ORIGINAL ARTICLE

Exploring the antioxidant potential of *Moringa oleifera* leaf extracts mitigating doxorubicin-induced cardiotoxicity in male rats

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ABSTRACT

Doxorubicin (DOX) is a commonly used drug in chemotherapy for cancer treatment. However, it can cause the threatening side effect of cardiotoxicity. This study investigates whether the hydro-alcoholic leaves of *Moringa oleifera* have any protective potential against DOX-induced cardiotoxicity. The phytochemical analysis showed that the plant extracts contained bioactive compounds with antioxidant activities. The DOX-treated group confirmed a significant increment in cardiac troponin I (cTnI) and proinflammatory cytokine interleukin-6 (IL-6) levels, which indicates damage to the cardiomyocytes and also inflammation. However, treatment with the *M. oleifera* extracts significantly inhibited DOX-induced cardiomyocyte damage, as indicated by the significantly low cTnI release. Furthermore, treatment with *M. oleifera* extracts further increased antioxidant activities, thereby decreasing oxidative stress and lipid peroxidation. Moreover, DOX was found to increase the IL-6 level, and treatment with *M. oleifera* extracts had a significant impact on the inhibition of IL-6 levels. These results indicate that the *M. oleifera* extracts have a cardioprotective effect and can play a role as an adjunct drug in mitigating DOX-induced cardiotoxicity, thus providing new prospects for the improvement of safety and efficacy in the treatment of cancer.

Key words: Antioxidant, cardiotoxicity, *Moringa oleifera*, oxidative stress, phytochemicals

INTRODUCTION

The plant kingdom provides us with most of the food and air we consume. In addition, it serves as the primary resource for deriving our medications, and its use has recently experienced a resurgence. Medicinal herbs have been widely used in ancient European and Asian medicine for centuries.[1]

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The widely utilized Moringaceae family includes *Moringa oleifera* Lam. Originating from the western and sub-Himalayan regions of India, Asia Minor, Arabia, and Africa, people now grow this plant in North and South America, the Caribbean, Cambodia, the Philippines, and Central America. *M. oleifera* is known as the Drumstick tree, Horseradish tree, Kelor tree, Benzolive tree, Mlonge tree, Sahjan tree, Marango tree, Mulangay tree, and Sajna tree.[2]

Since ancient times, *M. oleifera* leaves have been employed in medicine and nourishment. *M. oleifera* is anti-inflammatory, antihypertensive, antioxidant, antidiabetic, antihyperlipidemic, and neuroprotectant.[3]

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Drug discovery research isolates strong bioactive metabolites to produce novel drugs. Herbs, such as phenolics and monoterpenes, contain biologically active medicines with physiological effects but are rare.[4] Several projects are studying the health impacts of bioactive chemical compounds. According to multiple studies, these chemicals reduce the incidence of cancer, chronic inflammation, and cardiovascular disease.[5] The cardioprotective activity of the compound was attributed to its antioxidant properties, which prevented lipid peroxidation and protected against histopathological and ultrastructural damage initiated by isoproterenol.[6] Quantification tests on all the main ingredients showed that the main phenolic contributions came from kaempferol and quercetin glucosides.[7]

Doxorubicin (DOX), which is also known by its brand name Adriamycin, is an effective chemotherapy medication that is extensively utilized for the management of diverse forms of cancer, including breast cancer, leukemia, and lymphoma. It prevents cancer cells from growing and spreading in the body.[8] We administer DOX intravenously, classifying it as an anthracycline antibiotic. It can cause side effects such as nausea, hair loss, fatigue, and an increased risk of infection. Despite these side effects, DOX remains an important and effective tool in the fight against cancer.[9]

This study addresses the research gap regarding medicinal herbs' specific bioactive compounds and health effects, focusing on *M. oleifera*. Cardioprotective properties of *M. oleifera* have been demonstrated in the literature but demand further study on the mechanisms and the potential application. The specific study objectives are to evaluate the antioxidant activity of *M. oleifera* and the effect of *M. oleifera* on the lipid peroxidation of the cardiac cells and histopathological changes caused by DOX and to evaluate the quantification of phenolic content. Through the above objectives, this study aims at producing knowledge related to the antioxidant potential of *M. oleifera* and its protective role against DOX-induced cardiotoxicity and thus may consequently pave the way for its use as an adjunct therapy during the treatment of cancers.

