**Mitigation of Doxorubicin-Induced Cardiovascular Damage Using *Commelina diffusa* (Dayflower) Extract in Wistar Rats**

**ABSTRACT**

This study determined the mitigation of doxorubicin-induced cardiovascular damage using *Commelina diffusa* extract in Wistar Rats. Twenty five adult male Wistar rats weighing 160 and 200g were purchased from the Biochemistry Animal House, University of Port Harcourt. The rats were acclimatized for weeks, giving free access to rat feed and water. The rats were then be divided into 5 groups, on basis of body weight into five groups of five rats per group. Group 1 and 2 served as normal and negative controls. Rats in group 3-5 were induced with doxorubicin and treated with the extract 166mg/kg, 250mg/kg, and 500mg/kg for 21 days. All analysis were carried out based on standard methods. The cardiac IL-6, C-reactive protein, CTn-I and CTn-T of the negative control were 153.06±0.03 pg/dl, 83.64±0.03 mg/ml, 472.25±0.03pg/mL, and 442.64±0.04pg/Ml respectively while those treated with extract at 500mg/kg for 21 days were 117.93±0.02pg/dl, 41.04±0.01mg/ml, 447.08±0.03pg/mL, and 410.72±0.03 pg/Ml respectively. The MDA and GSH levels of the negative control were 23.83±0.02 mmol/l and 73.63±0.03 μg/mg protein respectively. The mean CAT and SOD activities of group 5 were 96.06±0.03 mg/promin and 9.04±0.03 mg/g respectively. The IL-6, C-reactive protein, CTn-I, CTn-T, CAT and SOD activities of group 5 treated at 500mg/kg for 21 days were significantly ameliorated. Aqueous extract of *Commelina diffusa* ameliorated cardiovascular damage facilitated by doxorubicin exposure, hence could serve as a herbal agent in the treatment of cardiovascular damage.

Keywords: Doxorubicin, *Commelina diffusa*, *cardio-neurohormone*, *oxidative stress biomarkers, Wistar rats*.

1. **INTRODUCTION**

Doxorubicin which is also called an anthracycline antibiotic, is widely applied in the treatment of cancers of different types including leukemia, lymphoma, and solid tumors. However, its therapeutic potential is counterbalanced by its adverse effect on the cardiovascular system (Xiaoxiao *et al*., 2023). The mechanism underlying DOX-induced cardiotoxicity involves the generation of reactive oxygen species (ROS), which leads to oxidative stress, mitochondrial damage, and myocardial cell death. Another mechanism is by inhibition of mitochondrial ATP production (Guanjing *et al.,* 2022).

The metabolism of the cardiovascular system is dependent high consumption of huge concentration of ATP for day after every day proper physiological activity (Bianchi, 2020). The heart of any healthy human, almost all ATP is produced by the oxidative metabolism of mitochondria and medicaments that thwarts mitochondrial responsibility might result in enfeeblement of ATP, which might ultimately lead to myocardial dysfunction (Verma *et al*, 2013). Mitochondrial bioenergetics is physiologically connected to myocardial substrate utilization, the mitochondrial respiratory chain, high-energy phosphate storage, transport and energy signalling pathways, including other physiological processes that are linked to mitochondrial structure and activity (Wu *et al*., 3016). Wu *et al*. (3016) showed that DOX-induced mitochondrial bioenergetic crumple (DiMBc) may be conciliated in several mannerisms, by disrupting the Krebs cycle, fatty acid β-oxidation, the respiratory chain, and oxidative phosphorylation, resulting in a bioenergy crisis that ultimately leads to cardiomyocyte necrosis.

 Impersonally, mitochondrial bioenergetic collapse has become a significant feature of doxorubicin-induce cardiotoxicity, whether at an early stage, intermediate stages, or in the long-term (Tscheschner *et al.,* 2019). Significant insight into the mechanisms of doxorubicin-induced mitochondrial bioenergetic enfeeblement might be supportive in the identification of new targets to develop novel strategies for the prevention of cardiotoxicity in any form.

*Commelina diffusa*, commonly known as the climbing or spreading dayflower, is a tropical herbaceous plant widely utilized in traditional medicine across various cultures. Wellington and ThankGod (2024) evaluated the aerial parts of *Commelina diffusa* for phytochemical characterization and they showed that it is endowed with eleven flavonoids with a total 133.41 mg/100g, seven terpenoids (45.72 mg/100g), nine phenolic acids (199.76 mg/100g), seven cyanogenic glycoside (194.99 mg/100g), twelve glycosides (128.57 mg/100g), fourteen alkaloids (35.48 mg/100g), fourteen lignans (265.7 mg/100g), eighteen saponins (82.9 mg/100g), twelve anthocyanins (57.22 mg/100g), thirteen anthraquinones (168.66 mg/100g), thirteen sterols (45.67 mg/100g), and essential oils (100%). Adegbite *et al*. (2023) demonstrated that *Commelina diffusa* extract enhances the activity of endogenous antioxidants such as superoxide dismutase (SOD) and catalase (CAT), reducing oxidative stress. Singh *et al.* (2018) showed that administration of Commelina diffusa extract in Wistar rats reduced heart weight, improved antioxidant enzyme activity, and decreased markers of lipid peroxidation. While Zhang *et al*. (2019) i**n a potential clinical applications of *Commelina diffusa* extract** demonstrated its antioxidant, anti-inflammatory, and anti-apoptotic properties, which reflects a novel adjunct therapy for patients undergoing chemotherapy, potentially reducing DOX-induced cardiotoxicity.

Commelina diffusa has been traditionally used in herbal medicine for its antioxidant, anti-inflammatory, and wound-healing properties. These properties suggest potential cardioprotective effects against oxidative stress and inflammation, which are major mechanisms of DOX-induced cardiotoxicity. While several investigations have explored the medicinal properties of Commelina diffusa, its specific role in protecting the heart from chemotherapy-induced dysfuction remains largely unexplored. Addressing this gap could uncover a novel, natural therapeutic option. **In bridging traditional knowledge with western medicaments**, evaluating Commelina diffusa in this context could assist providing scientific credence for its traditional use and contribute to integrating herbal medicine into mainstream healthcare.

**2. MATERIALS AND METHODS**

**2.1 Chemical/Reagents**

The reagents adopted in this work were bought from commercial industries and the manufacturers’ standard methods and procedure were strictly adhered with respect to this research.

**2.2 Source and Identification of Plant**

*Commelina diffusa* were obtained from Toru-Orua and Ebedebiri Communities, in Sagbama Local Government Area of Bayelsa State, Nigeria. The plant sample was identified and authenticated at the Herbarium Unit of the Department of Agriculture, University of Africa Toru-Orua. The sample was registered with Voucher Number UAT/A/3011.

**2.3 Source of Experimental Wistar Albino Rats**

Twenty five (25) adult male Wistar rats weighing 160 and 200g were purchased from the Biochemistry Animal House, University of Port Harcourt. The rats were acclimatized for weeks , giving free access to rat feed and water. They were given standard animal feed manufactured by Grand Cereals and Oil Mills Ltd., Yenagoa, and water *ad libitum*. They were treated following the principles and standard protocols for the use of laboratory animals for experiments.

