

Ameliorative Potential of Different Doses of Indol-3-carbinol on Doxorubicin-induced Cardiotoxicity in Mice

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Abstract Background: Doxorubicin (DOX) is a commonly used chemotherapeutic agent that is associated with serious dose-limiting cardiotoxicity. This cardiotoxicity was attributed to various mechanisms including induction of oxidative stress and inflammation together with inhibition of apoptosis. Indole-3-carbinol (I3C) is a phytochemical that was suggested to have potent anti-oxidant and anti-inflammatory properties. *Aim:* It was to detect the possible ameliorative effects of different doses of I3C on doxorubicin-induced cardiotoxicity in mice. *Methods:* Eighty mice were divided into four equal groups: control untreated group; DOX group; DOX + I3C 1000 ppm group and DOX + I3C 2000 ppm group. Survival rate, serum creatine kinase (CK-MB), lactate dehydrogenase (LDH) and troponin I were measured. Also, tissue malondialdehyde (MDA), tissue catalase (CAT), tissue glutathione peroxidase (GPx), and tissue tumor necrosis factor alpha (TNF- α) were determined. Parts of the heart were subjected to histopathological examination. *Results:* I3C produced dose-dependent significant increase in the survival rate, tissue GPx and CAT with significant decrease in serum CK-MB, LDH, troponin I, tissue MDA and TNF- α and improved the histopathological and immunohistochemical changes compared to DOX-treated group. *Conclusion:* I3C- in a dose dependent manner- had a protective effect against doxorubicin-induced cardiotoxicity in mice.

Keywords: indole-3-carbinol, doxorubicin, cardiotoxicity, mice

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1. Introduction

The use of the traditional anticancer agents such as 5-fluorouracil, methotrexate, adriamycin and cisplatin was faced by their harmful adverse effects. [1] In an attempt to decrease the effective chemotherapeutic dose and thereby side effects, various approaches were investigated. One of them was the search for natural compounds with anticancer properties that can be used in combination with the traditional anticancer agents [2].

The anthracycline anticancer drug doxorubicin (DOX) is an effective and frequently used chemotherapeutic agent for various malignancies. They target topoisomerase II (Top2), binding to both DNA and Top2 to form complexes that trigger cell death. [3] The major adverse effect of DOX is cardiotoxicity, which may limit its use and, once developed, carries a poor prognosis. [4] There are multiple proposed mechanisms of anthracycline-induced cardiotoxicity. However, the most widely cited mechanism is the formation of reactive oxygen species (ROS) leading to oxidative stress which results in

mitochondrial dysfunction, cellular membrane damage, and cytotoxicity. [5] Other factors contributing to DOX-induced cardiotoxicity include dysregulation of calcium metabolism, induction of the expression of proinflammatory cytokines, adrenergic dysfunction and selective inhibition of cardiomyocyte-specific genes expression. [6] The presently available treatment of DOX cardiotoxicity does not appear to improve prognosis. Thus, many preventive treatments have been proposed. [5]

Indole-3-carbinol (I3C) is one of the phytochemicals that was shown to have potent antioxidant and anti-inflammatory properties. [7] Recent studies showed that I3C has beneficial effects on lipid metabolism that could be of great value for prevention of DOX-induced cardiotoxicity. [8,9] 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. [10,11] The aim of this study was to detect the possible ameliorative effects of different doses of I3C on DOX-induced cardiotoxicity in mice and the possible underlying mechanisms of these effects.

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.

2.2. Classification of Animals

In this study, we used eighty BALB/c mice weighing about 18–25 grams. All the experiments were conducted according to the National Research Council's guidelines. Animal handling was followed according to Helsinki declaration of animal ethics. The animals were divided into four equal groups as follows:

Group (1): is the control group, received intraperitoneal injection of normal saline once weekly for 4 weeks.

Group (2): DOX was given by intraperitoneal injection in a dose of 4 mg/kg body weight once weekly for 4 weeks. [12]

Group (3): Mice were put on diet containing 1000 ppm I3C one week before DOX administration and continued for 7 weeks. [13]

Group (4): Mice were put on diet containing 2000 ppm I3C one week before DOX administration and continued for 7 weeks. [14]

2.3. Recording of the Survival Rate

The day of the first injection of DOX was considered as the zero point of the experiment for recording and analysis of the survival rate weekly for 6 weeks (By recording the number of the survived mice in each group at the end of each week).

