

# Synergistic Therapeutic Effect of L-Carnitine Nanoparticles and *Moringa Oleifera* Against Doxorubicin Induced Cardiac Toxicity in Male Rats: Biochemical and Histological Study

Bosy Azmy Abd El-Motelp<sup>1,\*</sup> 

<sup>1</sup> Faculty of Women for Arts, Science and Education, Zoology Department, Ain Shams University, Cairo, Egypt

\* Correspondence: [Bosy.azmy@women.asu.edu.eg](mailto:Bosy.azmy@women.asu.edu.eg) (B.A.A.E.M.);

Scopus Author ID 568050400

Received: 30.12.2021; Accepted: 28.01.2022; Published: 27.03.2022

**Abstract:** Doxorubicin (DOX) is a powerful chemotherapeutic agent. However, dose-dependent cardiotoxicity may limit its clinical applicability. This study was directed to explore the possible therapeutic effects of L-Carnitine Nanoparticles (LCNPs) and *Moringa Oleifera* extract (MO) against doxorubicin-induced cardiac toxicity in male rats. Fifty rats were allocated into two main groups: the first served as a normal control group (con.) (n=10). The remaining 40 rats were given doxorubicin at a cumulative dose of 20 mg/kg b. wt. to induce cardiotoxicity. The Dox-treated rats were subdivided into 4 groups (n = 10/group) to be orally administered with 1) 0.9% normal saline; 2) MO at 400 mg/kg, b. wt./day; 3) LCNPs at 50 mg/kg b. wt./ day for four weeks; 4) MO+LCNPs group as previously indicated doses. The results demonstrated that DOX treatment raised MDA, NO, HO-1, and NF-kB levels while decreasing TAC activity. In addition to increased serum cardiac levels of CRP, CK, LDH, ALT, and Troponin-1 and Endothelin-1. LCNPs or MO treatment alleviated these changes. Whereas co-administration of LCNPs and MO reversed all these parameters. DOX treatment caused severe histopathological alterations in the cardiac tissues, corrected by LCNPs and MO treatment. In conclusion, these data imply that treating with LCNPs and MO in combination provides greater cardioprotection against DOX-induced cardiotoxicity in rats via repressing oxidative stress and boosting antioxidant status.

**Keywords:** L-Carnitine nanoparticles; *Moringa oleifera*; doxorubicin; cardiotoxicity; troponin-1; endothelin-1.

© 2022 by the authors. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Doxorubicin is an anthracycline anticancer drug used to treat various diseases, including leukemia, lymphoma, sarcoma, and breast cancer, and it plays an important role in patient therapy and survival [1]. Even though the pathophysiology of doxorubicin-induced cardiotoxicity is multifaceted, free radical-mediated lipid peroxidation, oxidative stress, inflammation, apoptosis, and mitochondrial abnormalities have all been shown to play a role in the onset and progression of cardiac dysfunction [2,3]. Doxorubicin activates nuclear factor kappa B (NF-B) and myocardial inflammatory mediator enzymes, causing the release of proinflammatory cytokines [4].

Since discovering the mechanisms of doxorubicin-produced cardiotoxicity, several treatment techniques aimed against oxidative stress, inflammation, oxidative damage to DNA, and apoptosis have been developed [5]. Many antioxidants, including L-carnitine (LC) have been found to exert cardioprotective properties. LC is an amino acid produced in the body. It plays a key role in transporting the long-chain fatty acids across the mitochondrial membrane in the cell, especially those that necessitate high energy, like the cardiac system [6,7]. It is required for ATP synthesis during  $\beta$ -oxidation of long-chain fatty acid in lipid metabolism [8]. L-carnitine supplementation has been proven in clinical investigations to improve myocardial fat metabolism by increasing the demand for free fatty acids and their metabolites, resulting in improved cardiac function [9]. Moreover, L-carnitine's antioxidant, anti-inflammatory, and antiapoptotic properties may be related to its regulatory role against cardiotoxicity [10].

However, utilizing synthetic antioxidants or plant extract alone to cure various diseases is still ineffective or slow, and they require more development to show their usefulness. Nanotechnology and its applications in science and technology have made significant progress recently [11,12]. Specificity, targeted distribution, controlled drug release, acceleration of dissolution, better absorption, and uplifting stability are all features that are strongly related to the structure and characteristics of the perfect nanomedicine [13]. These properties allow the medicine to be properly targeted and distributed to tissues, limiting toxicity to organs, specifically the heart, and maximizing the drug's efficacy [14].

On the other hand, medicinal plants, such as *Moringa oleifera* (MO), are useful because their bioactive ingredients affect several biological signaling pathways [15,16]. It contains several natural antioxidant compounds, such as flavonoids, ascorbic acid, carotenoids, and phenolics, and several parts, including leaves, roots, seed, bark, fruit, flowers, and immature pods, are thought to act as cardiac and circulatory stimulants and to have antitumor, antiepileptic, antipyretic, anti-inflammatory, antispasmodic, antiulcer, diuretic, antihypertensive, cholesterol-lowering, antioxidant, It has the potential to work in combination with medicinal medications to improve treatment outcomes [17,18].

Based on the previous layout, this research focuses on studying the therapeutic effect of LCNPs and MO in an experimental model of DOX-induced cardiotoxicity in adult male albino rats.

## 2. Materials and Methods

### 2.1. Chemicals and drugs.

Doxorubicin (Adricin) (Dox) was bought from EIMC United Pharmaceuticals, Egypt. Nano L-carnitine (47nm) was acquired from NANOSHEL LLC chemical company Wilmington, Delaware, USA. All of the other chemicals and reagents were of analytical grade.

### 2.2. Experimental animals.

Fifty adult male albino rats weighing 160–200 g were delivered from a breeding stock maintained in the Animal House of the National Research Centre, Giza, Egypt. The animals were acclimatized for two weeks before experimentation in an air-conditioned atmosphere at a temperature of 25 °C with alternating 12-hour light and dark cycles and allowed free access to standard diet pellets and water (Wadi El Kabda Co., Cairo, Egypt). The Ethical Committee approved the animal use and experimental design of the Medical Research of the National

Research Centre, Egypt (approval no. 14023), in accordance with the National Institutes of Health guidelines for the care and use of Laboratory Animals.

### 2.3. Collection of plant material.

*Moringa oleifera* (family: Moringaceae) was obtained from the Egyptian Scientific Society for Moringa at the National Research Centre in Dokki, Giza, where it has been recognized and stored at the Horticulture and Crops, Technology Department with a voucher specimen number (Voucher No. MO19).

### 2.4. Preparation of *Moringa oleifera* Lam. ethanol extracts.

*Moringa Oleifera* (MO) leaves were air-dried and ground into a coarse powder. After that, the powdered plant was macerated for 48 hours in 100% ethanol before being filtered. The ethanol extract was concentrated and allowed to evaporate at a temperature between 40 and 45°C to avoid denaturation of active components. The concentrated extract was diluted with polysaccharides and refrigerated until it was used in the biological study.

### 2.5. Induction of cardiotoxicity in rats.

The rats received intraperitoneal injections of DOX (5 mg/kg) once a week for a total of 4 consecutive weeks, attaining a cumulative dose of 20 mg/kg to induce cardiotoxicity, according to Mantawy *et al.* [19]. DOX-induced cardiotoxicity was confirmed by the increased serum creatine kinase (CK) and lactate dehydrogenase (LDH) levels in rats.