**2.4 Animals and Approval from Animal Ethical Committee**

Healthy adult male Wistar albino rats (160-200g) were used for all investigations. The animals were maintained under standard husbandry conditions in the animal house of ‘College of Health Sciences, University of Africa Toru-Orua, Nigeria (temperature 25 ± 2 °C) in a natural light-dark cycle and fed with standard rodent diet and water ad libitum. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of UAT/CEREMAD/REC/MM72/011 .Ref. No. UAT20/03/01/1377.

**2.5 Experimental Design**

Twenty five (25) Wistar rats weighing between 160 and 200g were used for this study. They were be purchased from the Biochemistry Animal House, University of Port Harcourt Choba and be acclimatized for 14 days, giving free access to rat feed and water. The rats were then be divided on basis of body weight into five groups of five rats per group and treated as shown in the table below.

Table 1 experimental design

|  |  |  |
| --- | --- | --- |
| Groups | Treatment | Duration |
| 1 | Normal control: Received rat feed H2O only, serving as normal control | 21 days |
| 2 | Negative control: Received one dose of 50mg/kg doxorubicin + rat feed+ H2O only | 21 days |
| 3 | Received 50mg Doxorubicin+166mg/kg *C. diffusa*+ rat feed+ H2O only | 21 days |
| 4 | Received 50mg Doxorubicin+ 250 mg/kg *C. diffusa*+ rat feed+ H2O only | 21 days |
| 5 | Received 50mg Doxorubicin+ 500 mg/kg *C. diffusa*+ rat feed+ H2O only | 21 days |

Exactly, 24 hours after the last day of oral treatment with the extract, the rats were humanly sacrificed through cervical dislocation, blood sample were be collected for biochemical assays. The heart tissue of the rats in each group were harvested and was cut into two equal halves. Half of the organs was homogenized for estimation of heart biomarkers while the other half was used for histological examination.

**2.6 Biochemical Determination**

**2.6.1 Determination of C-reactive protein (CRP), Interluikin-6 (IL-6), Cardiac Troponins (**

**cTnI and cTnT) Levels**

The indices observed for cardio-neuro hormones were interleukin 6 (IL-6), c-reactive protein (CRP), cardiac troponin I and T (CTn-I and T). Plasma IL-6 and cardiac troponin I and T were determined using enzyme-linked immunosorbent assays (ELISA), based on antigen-antibody interactions, where specific antibodies bind to cardiac troponins. A detectable enzyme-linked reaction reveals the presence and concentration of the target biomarker. In this method, a chromogenic substrate (TMB - 3,3',5,5'-tetramethylbenzidine) was used, which produce color complexes at 450 nm, that are proportional to the troponin I and T concentrations as described by Chaulin (2021).

**2.6.2 Determination of Superoxide Dismutase (SOD) and GPx Activities**

SOD activity can be measured using spectrophotometric assays that monitor the enzyme's ability to inhibit the reduction of specific substrates. One common method involves the reduction of cytochrome c, where SOD competes with cytochrome c for superoxide radicals, leading to a decrease in absorbance at 550 nm. Automated versions of this assay have been developed to enhance efficiency ([Wheeler](https://pubmed.ncbi.nlm.nih.gov/?term=Wheeler+CR&cauthor_id=2327564) *et al.*, 1990).

**2.6.3 Determination of Catalase (CAT) Activity**

CAT activity was typically assessed by measuring its capacity to decompose hydrogen peroxide (H₂O₂). A spectrophotometric method, which involves incubating the sample with H₂O₂ and then adding a cobalt-bicarbonate reagent to react with the remaining H₂O₂, forming a colored complex measurable at 440 nm as defined by Mahmoud (2018).

**2.6.4 Determination of MDA Level**

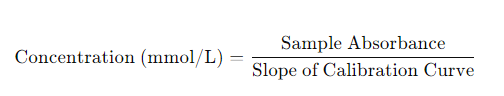
MDA is a byproduct of lipid peroxidation and is commonly measured using the Thiobarbituric Acid Reactive Substances (TBARS) assay, which relies on spectrophotometry. The plasma MDA react with **thiobarbituric acid at 95°C** for **30 minutes** to form a pink-colored **MDA-TBA adduct, that is proportional to the concentration of MDA.** The pink color complex was measured at **532 nm** using a spectrophotometer as described by Miruna *et al*. (2010).

### ****2.6.5 Determination of GSH Level****

The plasma GSH was estimated using **Ellman’s Reagent (DTNB) assay, which** is the most common spectrophotometric method for GSH quantification. In this method, the plasma GSH react with **5,5'-dithiobis(2-nitrobenzoic acid) (DTNB)** to produce a yellow-colored product which proportional to the level of GSH in the sample as described by Miruna *et al*. (2010).

**2.6.6 Determination of Cardiac Electrolyte Levels**

All cardiac electrolytes were estimated using spectrophotometric methods, using sodium tetraphenylborate, arsenazo III, ranyl zinc acetate, and reagents as described by.. The calibration curve was used to determine the concentration of each electrolyte based on the measured absorbance using the general relationship below (**Bishop *et al*., 2013)**:



**2.7 Histopathological Analysis of Heart Tissue**

The heart tissue isolated from sacrificed Wistar rats was fixed in 10% formalin, then after processing embedded in paraffin wax. Paraffin sections were made at 5 mm and stained with hematoxylin and eosin. The slides were studied under a light microscope and captured the magnified images of heart tissue structure for further study.

**2.8 Statistical Analysis**

All Data are represented as means ± error of mean (M±E) and were analyzed using Statistical Package foe Social Sciences (SPSS) for window version 20.0 USA. Descriptive statistics was done by one way analysis of variance (ANOVA) and multiple comparison was done using Turkey Post hoc at (p≤0.05) confidence interval.

**3. RESULTS**

**3.1 Effect of** **Aqueous Extract of the Aerial Parts of *Commelina Diffusa* on Homogenate Cardio-Neuro Hormones on Doxorubicin-Induced Cardiovascular Damage in Wistar Rats**

Table 2 indicates the mean plasma levels of c-reactive protein (CRP), interluikin-6 (IL-6), and cardiac troponin I and T (CTn- I and T) levels of doxorubicin-induced cardiotoxicity in Wistar rats. All assayed indices were discussed in comparison to the normal and negative control as shown in Table 2.

Table 2 Effect of aqueous extract of the aerial parts of *Commelina diffusa* on homogenate cardio-neuro hormones in doxorubicin-induced cardiovascular damage in Wistar rats (n=5)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Group | IL-6  (pg/dl) | CRP  (mg/ml) | CTn-I  (pg/mL) | CTn-T  (pg/Ml) |
| N/Control | 4.30±0.17f | 11.55±0.03 f | 183.25±0.02 f | 291.45±0.03 f |
| Ne/Control | 153.06±0.03e | 83.64±0.03e | 472.25±0.03 e | 442.64±0.04e |
| 50mg/kg Dx+ 166mg/kg CD 21 days | 148.75±0.04h | 75.84±0.03 h | 451.85±0.03 h | 437.83±0.03 h |
| 50mg/kg Dx+ 250mg/kg CD 21 days | 137.04±0.04 h | 64.36±0.03 h | 447.27±0.03 h | 421.94±0.03 h |
| 50mg/kg Dx+ 500mg/kg CD 21 days | 117.93±0.02 h | 41.04±0.01 h | 447.08±0.03 h | 410.72±0.03 h |

Definition of symbols: IL-6= Interleukin 6, CRP= C-reactive protein, CTn-I= Cardiac troponin I, CTn-T= Cardiac troponin T. Values are reported as mean ± standard error of mean (M±SEM) (n =5). Values bearing superscript (“e”) were significantly different (p≤ 0.05) from the normal control down the groups. Values with similar superscript (“h”) were not significantly (p≤ 0.05) different from the negative control down the groups.

