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ORIGINAL ARTICLE

Goniothalamin-Mediated Amelioration of Doxorubicin-Induced Myocardial Damage and Regulation of Nuclear Factor-κB/HO-1/NQO-1 Signaling Biomarkers in Cardiotoxic Rats

Chong Liu, Qiuju Wang[1]

Department of Cardiology, Chengwu People's Hospital, Heze 274200, [1]Foreign Joint Office, Chengwu People's Hospital, Heze, China

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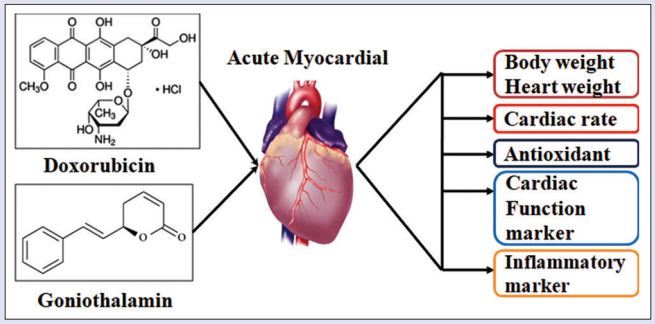
ABSTRACT

Background: Chronic use of doxorubicin (DOX) as an anticancer and antineoplastic agent has a chief jeopardy of cardiotoxicity. About 10% of the treated population has logged to cause cardiac damage. **Objectives:** This study principally engrossed on inspecting the effective cardioprotective activity of goniothalamin (GTN) against DOX-induced cardiotoxic rats. **Materials and Methods:** Group I – control, Group II – inducer (DOX) alone (2.5 mg/kg body weight [BW]) given on alternate days, Group III – DOX + GTN (2.5 mg/kg BW + 200 mg/kg BW), and Group IV – GTN alone (200 mg/kg BW). First, it employed its protective effects over the isolated cardiac tissues in which the status of HO-1 and NQO-1 were upregulated. **Results:** GTN administered with DOX induced rats were showed the increased the status of antioxidant levels, elevation of reactive oxygen species, which is also reduced the inflammatory and stress markers contributing to its cardio protective activity. Furthermore, GTN also downregulated the mRNA expression status of inflammatory markers and HO-1, NAD (P) H, and NQO-1 in DOX-induced rats, thereby weakening the cardiac damage. **Conclusion:** GTN is an effective protective agent against DOX-induced cardiotoxicity in rats.

Key words: Antioxidant, cardiotoxicity, doxorubicin, goniothalamin, nuclear factor-κB pathway

SUMMARY

- Goniothalamin (GTN) enlarged the antioxidant activity of doxorubicin (DOX)-induced cardiac rats.
- GTN also repressed the mRNA expression levels of inflammatory markers and HO-1, NAD (P) H, and NQO-1 in DOX-induced rats.



Abbreviations used: W: Body weight; DOX: Doxorubicin; TOP II: Topoisomerase II; NF-κB: Nuclear factor-κB; BP: Blood pressure; CAT: Catalase; DAP: Diastolic arterial pressure; GPx: Glutathione peroxidase; SOD: Superoxide dismutase; GSH: Glutathione; GTN: Goniothalamin; GR: Glutathione reductase; GST: Glutathione-S-transferase; CK: Creatine kinase; BNP: B-type natriuretic peptide; HR: Heart rate; LDH: Lactate dehydrogenase; SAP: Systolic arterial pressure; AST: Aspartate transferase; CK-MB: Creatine kinase-muscle/brain; MYO: Myoglobin; PETIA: Particle-enhanced turbidimetric immunoassay; MCP-1: Monocyte chemotactic protein-1; MAP: Mean arterial pressure; INF-γ: Interferon-gamma; cTnI: Cardiac troponin I; HW: Heart weight; LPO: Lipid peroxidase.

