Ameliorating and anti-inflammatory role of *Balanites aegyptiaca* aqueous extract on Doxorubicin-induced hepatotoxicity in male Wistar rats

Hanaa F.A. Elkareem[a], Abd El-Naby I. Essawy[a], Mahmoud Ashry[b], Manal A. Badawi[c], Khaled G. Abdel-Wahhab[d]

[a]Department of Chemistry, Faculty of Science, Fayoum University, Fayoum, [b]Department of Zoology, Faculty of Science, Al Azhar University, Assiut, Departments of [c]Pathology, [d]Medical Physiology, National Research Centre, Giza, Egypt

Correspondence to Khaled G Abdel-Wahhab, Department of Medical Physiology, National Research Centre, Giza 12622, Egypt.
Tel: +20 111 091 7108;
e-mail: kgm194@yahoo.com

Received: 5 January 2021
Revised: 22 February 2021
Accepted: 17 March 2021
Published: 16 June 2021

Egyptian Pharmaceutical Journal 2021, 20:157–165

Background and objective
Doxorubicin (Doxo) is an antibiotic that used in cancer treatment, with many complications like hepatotoxicity. The objective of this study was to explore the ameliorative effect of *Balanites aegyptiaca* aqueous extract (BAE) against Doxo-induced hepatotoxicity in male rats.
Materials and methods
Adult male Wistar rats (140–160 g) were randomly divided into six groups (10 animals each) as follows: group I, normal rats act as a control group; group II, rats ingested with BAE (200 mg/kg) for 4 weeks; group III, rats intoxicated (intraperitoneal) with Doxo (0.5 mg/kg) for 4 weeks; group IV, rats ingested with BAE in combination with Doxo injection for 4 weeks; group V, rats ingested with BAE for 4 weeks before Doxo injection for another 4 weeks; and group VI, rats ingested with BAE for 4 weeks after Doxo injection.
Results
The results revealed all BAE regimens succeeded to decrease the hepatotoxicity induced by Doxo. This was evidenced by the significant reduction of serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin, urea, creatinine, tumor necrosis factor alpha, and interleukin-1beta levels, as well as hepatic malondialdehyde and nitric oxide levels. Moreover, a marked increase was observed in serum protein and albumin levels, as well as hepatic-reduced glutathione, superoxide dismutase, and catalase values. The obvious histopathological regenerations came in line with both serum and tissue biochemical findings. The Doxo-BAE combined regimen exhibited the highest potential of amelioration.
Conclusion
As a promising supplement, BAE exhibited hepatoprotective potential against Doxo-induced hepatic injuries; this could be mechanized through its antioxidant and radical scavenging exhibition of its bioactive constituents.

Keywords:
Balanites, cancer, Doxorubicin, hepatotoxicity, rats

Egypt Pharmaceut J 20:157–165

1687-4315

Introduction

Cancer is one of the most of common causes of death worldwide [1], and there are many anticancer therapies, such as Doxorubicin (Doxo), which result in various adverse effects [2]. Doxo has been documented in the treatment of several human neoplasms; however, owing to its toxic effects on several organs (heart, liver, and kidney) and its hematologic and testicular toxicity, the use of Doxo in clinical chemotherapy is limited [3]. Doxo binds to DNA and stabilizes the topoisomerase II complex, thus inducing apoptosis, mainly in cancer cells, by blocking the cell cycle [4]. However, its clinical utility in cancer care has been limited by the toxic effects of Doxo [5]. Oxidative stress and free radical development have been suggested to be involved in both Doxo's antineoplastic and toxic effects [6]. Several studies have concluded that Doxo semiquinone creates free radical formation, such as superoxide and hydrogen peroxide radicals [7,8].

Necrosis, steatosis, fibrosis, cholestasis, and vascular injury can be caused by hepatotoxicity [9]. During cancer chemotherapy treatment, liver damage is represented by not only hepatotoxic anticancer drugs but also antibiotics, analgesics, antiemetics, or other drugs. Pre-existing medical conditions, tumors, immunosuppression, hepatitis viruses and other infections, and nutritional deficiencies or complete

parenteral nutrition may affect the vulnerability of the host to liver injury. It is also difficult to relate hepatic damage to a toxic reaction [10,11]. Although the liver performs many metabolic functions, there are no appropriate quantitative markers used in normal practice for liver function. The stage or definition of acute hepatotoxicity is based primarily on liver biopsy [12]. There are several drugs that may trigger liver damage, but most hepatotoxic drug reactions are either idiosyncratic through immunological mechanisms or by host metabolic response variations [13]. Usually, all these reactions are not dose dependent. Pre-existing liver disease, in general, has no effect on the elimination and toxicity of the majority of medicines [14,15].

Over 4000 years ago, herbal medicines were used around the world for the treatment of different diseases owing to the presence of beneficial chemical elements in them. In essence, plants' medicinal potential lies in their phytochemical constituents, which when applied to the human body, generate a definite pharmacological action. In medicinal plants, leaves, vegetables, and fruits that have their own defense mechanisms and defensive ions from different diseases, phytochemicals occur naturally [16].

Balanites aegyptiaca (BA) is a wild plant that is popular in Egypt. It is regarded as the Date of the Desert. It includes 1.5% protein and 37% sugars, as well as 15% organic acids in the fruit mesocarp. The phytochemical composition of the various sections of the plant shows that, in addition to fatty acids and sterols, it contains high concentrations of saponins and moderate amounts of tannins, flavonoids, and cardiac glycosides [17–19]. BA stem bark aqueous extract protected the hepatocytes in biliary duct ligated rats, shown by dose-reliant reduction in serum bilirubin levels [20–22]. The desert fruit mesocarp shows increased hepatotoxicity caused by Doxo in rats [23]. The purified fractions of BA own considerable antioxidant [24] and anti-inflammatory [25] activities. The main objective of this study was to explore the ameliorative potential of *Balanites aegyptiaca* aqueous extract (BAE) against Doxo-induced hepatic, immunological, and oxidative deterioration.

Materials and methods

This study dealt with the aqueous extract of the herbs rather than that of organic solvents; this is owing to the possible effects of the organic solvents on the conformational and configurationally structure of the extract components. *B. aegyptiaca* is purchased from the stores of Abd El-Rahman Harraz (Bab El-Khalk Zone, Cairo, Egypt).