### 2.6. Experimental design.

The animals were allocated mainly into 2 groups, the first group served as the normal control group (con.) (n=10) and was given normal saline *via* an oral gavage tube. While the remaining 40 rats were subjected to doxorubicin to induce cardiotoxicity at a cumulative dose of 20 mg/kg b.wt., after 28 days, the Dox-treated rats were subdivided into 4 co-treatment groups (n = 10/group) to be orally administered with (1) 0.9% normal saline solution and served as positive control group, (2) *Moringa oleifera* (400 mg /kg, b.wt.) [20] and served as Dox + MO group: (3): L-Carnitine Nanoparticles (50 mg/kg b.wt.) [21] and served as DOX+ LCNPs group; (5) Dox+MO+LCNPs group: received MO and LCNPs as previously indicated doses. All treatments were administered daily for four weeks. After the experimental period finalization, all groups were kept fasting for 12 h before blood sampling. To this end, blood was collected in dry clean test tubes under diethyl ether anesthesia from the retro-orbital venous plexus in clean centrifuge tubes and allowed to coagulate at room temperature. Serum samples were centrifuged at 1800×g for 15 minutes at 4°C using a cooling centrifuge for biochemical measurements and kept at -20°C until analysis. Heart weights (HW) were measured. Heart tissues were collected and homogenized in phosphate buffer saline (PBS). The homogenate was then subjected to biochemical analysis. Some heart specimens were fixated in 10% buffered formalin for histopathological analysis.

### 2.7. Biochemical estimations.

Cardiac malondialdehyde (MDA) content was determined by colorimetric method using a kit purchased from Eagle Biosciences, Inc., USA, according to the method of Satoh

[22]. Cardiac nitric oxide (NO) was performed by ELISA technique using a kit purchased from Bender MedSystems, GmbH, Vienna, Austria, corresponding to the manufacturer's instructions. Cardiac Heme Oxygenase 1 (HO1) level was determined by the ELISA technique purchased from Abcam (ab207621) according to the manufacturer's instructions. Cardiac nuclear factor kappa B (NF- $\kappa$ B) level in cardiac tissue was determined by the ELISA technique using rat nuclear factor kappa B ELISA kit purchased from Abcam (ab176648) according to the manufacturer's instructions. Cardiac Total Antioxidant Capacity (TAC) was determined using the ELISA technique using a kit purchased from Cell Biolabs, Inc, San Diego, USA, according to Campbell *et al.* [23]. Serum C-reactive protein (CRP) was determined by CRP-HS (ab99995 Immunoassay) kit purchased from Abcam, according to Whicher [24]. Serum Creatine Kinase (CK) was determined by a kit bought from Diagnosticum Zrt., according to Mathieu *et al.* [25]. Serum lactate dehydrogenase (LDH) was assessed by ELISA technique using kits purchased from MyBioSource Inc. (San Diego, CA, USA) according to the methods of Young [26]. Serum alanine aminotransferase (ALT) activity was measured by the colorimetric technique using Salucea kit (The Netherlands) according to the way described by Young [27]. Serum cardiac Tropinin-1 and Endothelin-1 were assessed by the ELISA technique purchased from Abcam (ab200016 and ab133030), respectively, according to the manufacturer's instructions. The cardiac total protein level was determined by the colorimetric method of Lowry *et al.* [28].

### 2.8. Histopathological investigation of heart sections.

The liver tissues were rinsed in running tap water after being fixed in formalin saline (10%) for 24 hours and then dehydrated with a progressive grade of ethyl alcohol (30, 50, 70, 90, and 100%). Specimens were cleared in xylene and embedded in paraffin (melting point 58-60 oC) for twenty-four 24 hours. Using a sledge microtome, paraffin wax tissue blocks were created for sectioning at 5 $\mu$ . The tissue sections were mounted on glass slides, deparaffinized, and stained with hematoxylin and eosin (H & E) stain [29] before being examined under a light microscope.

### 2.9. Statistical analysis.

In the current study, results are indicated as the mean  $\pm$  S.E of the mean for the ten rats in each group. Statistical Package for the Social Sciences program (SPSS), version 20.0 (SPSS Inc., USA), was used to compare significance between every two groups. The difference was considered statistically significant at  $p \leq 0.05$ .

## 3. Results and Discussion

### 3.1. Effect of LCNPs and MO on the levels of cardiac MDA, TAC, NO, HO-1 and NF- $K\beta$ in male rats.

The results of MDA, NO, HO-1, NF-  $K\beta$ , and TAC in the control and other groups' cardiac tissue have been represented in Table 1. The levels of MDA, NO, HO-1, and NF-  $K\beta$  were significantly increased ( $p < 0.05$ ) in the DOX group compared to the normal control group. At the same time, the level of TAC activities was significantly decreased ( $P < 0.05$ ) in the DOX groups compared to the normal control group. On the other hand, treatment with either MO or LCNPs exerted a significant reduction ( $P < 0.05$ ) in the cardiac levels of MDA, NO, HO-1, and

NF- $\kappa$ B associated with significant increment ( $P < 0.05$ ) in the activities of TAC compared to the untreated DOX group (Table 1). The data, however, display that after treatment with a combination of LCNPs and MO significantly ( $P < 0.05$ ) restored, the levels of MDA, NO, HO-1, NF-  $\kappa$ B, and TAC to normal levels compared to the DOX untreated group (Table 1).

**Table 1.** Effects of LCNPs, MO, and their combination on the levels of rat cardiac MDA, TAC, NO, HO-1, and NF-  $\kappa$ B in different treated groups.

	Con	DOX	DOX+MO	DOX+LCNPs	DOX+MO+LCNPs
MDA (n mol/g)	8.66± 0.84	66.00± 4.42 <sup>a</sup>	39.16± 1.63 <sup>b</sup>	29.01±1.02 <sup>b</sup>	18.66± 0.57 <sup>b</sup>
NO( $\mu$ mol/g)	63.01± 3.40	151.33± 4.87 <sup>a</sup>	120.83± 3.56 <sup>b</sup>	85.50± 3.78 <sup>b</sup>	69.03± 2.20 <sup>b</sup>
HO-1(pg/mg)	0.31± 0.02	0.69± 0.06 <sup>a</sup>	0.50± 0.03 <sup>b</sup>	0.40± 0.02 <sup>b</sup>	0.34± 0.01 <sup>b</sup>
NF- $\kappa$ B( $\mu$ g/g)	99.83± 4.30	313.02± 6.79 <sup>a</sup>	290.33± 4.25 <sup>b</sup>	242.33± 3.73 <sup>b</sup>	140.66± 4.14 <sup>b</sup>
TAC (m mol/g)	51.33± 2.70	12.66± 0.80 <sup>a</sup>	23.16± 1.49 <sup>b</sup>	41.00± 1.34 <sup>b</sup>	53.66± 2.14 <sup>b</sup>

Data are expressed as mean ± SE of 10 rats/group.

a: Significant change at  $P < 0.05$  compared to the negative control group.

b: Significant change at  $P < 0.05$  compared to the untreated DOX group.

### 3.2. Effect of LCNPs and MO on the activities of serum cardiac marker enzymes in male rats.

Data in Table 2 illustrated the effect of treatment with LCNPs and ethanolic extract of MO on the activities of serum cardiac enzymes in DOX-treated rats. DOX group showed significant elevation ( $P < 0.05$ ) in serum CRP, CK, LDH, and ALT activity concerning the negative control group. Treatment with either MO or LCNPs separately showed a significant decrease ( $P < 0.05$ ) in serum CRP, CK, LDH, and ALT activity compared to the untreated DOX group. However, combined treatment with MO and LCNPs led to the normalization of all serum cardiac markers compared to the healthy control group.

**Table 2.** Effects of LCNPs, MO, and their combination on the activities of rat serum CRP, CK, LDH and ALT enzymes in different treated groups.