Correspondence:

Prof. Qiuju Wang,
Chengwu People's Hospital, Heze 637000, China.
E-mail: wqj0042@sina.com
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INTRODUCTION

Anthracycline antibiotic group of doxorubicin (DOX) fits to the secondary metabolite produced by *Streptomyces peucetius* var. *caesius*.[1] It is extensively used for treating multiple assortments of tumors and hematological malignancies affected in both adults and infants. DOX is an effective chemotherapeutic agent that induces severe cardiotoxicity myocardial dysfunction even from heart failure to death, which relics a challenge.[2,3] It exerts its anticancer effects by blocking topoisomerase II (TOP II) in cancer cells and causing irreversible heart injury with functional cardiac cells by apoptosis.[4] However, it has a dissimilar mechanism of action that is not comparative to cardiac dysfunction as it causes cardiac toxicity though TOP II is not articulated in cardiac cells.[5,6] However, further reports advise that elevated free radicals and cell death in cardiomyocytes contribute to DOX-induced cardiotoxicity.[7] As the underlying pathological mechanism remains unknown, it is more tough to measure or envisage its adverse effects in patients.[8,9]

DOX treated rats were showed the chronic oxidative stress and elevated the nuclear factor-kB (NF-kB), and tumour necrosis TNF-alpha to the cardiac cells inducing its damage.[10] Cardiac damage due to the DOX treatment persuades apoptosis through mitochondria, increasing cyt-c release and prompting caspase-3-induced cell death.[11-14] HO-1, NQO1, and superoxide dismutase (SOD) are the downstream effectors of Nrf2 due to chronic oxidative stress.[15] Even though there are many effective

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antioxidant agents, they have side effects of cardiac toxicity; there is a necessity for therapeutic agents to treat DOX-induced toxicity.[16]

Goniothalamin (GTN) is a styryl lactone, isolated from the *Goniothalamus* species of the Annonaceae family. They possess valuable properties according to clinical aspects, such as anticancer, anti-inflammatory, and antineoplastic properties.[17-19] Compared with DOX, GTN displayed no toxicity against liver cells with specific cytotoxicity against cancer cells. Although numerous natural phytochemicals isolated from plants have a critical role in cancer treatment, many of the molecular mechanisms governing the anticancer effects remain uncharted. Different results also portray maybe their anticancer effects were attributed to the apoptosis-induced cell death.[20] Owing to its outstanding pharmacological properties, we highlight that it could be an effective cardioprotective agent.

This study were investigated that the cardiopreotective effect of GTN against DOX-induced heart damage by reduced the cardiac stress by induced anti-oxidative enzymes, thereby reguting NFkB/HO-1/NQO-1 signalling pathways. We accentuate that GTN may be a protective agent bypassing and suppressing the cardiac toxicity induced by DOX-treated rats.

MATERIALS AND METHODS

Chemicals

DOX, GTN, and other chemicals for the study were also gained from Sigma-Aldrich Chemicals, St. Louis, MO, USA.

Animals

Male Wistar rats (6–7 weeks old) weighing about 190–220 g, were selected and the animals were maintained with controlled humidity (65% ± 5%) and temperature (25°C ± 2°C) in a sterile room. The rats were sustained under 12-h light and dark cycles and kept on standard pellet diet and fresh water *ad libitum*. As per the Institutional Animal Ethics Committee's norms, the study was permitted by the Chengwu People's Hospital Animal Ethical Committee (Approved No: HZCW-827).

Animal experimental design

All experimental rats were considered into four groups covering 6 rats each, i.e., Group I – control, Group II – inducer (DOX) alone (2.5 mg/kg body weight [BW]) given on alternate days, Group III – DOX + GTN (2.5 mg/kg BW + 200 mg/kg BW), and Group IV – GTN alone (200 mg/kg BW). All animals were forfeited by cardiac puncture at the termination of the experiment. The blood was extracted via cardiac puncture, centrifuged to extract the serum for 20 min, and processed for further biochemical analysis at −20°C. The BW and heart weight (HW) of the animals were chronicled. In addition, blood pressure (BP) determinants were analyzed using the non-invasive electronic tail-cuff technique.