3.2 **Effect of Aqueous Extract of The Aerial Parts of *Commelina Diffusa* on Enzyme Biomarkers of Heart Homogenate in Doxorubicin-Induced Cardiovascular Damage in Wistar Rats**

Table 3 shows the mean plasma effect of aqueous extract of the aerial parts of *Commelina diffusa* on enzyme biomarkers of heart homogenate in doxorubicin-induced cardiovascular damage in Wistar rats. The result in Table 3 were presented in comparison to the negative control for better interpretation and understanding

Table 3 Effect of aqueous extract of the aerial parts of *Commelina diffusa* on enzyme biomarkers of heart homogenate in doxorubicin-induced cardiovascular damage in Wistar rats (n=5)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Group | ALT  (U/L) | AST  (U/L) | ALP  (U/L) | LDH  (U/L) | CK  (U/L) |
| N/Control | 66.17±0.02f | 184.24±0.03f | 91.19±0.01 f | 194.43±0.02f | 178.95±0.03 f |
| Ne/Control | 157.24±0.04e | 319.44±0.03e | 137.64±0.04e | 282.54±0.02e | 261.26±0.03e |
| 50mg/kg Dx+ 166mg/kg CD 21 days | 147.37±0.02h | 321.52±0.02h | 135.94±0.04h | 279.85±0.03 h | 259.04±0.01 h |
| 50mg/kg Dx+ 250 mg/kg CD 21 days | 136.73±0.04 h | 316.46±0.03h | 131.05±0.01h | 279.02±0.03h | 256.86±0.04 h |
| 50mg/kg Dx+ 500mg/kg CD 21 days | 181.85±0.04 h | 301.35±0.02h | 127.94±0.04h | 276.94±0.05 h | 256.17±0.03 h |

Values are reported as mean ± standard error of mean (M±SEM) (n =5). Values bearing superscript (“e”) were significantly different (p≤ 0.05) from the normal control down the groups. Values with similar superscript (“h”) were not significantly (p≤ 0.05) different from the negative control down the groups.

**4.3 Effect of Aqueous Extract of the Aerial Parts of *Commelina Diffusa* on Oxidative Stress Biomarkers of Heart Homogenate in Doxorubicin-Induced Cardiovascular Damage in Wistar Rats**

Table 4 shows the meaneffect of aqueous extract of the aerial parts of *Commelina diffusa* on oxidative stress biomarkers of heart homogenate in doxorubicin-induced cardiovascular damage in Wistar rats. The information on the table were written in comparison to the negative and normal control for easy understanding.

Table 4 Effect of aqueous extract of the aerial parts of *Commelina diffusa* on oxidative stress biomarkers of heart homogenate in doxorubicin-induced cardiovascular damage in Wistar rats (n=5)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Group | MDA  (mmol/l) | GSH  (μg/mg protein) | GPX  (lU/g) | CAT  (mg/promin) | SOD  (mg/g) |
| N/Control | 3.17±0.02 f | 73.63±0.03f | 77.38±0.01f | 119.82±0.02f | 51.74±0.02f |
| Ne/Control | 23.83±0.02e | 73.63±0.03e | 51.35±0.04e | 51.35±0.04e | 3.72±0.03e |
| 50mg/kg Dx+ 166mg/kg CD 21 days | 36.24±0.04h | 28.05±0.02h | 68.47±0.03h | 68.47±0.03 h | 7.83±0.03h |
| 50mg/kg Dx+ 250 mg/kg CD 21 days | 36.04±0.02h | 28.18±0.01h | 83.63±0.02h | 83.63±0.02h | 8.65±0.04 h |
| 50mg/kg Dx+ 500mg/kg CD 21 days | 35.84±0.02h | 28.33±0.02h | 96.06±0.03h | 96.06±0.03 h | 9.04±0.03 h |

Values are reported as mean ± standard error of mean (M±SEM) (n =5). Values bearing superscript (“e”) were significantly different (p≤ 0.05) from the normal control down the groups. Values with similar superscript (“h”) were not significantly (p≤ 0.05) different from the negative control down the groups.

**4.5 Effect of Aqueous Extract of the Aerial Parts of *Commelina Diffusa* on Cardiac Electrolytes in Doxorubicin-Induced Cardiovascular Damage in Wistar Rats**

Table 5 presents the mean effect of aqueous extract of the aerial parts of *Commelina diffusa* on cardiac electrolytes in doxorubicin-induced cardiovascular damage in Wistar rats. The electrolytes in the extract treated groups were arranged in Table 5 in comparison to the normal and negative control.

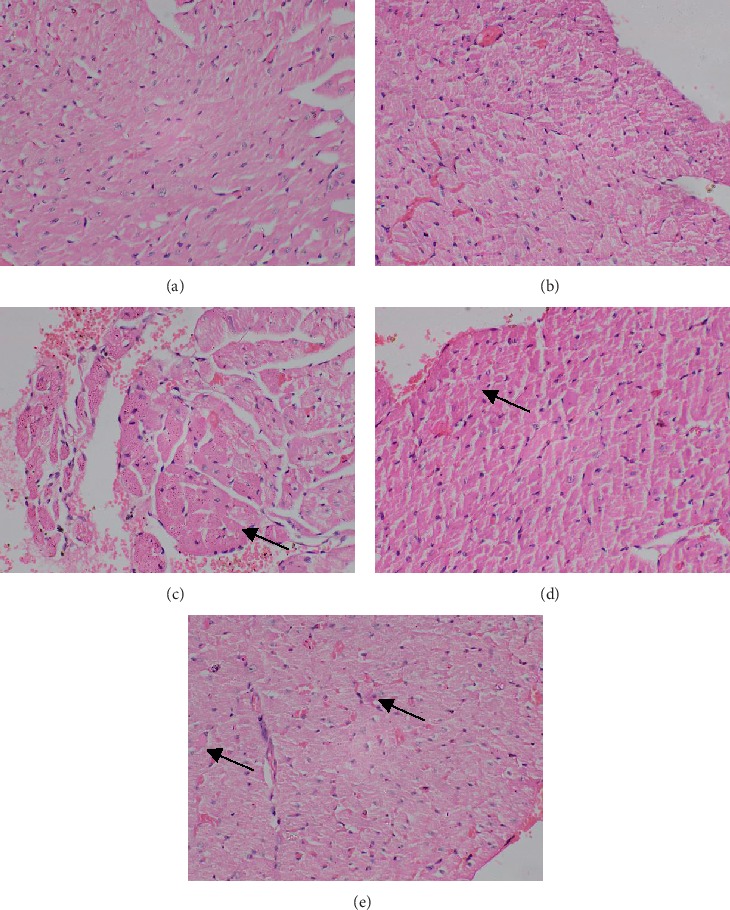
Table 5 Effect of aqueous extract of the aerial parts of *Commelina diffusa* on cardiac electrolytes in doxorubicin-induced cardiovascular damage in Wistar rats (n=5)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Group | K+ (g/L) | Ca2+  (mmol/l) | HCO32-  (mmol/l) | Na+  (mmol/l) |
| N/Control | 7.56±0.04f | 91.25±0.03 f | 41.75±0.03f | 200.03±0.02f |
| Ne/Control | 0.85±0.04e | 19.37±0.02e | 6.23±0.03 e | 91.16±0.03e |
| 50mg/kg Dx+ 166mg/kg CD 21 days | 1.57±0.02h | 27.62±0.02 h | 9.23±0.02 h | 95.71±0.05 h |
| 50mg/kg Dx+ 250 mg/kg CD 21 days | 1.94±0.03 h | 27.73±0.03 h | 9.23±0.02 h | 97.14±0.02 h |
| 50mg/kg Dx+ 500 mg/kg CD 21 days | 2.05±0.02 h | 27.94±0.03 h | 9.88±0.03 h | 97.76±0.03 h |

Values are reported as mean ± standard error of mean (M±SEM) (n =5). Values bearing superscript (“e”) were significantly different (p≤ 0.05) from the normal control down the groups. Values with similar superscript (“h”) were not significantly (p≤ 0.05) different from the negative control down the groups.

