphPad-8 software was used to perform the statistical analysis using one-way ANOVA and DMRT assays. Note: "#" represents $p < 0.01$ when compared to control and "##" represents $p < 0.05$ when compared to the doxorubicin-induced cardiotoxic rats.

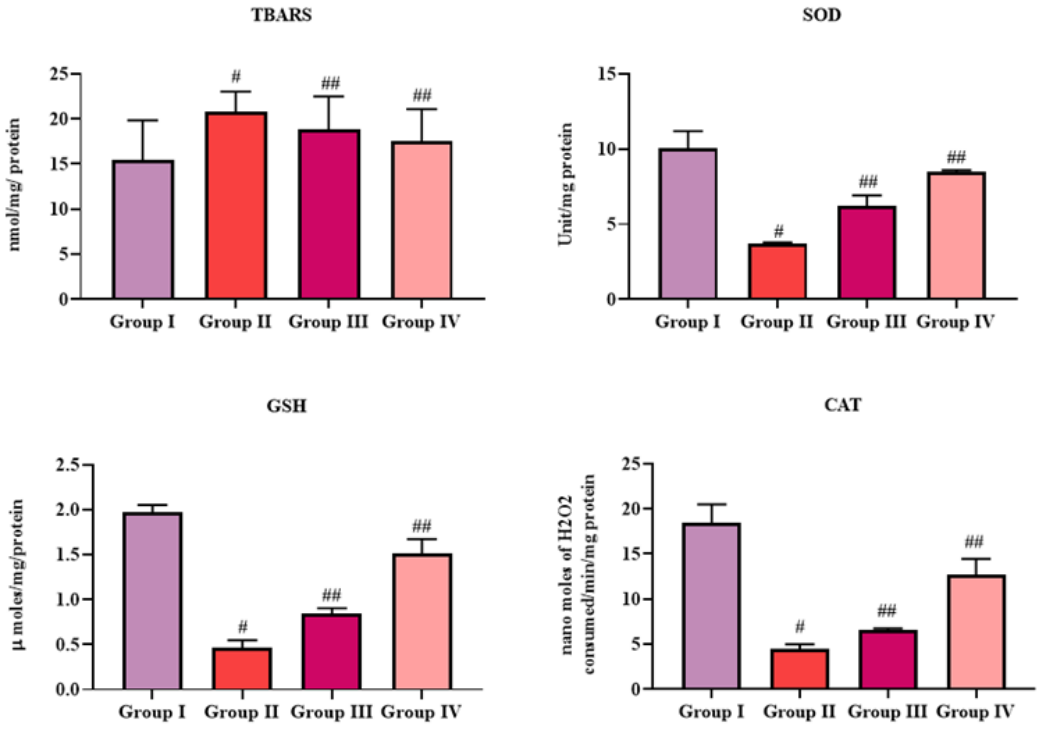

Figure 3: Effect of triptonide on the antioxidants in the heart tissues of doxorubicin-induced cardiotoxic rats.

The levels of TBARS were decreased and antioxidants were increased by the triptonide treatment in the doxorubicin-induced rats. Values are given as the mean ± SD of three triplicate assays. The Prism GraphPad-8 software was used to perform the statistical analysis using one-way ANOVA and DMRT assays. Note: "#" represents $p < 0.01$ when compared to control and "##" represents $p < 0.05$ when compared to the doxorubicin-induced cardiotoxic rats.

