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Original Research Article

Targeting Inflammation, Oxidative Stress, and Apoptosis by Indole-3-Carbinol to Ameliorate Doxorubicin- Induced Cardiotoxicity in Mice

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Abstract: ***Background***: Doxorubicin (DOX) is an effective antineoplastic agent of the anthracycline group. However, as with most anticancer drugs, they cause some toxic effects, including major cardiotoxicity. Oxidative stress, inflammation and apoptosis contribute to the pathological basis of doxorubicin (DOX)-induced cardiotoxicity. Indole-3-carbinol is a cruciferous-derived phytochemical, with potential anti-inflammatory and antioxidant effects. ***Purpose*:** Our present study aimed to investigate the protective effects of I3C against DOX- induced cardiotoxicity in mice by Targeting Inflammation, Oxidative Stress, and Apoptosis. ***Methods*:** BALB/c mice were subjected to DOX (4 mg/kg/day, i.p.) once weekly on days 0, 7, 14, 21 (for 21 days) to generate DOX- induced cardiotoxicity. Indole-3-carbinol was administered daily orally in the diet at two dose levels; 1000 ppm and 2000 ppm for 7 days before and 42 days after first injection of DOX. Serum creatine kinase (CK-MB), lactate dehydrogenase (LDH) and troponin I (cTn-I) levels activities, which are cardiac function markers were determined. Also, the levels of malondialdehyde (MDA) was assessed and enzyme activities of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx) were assessed in the heart tissues to determine the protective effect of Indole-3-carbinol against oxidative stress. To determine the anti-inflammatory effect, the levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) were assessed in the heart tissues. Parts of the heart were subjected to histopathological and immunohistochemical examinations. ***Results*:** Indole-3-carbinol in a dose-dependent manner was found to have the ability to mitigate the harmful effects of DOX on myocardial muscles with significant decrease in serum levels of CK-MB, LDH and cTn-I. In addition, Indole-3-carbinol significantly inhibited DOX-induced cardiac oxidative stress as seen in the reduced level of MDA and increased SOD, CAT, GR and GPx cardiac tissue levels; Indole-3-carbinol significantly reduced inflammatory mediators TNF-α and IL-6 levels and inhibited cardiac apoptosis by modulating Caspase 3 cardiac tissue levels. Moreover, Indole-3-carbinol, in a dose-dependent manner, had the ability to combat the histopathological and immunohistochemical changes induced by doxorubicin in cardiomyocytes. ***Conclusion*:** These results demonstrate that administration of I3C in a dose dependent manner prevents heart injury induced by DOX via its antioxidant, anti-inflammatory, and antiapoptotic activities.

Keywords: Cancer, doxorubicin, heart, cardiotoxicity, oxidative stress, apoptosis, inflammation, indole-3-carbinol, mice.

1. INTRODUCTION

Doxorubicin (DOX) is one of the most widely prescribed antineoplastic drugs introduced over the past 50 years and remains the cornerstone for other targeted agents in standard tumor chemotherapy regimens [1-3]. It is used in the treatment of several types of human malignancies including hematological malignancies, solid tumors, soft-tissue sarcomas and breast carcinoma [4, 5]. The therapeutic potential of DOX is achieved through the processes of intercalating into DNA, inhibiting topoisomerase II, preventing DNA and RNA synthesis [6]. However, its clinical applications are

relatively restricted due to its detrimental side effects that include cardiotoxicity [7]. This toxic damage to the cardiomyocytes induced by DOX can lead to the development of tachycardia, arrhythmia, pericarditis, myocarditis, left ventricular function transient depression, late-onset refractory cardiomyopathy, and, eventually, congestive heart failure [8, 9]. DOX cardiotoxicity is usually accompanied by raised troponin, creatine kinase isoenzyme MB (CK-MB), and lactate dehydrogenase (LDH) levels in the serum [10].

The mechanism that mediates doxorubicin-induced cardiotoxicity is unclear; however, it might be related to oxidative stress, produced by increased levels of free radicals [11], intracellular iron [12, 13], and decreased levels of antioxidants [14]. This oxidative stress causes increased intracellular calcium [14], and acceleration of lipid peroxidation [15, 16]. Moreover, increased ROS level suppresses the expression of nuclear factor erythroid 2-related factor (Nrf2) (Figure. 1), which increases the cellular susceptibility to oxidative stress and apoptosis [17]. Due to great importance of DOX in cancer chemotherapy, it is essential to reduce its toxicity to normal cells, a goal that can be achieved by concurrent administration of free radical scavenging agents such as antioxidants [18]. Thus, attenuating oxidative stress, inflammation and apoptosis is a potential therapeutic strategy against DOX-induced toxicity [19]. Therefore, cardioprotection during DOX treatment is required to reduce the incidence of DOX-induced heart damage; hence, it is necessary to discover new drugs that can be utilized as cardioprotective agents with DOX therapy. In this regard, natural products remain an attractive source of bioactive lead compounds that can tackle this problem [20]. With regards to disease treatment and prevention, natural products are still considered one of the best sources of novel bioactive molecules. Phytoconstituents are considered a source of bioactive compounds that could lead to new drugs.

Scientific studies show that substances derived from natural plants, such as flavonoids and isoflavones, can reduce mortality from cancer and cardiovascular disease [21]. Indole-3-carbinol (I3C) is a small molecule derived from the genus Brassica (e.g., cabbage, cauliflower, broccoli, Brussels sprouts, and daikon) [22]. It is one of the phytochemicals that was shown to have antioxidant activities and anti-inflammatory properties [23]. Apart from its antioxidant activities, it has anticancer properties by interrupting cancer cell cycles, promoting cell apoptosis, controlling cell division and angiogenesis deregulation of cancer cells [24]. Recent studies showed that I3C has beneficial effects on lipid metabolism that could be of great value for prevention of DOX-induced cardiotoxicity [25, 26]. Previous studies have reported the toxicity profile and protective efficacy of natural compound indole-3- carbinol (I3C) against doxorubicin (DOX)-induced genotoxicity and cardiotoxicity in normal mice [27]. Moreover, other studies reported that I3C might prevent cardiac remodeling via activation of AMP kinase enzyme leading to improvement of the myocardial functions and modulation of the expression of the genes that are responsible for the production of the hypertrophic and fibrotic markers with regeneration of the damaged myocardial tissues which significantly decreases the activity of the cardiac enzymes such as lactate dehydrogenase and creatine phosphokinase [28, 29].

Therefore, the present study was designed to investigate I3C possible chemoprotective effect on doxorubicin-induced cardiotoxicity through targeting of inflammation, oxidative Stress, and apoptosis in mice.