	Con	DOX	DOX+MO	DOX+LCNPs	DOX+MO+LCNPs
CRP(mg/L)	17.50± 1.51	66.50± 4.69 <sup>a</sup>	47.66± 3.11 <sup>b</sup>	30.16± 3.48 <sup>b</sup>	23.66± 3.25 <sup>b</sup>
CK (mg/dl)	150.00± 8.58	349.16± 13.53 <sup>a</sup>	259.83± 11.14 <sup>b</sup>	204.00± 7.93 <sup>b</sup>	176.66± 6.32 <sup>b</sup>
LDH(mg/dl)	236.66± 5.14	474.00± 7.84 <sup>a</sup>	377.83± 6.91 <sup>b</sup>	290.16± 6.70 <sup>b</sup>	259.83± 3.85 <sup>b</sup>
ALT(mg/dl)	22.83±2.71	76.33± 4.41 <sup>a</sup>	62.50± 3.20 <sup>b</sup>	47.16± 3.25 <sup>b</sup>	34.16± 2.83 <sup>b</sup>

Data are expressed as mean ± SE of 10 rats/group.

a: Significant change at  $P < 0.05$  compared to the negative control group.

b: Significant change at  $P < 0.05$  compared to the untreated DOX group.

### 3.3. Effect of LCNPs and MO on the levels of serum Troponin-1 and Endothelin-1 in male rats.

Table 3 represented the impact of dealing with LCNPs, MO extract on serum levels of Troponin-1 and Endothelin-1 in normal and DOX treated rats. The DOX group showed significant elevation ( $P < 0.05$ ) in serum Troponin-1 and Endothelin-1 levels relative to the control group.

**Table 3.** Effects of LCNPs, MO and their combination on the levels of rat serum Troponin-1 and Endothelin-1 in different treated groups.

	Con	DOX	DOX+MO	DOX+LCNPs	DOX+MO+LCNPs
Troponin-1 (pg/mL)	0.46± 0.03	0.82± 0.04 <sup>a</sup>	0.60± 0.03 <sup>b</sup>	0.51± 0.02 <sup>b</sup>	0.48± 0.01 <sup>b</sup>
Endothelin-1 (pg/mL)	0.08± 0.01	1.02± 0.04 <sup>a</sup>	0.56± 0.03 <sup>b</sup>	0.34± 0.02 <sup>b</sup>	0.12± 0.02 <sup>b</sup>

Data are expressed as mean ± SE of 10 rats/group.

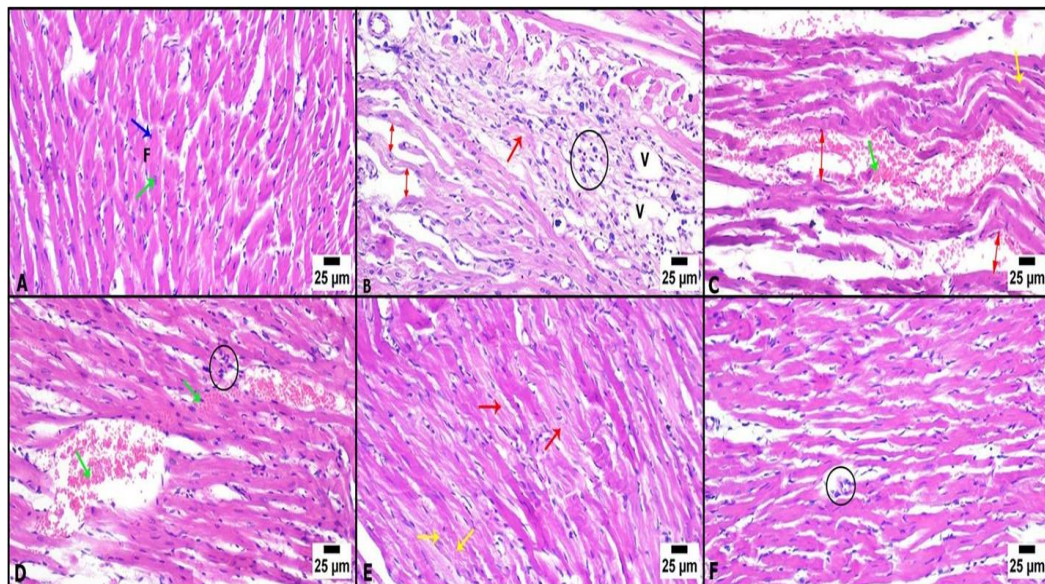
a: Significant change at  $P < 0.05$  compared to the negative control group.

b: Significant change at  $P < 0.05$  compared to the untreated DOX group.

Treatment with either MO or LCNPs exerted a significant reduction ( $P<0.05$ ) in the levels of serum Troponin-1 and Endothelin-1 in comparison to DOX untreated control (Table 3). Whereas combined treatment with MO and LCNPs showed more superior therapeutic effects and a substantial decrease ( $P<0.05$ ) in serum levels of Troponin-1 and Endothelin-1 in comparison to healthy control rats (Table 3).

### 3.4. Histological examination.

They examined heart sections from rats of control, MO, LCNPs, and MO+ LCNPs groups revealed normal histological structures of the cardiac myofibers represented as normally arranged cardiomyocytes that appeared cylindrical and branching with acidophilic sarcoplasm and centrally located oval nuclei (Figure 1A). At the same time, the heart sections from the DOX-treated group showed severe cardiac pathological changes represented by multifocal areas of mononuclear inflammatory cells infiltration between the myocardial fibers accompanied by wide vacuolar degeneration and necrosis of the adjacent myocytes. Besides, increased wavy myocardial fibers lead to contraction and large areas of hemorrhage, filling the wide spaces between cardiac myofibers (Figure 1B, C). The heart section from the MO group showed moderate improvement in the form of a few inflammatory areas and hemorrhages reaching the fibers compared to the DOX-treated group (Figure 1D). Moreover, fewer histopathological signs were observed in cardiac sections treated with LCNPs demonstrated by myocardial degeneration and few wavy fibers (Figure 1E). On the other hand, the examined heart sections from received rats MO and co-administered with LCNPs revealed marked improvement displayed by apparently normal cardiomyocytes with few inflammatory cells (Figure 1F).



**Figure 1.** Photomicrographs of rat cardiac tissue (HE-400X), (A) Normal control showing normal cardiac structure, myocardial fibers (F), central disc nucleus (green arrow), endothelial nucleus (blue arrow), (B) group treated with DOX revealing severe cardiac pathological changes lymphocytic inflammation (black circle), wide vacuolar degeneration (V), depositions of extracellular matrix (red arrow), wide spaces between myocardial fibers due degeneration (red double headed arrows) and myocardial degeneration that looked pale, (C) group treated with DOX revealing wavy myocardial fibers (yellow arrow) leading to contraction, hemorrhage filling the wide spaces between fibers (green arrow), (D) group treated with MO posting less pathological features, a few number of inflammatory cells (black circle), hemorrhage reaching the fibers (green arrow), (E) group treated with LCNPs displaying less pathological sign, myocardial degeneration (red arrows), wavy fibers (yellow arrows), (F) group treated with MO+LCNPs showing marked improvement and a few inflammatory cells (black circle).

### 3.5. Discussion.

This recent study presented promising findings of L-carnitine nanoparticles and *Moringa oleifera* ethanolic extract, a hopeful candidate for mitigating cardiotoxicity. It has been commonly well-thought-out that DOX is one of the most effective and widely used anticancer drugs with significant cardiotoxicity [1].

In our study, DOX administration caused cardiotoxicity to normal rats markedly by increased levels of pro-oxidant (MDA, NO, HO-1) and proinflammatory cytokines marker (NF- $\kappa$ B) in the cardiac tissue reflected by a significant reduction of total antioxidant capacity (TAC) when compared to the normal control group. This result was consistent with Kiss *et al.* [30], who confirmed that oxidative stress and mitochondrial damage play an essential role in DOX-induced myocardial injury, producing a larger amount of oxidation products, causing obvious damage to mitochondria, and ultimately leading to cardiomyocyte damage [31]. Furthermore, earlier studies have shown that mitochondrial dysfunction is one of the pathogenic pathways linked to the cardiotoxic action of Dox. It leads to a rise in ROS (reactive oxygen species) production and mitochondrial depolarization [32].

