

Improving Captopril cardioprotective activities against Doxorubicin induced cardiotoxicity by co-administration with allicin**Esam M. Aboubakr^{a*}, Mohammed I. Rushdi^b, Elmoiz Yousif Ali Babekir^c**^a Department of Pharmacology and Toxicology, Faculty of Pharmacy, South Valley University, Qena, 83523, Egypt^b Department of Pharmacognosy, Faculty of Pharmacy, South Valley University, Qena, 83523, Egypt^c Medicine and Adult Critical Care Medicine, Ibn Sina National College for Medical Studies, Jeddah 21589, Saudi Arabia.**Abstract****Background:** Doxorubicin (DOX) is an anticancer drug with significant clinical implications; however its utilization is limited due to its significant adverse effects especially on the cardiac muscle.**Objectives:** The current study aimed to examine improving the cardio-protective activity of Captopril (CAP) against Doxorubicin (DOX) induced cardiotoxicity via co-administration with allicin and investigate the potential underlying mechanisms.**Materials and methods:** 40 Sprague dewily rats were allocated into five groups, consisting of a Control group, DOX group, DOX + CAP group, DOX + allicin group and DOX + CAP + allicin group. Biochemical and histological evaluations of the myocardial tissue were conducted. The study utilized myocardium specimens to determine the levels of lipid peroxide product (MDA) and superoxide dismutase (SOD) in addition to reduced glutathione (GSH), while the inflammatory mediators IL-1 β and TNF- α in addition to cardiac troponin I and creatine kinase were determined in rats' sera.**Results:** either CAP or allicin administration resulted in a significant reduction in the biochemical indicators of cardiotoxicity. Both CAP and allicin ameliorate Dox impact on inflammation and oxidative stress markers, whereas allicin showed higher impact than CAP. However, the combined administration of CAP with allicin produced much more ameliorative effects. Histopathological, the concurrent administration of both medications produced higher effect to mitigate DOX-induced myocardial damage than use any of them solely.**Conclusion:** this study revealed that the cardioprotective activities of CAP can be greatly enhanced by co-administration with allicin via dramatically reduction in both oxidative stress and inflammatory status that contribute to DOX induced cardiotoxicity.**Keywords:** Cardiotoxicity; Doxorubicin; Oxidative stress, Allicin, Inflammation.**DOI:** 10.21608/svuijm.2023.248450.1735***Correspondence:** esam_pharma@svu.edu.eg**Received:** 17 November, 2023.**Revised:** 4 December, 2023.**Accepted:** 4 December, 2023.**Published:** 4 December, 2023**Cite this article** as: Esam M. Aboubakr, Mohammed I. Rushdi, Elmoiz Yousif Ali Babekir (2024). Improving Captopril cardioprotective activities against Doxorubicin induced cardiotoxicity by co-administration with allicin. *SVU-International Journal of Medical Sciences*. Vol.7, Issue 1, pp: 49- 61 .

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Introduction

Doxorubicin utilization in cancer treatment was initiated throughout the late 1960s. This compound has been recognized as a highly effective class of broad-spectrum anticancer anthracycline antibiotics, used in the treatment of different types of cancer, including lymphoma, soft-tissue sarcoma, leukemia, and solid tumors, the administration of DOX can be either as a monotherapy or in combination with other anticancer drugs (Arcamone, 2012; Bin Jordan et al., 2020; Tacar et al., 2013). The clinical application of DOX is significantly limited by its severe side effects; mainly cardiotoxicity, and renal toxicity, which eventually results in congestive heart failure and advanced nephrotoxicity (Duarte et al., 2023; van der Zanden et al., 2021; Wu et al., 2016).

The exact mechanism of cardiotoxicity induced by DOX is not fully explored. Nevertheless, different studies have found that iron anthracycline free radical formation is one of the main mechanisms that produce its myocardial damage (Kong et al., 2022; Songbo et al., 2019). DOX administration can induce severe inflammation in different organs including liver, intestine, kidney, and blood vessels, in addition severe cardiac inflammation. DOX administration intensely increase the proinflammatory cytokines levels, such as IL-6, IL1 and TNF α , meanwhile, blocking this effect can alleviate to great extent its induced tissue damage (Bhagat et al., 2022; Bin Jordan et al., 2020).

The significance of angiotensin II, the primary mediator of the renin-angiotensin system, has been determined as being of significant importance in the development of various cardiovascular ailments (Rajaram, 2021; Thangavel et al., 2021). Studies have indicated that angiotensin-converting enzyme inhibitors

(ACEIs) can produce a beneficial effect in mitigating the cardiotoxicity induced by DOX. Nevertheless, the precise mechanisms by which ACEIs and ARBs exert their effects and their respective efficacies in DOX-induced cardiotoxicity remain need further studies. Hence, additional empirical investigations are required to validate their effectiveness and define their precise method of operation (Gao et al., 2023; Umadat and Gharacholou, 2022).

