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Moringa Oleifera Ameliorates Cardiotoxicity and Improves Antioxidants in Breast Cancer- induced Rats Treated with Doxorubicin: A Preliminary Study

Rehab A.M. Gouda*, Abeer G. Ali Hassan, Yasmina K. Mahmoud, Ibrahim A. Ibrahim

Department of Biochemistry, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.

***Correspondence**
Corresponding author: Rehab A.M. Gouda
E-mail address:rehab.2021_psg@vet.suez.edu.eg

Abstract

Moringa Oleifera (MO) is a miracle plant of huge medical significance. It could be used to suppress the aggressiveness of various tumors as well as ameliorate the consequences of chemotherapeutic agents. In the present study, 49 albino rats were used to evaluate the effect of MO nanoparticles (MONPs) in DMBA-induced breast cancer rats (BC-induced) treated with doxorubicin (DOX). Cardiotoxicity, antioxidant markers, and protein profile were evaluated. Serum and mammary glands samples were collected for both biochemical and histopathological examinations. Rats were classified into control and BC-induced rats. The last group was further divided into 6 groups to evaluate the synergistic and prophylactic effects of MONPs. There was a significant reduction in the levels of tumor, and cardiotoxicity markers with a significant increase in the antioxidants/oxidants and proteins profile in BC-induced rats treated synergistically or/and prophylactically with MONPs and DOX. In conclusion, the prophylactic use of MONPs and synergistic use of MONPs and DOX induce a magnificent resistance against cardiotoxicity induced by doxorubicin and ameliorate the aggressiveness of breast cancer as well as the oxidative stress induced in rats.

KEYWORDS
Antioxidants, Cardiotoxicity, *Moringa Oleifera*, Nanoparticles, Tumor markers

INTRODUCTION

Moringa Oleifera (MO) is a known plant called the "miracle tree" has gained huge importance in present years due to its potential medicinal applications. Traditionally, the plant has been used for centuries in Ayurvedic medicine for its huge health benefits (Pareek *et al*., 2023). The approved therapeutic potentialities of MO include antioxidant, anti-inflammatory, antimicrobial, antidiabetic, anticancer, antianemia and hepatoprotective (Jikah and Edo, 2023; Rotella *et al*., 2023). Along with its various therapeutic potentials; MO has a great nutritive value as it contains high quantities of vitamins "A, C, and E" and various minerals with high content of "Ca and Fe" (Trigo *et al*., 2023). Cancer -a complex and devastating disease- has accounted as one of the highest causes of mortality worldwide. Its impact is felt not only by individuals and their families but also by societies and healthcare systems globally (Bray *et al*., 2018). This staggering figure emphasizes the widespread nature of cancer's impact on populations around the world. Furthermore, it is expected that by 2040, the number of new cancer patients will rise to over 29 million annually (Bray *et al*., 2018). These projections indicate a significant increase in cancer incidence and highlight the urgent need for effective prevention strategies and improved treatment options. Breast cancer (BC) is a prevalent and life-threatening disease that affects millions worldwide. Over the years, extensive research has been conducted to better understand the causes, risk factors, and treatment options for breast cancer (Eliassen *et al*., 2010). Doxorubicin (DOX) is widely used globally as a standard treatment option for various cancers. It has been included in the list of WHO as an essential medicine since 1985 (Speth *et al*., 1988). DOX is commonly and widely included in the treatment of BC at different stages (early-stage or metastatic). Its usage may depend on tumor characteristics, patient age, overall health status, and treatment guidelines specific to each country. However, its clinical usage is often restricted due to its resistance and its adverse consequences on normal tissues like cardiotoxicity, hepatotoxicity, and nephrotoxicity. These are the major challenges facing its wide use as an anticancer agent (Carvalho *et al*., 2009; Pugazhendhi *et al*., 2018; Stevens *et al*., 2023; Chen *et al*., 2023; Lykhova *et al*., 2023). Multiple formulations have emerged to overcome the deleterious effects of DOX; like Nano formulations (Sarkar *et al*., 2023) and liposomal formulations (Aloss and Hamar, 2023). One area of particular interest is the potential protective effect of moringa against DOX toxicity. Several studies have investigated the potential role of moringa in mitigating DOX-induced toxicity. These studies have demonstrated that MO extracts or their bioactive components possess remarkable anti-inflammatory as well as antioxidant activities helping in declining the oxidative stress (OS) and inflammation contributed by DOX (Quagliariello *et al*., 2022; Ranote *et al*., 2022; Patintingan *et al*., 2023). Although, the preclinical studies have approved promising protective effects of MO against DOX toxicity, further investigations are needed to establish its safety and efficacy in many experimental models. Clinical trials evaluating the potential