At the end of the study, all mice were sacrificed and blood samples were obtained and centrifuged for determination of serum creatine kinase (CK-MB) according to Hess et al. [15], serum lactate dehydrogenase (LDH) according to Buhl and Jackson [16] and serum troponin I using ELISA kits purchased from Sigma Aldrich Co. according to the instructions of the manufacturer. Parts of the cardiac tissues were homogenized for determination of tissue catalase (CAT) according to Abei method [17], tissue malondialdehyde (MDA) according to Uchiyama and Mihara [18] and tissue tumor necrosis factor alpha (TNF- α) using mouse ELISA kits supplied by RayBiotech, Inc. according to the instructions of the manufacturer. Tissue glutathione peroxidase (GPx) was determined in the supernatant using BIOXYTECH GPx-340TM Assay kit produced by OXIS International, Inc., USA. The GPx assay was based on the oxidation of NADPH to NADP⁺, which was accompanied by a decrease in the absorbance at 340 nm. [19]

2.4. Assay of Tissue Caspase-3 Activity

A piece of the heart tissue was homogenised and proteins were extracted and stored at -80 °C. 100 μ g of heart 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 μ M of the peptide substrate N-acetyl-Asp-Glu-Val-Asp-p-nitroanilide (Ac-DEVD-pNA) 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.

2.5. Histopathological Examination

The heart sections were prepared, fixed in 10 % formalin, embedded in paraffin wax, stained with hematoxylin and eosin (H & E) and examined under light microscope.

2.6. Statistical Analysis

The data obtained were subjected to one way ANOVA and Tukey's multiple comparison test. Data were presented as mean \pm S.E.M. Differences between the means of different groups were considered significant at a level of p-value less than 0.05.

3. Results

3.1. Effect of Different Treatments on the Survival Rate (Figure 1)

Administration of DOX resulted in significant decrease in the survival rate compared to the control group. Administration of I3C resulted in significant increase in the survival rate compared to DOX-treated group. This increase was significant in the group that received 2000 ppm I3C compared to the group that received 1000 ppm I3C.

3.2. Effect of Different Treatments on Serum CK-MB, LDH and Troponin I (Table 1)

Administration of DOX resulted in significant increase in serum CK-MB, LDH and troponin I compared to the control group. Administration of I3C resulted in significant decrease in serum CK-MB, LDH and troponin I compared to DOX-treated group which was more significant in the group that received 2000 ppm I3C compared to the group that received 1000 ppm I3C.

3.3. Effect of Different Treatments on the Antioxidant Status (Table 2)

Administration of DOX resulted in significant decrease in tissue GPx and CAT with significant increase in tissue MDA compared to the control group. Administration of I3C resulted in significant increase in tissue GPx and CAT with significant decrease in tissue MDA compared to DOX-treated group. This improvement in the antioxidant status was significant in the group that received 2000 ppm I3C compared to the group that received 1000 ppm I3C.

3.4. Effect of Different Treatments on Tissue TNF- α (Table 2)

Administration of DOX resulted in significant increase in tissue TNF- α compared to the control group. Administration of I3C resulted in significant decrease in tissue TNF- α compared to DOX-treated group which was

more significant in the group that received 2000 ppm I3C compared to the group that received 1000 ppm I3C.

