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Original Article

# Doxorubicin-induced Chronic Heart Failure is Alleviated by Gentiopicroside by Inhibiting Oxidative Stress and Inflammation

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## ABSTRACT

**Aim/Background:** To study the Oxidative stress and inflammation inhibited by Gentiopicroside in chronic heart failure induced by doxorubicin. **Materials and Methods:** The procured healthy rats were first divided into four groups group I was maintained as healthy control and the others were subjected to the induction of doxorubicin-induced chronic heart failure (Dox-CHF) by2.5 mg/kg/day DOX by i.p. on alternate days for 2 weeks. Then the Dox-CHF rats were further divided into three groups, group II was used as the Dox-CHF control rats, the group III and group IV were administered with Gentiopicroside (25 mg/kg b.wt and 50 mg/kg b.wt, respectively) for 2 weeks. The heart tissue and body weight, Serum Lipids, Lipid Peroxidation and Antioxidants, Biomarkers enzymes for Cardiac Injury such as Aspartate Aminotransferase (AST), Creatine Kinase (CK), and Lactate Dehydrogenase (LDH)), Immunological Biomarkers such as Heart-type fatty acid-binding protein H-FABP, Glycogen Phosphorylase isoenzyme BB (GP-BB), and Creatine Kinase-MB isoenzyme (CK-MB), Inflammatory Interferon-γ (INF-γ), and Monocyte Chemotactic Protein-1 (MCP-1)) and pro-inflammatory (TNF-α and IL-6) levels were estimated at week 0 and week 2. **Results:** The cardioprotective properties of Gentiopicroside were evaluated in a Doxorubicin-induced cardiomyopathy model. Gentiopicroside was highly effective in combating the cardiotoxic attributes manifested by DOX at 2.5 mg/kgs, such as abnormal hemodynamic parameters, oxidative stress, and inflammation. Based on the reduced cardiotoxicity biomarkers, it was capable of reducing serum lipids, enhancing host antioxidant expression, and suppressing lipid peroxidation. According to the results of this study, Gentiopicroside reduces oxidative stress and inhibits inflammatory responses in DOX-administered individuals, suppressing cardiotoxicity. **Conclusion:** All the test results concluded that Gentiopicroside can treat DOX-induced cardiotoxicity.

**Keywords:** Gentiopicroside, Doxorubicin, Cardiotoxicity, Oxidative stress, Anti-inflammatory.

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## INTRODUCTION

Anthracyclines are a group of antibiotics isolated from *Streptomyces* spp. Which include many effective chemotherapeutic drugs for the treatment of various cancers such as leukemia, lymphomas, and malignant tissues. Their therapeutic activity is believed to be due to their DNA modifying activities such as topoisomerase-II binding, DNA intercalation, DNA adduct formation, and Reactive Oxygen Species (ROS) production.[1] Anthracyclines are known to be among the most effective anti-cancer drugs ever developed, of which Doxorubicin (DOX) and Daunorubicin (DNR) were the first anthracyclines isolated from the pigment produced by *Streptomyces peucetius*.[2] Despite this, anthracyclines cause many adverse side effects during chemotherapy. In this study, we examined the effect of the chemotherapy drug Doxorubicin (DOX) on chronic heart failure. This drug has been reported to cause acute nausea, vomiting, alopecia baldness, stomatitis, gastrointestinal disturbances, neurologic disturbances, cumulative cardiotoxicity, and bone marrow aplasia. These are some of the most common side effects that make survival through chemotherapy challenging for the victim. Cardiotoxicity of anthracyclines has been the most reported effect of them all.[3]

Epirubicin, a derivative of DOX developed by epimerization of the daunosamine carbon of DOX, modified the molecule

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to be more pharmacokinetic, introducing characteristics such as increased distribution, and shorter half-life. Despite DOX's beneficial therapeutic effects, its cardiotoxicity cannot be eliminated.[4] Anthracyclines, like DOX, cause both acute and chronic cardiotoxicity. Due to the acute cardiotoxicity of this drug, hypotension, arrhythmias, pericarditis, and tachycardia manifest. Chronic cardiotoxicity, however, presents more severe health conditions identified by congestive heart failure, and progressive left ventricular dysfunction which can lead to extreme cases that are fatal.[5] The Centers for Disease Control and Prevention (CDC) estimates about 1 million cancer patients receive chemotherapy annually in the USA in 2018.[6] Doxorubicin-induced cardiomyopathy ranges from 1.7% to about 6.8% of the total prevalence of cardiomyopathy cases overall. Studies point out that the severity and magnitude of cardiac dysfunction are directly associated with the cumulative dose of the drug received by the patient.[7] It is also suggested that the maximum dosage should not surpass 550 mg/m$^2$ of body surface area.[8]