Histopathology analysis

Before exposing to the histopathologic analysis, the heart was isolated from the induced and treated animals. Then, the heart detached was fixed with neutral formalin buffer (10%) and embedded with paraffin wax. After fixation, the tissues were sliced around 4–6 mm in size and stained with hematoxylin-eosin (H and E) and analyzed under a microscope.

Homogenate preparation

A cardiac puncture sacrificed animals after the experiment; the heart tissues were exposed to molecular and biochemical examination. Heart tissues were homogenized by a 10% tissue homogenization lysis buffer of pH 7.6, and the lysates were conserved at − 20°C for future use. Heart tissues were homogenized by a 10% tissue homogenization lysis buffer of pH 7.6, and the lysates were preserved at − 20°C for future use.

Biochemical evaluation

The cell lysates were exposed to biochemical analysis after homogenization. The TBARS production was established per Ohkawa *et al.* 1979. The levels catalase (CAT), GPx, SOD, glutathione (GSH), glutathione reductase (GR), and glutathione-S-transferase (GST) were examined as per the method Sinha,[21] Rotruck *et al.*,[22] Kakkar *et al.* (1984) technique, Omura and Sato (1964) technique, Carlberg and Mannervik (1985),[23] and Habig *et al.* (1974),[24] respectively.

Enzyme levels such as creatine kinase (CK), lactate dehydrogenase (LDH), and aspartate transferase (AST) were measured in the serum by a Hitachi automated analyzer. The CK-muscle/brain (CK-MB) levels were restrained by using a colorimetric test kit (Cat. No. KT-12247) procured from Spinreact, Girona, Spain. The serum levels of MYO were measured using a particle-enhanced turbidimetric immunoassay (PETIA) kit (Cat. No. KT-60345) from Kamiya Biomedical Company, Washington, USA.

Determination of cardiac, cytokines and inflammatory mediators by ELISA method

The cytometric bead array kits were bought from Thermo Fisher Scientific, Waltham, MA, USA, and assessed the serum cytokine levels, including MCP-1 (Cat. No. BD-558342) and interferon-gamma (INF-γ) (Cat. No. BDB-558305) as per manufacturer protocol. In addition, the cytokines HFABP (Cat. No. ab-242240), GP-BB (Cat. No. ab-267585), Cardiac troponin I (cTnI) (Cat. No. ab-246529), BNP (Cat. No. ab-108816), and transforming growth factor-β (TGF-β) (Cat. No. ab-119558) levels were measured by using the enzyme-linked immunosorbent assay (ELISA) kits procured from Abcam, Cambridge, MA, USA, as per manufacturer protocol.

mRNA expression levels of cardiac and stress markers

Inflammatory and cardiac stress markers of the mRNA expression levels of such as NF-κB, IL-1β, TNF-α, HO-1, NAD (P) H, and NQO-1 were surveyed reverse transcription-polymerase chain reaction. The quantitative RT-PCR (Applied Biosystems) mixture system was set subsequent to the manufacturer protocol. The primers were acquired from Integrated DNA Technologies, US, as shown in Table 1. Each reaction was completed in triplicates, and the Δct method was employed to recognize the fold changes.

Statistical analysis

Data were examined by analysis of variance and *post hoc* test Least Significant Difference (LSD) regarding the ethylene glycol group using GraphPad software and articulated in mean ± standard deviation. The data from us were measured significantly if $P < 0.05$.

RESULTS

Effect of goniothalamin on body weight and heart weight

In DOX-induced rats presented significantly ($P < 0.05$) condensed BW and HW compared to the control. The effect of GTN on the experimental animals has recuperated the BW and HW to the average levels. There was no momentous difference distinguished in the BCP- alone treated group versus the control [Figure 1].