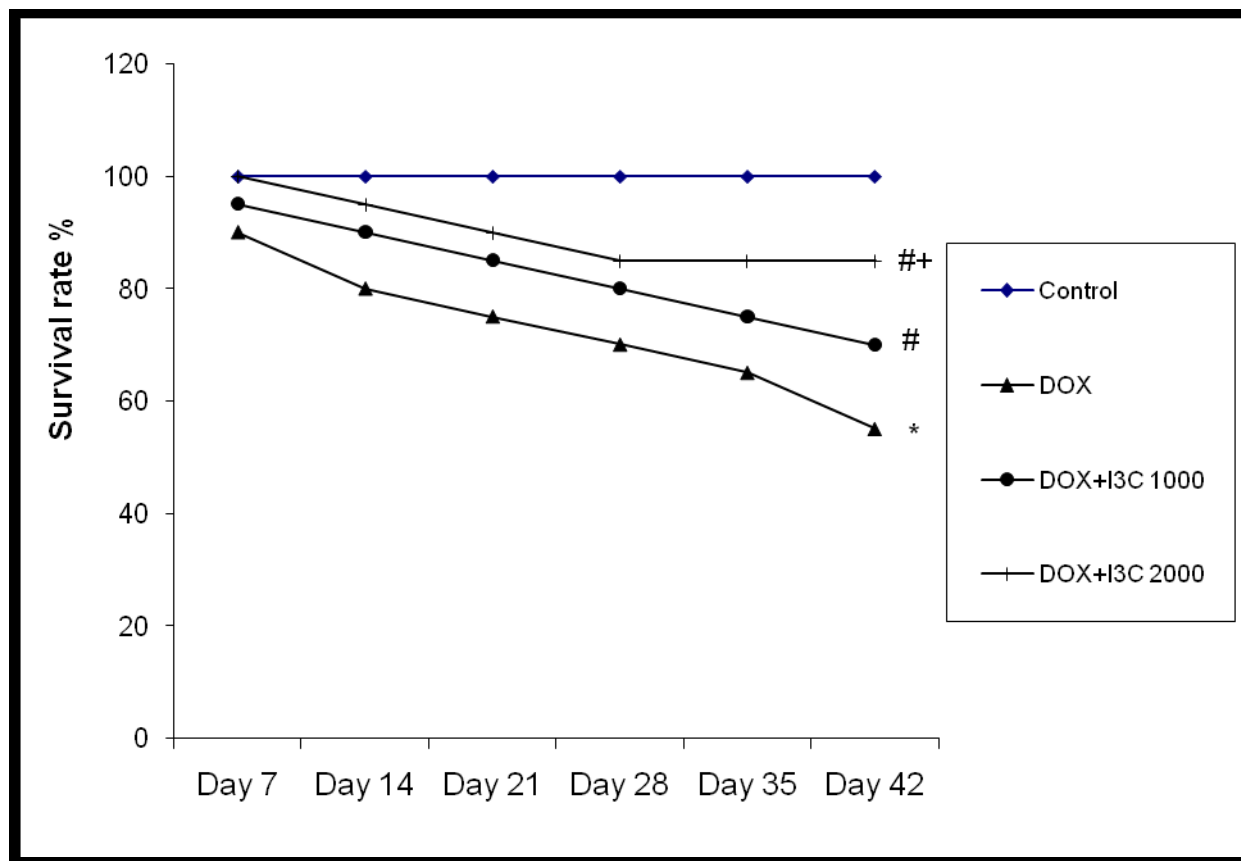


Figure 1. Effect of different treatments on the survival rate

Values were represented as mean \pm SEM.

* Significant compared to the control group.

Significant compared to DOX group

+ Significant compared to DOX + I3C 1000 ppm group.

Table 1. Effect of different treatments on serum CK-MB, LDH and troponin I in the studied groups

	Control	DOX	DOX+I3C 1000	DOX+I3C 2000
Serum CK-MB (U/L)	82.33 \pm 2.55	142.63 \pm 8.41 *	108.12 \pm 4.12#	95.21 \pm 7.8##
Serum LDH (U/L)	901.1 \pm 14.5	6065.4 \pm 98.2*	4015.5 \pm 66.1#	2821.6 \pm 65.5##
Serum troponin I (ng/ml)	1.31 \pm 0.07	3.72 \pm 1.6*	2.34 \pm 1.1#	1.82 \pm 0.08##

* Significant compared to the control group.

Significant compared to DOX group.

+ Significant compared to DOX + I3C 1000 group.

Table 2. Effect of different treatments on tissue GPx, CAT, MDA, TNF- α and caspase 3 activity in the studied groups

	Control	DOX	DOX+I3C 1000	DOX+I3C 2000
Tissue GPx (U/g tissue)	0.87 \pm 0.02	0.54 \pm 0.03*	0.67 \pm 0.05#	0.76 \pm 0.05##
Tissue CAT (U/mg protein)	48.3 \pm 3.12	16.6 \pm 0.8*	29.3 \pm 1.3#	39.37 \pm 1.6##
Tissue MDA (nmol/g protein)	6.3 \pm 0.5	9.88 \pm 0.78*	7.85 \pm 0.67#	7.16 \pm 0.68##
Tissue TNF- α (pg/mg protein)	236.3 \pm 11.76	868.6 \pm 38.03*	464.1 \pm 15.9#	405.65 \pm 16.6##
Tissue caspase 3 activity (nmol/mg protein/min)	8.81 \pm 0.54	4.32 \pm 0.25*	6.64 \pm 0.3#	7.53 \pm 0.41##

* Significant compared to the control group

Significant compared to DOX group

+ Significant compared to DOX + I3C 1000 group

3.5. Effect of Different Treatments on Tissue Caspase 3 Activity (Table 2)

Administration of DOX resulted in significant decrease in tissue caspase 3 activity compared to the control group. Administration of I3C resulted in significant increase in tissue caspase 3 activity compared to DOX-treated group.

