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Research Article

Ameliorative Effect of Almond Oil Against Doxorubicin-Induced Cardiotoxicity in Mice Via Downregulation of TLR4 Gene Expression, Lowering NF-κB and TNF-α Levels

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Abstract | Doxorubicin is a chemotherapeutic drug broadly used for the treatment of a wide range of malignancies. Many studies have shown that natural compounds derived from plants have medicinal and antioxidant properties. The purpose of the current investigation was to investigate if almond oil could protect male mice against doxorubicin-induced cardiotoxicity. The experimental mice were divided into three groups; control group: received 0.9 percent saline, doxorubicin group: Mice were intraperitoneal injected with doxorubicin (5 mg/kg) ithree times over a period of two weeks (dose every five days) and almond oil group: Mice were administered almond oil orally (2.26 g/kg) using oral gavage daily over a period of three weeks, one week prior to the doxorubicin injection and two weeks along with doxorubicin injection. The effects and mechanisms of doxorubicin on superoxide dismutase activities were elucidated by molecular docking studies. Treatment with almond oil attenuated lipid peroxidation, improved superoxide dismutase (SOD) activities that are associated with doxorubicin administration. Also almond oil considerably modulated the gene expression of toll like receptor 4 (TLR4) and lowers the serum levels of both nuclear factor κB (NF-κB) and tumor necrosis factor α (TNF-α). The elevated levels of Creatine Kinase - MB (CK-MB), Lactate Dehydrogenase (LDH), and Troponin-1 induced by doxorubicin injection were neutralized by almond oil supplementation. Almond oil ameliorated all the histological alterations caused by doxorubicin. The administration of almond oil with doxorubicin modulated the doxorubicin-induced changes in serum and cardiac tissue in mice due to its anti-inflammatory and antioxidant properties.

Keywords | Doxorubicin, Medicinal plant, Transmembrane receptors, Heart toxicity, Oxidative stress, Cytokines

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INTRODUCTION

Doxorubicin is an anthracycline antibiotic. It is also known as adriamycin. Doxorubicin is commonly used in the chemotherapy of breast cancer, lung cancer, leukemia and solid tumors (Xinyong et al., 2020). This cardiotoxicity has serious implications, resulting in a bad prognosis and deaths for up to 61% of patients (Wouters et al., 2005).

Previous studies stated that the doxorubicin induced cardiac toxicity involves generation of huge amounts of reactive oxygen and reactive nitrogen species (ROS and RNS) which can obviously cause cardiac toxicity (Gilliam and

Clair, 2011). Also doxorubicin promotes the peroxidation of phospholipids in heart and respiratory chain uncoupling which resulting in cardiac mitochondrial metabolic dysfunction and cell death (Koleini et al., 2019).

Toll-like receptors (TLRs) are a type of transmembrane receptors resembling those encoded by the Drosophila Toll gene revealed by Medzhitov et al. (1997). Previous investigations have exhibited that TLRs are also expressed in cardiac myocytes and cardiovascular endothelial cells (Zhang et al., 2015). TLRs are conservative transmembrane receptor proteins, that include extracellular, transmembrane, and intracellular toll interleukin receptor (TIR) proteins. They are all type I transmembrane receptors (Xinyong et al., 2020). TLR4 is a component of the innate immunity and initiate inflammatory response by promoting nuclear factor kappa B (NF-κB) (Khames et al., 2019). Subsequently, several studies have explained that TLR4-mediated signalling pathways are implicated in myocardial injury. Remarkably, treatment of mice with TLR4 antagonist considerably reduced the myocardial infarction (Shimamoto et al., 2006).

Herbal medicines, stemmed from plant extracts, have received great care as complementary therapy or alternative medicines. Herbal medicines are being consumed to treat a wide diversity of clinical diseases (Gupta et al., 2004). Some natural compounds having antioxidant characteristics may help to reduce the partial or total oxidative stress and can play an important role in maintaining health when taken as a supplement or ingested as part of a normal diet (Li et al., 2013). Because of its fewer adverse effects, ease of use, and low cost, a lot of research is being done on the use of herbal compounds as natural antioxidants nowadays (Riaz et al., 2011).