Moreover, Valls-Belles *et al.* [33] revealed that enhanced lipid peroxidations and free radical production in the heart are the main cause of DOX-induced cardiotoxicity. DOX may form a DOX-Fe complex with iron, resulting in iron cycling between Fe (II) and Fe (III) forms and considerable ROS production. As a result, chronic inflammation develops, leading to several problems. In addition, Some mediators, such as MDA, ROS, NO, HO-1, IL-6, and TNF-, are known to be released by activated macrophages as a consequence of severe oxidative stress and inflammation [34]. Likewise, the observed rise in NO levels could be attributable to  $\beta$ -adrenergic stimulation, which increases the protein expression of inducible nitric oxide synthase (iNOS), causing nitrosative stress and the production of peroxynitrite (ONOO-) radicals [35,36].

Furthermore, Some evidence indicates that DOX may enhance inflammatory response in the myocardium and vasculature by activating nuclear factor kappa-B (NF- $\kappa$ B) signaling, which may contribute to the secretion of inflammatory cytokines or chemokines like interleukin 1 (IL-1), IL-6, IL-18, and tumor necrosis factor (TNF-) [37]. Furthermore, Thandavarayan *et al.* [38] found that increased ROS generation causes oxidative stress, a significant factor in the onset and progression of DOX-induced cardiac dysfunction. DOX induces cardiac oxidative stress and cardiotoxicity by reducing myocardial antioxidants, disturbing calcium handling, and increasing superoxide production accompanied by releasing inflammatory cytokines [39,40]. Our findings support this theory, as the increase observed in (MDA, NO, HO-1, and NF- $\kappa$ B) levels could be the reason for myocardial dysfunction that resulted in depletion of total antioxidant capacity, thereby cardiac damage of DOX-injected rats.

Moreover, the current study results clearly showed that DOX treatment was markedly up-regulated the serum levels of CRP, CK, LDH, ALT, and serum troponin-1 and endothelin-1 versus the control group. This rise in CRP, CK, LDH, and ALT levels is linked to oxidative stress and releasing reactive oxygen species (ROS) due to DOX treatment, leading to cardiac damage development [41,42]. Consistently with our results, several earlier studies have linked the deterioration of membrane integrity in cardiac cells to the release of free radical-mediated lipid peroxidation, which induces enzyme leakage. The release of these enzymes into the bloodstream causes their levels in the serum to rise, indicating myocyte damage [43,44].

Furthermore, CRP, CK, LDH, and ALT levels in the serum have been employed as diagnostic indications of myocardial injury. Creatine kinase serves an important part in cellular energy metabolism. It's a dimer of two polypeptide chains made up of M and/or B subunits that unite to form the CK-MB (noticed predominantly in cardiac muscle), CK-MM (found in striated muscle), and CK-BB (observed in the brain, stomach, and other places) Isoenzymes. Troponin (Tn) is a family of three controlling proteins that play a role in muscular contraction in skeletal and cardiac muscles. Also, Endothelin-1 is a powerful vasoconstrictor produced by endothelial cells in the blood vessels. After the Dox treatment, the levels of endothelin-1 increase in cardiac muscle cells and cause hypertrophic cardiomyopathy [45]. So, Increased generation of free radicals may exacerbate the inflammatory cascade, leading to damage of these enzymes, thereby myocardial dysfunction [46,47].

Interestingly, the results from our study have confirmed that treatment of DOX group with MO, LCNPs, or both led to a significant decrement in the levels of pro-oxidant markers MDA, NO, HO-1, and inflammatory marker (NF-  $\kappa$ B) while strongly enhancing the levels of TAC while compared to DOX group, indicating that both MO and LCNPs exerting cardioprotective impact against DOX toxicity. These results are in accordant with prior reports which demonstrated that pretreatment with MO extract restores MDA and GSH levels to near-control levels, most likely through direct antioxidant activities or as an efficient free radical scavenger, or by protecting from lipid peroxidation and ROS by replenishing the GSH pool and decreasing ROS production [48,49]. Moreover, The observed decrease in oxidative stress markers and down-regulation of proinflammatory marker NF- $\kappa$ B following MO administration may have been attributed to its phytochemical ingredients that are shown to have antioxidant and anti-inflammatory activities, including caffeic acid and methyl gallate and catechin [50,51]. It also contains gallic acid, which was reported to have antioxidant, anti-inflammatory activities down-regulating the release of inflammatory mediators, including TNF- $\alpha$ , NF-  $\kappa$ B [52,53].

Likewise, the ability of MO to combat oxidative stress can be related to the strong antioxidant activity of its leaves, flowers, and seeds because of ascorbic acid, flavonoids, phenolics, carotenoids, quercetin, kaempferol, and phenolic acids [54,55]. It's also because of the existence of isothiocyanates, polyphenols, and rutin in leaves [56]. Quercetin is essential as it contains phenolic hydroxyl groups that have antioxidant action and greatly suppress the production of reactive oxygen species (ROS) [57]. Similarly, luteolin has potent antioxidant properties, including DNA protection, free radical scavenging, and anti-inflammatory [58].

Furthermore, our findings showed that LCNPs treatment caused a significant reduction in the levels of oxidative stress markers MDA, NO, HO-1 reflected by a significant increase in the levels of TAC as compared to the DOX group. This results in coincidence with previous works confirmed that L-carnitine nanoparticles (LCN) therapy reduced the hepatic level of the antioxidant enzyme GSH, as evidenced by lower levels of GSSG, NO, and MDA. They attributed this to the antioxidant capabilities of L-carnitine (LC) and its acyl derivatives, which block xanthine oxidase (Pauly and Pepine [59] and Radwan and Abd El-Motelp [21]. Also, Guan *et al.* [60] noted that L-carnitine exerts an antioxidant action by protecting against lipid peroxidation of membrane phospholipid and eliminating extracellular toxic acetyl-coenzyme A responsible for mitochondrial ROS.

Moreover, Li *et al.* [61] and Gulcin [62] established that LC could be used to eliminate free radicals, reduce oxidative stress, regulate nitric oxide, cellular respiration, and the activity of enzymes involved in oxidative damage defense, as well as chelate metal ions (e.g., ferrous)



that accelerate ROS formation. Our findings support this hypothesis, as the considerable reduction in the cardiac levels of MDA, NO, HO-1, and NF- $\kappa$ B, and the restoration of TAC activity, are obvious indicators that LCNPs have an antioxidant effect that prevents oxidative damage.

However, the mechanism by which LCNPs caused cardioprotection against cardiotoxicity could be linked to the free radical scavenging activity, antioxidant, anti-inflammatory, and antiapoptotic properties of L-carnitine [7]. It protects cardiac and endothelial cells from oxidative stress by reducing membrane lipid peroxidation, protecting the mitochondrial membrane, which is critical for mitochondrial ATP production, activating antioxidant enzymes, and synthesizing antioxidant molecules like reduced glutathione, thereby preventing apoptosis [63,64].

Furthermore, the present study demonstrated that treatment of DOX group with MO, LCNPs, or their combination caused a significant decline in the serum levels of CRP, CK, LDH, and ALT associated with a significant reduction in serum levels of troponin-1 and endothelin-1 as compared to DOX group, indicating that both of MO and LCNPs has a protective effect on the heart by reducing myocardial damage and thus limiting the leakage of these enzymes from the myocardium [65]. Similar results were reported in previous studies, which demonstrated that MO is a cardioprotective agent, as it hinders various cardiovascular impairments [66,67]. The cardioprotective properties of ingredients isolated from MO leaves prevent the disruption of cardiac myofibrils and improve cardiac contractile function [68]. Moreover, the favorable effect of dietary MO supplementation on animal performance may be ascribed to calcium, magnesium, sodium, potassium, copper, iron, manganese, zinc,  $\alpha$ -tocopherol,  $\beta$ -carotene, and ascorbic acid, as well as polyunsaturated fatty acid and some bioactive components of *Moringa* [69].