Table 1: List of primers and their respective sequences

Primer name	Forward sequence	Reverse sequence
TNF-α	5′-GCCTATGTCTCAGCCTCTTCTC-3′	5′-GGCCATTTGGGAACTTCTCATC-3′
IL-1β	5′-TCCCATTAGACAACTGCACTAC-3′	5′-GCTCATGGAGAATATCACTTGTTG-3′
HO-1	5′-CAACATTGAGCTGTTTGAGGAG-3′	5′-GTG TCTGGGATGAGCTAGTG-3′
NQO-1	5′-ACCTGGTGATATTTCAGTTCCC-3′	5′-AGTGGTGATAGAAAGCAAGGTC-3′
NF-κB	5′-AGTTGAGGGGACTTTCCCAGCC-3′	5′-AGACCCCCAGTGCCATCAAT-3′
GAPDH	5′-TGAACGGATTTGGCCGTATTG-3′	5′-CTTGACTGTGCCGTTGAATTTG-3′

TNF-α: Tumor necrosis factor-alpha; IL-1β: Interleukin-1β; HO-1: Heme oxygenase-1; NF-κB: Nuclear factor-κB; NQO-1: NAD(P)H:quinone oxidoreductase; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

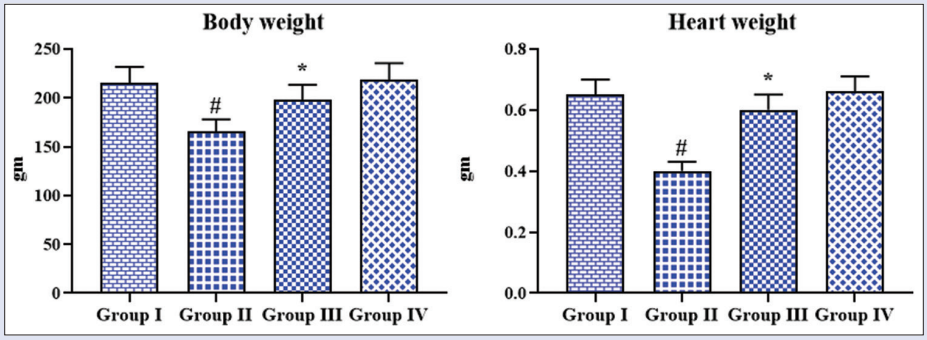

Figure 1: Effect of goniothalamin on changes of HW to BW. Bars are expressed as mean ± standard deviation for six rats in each group. Values did not share a familiar superscript note (#, *) between the groups at $P < 0.05$ (DMRT)

Effect of goniothalamin on cardiac rate and blood pressure

Compared to control rats, DOX-induced animals had signs of bradycardia. As confirmed in Group III animals, the HR variations were improved by GTN administration. Compared to control rats, significant reductions in all BP indices, namely systolic arterial pressure (SAP), mean arterial pressure (MAP), and diastolic arterial pressure (DAP), were detected in DOX-treated animals. As shown in Figure 2, treatment with GTN improved MAP, SAP, and DAP levels relative to DOX-induced rats.

Effect of goniothalamin on antioxidant enzymes

DOX-induced rats show strangely augmented TBARS and depletion in the function of antioxidant enzymes levels such as SOD, CAT, GSH, GST, GR, and GPx in Group II rats. Conversely, oral treatment of GTN (Group III) efficiently improves antioxidants, thereby plummeting LPO levels in DOX-induced cardiomyopathy rats. However, GTN-alone treatment has no noteworthy fluctuations in antioxidant levels compared to control [Figure 3].

Effect of goniothalamin on levels of cardiac marker enzymes

The levels of antioxidant and cardiac markers, were analyzed such as AST, GP-BB, CK-MB, Myo, H-FABP, CK, and LDH, in experimental animals [Figure 4]. Group II animals stated substantial deviations in GP-B, H-FABP, CK-MB, CK, LDH, Myo, and AST markers compared with control rats. Compared with test animals, treatment with GTN (200 mg/kg BW) pointedly declined all these marker enzyme levels. There are no important differences in cardiac markers found between the control and rats treated with GTN.