Alliin represents the main bioactive ingredients obtained from garlic extract, studies have suggest that alliin administration may have a potential role in the prevention of specific types of malignancies, as well as in the reduction of blood sugar levels, blood pressure and cholesterol levels, additionally its consumption could facilitate muscle recovery and provide protection against infections (Marón et al., 2020; Salehi et al., 2019). Alliin has attracted considerable interest owing to its potent antioxidant properties in mitigating oxidative stress. A studies conducted by researchers emphasized the efficacy of alliin and its precursor, alliin, as antioxidants inside the Fenton oxygen-radical producing system through their interaction with enzymes that contain thiol groups (Aboubakr et al., 2023; Nadeem et al., 2021). This interaction implies that alliin has the potential to efficiently counteract detrimental oxygen radicals and protect cells from oxidative injury. The antioxidant capabilities of alliin are thought to be a result of its capacity to inhibit the activity of hydroxyl and superoxide radicals, which are reactive species that play a role in oxidative stress (Chan et al., 2013; Okada et al., 2006).

The relationship between the angiotensin-oxidative stress axis and mitochondrial dysfunction plays a crucial role in the development of cardiovascular

comorbidities, including obesity, insulin resistance, metabolic syndrome, and inflammation (Cooper et al., 2007; Zablocki and Sadoshima, 2013). In this context, the mitigation of oxidative stress is a fundamental mechanism for the observed beneficial effects of oxidative stress controllers. In accordance with the observed mitochondrial ultrastructural characteristics, scientists conducted a study that revealed a notable reduction in NADPH oxidase activity, as well as the induction of heat shock protein 70 (HSP70) subsequent to allicin administration. Allicin has been found to provide protection against glutamate-producing oxidative stress in a model of spinal cord neurons via modulating the HSP70 pathway (Nguyen Dinh Cat et al., 2013; Wang et al., 2013).

Taken all together, the present study was conducted to examine the potentiality to enhance the cardio-protective properties of CAP against DOX induced myocardial toxicity.

Materials and Methods

Disodium hydrogen phosphate, allicin, captopril, CCl₄ and thiobarbituric acid, and sodium dodecyl sulfate (SDS) were purchased from Sigma-Aldrich (Saint Louis, MO, United States).

Forty male Sprague dewily rats, weighing 180–200 g, were purchased from the National Research Centre (NRC) in Giza, Egypt., and housed in well-ventilated cages, at room temperature $26 \pm 2^\circ\text{C}$ and humidity of $58 \pm 5\%$ under 12 hr dark-light cycle for 10 days before the experiment. The experimental protocols follow the Guidelines used for Animal Experimentation and approved by the ethical committee of the Faculty of Pharmacy at South Valley University, Egypt, under approval number (P.S.V.U 200/23). Animals were fed standard diet with free access to water ad libitum. All rats were divided randomly into

five groups, animal groups comprise eight animals each as follows:

- Control group: orally administered 1 mL of 0.9% sodium chloride on days 8, 10, 12, 15, 17, and 19 by gavage tube from the begin of the study.
- DOX group: Rat's i.p injected with DOX (3 mg/kg) on days 8, 10, 12, 15, 17 and 19, which applied for all animal groups except the control group (Aziz, 2021).
- CAP group: rats treated with CAP orally 25 mg/kg on daily base for 21days in combination with DOX treatment protocol (Tomaz de Castro et al., 2021).
- Allicin group: rats treated with allicin orally 14 mg/kg on daily base for 21days in combination with DOX treatment protocol (Cui et al., 2020).
- CAP + Allicin group : rats treated with CAP orally 12.5 mg/kg + allicin orally 7 mg/kg on daily base for 21days in combination with DOX treatment protocol.

At the end of study rats were killed under ether anesthesia, Cardiac puncture was performed to collect blood samples. The collected blood was then subjected to centrifugation to separate the sera. The separated sera were refrigerated at -20°C to be utilized for further assessments, whereas sections of the cardiac muscle were kept in formalin, while the other parts were homogenized in cold phosphate buffe solution, followed by centrifugation at 3000 rpm for 15 min and the supernatant was collected and frozen at -80°C to be used in further determinations.