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benefits of MO supplementation alongside DOX treatment are warranted to determine its optimal dosage and duration. Recently, Nano formulations of MO (preparation of moringa extracts or compounds at the nanoscale level, typically ranging from 1 to 100 nanometers) have been explored to enhance their bioavailability and therapeutic efficacy (Abd-Rabou *et al*., 2016). One significant advantage of Nano formulations is their ability to improve the solubility and stability of bioactive compounds found in moringa (Alven *et al*., 2021). Furthermore, Nano formulations can also enhance the targeted delivery of moringa's bioactive constituents to specific cells or tissues (Fong *et al*., 2022). However, *Moringa Oleifera* nanoparticles (MONPs) hold great promise in enhancing its medicinal properties and therapeutic applications, further investigations are still required to understand the long-term effects of these Nano formulations. This gives us the impetus to fill gaps and continue to study the biochemical and molecular biological properties of MO Nano formulations in cancer biology. In the present study, we aimed to spot the light on the protective effect of MONPs on the deleterious consequences of DOX in a BC-induced rats model through evaluation the efficacy of MONPs to alleviate the cardiotoxic effect of DOX along with evaluation of its ability to potentiate DOX therapeutic effect and antioxidants in affected rats.

MATERIALS AND METHODS

Animals

Forty-nine female albino rats of weight ranged 200-250 g. The rats were accommodated in the experimental conditions for 21 days before starting the experimental procedures. They were kept in stainless steel cages and allowed to free access to standard diet and water ad-libitum with an ambient environment with a temperature range of 21-25°C, a relative humidity range of 55-60%, and a 12-hour light-dark cycle. The experimental work was approved by the Institutional Animal Care and Use Committees Suez Canal University (SCU-IACUC).

Chemicals

Doxorubicin (>99% purity) (Sigma-Aldrich, Louis, MO, USA) and DMBA (Santa Cruz Biotechnology, Inc., Dallas, Texas, catalog # 57–97-6) have been used in the given experiment.

Synthesis and characterization of M. Oleifera Nanoparticles (MONPs)

Preparation of aqueous extract

Green and healthy leaves of MO were collected. The collected leaves were washed with water four times and dried by using a clean and sterile filter paper then the unneeded parts of the leaves were excluded. After drying, leaves are ground in a grinder (WAHL, USA) and then stored in specialized sealed containers until its use. About 150 g of the leaves powder was mixed with a 1000 mL of distilled water, and then warmed for 120 minutes at 50°C, then was centrifugated in an ordinary centrifuge (Hettich, Germany). The upper supernatant was collected in a rotary evaporator (IKA, England) to collect the dry matter and used to formulate the nanosolution (Ali-Shtayeh and Abu Ghdeib, 1999).

Synthesis of *M. Oleifera* nanoparticles (MONPs)

One-pot green synthesis method was applied for MONPs synthesis. 90 mL of 1 mM silver nitrate were added to 10 mL 1% MLAE which incubated for nine hours in an incubator at 60°C with regular agitation. The MONPs formation was monitored by UV-Vis spectrometry. The MONPs were collected by centrifuging at 6500 rpm for 35 minutes, then particles were freeze-dried to obtain the dried form of MONPS. Characterization of MONPs were applied by scanning electron microscope (Leo Supra 50 VP, Zeiss) and transmission electron microscope (Libra 120 electron microscope, Zeiss). The given protocol was according to Medda *et al*. (2014) with modifications. The characterization criteria for MONPs have been applied with modifications according to Mohammed and Hawar (2022).