Effect of goniothalamin on inflammatory markers

The results of cytokine production are shown in Figure 5. The results exposed that DOX-induced animals displayed a considerable surge in MCP-1, INF-γ, TGF-β, BNP, and cTnI. On the contrary, inflammatory markers in GTN-treated animals had a noteworthy downregulation effect. In addition, non-significant upsurges in inflammatory markers have been recognized in GTN-treated rats compared to the controls.

Histopathological changes in cardiac tissue

As evidenced by pathological variations in the heart anatomy with extreme leukocyte infiltration and necrosis, the rats administered with DOX (Group II) advanced a severe heart dysfunction [Figure 6]. Group III rats display enhanced cardioprotection as inspected in the hearts of DOX-induced rats due to the absenteeism of complicated pathological adjustments. The standard tissue architecture of control was seen in animals treated with GTN alone (Group IV).

Reverse transcription-polymerase chain reaction expression of inflammatory stress markers

The production of inflammatory stress markers such as NF-κB, IL-1β, and TNF-α has been augmented in rats induced with DOX compared to control rats [Figure 7]. Condensed inflammatory marker expression was meaningfully contemporary when treated with GTN. A reduction in HO-1 and NQO-1 levels has also been detected, as per the control-assessed DOX-induced rats' results. There was an enlarged status of HO-1 and NQO-1 expression in GTN-treated rats.

DISCUSSION

By redox cycling, DOX can produce reactive oxygen species (ROS) causing an imbalance in the endogenous enzymes accountable for antioxidant activity.[25,26] However, heart failure is due to the apoptosis of cardiomyocytes unswervingly or indirectly related to oxidative stress.[27] Thus, the two chief means of DOX-mediated cardiotoxicity are anti-oxidative and anti-apoptotic therapeutic agents. Lower expression in the BP related to the untreated control group in the DOX-induced animals decides with the earlier reports due to cardiac development.[28]

CK-MB and troponins I have been concerned in myocardial damage, which is released to the blood circulation and serves as an indicator for myocardial damage identification.[29] The levels of MCP-1, INF-γ, AST, CK-MB, LDH, CK, and cTnI were augmented in DOX-induced animals, which have been later assuaged due to the action of GTN. Alterations in LDH and CK-MB levels may be due to the necrotic lesions with disruption in the membrane's integrity developed in the DOX-induced rats, released to the blood circulation on the onset of myocardial damage.[30,31] In the present study, GTN-treated animals have conserved the myocardial membrane's integrity, thereby keeping their level substantially equal to the controls. They have augmented ROS generation, which has been broadly occupied in the myocardial damage-inducing formation of myocardium malondialdehyde (MDA) formation, signifying the amplified accumulation of lipid peroxidase (LPO). Besides, the GTN reduced LPO and TBARS levels, tumbling ROS generation levels, which has useful effects over reducing oxidative stress.[2,32]

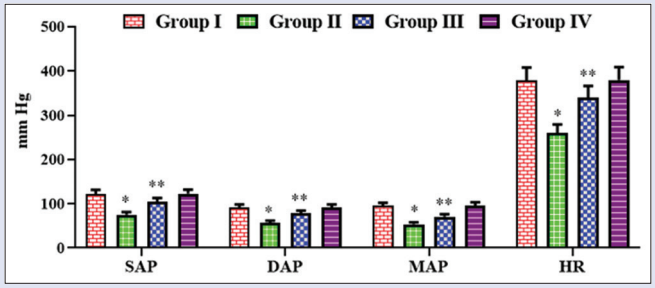

Figure 2: Effect of goniothalamin on hemodynamic parameters. Bars are expressed as mean ± standard deviation for six rats in each group. Values did not share a familiar superscript note (*, **) between the groups at $P < 0.05$ (DMRT)