**4.6 Effect of Aqueous Extract of *Commelina diffusa* on Cardiac Histology in Doxorubicin-Induced Cardiovascular Damage in Rats**

Plate 1-5 shows the effect of aqueous extract of the aerial parts of *Commelina diffusa* on cardiac histology in doxorubicin-induced cardiovascular damage in rats



Photomicrographs of subendocardial region of rat myocardium (H & E, ×400).

Plate 1: Normal control showing normal cardiac morphology

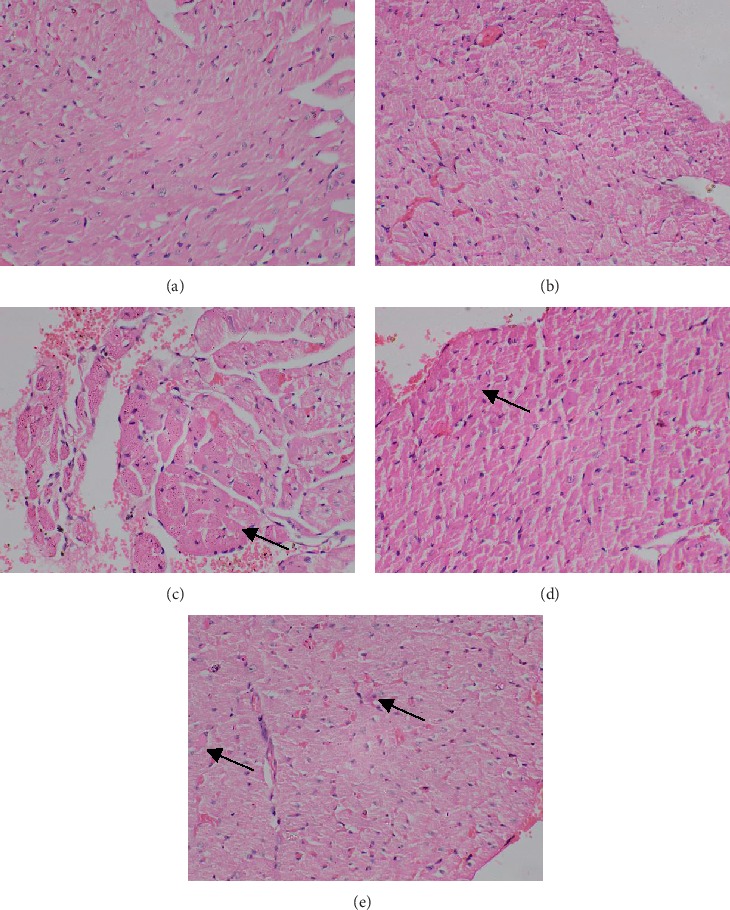


Plate 2: Negative control, showing a large necrosis region area of myocytes with hypereosinophilic cytoplasm and nuclear changes in cell death including pyknosis and karrheorhexis indicated by the black arrow head

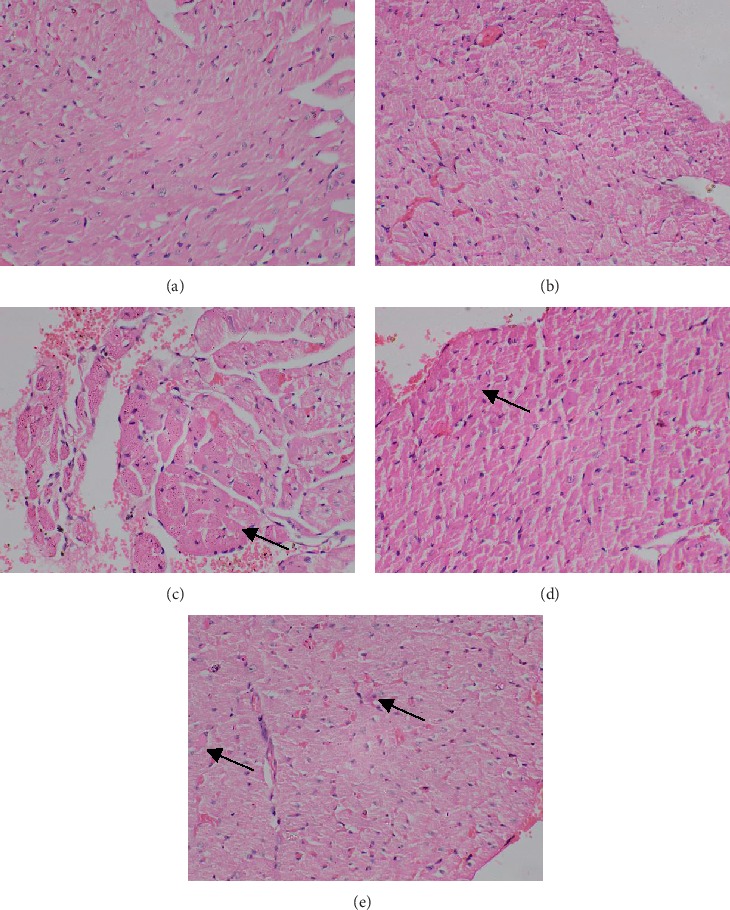


Plate 3: Doxorubicin-induced and treated with 166mg/kg *Commelina diffusa* extract for 21 days, showing mild changes of myocytes, hypereosinophilic cytoplasm and nuclear cell death.

