http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2022.270.281

Research Article
Cardioprotective and Antioxidant Efficiency of *Balanites aegyptiaca* Extract Against Doxorubicin® Complication

[1,2]Heba F. Gomaa, [3]Khaled G. Abdel-Wahhab, [4]Mahmoud Ashry and [5]Doaa Galal EL-Sahra

[1]Department of Zoology, Faculty of Science, Ain-shams University, Cairo, Egypt
[2]Department of Biology, College of Sciences and Arts-Scientific Department, Qassim University, Saudi Arabia
[3]Department of Medical Physiology, National Research Centre, Giza, 122622, Egypt
[4]Department of Zoology, Faculty of Science, Al-Azhar University, Assuit, Egypt
[5]Modern University for Technology and Information, Cairo, Egypt

Abstract

Background and Objective: The use of Doxorubicin® (Doxo) in the treatment of different tumours is restricted due to its cardiotoxicity. The objective of this study was to determine the protective effect of *Balanites aegyptiaca* extract against cardiotoxicity induced by Doxorubicin® in male rats. **Materials and Methods:** Adult male rats (140-160) were parted into 6 groups (10 animals each) as follows: Group (1) Normal rats the control, group (2) Rats were administered BAE (200 mg kg^{-1}) orally for 4 weeks, group (3) Rats were treated IP with the anticancer drug (Doxorubicin®) at the dose of (0.5 mg kg^{-1}) for 4 weeks, group (4) Administrated orally with BAE in combination with Doxo injection for 4 weeks, group (5) Rats orally with BAE before intoxication with Doxo for 4 weeks and finally group (6) Animals post-administration of BAE for 4 weeks after intoxication with Doxo. After 4 weeks of injections. **Results:** Revealed that BAE succeeded to decline the Doxorubicin cardiotoxicity, this was evidenced by the significant reduction of serum LDH, CK-MB, total cholesterol, triglycerides, HDL, TNF-α, IL-1β and IL-6 as well as cardiac MDA and nitric oxide levels coupled with marked improvement in serum LDL, PON1 as well as cardiac GSH, SOD and CAT. Moreover, the BAE induced prominent regeneration of the cardiac muscle. **Conclusion:** *Balanites aegyptiaca* extract may be a promising cardio-protector against Doxorubicin® toxicity mediated through their antioxidant and radical scavenging activities.

Key words: Cardiotoxicity, Doxorubicin®, *Balanites aegyptiaca*, paraoxonase-1, pathology, GC-mass, inflammatory cytokines

Citation: Gomaa, H.F., K.G. Abdel-Wahhab, M. Ashry and D.G. EL-Sahra, 2022. Cardioprotective and antioxidant efficiency of *Balanites aegyptiaca* extract against Doxorubicin® complication. Pak. J. Biol. Sci., 25: 270-281.

Corresponding Author: Heba F. Gomaa, Department of Zoology, Faculty of Science, Ain-shams University, Cairo, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Anthracyclines are a group of widely used potent chemotherapeutic agents for the treatment of multiple solid and hematologic malignancies. Cardiomyocyte is the main cellular target of anthracycline, as its destruction results in the progressive development of cardiac dysfunction[1,2]. Doxorubicin (Adriamycin) is one of the anthracyclines that can induce cardiotoxicity in both acute and chronic forms. Acute cardiotoxicity has been described as arrhythmia and transient left ventricular dysfunction but these are rare as compared to chronic cardiotoxicity. High cumulative doses of anthracyclines can lead to left ventricular systolic dysfunction and heart failure[1,2].

Many scientists explained Doxorubicin cardiotoxicity, one of them was that Doxo-induced DNA double-strand breaks. It causes dysfunction and apoptosis of mitochondria by disrupting the neuregulin/ErbB signalling[1,2]. The most reliable one is oxidative stress induced by reactive oxygen species resulting in cardiac dysfunction[1,2].

Also, Doxorubicin-induced mitochondrial dysfunction via inhibition of protein expression of electron transport chain resulting in energy depletion of cardiac muscle and then cell death[3-5].

Medicinal plants are of great importance to health worldwide. They have many effects such as, antibacterial, antifungal, antiviral, anti-helminthic, anti-allergic, anti-carcinogenic and larvicidal[6]. These medicinal values are due to the presence of chemical substances with therapeutic activity such as tannins, oils, gums. In addition the knowledge of the chemical constituents of plants. Pharmacologists, microbiologists, botanists and natural products chemists are combing the earth for phytochemicals that could be developed for the treatment of various diseases. Many modern drugs have been derived from plants[7].

Balanites aegyptiaca (BA) is an Egyptian wild plant from the family Zygophyllaceae. This plant is native to Africa and Middle East, it has many synonyms, such as the desert date in English and lalob, hidjihi and heglig in Arabic[8,9].

Many articles, reported that the fruit mesocarp of BA has high amounts of the antioxidants such as saponins and moderate concentrations of tannins, flavonoids and cardiac glycosides[3,7].

Some of the identified anti-diabetic bioactive compounds are steroidal saponins, phenolic compounds like vanillic and syringic acids as well as flavonoids (rutin) and isorhamnetin in fruit[9,10], Isorhamnetin, rutinoside and glycine quercetin from plant seed extracts have been recorded.

Reports revealed that leaves are rich in coumarins and quercetin, while the stem bark has alkaloids and coumarins. On the contrary, the fruit contains rutins and seed kernel among others[11].

This study aims to investigate the ameliorative effects of *Balanites aegyptiaca* (BA) aqueous extract against cardiotoxicity induced by Doxorubicin®.

MATERIALS AND METHODS

Study area: This study was carried out from January-July, 2020 at the Department of Medical physiology and animal colony, National Research Centre, Egypt.

Aqueous extraction: The dry fruits of *Balanites aegyptiaca* were purchased from the stores of Abd El-Rahman Harraz (Bab El-Khalk zone, Cairo, Egypt). *Balanites aegyptiaca* Aqueous Extract (BAE) was carried out according to the previous method[10], the dry extract was stored in a dark bottle at -20°C until usage.

***In vitro* determinations of the extract:** The percentage of extracts' yield was calculated according to Abdel-Wahhab *et al.*[11], total phenolics content (TPC) of the extract was estimated as catechin equivalents according to the method of Jayaprakasha and Rao[12], the extract radical scavenging activity (RSA) to quench DPPH radical was determined using the method of Nogala-Kalucka *et al.*[13], Phytochemical analysis of the extract was evaluated as described by Rajasekar and Elango[14].

Chemical composition identification of the aqueous extract: LC/MS/MS,4000 Qtrap Applied Biosystems, (Quadrupole/linear ion trap mass spectrometer) and the liquid chromatography (Agilent Technologies, Palo Alto, CA, USA) system coupled with an Electrospray Ionization Mass Spectrometer (ESI-MS/MS) detector were employed to identify the chemical composition of the aqueous extract[15].

