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Ameliorative effect of gallic acid on doxorubicin-induced cardiac dysfunction in rats

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Abstract

Background: The use of doxorubicin (DOX) as an anti-neoplastic agent has been greatly limited because of the myriad of toxic sequelae associated with it. The aim of this study was to assess the protective effects of gallic acid (GA) on DOX-induced cardiac toxicity in rats.

Methods: Sixty male rats (Wistar strain) were used in this study. They were divided into six groups (A–F) each containing 10 animals. Group A was the control. Rats in Groups B, C, and D were treated with DOX at the dosage of 15 mg/kg body weight i.p. Prior to this treatment, rats in Groups C and D had been treated orally with GA for 7 days at the dosage of 60 and 120 mg/kg, respectively. Animals from Groups E and F received only 60 and 120 mg/kg GA, respectively, which were administered orally for 7 days.

Results: The exposure of rats to DOX led to a significant ($p<0.05$) decrease in the cardiac antioxidant defence system and elevation of creatine kinase myocardial band and lactate dehydrogenase. The electrocardiography results showed a significant decrease in heart rate, QRS, and QT-segment prolongation. GA alone improved the antioxidant defence system.

Conclusions: The GA pretreatment significantly alleviated GA-associated ECG abnormalities, restored the antioxidant status and prevented cardiac damage.

Keywords: antioxidant; cardiotoxicity; doxorubicin; electrocardiogram; gallic acid; serum biomarkers.

Introduction

Chemotherapy, either as a primary therapy or as adjuvant for cancers, carries a risk of adverse effects that might result in unfavourable sequelae for the patient [1]. For the treatment of various malignancies, the quinine-containing anthracycline antibiotic, doxorubicin (DOX) is an effective and widely used drug [2]. However, its use in chemotherapy has been largely limited by its toxicity, which causes both cardiac and renal dysfunctions [3]. DOX is a prototype of the reactive oxygen species (ROS)-producing chemotherapeutic drug and, in the presence of molecular oxygen, it has the ability to generate the highly reactive superoxide radical ($O_2^{\cdot-}$) via the redox recycling of the quinone moiety [4].

The mechanisms of DOX-induced toxicity include the increased oxidative stress, interaction with DNA, the inhibition of topoisomerase II in DNA replication and the inhibition of DNA and RNA biosynthesis [5]. In addition, damage to the heart and other organs may adversely modulate renal perfusion, thereby altering the xenobiotic detoxification process and contributing to the DOX-induced nephropathy [6]. To date, no single chemical has been found to reduce the deleterious effects of DOX, and this has led to the search for effective and safe antagonists to DOX-induced toxicity [7].

Gallic acid (GA), a poly-hydroxyphenolic compound, is largely distributed in various plants, fruits and foods.

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Similarly, it occurs naturally in various types of lands and aquatic plants [8]. GA is a strong anti-carcinogenic, anti-mutagenic and anti-inflammatory agent [9]. GA and its derivatives have been found to be strong antioxidants that can scavenge free radicals, such as superoxide anions, hydrogen peroxide (H_2O_2), hydroxyl radicals and hypochlorous acid [9]. The free radical scavenging activity of GA has been suggested as a probable mechanism for its ability to ameliorate oxidative stress [10, 11]. Natural products with antioxidant properties may, therefore, be useful in improving the therapeutic index of DOX. In this study, we investigated the ameliorative effect of GA against DOX-induced cardiotoxicity and oxidative stress in an experimental animal model.

Materials and methods

Chemicals

In this study, DOX, an anticancer agent, glutathione, 1,2 dichloro-4-nitrobenzene (CDNB), thiobarbituric acid (TBA), epinephrine, xylenol orange, potassium hydroxide, sodium hydroxide, glucose-6-phosphate and H_2O_2 were purchased from Sigma Chem., Co. (London, UK). CK-MB and LDH (Kit) were obtained from Randox Laboratories (Crumlin, UK). All other chemicals were of analytical grade and were obtained from British Drug Houses (Poole, UK).

Experimental animals

A total of 60 adult male Wistar rats, weighing 180–200 g, were randomly divided into six groups each containing 10 animals. The rats were fed with commercial rat chow and water supplied liberally. They were subjected to a photoperiod of about 12 h light/12 h darkness and acclimatised for a week prior to pretreatment with GA and administration of DOX a earlier described [1]. As for ethical approval, the research related to animal use has complied with all the relevant national regulations and institutional policies for the care and use of animals.