This increase was significant in the group that received 2000 ppm I3C compared to the group that received 1000 ppm I3C.

3.6. Histopathological Findings

Administration of DOX resulted in diffuse infiltration with inflammatory cells (Mostly mature lymphocytes),

swelling of the myocardial fibers with interstitial fibrosis (Figure 2b). This picture was significantly improved in mice given I3C in a dose-dependent manner evidenced by

decreased number of the inflammatory cells, disorganization of myocardial fibers and interstitial fibrosis (Figure 2c,d).

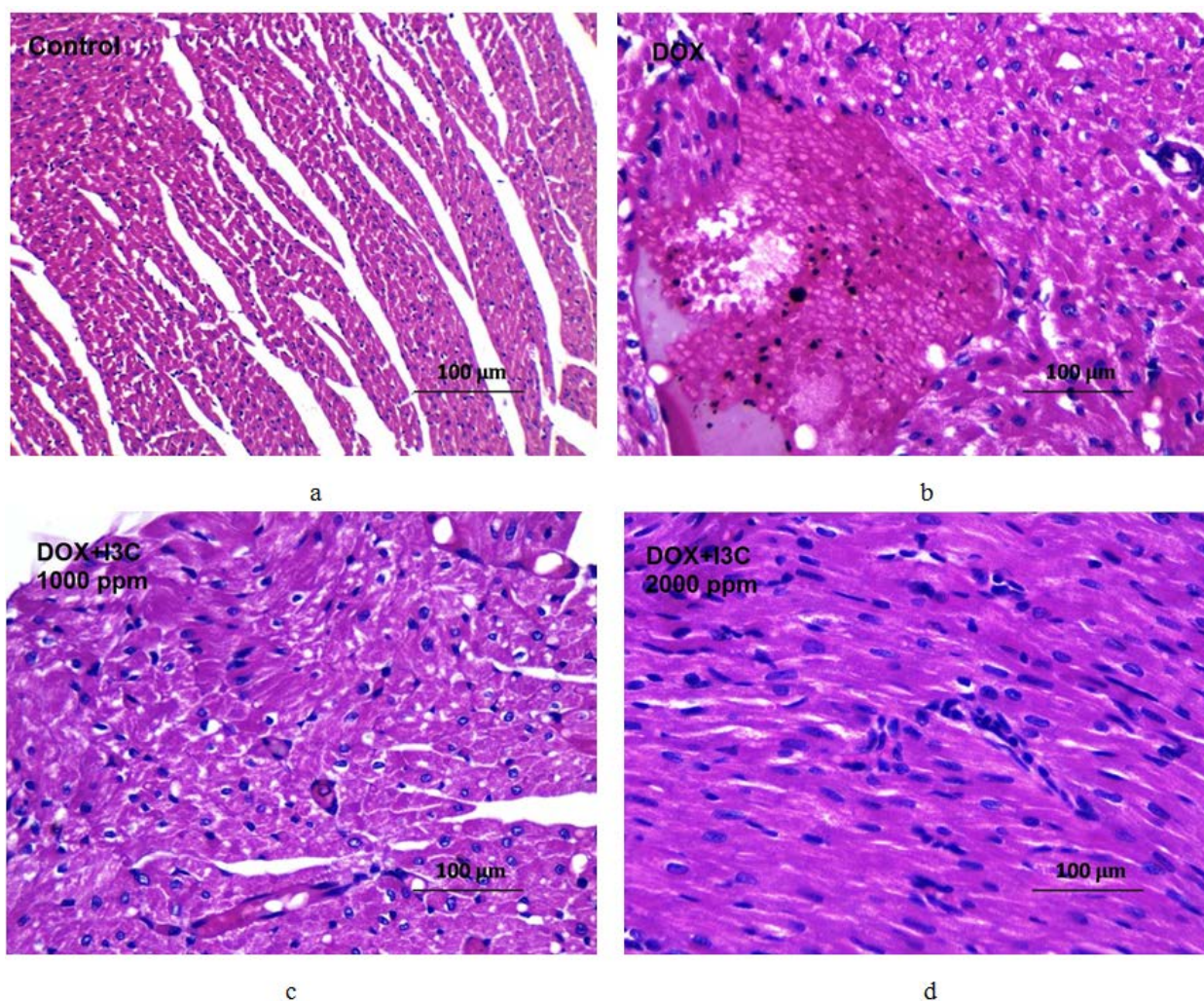


Figure 2. A photomicrograph of the cardiac sections from **a)** the control group showing normal morphology of the cardiac tissues (H&E $\times 250$); **b)** DOX group showing inflammatory cellular infiltration among cardiac muscle cells (Mostly mature lymphocytes), swelling of the myocardial fibers with interstitial fibrosis (H&E $\times 400$); **c)** DOX+I3C 1000 ppm group showing decreased inflammatory cellular infiltration among cardiac muscle cells with decreased swelling of the myocardial fibers (H&E $\times 400$); **d)** DOX+I3C 2000 ppm group showing marked improvement in the inflammatory cellular infiltration and disorganization of myocardial fibers (H&E $\times 400$)

4. Discussion

Doxorubicin (DOX) is one of the most effective anti-cancer agents. However, its use is associated with adverse cardiac effects, including cardiomyopathy and progressive heart failure. [20,21] Several hypotheses have been advanced to explain DOX cardiac side effects. They attributed DOX-cardiotoxicity to formation of reactive oxygen species (ROS) leading to oxidative stress, increased expression of the proinflammatory cytokines leading to serious cytotoxicity and modulation of the pathways of apoptosis. [5]

In the present study, administration of DOX resulted in significant decrease in the survival rate, serum CK-MB, LDH and troponin I compared to the control group. These results were in agreement with Koul et al. [22] and Osman et al. [23] 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. These biochemical

data were confirmed by the histopathological changes induced by DOX where the cardiac tissues showed swollen cardiac muscle fibers, interstitial edema and inflammatory infiltration. These changes were ameliorated with administration of I3C in a dose-dependent manner. 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. [24,25]