Balanites aegyptiaca aqueous extract preparation

BAE was carried out according to the method of Berkovich *et al.* [26]. First, the dry leaves of *B. aegyptiaca* were powdered and kept dry in an air-tight container before the extraction. Second, the aqueous extract of the powdered herb was prepared in the laboratory by mixing 50 g of the dry-powdered leaves with 500-ml boiling distilled water for 15 min. The mixture was then filtered through sterile Whatman filter paper number 42 (Whatman International Ltd, Maidstone, England) using a Buchner funnel. In Aroma and Flavoring Department, National Research Centre, the filtrate was subjected to lyophilization process through freeze drier (Snijders-Scientific, Tilburg, the Netherland) under pressure of 0.1–0.5 mBar and temperature of −35 to 41°C. The dry extract was stored in a dark bottle at −20°C until usage.

Determination of total extract yield

The combined extracts were transferred to a quick fit round bottom flask with known weight (W1), freeze dried, and weighted again (W2), and finally, the yield was calculated from the following formula: [extract yield (g/g crude herb)=(W2–W1)/W3], where, W1 is the weight of clear and dry quick fit flask (g), W2 is the weight of the flask after lyophilization (g), and W3 is the weight of the crude powdered herb (g).

Determination of total phenolic content

Phenolic content of the balanites aegyptiaca aqueous extract (BEE) was performed by dissolving five mg of the extract in 10 ml mixture of acetone and water (6:4 v/v). Then, a sample of 0.2 ml was mixed with 1.0 ml of Folin-Ciocalteu reagent (10 fold diluted) and 0.8 ml of sodium carbonate solution (7.5%). After 30 min at room temperature, the absorbance was measured at 765 nm using Cary 100 ultraviolet–visible spectrophotometer. Estimation of phenolic compounds as catechin equivalents was carried out using the standard curve [27].

DPPH radical scavenging activity

The capacity of antioxidants of BEE to quench DPPH (1,1 Diphenyl 2 Picrylhydrazyl) radical scavenging activity radical was determined as previously described by Nogala-Kalucka *et al.* [28]. In this method, certain amount of the crude extract was dissolved in methanol to obtain a concentration of 200 ppm. A volume of 0.2 ml of this solution was completed to 4 ml by methanol, and 1-ml DPPH

solution (6.09×10^{-5} mol/l), in the same solvent, was then added. The absorbance of the mixture was measured at 516 nm after 10-min standing. The reference sample (blank) was 1 ml of DPPH solution and 4 ml of methanol. Triplicate measurements were made, and the percentage of radical scavenging activity was calculated according to the following equation:

$$\mathrm{RSA}(\%) = \frac{\left(\mathrm{A}_{\text{control sample}} - \mathrm{A}_{\text{sample extract}}\right)}{\mathrm{A}_{\text{control sample}}} \times 100.$$

Animal and experimental design

This study was conducted on adult male Wistar albino rats (140–160 g) obtained from Animal Colony, National Research Centre, Cairo, Egypt. The animals were housed in suitable plastic cages for 1 week for acclimation. Excess tap water and standard rodent pellets [20.3% protein (20% casein and 0.3% DL-methionine), 5% fat (corn oil), 5% fibers, 3.7% salt mixture, and 1% vitamin mixture, obtained from Meladco Company, El-Obour City, Cairo, Egypt] were always available. 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). After animals were acclimatized with the experimental room conditions, they were randomly divided into six groups (10 animals each): group I, normal rats daily administrated 0.4 ml/kg saline by oral intubation for 4 weeks and act as control; group II, animals subjected to daily oral administration of BAE (200 mg/kg) for 4 weeks; group III, animals subjected to intraperitoneal injection of the anticancer drug (Doxo) at dose of 0.5 mg/kg for 4 weeks; group IV, rats administrated orally with *B. aegyptiaca* extract in combination with Doxo injection for 4 weeks; group V, rats orally administrated with *B. aegyptiaca* extract before intoxication with Doxo for 4 weeks; and finally, group VI, animals postadministration of *B. aegyptiaca* extract for 4 weeks after intoxication with Doxo.

Blood and tissue sampling

At the end of the treatment period (4 weeks), rats were weighed and then fasted overnight. Following anesthesia (inhalation with diethyl ether), blood specimens were withdrawn from the retro-orbital plexus using heparinized and sterile glass capillaries; whole blood specimens were cool centrifuged at 3000 rpm for 10 min and the sera were separated, divided into aliquots, and stored at −80°C till biochemical measurements, which were carried out immediately. Then after blood collection, the animals were killed soon, and then the liver of each animal was dissected out. One part of the liver of each animal was washed in saline, dried, rolled in a piece of aluminum foil, and stored at −80°C for either biochemical determinations. Another portion of the liver was soaked in formalin-saline (10%) buffer for histopathological processing and microscopic examination.

Serum biochemical and immunological measurements

Serum aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), and alkaline phosphatase (ALP) activities were determined photometrically using reagent kits purchased from Human Gesell Schaft fur Biochemical und Diagnostic mbH, Germany. Serum urea, creatinine, bilirubin, albumin, and total protein levels were determined using reagent kits purchased from DiaSys Diagnostic Systems GmbH, Germany. Using ELISA (Dynatech Microplate Reader Model MR 5000, Texas, USA), serum tumor necrosis factor alpha (TNF-α) and interleukin-1beta (IL-1β) levels were measured using reagent kits purchased from SinoGeneClon Biotech Co. Ltd (Hang Zhou, China).

Tissue homogenization and oxidative stress markers

A specimen from the liver organ was homogenized in ice-cold phosphate buffer (50 mM, pH 7.4) to give 10% homogenate (w/v); the homogenate was centrifuged at 5000 rpm for 20 min to remove the nuclear and mitochondrial fractions. The supernatant was divided into aliquots and stored at −80°C till the biochemical measurements. Hepatic glutathione (GSH), nitric oxide (NO), superoxide dismutase (SOD), and catalase (CAT) values were estimated using reagent kits obtained from Biodiagnostic (Dokki, Giza, Egypt). Hepatic malondialdehyde (MDA) (the most abundant individual aldehyde resulting from lipid peroxidation breakdown in biological systems and used as an indirect index for lipid peroxidation) level was determined chemically as described by Ruiz-Larrea *et al.* [29] through MDA reaction with thiobarbituric acid forming a pink complex that can be measured photometrically.