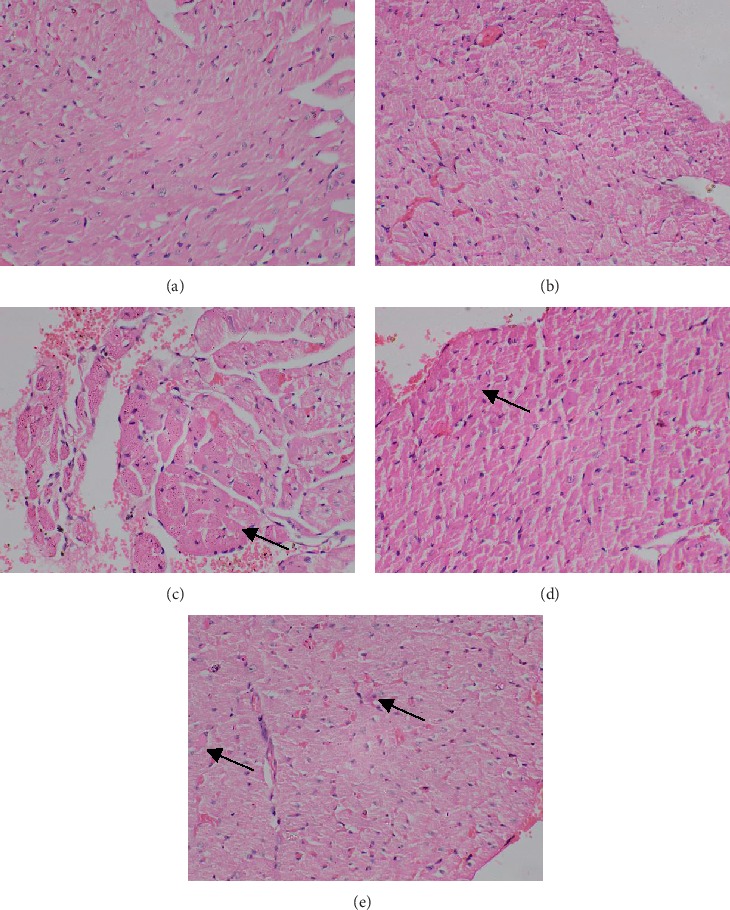


Plate 4: Doxorubicin-induced cardiovascular damage treated with 250mg/kg *Commelina diffusa* extract for 21 days, showing a smaller area of early changes of necrosis,

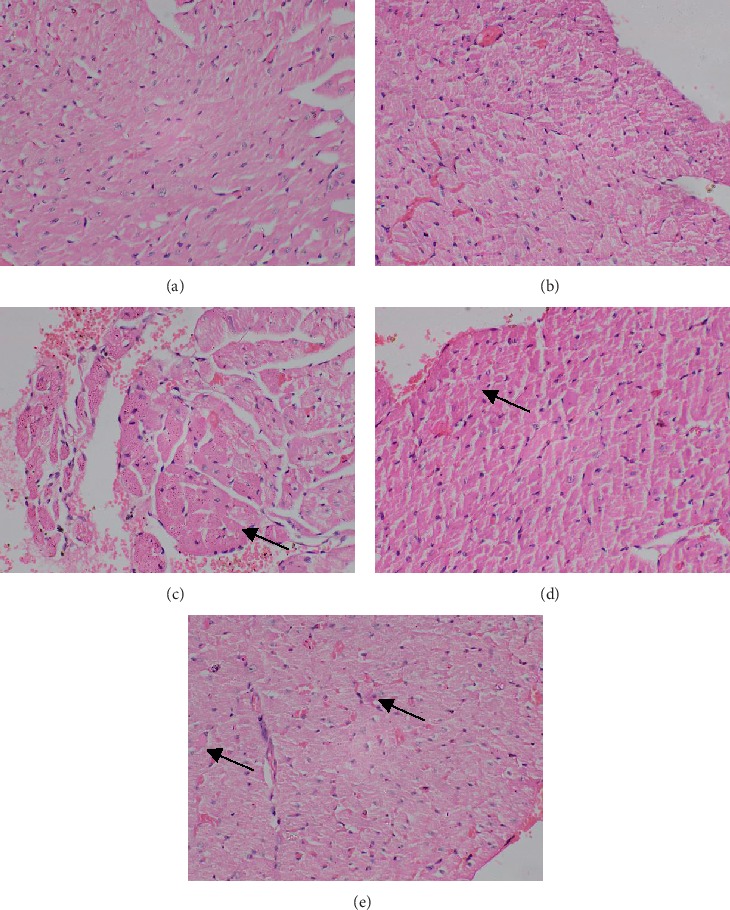


Plate 5: Doxorubicin-induced cardiovascular damage treated with 500mg/kg *Commelina diffusa* extract for 21 days, showing occasional cells with few necrotic cells of subendocardium.

**5. DISCUSSION OF FINDINGS**

In Table 2, the mean homogenate IL-6 level of the negative control was significantly raised after intraperitoneal administration of doxorubicin when compared to the normal control. The high IL-6 level noticed in the negative control points to cardiovascular toxicity due to doxorubicin administration. Treatment of rats with *Commelina diffusa* extract at 166mg/kg body weight for 21 days, significantly reduced the mean homogenate IL-6 concentration in comparison to the negative control. The mean homogenate IL-6 levels was considerably lower than the negative control after treatment with *Commelina diffusa* extract at 250mg/kg body weight for 21 days. More so, the mean homogenate IL-6 levels of rats treated with the extract at 500mg/kg body weight for 21 days was significantly reduced in comparison to the negative control value (Table 2). Aqueous extract of the aerial parts of *Commelina diffusa* at 500mg/kg body weight elicited a more significant effect on the IL-6 level followed by dose at 250mg/kg body weight while the least was dose at 166mg/kg body weight (Table 2). The effect produced by *Commelina diffusa* extract in this study is similar to that reported by Alaaeldin *et al.* (2021) on polyphenolic-enriched olive leaf extract attenuated doxorubicin-induced cardiotoxicity in rats via suppression of oxidative stress and inflammation.

Refli *et al.* (2020) examined the anti-inflammatory effects of bay leaf extract on CRP and myeloperoxidase (MPO) levels in a rat model of myocardial infarction. The results indicated that administration of the extract significantly reduced CRP and MPO levels in the heart tissue of rats with induced myocardial infarction, suggesting its potential cardioprotective properties. The mean homogenate C-reactive protein concentration of the negative control rats were significantly higher than that of the normal control rats. The significantly increased mean homogenate c-reactive protein levels observed in the negative control rats points to severe necrosis of cardiac tissues facilitated by exposure to 50mg/kg body weight of doxorubicin. The mean homogenate c-reactive protein level of rats treated with *Commelina diffusa* extract at 166m/kg body weight for 21 days was significantly reduced when compared to the negative control only. Treatment with the extract at 250mg/kg body weight for 21 days resulted in significant decreases on the mean homogenate c-reactive protein level in comparison to the negative control. *Commelina diffusa* extract at 500mg/kg body weight after oral treatment significantly decreased the mean homogenate c-reactive protein level in comparison to the negative control value (Table 2). This ameliorative effect elicited by *Commelina diffusa* aqueous extract in this study is related to that showed by Bisi et al. (20190 on Cardioprotective effects and antioxidant status of *Andrographis paniculata* in isoproterenol-induced myocardial infarction in rats.

[Saravanan](https://pubmed.ncbi.nlm.nih.gov/?term=Saravanan+G&cauthor_id=21962802) *et al*. (2013) evaluated the protective role of Amaranthus viridis Linn on isoproterenol (ISO)-induced myocardial infarction in rats. Subcutaneous injection of ISO led to significant increases in serum marker enzymes and cardiac troponin levels, indicating myocardial damage. Oral treatment with A. viridis extract (100, 200, and 300 mg/kg body weight) for 45 days resulted in a significant cardioprotective effect by lowering serum marker enzymes and cardiac troponin levels. The 300 mg/kg dose was particularly effective, bringing all parameters near normal levels. The mean homogenate cardiac troponin I and T of the negative control were significantly increased when compared to the normal control. The observed significant increases on the mean homogenate cardiac troponin I and T levels in comparison to the normal control is reflective severe damage to the heart due to intraperitoneal administration of 50mg/kg body weight of doxorubicin once. Treatment with aqueous extract of the aerial parts of *Commelina diffusa* at 166, 250, and 500mg/kg body weight for 21 days significantly reduced the mean homogenate cardiac troponin I and T concentration when compared to the negative control. The extract 500mg/kg body weight yielded a more ameliorative effect on the cardiac troponin I and T level, next was dose at 250mg/kg body weight while the least was dose at 166mg/kg body weight (Table 2). The significant reduction observed on the mean cardiac troponin I and T in comparison to the negative control is indicative that Commelina diffusa extract at 166, 250, and 500mg/kg body weight reduced the toxicological impact of doxorubicin exposure on plasma cardiac troponin I and T. These significant decreases on the mean cardiac troponin I and T disagree with the finding of Daniel et al. (2023), whom in their study on evaluation of cardiopreventive effects of *Ximenia americana* (Linn.) and *Pappea capensis* (Eckl. and Zeyh.) leaf aqueous extracts in rat models with myocardial infarction, reported that *Ximenia americana*  and *Pappea capensis* showed that both crude plant extracts caused increases on cardiac troponin I and T.