Study design: Adult male 60 rats (140-160 g) from animal colony, National Research Centre, Cairo, Egypt. All animals received human care in compliance with the standard institutional criteria for the care and use of experimental animals according to the NRC ethical committee (FWA 00014747). The animals were randomly parted into 6 groups (10 animals each), group (1) Control normal rats daily administrated (0.4 mL kg^{-1}) saline via oral intubation for 4 weeks, group (2) Animals subjected to daily oral Administration of BAE (200 mg kg^{-1}) for 4 weeks, group (3) Animals received an intraperitoneal injection with

Doxorubicin® at a dose of 0.5 mg kg^{-1} for 4 weeks, group (4) Administrated orally with BAE together with Doxorubicin® injection for 4 weeks, group (5) Rats orally with BAE before intoxication with Doxorubicin® for 4 weeks and finally group (6) Animals treated with BAE for 4 weeks after intoxication with Doxorubicin® for 4 weeks.

Blood and tissue sampling: At the end treatment, the animals were sacrificed, the sera were separated and stored at -80°C till biochemical measurements were carried out. The heart of each animal was dissected out and divided longitudinally, the first half was stored at -80°C for determinations for biochemical analysis, the second half was kept in formalin-saline (10%) buffer for histopathological processing.

Serum biochemical determinations: Biochemical measurements were carried out spectrophotometrically as serum CK-MB and LDH activities were determined according to the Pepato *et al.*[16], using reagent kits purchased from Spectrum Diagnostic System MDSS GmbH, Egypt. Serum total cholesterol, triglycerides, LDL and HDL levels were determined colourimetrically, using reagent kits purchased from Diagnostic System GmbH, Germany. Cardiac GSH and NO levels as well as SOD and CAT activities were estimated using kits obtained from Biodiagnostic, Dokki, Giza, Egypt. Serum paraoxonase-1 (PON-1) activity was determined according to the kinetic spectrophotometric chemical method described by Charlton-Menys *et al.*[17]. Using ELISA (Dynatech Microplate Reader Model MR 5000, 478 Bay Street, Suite A213 Midland, ON, Canada), serum TNF-α, IL-1β and IL-6 concentrations were measured using rats ELISA reagent kits (SG-10057, SG-10179 and SG-10127, respectively) purchased from Sino Gene Clon Biotech Co., Ltd, Hang Zhou and China.

Cardiac oxidative stress markers: Cardiac MDA level was determined chemically as described by Ruiz-Larrea *et al.*[18]. Cardiac nitric oxide (NO) and reduced glutathione (GSH), as well as superoxide dismutase (SOD) and catalase (CAT), were estimated spectrophotometrically using reagent kits obtained from Biodiagnostic, Giza, Egypt.

Histopathology: Cardiac samples with 5 µm thickness were stained with Hematoxylin and Eosin.

Statistical analysis: One way analysis of variance followed by Duncan *post hoc* test was performed on data using a Statistical Analysis System (SAS) program software, copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA.

RESULTS

The yield amount, total phenolic content and radical scavenging activity of the *aqueous extract of Balanites aegyptiaca* are shown in Fig. 1. The aqueous extract of *Balanites aegyptiaca* contained 8 saponin compounds, 6 of which were with molecular weights of 1.196, 1.064, 1.210, 1.224, 1.078 and 1.046 KDa, the third one (1.210 KDa) was the main saponin (36%). Also, the extract was found containing 6 diosgenin glucosides (di-, tri-and tetra-glucosides), 25D-spirosta-3, 5-diene and 3β-chloro-25D-spirost-5-ene and balanitin-1-8 (Table 1).

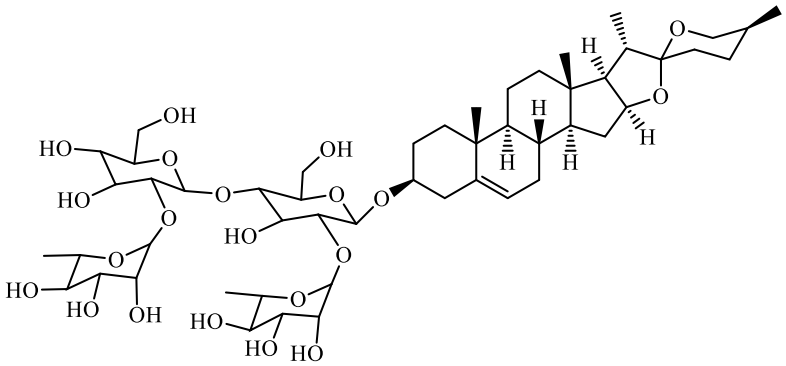
Doxorubicin treatment intraperitoneally at a dose of 0.5 mg for 4 weeks resulted in a significant elevation in LDH and CK-MB activity as compared to the values of the control group. While the co-administration of BAE with Doxorubicin-induced significant protection against Doxorubicin-induced deteriorations in the mentioned parameters (LDH and CK-MB activity) than that resulted from its administration in groups 5 and 6 (Table 2).

Doxorubicin® injection resulted in a significant rise in the levels of serum total cholesterol, triglycerides, LDL-cholesterol coupled with a significant decline in the level of serum HDL-cholesterol when compared with the control group. Interestingly, the administration of BAE either as co-treatment with Doxo or as a protector (before Doxo-treatment) or as a treatment (after Doxo-treatment) succeeded to improve all lipid profile levels compared to that of the Doxorubicin-injected group but the most prominent effect in the lipid profile resulted from co-treatment of BAE-Doxo (Table 3).

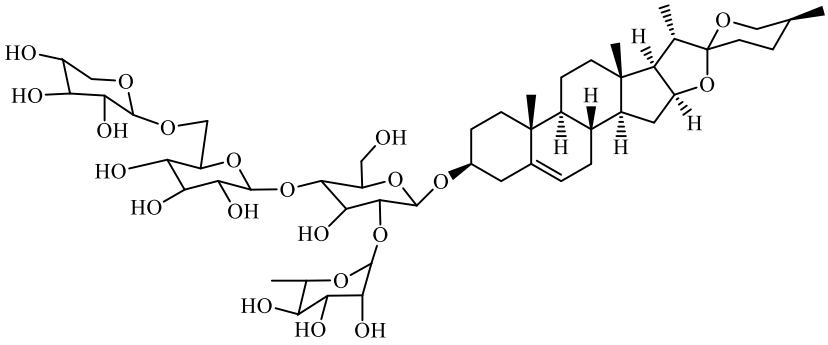
Doxorubicin treatment revealed a significant increase in the levels of cardiac MDA and NO and a significant decline in the levels of cardiac GSH, activities of SOD and CAT when compared with results of the control group. The co-administration of BAE-DOX showed a significant improvement in the levels of cardiac MDA, NO, GSH and activities of cardiac SOD and CAT than results from its administration before Doxo (protector) or after Doxo-treatments (treatment) (Table 4).