Histopathology

Paraffin sections of 5-μm thick were stained with hematoxylin and eosin [30] and investigated by light microscope.

Statistical analysis

The obtained were subjected to one-way analysis of variance followed by Duncan post-hoc test at levels of *P* value less than or equal to 0.05 according to Steel and

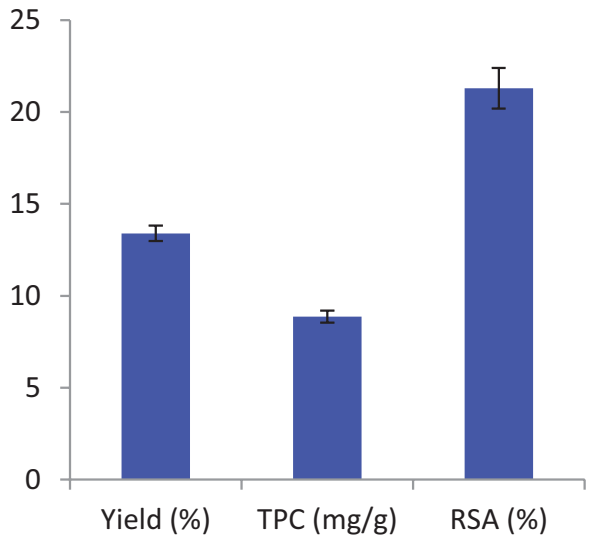
Torrie [31] using a statistical analysis system (SAS) program software, copyright 1998 by SAS Institute Inc. (Cary, North Carolina, USA).

Results

In vitro results illustrated that BAE gives a valuable yield amount: total phenolic content and radical scavenging activity (Fig. 1).

The data obtained indicated that Doxo injection induced significant elevations in serum ALAT, ASAT, ALP, urea, creatinine, and bilirubin values coupled with marked decrease in total protein and albumin values as compared with the corresponding values of the control group. Interestingly, treatment of rats with both BAE and Doxo-intoxication (at three different regimens) significantly antagonized the mentioned Doxo-deteriorating effect; this was monitored from the marked reduction in serum ASAT, ALAT, ALP, urea, creatinine, and bilirubin associated with significant rise in serum total protein and albumin level in comparison with the corresponding values of the Doxo-intoxicated group; treatment of the animals with BAE and Doxo at the same time recorded the highest ameliorating potential (Tables 1 and 2).

Figure 1



The yield (%), total phenolic content (mg/g), and radical scavenging activity (%) of BAE (mean of three replicated). BAE, *Balanites aegyptiaca* aqueous extract.

With respect to results of the hepatic oxidative status (Table 3), the present study showed that Doxo-intoxication increased the oxidative voltage of the hepatic tissue (which was evidenced through the significant increase of hepatic MDA and NO levels) and markedly weakened the antioxidant barrier (monitored from the remarkable decrease of liver GSH level and SOD and CAT activities) in comparison with that of control group. Favorably, BAE administration besides Doxo regimen-dependent improvement, as hepatic MDA and NO levels significantly decreased while the values of hepatic GSH, SOD, and CAT were raised up in comparison with the Doxo-intoxicated animals. BAE and Doxo together recorded the greatest antioxidant capacity.

Table 3 shows a significant increase occurred in serum TNF-α and IL-1β levels after Doxo-intoxication compared with that of the control group. In a promising manner, different regimens of Doxo and BAE treatments exhibited a valuable anti-inflammatory efficiency that was achieved from significant downregulation of serum TNF-α and IL-1β levels compared with Doxo-intoxicated animals; similarly, administration of Doxo together with BAE recorded the highest anti-inflammatory activity (Table 4).

Regarding histopathological examinations of the liver sections of control, Doxo-intoxicated and BAE-treated animals are illustrated and explained in Figs 2–7.

Table 1 Serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, creatinine and urea levels of control, Doxorubicin-intoxicated and *Balanites aegyptiaca* aqueous extract-treated animals

	ALAT (U/l)	ASAT (U/l)	ALP (U/l)	Creatinine (mg/dl)	Urea (mg/dl)
Control	83 ± 4^{B}	118 ± 11^{CD}	159 ± 6^{B}	0.7 ± 0.04^{B}	39 ± 1.5^{C}
BAE	78 ± 6^{B}	114 ± 10^{D}	159 ± 7^{B}	0.7 ± 0.2^{B}	37 ± 2.3^{C}
Doxo	130 ± 4^{A}	225 ± 13^{A}	297 ± 30^{A}	1.2 ± 0.1^{A}	127 ± 6.8^{A}
BAE+Doxo	75 ± 10^{B}	147 ± 8^{C}	141 ± 8^{B}	0.8 ± 0.04^{B}	81 ± 5.2^{B}
BAE→Doxo	88 ± 9^{B}	188 ± 6^{B}	186 ± 7^{B}	0.9 ± 0.1^{B}	97 ± 7.2^{B}
Doxo→BAE	89 ± 6^{B}	180 ± 7^{B}	165 ± 9^{B}	0.9 ± 0.03^{B}	95 ± 3.5^{B}

Data are presented as mean±SE; the data were statistically treated with one-way analysis of variance followed by post-hoc (Duncan) test at *P* value less than or equal to 0.05. Within each column, means with superscript different letters are significantly different. ALAT, alanine aminotransferase; ALP, alkaline phosphatase; ASAT, aspartate aminotransferase; BAE, *Balanites aegyptiaca* aqueous extract; Doxo is Doxorubicin.