In line with prior results, preeminent levels of GP-BB, myoglobin, and H-FABP reminiscent of cardiac damage as a biomarker were pragmatic in DOX-treated cardiotoxic rats serum in this research.[33,34] GTN therapy has also significantly diminished the serum levels in DOX-induced rats. By converting superoxide radicals to H_2O_2, CAT can eventually convert to oxygen and water; the functions of SOD are a pivotal role in protecting against oxidative damage. Augmented

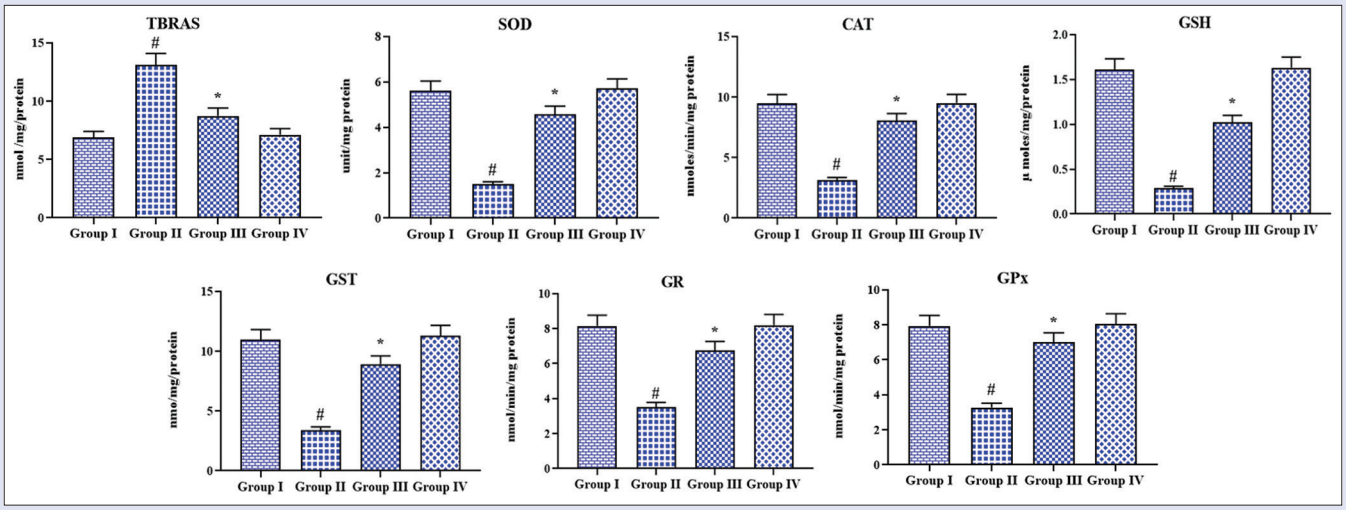

Figure 3: Effect of goniothalamin on levels of and LPO and antioxidant enzymes in cardiac markers in cardiac tissues. Bars are expressed as mean ± standard deviation for six animals in each group. Values do not share a familiar superscript note (#, *) between the groups at $P < 0.05$ (DMRT)