Almond oil, comes from *Oleum amygdalae*, is widely used in folk medicine as an anti-inflammatory, wound healer, and treatment for rheumatic and muscle pains (Torres et al., 2016)[a]. Chemically, almond oil is a water insoluble, rapidly emulsifiable ester that is non-toxic, non-irritating, non-sensitizing, and non-comedogenic. According to a previous study, almond oil has immune-boosting and hepatoprotective properties (Ahmad, 2010). Proteins (amandine) and minerals including calcium and magnesium are found in almond oil. It also contains vitamin E and D, as well as being high in unsaturated fats, low in saturated fats, and cholesterol-free (Barku Atsu et al., 2012). Current pharmacological investigations revealed that sweet almond has several biological activities including prebiotic, antioxidant, antimicrobial, anticancer, anti-inflammatory, cardiometabolic protection, hepatoprotective, anxiolytic, sedative–hypnotic, nootropic, and nervous-improving effects (Karimi et al., 2020).

The current study aims to ascertain if almond oil can protect mice from doxorubicin-induced cardiotoxicity as there is no previous literature have revealed the cardio-protective effect of almond oil against doxorubicin.

MATERIALS AND METHODS

Chemicals

Doxorubicin Hydrochloride (Mylan S.A.S., Saint-Priest, France) vials (2mg/ml) were purchased from (El-Borg pharmacy, Beni-Suef, Egypt). Almond oil was purchased from Harraz for food industry and natural products Co, Ahmed Maher St. Bab Alkhalq, Cairo, Egypt. Malondialdehyde (MDA) and Superoxide dismutase (SOD) commercial kits were provided by Biodiagnostic Company, Cairo, Egypt. LDH commercial kit was purchased from Spinreact Company, Spain. ELISA kits which are used for determination of NF-κB and TNF-α levels were provided by Ray Biotech, USA. Catalog No. CSB-E12108m and CSB-E04741m respectively. ELISA kits which are used for estimation of serum levels of creatine kinase MB and cardiac troponin-1 were supplied by Cusabio Company. Catalog No. CSB-E14404m and CSB-E08421m respectively.

Animals and treatments

Thirty male mice, 10 weeks of age, were obtained from private laboratory animal farm, Beni-suef governorate, Egypt. Mice were given balanced commercial diet and water ad libitum and housed at room temperature of 25 °C, 45 % humidity, and a 12:12h light: dark cycle. All experimental procedures were carried out in line with the guide for the care and use of laboratory animals and in agreement with the Research Ethical Committee of Faculty of Veterinary Medicine, Beni-Suef University, Egypt (approval number: 021-176).

One week after acclimatization, the mice were equally divided into 3 experimental groups of 10 mice each:
Control group: Mice were used as control and were given identical amounts of 0.9 percent saline intraperitoneally.
Doxorubicin group: Mice were intraperitoneal injected with doxorubicin (5 mg/kg) (Qi et al., 2020) in three injections over a period of two weeks (dose every 5 days).
Almond oil group: Mice were administered almond oil orally (2.26 g/kg body weight) (Kato et al., 2019) using oral gavage daily over a period of three weeks, one week prior to the doxorubicin and two weeks along with doxorubicin administered.

Sampling and Biochemical assays

After 4 days from the final dose of doxorubicin injection, collection of blood samples via retro-orbital bleeding using light ether anesthesia. Centrifugation of clotted blood

samples were done at 3000 rpm for 15 minutes for serum separation. The obtained sera were preserved at – 20 ºC till use.

Specimens collection

The mice were euthanized by cervical dislocation. The heart was excised and rinsed by physiological saline. The heart samples were partitioned into three portions; the first portion (0.5) was homoginized for estimation of oxidant/antioxidant parameters, the second portion was used for detection of TLR4 gene expression and the third one was fixed in formalin 10 % for histopathological examination.

Estimation of biochemical parameters

Cardiac tissue injury biomarkers: Serum LDH activity was measured according to Bais and Philcox (1994). Levels of creatine kinase MB and cardiac troponin-1 in serum were assessed by ELISA according to the manufacturer's directions.