Doxorubicin is widely used along with cyclophosphamide, taxanes, and 5-Fluorouracils (5-FU) as an adjuvant during chemotherapy against solid tumors.[6] Its chemotherapeutic action is a function of topoisomerase poisoning, ROS production, and inducing apoptosis.[9] The structure of DOX possesses a quinone moiety that favours the redox reaction of NADPH, which produces free radicals by donating unpaired electrons to oxygen. These free radicals are capable of modifying essential biomolecules, thus inducing oxidative stress. This is one of the significant mechanisms responsible for causing cardiotoxicity to the recipient.[10]

It is currently possible to prevent cardiotoxicity resulting from DOX by using methods such as (a) continuous infusion process, (b) liposome-mediated targeted delivery of DOX, (c) administering cardio-protective agents such as dexrazoxane as well as DOX simultaneously, and (d) treating with DOX and antioxidants such as Erythropoietin (EPO) and Thrombopoietin (TPO).[11] However, there are no specific guidelines for the management of DOX-induced cardiotoxicity. Supportive clinical studies are required to develop an alternative pharmacological product that can be used in the diverse patient population who receive DOX chemotherapy.[9]

Gentiana plants and Swertia plants are traditionally used for their anti-inflammatory, choleretic, and hepatoprotective properties.[12] According to previous reports, the plants were a significant source of bitter secoiridoid glucosides.[13] Gentiopicroside, another member of this family, also exhibits similar properties.[14,15] However, this valuable compound has not been extensively examined in terms of its cardioprotective properties. This investigation intends to study the cardioprotective characteristics of Gentiopicroside by inducing cardiomyopathy using Doxorubicin-a frequently reported comorbidity among cancer patients who have undergone chemotherapy.

# MATERIALS AND METHODS

## Chemicals

Pure analytical grade Doxorubicin and Gentiopicroside were purchased from Sigma-Aldrich (USA) for this study. All the kits for biomarker estimation were performed using commercial kits purchased from BioVision (California, USA), Origene (Maryland, USA) and Raybiotech (Georgia, USA).

## Experimental Animals

The experimental animals adopted for this investigation were handled as per the Institutional animal ethics guidelines. Male Wistar rats weighing approximately 250g each were acclimatized in separate enclosures with 12-hr of equal light and dark cycles, at 22°C ± 2°C controlled temperatures. All the rats were fed with standard sterile pellet feed with water *ad libitum*. The animals were acclimatized for a week before initiating the treatment.

## Experimental Plan

The rats were divided into 4 groups of 6 animals that were housed separately in each enclosure for 14 days. The Groups were numbered serially from I to IV and received treatment as mentioned below:

**Group I** – Reserved as Control. Administered with standard pellet feed only and was allowed access to water *ad libitum*.

**Group II** – 2.5 mg/kg/day DOX i.p. on alternate days for 2 weeks.

**Group III**– 2.5 mg/kg/day DOX i.p. with 50 mg/kg/day Gentiopicroside p.o. for 2 weeks.

**Group IV**– 50 mg/kg/day Gentiopicroside p.o. for 2 weeks.

After 14 days of treatment, the animals were sacrificed by cervical dislocation and decapitation. Serum was separated from the blood samples collected from all rats and stored at -20°C until further experiments. The heart of all rats was excised, rinsed with ice-cold saline, weighed and stored at -20°C until further assays.

## Blood and Tissue Sample Collection

Blood specimens were collected to separate the sera; the collected blood samples were allowed to coagulate in anti-coagulant free tubes. The specimens were centrifuged at 3000 rpm at 4°C for 20 min. The separated sera were used to estimate the biochemical parameters targeted in this study.

The heart was excised from individual rats, rinsed with saline and weighed. The weighed heart was stored as a homogenate as well as whole heart tissue, at -80°C until further study. Tissue homogenate was prepared by homogenizing the heart tissue sections with ice-cold PBS to achieve a 10% w/v solution. The homogenized solution was centrifuged at 3000 rpm/4°C/10 min to segregate a clear supernatant from the solids. This supernatant was as homogenate at -80°C for further analysis.