Histopathological procedures Rat's myocardia were immersed in a 10% formalin solution at a temperature of 28°C for 24 hours. Subsequently, the cardiac tissues were fixed in paraffin wax and sliced into sections with a thickness of 4 μm . The H&E staining procedure was conducted in

accordance with the established protocol, involving the addition of a 0.8% hematoxylin solution for a duration of 5 minutes, followed by the application of a 0.35% eosin stain for 3 minutes at a temperature of 28 °C. The examination of cardiac tissue architecture was conducted using an Olympus light microscope at a magnification of 200 x. The myocardial injury score was performed by pathologists who were independent of the study.

Masson's trichrome staining technique: The cardiac tissues, which were embedded in paraffin, were sectioned into consecutive slices measuring 4 µm in thickness. These sections were then subjected to staining using Masson's trichrome stain, which incorporates a blue aniline dye. The sections were subsequently subjected to analysis by optical microscopy at a magnification of 200 x.

Total protein determination: The cardiac protein content was quantified in tissue homogenates using Bio-Rad protein assay kit (California 94547, USA) and following the manufacturer instructions.

Catalase determination: The purpose of this test is to assess the cardiac catalase antioxidant activity, serves to protect the heart tissue from oxidative harm by facilitating the conversion of hydrogen peroxide into water and oxygen. The assay quantifies the residual concentration of hydrogen peroxide following the catalytic activity of catalase on samples of cardiac tissue homogenate (Aboubakr et al., 2013; Qin et al., 2010).

Super oxide dismutase determination: The concentration of SOD was determined as previously reported methods using Biodiagnosics (Cairo, Egypt) assay kit.

Reduced glutathione determination: The levels of glutathione (GSH) in homogenized cardiac tissue were quantified as previously reported methods (Aboubakr et al., 2017; Ellman, 1959) by using a

commercially available kit provided by Biodiagnosics (Cairo, Egypt), in accordance with the instructions provided by the manufacturer.

Determination of malondialdehyde (MDA) content: The quantification of cardiac lipid peroxidation in the tissue was conducted using the assessment of malondialdehyde (MDA) levels. The methodology relies on the chemical interaction between MDA and thiobarbituric acid under acidic conditions at a temperature of 95 °C, yields a pink-colored product that can be quantified by measuring its absorbance at a wavelength of 532 nm (Aboubakr et al., 2022; Niehaus and Samuelsson, 1968).

Enzyme-linked immunosorbent assay: The ELISA method was employed to measure the plasma concentrations of the inflammatory mediators), tumor necrosis factor-alpha (TNF- α) with Catalog # MTA00B-1 and interleukin-1 beta (IL-1 β) with catalog # MLB00C-1, in accordance with the instructions provided by the manufacturer (R&D Systems, Minneapolis, USA.)

Statistical analysis

The mean \pm standard deviation (SD) was used to express all parameters in the current study. The acquired findings were subjected to statistical analysis using a One-way Analysis of Variance (ANOVA) test, followed by Tukey's test. The statistical significance was assessed to be $P < 0.05$.

Results

Hematoxylin and eosin (H&E) staining

The control group exhibited normal cardiac fiber structure with typical striations. The group treated by DOX a had distinct patterns of cardiac fiber separation, the presence of vacuolated cells, edema, and a high concentration of inflammatory cells dispersed around blood vessels, while the smooth muscle tissue displayed degenerative changes. The group treated with CAP

exhibited a moderate disruption in the striations of the heart muscle, mild vacuolization in certain cardiac cells, and an increase in the presence of inflammatory cells when compared to the control group. The bundles of smooth muscle in the heart tissue exhibited moderate degeneration, characterized by a pale and eosinophilic cytoplasm associated with mild

inflammatory cells infiltration was observed in allicin treated group. On the other hand, the group which received DOX + CAP + allicin had a significant decrease in the myocardial cytoplasm granularity, the cardiac smooth muscle did not exhibit any signs of degeneration, and the distribution of inflammatory cells was observed to be minimal, as depicted in (Fig.1).