Doxorubicin-treatment intraperitoneally showed a significant increase in the inflammatory cytokines TNF-α, IL-1β, IL-6 and a significant decline in the levels of serum PON1 activity when compared with results of the Doxo-treated group. Interestingly, the co-administration of Doxo with BAE

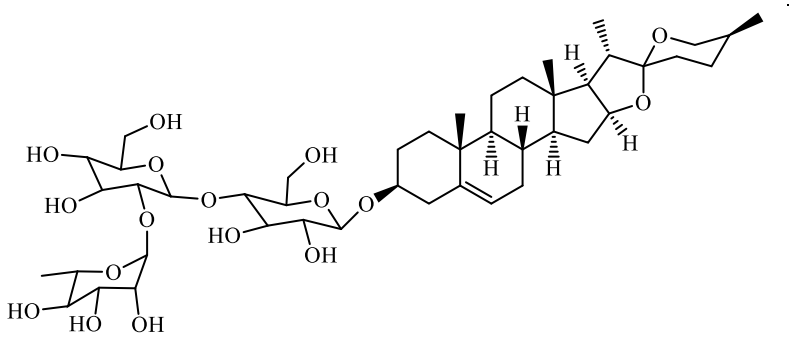
Balanitine-1



Balanitine-2



Balanitine-3



Balanitine-4

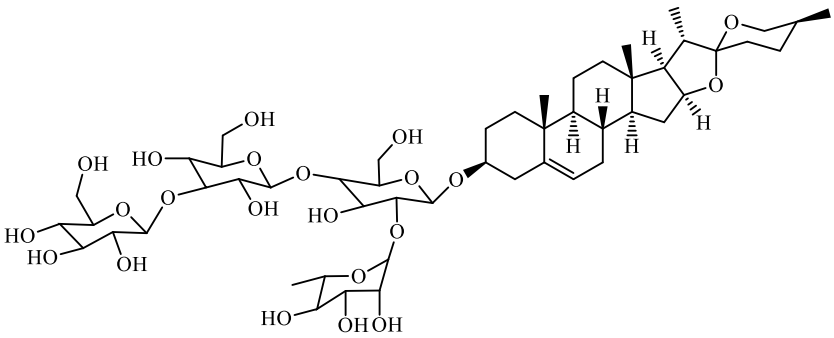
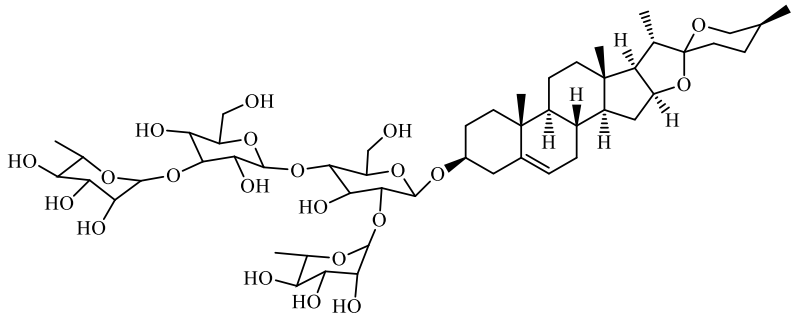
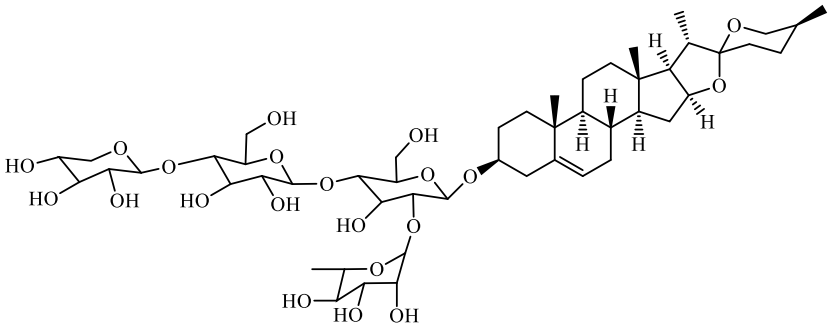


Fig. 1: Continue

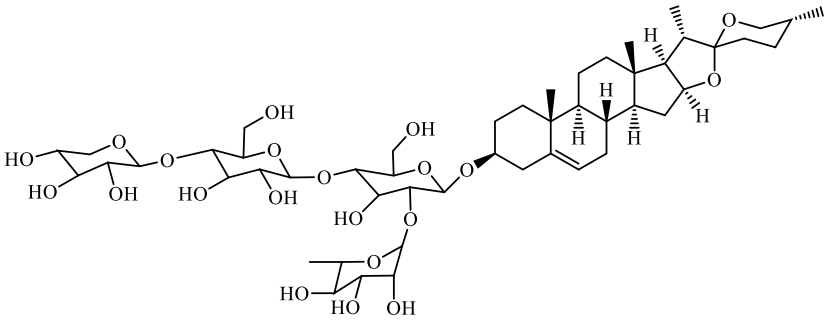
Balanitine-5



Balanitine-6



Balanitine-7



Balanitoside

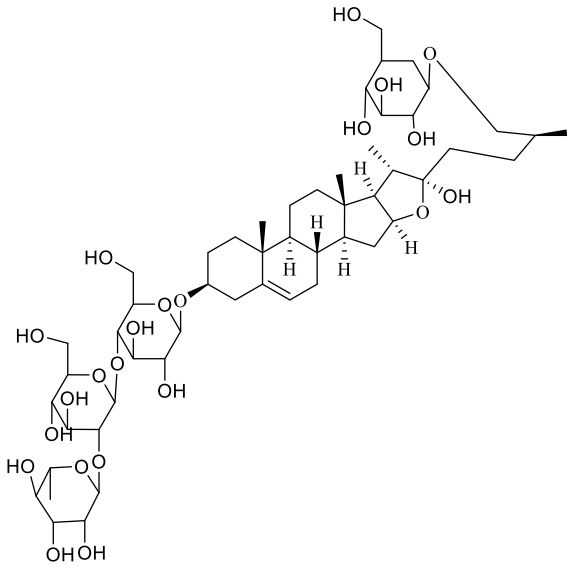


Fig. 1: Major saponin compounds were identified in BAE

Table 1: Phytochemical analysis of constituents found in *Balanites aegyptiaca* aqueous extract