### Estimation of Heart and Body Weight

Every rat was weighed before and after the treatment period. Post-treatment, the rats were sacrificed, and their hearts were excised to measure their heart weight. The observed data were recorded for comparative studies.

### Hemodynamic Parameters

On the 14th day of treatment, tail-cuff plethysmography was performed with a pressure meter. This was performed to estimate the Heart Rate (HR), Systolic Arterial Pressure (SAP), Diastolic Arterial Pressure (DAP), and Mean Arterial Pressure (MAP) of all experimental rats including individuals from the control group. The observed values were recorded.

### Serum Lipids

The separated sera were taken for the estimation of Total Cholesterol (TC), Triglyceride (TG), Low-density Lipopolysaccharides (LDL), Very Low-Density Lipopolysaccharides (VLDL), High-Density Lipopolysaccharides (HDL-chol), and the Atherogenic Index (AI), respectively. Serums TC, TG, HDL-chol were estimated according to the method prescribed by Burstein and Richmond.[16,17] Atherogenic Index (AI) was calculated using the formulae:

$$AI = (LDL\text{-}chol/HDL\text{-}chol) \quad \ldots\ldots\ldots\ldots (1)$$

### Lipid Peroxidation and Antioxidants

The presence of lipid peroxidation was monitored through the estimation of Thiobarbituric Acid Reactive Substances (TBARS). These are low molecular weight markers produced as a result of lipid peroxidation due to oxidative stress.[18] Lipid peroxidation was monitored by targeting TBARS. The assay was performed with commercially purchased kits following the manufacturer's protocol (BioVision (California, USA)). Oxidative stress present in the body was estimated through the quantification of antioxidants according to the given authors-*Superoxide dismutase* (SOD),[19] *Catalase* (CAT),[20] *Glutathione peroxidase* (GPx),[21] Glutathione (GSH),[22] *Glutathione reductase* (GR),[23] and *Glutathione S-transferase* (GST),[24] from the tissue homogenate specimen, following the instructions provided in the commercial kit (BioVision (California, USA)).

### Biomarkers for Cardiac Injury

Post-treatment symptoms of cardiac injury were investigated by estimating the levels of *Aspartate aminotransferase* (AST), *Creatine kinase* (CK), and *Lactate dehydrogenase* (LDH) present in the serum of all the rats. The manufacturer's protocol was followed to estimate the above-mentioned parameters using the purchased kits (Origene (Maryland, USA)).

### Immunological Biomarkers

Heart-type fatty acid-binding protein H-FABP, *Glycogen Phosphorylase* isoenzyme BB (GP-BB), and *Creatine Kinase-MB* isoenzyme (CK-MB) indicate the manifestation of acute cardiotoxicity. These markers aid the early diagnosis of the clinical condition. These markers were assessed from the sera of all rats using the commercial kit protocol (Raybiotech (Georgia, USA)).

### Inflammatory markers

The presence of inflammation was analyzed by estimating the presence of pro-Inflammatory mediators including Interferon-γ (INF-γ), and Monocyte Chemotacticprotein-1 (MCP-1) in the tissue homogenate by ELISA using procured commercial kits following the kit insert provided (BioVision (California, USA)).

### Pro-inflammatory Cytokines

TNF-α and IL-6 were assessed from the serum samples collected using the diagnostic kits following the instructions provided by the manufacturer (BioVision (California, USA)) to study the dynamics of inflammation among the rat groups.

### Statistical Analysis

All experimental values have been expressed as Mean ± S.E.M. All results have been analyzed through one-way Analysis of Variance (ANOVA). *Pos hoc* test for group comparison was performed in Tukey using SPSS for Windows. Differences have been considered significant at $p<0.05$.

## RESULTS

### Heart and Bodyweight

The heart and body weight differed significantly among all the groups in the experiment. The highest body and heart weight was observed in the Control Group I and the lowest weight was observed for Group II which received 2.5 mg/kg DOX alone. Group IV showed minimal deviation from the Control Group I that was administered with 50 mg/kg of Gentiopicroside only. Group IV rats presented a reduced body weight however it was seen to be substantially higher than that of Group II as evident from the height in the bars for both heart and body weight (Figure 1). Group III animals were observed with a lower-body and heart weight than Group IV but the weight was seen to be much higher than Group II rats.