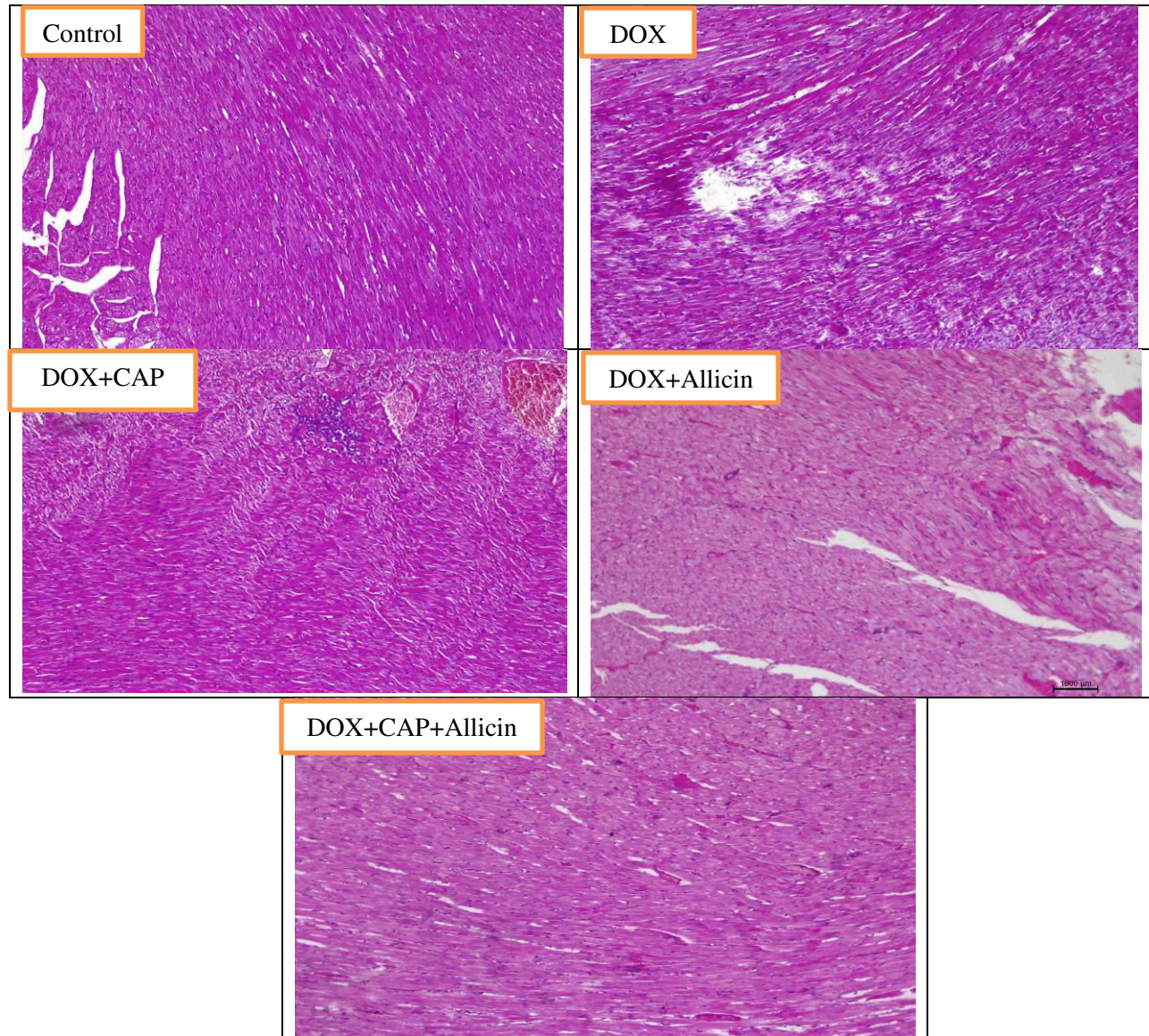


Fig.1. The effects of CAP, Allicin, and CAP + Allicin on the histopathological features of the cardiac muscle of DOX treated animals examined by H&E stain.

Masson's trichrome staining results

The histological analysis of cardiac tissue sections from various treated groups was conducted using Masson's trichrome staining. The investigation revealed that the control group exhibited a minimal presence of collagenous fiber adjacent to blood vessels. The group of individuals treated by DOX exhibited a conspicuous and compact accumulation of collagen surrounding nearly all blood arteries and various regions of heart tissue in addition to fibrosis was observed in both the perivascular and

interstitial areas of the myocardium. The group that received DOX + CAP treatment exhibited a moderate level of collagen deposition, accompanied by the presence of perivascular fibrosis in many instances. The group that received DOX + allicin treatment exhibited a decrease in cardiac fibrosis, characterized by a modest presence of collagen surrounding the blood vessels in the heart. Moreover, the group treated by DOX + CAP + allicin showed nearly normal myocardial fibers, as depicted in (Fig.2).