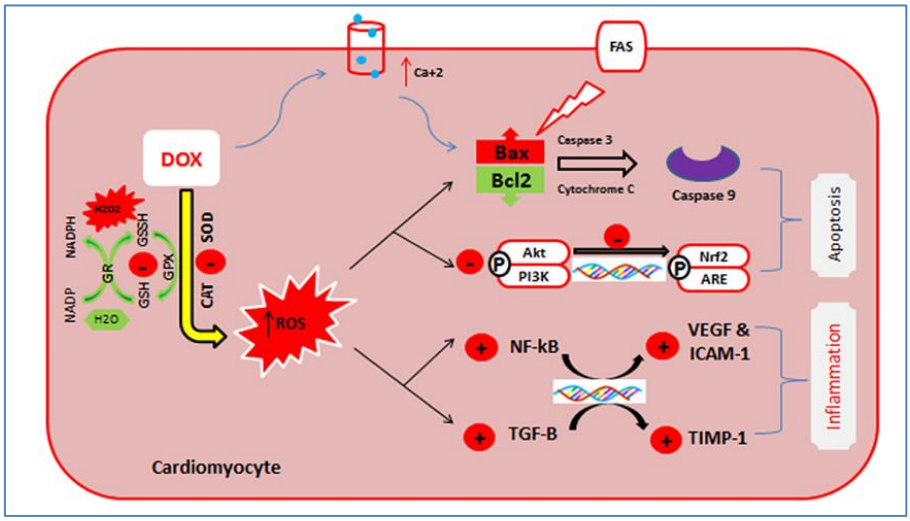

Figure 1: Shows the pathogenic effects of DOX on the molecular level and the central role of reactive oxygen species in DOX-induced cardiomyopathy. Abbreviations: ARE: Antioxidant response element, CAT: Catalase, GPx: Glutathione Peroxidase, DOX: Doxorubicin, GR: Glutathione Reductase, ICAM-1: Intracellular adhesion molecule- 1, NADP: Nicotinamide Adenine Dinucleotide Phosphate, NF-kB: Nuclear Factor-Kappa B, Nrf: NF-E2-related factor-2, ROS: Reactive oxygen species, SOD: Superoxide dismutase, TGF-B: Transforming growth factor B, TIMP-1: Tissue inhibitor of matrix metalloproteinase-1, VEGF: Vascular endothelial growth factor [30].

2. MATERIALS AND METHODS

2.1. Drugs Used

Indole-3 carbinol (I3C) was purchased from Sigma Aldrich Co. and administered daily orally in diet. Doxorubicin (DOX) was commercially available in powder form for injection purchased from Carlo Erba, Turkey. It was dissolved in normal saline and administered by intraperitoneal injection once weekly for 4 weeks [31].

2.2. Animals

BALB/c mice weighing about 20–25 grams, obtained from the animal house of the faculty of medicine, Tanta University, Egypt. Animals were kept in individual metabolic cages at 22 ºC, 55% relative humidity and 12/12 hours light-dark cycle through the whole period of the study. The protocol of this study was conducted following the Helsinki declaration of animal ethics [32], and was approved by the Research Ethics Committee of Faculty of Medicine, Tanta University.

2.3. Experimental Design

Eighty BALB/c male mice were used in this study. Except for mice in the control group (group 1), each mouse was given Doxorubicin (DOX) (4 mg / kg, i.p.) once weekly on days 0, 7, 14, 21 (for 21 days) [31].

The day of the first injection of DOX was considered as the zero point (day 0) of the experiment. The total period of the experiment was 49 days (7 days before and 42 days after first injection of DOX). The animals were randomly divided into four equal groups (20 mice per each group) as follows:

Group 1: Control group, in which mice received intraperitoneal (i.p.) injection of 0.5 ml normal saline once weekly for 4 weeks on days 0, 7, 14, 21 (for 21 days). Group 2: Doxorubicin (DOX) group, in which mice received DOX (4 mg/kg, i.p.) once weekly for 4 weeks on days 0, 7, 14, 21 (for 21 days) [31].

Group 3: Indole-3-carbinol (small dose) and Doxorubicin (I3C 1000 ppm+ DOX) combination group, in which mice were put on a diet containing 1000 ppm I3C starting seven days before and continued for 42 days after first injection of DOX [33].

Indole-3-carbinol (large dose) and Doxorubicin (I3C 2000 ppm+ DOX) combination group, in which mice were put on a diet containing 2000 ppm I3C starting seven days before and continued for 42 days after first injection of DOX [34]. At the end of the study, 42 days after first injection of DOX. The blood samples were collected. Mice were sacrificed and their hearts were excised for further investigation.

2.4. Assessment of Cardiac Function Tests in Blood Samples

At the end of the experimental period, blood was withdrawn from the orbital sinus of mouse under light ether anesthesia. Serum was separated immediately by centrifugation at 4000 rpm for 10 minutes, which was utilized for assessment of lactate dehydrogenase (LDH) using kits supplied by STANBIO, USA according to Buhl and Jackson [35]. Kits purchased from STANBIO, USA, were utilized for quantification of the levels of serum creatine kinase (CK-MB) [36], and serum troponin I (cTn-I) using ELISA kits purchased from Sigma Aldrich Co. according to the instructions of the manufacturer.

2.5. Processing and Preparation of Cardiac Tissues

Mice were euthanized and cardiac tissues were immediately extracted out and freed from the adjacent tissues, washed with cold saline to remove any excess blood, blotted to dry on filter paper and then weighed. A portion of the extracted tissues was homogenized by a Branson sonifier (250, VWR Scientific, Danbury, CT, USA) and the homogenate was centrifuged at 3000 rpm for 10 min. The resulting supernatant was utilized for exploration of the levels of the biochemical parameters in the specimens of cardiac tissues. The other portion of the cardiac tissue was processed for further histopathological and immunohistochemical examinations.

2.6. Evaluation of Oxidative Stress Parameters Content in Cardiac Tissues

The intracellular antioxidants in cardiac tissue were measured, such as catalase (CAT) according to Higgins *et al.,* [37], Superoxide dismutase (SOD) according to Marklund and Marklund [38], glutathione reductase (GR) using kits supplied by Sigma Aldrich Co., USA, according to the instructions of the manufacturer, glutathione peroxidase (GPx) was determined using BIOXYTECH GPx-340TM Assay kit produced by OXIS International, Inc., USA according to Rotruck *et al.,* (1973) [39].

2.7. Lipid Peroxidation (LPO) Assay

The malondialdehyde (MDA) content was estimated to evaluate the peroxidation of lipids. Levels of MDA in cardiac tissue were measured using Uchiyama and Mihara method [40], according to the manufacturer's directions. This method depends on the fact that MDA reacts with TBA producing thiobarbituric acid reactive substance (allegedly a $[TBA]_2$ – Malondialdehyde adduct) a pink chromogen.

2.8. Assessment of Cardiac Inflammatory Markers, Interleukin 6 (IL-6) and Tumor Necrosis Factor-Alpha (TNF-A).

Cardiac tissue interleukin-6 (IL-6) levels were quantified using ELISA kits purchased from Sigma chemical Co., according to the instructions of the manufacturer. Assay of cardiac tissue tumor necrosis

factor alpha (TNF-α) levels was executed using mouse ELISA kits supplied by Ray Biotech, Inc., according to the instructions of the manufacturer.