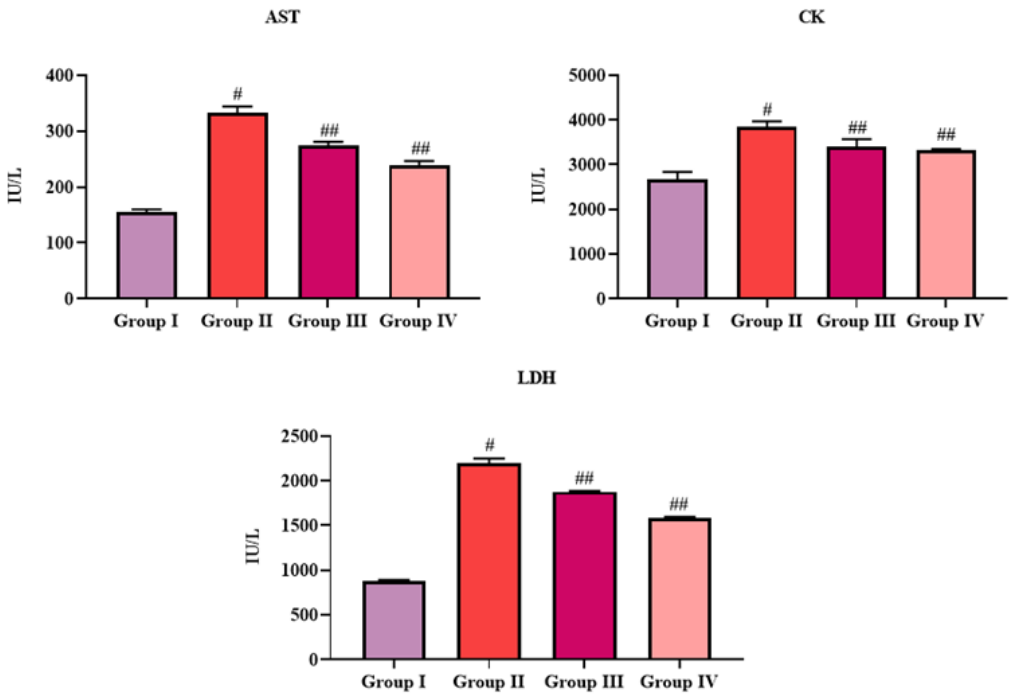

Figure 4: Effect of triptonide on the levels of serum cardiac biomarkers in the doxorubicin-induced cardiotoxic rats.

The triptonide treatment remarkably reduced the activities of the serum cardiac biomarker enzymes in the doxorubicin-induced rats. Values are given as the mean ± SD of three triplicate assays. The Prism GraphPad-8 software was used to perform the statistical analysis using one-way ANOVA and DMRT assays. Note: "#" represents $p < 0.01$ when compared to control and "##" represents $p < 0.05$ when compared to the doxorubicin-induced cardiotoxic rats.