Table 2 Serum total bilirubin, direct bilirubin, total protein and albumin levels of control, Doxorubicin-intoxicated and *Balanites aegyptiaca* aqueous extract-treated animals

	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Total protein (g/dl)	Albumin (g/dl)
Control	0.039 ± 0.002^{B}	0.16 ± 0.02^{A}	6.4 ± 0.1^{B}	3.8 ± 0.09^{A}
BAE	0.035 ± 0.002^{B}	0.15 ± 0.01^{A}	6.6 ± 0.3^{AB}	4.0 ± 0.1^{A}
Doxo	0.063 ± 0.002^{A}	0.201 ± 0.01^{B}	4.9 ± 0.2^{C}	2.9 ± 0.2^{C}
BAE+Doxo	0.049 ± 0.004^{B}	0.163 ± 0.02^{C}	7.4 ± 0.3^{A}	3.9 ± 0.1^{AB}
BAE→Doxo	0.061 ± 0.002^{A}	0.19 ± 0.01^{A}	5.5 ± 0.3^{C}	3.6 ± 0.2^{BC}
Doxo→BAE	0.059 ± 0.007^{A}	0.18 ± 0.01^{AB}	5.6 ± 0.2^{A}	3.5 ± 0.2^{ABC}

Data are presented as mean±SE; the data were statistically treated with one-way analysis of variance followed by post-hoc (Duncan) test at *P* value less than or equal to 0.05. Within each column, means with superscript different letters are significantly different. BAE, *Balanites aegyptiaca* aqueous extract; Doxo is Doxorubicin.

Table 3 Hepatic oxidative stress markers of control, Doxorubicin-intoxicated and *Balanites aegyptiaca* aqueous extract-treated animals

	MDA (mmol/g T)	NO (mmol/g T)	GSH (mg/g T)	SOD (U/g T)	CAT (U/g T)
Control	87 ± 1.9^{A}	736 ± 29^{A}	6.5 ± 0.4^{A}	5.8 ± 0.2^{D}	154 ± 3.6^{E}
BAE	90 ± 2.1^{A}	740 ± 30^{A}	6.6 ± 0.4^{A}	5.5 ± 0.2^{D}	149 ± 3.5^{E}
Doxo	46 ± 1.3^{A}	468 ± 20^{E}	4.1 ± 0.4^{D}	16.5 ± 1.6^{A}	627 ± 14^{A}
BAE+Doxo	77 ± 1.8^{D}	692 ± 26^{B}	5.5 ± 0.3^{B}	7.49 ± 0.4^{C}	241 ± 3.2^{D}
BAE→Doxo	58 ± 1.3^{C}	560 ± 21^{D}	4.5 ± 0.3^{D}	12.53 ± 0.8^{B}	558 ± 13^{B}
Doxo→BAE	61 ± 1.3^{C}	600 ± 23^{C}	5.6 ± 0.3^{C}	10.65 ± 0.9^{B}	427 ± 12^{C}

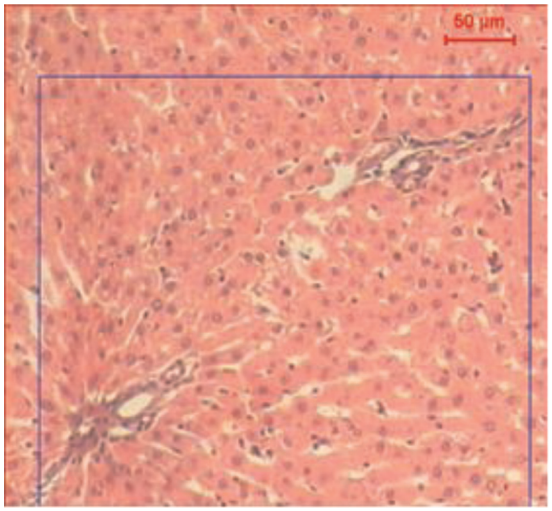
Data are presented as mean±SE; the data were statistically treated with one-way analysis of variance followed by post-hoc (Duncan) test at *P* value less than or equal to 0.05. Within each column, means with superscript different letters are significantly different. BAE, *Balanites aegyptiaca* aqueous extract; CAT, catalase; GSH, glutathione; Doxo is Doxorubicin; MDA, malondialdehyde; SOD, superoxide dismutase.

Table 4 Serum tumor necrosis factor alpha and interleukin-1beta levels of control, Doxorubicin-intoxicated and *Balanites aegyptiaca* aqueous extract-treated animals

	TNF-α (pg/ml)	IL-1β (pg/ml)
Control	23 ± 2.1^{D}	376 ± 12^{CD}
BAE	21 ± 0.9^{D}	365 ± 10^{D}
Doxo	63 ± 2.8^{A}	775 ± 16^{A}
BAE+Doxo	31 ± 1.1^{C}	403 ± 18^{C}
BAE→Doxo	48 ± 1.5^{B}	636 ± 13^{B}
Doxo→BAE	42 ± 1.8^{B}	598 ± 13^{B}

Data are presented as mean±SE; the data were statistically treated with one-way analysis of variance followed by post-hoc (Duncan) test at *P* value less than or equal to 0.05. Within each column, means with superscript different letters are significantly different. BAE, *Balanites aegyptiaca* aqueous extract; Doxo is Doxorubicin; IL-1β, interleukin-1beta; TNF-α, tumor necrosis factor alpha.

Figure 2

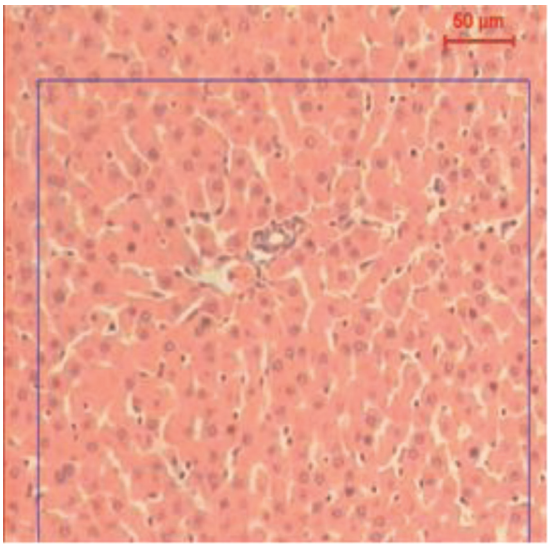


Microscopic section of control hepatic tissue showed normal portal tract with normal periportal area (hematoxylin and eosin, ×100).