Protein
Sugars
Inorganic acids
3-rutinoside
3-rhamnogalactoside
Diosgenin
23/22S epimers
(26-(O-β-D-glucopyranosyl)-3-β-[4-O-(β-D-glucopyranosyl)-2-O-(α-L-rhamnopyranosyl)-β-D-glucopyranosyloxy]-22,26-dihydroxyfurost-5-ene)
Saponin (xylopyranosyl derivative)
Balanitoside (furostanol glycoside)
6-methyldiosgenin
Balanitin1-8 (mostly 3 is spirostanol glycoside)
Balanitin-6 and -7
Diosgenyl saponins
Pregnane glycosides
(pregn-5-ene-3β,16β,20(R)-triol3-O-(2,6-di-O-α-l-rhamnopyranosyl)-β-d-glucopyranoside (balagyptin)
pregn-5-ene-3β,16β,20(R)-triol 3-O-β-d-glucopyranoside

Table 2: Serum LDH and CK-MB activity of control, doxorubicin®-intoxicated and BAE-treated male Albino rats

	(U L^{-1})	
Control	1145±56^{b}	729±73^{c}
BAE	1102±50^{b}	718±72^{c}
Doxo	1665±53^{a}	1172±99^{ab}
BAE+Doxo	1196±55^{b}	737±89^{c}
BAE→Doxo	1332±61^{b}	1002±93^{a}
Doxo→BAE	1270±34^{b}	846±87^{b}

Data are presented as Mean±Standard error, within the same column, means with superscript different letters are significantly different at $p \leq 0.05$ using one way ANOVA followed by Duncan *post hoc* test, BAE: *Balanites aegyptiaca* aqueous extract and Doxo: Doxorubicin®

Table 3: Serum cholesterol, triglycerides, HDL-C and LDL-C levels of control, doxorubicin®-intoxicated and BAE-treated male Albino rats

Groups	Cholesterol (mg dL^{-1})	Triglycerides (mg dL^{-1})	HDL (mg dL^{-1})	LDL (mg dL^{-1})
Control	93±3.4^{e}	121±13^{c}	29±1.6^{a}	28±3.3^{d}
BAE	92±6.6^{de}	110±18^{c}	29±2.7^{a}	26±2.6^{d}
Doxo	207±7.1^{a}	224±11^{a}	21±1.7^{c}	68±2.5^{a}
BAE+Doxo	118±2.6^{c}	134±8.7^{c}	27±1.2^{a}	41±1.7^{c}
BAE→Doxo	146±8.7^{b}	198±21^{ab}	23±0.8^{a}	60±2.9^{ab}
Doxo→BAE	128±8.8^{cd}	177±13^{b}	24±1.1^{a}	52±1.6^{b}

Data are presented as Mean±Standard error, within the same column, means with superscript different letters are significantly different at $p \leq 0.05$ using one way ANOVA followed by Duncan *post hoc* test, BAE: *Balanites aegyptiaca* aqueous extract and Doxo: Doxorubicin®

Table 4: Oxidative stress of cardiac muscle of control, doxorubicin®-intoxicated and BAE-treated male Albino rats

Groups	MDA (mmol g^{-1} tissue)	NO (mmol g^{-1} tissue)	GSH (mg g^{-1} tissue)	SOD (U g^{-1} tissue)	CAT (U g^{-1} tissue)
Control	61.4±2.8^{e}	28.34±0.9^{d}	3.7±0.5^{a}	47904±365^{a}	45.8± 0.8^{a}
BAE	59.33±2.6^{e}	27.22±0.9^{d}	3.9±0.5^{a}	49820±246^{a}	44.1±0.8^{a}
Doxo	104.1±4.8^{a}	71.17±2.2^{a}	1.9±0.6^{c}	21928±1774^{c}	21.2±0.7^{c}
BAE+Doxo	74.1±2.67^{d}	40.11±1.2^{c}	3.1±0.5^{a}	39430±223^{a}	34.1±0.7^{b}
BAE→Doxo	97.0±4.4^{b}	68.83±1.9^{a}	2.1±0.4^{c}	28119±188^{b}	23.9±0.7^{c}
Doxo→BAE	86.0±4.0^{c}	51.19±1.7^{b}	2.6±0.5^{b}	30286±195^{b}	31.1±0.8^{b}

Data are presented as Mean±Standard error, within the same column, means with superscript different letters are significantly different at $p \leq 0.05$ using one way ANOVA followed by Duncan *post hoc* test, BAE: *Balanites aegyptiaca* aqueous extract and Doxo: Doxorubicin®

Table 5: Serum levels of TNF-α, IL-1β, IL-6 and PON1 activity of control, doxorubicin®-intoxicated and BAE-treated male Albino rats

Groups	IL-6 (pg mL^{-1})	IL-1β (pg mL^{-1})	TNF-α (pg mL^{-1})	PON1 (IU L^{-1})
Control	305±14^{d}	451±15.4^{c}	32.1±4.1^{d}	647±19^{a}
BAE	297±11^{d}	441±14.5^{c}	29.4±2.9^{d}	656±21^{a}
Doxo	635±17^{a}	930±21.7^{a}	88.2±3.8^{a}	362±14^{d}
BAE+Doxo	399±15^{a}	738±23.4^{b}	43.4±4.1^{c}	521±23^{b}
BAE→Doxo	522±11^{b}	771±25.1^{b}	58.4±3.8^{b}	402±14^{c}
Doxo→BAE	454±20^{c}	763±17.9^{b}	67.8±2.5^{b}	444±18^{c}

Data are presented as Mean±Standard error, within the same column, means with superscript different letters are significantly different at $p \leq 0.05$ using one way ANOVA followed by Duncan *post hoc* test, BAE: *Balanites aegyptiaca* aqueous extract and Doxo: Doxorubicin®

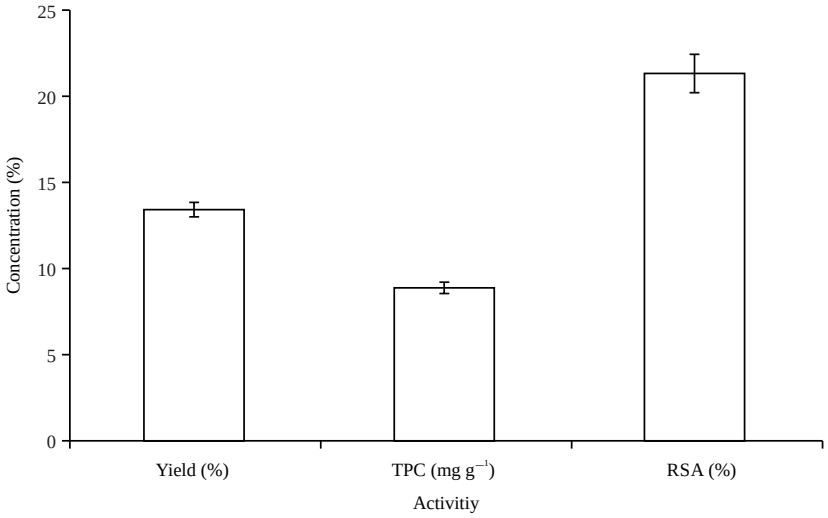


Fig. 2: Yield (%), total phenolic content (mg g^{-1}) and radical scavenging activity (%) of BAE