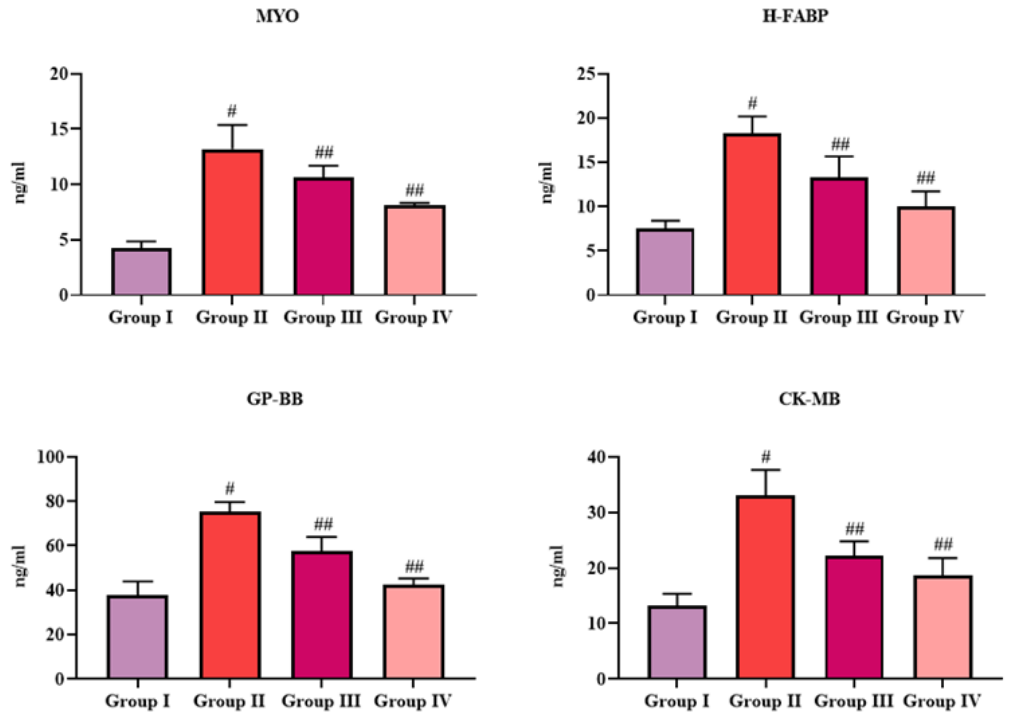

Figure 5: Effect of triptonide on the cardiac biomarker levels in the serum of doxorubicin-induced cardiotoxic rats.

The levels of cardiac biomarkers were effectively reduced in the serum of triptonide-treated cardiotoxic rats. Values are given as the mean ± SD of three triplicate assays. The Prism GraphPad-8 software was used to perform the statistical analysis using one-way ANOVA and DMRT assays. Note: "#" represents $p < 0.01$ when compared to control and "##" represents $p < 0.05$ when compared to the doxorubicin-induced cardiotoxic rats.

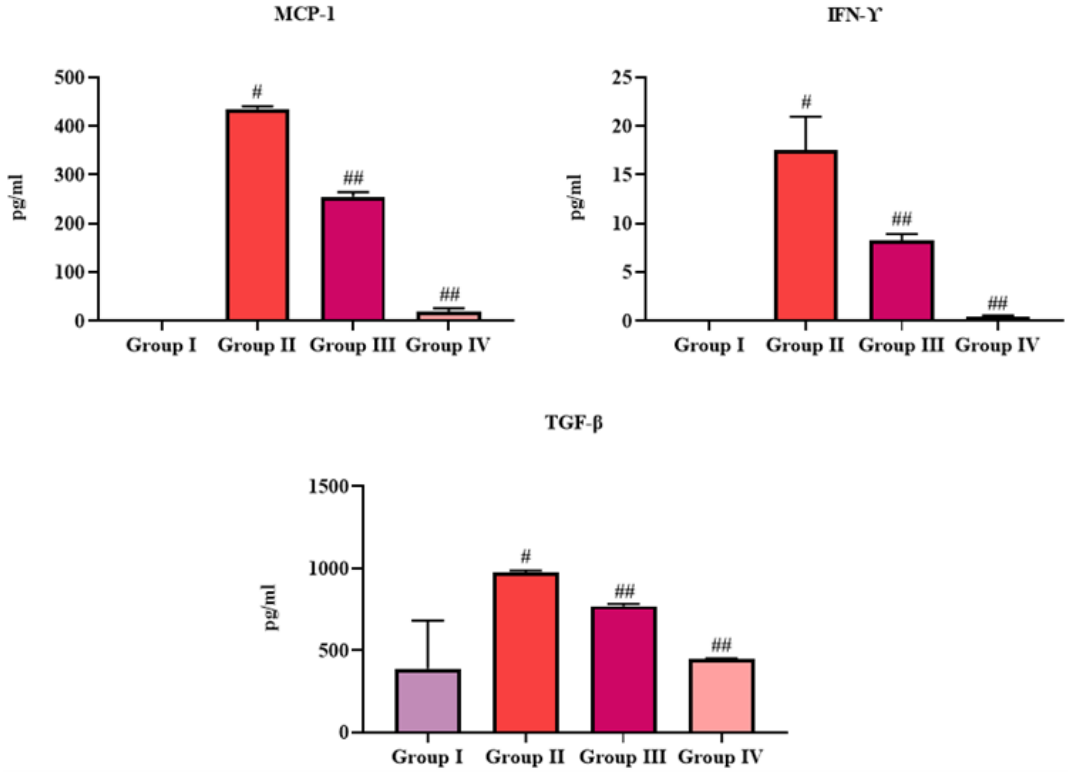

Figure 6: Effect of triptonide on the inflammatory marker levels in the serum of doxorubicin-induced cardiotoxic rats.