Experimental design

The animals were divided randomly into six groups of 10 animals per group and treated as follows:

- Group A (Control): Received distilled water for 8 days.
- Group B: Received (DOX) 15 mg/kg i.p. on day 8.
- Group C: Pretreated with 60 mg/kg GA orally for 7 days prior to the administration of DOX 15 mg/kg i.p. on day 8.
- Group D: Pretreated with 120 mg/kg GA orally for 7 days prior to the administration of DOX 15 mg/kg i.p. on day 8.
- Group E: Received 60 mg/kg GA orally alone for 7 days.
- Group F: Received 120 mg/kg GA orally alone for 7 days.

Electrocardiography

The electrocardiographic evaluation of the rats was done using a 6/7 lead computer ECG machine, EDAN 1010. The ECG machine was set at 50 mm/s paper speed and 10 mm/mv voltage calibrations. The procedure was carried out, as described by Calderon et al. [12]. Briefly, each rat was gently restrained on the right lateral recumbency. The limbs were held parallel to each other and vertical to the long axis of the body. The electrode wires were connected to the skin using the attached alligator clips. The electrocardiographic gel was used to improve contact between the electrode and the skin.

Blood pressure measurement

At the end of the treatment period, indirect blood pressure parameters, including systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MAP), were determined without anaesthesia, by tail plethysmography using an electrosphygmomanometer (CODA, Kent Scientific, USA). The average of at least nine readings, recorded in the quiescent state, following acclimatisation, was taken per animal.

Blood collection and preparation of tissues for biochemical assays

The rats were anaesthetised using xylazine/ketamine (v/v) at a dosage of 0.1 mL/100 g prior to blood collection. They were sacrificed by cervical dislocation, after which blood samples were collected by retro-orbital venous plexus into a sterilised plain sample bottles and allowed to coagulate for 30 min. The blood samples were then centrifuged for 10 min at 4000 *g* using a bench centrifuge. The clear serum was collected and frozen until use.

Preparation of cardiac homogenates

Excised hearts were rinsed in ice-cold 1.15% KCl, after which they were blotted on filter paper and weighed. They were then minced with scissors in four volumes of ice-cold 0.1 M phosphate buffer, pH 7.4 and homogenised. The resultant homogenates were centrifuged at 10,000 *g* at 4 °C for 10 min. The supernatant post mitochondrial fractions (PMFs) were collected and processed for subsequent biochemical analyses.

Biochemical assays

The production of NO was evaluated by measuring the level of nitrite (an indicator of NO) in the cardiac tissues using Griess reagent as described [13, 14]. The amounts of nitrite in supernatants were measured following the Griess reaction by incubating equal volumes of sample with the Griess reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride; 1% sulfanilamide in 5% phosphoric acid; 1:1) at room temperature for 20 min. The absorbance at 550 nm (OD 550) was measured spectrophotometrically. Nitrite concentration was calculated by comparison with the OD 550 of a standard solution of

known sodium nitrite concentrations. Serum myeloperoxidase (MPO) activity, an indicator of polymorphonuclear leukocyte accumulation, was determined according to the modified method of Xia and Zweier [15]. The activity of catalase (CAT) was determined according to the method of Shinha [16]. Superoxide dismutase (SOD) was determined by measuring the inhibition of the auto-oxidation of epinephrine at pH 7.2 at 30 °C as described by Misra and Fridovich with a previously described modification [17–19]. Glutathione-S-transferase (GST) was estimated by following the method of Habig et al. [20] using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate. Reduced glutathione (GSH) was determined at 412 nm using the method described by Beutler et al. [21]. The generation of H_2O_2 was determined as earlier described [22]. Lipid peroxidation was determined by measuring the formation of the thiobarbituric acid reactive substances (TBARS) according to the method described by Olszewska-Słonina et al. [23]; in addition, malondialdehyde (MDA) level was computed by using the formula $\Sigma = 1.56 \times 105\ M^{-1}CM^{-1}$. Glutathione peroxidase (GPx) activity was measured according to Wendel et al. [24]. The activities of creatine kinase myocardial band (CK-MB) and lactate dehydrogenase (LDH) were determined with the aid of manual kits following the manufacturer's instructions. The sulfhydryl (total thiol) and non-protein thiol (NPT) contents were determined as described by Ellman [25] Protein concentration was determined as described by Lowry et al. [26].

Histopathology

Small pieces of heart tissues were collected in 10% formalin for proper fixation. The tissues were processed and embedded in paraffin wax. Sections with thicknesses of of 5–6 µm were made and stained with haetoxylin and eosin for histopathological examination [27]. The sides were coded before examination with light microscope by the investigators who were blinded to the control and treatment groups.