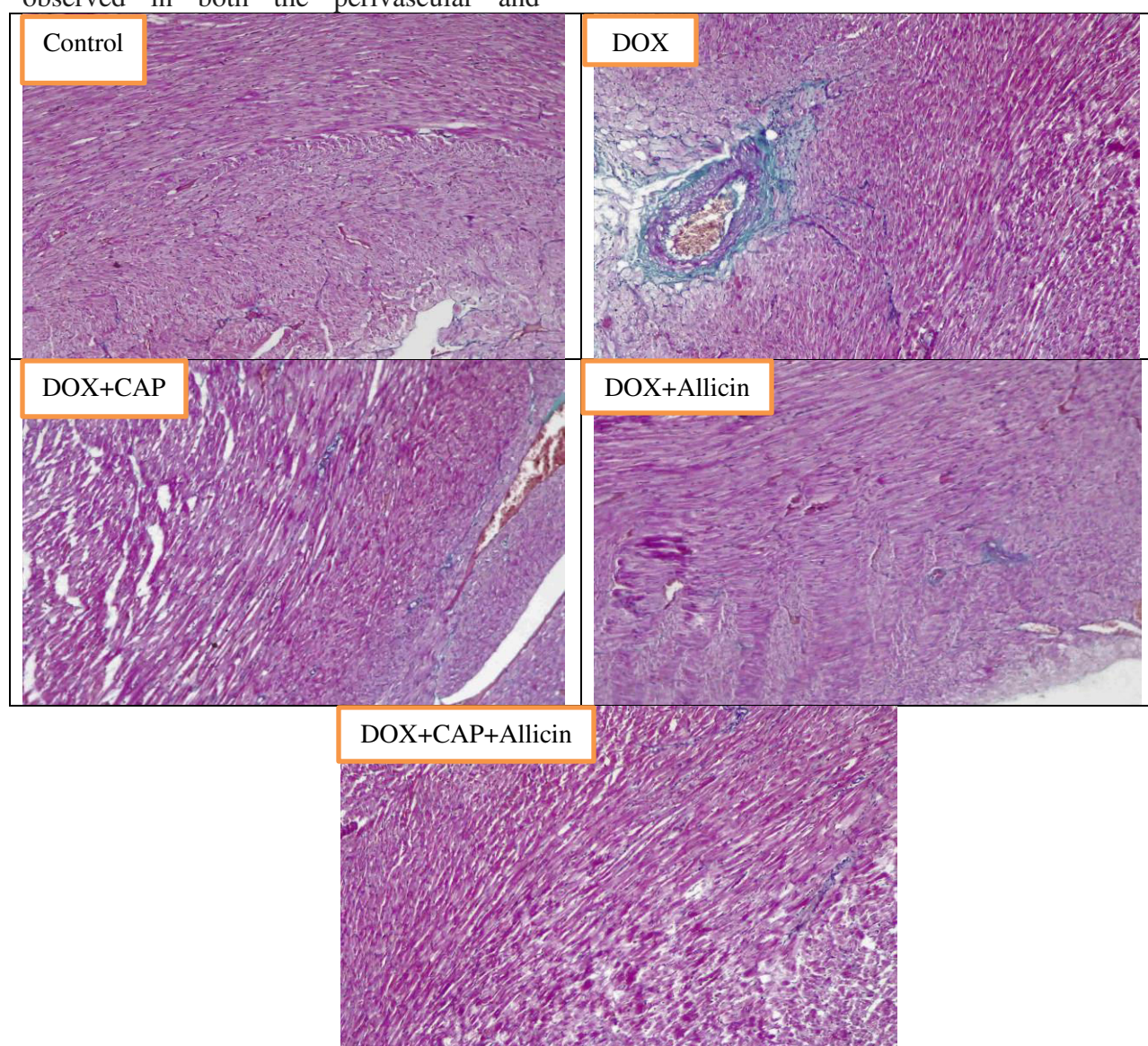


Fig.2.The effects of CAP, Allicin, and CAP + Allicin on the fibrotic changes of the cardiac muscle of DOX treated animals examined using Masson trichrome stain

Effect of CAP and/or allicin on Serum Level of Troponin I and CPK

In the DOX-treated group, a significant increase (P -value < 0.05) in the level of troponin I and CPK compared to the control group (2.3 ng/ml and 184.3 U/L respectively). The combination of CAP with allicin produced a significant (P value $<$

0.05) decrease of both Troponin I and CPK (1.1 ng/ml and 112 U/L respectively) compared to the DOX group. Each of CAP and allicin individual administration also significantly (P -value < 0.05) decreased the level compared to DOX group (1.5 ng/ml and 135 U/L respectively) and (1.58 ng/ml and 140 U/L respectively) (**Fig.3**).