2.9. Evaluation of the Histopathological Changes in Cardiac Tissues

Specimens of the cardiac tissues were fixed in 10% formaldehyde solution and then put in paraffin blocks. After that, these specimens were deparaffinized by xylene, hydrated in alcohol, stained with hematoxylin for 10 min, and then counterstained with 1% eosin solution (H & E). These sections were examined by using light microscope to assess the histopathological changes. This examination was carried out by a pathologist in a blind manner.

2.10. Assessment of Cardiac Tissues Caspase-3 Activity: Immunohistochemical Staining of Cardiac Tissues for Assessment of Caspase-3

A piece of the cardiac tissues was homogenized and proteins were extracted and stored at -80 °C. 100 µg of tissue extract in the assay buffer (50 mM HEPES, pH 7.4, 100 mM NaCl, 0.1% CHAPS,10 mM dithiothreitol,1m M EDTA,10% glycerol) was added to 100 µM of the peptide substrate N-acetyl–Asp–Glu–Val–Asp–ρ-nitroanilide (Ac–DEVD–ρNA) and incubated at 37 °C for 1 hour. Cleavage of the substrate was monitored every 30 minutes up to 2 hours at 405 nm and the enzyme activity was expressed as nmol/min/mg protein.

2.11. Statistical Analysis of the Obtained Data

For statistical analysis, the Statistical Package for the Social Sciences (SPSS) version 16.0 was used. Parameters were shown with mean ± Standard error of mean (SEM). Multiple comparisons were performed using one way analysis of variance (ANOVA) and nonparametric followed by Tukey-Kramer test for post hoc analysis, as appropriate. Unpaired t-test and Mann-Whitney test were used to compare between two different treatment groups. Differences between the means of the different groups were considered significant at a level of p-value < 0.05.

3. RESULTS

3.1. Indole-3-Carbinol (I3C), in A Dose-Dependent Manner, Combatted the Changes Induced by Doxorubicin in Cardiac Function Tests.

Doxorubicin-treated mice exhibited significant increase in serum CK-MB, LDH and troponin I, relative to the control group. Administration of I3C/DOX combination **was** found to have the ability to elicit significant decrease in these parameters, when compared to mice treated with doxorubicin alone. The improvement in the cardiac function tests was more pronounced in mice that received I3C 2000 ppm, compared to the group that received I3C 1000 ppm (Figures.2-4).

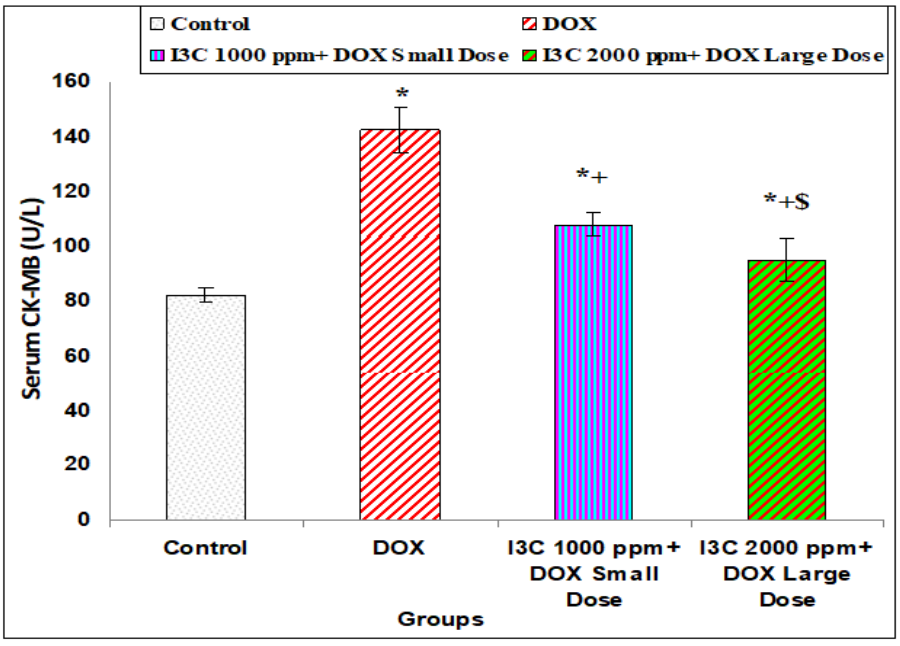

Figure 2: Effect of different treatments on serum CK-MB in the studied groups.

*Significantly different from the control group (P< 0.05); + Significantly different from DOX group (P< 0.05); $ Significantly different from DOX+I3C 1000 group (P< 0.05)

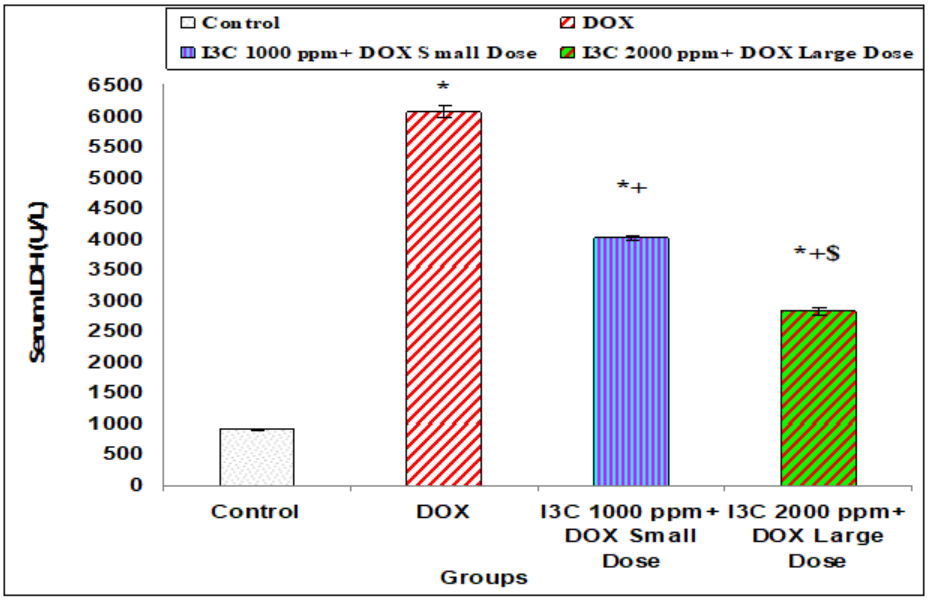

Figure 3: Effect of different treatments on serum LDH in the studied groups.
*Significantly different from the control group (P< 0.05); + Significantly different from DOX group (P< 0.05); $ Significantly different from DOX+I3C 1000 group (P< 0.05)