In the present study, administration of DOX resulted in significant decrease in tissue GPx and CAT with significant increase in tissue MDA compared to the control group. These results were in the same line with Octavia et al. [5] who attributed DOX induced cardiotoxicity to generation of reactive oxygen species leading to oxidative stress. This results in impaired mitochondrial function, cellular membrane damage, and cytotoxicity. [26] These changes were ameliorated with administration of I3C in a dose-dependent manner,

probably due to the antioxidant effect of I3C through its ability to act as a scavenger of free radicals and to induce the activity of various antioxidant enzymes such as CAT, superoxide dismutase and GPx. [27,28]

In the present study, administration of DOX resulted in significant increase in tissue TNF- α compared to the control group. This was in the same line with Riad et al. [29] who reported that there is a strong association between oxidative stress and cardiac inflammatory responses including cytokine release after doxorubicin treatment. 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. [30] This effect was ameliorated with the administration of I3C in a dose-dependent manner which was in accordance with Tsai et al. [31] 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-10 (IL-10), possibly through affection of gene expression of these mediators.

Caspase 3 is a protein that plays a key role in the execution-phase of apoptosis. [32] DOX administration resulted in significant decrease in caspase 3 activity compared to the control group which was in agreement with other studies that reported that DOX may affect the normal pathways of apoptosis in the cardiac tissues resulting in cardiomyocyte damage. [33] This picture was improved with administration of I3C in a dose-dependent manner manifested by significant increase in caspase 3 expression compared to DOX-treated group. Choi et al. [34] suggested that I3C induces apoptosis through p53 and activation of caspase pathways in various body tissues. Moreover, inactivation of Akt and nuclear factor-kappa B was suggested as a key event in I3C-induced apoptosis. [35]

5. Conclusion

I3C- in a dose dependent manner - had a protective effect against DOX-induced cardiotoxicity in mice. These effects can be attributed to the anti-proliferative, antioxidant and anti-inflammatory properties of I3C together with its ability to induce apoptosis in the cardiac tissues. So, I3C might represent a new adjuvant line of treatment to prevent DOX-induced cardiotoxicity.

Conflict of Interest

The authors have no conflict of interest to declare.

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