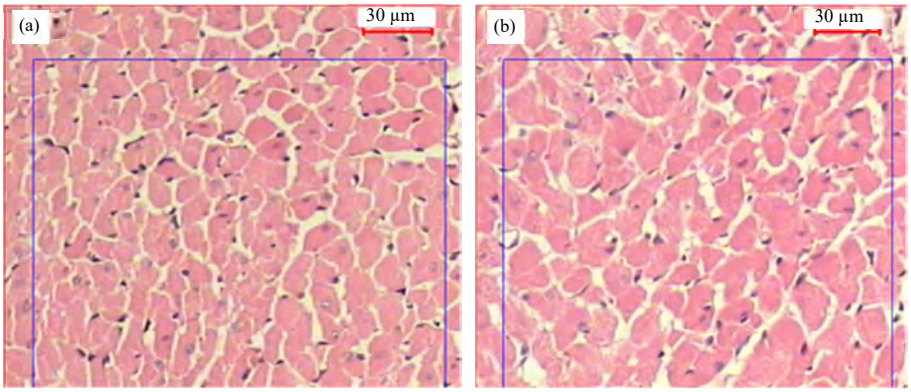


Fig. 3(a-b): Histological assessment of cardiac tissue of rats, (a) Micrograph of control group cardiac muscle cells which are roughly rectangular, with a single central nucleus with a preserved structure and (b) Micrograph of BAE cardiac muscle cells with normal appearance (H and E, ×200)

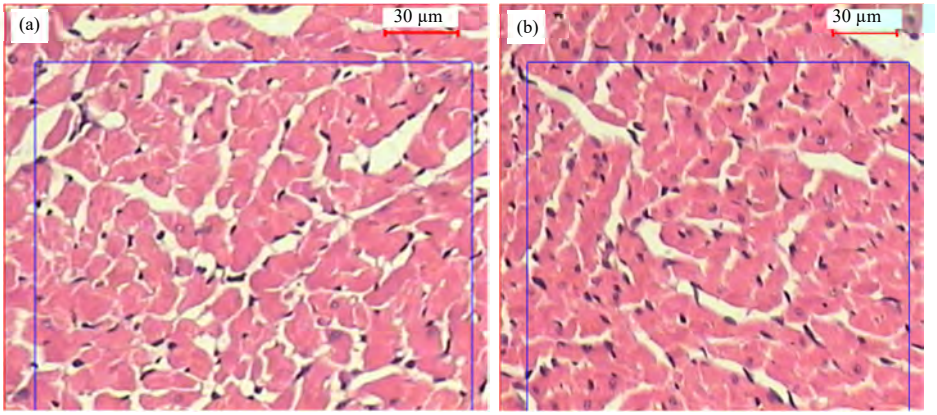


Fig. 4(a-b): Histological assessment of cardiac tissue of rats of Doxo-treated group, (a) Micrograph of Doxo group cardiac muscle cells with cytoplasmic vacuolization and (b) Micrograph of Doxo group cardiac muscle cells showed muscle degeneration (H and E, ×200)

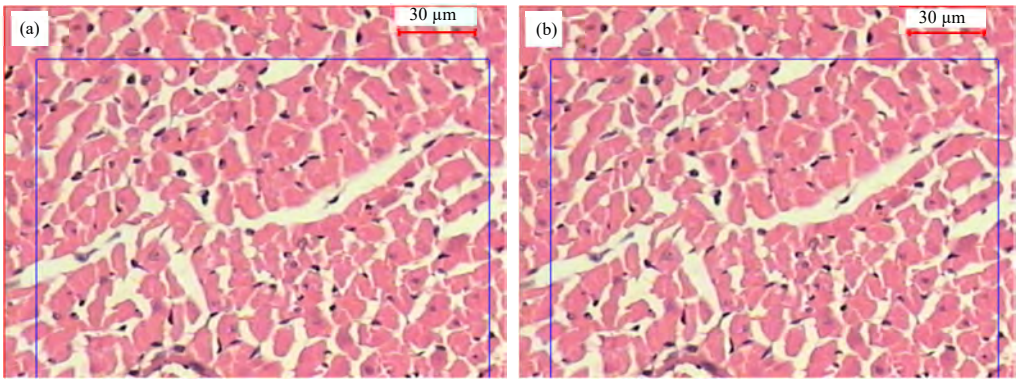


Fig. 5(a-b): Histological assessment of cardiac tissue of rats of (a) Doxo with BAE and (b) BAE then Doxo group (a) Micrograph of Doxo with BAE cardiac muscle cells with normal appearance and (b) Micrograph of BAE then Doxo cardiac muscle cells with normal appearance (H and E, ×200)

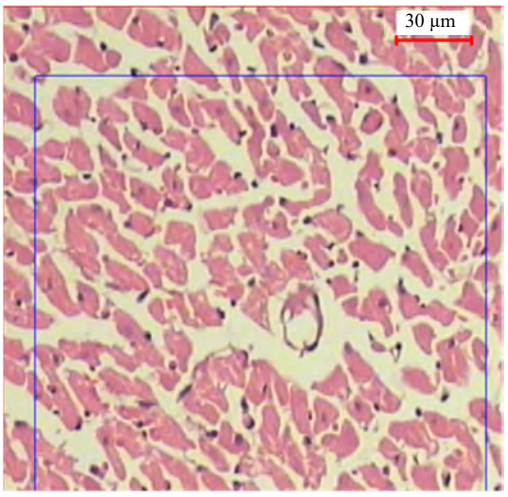


Fig. 6: Histological assessment of cardiac tissue of rats of Doxo then BAE group
Micrograph of Doxo then BAE cardiac muscle cells with cytoplasmic vacuolization (H and E, ×200)

showed a highly significant improvement in the levels of serum inflammatory cytokines TNF-α, IL-1β, IL-6 and serum PON1 activity when compared to results of BAE→Doxo-treated animals or Doxo→BAE-treated animals (Table 5).

Histopathological investigation: Histopathological assessment of cardiac tissues of rats of the different groups showed that control rats exhibited normal architecture and histology of cardiac tissues (Fig. 3a), similarly, BAE-administrated animals showed normal cardiomyocytes that were similar to those of the control group (Fig. 3b). In contrast, Doxo-administrated animals showed cytoplasmic vacuolization and marked degeneration (Fig. 4a-b). Interestingly, treatment of rats with Doxo combines with BAE or those administrated with BAE before Doxo showed cardiac muscle cells with normal appearance (Fig. 5a-b), while the animal's group that intoxicated with Doxo before being treated with BAE still performing a cytoplasmic vacuolization in cardiac muscle cells (Fig. 6).