Statistical analysis

All values are expressed as mean ± SD. The test of significance between groups was estimated one-way ANOVA with Dunnett's post-test. The level of significance was taken as $p < 0.05$.

Results

Cardiac antioxidant defence system

The administration of DOX led to a significant ($p < 0.05$) reduction in the activities of cardiac CAT, SOD, GST and GPx together with the significant ($p < 0.05$) reduction in the GSH content compared with the control group (Table 1). However, animals pretreated with GA prior to DOX administration showed significant ($p < 0.05$) improvements in the antioxidant activities of CAT, GST, GPx and GSH contents, respectively (Table 1). Furthermore, GA pretreatment alone significantly ($p < 0.05$) increased GSH content at 60 mg/kg relative to the control and DOX-only treatment groups (Table 1).

Cardiac markers of oxidative stress

As shown in Table 2, the DOX administration significantly reduced the protein thiol relative to the control, whereas pretreatment GA and those that received GA alone showed significant increase in protein thiol content (Table 2). In another experiment, the levels of non-protein thiol declined significantly following DOX administration in comparison to the control (Table 2). Similarly, pretreatment with GA alone caused a significant increase in the content of non-protein thiol (Table 2). Our results showed that the cardiac nitric oxide (NO) levels reduced significantly in DOX-only treated rats compared with the control (Table 2). Further, pretreatment GA before DOX and GA pretreatment alone caused significant improvements in NO bioavailability (Table 2). The cardiac MDA increased significantly in DOX-only treated rats compared with the control, whereas

Table 1: Effects of GA on the antioxidant systems in the cardiac tissues of rats exposed to DOX.

Treatment group	CAT, µmol of H_2O_2 consumed/min/mg protein	SOD, units/mg protein	GSH, µmol/g tissues	GST, µmol CDNB-GSH complex formed/min/mg protein	GPx, units/mg protein
Group A	54.9 ± 1.7	22.62 ± 1.16	74.10 ± 1.18	0.32 ± 0.04	1679.1 ± 84.0
Group B	51.4 ± 1.2[a]	20.70 ± 1.25[a]	72.34 ± 0.90[a]	0.21 ± 0.02[a]	1525.4 ± 47.2[a]
Group C	53.3 ± 7.3[b]	21.42 ± 0.23	74.50 ± 0.25[b]	0.19 ± 0.03[a]	1551.8 ± 27.9[a]
Group D	53.1 ± 4.5[b]	21.83 ± 0.58	78.12 ± 7.25[a,b]	0.33 ± 0.05[b]	1652.5 ± 84.1[b]
Group E	54.1 ± 1.1	21.97 ± 0.66	78.0 ± 6.30[c]	0.31 ± 0.05	1593.0 ± 34.9
Group F	53.8 ± 1.1	22.47 ± 0.47	72.69 ± 0.24	0.30 ± 0.06	1615.8 ± 38.5

The results are shown as mean ± SD for each group of 10 rats per group, [a]$p < 0.05$ compared with the distilled water control group A, [b]$p < 0.05$ compared with the DOX-treated group B. Group A (control), received distilled water for 8 days; Group B, received DOX 15 mg/kg i.p. on day 8; Group C, pretreated with 60 mg/kg GA orally for 7 days prior to the administration of DOX 15 mg/kg i.p. on day 8; Group D, pretreated with 120 mg/kg GA orally for 7 days prior to the administration of DOX 15 mg/kg i.p. on day 8; Group E, received 60 mg/kg GA orally alone for 7 days; Group F, received 120 mg/kg GA orally alone for 7 days.

Table 2: Effects of GA on the cardiac markers of the oxidative stress and inflammation.

Treatment groups	Protein thiol, nmol/mg protein	Non-protein thiol, nmol/mg protein	Nitric oxide, µmol/mg protein	MDA, µmol of MDA formed/mg protein	H_2O_2 µmol/mg protein	Myeloperoxidase activity, U/mg protein
Group A	1.47±0.11	1.05±0.10	0.35±0.06	6.55±0.45	13.2±0.37	4.0±0.9
Group B	1.32±0.08[a]	0.86±0.06[a]	0.31±0.01[a]	12.37±2.26[a]	14.0±0.35[a]	5.5±1.7[a]
Group C	1.44±0.09[b]	0.92±0.03[a]	0.53±0.04[a,b]	7.13±0.85[b]	13.65±0.29[b]	5.4±1.6[a]
Group D	1.44±0.06[b]	1.01±0.03[b]	0.41±0.04[a,b]	7.24±0.64[a,b]	13.94±0.13[a]	5.0±2.2[b]
Group E	1.49±0.16	1.05±0.04	0.42±0.05[a]	6.8±1.50	13.6±0.82	4.9±1.1
Group F	1.91±0.16[a]	1.08±0.03	0.34±0.08	6.40±0.41	13.2±0.37	4.4±0.6