### Hemodynamic

The rats were assessed for physiological parameters to study the condition of the heart on the 14th day of the treatment. SAP, MAP, HR, and DAP were quantified and the following observation was made. SAP was observed to be highest in Group IV when compared to the Control Group I and the pressure was observed to decrease upon shifting towards Group III and Group II (Figure 2). Group III which received a combination of DOX and

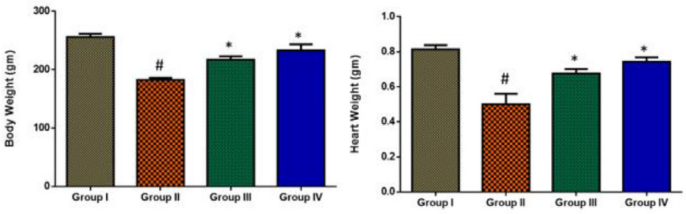


**Figure 1:** Body and Heart weight were compared to confirm the effect of DOX and check the ameliorative activity of Gentiopicroside in the experimental animals. The body and heart weight can be observed to be higher for Group III than for Group II.

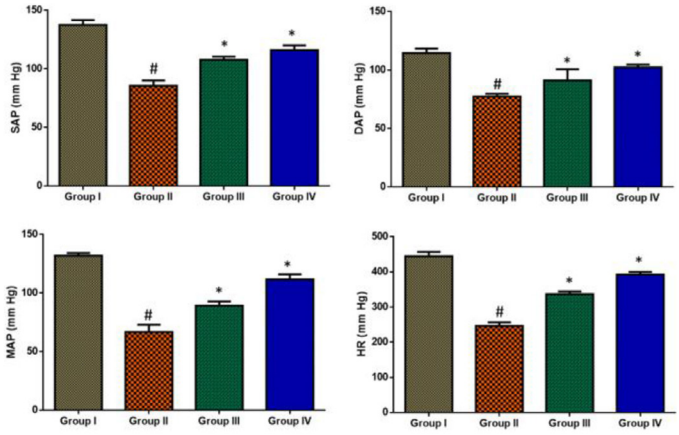


**Figure 2:** Assessment of hemodynamic parameters-SAP, DAP, MAP and HR, done to confirm the presence of cardiac injury. Group III treated with DOX and Gentiopicroside presents a lower degree of injury depicted by the higher bar than that of Group II.

Gentiopicroside showed a substantially elevated SAP than Group II. Group IV that received Gentiopicroside alone presented minimal pressure difference from that of Group I and Group III. DAP showed similar dynamics as observed for SAP, but the pressure difference between that of Group III and IV was significant. The trend in the DAP graph was observed to decrease upon moving from Group IV to Group II. The Controls showed a much-elevated DAP level than the other three experimental groups.

MAP or mean arterial pressure when compared among the group furnished coexisting results with that of the previous parameters SAP and DAP. A similar increasing trend in MAP was seen when the treatment shifted from DOX to Gentiopicroside as evident in the bar height difference observed among the experimental Groups II to IV. The MAP levels of Group IV were quite similar to that of Group I. Heart rate in Group II (which was administered with 2.5 mg/kg of DOX) was observed to be reduced most among all the other experimental groups. The Controls showed the most elevated HR, followed by Group IV and Group III, which received 50 mg/kg of Gentiopicroside.

## Cardiac Markers

Cardiac markers for cardiotoxicity including-TC, TG, LDL, and VLDL, were estimated in the serum samples of all experimental animals. There was a similar inclination in the graph was observed in all the 4 parameters mentioned above. The serum lipid levels decreased from Group II to Group IV, where Group IV showed the lowest lipid levels when compared to the Control Group I and Group II presented the highest. Group III which received both DOX and Gentiopicroside showed a notable demotion of lipids when compared to Group II.

HDL-cholesterol levels differed in the trend from that of the previous parameters. The highest value of HDL-cholesterol was seen in Group IV when compared to the Controls as seen in Figure 3, and the levels reduced while shifting to Group III and II. Atherogenic Index (AI) was also estimated to study the manifested cardiotoxicity which showed the highest index value of >7 for Group II and the lowest <4 was observed for Group IV upon comparing with the Control Group I. Group III showed a lower AI of <6.

## Lipid Peroxidation and Antioxidants

TBARS was assessed to investigate the lipid peroxidation episode within the experimental rats. The recorded values revealed a high TBARS concentration in Group II and the concentration reduced in Groups III and IV. The Control Group I showed the least value depicting the normal value.