DISCUSSION

Although chemotherapy showed great beneficial effects in the treatment of numerous types of tumours, it may induce severe toxic side effects. There are many kinds of anticancer drugs such as Doxorubicin® (Doxo), which is used in the treatment of solid and hematologic tumours[19]. Doxo is a quinone compound that was reduced by oxidoreductases within the cell including NADPH oxidase, xanthine oxidase and mitochondrial electron transport chain enzymes, especially complex I. Reduction and oxidation reaction produced semiquinone compound that interacts with oxygen to form the superoxide anion. Doxo has a great affinity towards cardiolipin, a negatively charged phospholipid that is located much in the inner mitochondrial membrane of cardiac cells. On the molecular level, Doxo interacted with DNA is the main cause of the cytostatic effect of anthracyclines, DNA binding and cross-linking, inhibition of topoisomerase II and induction of apoptosis are the mechanisms of Doxo-induced toxicity. Doxorubicin inhibited mitochondrial respiration and induced mitochondrial bioenergetics failure[20]. Another cause of oxidative stress and production of ROS is the ability of Doxorubicin to chelate free iron forming Doxorubicin-iron complexes which promotes oxidative stress[21].

A large number of plants worldwide have revealed strong antioxidant activity and strong scavenger activity against free radicals[6,21,22]. Flavonoids are a group of polyphenolic

compounds with known free radical scavenging, hydrolytic and oxidative enzyme inhibition and anti-inflammatory properties[7,22,23].

Doxorubicin treatment 0.5 mg kg^{-1} b.wt., intraperitoneally into adult male Albino rats induced significant elevations in LDH and CK-MB activities, these findings correlate with the previous reports which suggest that Doxo-induced oxidative stress can lead to lipid peroxidation which is accompanied by the release of these enzymes into serum[1,24].

The administration of Doxo resulted in a significant elevation in the levels of cardiac NO and MDA coupled with decreased cardiac GSH, SOD and CAT compared to the control group, these results are in line with those of[1,25,26], these results may be due to oxidative damage in heart tissue that resulted in lipid peroxidation with the concomitant production of MDA and reduction in GSH content. The increase in NO level can be explained by the ability of Doxo to mediate the induction of expression of nitric oxide synthase (NOS) and thus, NO release in the heart[26]. Previous studies have suggested that endothelial cell stimulation with calcium mobilizing agents stimulates and dissociates the eNOS bound membrane. Because Doxo-induced toxicity is mediated by intracellular H_2O_2 as well as calcium influx, Doxo treatment causes an increase in eNOS transcription and protein activity in aortic endothelial cells and thus NO synthesis.

The overproduction of ROS, caused by Doxo-injection, accounted for this decline in GSH content, as these species are detoxified by endogenous antioxidants mainly GSH causing their cellular stores to be depleted[1-3,6,7,27,28]. The observed decrease in cardiac GSH content could also be due to the enhanced activities in GSH metabolizing enzymes and SOD as shown in the present study.

BAE showed potent free radical scavenging activity. This potent antioxidant activity was attributed to the presence of phytochemical compounds such as, furanocoumarins, quercetins, coumarins, glycosides, saponins, alkaloids and flavonoids, which are responsible for its[29] treatment with *Balanites aegyptiaca* extract showed a reduction in circulating CK-MB levels with a simultaneous increase in endogenous antioxidant components (SOD, GSH and CAT). Concomitantly a decrease in the extent of lipid peroxidation was also observed[6].

High blood lipid levels accelerate atherosclerosis and are thought to be an important risk factor for myocardial infarction[6,10,29]. Doxorubicin® injection resulted in a significant elevation of the lipids level in blood and induced hyperlipidemia. Experimental hypertriglyceridemia in Doxorubicin®-injected rats might be due to a decline in the lipoprotein lipase activity in the myocardium leading to lesser uptake of triglycerides from the blood circulation. These results are in agreement with the report of[29,30].

Doxo-treatment resulted in an elevation of CK-MB activity due to damaged membranes of cardiomyocytes indicating cardiotoxicity. *Balanites aegyptiaca* extract was found to inhibit the Doxo-induced CK-MB release in the serum of rats. The following study has revealed that *Balanites aegyptiaca* extract treatment led to inhibition of CK-MB release in a dose-dependent manner. This inhibition of serum CK-MB activity with *Balanites aegyptiaca* extract is splendidly correlated with histopathological studies of rat myocardium[30,31].

Cardioprotective activity of *Balanites aegyptiaca* extract was further supported by increased myocardial antioxidant enzyme activity and decreased extent of lipid peroxidation[32].

Administration of *Balanites aegyptiaca* extract in combination with Doxorubicin® reduced the elevation in the levels of total cholesterol, triglycerides and LDL-Cholesterol induced and prominently raised the HDL-Cholesterol level. The probable mechanism responsible for this action may be due to the decrease in the biosynthesis of cholesterol in the liver and heart or by inhibiting enzymes responsible for the synthesis of cholesterol under the influence of the hypolipidemic constituents of *Balanites aegyptiaca*. The reduction of total lipids, cholesterol and triglycerides in the present study may be attributed to the increased clearance and decreased production of the major transporters of endogenously synthesized total cholesterol and triglycerides. All these observations indicated the hypolipidemic effect of *Balanites aegyptiaca* extract[32].

The obtained data showed that treatment of adult male albino rats with aqueous extract *Balanites aegyptiaca* reduced the proinflammatory cytokines and chemokines, including tumour necrosis factor-(TNF-α), Interleukin-1β (IL-1β) and Interleukin-6 (IL-6) levels coupled with increased PON1 compared to the control group, evidencing the biological safety of extract. These findings are in agonist with the finding of Zhang *et al*.[33]. In contrast, the present study pointed that IP injection of Doxorubicin® only induced a significant elevation in TNF-α, IL-1β and IL-6 matched with a significant reduction in the PON1. These findings are in constituent with the reports of Zhang *et al*.[33].

Moreover, the present study revealed that administration of the rats with the aqueous extract of *Balanites aegyptiaca* before, after and with companied Doxorubicin® resulted in a significant decrease in TNF-α, IL-1β and IL-6 levels matched with a significant enhancement of PON1 compared to the

Doxorubicin®-intoxicated group. These findings are concomitant with the reports of previous studies[6,34].

The mechanisms of Doxorubicin® mediated cell death include oxidative stress, apoptosis, intracellular calcium dysregulation, topoisomerase II poisoning, DNA adducts formation and ceramide overproduction[29,30]. Over-production of reactive oxygen can result in not only direct organ injury but also exacerbation of the inflammatory reaction simultaneously. The release of pro-inflammatory cytokines and chemokines, including Tumour Necrosis Factor-(TNF-α), Interleukin-1β (IL-1β) and Interleukin-6 (IL-6), the most important cytokines mediating the inflammatory response, normally triggers beneficial host innate immune response to confining tissue damage[6,33].