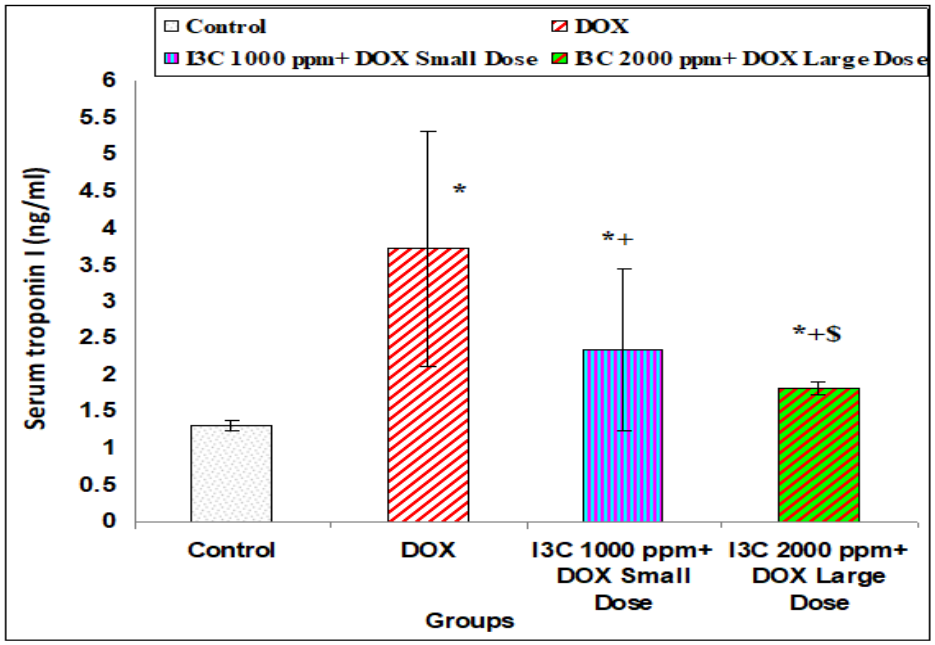

Figure 4: Effect of different treatments on serum troponin I in the studied groups.
*Significantly different from the control group (P< 0.05) ; + Significantly different from DOX group (P< 0.05); $ Significantly different from DOX+I3C 1000 group (P< 0.05)

3.2. Indole-3-Carbinol (I3C) Augmented the Antioxidant Defense Mechanisms of Cardiac Tissues in Doxorubicin-Treated Mice.

The group that was injected with doxorubicin alone exhibited significant decrease in CAT, SOD, GR and GPx levels in cardiac tissues, compared to the control group. Administration of I3C/DOX combination elicited significant increase in CAT, SOD, GR and GPx levels in cardiac tissues relative to mice treated with doxorubicin alone. These changes were more evidenced with I3C 2000 ppm, compared to the group that received I3C 1000 ppm (Figures. 5-8).

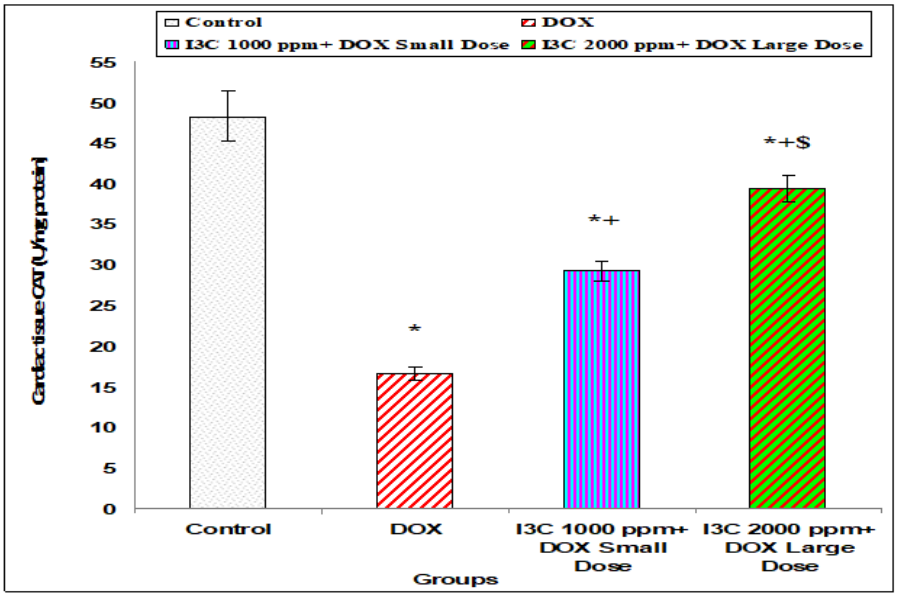

Figure 5. Effect of different treatments on cardiac tissue CAT **in the studied groups.**
* Significantly different from the control group (P< 0.05) ; + Significantly different from DOX group (P< 0.05); $ Significantly different from DOX+I3C 1000 group (P< 0.05)

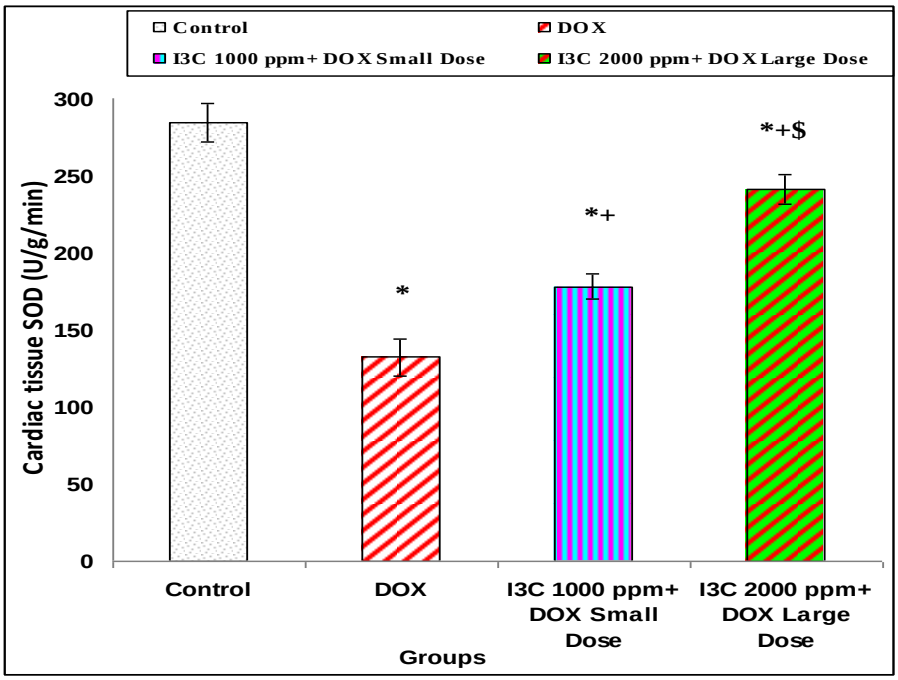

Figure 6: Effect of different treatments on cardiac tissue SOD **in the studied groups**.
*Significantly different from the control group (P< 0.05); + Significantly different from DOX group (P< 0.05); $ Significantly different from DOX+I3C 1000 group (P< 0.05)