Antioxidants SOD, CAT, GPx, GST, GR and GSH were quantified post the treatment with DOX and Gentiopicroside respectively. The recorded data showed an analogous inclination for all the parameters evident in Figure 4. The antioxidants listed above were minimal in Group II and the levels saw an inclination in Group III and IV. The highest values were depicted by Group I Control rats. Group III and IV presented a substantially increased concentration of antioxidants in comparison to that Group II. This is evident from the graphs illustrated in Figure 4.

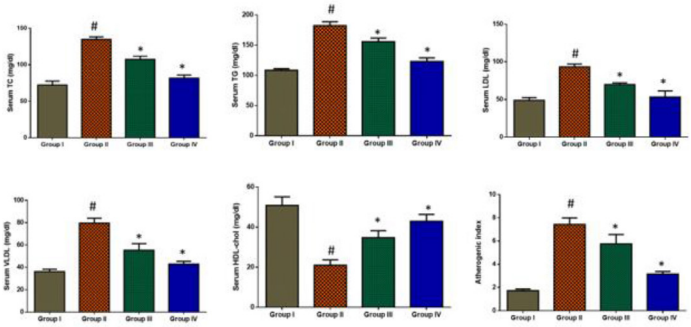

**Figure 3:** Serum lipid profiling and atherogenic index were performed to assess the inflammation present in the heart. Group III presents lower lipid levels than Group II, except for HDL-cholesterol. This explains the lower magnitude of damage caused due to DOX administration when compared to Group II.

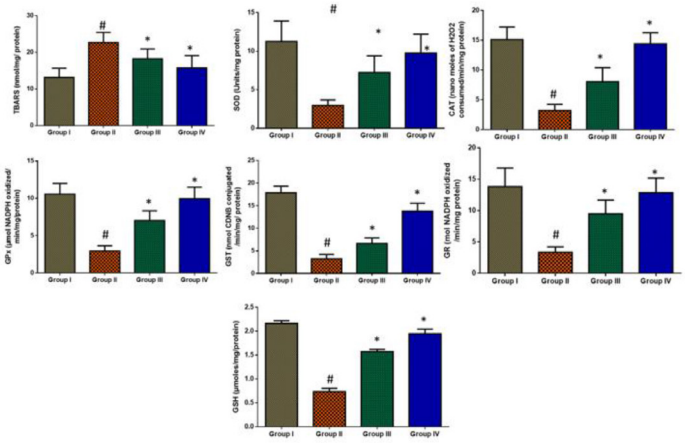


**Figure 4:** Assessment of lipid peroxidation through the biomarker TBARS. The high value of peroxidation is indicated by the height of the bar as seen in Group II. Group III shows a shorter bar than Group II, indicating the suppression of cardiotoxins. The concentration of antioxidants was also elevated by Gentiopicroside was visible in Group III when compared to controls and Group II data.

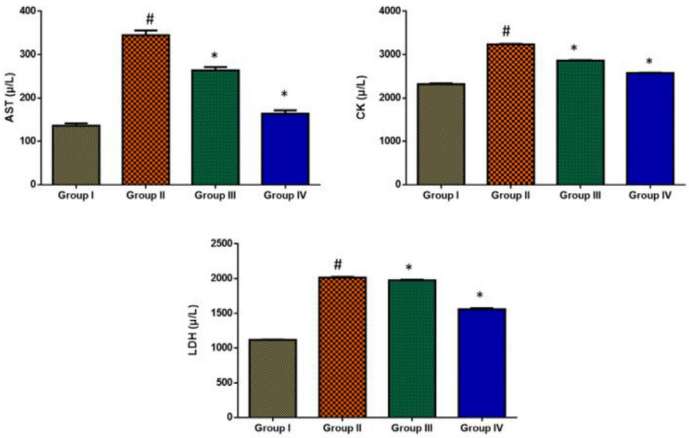


**Figure 5:** The cardiac injury was investigated through the direct estimation of biomarkers-AST, CK and LDH. A lower level of these markers indicates a healthier condition of the heart as indicated by Control Group I. Gentiopicroside is capable of suppressing the level of these cardiac markers.