Oxidant/antioxidant biomarkers: Oxidant-antioxidant levels in heart tissue was evaluated by determining the levels of MDA and SOD activity in heart tissue homogenate. According to Garcia et al. (2005) and Flohe and Otting (1984) respectively.

Determination of serum levels of TNF-α and NF-κB: The serum levels of TNF-α and NF-κB were measured by ELISA according to the manufacturer's directions.

Detection of TLR4 gene expression by real time-polymerase chain reaction (RT-PCR): According to the manufacturer's instructions, total RNA was extracted from the heart using RibozolTM RNA Extraction Reagents with the code N580 (AMRESCO, LLC Corporate Headquarters, 28,600 Fountain Parkway, Solon, OH 44,139, USA). A UV spectrophotometer «Hitachi spectrophotometer, Model U-2000, Hitachi Ltd. Tokyo, Japan» was used to quantify the concentration of RNA.

Synthesis of DNA. Five grams of RNA were reverse transcribed and denatured at 70 degrees Celsius for two minutes using oligonucleotide (dT)18 primer (final concentration, 0.2 mM). On ice, denatured RNA was added to a reverse transcription mixture comprising 50mM KCl, 50mM Tris HCl (pH 8.3), 0.5mM deoxyribonucleotide triphosphate (dNTP), 1 U/mL RNase inhibitor, 3mM MgCl2, , and 200 units of moloney murine leukaemia virus reverse transcriptase. The reaction tube was kept at 42°C for 1 hour before being heated to 92°C to stop it.

RT-PCR. For real-time quantitative PCR, 5 µL of first-strand cDNA were mixed with 12.5 µL of 2x SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and 200 ng of each primer in a total volume of 25 µL, as shown in Table 1. On the step one plus real-time PCR system, PCR reactions consisting of 95 °C for 10 minutes (1 cycle), 94 °C for 15 seconds, and 60 °C for 1 minute (40 cycles) were performed (Applied Biosystems). The ABI Prism 7500 sequence detection system software was used to evaluate the data, and PE Biosystems' v1.7 Sequence Detection Software was used to quantify it (Foster City, CA). The relative expression of the genes under investigation was calculated. The comparative threshold cycle approach was used to calculate the relative expression of the genes investigated. All results were normalized to beta-actin genes, and all of these steps were carried out according to Livac and Schmittgen, (2001).

Table 1: Primers sequences used for mRNAs amplification encoding TLR4 by quantitative RT- PCR.

mRNA	Sequences (5`→3`)	Accession no./ References
TLR4	Forward primer: GTTCTCT-CATGGCCTCCACT Reverse primer: GGAAC-TACCTCTATGCAGGGAT	NM_021297 de Vicente et al. (2020)
β-actin	Forward primer: AT-GAGCCCCAGCCTTCTC-CAT Reverse primer: CCAGC-CGAGCCACATCGCTC	NM_007393 Zhang et al., 2019

Histopathological examination

Heart samples were dissected from all studied mice of all groups, directly immersed in 10% formalin fixative for 48hrs. The specimens of the myocardium were subjected to the routine histological technique and the tissue slides were stained with hematoxylin and eosin stain (H & E) according to Bancroft and Gamble (2008). The stained sections were checked and photographed by LEICA (DFC290 HD system digital camera, Heerbrugg, Switzerland). Scoring of tissue histopathological injury was applied according to Cove-Smith et al. (2014).

Statistical analysis

All results were statistically assessed by one-way analysis of variance (ANOVA) using SPSS software, version 22 (Chicago, USA) then comparisons by using the Tukey post hoc test. The data were exhibited as a mean ± standard error of the mean (SE). The differences were considered statistically significant at $p < 0.05$.