Induction of breast cancer

Breast cancer was induced in experimental rats by a fresh single dose of 20 mg/mL of DMBA diluted in oil was administered per os using gavage according to the technique prescribed by Liu *et al*. (2017). Rats were monitored weekly from 4th week after DMBA administration, to check BC progress. The first observed tumor growth was after the 10th week, whereas by the 14th week, the tumor growth was observed in all induced rats (Figure 1).

Treatment with Doxorubicin

All experimental rats were subjected to fasting overnight with allowing for free access to drink water before beginning the treatment. A dose of DOX equals 11.5 mg/kg was intravenously administrated every week according to Wu *et al*. (2021).

Experimental design

Animals were divided into 7 groups, each group containing 7 rats, and these groups were divided into two main groups healthy animals and breast cancer-induced rats. Groups were treated orally using a metallic stomach tube daily for the all period of the experimental design. The main groups were divided randomly as follows: Healthy rats; group 1; Negative control group, animals were fed with standard food pellets and tap water and did not receive any type of treatment. Breast cancer-induced rats; were the remaining six groups as the following; group 2; breast cancer-induced rats did not receive any treatments, Group 3; Breast cancer-induced rats treated with DOX for 30 days, Group 4; Breast cancer-induced rats treated with DOX plus (MONPs) for 30 days, group 5; Breast cancer-induced rats received (MONPs) for 30 days before induction of cancer, Group 6; Breast cancer-induced rats received (MONPs) for 30 days before induction of cancer then received DOX after induction of breast cancer for 30 days and Group 7; Breast cancer-induced rats received (MONPs) 30 days before induction of breast cancer then received DOX plus MONPs for 30 days after induction of breast cancer.

Sampling

Blood samples were aspirated from the orbital venous plexus, left for coagulation in plain tubes, and then centrifuged at 3,500 rpm for 15 minutes for separation of serum samples. The serum samples were preserved at -20°C until used for biochemical determinations. The protocol for blood samples collection, preparation, and storage was applied according to Zheng and Cai (2019).

Mammary glands samples were taken from 4 glands and were used for histopathological examination according to Lee *et al*. (2015).

Histopathological examination

The entire mammary glands were dissected and flushed with chilled isotonic saline, samples representing all glands parts were fixed in 10% formalin solution and then imbedded in paraffin. All histopathological examinations were performed on 5-µm sections cut on a rotary microtome and detected onto the microscope. Hematoxylin and eosin staining technique was performed on mammary glands sections. The protocol was applied according to Lee *et al*. (2015) with modifications.

Biochemical determinations

Tumor markers: Cancer antigen 15-3 (CA 15-3), carcinoembryonic antigen (CEA), and cancer antigen 27-29 (CA 27-29) have been determined according to the manufacturer's instructions of ELISA Kit (LifeSpan BioSciences, Seattle, WA, USA). Cardiac biomarkers: cardiac troponin T (cTnT) and cardiac troponin I (cTnI) have been determined according to the manufacturer's instructions of Roche Diagnostics GmbH (Sandhofer Strasse 116, D-68305 Mannheim, Canada) with references 07007302 and 05094810 respectively. While creatine kinase-MB (CK-MB), aspartate transaminase (AST), and lactate dehydrogenase (LDH) have been determined according to the manufacturer's instructions of (BIOLABO, Les Hautes Rives 02160 Maizy, France) with references 97217, 92025, and 92111 respectively. Antioxidants and oxidants markers (Reduced glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA)) have been determined according to manufacturer's instructions of (Northwest life science specialties, Vancouver, USA) with product keys NWK-GSH01-1, NWK-SOD02, and NWK-MDA01 respectively. Serum proteins profile (Total proteins, Albumin, and globulins) has been determined according to the manufacturer's instructions of (BIOLABO, Les Hautes Rives 02160 Maizy, France) with references LP87016, 80002, and 098100 respectively.

Statistical analysis

The data were presented as mean ± SEM. One-way ANOVA was applied to make a statistical comparison among the obtained means of the data among experimental groups. Duncan's test was used as a post hoc test to figure out the significant differences among groups. SPSS version 28 was chosen for statistical analysis. A significance level of $P < 0.05$ was considered statistically significant.