## Cardiac Enzymes

Manifestation of myocardial infarction was investigated through the estimation of cardiac enzymes AST, CK and LDH. The sera of the experimental animals revealed a high concentration of all three cardiac enzymes in Group II (Figure 5). The concentration, however, was observed to reduce in experimental Groups III and IV, when compared with the Control which showed the least concentration for all 3 enzymes. Group III and IV presented comparable results, in which Group III showed a higher value for the enzymes than Group IV.

## Biochemical and immunological parameters

H-FABP, CK-MB and GP-BBP, were monitored to study the heart condition post-treatment period. It was noted that an elevation in the concentration biomarkers in Group II. The

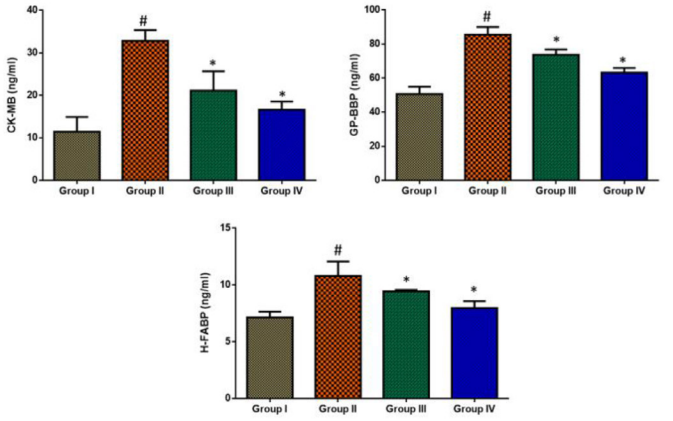


**Figure 6:** Immunological markers assessed that aid the early diagnosis of cardiac injury due to DOX. All three markers depict the ameliorative effect of Gentiopicroside as evident from the reduced height in the bars of Group III when compared to Group II and control.

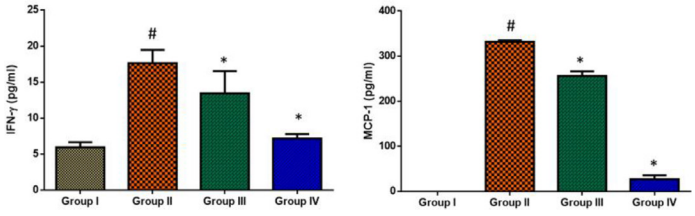


**Figure 7:** Inflammatory markers INF-γ and MCP-1 are estimated to study the inflammation produced by DOX. Gentiopicroside was able to inhibit the inflammation to a substantial extent as displayed by Group III.

concentration declined in Group III and Group IV (Figure 6). The least concentration was recorded in the Control group I. Group IV produced similar readings to Group I for CK-MB and H-FABP. Though Group III was treated with DOX a reduced level of enzymes was recorded when compared to Group II.

## Inflammatory Markers

The presence of inflammation was assessed through the quantification of INF-γ and MCP-1. The control rats did not express MCP-1, as evident from Figure 7. Group II revealed a significantly high concentration of MCP-1. Conversely, Group III and Group IV showed a diminishing value for the same. INF-γ was also noted to be concentrated in Group II. Groups III and IV treated with Gentiopicroside showed suppression in INF-γ levels as shown in the Figure 7.

## Pro-inflammatory Cytokines

ELISA results for the estimation of TNF-α and IL-6 illustrated a reduction in concentration from Group II to Group IV serially. Group IV specimens furnished the lowest concentration while Group II was observed to be at the highest level when compared with the Control (Figure 8).

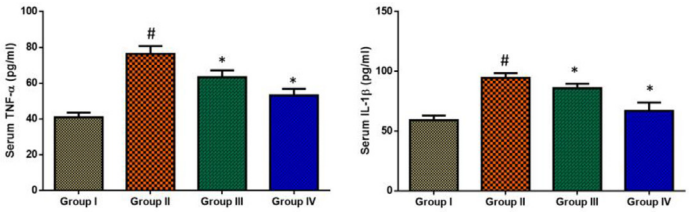


**Figure 8:** Pro-inflammatory cytokines were estimated to compare the effect of Gentiopicroside treated rats with DOX-induced cardiotoxicity. Group III bar indicates the suppression of cytokines upon administration of Gentiopicroside, explaining the cardio-protective activity.