However, liver enzyme biomarkers are essential diagnostic tools for probing, monitoring, and evaluating the physiological state of the liver. ALT, AST, ALP, GGT, and LDH provide valuable insights into the extent and type of liver injury (Lala *et al*., 2021). Pureti *et al*. (2020) showed that intraperitoneal administration of doxorubicin enhances the production of reactive oxygen species (ROS), leading to lipid peroxidation and depletion of antioxidant defenses such as superoxide dismutase (SOD) and catalase (CAT). This oxidative imbalance results in hepatocyte injury. Additionally, DOX activates inflammatory pathways, increasing levels of cytokines like tumor necrosis factor-alpha (TNF-α) and interleukins (IL-1β, IL-6), further exacerbating liver damage (Pureti *et al*., 2020). Administration of flavonoid extract, significantly attenuated ROS production, lipid peroxidation, and inflammatory markers in DOX-treated rats and restored antioxidant enzyme activities and improved histological architecture of the liver. Also, extract from this plant demonstrated protective effects by decreasing serum transaminases and increasing antioxidant defense enzymes while wistopathological analysis showed reduced liver inflammation and necrosis in DOX-treated rats pretreated with *Solanum torvum* (Adil *et al.,* 2020). In this study, the mean homogenate ALT, AST, and ALP activities of the negative control in Table 3 were significantly increased following intraperitoneal administration of 50mg/kg body weight of doxorubicin once, when compared to the normal control. The significant increases observed on the mean homogenate ALT, AST, and ALP activities of the negative control is indicative of cardiovascular damage due to doxorubicin exposure. Aqueous extract of *Commelina diffusa* at 166, 250, and 500mg/kg body weight upon oral administration for 21 days, significantly decreased the mean homogenate ALT, AST, and ALP activities in comparison to the negative control (Table 3). *Commelina diffusa* extract at 500mg/kg body weight elicited a more ameliorative potential on the ALT, AST, and ALP activities, followed by dose at 250mg/kg body weight while the least was 166mg/kg body weight (Table 3). Elevation in LDH activities regarding probing for liver proper functioning is reflective of compromise in the functional capacity of the liver due to exposure to toxicants or chemical agents (Shun *et al*., 2024). The lactate dehydrogenase activity of the negative control rat evaluated in this study was significantly raised when compared to the normal control rats. The significantly increased lactate dehydrogenase activities observed in the negative control reflects damage to the heart due to exposure to doxorubicin at 50mg/kg body weight. Aqueous extract of the aerial parts of *Commelina diffusa* at 166, 250, and 500mg/kg body weight upon treatment for 21 days, significantly reduced the mean homogenate lactate dehydrogenase (LDH) activities when compared to the control. Also, the mean creatin kinase activity of the negative control rats was significantly higher than that of the normal control rats. The significantly increased creatin kinase activities noticed in the negative control suggest compromise in the cardiovascular function. However, treatment with *Commelina diffusa* extract at 166, 250, and 500mg/kg body weight yielded significant decreases on the mean homogenate creatin kinase (CK) activity when compared to the negative control value. *Commelina diffusa* extract at 500mg/kg yielded a more significant ameliorative potential on the mean homogenate lactate dehydrogenase and creatin kinase activities, followed by 250mg/kg body weight while the least was 166mg/kg body weight.

Meanwhile, Aml (2022) showed that intraperitoneal administration of doxorubicin facilitated increases MDA levels in cardiac tissues, signifying enhanced lipid peroxidation and oxidative stress. According to Asmaa and Yasser (2018), associated with compromised cardiac function and structural damage. Elevated MDA levels have also been observed in liver and kidney tissues following doxorubicin treatment, indicating oxidative damage in these organs (Asmaa and Yasser, 2018). In this study, Table 4 indicates the effect of *Commelina diffusa* extract on the mean oxidative stress biomarkers of heart homogenate in doxorubicin-induced cardiovascular damage in rats. The mean MDA level of the negative control was significantly increased after intraperitoneal administration of 50mg/kg body weight of doxorubicin once, when compared to the normal control. The significantly increased mean MDA level observed in the negative control is indicative of lipid peroxidation due to increased oxidative stress due to doxorubicin administration. Commelina diffusa extract at 166, 250, and 500mg/kg body weight upon oral treatment for 21 days, reduced the mean MDA level in comparison to the negative control. Ahmed *et al*. (2018) reported a 40% decrease in plasma GSH levels in rats treated with 5 mg/kg DOX weekly for 4 weeks. Similarly, Sharma *et al.* (2020) demonstrated a time-dependent reduction in plasma GSH following DOX injection, correlating with increased markers of lipid peroxidation. Antioxidants of bio-compounds from medicinal plants such as N-acetylcysteine (NAC), vitamin E, and curcumin elicit promising results in preserving plasma GSH levels and reducing oxidative damage in rats. The mean homogenate GSH level of the negative control was significantly decreased after intraperitoneal administration of doxorubicin when compared to the normal control rats (Table 3). The significantly decreased GSH level in the negative control points to compromise in the antioxidant defense mechanism in the rats facilitated by doxorubicin administration (Table 3). Treatment with aqueous extract of the aerial parts of Commelina diffusa at 166, 250, and 500mg/kg body weight for 21 days significantly increased the mean GSH levels in comparison to the negative control. El-Bahr *et al.* (2021) found that co-administration of NAC with DOX restored plasma GSH levels to near-normal values, improving overall antioxidant capacity which is similar to the effect elicited by Commelina diffusa extract in doxorubicin-induced cardiovacluar damage in this study. Jinping *et al*. (2008) showed that DOX administration can lead to a decrease in GPx activity, contributing to increased oxidative damage. In GPx1-deficient mice demonstrated heightened susceptibility to DOX-induced cardiotoxicity, underscoring the protective role of GPx against oxidative stress in cardiac tissue Jinping *et al*. (2008). Additionally, exercise training has been Kanter *et al*. (2022) indicated that doxorubicin modulate CAT activity, potentially mitigating DOX-induced cardiotoxicity. Mice subjected to a swim training program exhibited elevated CAT activity, which correlated with reduced cardiac damage following DOX administration. The mean homogenate GPx, CAT, and SOD activities of the negative control were significantly reduced when compared to the normal control values (Table 4). Intraperitoneal administration of doxorubicin significantly caused decreases on the GPx, CAT, and SOD activities through increased oxidative stress (Table 4). Treatment Commelina diffusa extract at 166, 250, and 500mg/kg body weight for 21 days significantly increased the mean homogenate GPx, CAT, and SOD activities when compared to the negative control (Table 4). The extract at 500mg/kg body wright yielded a more antioxidant capacity in comparison to 166 and 250mg/kg doses.

