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

**ABSTRACT**

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, the mechanism underlying doxorubicin-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. 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, given free access to rat feed and water. The rats were then divided into 5 groups on the basis of body weight. Five groups of five rats per group. Groups 1 and 2 served as normal and negative controls. Rats in groups 3-5 were induced with doxorubicin and treated with the extract at 166mg/kg, 250mg/kg, and 500mg/kg for 21 days. All analyses were carried out based on standard methods. All Data are represented as means ± error of mean (M±E) and were analyzed using Statistical Package for Social Sciences (SPSS) for Windows 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. 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**

Cardiovascular diseases (CVDs) constitute the number one cause of mortality at the global level, representing 30% of all global deaths. Therefore, finding ways to reduce deaths due to CVDs remains an important public health goal (Sulaiman et al., 2021). 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 concentrations of ATP for day after every day proper physiological activity (Bianchi, 2020). In the heart of any healthy human, almost all ATP is produced by the oxidative metabolism of mitochondria and medicaments that thwart mitochondrial responsibility might result in the 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*., 2016). Wu *et al*. (2016) 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. Research suggests that the Akt pathway is an important pathway for cell survival, and it prevents cardiac muscle cell apoptosis by inhibiting the pro-apoptotic factors like Bad. Moreover, Dox downregulates the Akt pathway and induces caspase-3, which causes apoptosis (Rawat et al., 2021).

Impersonally, mitochondrial bioenergetic collapse has become a significant feature of doxorubicin-induced 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.

Phosphocreatine (PCr) is the product of creatine phosphorylation by creatine kinase (CK) and serves as an important substrate for the synthesis of adenosine triphosphate (ATP) by phosphocreatine kinase. The superior efficacy of exogenous PCr administration in the cardioprotective terms is observed in numerous clinical applications of cardiovascular diseases, including heart surgery, acute myocardial infarction, chronic and acute heart failure, viral myocarditis and DOX-induced cardiotoxicity (Wang et al., 2021). Furthermore, Carvacrol (CA), chemically known as 2-methyl-5-isopropylphenol (C10H14O), is a phenolic monoterpene found in the essential oils of various aromatic plants in the Lamiaceae family (Retnosari et al., 2024). CA demonstrates promise in mitigating the development and progression of cardiovascular diseases. In a research study, four series of compounds, comprising conjugates of CA with 3HA (1–12), were synthesized and evaluated in a DOX-induced H9c2 cell death model aimed at developing novel and potent cardioprotective agents. Among these, 6 exhibited promising outcomes, significantly enhancing the viability of DOX-treated H9c2 cells (Retnosari et al., 2024).

*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 the administration of Commelina diffusa extract in Wistar rats reduced heart weight, improved antioxidant enzyme activity, and decreased markers of lipid peroxidation. Zhang *et al*. (2019), i**n potential clinical applications of *Commelina diffusa* extract,** demonstrated its antioxidant, anti-inflammatory, and anti-apoptotic properties, which reflect 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 dysfunction 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 in 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 procedures 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, given 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 the 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.

**2.5 Experimental Design**

Twenty-five (25) Wistar rats weighing between 160 and 200g were used for this study. They were purchased from the Biochemistry Animal House, University of Port Harcourt Choba and acclimatized for 14 days, giving free access to rat feed and water. The rats were then divided on the 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 humanely sacrificed through cervical dislocation, blood samples were collected for biochemical assays. The heart tissue of the rats in each group was harvested and cut into two equal halves. Half of the organs were homogenized for estimation of heart biomarkers, while the other half were 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 produced 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, forms 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 reacts 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. 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 for Social Sciences (SPSS) for Windows 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 results 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 was 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**

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 were 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 were 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 a dose at 250mg/kg body weight, while the least was a 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 was 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 in 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 in the mean homogenate cardiac troponin I and T levels in comparison to the normal control is reflective of 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, extracts from this plant demonstrated protective effects by decreasing serum transaminases and increasing antioxidant defense enzymes, while histopathological 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 in the mean homogenate ALT, AST, and ALP activities of the negative control are 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 a 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 reflect 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 a compromise in the cardiovascular function. However, treatment with *Commelina diffusa* extract at 166, 250, and 500mg/kg body weight yielded significant decreases in 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 the intraperitoneal administration of doxorubicin facilitated increased 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 a 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 cardiovascular 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. 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 shown to modulate CAT activity, potentially mitigating DOX-induced cardiotoxicity (Kanter *et al*., 2022). 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 weight yielded a higher 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 is 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 the decline in 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 yielded a more significant ameliorative effect on the assayed cardiac electrolytes, followed by a 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 the early detection of cardiotoxicity. 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 in group 2-5 resulted in significant alteration in the subendocardial region of the 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, it could serve as a source of new herbal therapy in the treatment of cardiovascular dysfunction. The significant improvement observed in the cardiac architecture after treatment with the aqueous extract of *Commelina diffusa* is similar to that reported by Vikas *et al.* (2015) on the 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 groups 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 group 2 (negative control). Doxorubicin also induced significantly increased plasma cardiac ALT, AST, and 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 the 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.**

**Ethical Approval**

Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of UAT/CEREMAD/REC/MM72/011 .Ref. No. UAT20/03/01/1377.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

**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

Rawat, P. S., Jaiswal, A., Khurana, A., Bhatti, J. S., & Navik, U. (2021). Doxorubicin-induced cardiotoxicity: An update on the molecular mechanism and novel therapeutic strategies for effective management. *Biomedicine & Pharmacotherapy*, *139*, 111708.

Wang, C., Hu, L., Guo, S., Yao, Q., Liu, X., Zhang, B., ... & Yang, X. (2021). Phosphocreatine attenuates doxorubicin-induced cardiotoxicity by inhibiting oxidative stress and activating TAK1 to promote myocardial survival in vivo and in vitro. *Toxicology*, *460*, 152881.

Retnosari, R., Oh-Hashi, K., Ugusman, A., Zainalabidin, S., Latip, J., & Oka, N. (2024). Carvacrol-conjugated 3-Hydroxybenzoic Acids: Design, Synthesis, cardioprotective potential against doxorubicin-induced Cardiotoxicity, and ADMET study. *Bioorganic & Medicinal Chemistry Letters*, *113*, 129973.

Retnosari, R., Abdul Ghani, M. A., Majed Alkharji, M., Wan Nawi, W. N. I. S., Ahmad Rushdan, A. S., Mahadi, M. K., ... & Latip, J. (2024). The Protective Effects of Carvacrol Against Doxorubicin-Induced Cardiotoxicity In Vitro and In Vivo. *Cardiovascular Toxicology*, 1-15.

Sulaiman, M. A., Dahiru, D., Jada, M. S., & Hayatu, A. I. (2021). Curative Effects of Aqueous and Ethanol Stem Bark Extracts of Vitex doniana on Doxorubicin-Induced Cardiotoxicity in Rats. *Journal of Complementary and Alternative Medical Research*, *13*(3), 8–17.