## DISCUSSION

With the remarkable advancements in medical discovery and development, the survival rate of cancer patients' post-chemotherapy has increased.[25] The statistics are expected to further illustrate an increase in the rate in the next few decades.[26] Though the phrases sound overwhelming, there still exist a large number of people who suffer severe cardiac ailments either during or years after their chemotherapy.[27] DOX is a major chemotherapeutic drug that has been used widely since its discoveryfor its high potential cytotoxic and anti-tumour characteristics.[28] However, the cardiac system is very susceptible to the cytotoxic character of this drug.[29]

Several alternative strategies have been suggested that either target limit the dosage or precisely deliver the cytotoxic drug to the cancerous tissue or co-administer cardioprotective agents that suppress the toxic character of DOX on healthy cardiocytes.[30] In this study, we have come up with an approach to use a naturally occurring phytochemical as a cardioprotective agent while administering DOX to the recipient. The phytochemical was screened for its cardio-protective properties against most cardiotoxic mechanisms presented by Doxorubicin (DOX).[31]

Upon administering 2.5 mg/kg/day of Doxorubicin (DOX) to healthy rats, for 14 days, it was observed that the Body and Heart weight reduced dramatically as evident in Group II. 50 mg/kg/day Gentiopicroside when administered together with DOX furnished a higher Body and Heart weight as seen in Group III. Gentiopicroside alone when given to the rats showed a negligible reduction. This explains that Gentiopicroside in combination with DOX is capable of exhibiting cardio-protective activity.

SAP, DAP, MAP, and HR functioning is seen in the Control Group I indicate the healthy state of the heart and any abnormality in this function indicates the presence of a cardiac injury.[32] DOX administered rats showed suppression in these functions as seen in Group II. Administering Gentiopicroside with DOX shows a lower suppression rate when compared to Group II individuals, indicating an ameliorated cardiac condition. The serum lipid profile and Atherogenic Index (AI) presented parallel data which illustrate the cardio-protective potential of Gentiopicroside in the presence of DOX.[33] It was seen that LDL, VLDL, TC and TG were suppressed in Group III akin to the Control. Lowered AI value for the same group also supports this data. A higher level of HDL-cholesterol in Group III also indicates that Gentiopicroside is capable of reducing the cardiotoxicity of DOX as the same was not witnessed in Group II when compared with the control.

Lipid peroxidation was augmented in Group II treated with 2.5 mg/kg of DOX, visible from the elevated TBARS concentration due to excessive ROS produced.[34] In Figure 5, Group I control shows a lower concentration of TBARS depicting a healthy state or condition. Group III and Group IV that received Gentiopicroside shows a lower concentration of TBARS than Group II, indicating the suppressive activity of Gentiopicroside. All antioxidants screened including SOD, CAT, GPx, GSH, GST, and GR present a similar increasing trend from Group II to Group III. This indicates that the concentration of antioxidants increased with the administration of Gentiopicroside, reducing the oxidative stress produced by ROS.[35] 50 mg/kg of the same was capable of increasing the level of antioxidants to twice the initial value as depicted by the bars of Group III.

Acute cardiotoxic condition of the heart due to DOX was assessed by quantifying the enzymes AST, CK and LDH. These enzymes were highly expressed in DOX treated Group II, however, a notable suppression in the concentration of the markers can be observed in Group III that received Gentiopicroside along with DOX and in Group IV that received Gentiopicroside alone. This indicates that cardiotoxicity was restrained upon treating the rats with Gentiopicroside.

H-FABP is a protein biomarker found within cardio-myocytes that catalyze fatty acid metabolism. This protein is capable of indicating cardiac injury even years after the prognosis with DOX and is studied to be extremely sensitive just hours after the myocardial injury.[36] GP-BB and CK-MB are biomarkers that aid in the early diagnosis of Acute Myocardial Infarction. The marker can detect the clinical condition just within 4 hr from occurrence within the victim.[37] Estimation of all 3 markers gave parallel results. The levels of the enzymes were elevated in Group II treated with 2.5 mg/kg of DOX and Groups III and IV that received Gentopicroside in combination with DOX and Gentiopicroside alone showed a depleting concentration for the enzymes. Group IV substantially reduced the marker levels, whereas Group III was intriguingly capable of producing a suppressed marker level in the presence of DOX.

The presence of inflammation due to the cardio-toxic effect of DOX was investigated by estimating the concentration of INF-γ and MCP-1 from the tissue homogenate of all rats in the study.[38] It was observed that INF-γ levels were the highest in Group II rats indicating the inflamed condition of the heart. This concentration was suppressed in Group III and Group IV. Group III and Group IV both received 50 mg/kg of Gentiopicroside and thus the suppressive activity can be identified due to the administration

of this phytochemical. Group III received Gentiopicroside in combination with 2.5 mg/kg DOX, thus, the suppressive activity can be seen to persist even in the presence of the drug.