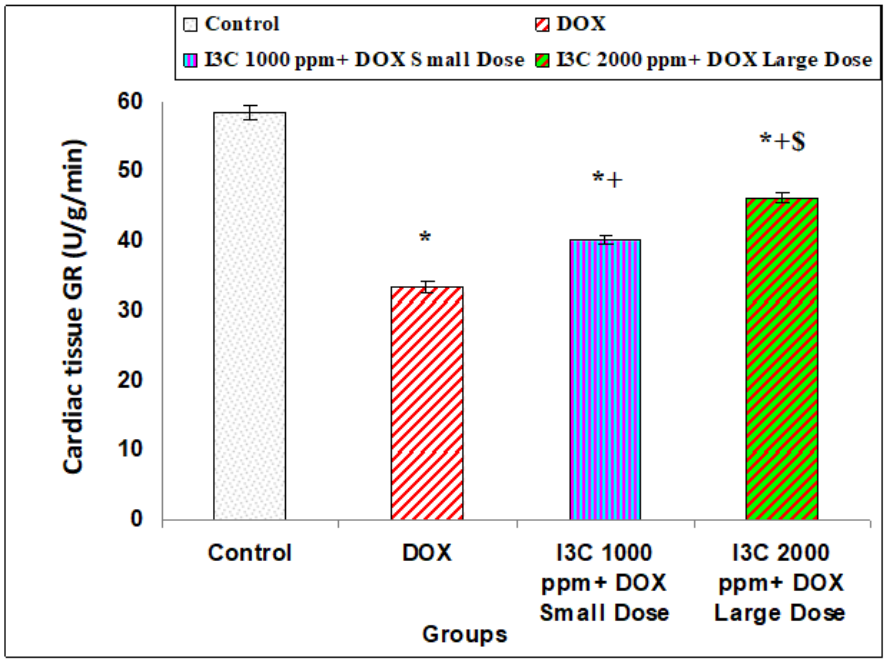

Figure 7: Effect of different treatments on cardiac tissue GR **in the studied groups.**
*Significantly different from the control group (P< 0.05); + Significantly different from DOX group (P< 0.05); $ Significantly different from DOX+I3C 1000 group (P< 0.05).

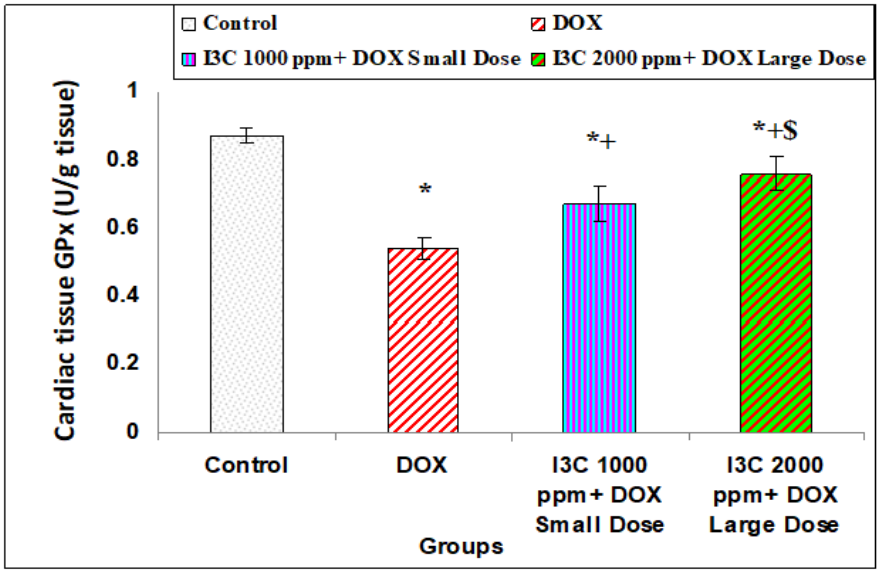

Figure 8: Effect of different treatments on cardiac tissue GPx **in the studied groups.**
*Significantly different from the control group (P< 0.05); + Significantly different from DOX group (P< 0.05); $ Significantly different from DOX+I3C 1000 group (P< 0.05)

3.3. Indole-3-Carbinol (I3C) Attenuates DOX-Induced LPO

As shown in figure. 9, induction of cardiotoxicity with DOX caused significantly increased MDA protein levels in comparison with the control group. Administration of I3C/DOX combination elicited significant decrease in MDA levels in cardiac tissues relative to mice treated with doxorubicin alone. These changes were more evidenced with I3C 2000 ppm, compared to the group that received I3C 1000 ppm.

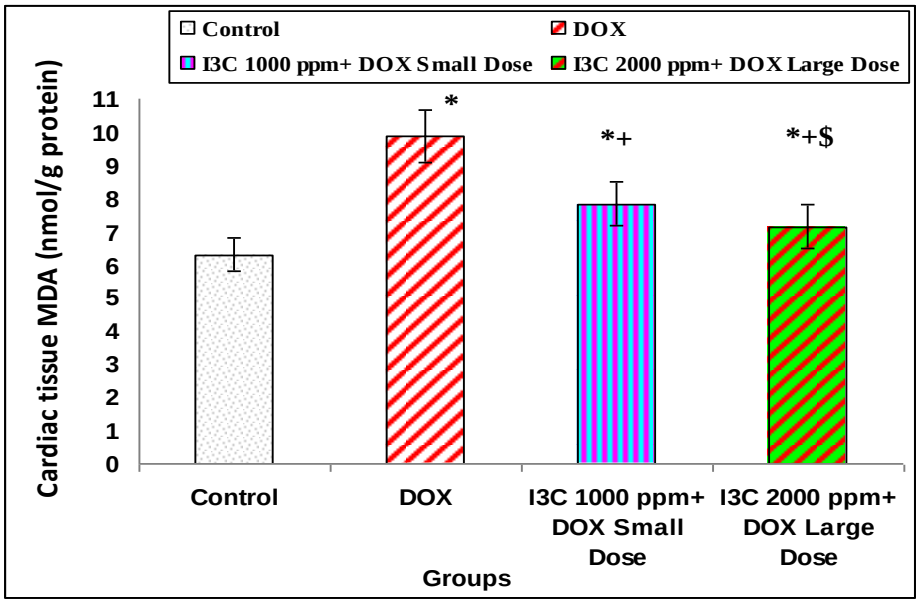

Figure 9: Effect of different treatments on cardiac tissue MDA **in the studied groups.**
* Significantly different from the control group (P< 0.05); + Significantly different from DOX group (P< 0.05)$ Significantly different from DOX+I3C 1000 group (P< 0.05)

3.4. Indole-3-Carbinol (I3C) Mitigated the Changes Induced by Doxorubicin (DOX) in Cardiac Tissue IL-6 and TNF-α.

The injected doxorubicin elicited significant increase in cardiac tissue IL-6 and TNF-α when compared with the control group. I3C/DOX combination, in a dose-dependent manner, had detrimental effects on IL-6 and TNF-α levels resulting in amelioration of the inflammatory processes compared to mice treated with doxorubicin alone, These changes were more evidenced with I3C 2000 ppm, compared to the group that received I3C 1000 ppm (Figures 10-11).