Discussion

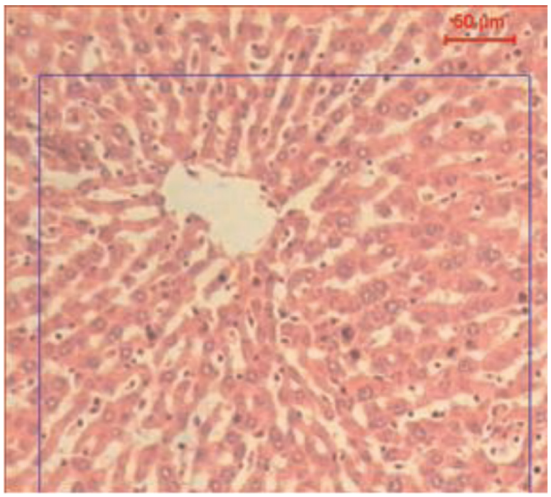
In different organ systems, including the liver, several medications used for cancer treatment are known to cause harmful adverse effects. For a long time, Doxo has been one of the most commonly used chemotherapy agents for the treatment of different cancers. However, the clinical application of this drug is complicated owing to its possible liver toxicity [32], which may also influence the metabolism and clearance of Doxo, because the liver is the main organ involved in the detoxification of Doxo. Such liver toxicity may be attributable to oxidative stress, apoptosis, and electron transport chain interference [33]. There is a need for a plan to reduce the adverse effects of anticancer drugs while maintaining their chemotherapeutic effectiveness. A large number of plant constituents worldwide exhibited strong antioxidant activity [34] and strong scavenger activity against free radicals [35]. Flavonoids in the polyphenolic compound group exhibit significant properties, including free radical scavenging, hydrolytic and oxidative enzyme inhibition, and

Figure 3



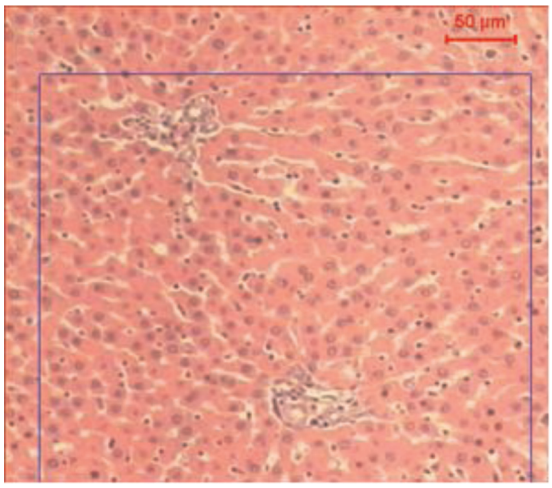
Microscopic section of hepatic tissue showed normal portal tract with normal periportal area (hematoxylin and eosin, ×100).

Figure 4



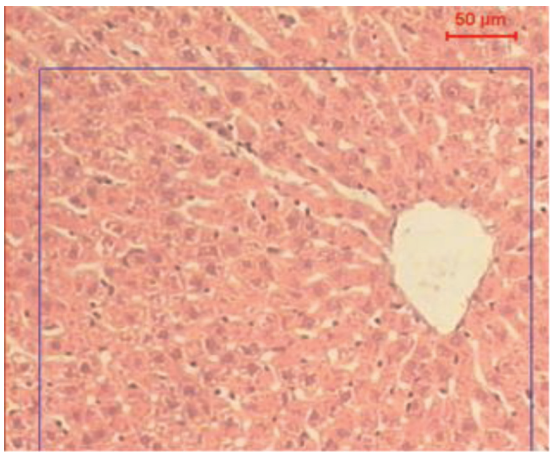
Microscopic section of doxorubicin-treated rat liver showing focal collection of mononuclear inflammatory cells, mainly in midlobular and centrilobular zone. Hepatocytes showed focal vacuolar degeneration with pycnotic nuclei (hematoxylin and eosin, ×100).

Figure 5



Microscopic section of hepatic tissue showing normal central vein with normal surrounding hepatocyte (hematoxylin and eosin, ×100).

Figure 6

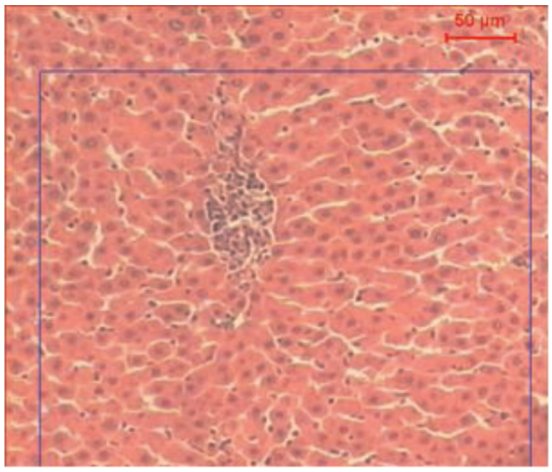


Microscopic section of hepatic tissue showing normal central vein with normal hepatocyte (hematoxylin and eosin, ×100).

anti-inflammatory action. Therefore, the present study aimed to explore the hepatoprotective and antioxidant property of BAE against Doxo-induced hepatotoxicity.

The obtained data showed that Doxo-injection induced significant elevations in serum ASAT, ALAT, and ALP activities, hepatic NO and MDA levels, coupled with decreased GSH, SOD, and CAT values compared with those of control group; these findings are in accordance with the finding of many previous studies [3,36,37]. Two distinct ways of Doxo's free radical formation have been described: the first way involves the formation of a free radical semiquinone by the action of several NADPH-dependent reductases producing Doxo's one-electron reduction to the corresponding Doxo-semiquinone. Redox cycling of Doxo-derived quinone-semiquinone produces superoxide radicals in the presence of oxygen. Second, Doxo-free radicals come from a nonenzymatic mechanism that involves iron reactions, as Fe^{3+} reacts with Doxo in a redox reaction, after which an electron is accepted by the iron atom and a Fe^{2+}/Doxo free radical complex is created that can then decrease oxygen to hydrogen. As a product of oxidative metabolism in rats, Doxo creates superoxide anion radicals, H_2O_2, and hydroxyl radicals. Oxidant damage at the cell level is attenuated by systemic battery antioxidants such as SOD, GSH, GPx, and CAT [38]. In line with this concept, the current study recorded that administration of BAE to

Figure 7



Microscopic section of rat liver with focal collection of mononuclear inflammatory cells (hematoxylin and eosin, ×100).