MATERIALS AND METHODS

M. oleifera leaves were harvested from Karbala trees in Iraq during March (2023). The study was completed within 3 months. Ruaa Ali Obeid identified all the samples. The leaves underwent a series of processing steps, including washing, shade drying, and grinding into a fine powder within a week. The powdered leaves were then used to prepare hydro-alcoholic extracts, which were analyzed using high-pressure liquid chromatography and chromatographic techniques to identify their phytochemical composition, including phenolic compounds, flavonoids, alkaloids, and terpenoids.

To obtain the pharmacologically active residues, two extraction methods were employed. In the first method, 100 g of powdered material was steeped in 70% ethanol for 2 days, followed by filtration and vacuum drying. In the second method, 100 g of powdered material was soaked in water for 2 days and then filtered and vacuum dried, yielding a dark green residue with pharmacological potential.

The study involved 42 male albino rats, aged 10–11 weeks, provided by the University of Karbala Faculty of Science. The rats were housed in a controlled environment with temperature and humidity control. They were given access to water and normal food *ad libitum*. Before the investigation began, a 2-week acclimation period was allowed to reduce stress.

Study design

A total of 42 male rats were divided randomly into six groups ($n = 7$ in each group).

1. Control group (Group 1): Rats received a natural diet and water during the whole period of the study
2. DOX group (Group 2; induced group): Rats received 4 mg/kg one time a week for 4 weeks by i.p. route (a cumulative dose of 15 mg/kg)
3. Water *Moringa* extract group (Group 3): Rats received 250 mg/kg daily for 4 weeks
4. Alcoholic *Moringa* extract (Group 4): Rats received 250 mg/kg daily for 4 weeks
5. DOX + water *Moringa* extract group (Group 5): Rats received 250 mg/kg of water *Moringa* extract daily for 4 weeks and 4 mg/kg weekly by i.p.
6. DOX + alcoholic *Moringa* extract group (Group 6): Rats received 250 mg/kg of alcoholic *Moringa* extract daily for 4 weeks and 4 mg/kg once a week for 4 weeks by i.p.

Tissue sample preparation

The hearts were excised, cleaned, weighed, and subsequently divided into apical and basal sections. The basal portion was rinsed with chilled saline to eliminate clots and then stored at −80°C. Upon thawing, each segment was weighed and homogenized in 0.1 M phosphate-buffered saline containing 1% Triton X-100 and a protease inhibitor cocktail, with a ratio of 1:10 (w/v). The homogenate was subjected to centrifugation at 5000 rpm and 4°C for 10 min. The resulting supernatants were subjected to malondialdehyde (MDA), superoxide dismutase (SOD), and total antioxidant capacity (TAC) testing using ELISA and colorimetric assay kits obtained from Elabscience, USA, following the provided guidelines by the manufacturer.

Ethics statement

Ethics Committee (Al-Manara College for Medical Sciences, Al-Amarah, 62001, Iraq), Ref. No.: 341SH Date: May 1, 2023.

RESULTS

It has shown experimental studies using rats with the existence of bioactive compounds with antioxidant activity. The rats that received *M. oleifera* extract showed a significant decrease in MDA levels, indicating a reduction in oxidative damage. The extract also increased the activity of the antioxidant enzyme SOD compared to the DOX control group alone and may suggest a protective role of the *M. oleifera* extract against oxidative damage in the heart.