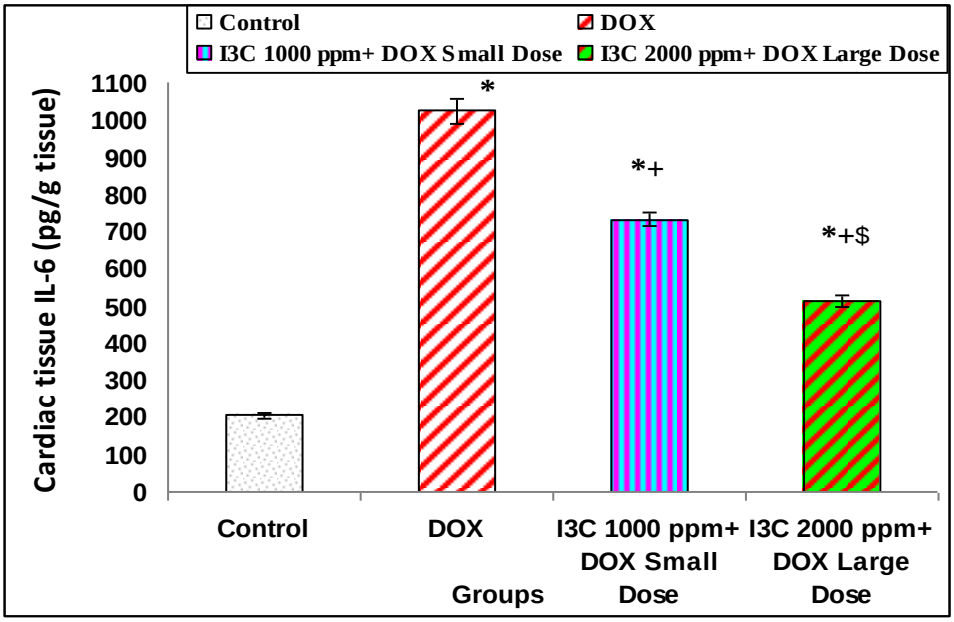

Figure 10: Effect of different treatments on cardiac tissue IL-6 in the studied groups.
*Significantly different from the control group (P< 0.05); + Significantly different from DOX group (P< 0.05); $ Significantly different from DOX+I3C 1000 group (P< 0.05)

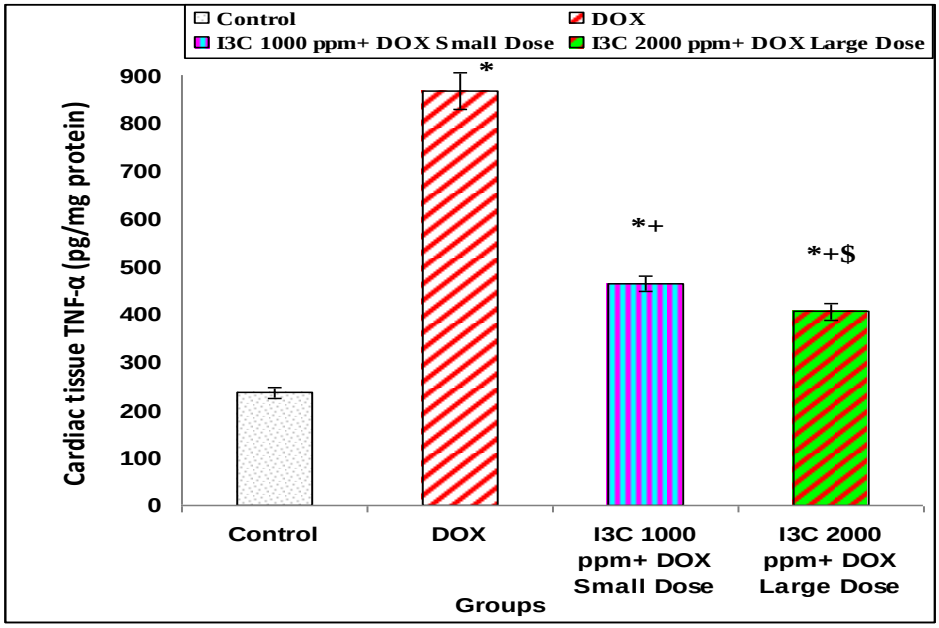

Figure 11: Effect of different treatments on cardiac tissue TNF-α in the studied groups.
*Significantly different from the control group (P< 0.05); + Significantly different from DOX group (P< 0.05); $ Significantly different from DOX+I3C 1000 group (P< 0.05)

3.5. Indole-3-Carbinol (I3C) Reduced the Histopathological Changes Induced by Doxorubicin in Cardiac Tissues.

Massive infiltration of cardiac tissues with different types of inflammatory cells with fragmentation of the myocardial fibers were observed in mice treated with doxorubicin alone (figure. 12b). Indole-3-carbinol administration induced significant reduction in inflammatory cellular infiltration with restoration of the normal architecture of the myocardial fibers (Figure. 12c, d). These favorable effects were more pronounced in the group treated with the high dose of I3C 2000 ppm (Figure .12d) relative to mice treated with 1000 ppm I3C (Figure .12c).