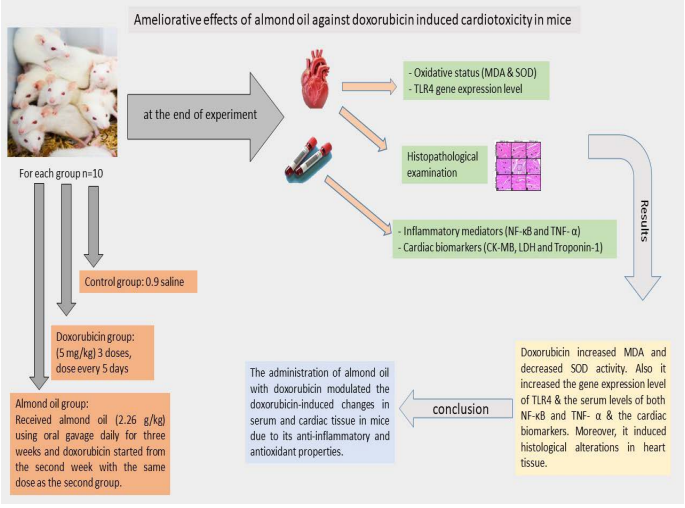
Insilico molecular docking

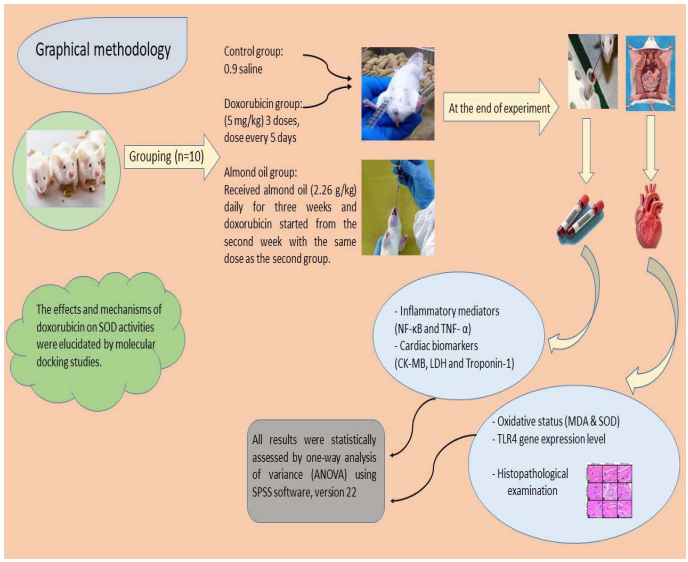
In silico computational protein-ligand docking was performed for SOD against doxorubicin structure by three steps. First, the preparation of the biological data. Second, the molecular docking procedures. Third, docking analysis

and results. Doxorubicin structure was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/compound/31703). Then, the toxicity prediction for it was done by the SwissADME database (http://www.swissadme.ch/) (Rajalakshmi et al., 2021). After that, Energy minimization was performed for it by the Swiss PDB Viewer program (spdbv) and the format was converted from pdb to pdbqt by Open Babel (Version 2.3.1, http://openbabel.org). On the other hand, the 3D structure of the SOD enzyme was obtained from AlphaFold Protein Structure Database (https://alphafold.ebi.ac.uk/entry/Q08420). The insilico molecular docking was conducted by Discovery Studio 2019 software by removing water molecules and adding the hydrogen atoms for the domain and then it was saved in PDBQT format. The performance of the grid box map was performed by AutoDock Vina at 1.00 Å spacing, points of numbers of 78 X × 50 Y × 94 Z Å grid focus dimensions, and center grid boxes dimensions of 3.447 X × 3.388 Y × -7.223 Z Å (Thakur and Pande, 2021).

RESULTS

Our data revealed that doxorubicin attenuated the antioxidant status of cardiac tissue in mice while pretreatment with almond oil considerably offsets the doxorubicin-induced oxidative stress indices as shown in Figure (1). Daily administration of almond oil significantly ameliorated the high level of TLR4 gene expression induced by doxorubicin group (Figure 2). The changes of serum levels of both NF-κB and TNF- α caused by doxorubicin and the improved effect of almond oil were revealed in Table (2). Adverse effect of doxorubicin injection on heart function was assessed in this work and the ameliorative effect of almond oil showed in Table (3). The role of almond oil in the amelioration of histopathological changes caused by doxorubicin administration was achieved in this study and presented in Figure (3). Figure (4) explain the in silico computational protein-ligand docking for SOD induced by doxorubicin.