The results are shown as mean±SD for each group of 10 rats per group. [a]$p < 0.05$ compared with the distilled water control group A, [b]$p < 0.05$ compared with the DOX-treated group B. Group A (control), received distilled water for 8 days; Group B, received DOX 15 mg/kg i.p. on day 8; Group C, pretreated with 60 mg/kg GA orally for 7 days prior to the administration of DOX 15 mg/kg i.p. on day 8; Group D, pretreated with 120 mg/kg GA orally for 7 days prior to the administration of DOX 15 mg/kg i.p. on day 8; Group E, received 60 mg/kg GA orally alone for 7 days; Group F, received 120 mg/kg GA orally alone for 7 days.

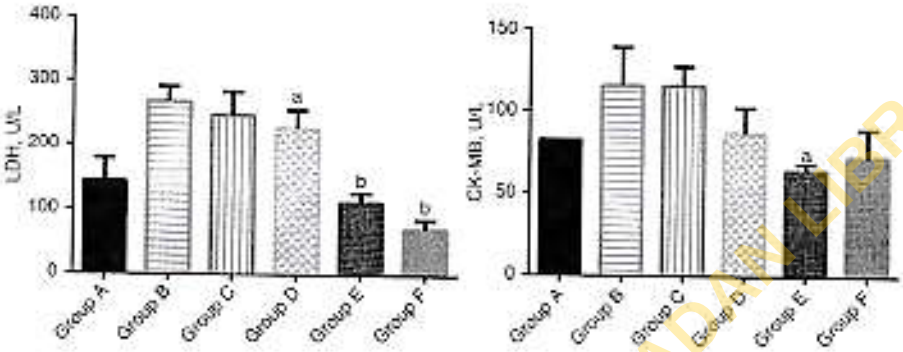

Figure 1: Effect of GA on cardiac marker enzymes in DOX-treated rats.
The results are shown as mean±SD for each group of 10 rats per group. [a]$p < 0.05$ compared with the distilled water control group A, [b]$p < 0.05$ compared with the DOX-treatment group B. Group A (control), received distilled water for 8 days, Group B, received doxorubicin (DOX) 15 mg/kg i.p. on day 8, Group C, pretreated with 60 mg/kg gallic acid (GA) orally for 7 days prior to the administration of DOX 15 mg/kg i.p. on day 8, Group D, pretreated with 120 mg/kg GA orally for 7 days prior to the administration of DOX 15 mg/kg i.p. on day 8, Group E, received 60 mg/kg GA orally alone for 7 days, Group F, received 120 mg/kg GA orally alone for 7 days.

GA (60 and 120 mg/kg) pretreated rats showed significant decline in cardiac MDA content (Table 2). Similarly, H_2O_2 generation followed the same pattern as that of cardiac MDA formation (Table 2). There was a significant increase in H_2O_2 generation following DOX intoxication in comparison with the control (Table 2). GA (60 and 120 mg/kg)-treated rats showed a significant decline in H_2O_2 generation close to the apparent values of the control group (Table 2).

Cardiac markers of inflammation and cardiac damage

The serum MPO activity also increased significantly in DOX-only treated rats compared with the control (Table 2). GA-pretreated rats had lower MPO activity compared with those that received DOX alone. The MPO values did not return to normal control values. The serum activities of both LDH and CK-MB were significantly elevated in DOX-only treated rats compared with the control (Figure 1). GA pretreatment significantly brought down the elevated values of these markers of cardiac damage. However, these values did not return to near normal values (Figure 1).

Electrocardiogram (ECG) and haemodynamic parameters

The ECG results showed that there was a significant increase in PR-wave, together with prolonged QT, QTc interval and QT segment in DOX-only treated rats (Figure 2). The aforementioned ECG changes could not be ameliorated with GA pretreatment (Figure 2). Again, DOX administration also led to significant increases in SBP, DBP and MAP compared with the control (Figure 3). However, the higher dose of GA (120 mg/kg) ameliorated the DOX-induced