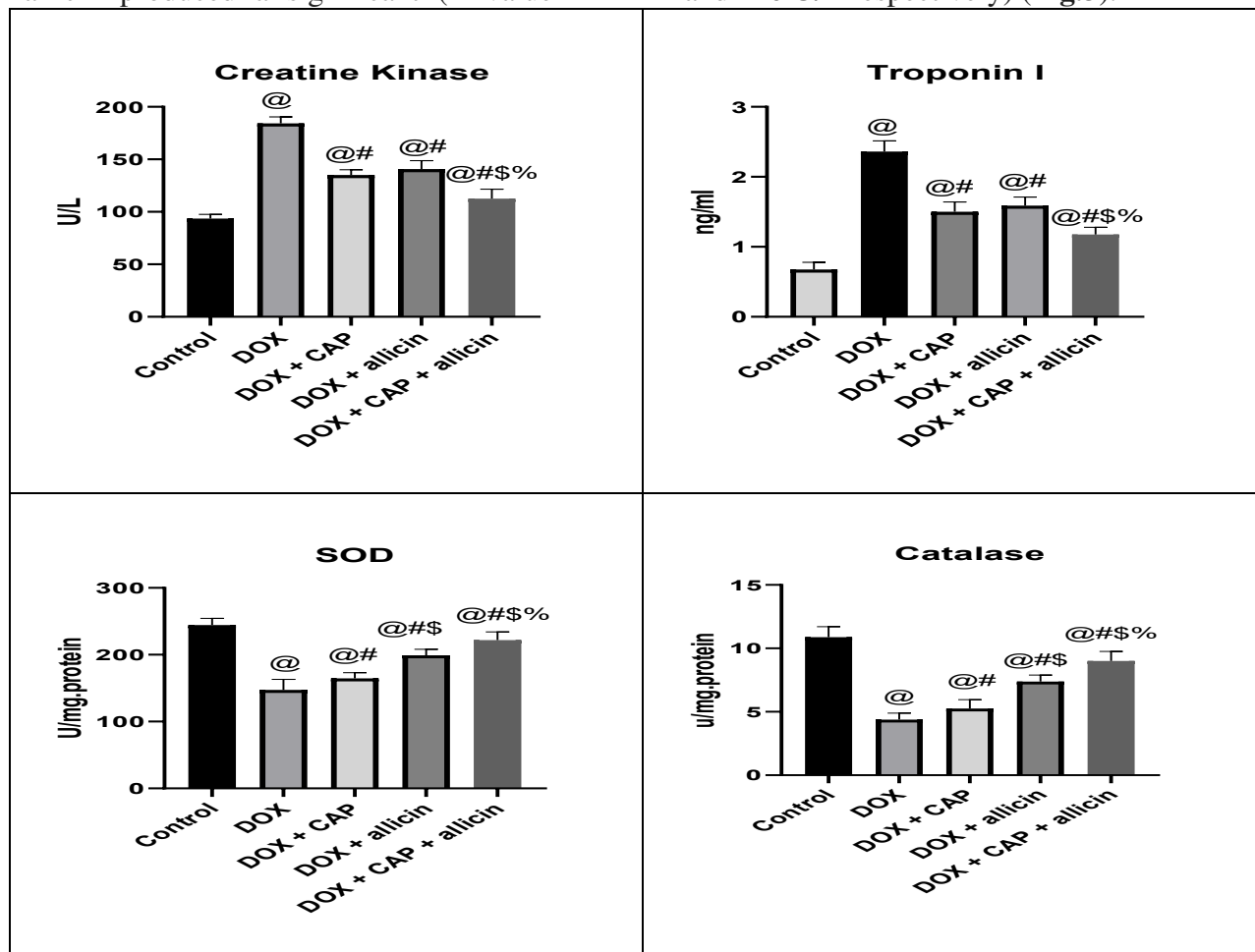


Fig.3 . Effect of CAP and allicin on the activities of creatine kinase, troponin 1, SOD and Catalase in the normal and DOX treated rats.

@ = significantly different compared to the control group, # = significantly different compared to DOX group, \$ = significantly different compared to the CAP group AND % = significantly different compared to allicin group. Results are presented as mean \pm SEM (n = 8).

Effect of CAP and/or allicin on antioxidant enzymes

The present study found a notable reduction in the levels of antioxidant enzymes, namely catalase and superoxide dismutase (SOD), in addition to reduced glutathione (GSH), in rats that were administered dox (147.2 U/mg.protein, 4.3 U/mg.protein and

8.3nmol/mg.protein respectively) comber to control group (10.8 U/mg.protein, 244 U/mg.protein and 19.25 nmol/mg.protein respectively). On the other hand, the administration of CAP through oral means demonstrated a moderate protecting against these enzymes depletion, resulting in values of 5.25 U/mg.protein, 164.5 U/mg.protein

and 10.6 nmol/mg.protein respectively, while allicin administration showed higher protective effect (7.3 U/mg.protein, 199 U/mg.protein and 12.5 nmol/mg.protein respectively). On the other hand, the combined administration of CAP + allicin showed the highest observed concentration of these enzymes between all DOX treated groups. (Fig.3).