DOX was tested for its effects on heart tissue oxidative stress, assessing MDA, TAC, and SOD. These levels were initially similar in the control and intervention groups. Compared to the control group, the treatment group had greater MDA and reduced TAC and SOD in cardiac tissue. *Moringa* extract (alcoholic and water) reduced cardiac MDA compared to DOX. The *Moringa* group had considerably increased cardiac TAC and SOD than the DOX group. Unlike the control group, *Moringa*-treated rats had significant changes in TAC and SOD concentrations but not MDA concentrations [Figure 1a-c].

Inflammatory parameters (interleukin-6 and tumor necrosis factor-alpha)

There was no significant change in tumor necrosis factor (TNF)-α and interleukin (IL)-6 levels between the control and *Moringa*-treated groups. DOX therapy substantially increased blood IL-6 and TNF-α levels compared to controls. Animals administered oleifera extracts showed considerably lower cardiac IL-6 and TNF-α levels than those given DOX alone. Compared to control groups, rats treated with extract exhibited substantial alterations in TNF-α and IL-6 levels [Figure 1d and e].

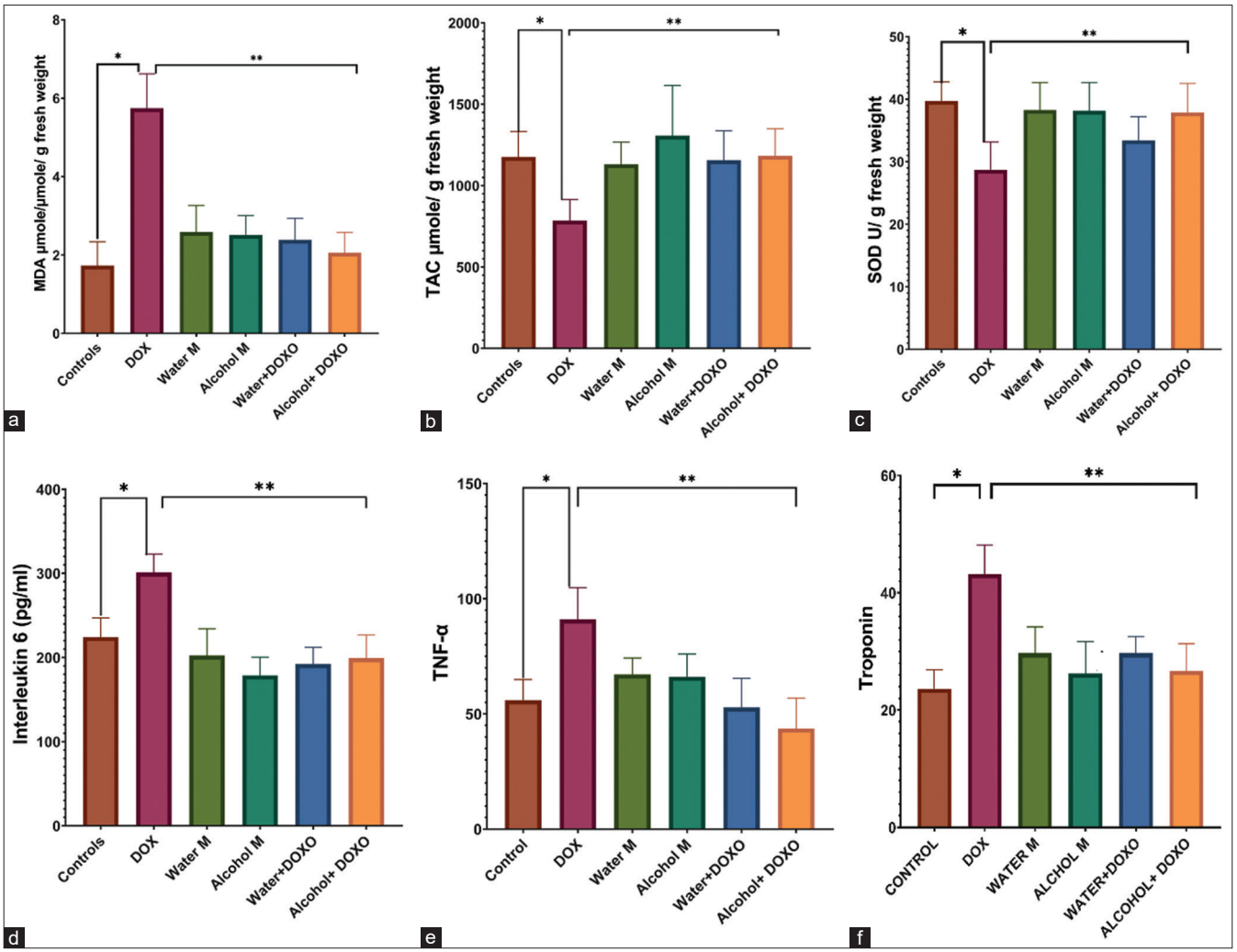

Figure 1: Effect of doxorubicin and *Moringa* extract on the (a) malondialdehyde of the cardiac tissue, (b) the total antioxidant Capacity (TAC), (c) the superoxide dismutase, (d) the (interleukin [IL]-6) levels, (e) the (IL-α) levels, and (f) troponin I (cardiac troponin I). The results of the statistical test using one-way ANOVA. The study found a significant difference ($P < 0.05$) in doxorubicin levels between the control group (*) and the treated group. In addition, there was a significant distinction ($P < 0.05$) across all treatment groups (**). MDA: Malondialdehyde, TAC: Total antioxidant capacity, SOD: Superoxide dismutase, IL: Interleukin, TNF: Tumor necrosis factor