More so, cardiac electrolyte homeostasis plays a pivotal role in maintaining electrocardiogram , and overall heart function. Doxorubicin effective against cancer but notorious for causing **cardiotoxicity,** particularly through heart oxidative stress, mitochondrial dysfunction, and ion imbalance which reduces cardiac K+ Ca2+, HCO32-, and Na+ levels ([Jiang](https://pubmed.ncbi.nlm.nih.gov/?term=Jiang+J&cauthor_id=7954093) *et al.,* 2014). DOX interferes with intracellular Ca²⁺ regulation in cardiomyocytes. Studies using guinea-pig heart myocytes have demonstrated that DOX exposure depresses Ca²⁺ transients and contractions, prolongs the time to peak Ca²⁺ transient, and delays Ca²⁺ sequestration (Kendall, 2017). In table 5, the mean homogenate K+ Ca2+, HCO32-, Na+ levels of the negative control were significantly decreased when compared to the normal control rats (Table 5). The significantly decreased mean homogenate K+ Ca2+, HCO32-, Na+ levels in the negative control points to decline electrocardiogram of the rats (Table 5). The mean K+ and Ca2+ levels were significantly increased after treatment with *Commelina diffusa* extract at 166, 250, and 500mg/kg body weight when compared to the negative control values (Table 5). The mean homogenate HCO32- and Na+ levels were also significantly increased after treatment with the extract at the stated doses for 21 days (Table 5). Aqueous extract of *Commelina diffusa* at 500mg/kg body weight yield a more significant ameliorative effect on the assayed cardiac electrolytes followed by dose at 250mg/kg body weight while the least was dosage at 166mg/kg (Table 5).

Dong-Sheng *et al.* (2022) assessed various biomarkers to detect cardiac injury in rats administered doxorubicin. Their results indicated significant oxidative damage in myocardial tissue, highlighting the potential of these biomarkers for early detection of cardiotoxicity. In Plate 1-5 shows the effect of aqueous extract of the aerial parts of *Commelina diffusa* on cardiac histology in doxorubicin-induced cardiovascular damage in rats. Cardiac architecture of the extract treated groups were compared to the normal and negative control. Intraperitoneal administration of doxorubicin to rats n group 2-5 resulted in significant alteration in subendocardial region of myocardium of the rats as observed in Plate 2 (negative control). Treatment with *Commelina diffusa* extract at 166mg/kg bw elicited very little regeneration of damaged heart tissue and inflammation when compared to the negative control. *Commelina diffusa* extract at 250 and 500mg/kg bw showed significant amelioration on damage cardiac tissues in comparison to the negative control (Plate 4 and 5). The significant regeneration of damaged cardiac tissue due to necrosis facilitated by doxorubicin exposure is reflective of the cardio-ameliorative potential of *Commelina diffusa* extract, hence could serve as a source new herbal therapy in the treatment of cardiovascular dysfunction. The significant improvement observed on the cardiac architecture after treatment with aqueous extract of Commelina diffusa is similar to those reported by Vikas *et al.* (2015) on cardioprotective effect of ellagic acid on doxorubicin induced cardiotoxicity in Wistar rats.

**5. CONCLUSION**

This study determined the mitigation of doxorubicin-induced cardiovascular damage using *Commelina diffusa* extract in Wistar Rats. Intraperitoneal administration of doxorubicin to rats in group 3-5 resulted in significantly increased mean plasma cardiac electrolytes, IL-6, c-reactive protein, CTn-I, CTn-T, and MDA levels in comparison to the group 2 (negative control). Doxorubicin also induced significantly increased plasma cardiac ALT, AST, ALP. LDH, and CK activities when compared to the negative control. Aqueous extract of *Commelina diffusa* at 250 and 500mg/kg significantly reduced the plasma cardiac IL-6, c-reactive protein, CTn-I, CTn-T, and MDA levels. The extract at the stated doses stimulated significant increases in plasma cardiac electrolytes and GSH levels as well as increased the CAT, SOD, and PGx activities. The extract at 250 and 500mg/kg mediated regeneration of heart tissues damaged by doxorubicin exposure. Aqueous extract of *Commelina diffusa* ameliorated cardiovascular damage facilitated by doxorubicin exposure, hence could serve as a herbal agent in the treatment of cardiovascular damage.

**COMPETING INTERESTS DISCLAIMER:**

**Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.**

**REFERENCES**

Bianchi, V. E. (2020). Impact of nutrition on cardiovascular function. *Current Problems in*

*Cardiology, 45*(1), 100391.

Wu, R., Wang, H.-L., **&** Yu, H.-L. (2016). Doxorubicin toxicity changes myocardial energy

metabolism in rats. *Chemico-Biological Interactions, 244,* 149–158.

Verma, M., Shulga, N.**, &** Pastorino, J. G. (2013). Retracted:sirtuin-4 modulates sensitivity to

induction of the mitochondrial permeability transition pore. *Biochimica Biophysica Acta, 1827*(1), 38–49.

Tscheschner, H., Meinhardt, E.**, &** Schlegel, P. (2019). Camkii activation participates in

doxorubicin cardiotoxicity and is attenuated by moderate Grp78 overexpression. *PLoS One, 14*(4), e0215992.

Guanjing, L., Xiaoping, W., Nannan, T., Jing, C., Weili, L., Yawen, Z., Jinchi, J., Qianbin, S.,

Yanyan, J., Wei, W., **&** Yong, W. (2022). Mechanisms and Drug Intervention for Doxorubicin-Induced Cardiotoxicity Based on Mitochondrial Bioenergetics. *Oxid Med Cell Longev*, *14,* 1-3

Xiaoxiao, L., Guomin, W., Shuai, W., **&** Jinyu, H. (2023). Bibliometric and visual analysis of

doxorubicin-induced cardiotoxicity. *Front Pharmacol, 14,* 1-5.

Adegbite, O. A., Akinmoladun, F. J., Olaleye, T. M., Oladele, J. O., Akinrinlola, O. T., & Oboh,

B. O. (2020). Protective effects of Commelina diffusa extract against doxorubicin-induced cardiotoxicity in Wistar rats. Journal of Ethnopharmacology, 250, 112484

Singh, D. K., Sharma, R., Patel, S., Verma, P., & Kumar, V. (2018). Phytochemical screening and

antioxidant potential of Commelina diffusa extract. Journal of Medicinal Plants Research, 12(3), 45-52.

Zhang, Y., Liu, X., Wang, J., Chen, Y., & Li, Q. (2019). Anti-inflammatory and antioxidant effects

of Commelina diffusa in cardiovascular protection. Phytomedicine, 60, 152957.

Wellington, E. O., **&** ThankGod, I. E. (2024). Phytochemical and Essential Oil Quantification of

the Aerial Parts of *Commelina diffusa. World Scientific News,* 194, 150-173

Chaulin, A. M. (2021). Diagnostic value of highly sensitive cardiac troponins and mechanisms of

their increase in serum and urine in arterial hypertension. *La Rivista Italiana della Medicina di Laboratorio, 17,* 99–107.

Miruna, S., Dan B., Claudia, M. G., Mihaela, I., **&** Constantin, P. (2010). Quantitative Analysis of

Malondialdehyde in Normal Human Plasma Using Fluorescence and the Standard Addition Method. *FARMACIA, 58*, 4.