Doxo-derived ROS could act as intrinsic stress that activates Mitogen-Activated Protein Kinases (MAPK), p38, JNK and NF-B pathways as well as intracellular p53 accumulation, leading to an increase in pro-inflammatory cytokines (TNF-α and IL-1β) and alteration in the ratio of pro-apoptotic proteins to anti-apoptotic proteins (e.g., Bax to Bcl-2), cytochrome C (Cyto C) release and Caspase-3 (C3) activation[33].

Animals treated with the *B. aegyptiaca* accompanied with Doxo showed significantly lower levels of these inflammatory markers compared with Doxo only. *B. aegyptiaca* inhibit inflammatory markers like TNF-α, IL-1β and IL-6 attributable to its inhibitory effect on the release of the inflammatory mediators TNF-α and IL-1β but also its antioxidant properties. In other words, the mechanism of the inhibitory effects, by which *B. aegyptiaca* protects against toxicity, may involve radical scavenging. The antioxidant activity of *B. aegyptiaca* may be due to its high content of vanillic acid, syringic acid and β-sitosterol which act as a free radical scavenger and enhance antioxidant activity[34].

This histopathological change in heart that intoxication of rats with Doxorubicin® showed high value of focal rarefactions and myocyte degeneration with cytoplasmic vacuoles and loss of myofibrils are in agreement with[27,34]. Doxorubicin®-administered cardiotoxicity shows myocardial necrosis with an accumulation of inflammatory cells, vacuolization and overall enlargement of the myocardium. The production of Reactive Oxygen Species (ROS) is considered to be a major factor in oxidative cell injury. *Balanites aegyptiaca* inhibited and ameliorated, in a duration dependant manner, the Doxorubicin®-induced cardiotoxicity in rats, possibly by blocking the formation of free radicals generated during Doxorubicin® metabolism or by the scavenging action of the free radicals, these results are following previous reports[34,35]. Histopathological investigations are in good agreement with biochemical changes, findings of the present study indicated that traditional plants extract showed a significant cardioprotective and curative activity against Doxo-induced cardiac injury.

CONCLUSION

The present study concluded *Balanites aegyptiaca* extract may be as promising as cardioprotection against Doxorubicin® in cardiotoxicity through their antioxidant and radical scavenging activities as they normalize lipid peroxidation mechanism and its related biochemical changes. The aqueous extract of *Balanites aegyptiaca* has an anti-inflammatory effect by improving the levels of interleukins to normal levels.

SIGNIFICANCE STATEMENT

This study discovered the effect of aqueous extract of *Balanites aegyptiaca* against cardiotoxicity, the extract can be useful for protection against Doxorubicin cardiotoxicity in male rats. This study will help researchers to uncover the critical areas of using an aqueous extract that many researchers were not able to explore.

ACKNOWLEDGMENTS

The authors would like to thank the colleagues at medical Physiology Laboratories and workers at the animal house colony, National Research Centre, Giza, Egypt.

REFERENCES

1. Ahmed, A.Z., S.M. Satyam, P. Shetty and M.R. D'Souza, 2021. Methyl gallate attenuates doxorubicin-induced cardiotoxicity in rats by suppressing oxidative stress. Scientifica, Vol. 2021. 10.1155/2021/6694340.
2. Bansal, N., M.J. Adams, S. Ganatra, S.D. Colan and S. Aggarwal *et al*., 2019. Strategies to prevent anthracycline-induced cardiotoxicity in cancer survivors. Cardio-Oncology, Vol. 5. 10.1186/s40959-019-0054-5.
3. Osataphan, N., A. Phrommintikul, S.C. Chattipakorn and N. Chattipakorn, 2020. Effects of doxorubicin induced cardiotoxicity on cardiac mitochondrial dynamics and mitochondrial function: Insights for future interventions. J. Cell. Mol. Med., 24: 6534-6557.
4. Elkareem, H.F.A., A.E.N.I. Essawy, M. Ashry, M.A. Badawi and K.G. Abdel-Wahhab, 2021. Ameliorating and anti-inflammatory role of *Balanites aegyptiaca* aqueous extract on doxorubicin-induced hepatotoxicity in male wistar rats. Egypt. Pharm. J., 20: 157-165.