The levels of inflammatory biomarkers were substantially decreased in the serum of triptonide-treated cardiotoxic rats. Values are given as the mean ± SD of three triplicate assays. The Prism GraphPad-8 software was used to perform the statistical analysis using one-way ANOVA and DMRT assays. Note: "#" represents $p < 0.01$ when compared to control and "##" represents $p < 0.05$ when compared to the doxorubicin-induced cardiotoxic rats.

and CK-MB, as shown in Figure 5. The concentrations of Myo, H-FABP, GP-BB, and CK-MB were significantly reduced in the rats given 25 mg/kg of triptonide and set back to near-normal levels. The 50 mg/kg of triptonide alone treated rats, which are similar to the control, did not show alterations in these markers (Figure 5).

Effect of triptonide on the inflammatory marker levels in the serum of doxorubicin-induced cardiotoxic rats

In the doxorubicin-induced cardiotoxic rats, the serum levels of inflammatory markers such as TGF-β, MCP-1, and INF-γ were significantly higher than in the control group, as revealed in Figure 6. However, the addition of 25 mg/kg of triptonide significantly reduced the levels of MCP-1, TGF-β, and INF-γ in the doxorubicin-induced rats. There were no differences in the serum status of MCP-1, TGF-β, and INF-γ between rats receiving 50 mg/kg of triptonide alone and control rats (Figure 6).

Effect of triptonide on the doxorubicin-induced histopathological alterations in the cardiac tissues

The cardiac tissues of control rats displayed normal histoarchitectures, as shown in Figure 7. In contrast, the doxorubicin-induced rats showed severe histological abnormalities such as rupturing of the heart muscle, deterioration of the myocytes, moderate hemorrhage, and myocyte necrosis. Triptonide (25 mg/kg) therapy significantly decreased the doxorubicin-induced cardiac tissue damage in rats (Figure 7). Rats given 50 mg/kg of triptonide alone and a control group did not exhibit any significant histological abnormalities in their heart tissues.

DISCUSSION

Although the long-term use of doxorubicin is restricted by its side effects, particularly its cardiotoxicity, it is still one of the most commonly prescribed chemotherapeutic medications in clinics. Though the precise mechanisms of doxorubicin-induced cardiotoxicity are still not fully understood. Doxorubicin-induced cardiotoxicity may be due to several mechanisms. These pathways include myocyte damage, ROS formation, intracellular Ca^{2+} dysregulation, and mitochondrial injury.[26] Doxorubicin preferentially builds up in the mitochondria of the heart and is linked to cardiac damage. For instance, doxorubicin can increase apoptosis and drastically decrease the activity of mitochondrial complex 1.[27] Following a two-week doxorubicin treatment, there have been reports of decreased heart weight, which were linked to parallel changes in cardiac function.[28] Our investigation found that DOX treatment caused a reduction in heart weight, which may have been caused by local tissue necrosis as observed in our study. This finding suggests that triptonide treatment can, at least