DISCUSSION

The chemotherapy medication DOX treats ovarian, breast, and gastrointestinal cancers. This medicine can cause allergic reactions, cardiac issues, hair loss, decreased blood cell count, nausea, vomiting, and urinary bladder discomfort. DOX causes cardiomyopathy, a serious adverse effect.

Effects of doxorubicin on cardiotoxic indices

Exposure to DOX resulted in cardiomyocyte injury, leading to a substantial elevation in cardiac troponin I (cTnI) levels when compared to the control group.[10] Recent research has reaffirmed the dependability of cTnI as a biomarker for anthracycline-induced cardiotoxicity.[11] DOX treatment increases serum troponin levels, indicating early and late cardiac damage.[12] DOX-induced myocardial injury and elevated blood troponin I activity may be linked to troponin I release from injured cardiac cells. Thus, troponin I detects heart damage.[13]

In this study, we established a mouse model for chronic cardiotoxicity caused by DOX to identify indicators for early cardiac tissue damage. All DOX-treated animals demonstrated considerably higher plasma cardiac troponin T levels than saline-treated controls, suggesting cardiac tissue damage.[14] We are examining troponin results in the remaining groups. DOX group comparisons. A significant change from the DOX group. Significant differences show that *Moringa* hydro and alcoholic extracts affect troponin concentration.

M. oleifera hydro-alcoholic leaf extracts' effects on (cTnI) DOX, an anthracycline antibiotic, and treat pediatric cancers. Sixty percent of treatments use anthracyclines. This study investigates using *M. oleifera* extract to reduce DOX-induced cardiotoxicity. *M. oleifera* therapy significantly improved DOX-induced cardiomyocyte damage. Compared to the DOX group, all *Moringa*-treated rats released less cTnI [Figure 1f].