RESULTS

Histopathology

Control group (group 1) rats' mammary glands (A) showed normal structures of cuboidal epithelial lining glandular tissue between adipose tissue. BC-induced non-treated group (group 2) (B) revealed trabecular pattern adenocarcinoma which is represented by hypercellularity, and pleomorphism of epithelial lining glands with mitotic figures. Moreover, some malignant nodules exhibited the presence of central necrosis. Group 3; (C) demonstrated coagulative necrosis of a large number of neoplastic glandular tissue besides the presence of fibrotic bands between destructed glands. Group 4; (D) showed intra-epithelial lymphocytic aggregates within proliferated glandular epithelium adjacent to apparent normal glands. Groups 5-7; (E) revealed regeneration of acini and duct in between adipose tissue (Figure 1).

Biochemical effect of MONPs on tumor markers in DMBA-induced breast cancer rats

Serum levels of the examined tumor markers; CA15-3, CEA, and CA27-29 were significantly declined in all rats treated with DOX or MONPs. Furthermore, the decline in tumor markers levels in groups synergistically treated with DOX and MONPs was clearer and more significant. The prophylactic effect of MONPs was significantly observed in groups treated with MONPs before the induction of breast cancer when compared with other groups. While there was no clear significant difference between the groups treated prophylactically with MONPs and then treated with MONPs or/and DOX (Table 1).

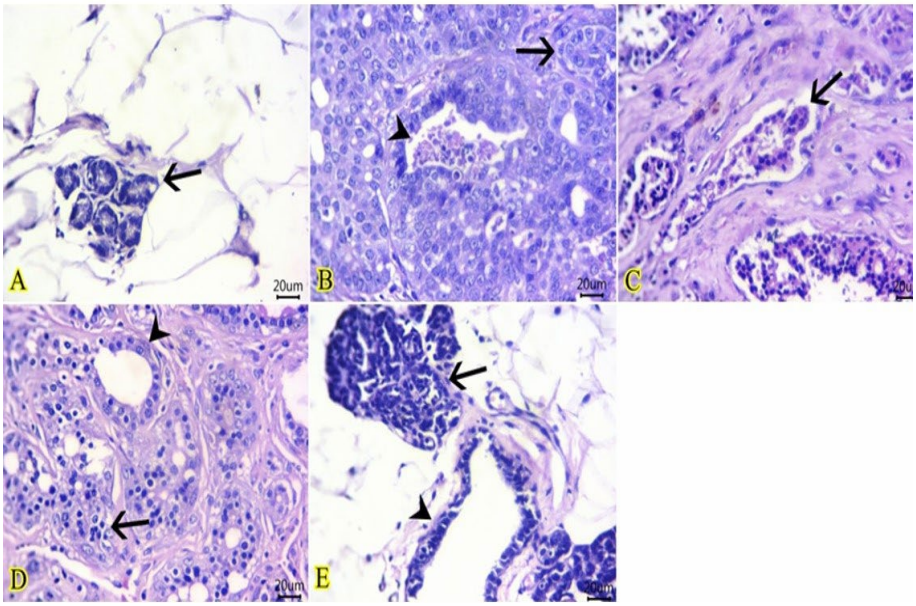

Figure 1. Photomicrograph of H&E stained sections of mammary glands from rats showing (A) normal structures of cuboidal epithelial lining glandular tissue (arrow). (B) trabecular pattern adenocarcinoma with hypercellularity, pleomorphism of epithelial lining glands (arrow), and central necrosis in some nodules (arrowhead). (C) coagulative necrosis of a large number of neoplastic glandular tissue (arrow). (D) intra epithelial lymphocytic aggregates within proliferated glandular epithelium (arrow) adjacent to apparent normal glands (arrowhead). (E) regeneration of acini (arrow) and duct (arrowhead) in between adipose tissue Scale bar 20µm.

Table 1. Biochemical effect of MONPs on tumor markers in DMBA-induced breast cancer rats.