Effect on lipid peroxidation

The impact of cap + allicin on lipid peroxidation was investigated in this study,

and it was found that malondialdehyde (MDA) concentration, a commonly used biomarker for assessing tissue lipid peroxidation, was significantly lowered in this group 123.3 μmole/gm.protein compare to other dox treated groups 205 μmole/gm.protein, 189 μmole/gm.protein and 151 μmole/gm.protein wherase the individual administration of these drugs produced a moderate effect (Fig.4)

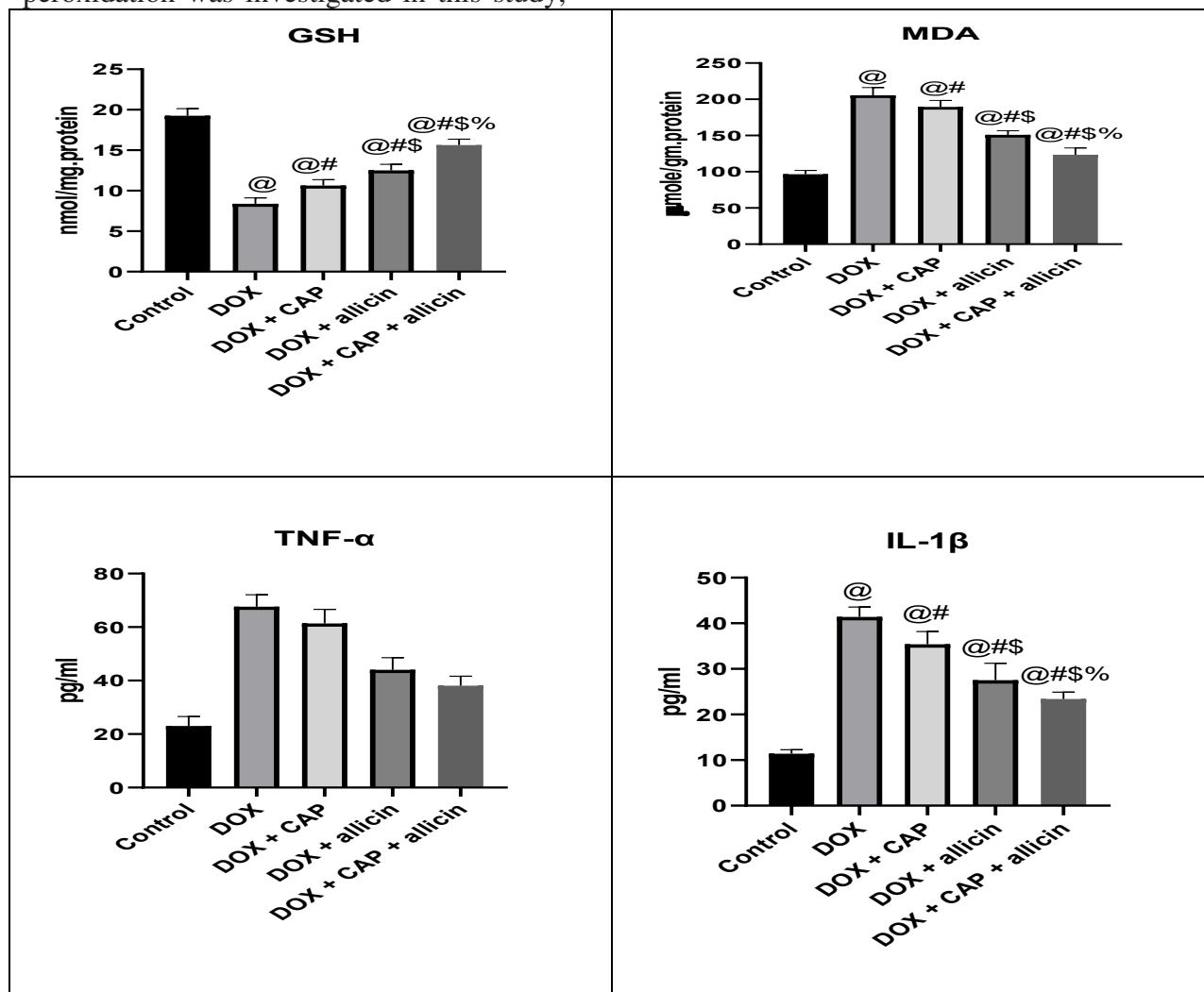


Fig.4 . Effect of CAP and allicin on the activities of GSH, MDA, TNF-α and IL-β in the normal and DOX treated rats.

@ = significantly different compared to the control group, # = significantly different compared to DOX group, \$ = significantly different compared to the CAP group AND % = significantly different compared to allicin group. Results are presented as mean ± SEM (n = 8).