DISCUSSION

Because doxorubicin is an important chemotherapeutic agent, it is important to lessen its toxicity due to continous use. In our research, we noticed that 5 mg/kg doxorubicin was harmful to mice cardiac tissue and 2.26 g/kg almond oil mitigated this effect.

The pathogenesis of doxorubicin-induced cardiotoxicity typically involves oxidative stress. The decreased levels of catalase and SOD in the heart tissue, as well as the quick inactivation of glutathione peroxidase in the myocardium by doxorubicin, make the myocardiam more vulnerable to oxidative injury (Minotti et al., 2004) and the high content of mitochondria in heart tissue where mitochondria are a major source of ROS and a target for doxorubicin action (Ghigo et al., 2016). These may be the reasons why doxorubicin is most toxic to the heart (Roca-Alonso et al., 2012). Mitochondrial enzymes convert doxorubicin to semiquinone once it enters cardiomyocytes. This process produces a lot of reactive oxygen species (ROS), like superoxide anion and hydrogen peroxide (de Carvalho et al., 2016). These findings were in line with that of Mauro et al., (2021) who use the same dose of doxorubicin (5mg/kg).

Table 2: Serum levels of NF-κB and TNF-α in the various studied mice groups.

Groups/ Parameters	Control group	Dox group	Dox-Al group
NF-κB (ng/ml)	0.93 ± 0.04 [a]	1.98 ± 0.03 [b]	1.58 ± 0.05 [c]
TNF-α (pg/mL)	18.67 ± 0.89 [a]	37.92 ± 1.93 [b]	27.58 ± 0.91 [c]

Values are represented as mean ± standard error. The different superscript letters mean a significant difference at ($P < 0.05$) between different groups in the same row.

Table 3: Changing in heart function biomarkers of different experimental groups.

Groups/Parameters	Control group	Dox group	Dox-Al group
Troponin-1 (pg/ml)	16.85 ± 1.51 [a]	50.75 ± 1.83 [b]	34.03 ± 1.35 [c]
CK-MB (mU/ml)	5.61 ± 0.50 [a]	17.21± 0.83 [b]	11.36 ± 0.47 [c]
LDH (U/L)	273.35 ± 7.25 [a]	602.46 ± 28.93 [b]	454.35 ± 18.69 [c]

Values are represented as mean ± standard error. The different superscript letters mean a significant difference at ($P < 0.05$) between different groups in the same row.

Table 4: Comparison between all studied groups, showed the score of the histopathological lesions caused by doxorubicin in comparison with normal control and almond oil treated groups in cardiac sections stained with H&E X100.

Lesions	Groups		
	Control	Dox group	Dox-Al group
Myocytic degeneration	-	+++	+
Hyalinosis	-	++	–/+
Vascular congestion	-	+++	++
Edema	-	++	+
Inflammatory cell infiltrations	-	+++	+
Myocardial gap	-	++	+

No changes (–), minimal (–/+), mild (+), moderate (++), and severe changes (+++).

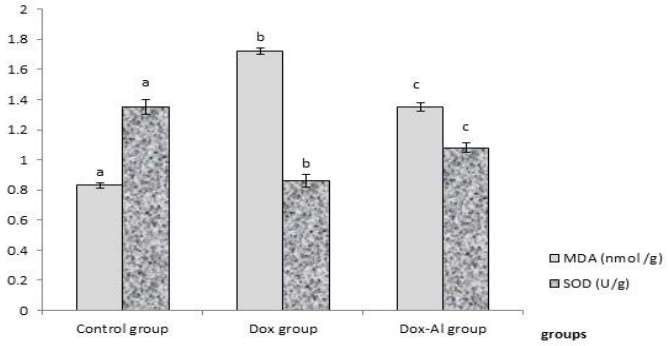


Figure 1: Changes in MDA levels and SOD activity in the heart of mice in different studied groups. The different superscript letters mean a significant difference at (P > 0.05) between different groups. Legend higand

in the current study, we observed that doxorubicin significantly decreased the level of myocardial MDA, which is acellular lipid damage marker and this is in agreement with the prior findings reported by Mathias et al. (2019) in addition, SOD activity was siginificantly decreased in doxorubicin treated mice in accordance with Zhang and Huang, (2017). We confirmed the inhibitory effect of