Groups	CA 15-3 (U/ml)	CEA (ng/ml)	CA 27-29 (U/ml)
Group 1	26.23±1.15^{c}	3.19±0.55^{c}	29.7.03±2.82^{b}
Group 2	48.46±4.515^{a}	6.15±1.34^{a}	57.33±2.03^{a}
Group 3	33.23±3.15^{b}	4.17±0.194^{b}	30.33±1.88^{bc}
Group 4	24.23±2.12^{c}	3.20±0.170^{c}	28.33±2.03^{b}
Group 5	23.23±1.13^{c}	3.01±0.38^{c}	27.26±2.05^{b}
Group 6	20.64±1.15^{d}	2.94±0.35^{d}	25.07±2.17^{bc}
Group 7	20.18±1.05^{d}	2.823±1.15^{d}	21.03±1.67^{bc}

Biochemical effect of MONPs on cardiac markers in DMBA-induced breast cancer rats

Serum levels of the examined cardiac markers; cardiac troponins (cTnT and I), CK-MB, AST, and LDH were significantly elevated in DOX treated in BC-induced rats when compared with other groups. Furthermore, there was a significant decline in the examined markers in synergistically and/or prophylactically treated groups with MONPs or/and DOX. While there was no clear significant difference between the groups treated prophylactically with MONPs and then treated with MONPs or/and DOX (Table 2).

Biochemical effect of MONPs on antioxidants/oxidants in DMBA-induced breast cancer rats

Serum levels of reduced glutathione (GSH) and superoxide dismutase (SOD) were significantly elevated in BC-induced rats which were synergistically or prophylactically treated with MONPs when compared with non-treated BC-induced rats. The serum levels of malondialdehyde (MDA) were significantly declined in BC-induced rats which were synergistically or prophylactically treated with MONPs and DOX (Table 3).

Biochemical effect of MONPs on serum proteins in DMBA-induced breast cancer rat

Serum levels of total proteins, albumin, and globulins were significantly declined in non-treated BC-induced rats when compared with other groups. Treated rats showed non-significant elevation in serum levels of total proteins, albumin, and globulins. Treatment with MONPs with DOX synergistically or prophylactically retains the A/G ratio in BC-treated groups when compared with the non-treated ones (Table 4).

Table 2. Biochemical effect of MONPs on cardiac markers in DMBA-induced breast cancer rats.

Group	cTn I (ng/ml)	cTn T (Pg/ml)	CK-MB (U/L)	AST (U/ml)	LDH (U/L)
Group 1	0.65±0.15^{d}	42.81±5.42^{d}	184.45±9.45^{d}	151.44±11.38^{d}	320.50±8.50^{d}
Group 2	1.29±0.11^{b}	182.90±9.70^{b}	683.20±18.70^{b}	411.20±18.60^{b}	511.90±26.50^{b}
Group 3	1.83±0.18^{a}	226.80±21.8^{a}	862.80±19.60^{a}	521.33±19.83^{a}	809.80±15.50^{a}
Group 4	1.26±0.09^{b}	167.60±11.60^{b}	582.50±9.10^{b}	378.54±16.1^{bc}	491.60±16.50^{b}
Group 5	0.94±0.13^{c}	88.40±7.50^{c}	312.80±10.60^{c}	225.28±13.53^{bc}	403.40±12.70^{c}
Group 6	0.79±0.023^{cd}	65.60±5.65^{d}	248.62±10.04^{c}	188.36±12.45^{bcd}	361.95±8.970^{c}
Group 7	0.68±0.123^{cd}	54.20±5.52^{d}	216.53±9.95^{cd}	169.90±9.45^{bcd}	341.23±9.05^{cd}

Table 3. Biochemical effect of MONPs on antioxidants/oxidants in DMBA-induced breast cancer rats.

Group	GSH (mmol/L)	SOD (U/ml)	MDA (µmol/L)
Group 1	3.99±0.16^{a}	5.95±0.16^{a}	21.03±1.82^{bc}
Group 2	1.80±0.06^{d}	2.42±0.17^{d}	27.33±2.03^{a}
Group 3	1.98±0.06^{c}	3.17±0.19^{c}	24.18±2.01^{a}
Group 4	2.08±0.06^{c}	4.20±0.17^{b}	22.61±1.92^{b}
Group 5	2.30±0.09^{b}	4.80±0.38^{b}	21.81±1.87^{b}
Group 6	2.58±0.08^{b}	4.94±0.35^{b}	21.42±1.84^{bc}
Group 7	2.85±0.09^{ab}	5.25±0.16^{a}	21.22±2.07^{bc}

Table 4. Biochemical effect of MONPs on serum proteins in DMBA-induced breast cancer rat.