Pro-inflammatory markers TNF-α and IL-6 have been quantified from the sera of all rats to study the regulatory effects of Gentiopicroside.[39] Both markers were elevated extremely in Group II animals that were administered with 2.5 mg/kg DOX alone. Rats that received Gentiopicroside showed a lower concentration of the markers in their sera. This included Groups III and IV. Group IV rats received no DOX and hence presented the lowest cytokine level when compared with the control group and the other two experimental groups. Group III that received DOX as well as Gentiopicroside intriguingly presented a significant suppression in the levels of the TNF-α and IL-6.

Thus, the results have disclosed a remarkable potential of the phytochemical Gentiopicroside to suppress the cardiotoxicity manifested by Doxorubicin in healthy Wistar rats. From the results, it is clear that Gentiopicroside is potentially cardio-protective even in the presence of DOX at a concentration of 2.5 mg/kg. Several plant-derived molecules have been previously screened for their cardio-protective properties in DOX-induced cardiotoxicity, but Gentiopicroside was yet to be explored.[40] Phytochemicals have been identified as a newer approach to DOX-induced cardiotoxicity management and have been reported to possess the potential to control this fatal adverse effect.[41]

## CONCLUSION

In this study, we used Doxorubicin as a model of cardiomyopathy. Gentiopicroside was characterized for its cardioprotective properties. According to the results of this study, Gentiopicroside can be used to treat DOX-induced cardiotoxicity. This study results conclude that Gentiopicroside suppresses cardiotoxicity in DOX-administered individuals by reducing oxidative stress and inhibiting inflammatory responses. The mode of action of the drug and its potential to regulate apoptosis in healthy cardiomyocytes will be examined through a mechanistic approach in the future. A preclinical study with chemotherapy patients will be necessary to confirm the molecule's cardioprotective properties in the future.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**DOX:** Doxorubicin; **CHF:** Chronic heart failure; **Dox-CHF:** Doxorubicin-induced chronic heart failure; **AST:** Aspartate aminotransferase; **CK:** Creatine kinase; **LDH:** Lactate dehydrogenase; **H-FABP:** Heart-type fatty acid-binding protein; **GP-BB:** Glycogen phosphorylase isoenzyme BB; **CK-MB:** Creatine kinase-MB isoenzyme; **INF-γ:** Inflammatory Interferon-γ; **MCP-1:** Monocyte chemotactic protein-1; **TNF-α:** Tumour necrosis factor α; **IL-6:** Interleukin 6; **PBS:** Phosphate buffer saline; **HR:** Heart rate; **SAP:** Systolic arterial pressure; **DAP:** Diastolic arterial pressure; **MAP:** Mean arterial pressure; **TC:** Total Cholesterol; **TG:** Triglyceride; **LDL:** Low-density lipopolysaccharides; **VLDL:** Very low-density lipopolysaccharides; **HDL:** Chol-High-density lipopolysaccharides; **AI:** Atherogenic Index; **SOD:** Superoxide dismutase; **CAT:** Catalase; **GPx:** Glutathione peroxidase; **GSH:** Glutathione; **GR:** Glutathione reductase; **GST:** Glutathione S-transferase; **TBARS:** Thiobarbituric acid reactive substances.

## SUMMARY

- The cardioprotective properties of Gentiopicroside were evaluated in a Doxorubicin-induced cardiomyopathy model.
- Gentiopicroside-treated rats showed a substantially increased concentration of antioxidants in comparison to DOX-CHF rats.
- Compared to DOX-CHF rats, Gentiopicroside-treated rats showed lower cardiac enzyme levels such as AST, CK, and LDH.
- Gentiopicroside-treated rats showed reduced levels of enzymes such as H-FABP, CK-MB, and GP-BBP compared to DOX-CHF rats.
- Gentiopicroside-treated rats showed suppression in Inflammatory Markers INF-γ levels and Pro-inflammatory Cytokines.
- According to the results of this study, Gentiopicroside can treat DOX-induced cardiotoxicity.
- As indicated in the study, Gentiopicroside reduces oxidative stress and inhibits inflammatory responses in DOX-administered individuals, suppressing cardiotoxicity.

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