Doxo-intoxicated rats significantly upregulated the hepatic SOD, CAT, and GSH values concomitant with a marked reduction in hepatic NO and MDA levels. These findings are concomitant with previous reports [23,39,40]. The cytotoxic free radicals, which are produced as a consequence of Doxo-injection, damage the cell membranes [41]; this explains the impaired permeability of hepatocytes that resulted in leakage of liver enzymes and their marked rise in blood circulation. Some chemotherapeutic agents give dose-dependent hepatotoxicity [42] or depending on the pattern of administration [43]. Hepatotoxicity after chemotherapy sometimes occurs as an unpredictable reaction of an idiosyncratic form owing to immunological mechanisms or changes in the metabolic response of the host [13]. Metabolism of anthracyclines occurs predominantly in the liver, and antioxidant capacities of the liver, including those provided for in the manufacture of GSH, can protect against free radical injury [42,44]. An important factor contributing to the extension of liver damage is the ability to repair the liver.

B. aegyptiaca is a common wild plant; its fruits contain 1.5% protein and 37% sugars, as well as 15% organic acids. The phytochemical composition of its various components has shown that, in addition to fatty acids and sterols, it comprises high concentrations of saponins, moderate amounts of tannins, flavonoids, and cardiac glycosides [17–19]. The aqueous extract-covered hepatocytes in biliary duct-linked rats showed a dose-dependent decrease in serum bilirubin levels [20–22]; also, its purified fractions possess considerable antioxidant [24] and anti-inflammatory [25] activities.

Coadministration of BAE with Doxo was also shown to improve Doxo toxicity in liver tissue, indicating functional improvement of hepatocytes as a result of BAE antioxidant effect. This antioxidant activity could be due to the presence of saponins and flavonoids which are known to exhibit anti-inflammatory and antioxidant activities. Administration of BAE was able to restore the impairment in tissue antioxidant battery, thus regenerating the hepatocytes function [24].

It was earlier reported that the BAE contains furanocoumarine and flavonoids like coumarins and quercetins, alkaloids, and coumarins in the stem-bark, whereas rutins were seen in the fruit among others [45–47]. Although the exact mechanism of Doxo-induced hepatotoxicity and nephrotoxicity remains unclear, it is thought that free radical formation, iron-dependent oxidative damage to biological macromolecules, membrane lipid peroxidation, and protein oxidation mediate the toxic effects [48–50]. Moreover, NO synthase may be responsible for the reductive activation of Doxo to its free radical semiquinone form, and the subsequent oxygen radical-mediated cellular damage.

The present study pointed that Doxo-injection resulted in induction of a significant elevation in serum TNF-α and IL-1β; this finding runs in constituent with some previous reports [51–53]. Favorably, different regimens of BAE administration besides Doxo resulted in a significant decrease in TNF-α and IL-1β in compare with the corresponding levels of Doxo-intoxicated group; our data come in parallel with the previous ones [54,55].

Oxidative stress, apoptosis, intracellular calcium dysregulation, topoisomerase II toxicity, DNA adduct formation, and ceramide overproduction are among the mechanisms of Doxo-mediated cell death [56]. Overproduction of reactive oxygen can lead not only to direct organ injury but also to simultaneous exacerbation of the inflammatory reaction. The release of proinflammatory cytokines, like TNF-α and IL-1β (the most significant inflammatory response mediating cytokines), normally causes host innate immune responses to harm a restricted tissue [57]. Inhibition of hydrogen inflammation is already meaningful, as its rapid gaseous diffuse ion makes it highly effective in reducing cytotoxic radicals and has been shown to protect against damage to various organs, including the brain, liver, heart, and lungs [58,59].Doxo-derived reactive oxygen species can serve as an intrinsic stress that activates the pathways of mitogen activated

protein kinases (MAPK), p38, JNK, and NF-kB, as well as intracellular p53 accumulation, resulting in increased proinflammatory cytokines (TNF-α and IL-1β) and changes in the ratio of pro-apoptotic to anti-apoptotic proteins (Bax to Bcl-2), release of cytochrome-C, and activation of caspase-3 [60]. The anti-inflammatory exhibition of BAE could be attributable to its inhibitory effect on the release of the inflammatory mediators TNF-α and IL-1β, as well as to its antioxidant properties. In other words, the mechanism of the cytokines-inhibitory effects of BAE may involve radical scavenging. It was stated that antioxidant activity of BAE may be due to its high content of vanillic acid, syrigic acid, and β-siststerol, which act as free radical scavenger and enhance antioxidant activity [61].

Histopathological examination showed that Doxo-intoxication led to unfavorable changes such as degenerations and pleomorphism in hepatocytes, proliferation in bile duct, cytoplasmic eosinophilia, parenchymal necrosis, congestion and thrombosis in central vein, and inflammation in portal space in liver tissue; this finding is in agreement with previous reports [37,62]. Interestingly, treatments of rats with BAE and Doxo (at different-regimens) significantly alleviated the deleterious effects resulted from Doxo. BAE may act as a cofactor in the synthesis of biological endogenous antioxidant material such as GSH and/or activate endogenous antioxidant enzymes such as SOD, CAT, GPx, and GST [63]. Histopathological investigations are in a good agreement with biochemical changes; findings of the present study indicated that BAE showed a significant regenerative and curative activity against Doxo-induced hepatic injury. The bioactive phytochemical constituents of BAE are responsible for the resultant anti-inflammatory and hepatoprotective activities; this could be performed through their antioxidant potentials.

Conclusion

BAE possesses a regenerative potential against Doxo-induced hepatic injuries; especially in case of Doxo/BAE coadministration regimen. BAE is a promising protecting agent against Doxo toxicities; this could be mechanized through its antioxidant and radical scavenging activities.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Wang B, Ma Y, Kong X, Ding X, Gu H, Chu T. NAD+ administration decreases Doxorubicin-induced liver damage of mice by enhancing antioxidation capacity and decreasing DNA damage. Chem Biol Interact 2014; 212:65–71.