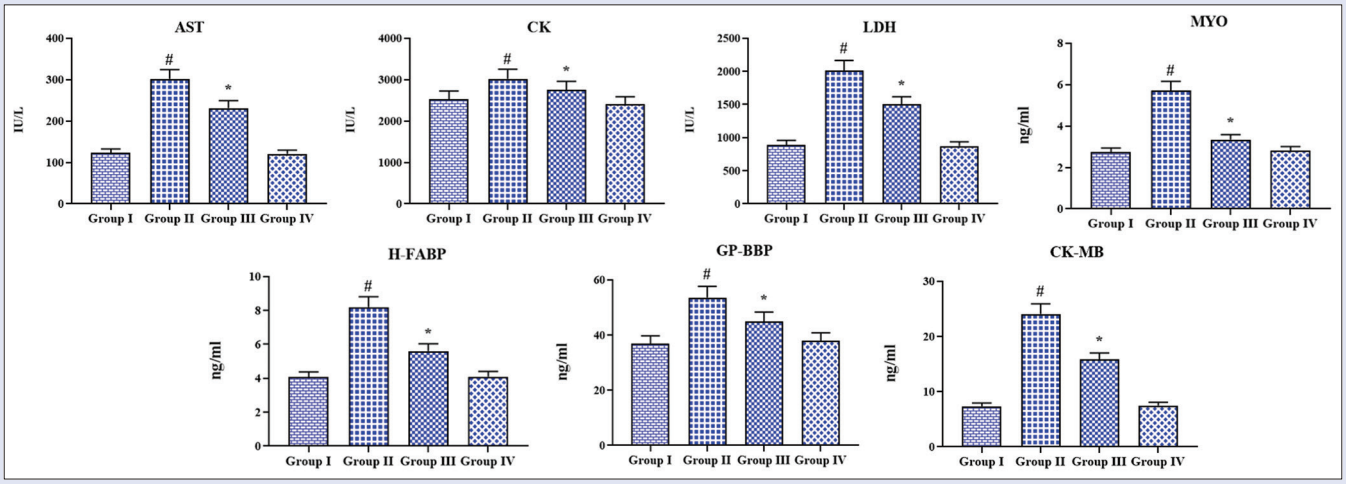

Figure 4: Effect of goniothalamin on status of the cardiac marker. Bars are expressed as mean ± standard deviation for six rats in each group. Values do not share a familiar superscript note (#, *) between the groups at $P < 0.05$ (DMRT)

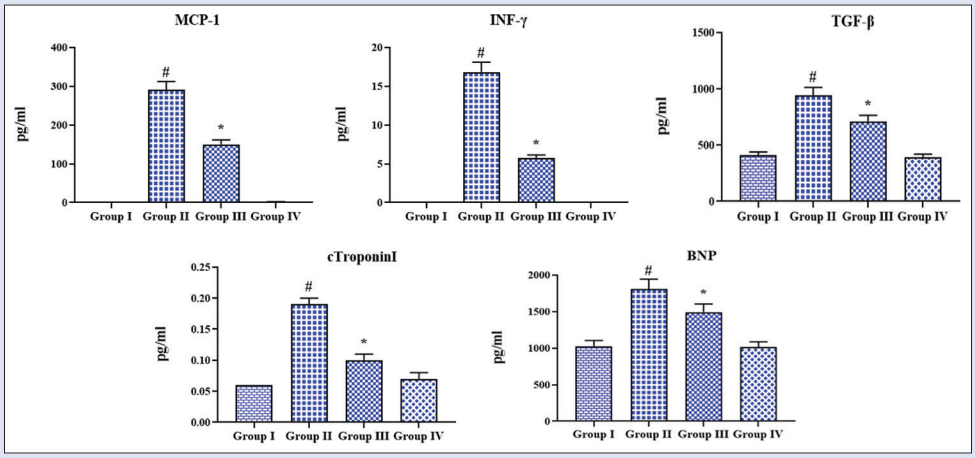

Figure 5: Effect of goniothalamin on inflammatory markers in control and experimental rats. Values do not share a familiar superscript note (#, *) between the groups at $P < 0.05$ (DMRT)

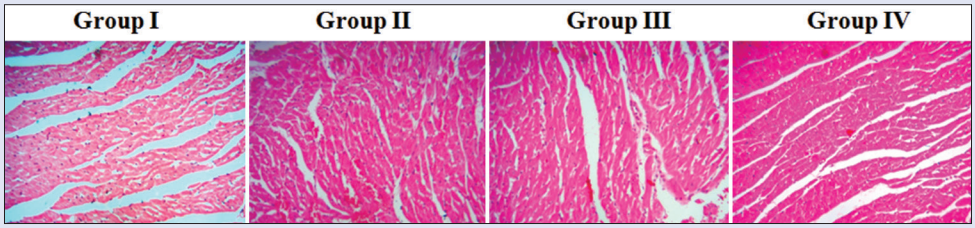

Figure 6: Histological sections of the cardiac tissues. Control (Group I), doxorubicin-induced (Group II), doxorubicin + goniothalamin (Group III), and goniothalamin alone (Group IV)