[Wheeler](https://pubmed.ncbi.nlm.nih.gov/?term=Wheeler+CR&cauthor_id=2327564), C.R., [Salzman](https://pubmed.ncbi.nlm.nih.gov/?term=Salzman+JA&cauthor_id=2327564), J.A., J. A.,  [Elsayed](https://pubmed.ncbi.nlm.nih.gov/?term=Elsayed+NM&cauthor_id=2327564), N.M.,   [Omaye](https://pubmed.ncbi.nlm.nih.gov/?term=Omaye+ST&cauthor_id=2327564), S.T., **&**  Korte, D.W. (1990).

Automated assays for superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activity

Mahmoud, H.H. (2018). Simple spectrophotometric assay for measuring catalase activity in

biological tissues. [*BMC Biochemistry*](https://bmcbiochem.biomedcentral.com/)*, 19,* 7.

**Bishop, M. L., Fody, E. P., & Schoeff, L. E.** (2013).Clinical Chemistry: Principles, Techniques,

and Correlations (7th ed.). Philadelphia, PA: Wolters Kluwer Health/Lippincott Williams & Wilkins.

Alaaeldin, A. H., Soha, O. H., Salsabil, H., Ali, A., & Ammr, A. (2021). Polyphenolic-enriched

olive leaf extract attenuated doxorubicin-induced cardiotoxicity in rats via suppression of oxidative stress and inflammation. *The Journal of Basic and Applied Zoology, 82*, 54.

Refli, H., Dharma, L., Gontar, A.S., & Zulfikri, M. (2020). The effect of bay leaf extract Syzygium

polyanthum (Wight) Walp. on C-reactive protein (CRP) and myeloperoxidase (MPO) level in the heart of rat model of myocardial infarction. *Med Glas (Zenica), 17*(1), 41-45.

Bisi O. A., Temitayo O. A., Ademola A. O., Temidayo O. O., Momoh A. Y., Aduragbenro D. A.,

Abiodun E. A., & Adeolu A. A. (2019). Cardioprotective effects and antioxidant status of Andrographis paniculata in isoproterenol-induced myocardial infarction in rats. *Journal of Medicinal Plants for Economic Development, 3*(1), 49

[Saravanan](https://pubmed.ncbi.nlm.nih.gov/?term=Saravanan+G&cauthor_id=21962802), G., [Ponmurugan](https://pubmed.ncbi.nlm.nih.gov/?term=Ponmurugan+P&cauthor_id=21962802), P., [Sathiyavathi](https://pubmed.ncbi.nlm.nih.gov/?term=Sathiyavathi+M&cauthor_id=21962802), M.,  [Vadivukkarasi](https://pubmed.ncbi.nlm.nih.gov/?term=Vadivukkarasi+S&cauthor_id=21962802), S., & [Sengottuvelu](https://pubmed.ncbi.nlm.nih.gov/?term=Sengottuvelu+S&cauthor_id=21962802), S.

(2013).Cardioprotective activity of Amaranthus viridis Linn: effect on serum marker enzymes, cardiac troponin and antioxidant system in experimental myocardial infarcted rats. *Int J Cardiol, 165*(3), 494-8.

Daniel, M.G., Patricia, M., & Matthew, P. N. (2023). Evaluation of cardiopreventive effects

of *Ximenia americana* (Linn.) and *Pappea capensis* (Eckl. and Zeyh.) leaf aqueous extracts in rat models with myocardial infarction. [*Future Journal of Pharmaceutical Sciences*](https://fjps.springeropen.com/)*, 9*, 14.

Lala, V., Goyal, A., & Minter, D. A. (2021). Liver function tests." StatPearls Publishing.

 Pureti, L.P., Kaviyarasi, R., Abilash, V.G. (2020). New molecular and biochemical insights of

doxorubicin-induced hepatotoxicity. *Life Science, 250*, 117599.

Adil, F.W., Summya, R., Shahzda, M., Rashid, M. A. A., Mohammad, R. K., Nazrul, H., Dhafer,

Y. A., Ajaz, A., Muneeb, R., Nazrul, H., Dhafer, Y. A., Ajaz, A., & Muneeb, U. R. (2020). Naringenin Regulates Doxorubicin-Induced Liver Dysfunction: Impact on Oxidative Stress and Inflammation*. Plant (Basel), 24*, 9(4), 550.

Shun, Y., Hongyu, C., Ting, T., Li, Z., Xingyue, Y., Xin, L., Zhiqiang, Y., Yongfeng, W., Jiaxin,

A., Guorong, W., Hai, J., & Biguang, T. (2024). Role of lactate and lactate metabolism in liver diseases (Review*). International Journal of Molecular Medicine, 24*, 5383

Aml, S. S. A. (2022). Potential protective effect of catechin on doxorubicin-induced cardiotoxicity

in adult male albino rats. *Toxicol Mech Methods, 32*(2), 97-105.

Asmaa, F. K., Yasser, S-El-S. (2018). All-trans-retinoic acid ameliorates doxorubicin-induced

cardiotoxicity: in vivo potential involvement of oxidative stress, inflammation, and apoptosis via caspase-3 and p53 down-expression. *Naunyn Schmiedebergs Arch Pharmacol, 391*(1), 59-70.

Ahmed, A., Khan, M. S., Jabeen, F., & Malik, A. (2018). Effect of doxorubicin on plasma GSH in

rats. Journal of Biochemical Toxicology, 32(5), e45562

Sharma, R., Gupta, A., Verma, P., & Singh, M. (2020). Antioxidant response in DOX-treated rats.

Pharmacology Reports, 72(3), 678-684.

Jinping, G., Ye, X., Ye-Shih, H., Xuwan, L., Chu, C.C., Xingshun, X., Hong, W., Ronald, H.,

Balvin, H.C. (2020). Glutathione peroxidase 1-deficient mice are more susceptible to doxorubicin-induced cardiotoxicity. *Biochim Biophys Acta, 1783*(10), 2020–2029.

Kanter, M.M., Hamlin, R.L., Unverferth, D. V., Davis, H.W., **&** Merola, A.J. (2022). Effect of

exercise training on antioxidant enzymes and cardiotoxicity of doxorubicin. *J Appl Physiol*, *59*(4), 1298-303.

[Jiang](https://pubmed.ncbi.nlm.nih.gov/?term=Jiang+J&cauthor_id=7954093), J., [Temma](https://pubmed.ncbi.nlm.nih.gov/?term=Temma+K&cauthor_id=7954093), K.,  [**&** Akera](https://pubmed.ncbi.nlm.nih.gov/?term=Akera+T&cauthor_id=7954093), T. (2014). Doxorubicin-induced changes in intracellular Ca2+

transients observed in cardiac myocytes isolated from guinea-pig heart. *Can J Physiol Pharmacol. 72*(6), 622-31.

Kendall, B. W. (2017). Adriamycin-induced interference with cardiac mitochondrial calcium

homeostasis. *Cardiovasc Toxicol, 7*(2), 101-7.

Dong-Sheng P., Bo, L., **&** San-Long, W. (2022). Evaluation of biomarkers for doxorubicin‑induced

cardiac injury in rats. *Experimental and Therapeutic Medicine, 24*, 712.

Vikas, S. W., Vishal, R. M., Arulmozhi, S., Subhash, L. B., **&**Kakasaheb, R. M. (2015).

Cardioprotective effect of ellagic acid on doxorubicin induced cardiotoxicity in Wistar rats. *Journal of Acute Medicine, 5,* 1, 1-8