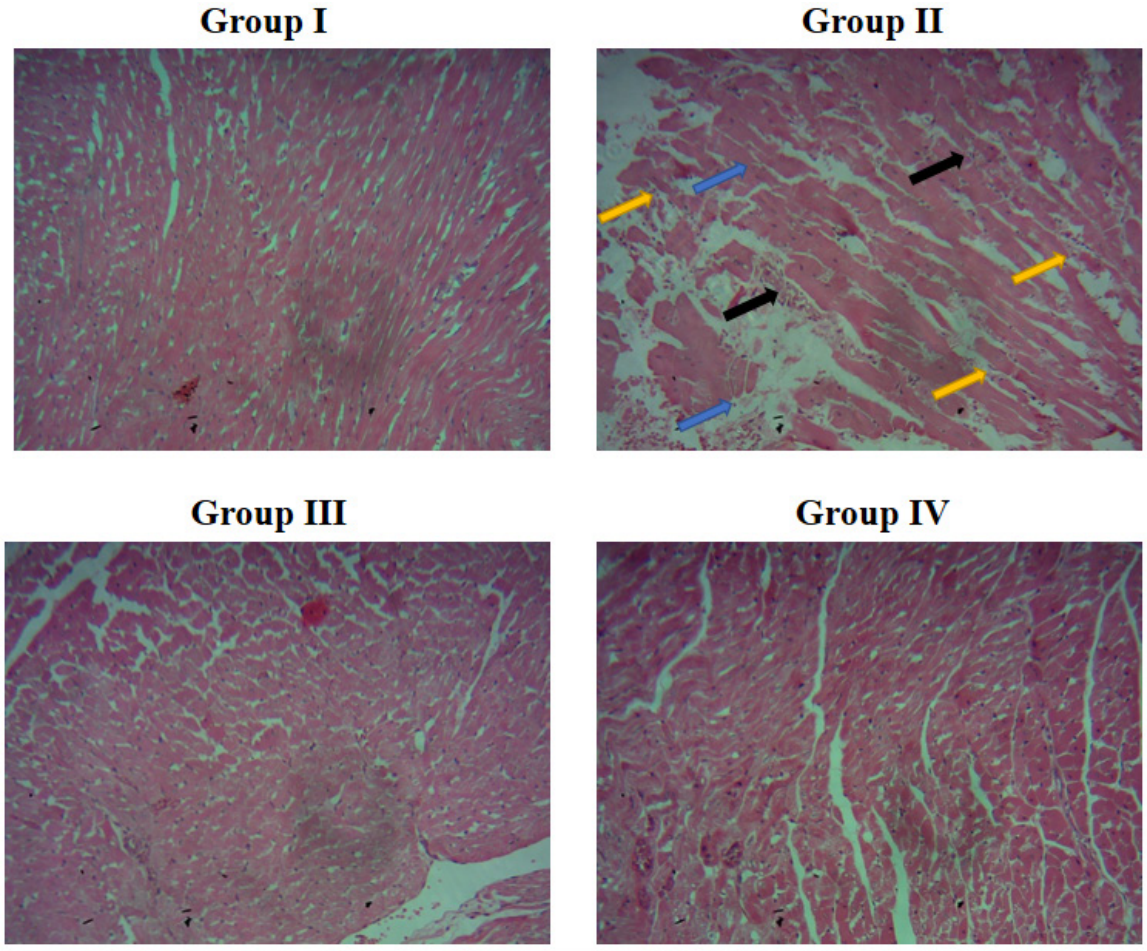

Figure 7: Effect of triptonide on the doxorubicin-induced histopathological alterations in the cardiac tissues.

Group I: The cardiac tissues of control rats displayed the typical histological structures. Group II: Cardiac tissues of doxorubicin-induced rats revealed the higher cardiac tissue damages (yellow arrows), infiltration of inflammatory cells (black arrows), and myocytes degeneration (blue arrows). Group III: The 25 mg/kg of triptonide treatment effectively reduced the doxorubicin-induced histopathological changes in the heart tissues of rats. Group IV: The 50mg/kg of triptonide alone treated rats showed no major histopathological alterations.

in part, reverse gross tissue morphological alterations brought on by DOX's cardiotoxic effects.

Hemodynamic parameters like SAP, MAP, and DAP have been mainly used to monitor the hemodynamic changes in patients with cardiac arrest. Hemodynamic monitoring is crucial for patients who have experienced cardiac arrest. Additionally, MAP or SAP have been used in the majority of investigations on hypotension incidents in patients with heart attacks for neuroprognostication.[29] In addition to MAP or SAP, DAP is being suggested as a promising predictive technique. According to a different study, DAP is better than SAP at determining the prognosis of cardiogenic shock.[30] When predicting risk in heart attack patients, DAP outperformed SAP or MAP, and among all hemodynamic measures, HR/DAP was the most reliable indicator of poor neurological outcomes at all time periods. DAP represents arterial compliance and vascular tone.[31] Our findings revealed that the blood pressure indicator index significantly improved in the doxorubicin-induced rats as a result of the triptonide treatment. In the doxorubicin-induced cardiotoxic rats, therapy with triptonide significantly improved the HR, SAP, DAP, and MAP, which suggests its supportive properties on the cardiac functions.