The impact of LCNPs in protecting myocardial integrity was seen by controlling the intra-mitochondrial percentage of acyl-CoA/CoA, which resulted in eliminating toxic compounds, conserving the mitochondrial membrane permeability, and encouraging the eradication of free radicals [70]. Additionally, L-carnitine supplementation has been proven in clinical investigations to improve myocardial fat metabolism by increasing the demand for free fatty acids and their metabolites, which improves cardiac function [9].

Moreover, this study showed that DOX rats displayed severe histological changes manifested by vacuolar changes in cardiac muscle fibers, mainly in the form of degeneration of myocardial tissue, cytoplasmic vacuolization of the cardiomyocytes, congestion of myocardial vessels, necrosis of the adjacent myocytes, myofibrillar loss and myocardial hypertrophy. These structural and cellular alterations are concordant with numerous prior studies on DOX-induced cardiomyopathy in rats [71-73]. However, MO or LCNPs and MO+ LCNPs treatment can attenuate the cardiac tissue lesions driven by DOX as indicated by slowing down myocardial inflammation and cardiac fibrosis pathogenesis compared to the untreated DOX group [74,75,36]. A similar cardioprotective effect for the MO and LCNPs has been previously reported, where cardiac histopathological changes induced by DOX were almost returned to normal myocardial architecture [76,77,65].

#### 4. Conclusions

The findings concluded that LCNPs and MO successfully prevented DOX-induced cardiotoxicity and cardiac dysfunction in rats. Additionally, the combination treatment would provide greater cardioprotection on both Physiological and structural levels by synergistically

suppressing cardiac oxidative biochemical markers and inflammatory cytokine markers while also increasing antioxidant status. These effects could be related to its antioxidant, radical scavenging activity, and anti-inflammatory properties. These findings suggest that LCNPs and MO could be promising drugs for treating cardiotoxicity.

## Funding

This research received no external funding.

## Acknowledgments

This research has no acknowledgment.

## Conflicts of Interest

The authors declare no conflict of interest.

## References

1. Cheng, X.; Liu, D.; Song, H.; Tian, X.; Yan, C.; Han, Y. Overexpression of Kininogen-1 aggravates oxidative stress and mitochondrial dysfunction in DOX-induced cardiotoxicity. *Biochemical and Biophysical Research Communications* **2021**, *550*, 142-150, <https://doi.org/10.1016/j.bbrc.2021.02.104>.
2. Wallace, K.B.; Sardao, V.A.; Oliveira, P.J. Mitochondrial determinants of doxorubicin-induced cardiomyopathy. *Circulation Research* **2020**, *126*, 926–941, <https://doi.org/10.1161/CIRCRESAHA.119.314681>.
3. Wan, Y.; Bo He, B.; Zhu, D.; Wang, L.; Huang, R.; Jing Zhu, J.; Wang, C.; Gao, F. Nicotinamide mononucleotide attenuates doxorubicin-induced cardiotoxicity by reducing oxidative stress, inflammation and apoptosis in rats. *Archives of Biochemistry and Biophysics* **2021**, *712*, <https://doi.org/10.1016/j.abb.2021.109050>.
4. Younis, N.S.; Elsewedy, H.S.; A.; Soliman, W.E.; Shehata, T.M.; Maged E. Mohamed, M.E. Geraniol isolated from lemon grass to mitigate doxorubicin-induced cardiotoxicity through Nrf2 and NF- $\kappa$ B signaling. *Chemico-Biological Interactions* **2021**, *347*, <https://doi.org/10.1016/j.cbi.2021.109599>.
5. Rawat, P.S.; Jaiswal, A.; Khurana, A.; Bhatti, J.S.; Navik, U. Doxorubicin-induced cardiotoxicity: An update on the molecular mechanism and novel therapeutic strategies for effective management. *Biomed. Pharmacother.* **2021**, *139*, <https://doi.org/10.1016/j.biopha.2021.111708>.
6. Abd-Elrazek, A.M.; Ahmed-Farid, O.A. Protective effect of L-carnitine and L-arginine against busulfan-induced oligospermia in adult rat. *Andrologia* **2018**, *50*, <https://doi.org/10.1111/and.12806>.
7. Aboubakr, M.; Elsayd, F.; Soliman, A.; Fadl, S.E.; El-Shafey, A.; Abdelhiee, E.Y. L-Carnitine and vitamin E ameliorate cardiotoxicity induced by tilmicosin in rats. *Environmental Science and Pollution Research* **2020**, *23*, 1–9, <https://doi.org/10.1007/s11356-020-08919-6>.
8. Al-Eisa, R.A.; Al-Salmi, F.A.; Hamza, R.Z.; El-Shenawy, N.S. Role of L-carnitine in protection against the cardiac oxidative stress induced by aspartame in Wistar albino rats. *PloS One* **2018**, *13*, <https://doi.org/10.1371/journal.pone.0204913>.
9. Elkomy, A.; Abdelhiee, E.Y.; Fadl, S.E.; Emam, M.A.; Gad, F.A.-M.; Sallam, A.; Alarifi, S.; Abdel-Daim, M.M.; Aboubakr, M. L-carnitine mitigates oxidative stress and disorganization of cytoskeleton intermediate filaments in cisplatin-induced hepato-renal toxicity in rats. *Frontiers in Pharmacology* **2020**, *11*, <https://doi.org/10.3389/fphar.2020.574441>.
10. Aziz, M.M.; Abd El Fattah, M.A.; Ahmed, K.A.; Sayed, H.M. Protective effects of Olmesartan and L-carnitine on doxorubicin-induced Cardiotoxicity in rats. *Can. Journal of Physiology and Pharmacology* **2020**, *98*, 183-193, <https://doi.org/10.1139/cjpp-2019-0299>.
11. Hussein, J.; El-Naggar, M.E.; Fouda, M.M.G.; Morsy, O.M.; Ajarem, J.S.; Almalki, A.M.; Allam, A.A.; Mekawi, E.M. The efficiency of blackberry loaded AgNPs, AuNPs and Ag@AuNPs mediated pectin in the treatment of cisplatin-induced cardiotoxicity in experimental rats. *International Journal of Biological Macromolecules* **2020**, *159*, 1084-1093, <https://doi.org/10.1016/j.ijbiomac.2020.05.115>.
12. Inoue, I.; Chiba, M.; Ito, K.; Okamoto, Y.; Suga, Y.; Kitahara, Y.; Nakahara, Y.; Endo, Y.; Takahashi, K.; Tagami, U.; Okamoto, N. One-step construction of ferritin encapsulation drugs for cancer chemotherapy. *Nanoscale* **2021**, *13*, 1875–1883, <https://doi.org/10.1039/D0NR04019C>.
13. Zhai, Z.; Cheng, Y.; Hong, J. Nanomedicines for the treatment of glaucoma: current status and future perspectives. *Acta Biomaterialia* **2021**, *125*, 41–56, <https://doi.org/10.1016/j.actbio.2021.02.017>.