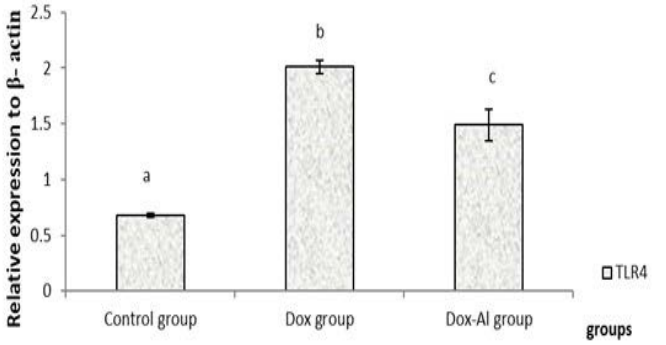


Figure 2: The relative expression levels of TLR4 gene in the various studied groups. The different superscript letters mean a significant difference at (P value less than 0.05) between different groups.

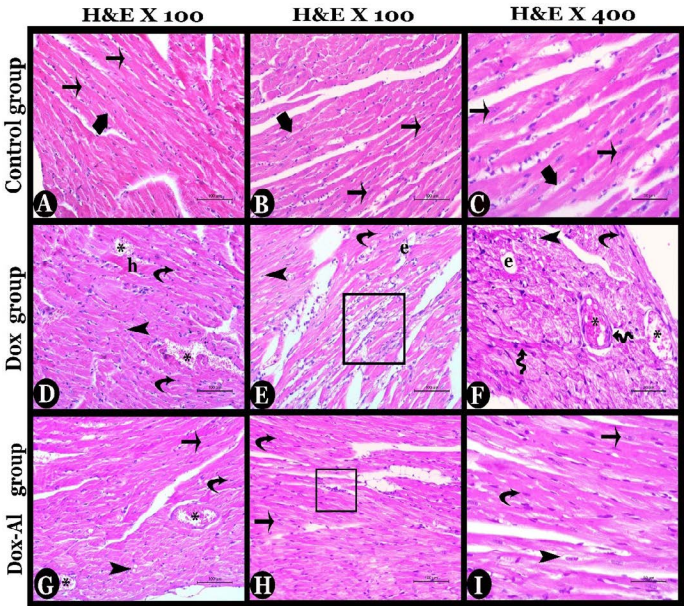


Figure 3: Photomicrographs of histolopathological sections of the heart tissues of all studied mice stained with H&E stain, 1st, and 2nd columns X100, and 3rd column X400: A, B & C) Control group showing normal cardiac architecture, normal arrangement of cylindrical branched myocytes (thin arrows) contained acidophilic cytoplasm and oval central vesicular nuclei. Note, narrow slits (thick arrows) between the myocytes contained fine connective tissue and normal blood vessels. D, E & F) Doxorubicin group showing degenerated condensed myocytes with pyknotic nuclei (curved arrows), deteriorated myocytes with vacuolated cytoplasm infiltrated by inflammatory cells (square), interfibrillar vacuolations (arrowheads), Hyalinosis (h), in addition to damaged blood vessels with congestion (*), proliferating fibroblast (zigzagged arrows) and interstitial edema (e). G, H & I) Almond oil-treated group showing mild degenerative changes appeared in form of few degenerated myocytes with pyknotic nuclei (curved arrows), interfibrillar vacuolations (arrowheads), few inflammatory cells infiltration (square) and vascular

degeneration with congestion (*), while most of myocytes appeared as normal (thin arrows).