Doxorubicin-induced cardiotoxicity has been explained by a variety of different mechanisms. The fundamental mechanism of doxorubicin cardiomyopathy was thought to be cardiac oxidative stress, as indicated by higher ROS generation. Higher amounts of ROS can cause mitochondrial malfunction, oxidative injury to macromolecules, and result in cell death.[32] This cardiotoxicity involves the binding of lipid peroxidation products like TBARS and MDA onto macromolecular targets, following oxidative stress and metabolic activation to a semiquinone. Doxorubicin treatment also reduces the antioxidant mechanisms.[33] The main thiol antioxidant within cells, GSH, is concurrently depleted as a result of this oxidative stress. Reduced GSH levels perform a pivotal function in the downregulation of GSH-Px brought on by doxorubicin. CAT is made of hemeprotein and is utilized to

scavenge generated ROS and protect tissues from free radical damage.[34]

Doxorubicin promotes oxidative stress by reducing the activity of antioxidant defense mechanisms. Our findings demonstrated that doxorubicin treatment in rats significantly increased lipid peroxidation, which was exhibited by a considerable augmentation in MDA and was also followed by a prominent drop in antioxidants in cardiac tissues, which is in agreement with earlier research.[35] These outcomes were expected because prior research had shown that doxorubicin-induced antioxidant molecule depletion, including GSH, and cardiac antioxidant enzyme exhaustion, including CAT and SOD, were caused by excessive consumption by doxorubicin-generated free radicals.[36] These free radicals reduce cardiac GSH levels and SOD and CAT activities and disrupt the antioxidant defense systems, which build up lipid peroxidation products in the heart tissues.[37] Several bioactive compounds with strong antioxidant activities have been shown to protect against the cardiac dysfunction caused by doxorubicin in animal models by their reversing effects on doxorubicin-mediated oxidative stress.[38] In agreement with these reports, our findings also revealed that triptonide treatment effectively reduced the TBARS and elevated the CAT, SOD, and GSH in the cardiac tissues of the doxorubicin-induced cardiotoxic rats. These outcomes suggested the strong antioxidant activities of the triptonide.

The excessive free radicals denature DNA and induce cellular proteins and lipid peroxidation. Due to this, the integrity of the membrane is compromised, and cardiac enzymes like LDH and CK-MB are released into the extracellular fluid from the cytoplasmic membrane.[39] As a result of the DOX-induced cardiotoxicity, the levels of LDH, CK, and AST were raised, as expected. The onset of cardiotoxicity often facilitates the risk of cardiac cell membrane damage and the subsequent release of myocardial enzymes into the blood. As a result, myocardial enzyme levels in the serum, including AST, LDH, and CK, are thought to be indications of cardiac injury.[40] Three cytosolic enzymes CK, AST, and LDH act as sensitive markers to assess the degree of cardiac damage. Higher concentrations of these enzymes in the serum are a sign of cellular injury and loss of cell membrane permeability.[41] Changes in LDH and CK levels may be caused by necrotic lesions with breakdown of membrane permeability generated in doxorubicin-induced rats and released into the bloodstream during the onset of cardiac injury.[42] Increased levels of LDH and CK-MB signify their release from the disrupted cardiomyocyte membranes into the bloodstream. CK is a recognized biomarker of muscle breakdown. Due to its short duration and excellent specificity, CK is mostly utilized to diagnose cardiac injury with recurring attacks within a short period of time.[43] It's interesting to note that triptonide treatment-maintained membrane integrity and blocked CK, AST, and LDH from leaking into the extracellular space.