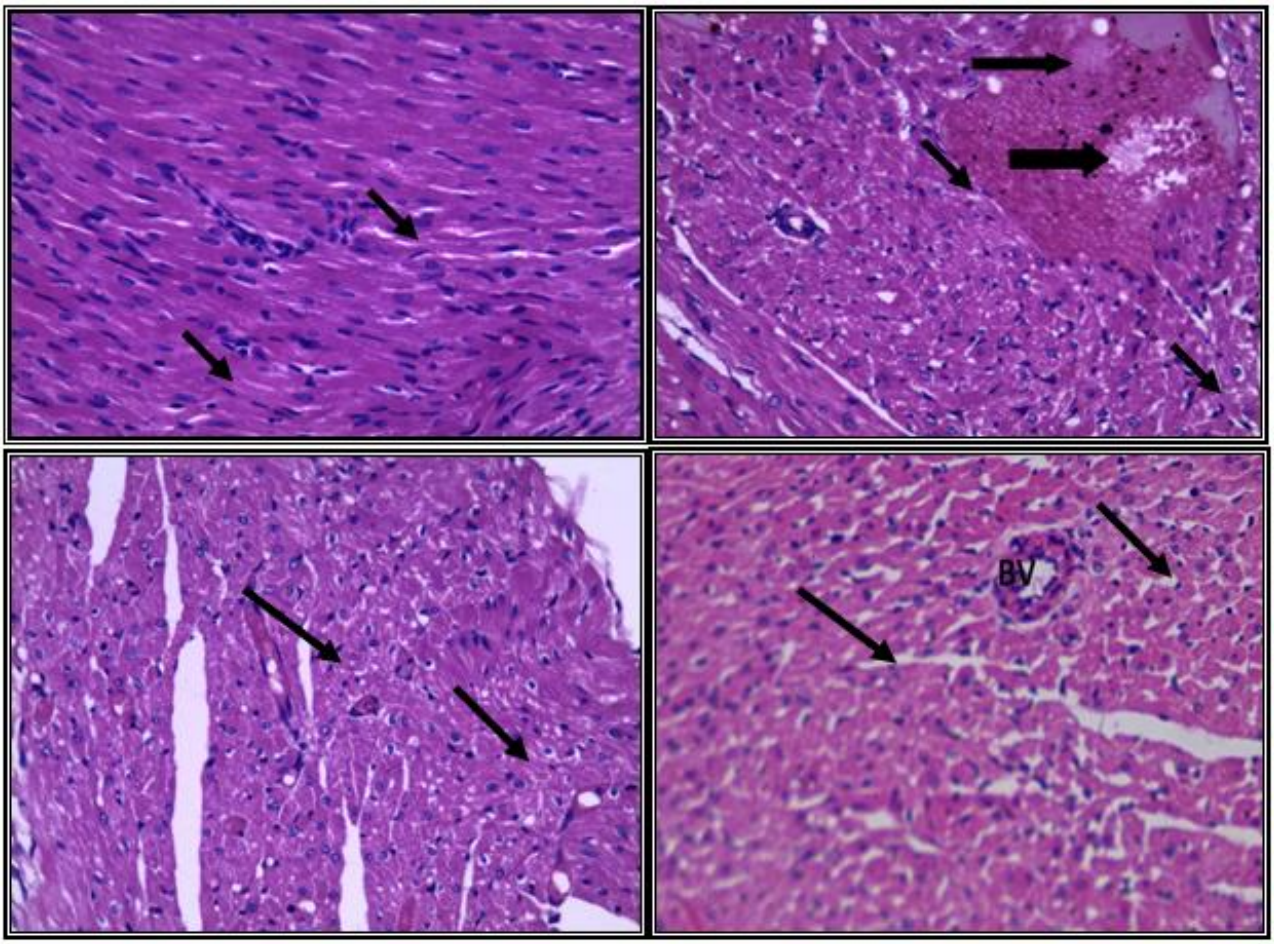

Figure 12: Sections of the cardiac tissues from a) control group showing normal morphology, consisting of striated muscle fibers (Arrows) and sparse connective tissue (H&E X 400), b) DOX group showing inflammatory cellular infiltration and widespread necrosis of cardiac tissues (H&E ×400); c) DOX+I3C 1000 ppm (small dose) group showing decreased inflammatory cellular infiltration and fibrosis among the cardiac muscle cells (Arrows) (H&E ×400), d) DOX+I3C 2000 ppm (large dose) group showing marked improvement in the inflammatory cellular infiltration (Arrows) among the cardiac muscle cells with decreased vascular congestion (BV) (H &E ×400).

3.6. Indole-3-Carbinol (I3C) Reduces DOX-Induced Caspase-3 Expression

To evaluate the effect of I3C in a probable DOX-induced apoptotic pathway, we examined expression levels of caspase-3 in cardiac tissues. Our results showed that expression of caspase-3 in cardiac tissue were markedly elevated in DOX-treated group compared to the control group. Administration of I3C/DOX combination elicited significant decrease in expression of caspase-3 in comparison to the DOX group alone. These changes were more evidenced with I3C 2000 ppm, compared to the group that received I3C 1000 ppm.

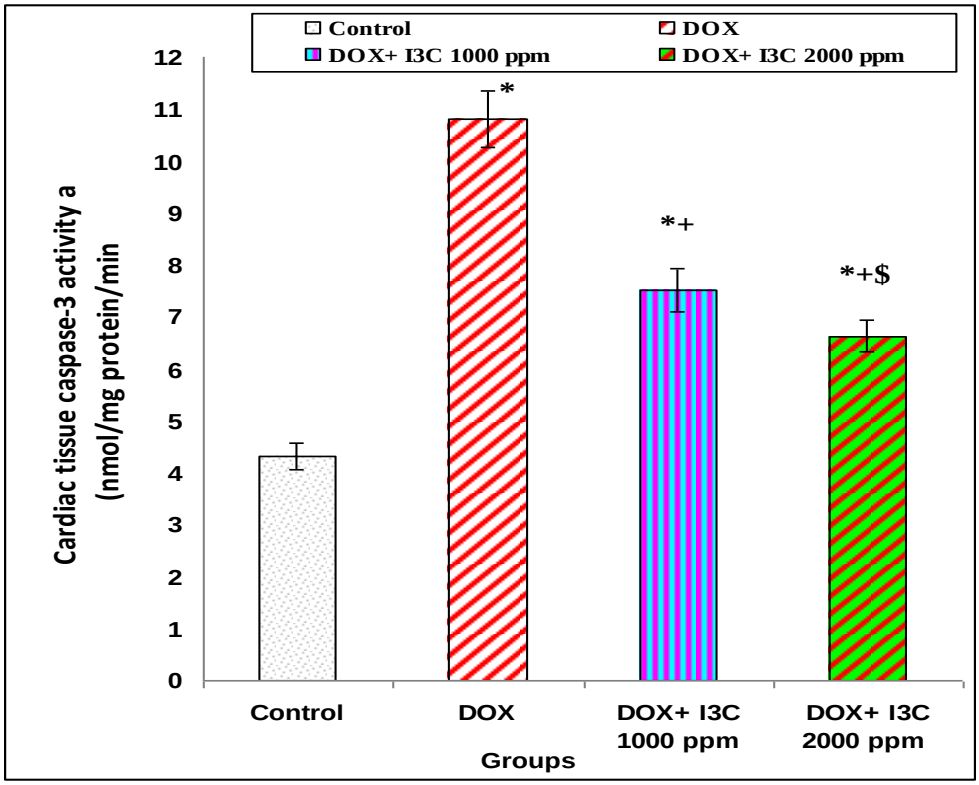

Figure 13; Expression levels of caspase-3 in cardiac tissues in the studied groups.
*Significantly different from the control group (P< 0.05); + Significantly different from DOX group (P< 0.05); $ Significantly different from DOX+I3C 1000 group (P< 0.05)

4. DUSCUSSION

It is well-known that DOX is a potent medication against tumor progression in the clinic, and significantly improves the survival rate of patients with cancer; however, many patients suffer from DOX-induced cardiac injury with left ventricular dysfunction and even heart failure, which severely restricts its clinical application [41, 42]. Multiple pathological processes, including the inflammatory response, oxidative stress, autophagy, apoptosis, pyroptosis, mitochondrial injury, and DNA damage, have been reported to be involved in the myocardial damage caused by DOX [41- 45]. Increasing studies have suggested that targeted therapy for these pathological processes is beneficial in mitigating DOX-induced cardiotoxicity [43].