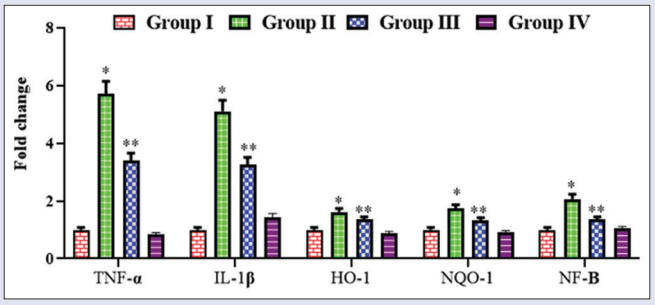

Figure 7: mRNA expression levels of cytokines, gene transcription, and antioxidant pathway regulators. Values do not share a familiar superscript note (#, *) between the groups at $P < 0.05$ (DMRT)

development of superoxide radicals condensed antioxidant activity and myocardium damage.[27]

A condensed substrate concentration, such as GSH, may be ascribed to lessened GST and GPx activities. The key cause for mitochondrial mechanism inequality is a diminution in the level of GSH.[35] GR is an energetic enzyme for the conservation of GSH levels, so it makes an essential role as a substrate for GST and GPx. In our study, in line with preceding results, DOX-treated rats have been revealed to have declined SOD, GSH, GST, GPx, CAT, and GR activities in the heart.[28] GTN has meaningfully amplified the mitochondrial levels of antioxidants mentioned above and the reduced LPO levels, highlighting its antioxidant activity, thereby having cardioprotective properties during ischemia. Similar to the GTN, catechin has also applied its cardioprotective effects over adriamycin-treated cardiotoxicity.[36]

In cultured cardiomyoblasts, DOX is a potent inducer of NF-κB, while the foremost pro-inflammatory mediator interferon-γ leads to DOX activity in the metabolic pathways.[37,38] The cut in GSH detected through ISO therapy could rise TNF-alpha production. Cell breakdown were increased by BNP production and TGF-β level in DOX-induced cardiotoxicity, measured the primary cardiomyopathy marker.[28] Increased production of ROS due to DOX triggering could have subsidized to overexpression of TNF-α, TGF-β, IL-1β, BNP, and NF-κB in our present data. The earlier research also shows the DOX-induced increased expression of these inflammatory mediators in line with the contemporary work.[39] Nrf2 functions as a vital downregulation of DOX-induced cardiomyopathy signifying the possibility that the Nrf2 target might be an effective drug strategy to lessen DOX-induced cardiotoxicity.[40,41] The levels of the mRNA expressions of TNF-α, TGF-β, IL-1β, BNP, HO-1, NQO-1, and NF-κB were also radically condensed in comparison to the control, which designates the cardioprotective nature of GTN.

CONCLUSION

In summary, it is apparent from the verdicts that GTN has strong cardioprotective activity in rats against DOX-induced cardiotoxicity by the lower appearance of the status of TNF-α, TGF-β, IL-1β, BNP, HO-1, NQO-1, and NF-κB. In addition, it also regulated the levels of cardiac, inflammatory stress and oxidative stress markers such as AST, GP-BB, CK-MB, Myo, H-FABP, CK, LDH and CP-1, INF-γ, TGF-β, BNP, and cTnI, respectively, in par with the levels of the animal control demonstrating the cardioprotective effects of GTN. Thus, GTN is an important protective agent against cardiotoxicity caused by DOX.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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