2 Kumral A, Giriş M, Soluk-Tekkeşin M, Olgaç V, Doğru-Abbasoğlu S, Türkoğlu Ü, *et al.* Beneficial effects of carnosine and carnosine plus vitamin E treatments on Doxorubicininduced oxidative stress and cardiac, hepatic, and renal toxicity in rats. Hum Exp Toxicol 2016; 35:635–643.

3 Barzanjeh SP, Roshan VD. Prior exercise training attenuated Doxorubicin-induced hepatic oxidative stress in Wistar rats: age-related differences. Hormozgan Med J 2017; 20:276–285.

4 Ozturk E, Kaymak E, Akin AT, Karabulut D, Unsal HM, Yakan B. Thymoquinone is a protective agentthat reduces the negative effects of Doxorubicin in rat testis. Human Exp Toxicol 2020; 39:1364–1373.

5 Fadillioglu E, Erdogan H, Sogut S. Protective effects of erdosteine against Doxorubicin-induced cardiomyopathy in rats. J Appl Toxicol 2003; 23:71–74.

6 Yagmurca M, Erdogan H, Iraz M. Caffeic acid phenethyl ester as a protective agent against Doxorubicin nephrotoxicity in rats. Clin Chim Acta 2004; 348:27–34.

7 Minotti G, Mancuso C, Frustaci A. Paradoxicol inhibition of cardiac lipid peroxidation in cancer patients treated with Doxorubicin. Pharmacologic and molecular reappraisal of anthracycline cardiotoxicity. J Clin Investig 1996; 98:650–661.

8 Singal PK, Li T, Kumar D. Adriamycininduced heart failure: mechanism and modulation. Mol Cell Biochem 2005; 207:77–86.

9 Ishak KG, Zimmerman HJ. Morphologic spectrum of drug-induced hepatic disease. Gastroenterol Clin North Am 1995; 24:759–786.

10 Benichou C. Criteria of drug-induced liver disorders: Report of an International Consensus Meeting. J Hepatol 1990; 11:272–276.

11 Maria VA, Victorino RM. Development and validation of a clinical scale for the diagnosis of drug-induced hepatitis. Hepatology 1997; 26:664–669.

12 Pratt DS, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. N Engl J Med 2000; 342:1266–1271.

13 Lee WM. Drug-induced hepatotoxicity. N Engl J Med 1995; 333:1118–1127.

14 Huet PM, Villeneuve JP, Fenyves D. Drug elimination in chronic liver diseases. J Hepatol 1997; 26:63–72.

15 Schenker S, Martin RR, Hoyumpa AM. Antecedent liver disease and drug toxicity. J Hepatol 1999; 31:1098–1105.

16 Supriya CS, Gowda KPS. Evaluation of hepatoprotective activity of extracts of *Balanites aegyptiaca* fruits in experimentally induced hepatic damage in rats. World J Pharma Pharma Sci 2017; 6:1271–1288.

17 Abdelaziz SM, Lemine FMM, Tfeil HO, Filali-Maltouf A, Boukhary AOMS. Phytochemicals, antioxidant activity and ethnobotanical uses of *Balanites aegyptiaca* (L.) Del. Fruits from the Arid Zone of Mauritania, Northwest Africa. Plants 2020; 9:401.

18 Al Ashaal HA, Farghaly AA, Abd El Aziz MM, Ali MA. Phytochemical investigation and medicinal evaluation of fixed oil of *Balanites aegyptiaca* fruits (Balantiaceae). J Ethnopharmacol 2010; 127:495–501.

19 Chothani DL, Vaghasiya HU. A review on *Balanites aegyptiaca* Del (desert date): phytochemical constituents, traditional uses, and pharmacological activity. Pharmacogn Rev 2011; 5:55–62.

20 Mohamed AH, Eltahir KE, Ali MB, Galal M, Ayeed IA, Adam SI, *et al.* Some pharmacological and toxicological studies on *Balanites aegyptiaca* bark. Phytother Res 1999; 13:439–441.

21 Jaiprakash B, Aland R, Karadi RV, Savadi RV, Hukkeri VI. Hepatoprotective activity of fruit pulp of *Balanites aegyptiaca*. Indian Drugs 2003; 40:296–297.

22 Abdel-Kader MS, Alqasoumi SI. Evaluation of the hepatoprotective effect of the ethanol extracts of *Solanum nigrum*, Cassiafistula, *Balanites aegyptiaca* and *Carthamus tinctorius* against experimentally induced liver injury in rats. Alex J Pharm Sci 2008; 22:47–50.

23 Salihu M, Nwozo SO, Oloyede BO. Aqueous extract of *Balanites aegyptiaca* del fruit mesocarp protects against CCl4-induced liver damage in rats. J Pharma Res Int 2013; 3:917–928.

24 Suky TMG, Parthipan B, Kingston C, Mohan PVR, Soris T. Hepatoprotective and antioxidant effect of *Balanites aegyptiaca* (L.) del against CCl4induced hepatotoxicity in rats. Int J Pharma Sci Res 2011; 2:887–892.

25 Gaur K, Nema RK, Kori ML, Sharma CS, Singh V. Anti-inflammatory and analgesic activity of *Balanites aegyptiaca* in experimental animal models. Int J Green Pharma 2008; 2:214–217.

26 Berkovich L, Earon G, Ron I, Rimmon A, Vexler A, Lev-Ari S. Moringa oleifera aqueous leaf extract down-regulates nuclear factor-kappaB and increases cytotoxic effect of chemotherapy in pancreatic cancer cells. BMC Complement Altern Med 2013; 13:212.

27 Jayaprakasha GK, Rao LJ. Phenolic constituents from lichen parmotrema stuppeum(NYI.) Hale and their antioxidant activity. Z Naturforsch C J Biosci 2000; 55:1018–1022.

28 Nogala-Kalucka M, Korczak J, Dratwia M, Lampart-szczapa E, Siger A, Buchowski M. Changes in antioxidant activity and free radical scavenging potential of rosemary extract and tocopherols in isolated rapeseed oil triacylglycerols during accelerated tests. Food Chem 2005; 93:227–235.

29 Ruiz-Larrea MB, Leal AM, Liza M, Lacort M, de Groot H. Antioxidant effects of estradiol and 2-hydroxyestradiol on iron- induced lipid peroxidation of rat liver microsomes. Steroids 1994; 59:383–388.