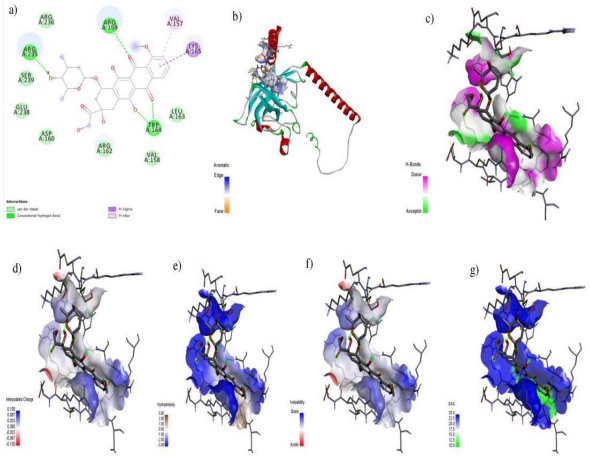


Figure 4: Explain the in silico computational protein-ligand docking for SOD induced by doxorubicin. The in silico docking analysis showed: a) The 2D chemical interaction of SOD- doxorubicin complex with four conventional hydrogen bonds, b): The PKS (PT domain) - interaction of SOD-doxorubicin complex, c): Hydrogen bond interaction of SOD- doxorubicin complex, d): The predicted charges of the SOD- doxorubicin complex, e): Hydrophobic interaction of SOD- doxorubicin complex, f): The ionizability of the SOD- doxorubicin complex and g): The SAS area in of the SOD- doxorubicin complex.

doxorubicin on SOD using the in silico docking analysis which illustrated the 2D chemical interaction between SOD- doxorubicin complex. The SOD- doxorubicin complex was stable due to the four conventional hydrogen bonds; two with tryptophan and two with arginine amino acids. Also, there were interactions with van der Waals in the amino acids leucine, valine, arginine, asparagine and glutamine beside one of pi-sigma interaction in the amino acid lysine and one pi-alkyl interaction in the amino acid valine. The SOD- doxorubicin complex showed a lot of donor H-bond interactions area in comparison to acceptor H-bond interactions area. Most of the interactions areas were neutral in charges when there were a few positive areas. Most of the interactions areas were hydrophilic and neutral in the ionizability areas with a few basic areas. Finally, most of the interactions areas were highly solvent accessible surface (SAS). Doxorubicin showed highly inhibition to SOD enzyme with binding energy -7.8, highly stable chemical reaction with covalent bonds and highly SAS interactions. Moreover, SOD may be inactivated directly by hydrogen peroxide and superoxide radicals (Yazar et al., 2002).

In addition, the increased level of myocardial MDA, which is a cellular lipid damage marker, is in agreement with prior findings reported by Mathias et al (2019).

Considering the chief role of oxidative stress in doxorubicin-induced cardiotoxicity, combining antioxidants with doxorubicin may mitigate its cardiotoxicity. In this study, almond oil significantly ameliorated the oxidant/oxidant status reinforcing its antioxidant properties. These findings were in line with those of Jia et al. (2011). Torres et al. (2016)[b] explained that these effects could be attributed to components of almond oil that have antioxidant properties as well as potential biological ingredients. Natural antioxidants, including minerals, vitamins, polyphenols and carotenoids are separated into two types, nutrient (e.g., vitamins) and non-nutrient (e.g., phenolics), with non-nutrient ones being more effective than nutrient antioxidants. These two types of antioxidants can be found in almonds (Alasalvar and Shahidi, 2009). Flavonoids and phenolic acids are abundant in almonds. Almond's high amount of phenolic compounds is thought to be responsible for its antioxidant properties. In vitro research revealed that almond flavonoids provide substantial cytoprotection against oxidative stress-induced apoptosis (Milbury et al., 2006).

TLRs are implicated in all types of cardiovascular disorders, according to numerous research. TLR4 is a lipopolysaccharide (LPS)-specific pattern recognition receptor that belongs to the type I transmembrane receptor group (Xinyong et al., 2020). The significant increase in the myocardial level of TLR4 gene expression in Dox group is in harmony with Birks et al. (2004). According to Xinyong et al. (2020), doxorubicin activation of TLR4 is triggered by oxidative stress, which causes cardiac cell injury by inducing cell membrane lipid peroxidation. This alters the structure of the membrane, compromising its integrity, altering the cell membrane structure, and resulting in the creation of a significant number of endogenous ligands and overflow, which activates TLR4. We observed that almond oil daily administration significantly down regulated the TLR4 gene expression when compared to doxorubicin group. We suggested that this amelioration is regarded to almond oil antioxidant activity.