Previous research has examined *M. oleifera*'s cardioprotective properties. Animal studies showed cardioprotection with numerous changes. These included decreasing blood cardiac biomarkers, enhancing cellular antioxidants and enzymes, minimizing cardiac necrosis, and lowering cardiac lipid peroxidation.[15]

Inflammatory interleukin-6

Immune system messenger cytokine IL-6. It mostly transports macrophages, dendritic cells, lymphocytes, and other inflammatory cells. Chronic hepatitis biomarker IL-6 is inflammatory. Local inflammation stimulates Kupffer cells and hepatocyte development. Hepatocyte cancer may result. Studies have linked some cytokines to heart disease progression. Cytokines may compromise left-ventricular remodeling and heart function. This is "cytokine theory for heart failure."[16] The Dox group showed higher cardiac IL-6 and TNF-β levels than the control group, supporting this idea. A study linked this cytokine surge to oxidative stress, which causes inflammation. For cardiac prevention and therapy, the research emphasizes cytokine monitoring.[17]

Effects of *Moringa oleifera* hydro-alcoholic leaves extracts on (interleukin-6)

M. oleifera extract decreased IL-6 in DOX-treated rats without further therapy [Figure 1d]. *M. oleifera* may prevent DOX-induced cardiotoxicity. Research indicates that *M. oleifera* extract reduces TNF-α and IL-6 levels in rats without DOX treatment, particularly in situations of ischemic brain damage. In rats, reducing proinflammatory markers prevented DOX-induced TNF-a and IL-6 increases. This minimized cerebral ischemia-induced brain damage. The negative effects of its phosphodiesterase inhibitor may explain these results.[18]

Effects of *Moringa oleifera* hydro-alcoholic leaves extracts on (malondialdehyde, superoxide dismutase, and total antioxidant capacity)

The study proves that *M. oleifera* leaves contain numerous antioxidant phytoconstituents. *M. oleifera* leaf extracts are known to contain a range of antioxidant compounds, including phenolic compounds such as flavonoids (such as kaempferol and quercetin), Vitamin C, beta-carotene, and Vitamin E. These phytoconstituents greatly enhance the intracellular antioxidant defenses of the rat testis and epididymis. The researchers attributed MOE's therapeutic efficacy to its flavonoid constituents' antioxidant properties.[19]

The reduction in SOD activity after DOX administration compared to the normal control group may be due to mitochondrial damage and OS reducing enzyme consumption. SOD converts superoxide radicals into hydrogen peroxide (H_2O_2) and prevents hydroxyl radicals from forming. This confirms,[20] which found that DOX treatment decreased SOD activity compared to the control group because of mitochondrial damage and enzyme usage during OS fight. SOD reduces free radicals and inhibits hydroxyl radical generation through Fenton's reaction by converting superoxide radicals to H_2O_2.

Bioactive substances including quercetin, kaempferol, isothiocyanates, and saponins provide the extract's antioxidant properties. Various bioactive substances scavenge free radicals, decrease oxidative stress, suppress inflammatory pathways, and activate antioxidant enzymes including SOD, catalase (CAT), and glutathione peroxidase (GPx). Saponins regulate cholesterol metabolism, isothiocyanates neutralize ROS, and phenolic substances suppress proinflammatory enzymes. *Moringa* extract reduces ROS to preserve cell homeostasis. The processes

and interactions of each bioactive component regarding *Moringa*'s antioxidant benefits require more study.

The present research uses two methods to reduce rat antioxidant status fluctuations. First, rats of the same strain and age were chosen to reduce genetic and age-related antioxidant status variations. This enables a more uniform study start. Second, researchers assess rats' antioxidant levels before the study. Biomarkers such as antioxidant enzymes (GPx, CAT, and SOD) and compounds (Vitamin C, Vitamin E, and glutathione) may be assessed. These assessments use each rat's baseline antioxidant levels to categorize or adjust statistically. These methods account for antioxidant status and improve research dependability.

CONCLUSION

The research found antioxidant phytochemicals in Iraqi hydro-alcoholic *M. oleifera* leaf extracts. To prevent DOX-induced cardiotoxicity, these extracts lower oxidative stress and improve antioxidant defenses. The data suggest that *M. oleifera* may be an antioxidant supplement for DOX-based chemotherapy patients. Research is needed to understand mechanisms and confirm results in clinical settings. The phytochemical research assessed Iraqi *M. oleifera* hydro-alcoholic leaf extracts' antioxidant properties on DOX-induced cardiotoxicity in male rats.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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