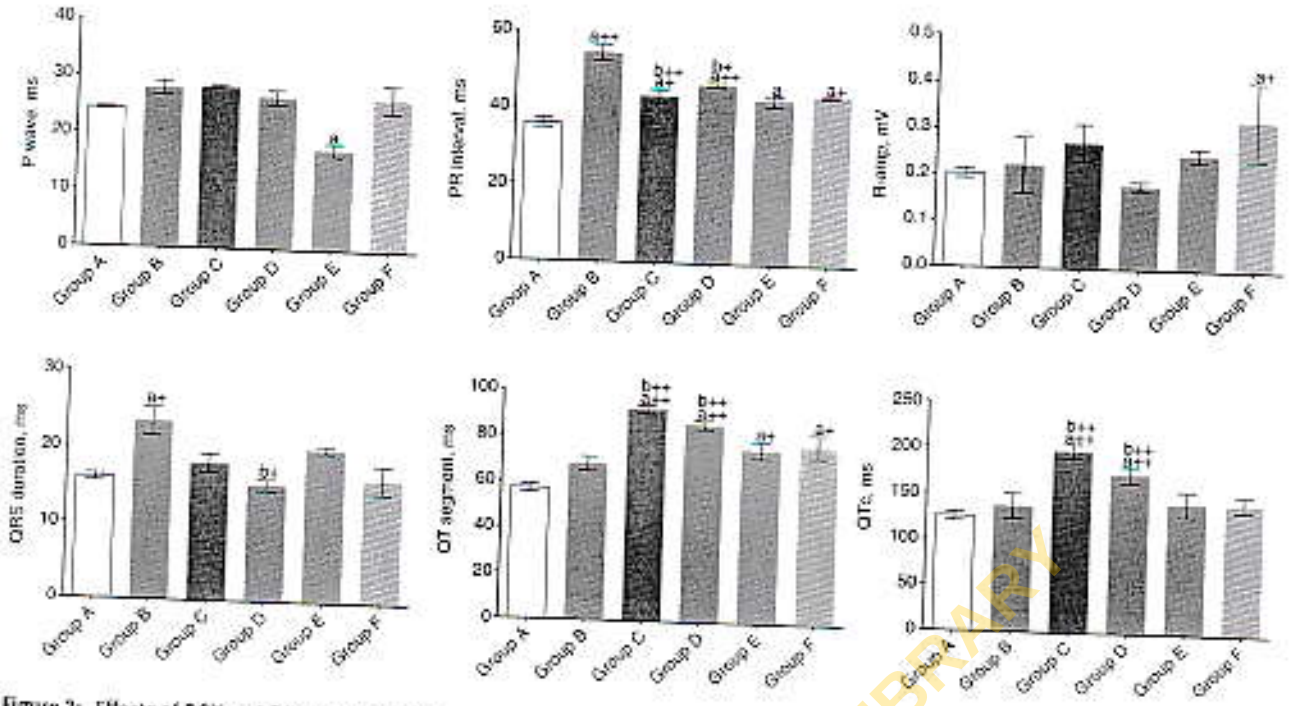

Figure 2: Effects of DOX and GA on Lead-II ECG.
a=*, a+=**, a++=***, b=*, b+=**, b=*** ('a' represents significance as compared with group A, 'b' represents significance as compared with group B).