14. Shi, J.; Kantoff, P.W.; Wooster, R.; Farokhzad, O.C. Cancer nanomedicine: progress, challenges and opportunities. *Nature Reviews Cancer* **2017**, *17*, 20–37, <https://doi.org/10.1038/nrc.2016.108>.
15. Madi, N.; Dany, M.; Abdoun, S.; Usta, J. Moringa oleifera's nutritious aqueous leaf extract has anticancerous effects by compromising mitochondrial viability in an ROS-dependent manner. *Journal of the American College of Nutrition* **2016**, *35*, 604–613, <https://doi.org/10.1080/07315724.2015.1080128>.
16. Mumtaz, M.Z.; Kausar, F.; Hassan, M.; Javaid, S.; Malik, A. Anticancer activities of phenolic compounds from Moringa oleifera leaves: in vitro and in silico mechanistic study. *Beni-Suef University Journal of Basic and Applied Sciences* **2021**, *10*, <https://doi.org/10.1186/s43088-021-00101-2>.
17. Pinto, C.E.M.; Farias, D.F.; Carvalho, A.F.U.; Oliveira, J.T.A.; Pereira, M.L.; Grangeiro, T.B.; Freire, J.E.C.; Viana, D.A.; Vasconcelos, I.M. Food safety assessment of an antifungal protein from Moringa oleifera seeds in an agricultural biotechnology perspective. *Food and Chemical Toxicology* **2015**, *83*, 1-9, <https://doi.org/10.1016/j.fct.2015.05.012>.
18. Hagoel, L.; Vexler, A.; Kalich-Philosoph, L.; Earon, G.; Ron, I.; Shtabsky, A.; Marmor, S.; Lev-Ari, S. Combined Effect of Moringa oleifera and Ionizing Radiation on Survival and Metastatic Activity of Pancreatic Cancer Cells. *Integrative Cancer Therapies* **2019**, *18*, <https://doi.org/10.1177/1534735419828829>.
19. Mantawy, E.M.; Esmat, A.; El-Bakly, W.M.; Salah Eldin, R.A.; El-Demerdash, E. Mechanistic clues to the protective effect of chrysin against doxorubicin-induced cardiomyopathy: plausible roles of p53, MAPK and AKT pathways. *Scientific Reports* **2017**, *7*, 1–13, <https://doi.org/10.1038/s41598-017-05005-9>.
20. Aja, P.M.; Ibiam, U.A.; Nwali, B.U.; Orji, O.U.; Edwin, N.; Afiukwa, C.A. Evaluation of effects of ethanol extracts of *Moringa oleifera* and *Cajanus cajan* leaves on lipid profiles, in alloxan-induced diabetic albino rats. *Global Journal of Biochemistry and Biotechnology* **2015**, *10*, 71–76.
21. Radwan, O.K.; Abd El-Motelp, B.A. Effectiveness of Nano L-Carnitine and Thyroxin in the management of hypothyroidism in male rats. *Journal of BioSciences and Applied Research* **2017**, *3*, 185-197.
22. Satoh, K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clinica Chimica Acta* **1978**, *90*, 37-43, [https://doi.org/10.1016/0009-8981\(78\)90081-5](https://doi.org/10.1016/0009-8981(78)90081-5).
23. Campbell, M.S.; Ouyang, A.; Krishnakumar, I.M.; Charnigo, R.J.; Westgate, P.M.; Fleenor, B.S. Influence of enhanced bioavailable curcumin on obesity-associated cardiovascular disease risk factors and arterial function: A double-blinded, randomized, controlled trial. *Nutrition* **2019**, *62*, 135-139, <https://doi.org/10.1016/j.nut.2019.01.002>.
24. Whicher, J. C-reactive protein. In: *Clinical Laboratory Diagnostics: use and assessment of clinical laboratory results/ed.* By Lothar Thomas. 1st edition. Frankfurt/Main **1998**; pp. 700-710.
25. Mathieu, M. et al. Recommendation pour la mesure de la concentration cataytique de la cratinine konase dans le serum humain. *Annales de Biologie Clinique* **1982**, 40-87.
26. Young, D.S. *Effects of disease on clinical laboratory tests.* 5th edition, AACC press, **2000**.
27. Young, D.S. *Effect of drugs on clinical laboratory tests.* 3rd ed. Washington DC: AACC Press, **1990**; pp. 6-12, <https://doi.org/10.1177/000456329703400601>.
28. Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **1951**, *193*, 265-75.
29. Bancroft, J.D.; Stevens, A.; Turner, D.R. *Theory and practice of histological techniques.* 4th ed. Churchill Livingstone Philadelphia PA USA **1996**; pp. 25-90.
30. Kiss, A.; Ráduly, A.P.; Regdon, Z.; Polgár, Z.; Tarapcsák, S.; Sturniolo, I.; El-Hamoly, T.; Virág, L.; Hegedűs, C. Targeting nuclear NAD (+) synthesis inhibits DNA repair, impairs metabolic adaptation and increases chemosensitivity of U-2OS osteosarcoma cells. *Cancers* **2020**, *12*, 1180. <https://doi.org/10.3390/cancers12051180>.
31. Abdelghffar, E.A.; Obaid, W.A.; Elgamal, A.M.; Daoud, R.; Mansour Sobeh, M.; El Raey, M.A. Pea (*Pisum sativum*) peel extract attenuates DOX-induced oxidative myocardial injury. *Biomedicine & Pharmacotherapy* **2021**, *143*, <https://doi.org/10.1016/j.biopha.2021.112120>.
32. Tian, Z.; Yang, Y.; Yang, Y.; Zhang, F.; Li, P.; Wang, J.; Yang, J. Zhang, P.; Yao, W.; Wang, X. High cumulative doxorubicin dose for advanced soft tissue sarcoma. *BMC Cancer* **2020**, *20*, <https://doi.org/10.1186/s12885-020-07663-x>.
33. Antonucci, S.; Di Sante, M.; Tonolo, F.; Pontarollo, L.; Scalcon, V.; Alanova, P.; Menabò, R.; Carpi, A.; Bindoli, A.; Rigobello, M.P.; Giorgio, M.; Kaludercic, N.; Di Lisa, F. The Determining Role of Mitochondrial Reactive Oxygen Species Generation and Monoamine Oxidase Activity in Doxorubicin-Induced Cardiotoxicity. *Antioxidants & Redox Signaling* **2020**, *34*, 531-550, <https://doi.org/10.1089/ars.2019.7929>.
33. Valls-Belles, V.; Torres, M.D.C.; Boix, L.; Muniz, P.; Gonzalez-SanJose, M.L.; Codoner-Franch, P.  $\alpha$ -Tocopherol, MDA-HNE and 8-OHdG levels in liver and heart mitochondria of adriamycin-treated rats fed with alcohol-free beer. *Toxicology* **2008**, *249*, 97–101, <https://doi.org/10.1016/j.tox.2008.04.010>.
34. Paulsen, Ø.; Laird, B.; Aass, N.; Lea, T.; Fayers, P.; Kaasa, S.; Klepstad, P. The relationship between proinflammatory cytokines and pain, appetite and fatigue in patients with advanced cancer. *PloS one* **2017**, *12*, <https://doi.org/10.1371/journal.pone.0177620>.
35. Li, D.; Qu, Y.; Tao, L.; Liu, H.; Hu, A.; Gao, F.; Sharifi-Azad, S.; Grunwald, Z.; Ma, X.-L.; Sun, J.-Z. Inhibition of iNOS protects the aging heart against  $\beta$ -adrenergic receptor stimulation-induced cardiac