The use of biomarkers is common for the early diagnosis of myocardial damage. Numerous cardiac indicators with varied degrees of sensitivity and specificity have been discovered so far for the early detection of cardiac injury. Numerous biomarkers, including Myo, CK-MB, and H-FABP, are utilized to identify cardiotoxicity. Within 3 hr after a cardiac injury, Myo is increased and fairly sensitive.[44] In the early phases of cardiac damage, CK-MB detection in serum is a sensitive signal of cardiac damage. CK-MB has been linked to myocardial damage because it is released into the bloodstream and acts as an indication of myocardial damage.[45] H-FABP is a cytoplasmic protein that is associated with fatty acid metabolism.[46] Following cardiac necrosis, it has been demonstrated that this new biomarker is secreted into the bloodstream.[47] During myocardial damage, the status of H-FABP is noticeably raised above its threshold level. H-FABP is a more accurate marker of cardiac damage than Myo since it is mostly expressed in cardiac tissue as opposed to skeletal muscle.[48] In the present investigation, Myo, H-FABP, GP-BB, and CK-MB serum levels were considerably elevated in rats treated with doxorubicin. These blood cardiac biomarkers' concentrations are rising, which is indicative of damaged myocardium and impaired heart architecture. These enzymes are finally released into the blood as a result of the rupture of cellular membranes in the heart tissues.[49,50] Triptonide treatment marginally depleted H-FABP, Myo, and GP-BB, despite the fact that higher levels of H-FABP, GP-BB, and Myo were seen in the doxorubicin-induced rats. These outcomes suggested the salutary properties of the triptonide on the doxorubicin-induced cardiotoxicity.

The onset of inflammation was highly regulated by the different cytokines and chemokines, such as TNF-α. Initiation and severity of chronic inflammation are also strongly correlated with other chemokines, such as MCP-1.[51] IFN-γ plays a pivotal role during the onset of the inflammatory response. Inflammatory cytokines and chemokines can be expressed in a synergistic manner when IFN-γ is present.[52] TGF-β is a cytokine that promotes fibrosis and regulates a number of fibrotic processes. For example, it can stimulate the proliferation of fibroblasts and myofibroblast differentiation, which results in the reduction of collagen and matrix proteins. TGF-β may also influence cell migration, death, differentiation, and proliferation.[53] In this work, the doxorubicin treatment revealed a marginal increase in the development of inflammatory markers such as MCP-1, IFN-γ, and TGF-β in the serum of rats. It's interesting to note that the triptonide treatment substantially reduced the levels of MCP-1, IFN-γ, and TGF-β, which indicates its anti-inflammatory properties. Furthermore, following DOX treatment, there was severe histological damage as evidenced by myocardial deterioration, inflammatory cell infiltration, and the disorganization of cardiac tissues. These outcomes supported earlier research findings.[54] The current findings of the histopathological analysis also suggested the therapeutic roles of triptonide. It effectively ameliorated the

doxorubicin-induced histological alterations in the cardiac tissues of the rats.

CONCLUSION

In conclusion, the antioxidant activities of triptonide and the stimulation of cardiac tissue antioxidant mechanisms are responsible for the therapeutic potential of triptonide against doxorubicin-induced cardiotoxicity. Additionally, it maintained the integrity of the heart tissue and regulated the biomarkers of cardiac function. Our study does have some limitations, though, we did not study the molecular mechanisms of triptonide and doxorubicin co-treatment. Future studies will require these factors to be taken into consideration. The salutary effects of triptonide on the molecular mechanisms that help prevent and treat drug-induced cardiotoxicity will also need to be studied in the future.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ATP: Triphosphate; **NO:** Nitric oxide; **CK:** Creatine kinase; **HR:** Heart rate; **SAP:** Systolic arterial pressure; **MAP:** Mean arterial pressure; **DAP:** Diastolic arterial pressure; **SOD:** Superoxide dismutase; **CAT:** Catalase; **LDH:** Lactate dehydrogenase.

SUMMARY

DOX treatment, there was severe histological damage as evidenced by myocardial deterioration, inflammatory cell infiltration, and the disorganization of cardiac tissues. Triptonide maintained the integrity of the heart tissue and regulated the biomarkers of cardiac function. It has significantly decreased the LDH, CK, and AST activities and the status of myoglobin, H-FABP, GP-BB, and CK-MB.

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