5. Carrasco, R., R.L. Castillo, J.G. Gormaz, M. Carrillo and P. Thavendiranathan, 2021. Role of oxidative stress in the mechanisms of anthracycline-induced cardiotoxicity: Effects of preventive strategies. Oxid. Med. Cell. Longevity, Vol. 2021. 10.1155/2021/8863789.
6. Montasser, A.O.S., H. Saleh, O.A. Ahmed-Farid, A. Saad and M.A.S. Marie, 2017. Protective effects of *Balanites aegyptiaca* extract, melatonin and ursodeoxycholic acid against hepatotoxicity induced by methotrexate in male rats. Asian Pac. J. Trop. Med., 10: 557-565.
7. Alansari, A.K.A. and S.A. Al-Thobaiti, 2021. *Balanites aegyptiaca* kernel extract (desert date) protects against diabetes-induced cardiomyopathy in rats: A histological and biochemical study. J. North Basic Appl. Sci., 6: 30-46.
8. Shafik, N.H., R.E. Shafek, H.N. Michael and E.F. Eskander 2016. Phytochemical study and antihyperglycemic effects of *Balanites aegyptiaca* kernel extract on alloxan induced diabetic male rat. J. Chem. Pharm. Res., 8: 128-136.
9. Murthy, H.N., G.G. Yadav, Y.H. Dewir and A. Ibrahim, 2021. Phytochemicals and biological activity of desert date (*Balanites aegyptiaca* (L.) Delile). Plants, Vol. 10. 10.3390/plants10010032.
10. Berkovich, L., G. Earon, I. Ron, A. Rimmon, A. Vexler and S. Lev-Ari, 2013. *Moringa Oleifera* aqueous leaf extract down-regulates nuclear factor-kappaB and increases cytotoxic effect of chemotherapy in pancreatic cancer cells. BMC Complementary Altern. Med., Vol. 13. 10.1186/1472-6882-13-212.
11. Abdel-Wahhab, K.G., F.A. Mannaa, M. Ashry, D.M. Khaled, L.K. Hassan and H.F. Gomaa, 2021. *Chenopodium quinoa* ethanolic extract ameliorates cyclophosphamide®-induced hepatotoxicity in male rats. Comp. Clin. Pathol., 30: 267-276.
12. Jayaprakasha, G.K. and L.J. Rao, 2000. Phenolic constituents from the lichen Parmotrema stuppeum (Nyl.) Hale and their antioxidant activity. Z. Naturforsch. C, 55: 1018-1022.
13. Nogala-Kalucka, K., J. Korczak, M. Dratwia, E. Lampart-Szczapa, A. Siger and M. Buchowski, 2005. Changes in antioxidant activity and free radical scavenging potential of rosemary extract and tocopherols in isolated rapeseed oil triacylglycerols during accelerated tests. Food Chem., 93: 227-235.
14. Rajasekar, S. and R. Elango, 2011. Estimation of alkaloid content of Ashwagandha (*Withania somnifera*) with HPLC methods. J. Exp. Sci., 2: 39-41.
15. Trivedi M.K., A. Branton, D. Trivedi, G. Nayak and C.D. Nykvist *et al*., 2017. Liquid chromatography-mass spectrometry (LC-MS) analysis of *Withania somnifera* (Ashwagandha) root extract treated with the energy of consciousness. Am. J. Quantum Chem. Mol. Spectrosc., 1: 21-30.
16. Pepato, M.T., R.H. Migliorini, A.L. Goldberg and I.C. Kettelhut, 1996. Role of different proteolytic pathways in degradation of muscle protein from streptozotocin-diabetic rats. Am. J. Physiol. Endocrinol. Metab., 271: E340-E347.
17. Charlton-Menys, V., Y. Liu and P.N. Durrington, 2006. Semiautomated method for determination of serum paraoxonase activity using paraoxon as substrate. Clin. Chem., 52: 453-457.
18. Ruiz-Larrea, M.B., A.M. Leal, M. Liza, M. Lacort and H. de Groot, 1994. Antioxidant effects of estradiol and 2-hydroxyestradiol on iron-induced lipid peroxidation of rat liver microsomes. Steriods, 59: 383-388.
19. Gyöngyösi, M., D. Lukovic, K. Zlabinger, A. Spannbauer and A. Gugerell *et al*., 2020. Liposomal doxorubicin attenuates cardiotoxicity via induction of interferon-related DNA damage resistance. Cardiovasc. Res., 116: 970-982.
20. Khalil, N.S.A., A.S. Abou-Elhamd, S.I.A. Wasfy, I.M.H.E. Mileegy, M.Y. Hamed and H.M. Ageely, 2016. Antidiabetic and antioxidant impacts of desert date (*Balanites aegyptiaca*) and Parsley (*Petroselinum sativum*) aqueous extracts: Lessons from experimental rats. J. Diabetes Res. Vol. 2016. 10.1155/2016/8408326.
21. Jaheed, E., A.H. Mohamed, N.M.F. Hassan, K.M.A. Mahran, S.M. Nasr and H.A.A. Abou-Zeina, 2019. Evaluation of the curative effect of *Balanites aegyptiaca* fruits ethanolic extract on haemonchosis experimentally induced in Egyptian Baladi goats: Phytoanalytical, parasitological and hematological studies. J. Parasitic Dis., 43: 638-650.
22. Abdelaziz, S.M., F.M.M. Lemine, H.O. Tfeil, A. Filali-Maltouf and A.O.M.S. Boukhary, 2020. Phytochemicals, antioxidant activity and ethnobotanical uses of *Balanites aegyptiaca* (L.) Del. fruits from the arid zone of Mauritania, Northwest Africa. Plants, Vol. 9. 10.3390/plants9030401.
23. Sadat-Hoseini, S.K. and V.D. Roshan, 2017. Is sub-chronic exercise in combination with medicinal nanoparticles a protective strategy against doxorubicin-induced hepatic oxidative stress and apoptosis in aging model rats?. Nanomed. J., 4: 224-231.
24. Qin, Y., T. Guo, Z. Wang and Y. Zhao, 2021. The role of iron in doxorubicin-induced cardiotoxicity: Recent advances and implication for drug delivery. J. Mater. Chem. B, 9: 4793-4803.
25. Mhya, D.H., K.M. Anigo, I.A. Umar and J.O. Alegbejo, 2018. Mechanisms and antidiabetic compounds of *Balanites aegyptiaca* delile plant: A mini-review. South Asian Res. J. Nat. Prod., 1: 1-9.
26. Safdar, M.N., T. Kausar, M. Nadeem, S. Jabbar and S. Ahmed 2017. Cardioprotective effect of mango and kinnow peel extracts on doxorubicin-induced cardiotoxicity in albino rats. B. Life Environ. Sci., 54: 219-235.

27. Sule, O.J., E.M. Arhoghro and P. Erigbali, 2017. Cardioprotective effect of *Commelina diffusa* leaf extract on doxorubicin induced cardiomyopathy in rats. World J. Pharm. Pharm. Sci., 6: 200-211.
28. Zilinyi, R., A. Czompa, A. Czegledi, A. Gajtko, D. Pituk, I. Lekli and A. Tosaki, 2018. The cardioprotective effect of metformin in doxorubicin-induced cardiotoxicity: The role of autophagy. Molecules, Vol. 23, No. 5. 10.3390/molecules23051184.
29. Syahputra, R.A., U. Harahap, A. Dalimunthe, M. Pandapotan and D. Satria, 2021. Protective effect of *Vernonia amygdalina* Delile against doxorubicin-induced cardiotoxicity. Heliyon, Vol. 7. 10.1016/j.heliyon.2021.e07434.
30. El-Moselhy, M.A. and A.A.K. El-Sheikh, 2014. Protective mechanisms of atorvastatin against doxorubicin-induced hepato-renal toxicity. Biomed. Pharmacother., 68: 101-110.
31. Saleem, M.T.S., M.C. Chetty and S. Kavimani, 2014. Antioxidants and tumor necrosis factor alpha-inhibiting activity of sesame oil against doxorubicin-induced cardiotoxicity. Ther. Adv. Cardiovasc. Dis., 8: 4-11.
32. Gao, Y., H. Yang, Y. Fan, L. Li, J. Fang and W. Yang, 2016. Hydrogen-rich saline attenuates cardiac and hepatic injury in doxorubicin rat model by inhibiting inflammation and apoptosis. Mediators Inflammation, Vol. 2016. 10.1155/2016/1320365.
33. Zhang, J., Q. Wu, S. Song, Y. Wan, R. Zhang, M. Tai and C. Liu, 2014. Effect of hydrogen-rich water on acute peritonitis of rat models. Int. Immunopharmacol., 21: 94-101.
34. Jaćević, V., V. Dragojević-Simić, Ž. Tatomirović, S. Dobrić and D. Bokonjić *et al*., 2018. The efficacy of *Amifostine* against multiple-dose doxorubicin-induced toxicity in rats. Int. J. Mol. Sci., Vol. 19. 10.3390/ijms19082370.
35. Supriya, C.S. and K.P.S. Gowda, 2017. Evaluation of hepatoprotective activity of extracts of *Balanties aegyotiaca* fruits in experimentally induced hepatic damage in rats. World J. Pharm. Pharm. Sci., 6: 1271-1288.