Group	Total proteins (g/dl)	Albumin (g/dl)	Globulins (g/dl)	A/G ratio
Group 1	7.90±0.23^{a}	4.60±0.3^{a}	3.30±0.82^{c}	1.39±0.08^{ab}
Group 2	5.60±0.29^{a}	2.20±0.17^{d}	3.40±0.93^{a}	0.65±0.03^{d}
Group 3	6.03±0.13^{ab}	3.17±0.19^{a}	2.86±0.88^{bc}	1.10±0.09^{b}
Group 4	6.87±0.38^{c}	3.20±0.17^{d}	3.67±0.83^{bc}	0.87±0.05^{c}
Group 5	7.01±0.44^{b}	4.01±0.38^{a}	3.00±0.85^{bc}	1.34±0.05^{ab}
Group 6	7.30±0.25^{ab}	4.40±0.35^{b}	2.90±0.87^{bc}	1.52±0.17^{a}
Group 7	7.40±0.25^{ab}	4.60±0.31^{a}	2.70±0.77^{bc}	1.70±0.03^{a}

DISCUSSION

In the present study, the prophylactic or/and synergistic use of MONPs and DOX was applied in BC-induced rats. Also, the ameliorative effect of MONPs on the cardiotoxicity resulted from DOX use has been lightened. In the present study, tumor markers have been used to monitor BC progression and to evaluate the possible synergistic and prophylactic use of MONPs with DOX. As well as histological sections have been examined to evaluate the progression of tumors in rats' mammary glands and the influences of both MONPs and DOX (Figure 1). It has been recorded that the consumption of MO lead to a marked reduction in the progress of multiple types of cancers including BC (Imran *et al*., 2019). Records that approved this concept revealed the anticancer activity of MO to its ability to increase the antioxidant capacity, anti-inflammatory effect, immune system support, nutritional value, and its contents of anti-tumor compounds (Moremane *et al*., 2023, Lim *et al*., 2020). CA 15-3, CEA, and CA 27-29 are common tumor markers used to monitor the progression of BC (Seale and Tkaczuk, 2022). CA 15-3 is commonly used as a tumor marker for breast cancer; its elevated serum level suggested the presence of breast cancer or its progression (AlGhamdi *et al*., 2023). In the present study, BC-induced non-treated rats have the highest CA 15-3 levels with a mean of 48.46 U/ml, followed closely by BC-induced rats treated with DOX only (Group 3) (33.23 U/ml). However, groups that treated prophylactically and synergistically with MONPs and DOX (groups 6 and 7) have the lowest CA 15-3 levels, with means of 20.64 U/ml and 20.18 U/ml, respectively. The higher levels in groups 2 and 3 might indicate more advanced