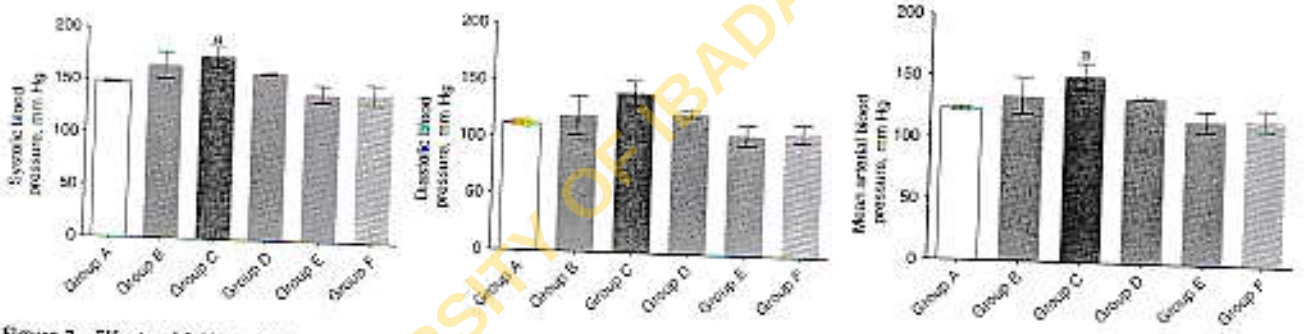

Figure 3: Effects of DOX and GA on systolic, diastolic and mean arterial blood pressure measurements.
'a' represents significance as compared with group A. The results are shown as mean ± SD for each group of 10 rats per group. $^{a}p < 0.05$ compared with the distilled water control group A, $^{b}p < 0.05$ compared with the DOX-treatment group B. Group A (control), received distilled water for 8 days. Group B, received DOX 15 mg/kg i.p. on day 8. Group C, pretreated with 60 mg/kg GA orally for 7 days prior to the administration of DOX 15 mg/kg i.p. on day 8. Group D, pretreated with 120 mg/kg GA orally for 7 days prior to the administration of DOX 15 mg/kg i.p. on day 8. Group E, received 60 mg/kg GA orally alone for 7 days. Group F, received 120 mg/kg GA orally alone for 7 days.

hypertension as indicated with higher values of SBP, DBP and MAP (Figure 3). Together, in all the markers assessed, GA at 120 mg/kg gave a better improvement in antioxidant defence system and reduction in the markers of oxidative stress and blood pressure parameters.

Histology

The rat hearts intoxicated with DOX at 15 mg/kg body weight i.p. (Group B) showed focal areas of infiltration by inflammatory cells and degeneration of myofibres. However, there are no visible lesions in the photomicrograph of the heart section in rats that were pretreated with GA or those that received GA alone (Figure 4).

Discussion

The toxicity of DOX was induced through the generation of the ROS and the amplification of mitochondrial dysfunction leading to oxidative stress [28]. The highly toxic ROS

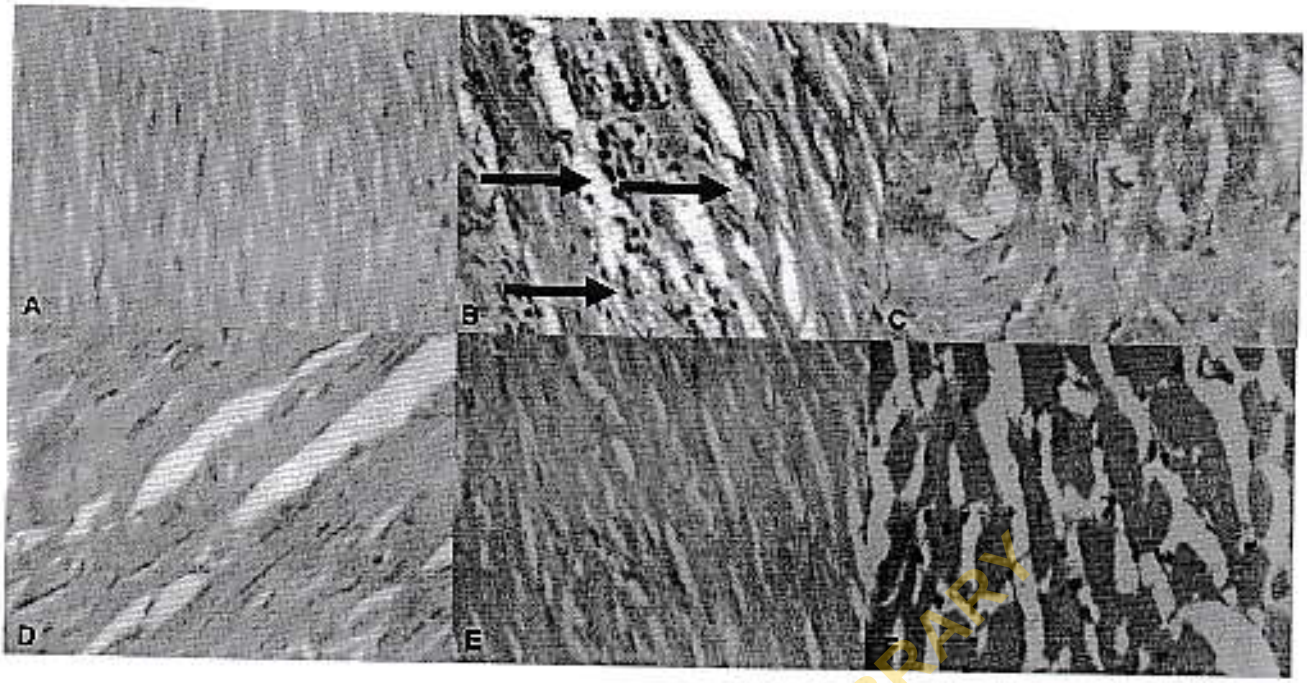

Figure 4: Histology results showing the effects of DOX and GA on cardiac tissue.
Group A (control), Rat hearts intoxicated with DOX at 15 mg/kg body weight i.p. Group B and Group C, pretreated with 60 mg/kg GA orally for 7 days prior to the administration of DOX 15 mg/kg, Group D, pretreated with 120 mg/kg GA orally for 7 days prior to the administration of DOX 15 mg/kg, Group E, received 60 mg/kg GA orally alone for 7 days, Group F, received 120 mg/kg GA orally alone for 7 days. Representative hematoxylin–eosin (H&E)-stained heart sections (×400 objectives).