The nuclear transcription factor NF-κB regulates the transcription of numerous genes, as a result, it is involved in immunology, inflammation, cell survival, and apoptosis. The inflammatory response induced by this transcription factor is mediated through the initiation and regulation of TNF, intercytokines and other genes (Hamerman et al., 2016). Moreover, Mann (2002) found that other pro-inflammatory cytokines and chemokines, in addition to TNF, are involved in the pathogenesis of heart failure. During doxorubicin treatment, TLR4 induced cardiac inflammation via the initiation of NF-κB in the nucleus,

leading to secretion and release of numerous bioactive substances that activate the innate immune response, including TNF- α and interleukins (Xinyong et al., 2020). these data confirms our results which revealed that the serum level of both NF-κB and TNF-alpha were increased significantly in doxorubicin group in comparison with control group in line with a previous study (Kumar et al., 2004). In the current study, almond oil pretreatment significantly ameliorated the elevated serum levels of both NF-κB and TNF- α induced by doxorubicin (Torres et al., 2016)[b]. Our data suggest a potential therapeutic effect of almond oil on inflammatory conditions induced by doxorubicin. Casas-Agustench et al. (2010) stated that almond is considered as one of the anti-inflammatory food, which contains some components, including antioxidants, L-arginine, magnesium, PUFAs, MUFAs, and dietary fibers.

The above-mentioned release of pro-inflammatory mediators and subsequent cardiac inflammation associated with doxorubicin treatment explained the cardiac dysfunction and tissue injury biomarkers that we found in mice injected by doxorubicin. In the present study, doxorubicin administration resulted in a significant increase in LDH, CK-MB and Troponin-1 serum levels, which indicate the development of cardiotoxicity in accordance with Zilinyi et al. (2018). Elevated levels of these biomarkers refer to their leakage from the damaged cardiomyocyte membranes into circulation and were shown previously to be indicators of cardiotoxicity (Hadi et al., 2012).

The current study revealed that daily administration of almond oil significantly attenuated the elevated LDH, CK-MB, and Troponin-1 levels induced by doxorubicin administration. Mathias et al. (2019) explained that lipid peroxidation disrupts the cellullar barrier, membrane pumps and cellular membrane channels which get worse cardiac function. Our results suggest that almond oil might alleviate the impaired cardiac injury markers and protect against doxorubicin-induced cardiotoxicity through its antioxidant properties.

The selenium is one of the minerals present in almond oil, it prevents cell damage by producing selenoproteins as antioxidant enzymes (Alasalvar & Shahidi, 2009). In addition, almond is one of the richest sources of vitamin E. The alpha-, beta-, gamma-, and delta-tocopherol and tocotrienol are some forms of vitamin E. Alpha (α) tocopherol is the major form of tocopherol found in almond (Çelik et al., 2019). Owing to the antioxidant activity of vitamin E, it has an effective role in the prevention and treatment of different diseases (Rizvi et al., 2014).

CONCLUSION

We postulated that almond oil improved the cardiac injury biomarkers associated with doxorubicin injection via attenuating the release of both NF-kB, TNF-alpha and down regulation of TLR4 as a result of its antioxidant activity. Therefore, it is advisable to be used concurrently with doxorubicin to reduce its cardiotoxicity. From this study we recommended following more clinical trials and meta-analyses to make definitive conclusion about the efficacy and therapeutic actions of almond.

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CONFLICT OF INTEREST

In relation to this manuscript, the authors state that they have no competing interests.

AUTHOR CONTRIBUTION

Doaa Shaaban: Conceptualization, Writing, reviewing and editing. Nema Sayed and Olfat Shehata: performed the experiment, laboratory works Supervision, reviewing and editing. Mai M Labib performed the bioinformatics work

NOVELTY STATEMENT

As far as we know, there is no previous literatures have revealed the cardioprotective effects of almond oil against the doxorubicin toxicity.

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