stages or more aggressive forms of breast cancer within those groups. On the other hand, CEA serum level was used as a potent marker for progression of BC (Wang *et al*., 2017). Its high serum level indicates a high cancer progression. Similar to CA 15-3, the higher levels in Groups 2 and 3 may indicate more advanced or aggressive forms of cancer within those groups. The 3rd tumor marker was CA-27-29; Group 2 has the highest CA 27-29 levels with a mean of 57.33 U/ml, followed by Group 3 (30.33 U/ml). Groups 6 and 7 have the lowest CA 27-29 levels, with means of 25.07 U/ml and 21.03 U/ml, respectively. CA 27-29 was primarily used as a tumor marker for breast cancer, its elevated levels can indicate the presence or progression of breast cancer, and it could be used for diagnosis, management, and classification of BC (Zubair *et al*., 2020, Beveridge, 1999). Similarly, to CA 15-3 and CEA; Groups 2 and 3 showed higher levels, suggesting more aggressive or advanced forms of BC. To the best of our knowledge, we are the first record for the effect of MONPs on the serum level of CA 15-3, CEA, and CA 27-29 in BC-induced models. Cardiac markers have been determined to evaluate the ameliorative effect of MONPs on DOX-induced cardiotoxicity in treated BC-induced rats. It has been known that the use of DOX as a chemotherapeutic agent in cancerous disease could lead to cardiotoxicity with cardiac dilatation and heart failure (Wu *et al*., 2023a). There were many records supported our concept regarding the potentiality of MO to reduce DOX-induced cardiotoxicity (Cheraghi *et al*., 2017; Quagliariello *et al*., 2022; Patintingan *et al*., 2023). Cardiac troponin I and T are widely accepted as the gold standard biomarkers for diagnosing of cardiotoxicity (Park and Jaffe, 2017; Mahmood *et al*., 2018; Dhingra *et al*., 2022). These markers have been extensively discussed in guidelines from organizations such as the American College of Cardiology (ACC) and the European Society of Cardiology (ESC). Both troponins have been approved as potent markers for DOX-induced cardiotoxicity progression (Prayogo *et al*., 2022; Dovganych *et al*., 2022). In the present study, group 3 has the highest cTn I level with a mean of 1.83 ng/ml, followed by group 2 (1.29 ng/ml) while group 7 has the lowest cTn I level with a mean of 0.68 ng/ml which explain the high ameliorative effect of MONPs. Elevated cTn I levels, especially in group 3, could indicate significant cardiac damage related to DOX therapy (Muneer *et al*., 2022). Similar to cTn I, Group 3 has the highest cTn T levels with a mean of 226.80 pg/ml, followed by Group 2 (182.9 pg/ml). Group 7 has the lowest cTn T levels with a mean of 54.20 pg/ml. the obtained results emphasized the DOX-induced cardiotoxicity and, at the same time approved the ameliorative effect of MONPs and cardioprotective agents against bad consequences of DOX therapy. Creatine kinase-MB (CK-MB), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) are also well-established markers used in diagnosing and monitoring cardiac conditions (Naderi *et al*., 2023). CK-MB is a cardiac-specific enzyme, and elevated levels can indicate myocardial damage. Group 3 has the highest CK-MB levels with a mean of 862.8 U/L, followed by Group 2 (683.2 U/L) while Group 7 has the lowest CK-MB levels with a mean of 216.53 U/L. The highest levels in Groups 2 and 3 are consistent with severe cardiac stress or injury. Group 3 has the highest AST levels with a mean of 521.33 U/ml, followed by Group 2 (411.2 U/ml). Group 7 has the lowest AST levels with a mean of 169.9 U/ml. AST is an enzyme found in heart and liver cells. Elevated AST levels, especially in Groups 2 and 3, can be indicative of cardiac damage. It has been recorded that MO could retain high levels of AST in many experimental models (Igwilo *et al*., 2013; Aliyu *et al*., 2021; Ibrahim Salih *et al*., 2022; Luo *et al*., 2022). Group 3 has the highest LDH levels with a mean of 809.8 U/L, followed by Group 2 (511.9 U/L). Group 7 has the lowest LDH levels with a mean of 341.225 U/L. LDH is a nonspecific marker of tissue damage, and elevated levels can be seen in various cardiac and non-cardiac conditions. The highest levels in Groups 2 and 3 suggest substantial tissue damage, including cardiac tissue. In the same line with AST results; It has been recorded that MO could retain high levels of LDH in many experimental models (Balakrishnan *et al*., 2019; Ndlovu *et al*., 2023; Patintingan *et al*., 2023). The obtained data suggested that Groups 2 and 3 are experiencing more severe cardiac stress or damage, as indicated by elevated levels of these cardiac biomarkers, while Group 7 has the least cardiac stress or damage among the groups. These findings may emphasize the potential use of MONPs as cardioprotective agents. The correlation between BC and induction of oxidative stress (OS) has been studied; the majority of data indicated high OS generation in BC conditions (Jelic *et al*., 2021, Wu *et al*., 2023b, Liu *et al*., 2023, Ghafoor, 2023). The provided data represents the levels of three biochemical markers: GSH (glutathione), SOD (superoxide dismutase), and MDA (malondialdehyde) across seven different experimental groups which have been used to evaluate the possible antioxidant effect of MONPs and its ability to reduce the OS-induced by BC. GSH is a crucial endogenous antioxidant that plays a vital role in protecting cells from oxidative damage (Lu, 2013). Control rats (Group 1) have the highest GSH levels with a mean of 3.99 mmol/L, followed by Group 7 (2.85 mmol/L). While Group 2 has the lowest GSH levels with a mean of 1.80 mmol/L. low levels of GSH, as seen in Group 2, suggest a potential impairment in the body's antioxidant defense mechanisms resulted from BC which was approved by Wu *et al*. (2023b). SOD is an enzyme that catalyzes the breakdown of superoxide radicals, a harmful product of oxidative stress (McCord and Edeas, 2005). Group 1 has the highest SOD levels with a mean of 5.95 U/ml, followed by Group 7 (5.25 U/ml). Group 2 has the lowest SOD levels with a mean of 2.42 U/ml. Higher levels of SOD, as observed in Group 1 and Group 7, may indicate a better ability of MONPs to counteract OS. MDA is a marker of lipid peroxidation and oxidative damage (Gawel *et al*., 2004). Group 2 has the highest MDA levels with a mean of 27.33 µmol/L, followed by Group 3 (24.18 µmol/L). Group 1 has the lowest MDA levels with a mean of 21.03 µmol/L. Elevated MDA levels, as seen in Group 2, suggest increased oxidative stress and potential damage to cellular structures. The obtained data suggested that Group 2 is experiencing higher OS, as indicated by lower GSH levels and higher MDA levels this may be due to the influence of BC. In contrast, Group 1 and Group 7 seem to have more robust antioxidant defenses, as seen by higher GSH and SOD levels. These findings may have to emphasize the high ability of MONPs to reduce OS which has been approved in many studies (Ercan *et al*., 2021; Ceci *et al*., 2022; Faheem *et al*., 2022; Moremane *et al*., 2023). Total proteome could be used to assess the progression and monitor BC cases (Sinha *et al*., 2023). Total protein levels can be influenced by various factors, including nutritional status, liver function, and inflammation (Schreiber *et al*., 1971; Barle *et al*., 1997; Tabata *et al*., 2023). In the present study, Group 7 has the highest total protein levels with a mean of 7.4 g/dl, followed by Group 6 (7.3 g/dl). Group 2 has the lowest total protein levels with a mean of 5.6 g/dl. Elevated levels, as seen in Groups 6 and 7, may suggest good nutritional and overall health status induced by MONPs administration (Falowo *et al*., 2018, Teixeira *et al*., 2014). Group 1 has the highest albumin levels with a mean of 4.6 g/dl, followed by Group 7 (4.6 g/dl) and Group 5 (4.01 g/dl). Group 2 has the lowest albumin levels with a mean of 2.2 g/dl. Albumin is primarily synthesized by the liver and is an important indicator of liver function and nutritional status (Lala *et al*., 2023). Low levels, as seen in Group 2, may suggest liver dysfunction or malnutrition. Group 4 has the highest globulin levels with a mean of 3.67 g/dl, followed by Group 3 (2.86 g/dl). Group 2 has the lowest globulin levels with a mean of 3.4 g/dl. Globulins are proteins that include antibodies and other components of the immune system (Finlayson, 1979). Elevated levels, as seen in Groups 4 and 3, may suggest an immune response or inflammation resulted from BC induction. Group 7 has the highest A/G ratio with a mean of 1.70, followed by Group 6 (1.517). Group 2 has the lowest A/G ratio with a mean of 0.647. The A/G ratio is used to assess the balance between albumin and globulins in the blood. A low ratio, as seen in Group 2, may suggest an imbalance in protein composition and could be indicative of certain medical conditions. The high level of A/G ratio in MONPs treated group may be due to the robust effect of MONPs which has been approved previously by Monir *et al*. (2020). The obtained data sug-

gested that Groups 6 and 7 have higher total protein levels and A/G ratios, indicating good nutritional status and a balanced albumin-to-globulin ratio. Group 2, on the other hand, exhibits lower total protein and albumin levels, suggesting potential nutritional deficiencies or liver dysfunction. Groups 3, 4, and 5 fall within intermediate ranges for these parameters. These findings may be used as markers for assessing the health and nutritional effects of MONPs in BC-induced rats.

CONCLUSION

Synergistic or/and prophylactic use of MONPs with doxorubicin reduce the aggressiveness of breast cancer, ameliorate the cardiotoxicity induced by DOX treatment, reduce the oxidative stress, and improve antioxidants and protein profile in breast cancer-induced rats.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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