react with cellular molecules, including nucleic acids, protein and lipids, resulting in cell damage. Due to its low level of antioxidants, such as SOD and CAT, compared with organs like the liver and kidney, the myocardium is highly susceptible to the deleterious activities of ROS. To reduce the effect of ROS on the heart, combining DOX with agents possessing free radical-scavenging properties would block the free radical-mediated toxicity and prevent its oxidative stress and tissue injury without affecting its antitumor activity.

GA is a strong antioxidant with the ability to confer cytoprotection against oxidative damage by scavenging free radicals. As a poly-phenolic compound, GA has the capacity to selectively inhibit the growth of some cancer cells without harming healthy cells [29]. The CAT, an enzymatic antioxidant present in the cytoplasm, aids the removal of H_2O_2 by breaking it down to water and oxygen molecule using either iron or manganese as co-factor. In this study, a significant ($p<0.05$) reduction in the CAT activity was recorded in rats that received DOX alone compared with the control. The reduction in the CAT activity indicates oxidative stress, as reported in previous studies [30]. The CAT activity in groups pretreated with GA (60 and 120 mg/kg) increased compared with the DOX-treated group. This shows that GA scavenged the generation of free radicals. Findings in this study are similar to earlier reports by Padma et al. [31] who reported that GA maintained CAT activity in the pretreated animals.

SOD participates in the first line of defence during oxidative stress by converting the superoxide anion radical (O_2^-) to H_2O_2, whereas GPx and CAT reduce H_2O_2 to water and oxygen, respectively. In addition, GST brings about the detoxification of toxic electrophiles (including metabolites of DOX) with the help of GSH as a co-factor to more soluble and less toxic metabolites, which can easily be excreted by the kidney [32]. In this study, the activity of SOD was reduced in tissues of the DOX-treated group compared with the control. This may be attributed to the level of free radicals generated, which might have overwhelmed the enzyme activity. However, the SOD activity increased in groups that received 60 and 120 mg/kg of GA compared with the DOX group indicating that GA had the ability to increase the activity of SOD.

Reduced glutathione (GSH) is a non-enzymatic free radical scavenger that prevents the generation of free radical from biological membranes [33]. The level of GSH was significantly decreased ($p<0.05$) in rats treated with 15 mg/kg DOX compared with the control. This might be due to a reduction in the level of GSH by DOX with a resultant enhancement of oxidative stress and membrane

lipid peroxidation. A significant increase ($p < 0.05$) was recorded in groups pretreated with 60 and 120 mg/kg GA in comparison with the DOX control group. This implies that, as an antioxidant, GA can maintain the level of GSH in the body [31].

Glutathione-S-transferase (GST) plays an important role in the detoxification of xenobiotics, drugs and carcinogens and thus protects the cells against redox cycling and oxidative stress. In this study, we observed a significant reduction ($p < 0.05$) in the activity of rats administered only with DOX compared with the control. Naturally, the heart has a low level of GST and an overwhelming generation of free radicals due to DOX might be responsible for the significantly low level of GST. However, the level of GST was significantly ($p < 0.05$) increased in rats pretreated with 120 mg/kg GA. This is probably due to the ability of GA to activate enzymatic antioxidant system thereby preventing the cardiotoxic effect of DOX. This result correlates with a study that showed the ability of GA in maintaining GST level in the heart [31].

GPx catalyses the breakdown of H_2O_2 and organic peroxides through the four selenium co-factors it contains. Compared with the control, the level of GPx was significantly decreased ($p < 0.05$) in DOX-treated rats and those treated with 60 mg/kg GA + DOX. The reduction in the GPx activity was probably due to the mopping up of GPx by free radicals generated by DOX. However, in rats that received 120 mg/kg GA + DOX, there was a significant increase ($p < 0.05$) in GPx in comparison with those treated with DOX alone, this is probably due to the ability of GA in maintaining endogenous antioxidants [34].