Scientific studies show that substances derived from natural plants, such as flavonoids and isoflavones, can reduce mortality from cancer and cardiovascular disease [21]. I3C is a small molecule derived from the genus Brassica (e.g., cabbage, cauliflower, broccoli, Brussels sprouts, and daikon) [22]. Administration of I3C may have numbers of beneficial effects, including antiinflammation, antioxidant, anti-virus, anti-angiogenesis, and promotion of tumor cell apoptosis [46- 48]. Hence, we aimed at investigating the possible protective actions of I3C due to DOX- induced cardiotoxicity in mice. More recent studies have suggested that doxorubicin affects the expression of certain genes related to the generation of ROS and the pro-inflammatory cytokines with the end result of distortion of the normal architecture and functions of cardiomyocytes [49]. This was in accordance with the data obtained from the current study where mice injected with doxorubicin exhibited significant deterioration in cardiac functions, represented by the change in the histological appearance where the cardiac tissues showed swollen cardiac muscle fibers, interstitial edema and inflammatory infiltration and the significant elevation in serum LDH, CK-MB, and troponin I, when compared to the control group. In addition, Koul *et al.*, [50] and Osman *et al.*, [51] have shown that, the elevation of the level of the different enzymes by DOX probably reflects that the drug induces cardiac toxicity, where LDH, CK-MB and troponin-I are rather specific for myocardial damage.

Rocca *et al.*, [2020] [52], have shown that, oxidative stress plays an important role in DOX-induced cardiotoxicity through the generation of free radicals that cause depletion of antioxidants, increasing lipid, protein, and nucleic acid peroxidation and disturbing mitochondrial function. Doxorubicin was proven to decrease antioxidants activity in cardiac tissues with subsequent increase in the generation of free radicals and ROS in cardiomyocytes, which subsequently impair myocardial functions [53]. In addition, Abdel-Daim *et al.*, [54], postulated that doxorubicin by its detrimental effects on the antioxidant

enzymes content of the myocardium may significantly increase the free radical production with subsequent augmentation of the effects of oxidative stress on cardiac tissues. Moreover, oxidative stress was proven to regulate ROS production which consequently affects the expression of the proinflammatory cytokines leading to serious cytotoxicity and modulation of the pathways of apoptosis [55]. This is in line with the results of the current work, the data showed that administration of DOX produced oxidative stress as seen in the elevated cardiac tissue level of MDA, depleted antioxidant enzymes, and induced the apoptosis and inflammatory cascade by increasing the caspase 3, TNF-α and IL-6 cardiac tissue levels compared to control group. These results are agreeable with previous studies affirming that DOX caused cardiac damage, oxidative stress, induced apoptosis and inflammation [56- 59]. One of the proinflammatory cytokines involved is TNF-α which is thought to mediate cardiac damage, cardiac remodeling and ventricular damage which were frequently encountered in DOX-induced cardiotoxicity [60], thus, DOX-induced cardiotoxicity was successfully established.

Numerous studies have demonstrated that excessive inflammation is one of the main features of DOX-induced acute cardiotoxicity [61-63]. Inflammatory cells are stimulated and proinflammatory cytokines are released following the administration of DOX, thereby amplifying the inflammatory cascade [63- 65]. In response to extracellular stimulation, NF-*κ*B P65 triggers the formation of inflammatory cytokines [66]. Multiple proinflammatory factors, including IL-6, and TNF-*α*, participate in the pathological process of DOX-elicited heart damage [61-69]. We next investigated the role of I3C in DOX-induced inflammation.

Our results demonstrated that I3C administration to doxorubicin-treated mice induced a dose-dependent significant decrease in serum CK-MB, LDH and troponin I enzymes activities where improved swollen cardiac muscle fibers, interstitial edema and inflammatory infiltration in cardiac tissues were seen. Moreover, I3C treatment was associated with relatively preserved cardiomyocytes and almost normal cardiac architecture, indicating a protective activity of I3C. These effects of I3C may be due to its inhibitory effect on cardiac remodeling which may be mediated by AMPK-α and extracellular signal-regulated kinases 1/2 signaling with regeneration of the damaged myocardial tissues which significantly decreases the activity of cardiac enzymes such as LDH, CK-MB and troponin-I. [28- 70]. Administration of I3C in a dose-dependent manner resulted in significant increase in the activity of various antioxidant enzymes; in addition I3C caused significant decline in malondialdehyde (MDA) levels and inflammatory mediators levels in cardiac tissue, when compared to mice treated with doxorubicin alone. These results illustrate that I3C could antagonize oxidative injury triggered by DOX. This was in accordance with the findings of Arnao *et al.*, [71], who threw light on the strong antioxidant properties of I3C through its ability to act as a scavenger of free radicals and to induce the activity of various antioxidant enzymes in cardiac tissue. In addition, Hajra *et al.*, [27], attributed these properties to the effect of I3C on oxidative stress by reducing ROS levels and lipid peroxidation as well as enhancing the levels of antioxidant enzymes. Moreover, Tsai *et al.*, [72], who found that I3C and the other cruciferous vegetable-derived compounds can suppress the production of inflammatory mediators, including nitric oxide (NO), TNF-alpha and interleukin-6 (IL-6), possibly through affection of gene expression of these mediators. Interestingly, the increase in tissue antioxidant activity induced by I3C was concomitantly associated with inhibition of the production of ROS, resulting in abrogation of its harmful effects on the cardiac tissues [Hajra *et al.*, [27)].

Cardiomyocytes apoptosis is another critical pathogenic mechanism in DOX-induced cardiomyopathy [73]. Many scholars believe that apoptosis is the major cause of cardiac dysfunction in DOX-induced myocardial damage [73], [43- 45]. In this regard, I3C was found in the current study to protect against the apoptosis of cardiac tissues in mice who received DOX. Consistent with previous literature [74-75]. DOX triggered apoptosis of cardiomyocytes in mouse hearts. However, the administration of I3C mitigated DOX-triggered cardiomyocyte apoptosis, as evidenced by the reduced proapoptotic proteins (Caspase3). Increased oxidative stress has been shown to promote apoptosis and antioxidants have been shown to inhibit this process [76]. Oxidative stress also is known to activate apoptosis- signal regulating kinase-1 (ASK1), which activates the c-Jun NH2-terminal kinase (JNK) and p38 MAPK pathways to induce apoptosis [77].

The generated findings in the current study highlight the importance of the antioxidant, anti-inflammatory, and antiapoptotic activities of I3C in mediating resistance to the cardiotoxic effects of DOX in mice.

5. CONCLUSION

DOX had the ability to induce cardiotoxicity which was ameliorated by I3C in a dose-dependent manner. The cardiotoxic effects of DOX were ameliorated after its concomitant administration with I3C. I3C protects against DOX-elicited inflammation, oxidative stress, apoptosis, and cardiac dysfunction, with its ability to prevent cardiac damage and regenerate the damaged cardiomyocytes. This might be due to its effects on oxidative stress by reducing ROS production as well as enhancing the activity of antioxidant enzymes in cardiac tissue, which is the center point of the pathogenic events that occur in the

myocardium of doxorubicin-treated mice. In addition, modulation the expression of the proinflammatory cytokines and apoptosis pathways.

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