- dysfunction and myocardial ischemic injury. *Journal of Surgical Basic Science* **2006**, *131*, 64–72, <https://doi.org/10.1016/j.jss.2005.06.038>.
36. Emran, T.; Chowdhury, N.I.; Sarker, M.; Bepari, A.K.; Hossain, M.; Rahman, G.M.S.; Reza, H.M. L-carnitine protects cardiac damage by reducing oxidative stress and inflammatory response via inhibition of tumor necrosis factor-alpha and interleukin-1beta against isoproterenol-induced myocardial infarction. *Biomedicine & Pharmacotherapy* **2021**, *143*, <https://doi.org/10.1016/j.biopha.2021.112139>.
  37. Mantawy, E.M.; El-Bakly, W.M.; Esmat, A.; Badr, A.M.; El-Demerdash, E. Chrysin alleviates acute doxorubicin cardiotoxicity in rats via suppression of oxidative stress, inflammation and apoptosis. *European Journal of Pharmacology* **2014**, *728*, 107–118, <https://doi.org/10.1016/j.ejphar.2014.01.065>.
  38. Thandavarayan, R.A.; Giridharan, V.V.; Arumugam, S.; Suzuki, K.; Ko, K.M.; Krishnamurthy, P.; Watanabe, K.; Konishi, T. Schisandrin B Prevents Doxorubicin Induced Cardiac Dysfunction by Modulation of DNA Damage, Oxidative Stress and Inflammation through Inhibition of MAPK/p53 Signaling. *PLoS one* **2015**, *10*, <https://doi.org/10.1371/journal.pone.0119214>.
  39. Das, O.; Ghosh, J.; Manna, P.; Sil, P.C. Taurine suppresses doxorubicin-triggered oxidative stress and cardiac apoptosis in rat via up-regulation of PI3-K/Akt and inhibition of p53, p38-JNK. *Biochemical Pharmacology* **2011**, *81*, 891–909, <https://doi.org/10.1016/j.bcp.2011.01.008>.
  40. Akolkar, G.; da Silva Dias, D.; Ayyappan, P.; Bagchi, A.K.; Jassal, D.S.; Salemi, V.M.C.; Irigoyen, M.C.; De Angelis, K.; Singal, P.K. Vitamin C mitigates oxidative/nitrosative stress and inflammation in doxorubicin-induced cardiomyopathy. *American Journal of Physiology-Heart and Circulatory Physiology* **2017**, *313*, H795-H809, <https://doi.org/10.1152/ajpheart.00253.2017>.
  41. Arinno, A.; Manechote, C.; Khuanjing, T.; Ongnok, B.; Prathumsap, N.; Chunchai, T.; Arunsak, B.; Kerdpoo, S.; Shinlapawittayatorn, K.; Chattipakorn, C.; Chattipakorn, N. Cardioprotective effects of melatonin and metformin against doxorubicin-induced cardiotoxicity in rats are through preserving mitochondrial function and dynamics. *Biochemical Pharmacology* **2021**, *192*, <https://doi.org/10.1016/j.bcp.2021.114743>.
  42. Potocnik, N.; Perse, M.; Cerar, A.; Injac, R.; Finderle, Z. Cardiac autonomic modulation induced by doxorubicin in a rodent model of colorectal cancer and the influence of fullerene pretreatment. *PLoS ONE* **2017**, *12*, <https://doi.org/10.1371/journal.pone.0181632>.
  43. Torres, V.M.; Simic, V.D. Doxorubicin-Induced Oxidative Injury of Cardiomyocytes – Do We Have Right Strategies for Prevention? In: *Cardiotoxicity of Oncologic Treatments*. Fiuza, M. (Ed.), Intech Open, London. **2012**.
  44. Afsar, T.; Razak, S.; Batoo, K.M.; Khan, M.R. Acacia hydasypica R. Parker prevents doxorubicin-induced cardiac injury by attenuation of oxidative stress and structural Cardiomyocyte alterations in rats, *BMC Complementary Medicine and Therapies* **2017**, *17*, 1–14, <https://doi.org/10.1186/s12906-017-2061-0>.
  45. Bien, S.; Riad, A.; Ritter, C.A.; Gratz, M.; Olshausen, F.; Westermann, D.; Grube, M.; Krieg, T.; Ciecholewski, S.; Felix, S.B.; Staudt, A.; Schultheiss, H.-P.; Ewert, R.; Völker, U.; Tschöpe, C.; Kroemer, H.K. The endothelin receptor blocker bosentan inhibits doxorubicin-induced cardiomyopathy. *Cancer Research* **2007**, *67*, 10428–10435, <https://doi.org/10.1158/0008-5472.CAN-07-1344>.
  46. Bipin, C.B. Cardioprotective effect of Aegle Marmelos Bael Against Doxorubicin induced Myocardial Toxicity in Albino Rats. *PHARMACY > Pharmacology* **2012**.
  47. D’Oria, R.; Schipani, R.; Leonardini, A.; Natalicchio, A.; Perrini, S.; Cignarelli, A.; Laviola, L.; Giorgino, F. The Role of Oxidative Stress in Cardiac Disease: From Physiological Response to Injury Factor. *Oxidative Medicine and Cellular Longevity* **2020**, *2020*, <https://doi.org/10.1155/2020/5732956>.
  48. Rakeshand, S.; Singh, V.J. In vivo antioxidant activity of M. oleifera leaf and pod extracts against carbon tetra chloride induced liver damage in albino mice. *Journal of Chemical and Pharmaceutical Research* **2010**, *2*, 275–283.
  49. Moyo, B.; Oyedemi, S.; Masika, P.J.; Muchenje, V. Polyphenolic content and antioxidant properties of Moringa oleifera leaf extracts and enzymatic activity of liver from goats supplemented with Moringa oleifera leaves/sunflower seed cake. *Meat Science* **2012**, *91*, 441–447, <https://doi.org/10.1016/j.meatsci.2012.02.029>.
  50. Rahman, N.; Jeon, M.; Kim, Y.-S. Methyl gallate, a potent antioxidant inhibits mouse and human adipocyte differentiation and oxidative stress in adipocytes through impairment of mitotic clonal expansion. *BioFactors* **2016**, *42*, 716–726. <https://doi.org/10.1002/biof.1310>.
  51. Hyun, K.H.; Gil, K.C.; Kim, S.G.; Park, S.-Y.; Hwang, K.W. Delphinidin Chloride and Its Hydrolytic Metabolite Gallic Acid Promote Differentiation of Regulatory T cells and Have an Anti-inflammatory Effect on the Allograft Model. *Journal of Food Science* **2019**, *84*, 920-930, <https://doi.org/10.1111/1750-3841.14490>.
  52. Badavi, M.; Mard, S.A.; Bayati, V.; Gholamreza, G. In vivo and in vitro evidence for the involvement of Nrf2-antioxidant response element signaling pathway in the inflammation and oxidative stress induced by particulate matter (PM10): the effective role of gallic acid. *Free Radical Research* **2019**, *53*, 210–225, <https://doi.org/10.1080/10715762.2018.1563689>.
  53. El-Shehawi, A.M.; Alkafafy, M.; El-Shazly, S.; Sayed, S.; Farouk, S.; Alotaibi, S.; Madkour, D.A.; Khalifa, H.K.; Ahmed, M.M. Moringa oleifera leaves ethanolic extract ameliorates high fat diet-induced obesity in rats. *Journal of King Saud University – Science* **2021**, *33*, <https://doi.org/10.1016/j.jksus.2021.101552>.