The H_2O_2 production was significantly increased ($p < 0.05$) in rats treated with DOX alone contrary to a report by Panchuk et al. who reported a significantly low level of H_2O_2 production after the administration of DOX in rats [35]. The increased production of H_2O_2 with the depletion of GPx, CAT and GSH contents enhances toxicity to cardiac myocytes. Though insignificant, rats pretreated with GA and DOX showed a decrease in H_2O_2 generation, which can be attributed to the ability of the antioxidant (GA) to prevent the deleterious effect of free radicals.

In the present study, the NO level was significantly increased ($p < 0.05$) in rats treated with DOX alone compared with the control. However, pretreatment with GA before the administration of DOX caused a significant decrease ($p < 0.05$) compared with the control. This could be due to the direct action of GA in donating its hydrogen atom, thereby stabilising NO. This causes a decrease in the level of free radicals formed, thus preventing oxidative damage. NO plays a significant role in both cardiac function and disease. The production of NO via constitutive NO synthase (NOS) serves a modulatory function in contractility and blood flow regulation.

The protein and non-protein thiol levels were significantly decreased ($p < 0.05$) in DOX-treated rats compared with the control group. However, treatment with GA caused a significant ($p < 0.05$) increase in the levels of both protein and non-protein thiols compared with the DOX-treated group.

MPO activity was significantly increased ($p < 0.05$) in animals treated with DOX, proving that DOX encouraged the elevation of MPO activity, as reported by Elberry et al. [36]. Though insignificant, the MPO activity was decreased in animals pretreated with GA compared with those treated with DOX alone. This is due to the ability of the poly-phenolic agents like GA to inhibit the enzymatic activity of peroxidases, thereby reducing the oxidative stress induced by the peroxidase by-products. MPO belongs to the heme peroxidase superfamily of enzymes and it generates a large number of oxidants and radical species that can initiate lipid peroxidation and promote the post-translational modifications of target proteins [37]. Secreted by neutrophils, monocytes and certain tissue macrophages, MPO, as part of its normal host defence mechanism, generates a variety of reactive oxygen and nitrogen species wthat are useful in destroying pathogens. These oxidants may also exert deliterious effects on the vasculature. The links that have been established among the MPO, oxidation and cardiovascular disease are suggestive of the fact that this enzyme may be clinically useful in assessing the status and outcome of cardiovascular diseases.

The increased serum activities of the CK-MB and LDH levels were observed in rats that received DOX alone compared with the control. This is a result of the oxidative stress induced by DOX, which led to the peroxidation of heart lipids accompanied by the release of CK-MB and LDH into the serum. This result agrees with studies previously reported elsewhere [38]. However, rats pretreated with GA showed a decrease in the levels of CK-MB and LDH compared with rats treated with DOX alone, demonstrating the ability of GA to protect against DOX-induced cardiac damage.

Histology results showed no visible lesions in the control group, whereas in group B, the heart section showed the focal areas of infiltration by the inflammatory cells and the degeneration of myofibres. This finding is similar to that of a past study, which reported that DOX caused damage to the muscle fibres [39]. The degeneration observed with inflammatory cell infiltration suggested an on-going injury state and this can be attributed to the effect of DOX. The free radicals formed by the action

of DOX led to cell damage. There were no visible lesions in the photomicrographs of the heart sections of rats in Groups C, D, E and F. This may be due to the ability of GA to scavenge the free radicals responsible for the alterations in cell viability.

ECG is a commonly used, non-invasive procedure for recording electrical changes in the heart. The waves in a normal record are named P, Q, R, S and T, in alphabetical order. In this study, a statistically significant ($p < 0.05$) decrease in heart rate with a rise in the P wave duration was recorded in the DOX-treated group compared with the control. In addition, the statistically significant ($p < 0.05$) increases in both the P-R interval and the QRS duration were recorded with the elevated QT intervals. These findings correlate with those of Shah et al. [38] who reported that DOX administration caused injury with evidence of QT prolongation, increase in QRS complex and decrease in heart rate. However, there was a decrease in P wave with a significant decrease in the PR interval and QRS duration in groups pretreated with GA. This is due to the ability of GA to restore the ECG changes to normal levels.

There was also an increase in SBP, DBP and MAP in the DOX-treated group as compared with the control group. However, there were minimal changes in the SBP, DBP and MAP of rats pretreated with GA, suggesting that GA can restore BP towards a normal value. This finding supports the report by Patel and Goyal [40], who concluded that treatment with GA reduced blood pressure and increased the heart rate.

In conclusion, this study showed that in alleviating the toxic effect of DOX, GA exerted its free radical scavenging ability and boosted the endogenous antioxidants in mopping up the ROS.

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