Effect on the inflammatory mediators

In the present investigation, the administration of DOX to rats produced a significant elevation ($P < 0.05$) in the levels of tumor necrosis factor-alpha (TNF- α) and interleukin-1 beta (IL-1 β), up to 67.6 and 41.3 pg/ml, respectively. In comparison, the control group which exhibited a concentrations of 23 and 11.3 pg/ml for TNF- α and IL-1 β , respectively. In contrast, the oral administration of the CAP resulted in a moderate reduction in the elevated TNF- α and IL-1 β , with levels decreasing to 61.3 and 35.3 pg/ml, respectively. The observed amounts of TNF- α and IL-1 β in the allicin treated group were 44 and 27.5 pg/ml respectively, while CAP + allicin group showed the lowest concentration between other dos treated group, (Fig.4).

Discussion

DOX is a double-edged sword used in treatment of different types of cancer, mostly due to its propensity to cause cardiotoxicity as a side effect, which is contingent upon the dosage administered (Dulf et al., 2023). The cytotoxic effects of DOX result in myocyte injury, ultimately contributing to the development of cardiomyopathy (Dulf et al., 2023). Research findings indicate that the occurrence of DOX-induced cardiotoxicity is influenced by various parameters, including the cumulative dosage of DOX, the specific dosing schedule, and the age of the individual (Rawat et al., 2021). The cardiotoxicity generated by DOX might manifest as either acute, subacute, or chronic. Acute cardiotoxicity generated by DOX typically manifests during a period of 2-3 days following DOX injection, with an estimated incidence rate of roughly 11%. Histological changes commonly observed in cases of acute cardiac damage generated by DOX encompass cytoplasmic vacuolation, as well as reduced density and discontinuity of myofibrils (Nishi et al., 2021), which

was in accordance with our findings in this study.

Oxidative stress is known to have a significant impact on certain clinical disorders, such as hypertension, pulmonary hypertension, diabetes, hepatic diseases and myocardial disorder (Hofni et al., 2023; Mansouri et al., 2023a; Ogura and Shimosawa, 2014). The primary factor contributing to cardiotoxicity is oxidative stress generated by DOX, which characterized by an imbalance in the generated amounts of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which in turn leads to the dysregulation of antioxidants, resulting in the destruction of subcellular structures and controlled cell death (Karabulut et al., 2021; Mansouri et al., 2023b). The cardiac muscle exhibits diminished expression of natural antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and glutathione (GSH) under cases of severe oxidative stress such as found by DOX administration, consequently the heart becomes more susceptible to damage (Moutabian et al., 2022; Songbo et al., 2019). In the present study CAP administration noticeably ameliorate DOX oxidative stress effect, while allicin produced higher significant ameliorative effect on the myocardial oxidative stress via inhibition of the antioxidant enzymes catalase and SOD depletion in addition to GSH upregulation, while the combined administration of CAP + DOX produced the higher observed protective activity against myocardial oxidative stress.

DOX has the potential to induce cardiotoxicity, cardiomyopathy, and congestive heart failure via inflammatory pathways, such as the upregulation of prostaglandin E2 and IL-1 β . Several prior investigations have demonstrated an elevation in monocyte chemotactic protein-1

(MCP-1), IL-8, NF- κ B and TNF- α levels in rats induced by DOX administration (Todorova et al., 2020; Yarmohammadi et al., 2021). The impact of DOX on adipose tissue has been shown to result in the activation of NF- κ B and the production of inflammatory cytokines. This, in turn, leads to an elevation in serum levels of total cholesterol, triglycerides, and low-density lipoprotein (Hu et al., 2021). The observed phenomenon may be attributed to the downregulation of peroxisome proliferator activated receptor gamma (PPAR γ) produced by DOX in adipose tissue, and can be attributed to a reduction in the clearance of circulating free fatty acids, an augmentation in the presence of macrophage cells, and the activation of NF- κ B and inflammatory cytokines (Bin Jordan et al., 2020), which was in agreement with our findings thus a severe inflammatory cells infiltration was observed in the cardiac tissue of DOX treated group accompanied by significant upregulation of both IL-1 β and TNF- α . This effect was mildly inhibited by CAP administration while allicin administration produced the higher ameliorative effect. On the other hand, the combined administration of CAP + allicin in rats treated by DOX, dramatically ameliorated DOX proinflammatory effect.

Conclusion

The present study has found that the addition of allicin to already well established cardioprotective drug CAP can significantly improve its therapeutic effect as a cardioprotective agent, specially in case of advanced myocardial damage produced by DOX (broadly used anticancer drug), via significantly improve its antioxidant and anti-inflammatory properties.

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