54. Gupta, R.; Mathur, M.; Bajaj, V.K.; Katariya, P.; Yadav, S.; Kamal, R.; Gupta, R.S. Evaluation of antidiabetic and antioxidant activity of *Moringa oleifera* in experimental diabetes. *Journal of Diabetes* **2012**, *4*, 164–171, <https://doi.org/10.1111/j.1753-0407.2011.00173.x>.
55. Ademiluyi, A.O.; Aladeselu, O.H.; Oboh, G.; Boligon, A.A. Drying alters the phenolic constituents, antioxidant properties,  $\alpha$ -amylase, and  $\alpha$ -glucosidase inhibitory properties of *Moringa oleifera* leaf. *Food Science and Nutrition* **2018**, *6*, 2123–2133, <https://doi.org/10.1002/fsn3.770>.
56. Tumer, T.B.; Rojas-Silva, P.; Poulev, A.; Raskin, I.; Waterman, C. Direct and indirect antioxidant activity of polyphenol-and isothiocyanate-enriched fractions from *Moringa oleifera*. *Journal of Agricultural and Food Chemistry* **2015**, *63*, 1505–1511, <https://doi.org/10.1021/jf505014n>.
57. Zakaria, N.; Khalil, S.R.; Awad, A.; Khairy, G.M. Quercetin reverses altered energy metabolism in the heart of rats receiving Adriamycin chemotherapy. *Cardiovascular Toxicology* **2018**, *18*, 109–119, <https://doi.org/10.1007/s12012-017-9420-4>.
58. El-Hawary, S.S.; El-sofany, R.H.; Abdel-Monem, A.R.; Ashour, R.S.; Sleem, A.A. Polyphenolics content and biological activity of *Plectranthus amboinicus* (Lour.) Spreng growing in Egypt (Lamiaceae). *Pharmacognosy Journal* **2012**, *4*, 45–51, <http://dx.doi.org/10.5530/pj.2012.32.9>.
59. Pauly, D.F.; Pepine, C.J. The role of carnitine in myocardial dysfunction. *American Journal of Kidney Disease* **2003**, *41*, S35–43, [https://doi.org/10.1016/S0272-6386\(03\)00115-X](https://doi.org/10.1016/S0272-6386(03)00115-X).
60. Guan, Y.; Zheng, X.; Yang, Z.; Li, S. Protective effects of L-carnitine upon testicular ischemia-reperfusion damage in rats. *Chinese journal of preventive medicine* **2009**, *89*, 1858–1861, <https://doi.org/10.1002/cbf.1355>.
61. Li, J.L.; Wang, Q.Y.; Luan, H.Y.; Kang, Z.C.; Wang, C.B. Effects of L-carnitine against oxidative stress in human hepatocytes: involvement of peroxisome proliferator-activated receptor  $\alpha$ . *Journal of Biomedical Science* **2012**, *19*, <https://doi.org/10.1186/1423-0127-19-32>.
62. Gulcin, I. Antioxidant and antiradical activities of L-carnitine. *Life Sciences* **2006**, *78*, 803–811, <https://doi.org/10.1016/j.lfs.2005.05.103>.
63. Surai, P.F. Antioxidant action of carnitine: molecular mechanisms and practical applications. *EC Veterinary Science* **2015**, *2*, 66–84.
64. Al-Eisa, R.A.; Al-Salmi, F.A.; Reham, Z.; Hamza, R.Z.; El-Shenawy, N.S. Role of L-carnitine in protection against the cardiac oxidative stress induced by aspartame in Wistar albino rats. *Plos One* **2018**, <https://doi.org/10.1371/journal.pone.0204913>.
65. Khalil, S.R.; Abdel-Motal, S.M.; Abd-Elsalam, M.; Abd El-Hameed, N.E.; Ashraf, A. Restoring strategy of ethanolic extract of *Moringa oleifera* leaves against Tilmicosin-induced cardiac injury in rats: Targeting cell apoptosis-mediated pathways. *Gene* **2020**, *730*, <https://doi.org/10.1016/j.gene.2019.144272>.
66. Fikriansyah, F.; Widiastuti, M.; Wulandari, N.; Tirtanirmala, P.; Murwanti, R. Cardioprotective effect of Kelor (*Moringa oleifera*) leaf ethanolic extract against doxorubicin-induced cardiotoxicity in rats. *Indonesian Journal of Cancer Prevention* **2015**, *6*, 53–57, <http://dx.doi.org/10.14499/indonesianjcanchemoprev6iss2pp53-57>.
67. Aekthamarat, D.; Pannangpetch, P.; Tangsucharit, P. *Moringa oleifera* leaf extract lowers high blood pressure by alleviating vascular dysfunction and decreasing oxidative stress in L-NAME hypertensive rats. *Phytomedicine* **2019**, *54*, 9–16, <https://doi.org/10.1016/j.phymed.2018.10.023>.
68. Panda, S.; Kar, A.; Sharma, P.; Sharma, A. Cardioprotective potential of N,  $\alpha$ -l-rhamnopyranosyl vincosamide, an indole alkaloid, isolated from the leaves of *Moringa oleifera* in isoproterenol induced cardiotoxic rats: In vivo and in vitro studies. *Bioorganic & Medicinal Chemistry Letters* **2013**, *23*, 959–962, <https://doi.org/10.1016/j.bmcl.2012.12.060>.
69. Gopalakrishnan, L.; Doriya, K.; Kumar, D.S. *Moringa oleifera*: A review on nutritive importance and its medicinal application. *Food Science and Human Wellness* **2016**, *5*, 49–56, <https://doi.org/10.1016/j.fshw.2016.04.001>.
70. Chao, H.-H.; Liu, J.-C.; Hong, H.-J.; Lin J.-W.; Chen, C.-H.; Cheng, T.-H. L-carnitine reduces doxorubicin-induced apoptosis through a prostacyclin-mediated pathway in neonatal rat cardiomyocytes. *International Journal of Cardiology* **2011**, *146*, 145–152, <https://doi.org/10.1016/j.ijcard.2009.06.010>.
71. Richard, C.; Ghibu, S.; Delemasure-Chalumeau, S.; Guillaud, J.-C.; Des Rosiers, C.; Zeller, M.; Cottin, Y.; Rochette, L.; Vergely, C. Oxidative Stress and Myocardial Gene Alterations Associated with Doxorubicin-Induced Cardiotoxicity in Rats Persist for 2 Months after Treatment Cessation. *Journal of Pharmacology and Experimental Therapeutics* **2011**, *339*, 807–814, <https://doi.org/10.1124/jpet.111.185892>.
72. Sheta, A.; Elsakkar, M.; Hamza, M.; Solaiman, A. Effect of metformin and sitagliptin on doxorubicin-induced cardiotoxicity in adult male albino rats. *Human & Experimental Toxicology* **2016**, *35*, 1–13, <https://doi.org/10.1177/0960327115627685>.
73. Shaker, R.A.; Abboud, S.H.; Assad, H.C.; Hadi, N. Enoxaparin attenuates doxorubicin induced cardiotoxicity in rats via interfering with oxidative stress, inflammation, and apoptosis. *BMC Pharmacology and Toxicology* **2018**, *19*, <https://doi.org/10.1186/s40360-017-0184-z>.
74. Gunjal, M.A.; Shah, A.S.; Wakade, A.S.; Juvekar, A.R. Protective effect of aqueous extract of *Moringa oleifera* Lam. stem bark on serum lipids, marker enzymes and heart antioxidants parameters in isoproterenol-induced cardiotoxicity in Wistar rats. *Indian Journal of Natural Products and Resources* **2010**, *1*, 485–492.

75. Aju, B.Y.; Rajalakshmi, R.; Mini, S. Protective role of Moringa oleifera leaf extract on cardiac antioxidant status and lipid peroxidation in streptozotocin induced diabetic rats. *Heliyon* **2020**, *5*, <https://doi.org/10.1016/j.heliyon.2019.e02935>.
76. Omori, Y.; Mano, T.; Sakata, Y.; Ohtani, T.; Takeda, Y.; Tamaki, S.; Kamimura, D.; Tsukamoto, Y.; Aizawa, Y.; Ikeya, Y. L-carnitine supplementation as treatment for cardiac fibrosis and heart failure with preserved ejection fraction. *Journal of Cardiac Failure* **2011**, *17*, <https://doi.org/10.1016/j.cardfail.2011.06.504>.
77. Gouda, A.S.; El-Nabarawy, N.A.; Ibrahim, S.F. Moringa oleifera extract (Lam) attenuates Aluminium phosphide-induced acute cardiac toxicity in rats. *Toxicology Reports* **2018**, *5*, 209–212, <https://doi.org/10.1016/j.toxrep.2018.01.001>.