30 Drury RAB, Wallington EA. Preparation and fixation of tissues. In: Drury RAB, Wallington EA, editors. Carleton's histological technique. 5. Oxford: Oxford University Press 1980. pp. 41–54.

31 Steel RG, Torrie GH. Principles and procedures of statistics and biometrical approach. 2nd ed. New York, Toronto, London: McGraw-Hill Book Company; 1960. pp. 71–117.

32 Nirwane A, Patil R. Effect of Punica granatumseeds on Doxorubicin-induced hepatotoxicity in Wistar. Rats J Pharma Res 2012; 5:4625–4628.

33 Jung KW, Won YJ, Kong HJ, Oh CM, Lee DH, Lee JS. Prediction of cancer incidence and mortality in Korea, 2014. Cancer Res Treat 2014; 46:124.

34 Baratto MC, Tattini M, Galardi C, Pinelli P, Romani A, Visioli F, Pogni R. Antioxidant activity of galloyl quinic derivatives isolated from P. lentiscus leaves. Free Radic Res 2003; 37:405–412. ?

35 Kumaran A, Karunakaran RJ. In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT-Food Sci Technol 2007; 40:344–352.

36 Rao AY, Sudhakar G. Hepatotoxicity assement in breast cancer patients receiving doxorubicin chemotherapy. J Dent Med Sci 2015; 14:74–77.

37 Jacevic V, Simic VD, Tatomirovic Ž, Dobric S, Bokonjic D, Kovacevic A, *et al.* The efficacy of amifostine against multiple-dose doxorubicin-induced toxicity in rats. Int J Mol Sci 2018; 19:2370.

38 Kalendera Y, Yelb M, Kalender S. Doxorubicin hepatotoxicity and hepatic freeradical metabolism in rats. The effects of vitamin E and catechin. Toxicology 2005; 209:39–45.

39 Ičević ID, Vukmirović SN, Srđenović BU, Sudji JJ, Djordjevic AN, Injac RM, Vasović VM. Protective effects of orally applied fullerenol nanoparticles in rats after a single dose of Doxorubicin. Hem Ind 2011; 65:329–337.

40 Mhya DH, Anigo KM, Umar IA, Alegbejo JO. Mechanisms and antidiabetic compounds of *Balanites aegyptiaca* delile plant: a mini-review. SARJNP 2018; 1:1–9.

41 Verret V, Namur J, Ghegediban SH, Wassef M, Moine L, Bonneau M, Pelage JP, Laurent A. Toxicity of Doxorubicin on pig liver after chemoembolization with doxorubicin-loaded microspheres: a pilot DNA-microarrays and histology study. Cardiovasc Intervent Radiol 2013; l36:204–212.

42 Joshi RK, Carbone P, Wang FC, Kravets VG, Su Y, Grigorieva IV, Nair RR. Precise and ultrafast molecular sieving through graphene oxide membranes. Science 2014; 343:752–754.

43 Boussios S, Pentheroudakis G, Katsanos K, Pavlidis N. Systemic treatment-induced gastrointestinal toxicity: incidence, clinical presentation and management. Ann Gastroenterol 2012; 25:106.

44 Grigorian A, O'Brien CB. Hepatotoxicity secondary to chemotherapy. J Clin Transl Hepatol 2014; 2:95–102.

45 Maor Y, Malnick S. Liver injury induced by anticancer chemotherapy and radiation therapy. Int J Hepatol 2013; 2013:815105.

46 Hozayen WG, Abou-Seif HS, Amin S. Protective effects of ruitn and/or hesperidin against Doxorubicin-induced hepatotoxicity. Int J Clin Nutr 2014; 2:11–17.

47 Quiles JL, Huertas JR, Battino M, Mataix J, Ramı? rez-Tortosa MC. Antioxidant nutrients and adriamycin toxicity. Toxicology 2002; 180:79–95.

48 Domitrović R, Jakovac H, Tomac J, Šain I. Liver fibrosis in mice induced by carbon tetrachloride and its reversion by luteolin. Toxicol Appl Pharmacol 2009; 241:311–321.

49 Ogawa Y, Kondo T, Sugiyama S, Ogawa K, Satake T, Ozawa T. Role of phospholipase in the genesis of Doxorubicin-induced cardiomyopathy in rats. Cancer Res 1987; 47:1239–1243.

50 Li Z, Lin H, Yang S, Diehl AM. Murine leptin deficiency alters Kupffer cell production of cytokines that regulate the innate immune system. Gastroenterology 2002; 123:1304–1310.

51 Motaal AA, Shaker S, Haddad PS. Antidiabetic activity of standardized extract of *Balanites aegyptiaca* fruits using cell based bioassays. Parmacogn J 2012; 4:20–24.

52 Gidey TK. Isolation and structure elucidation of flavonoids from leaves extract of *Balanites aegyptiaca* delile. Int J Mod Chem Appl Sci 2016; 3:446–449.

53 Lahoti S, Patel T, Thekkemadom D, Beckett V, D Ray S. Doxorubicin-induced in vivo nephrotoxicity involves oxidative stress-mediated multiple pro-and anti-apoptotic signaling pathways. Curr Neurovasc Res 2012; 9:282–295.

54 Aryal M, Arvanitis CD, Alexander PM, McDannold N. Ultrasound-mediated blood-brain barrier disruption for targeted drug delivery in the central nervous system. Adv Drug Deliv Rev 2014; 72:94–109.

55 Karanovic D, Grujic-Milanovic J, Miloradovic Z, Ivanov M, Jovovic D, Vajic UJ, Mihailovic-Stanojevic N. Effects of single and combined losartan and tempol treatments on oxidative stress, kidney structure and function in spontaneously hypertensive rats with early course of proteinuric nephropathy. PLoS ONE 2016; 11:1016–1706.

56 Pugazhendhi A, Jebakumar TN, Edison I, Velmurugan BK, Jacob JA, Karuppusamy I. Toxicity of Doxorubicin (Dox) to different experimental organ systems. Life Sci 2018; 200:26–30.

57 Argun M, Üzüm K, Sönmez MF, Özyurt A, Karabulut D, Soyersarıca Z, *et al.* Cardioprotective effect of metformin against Doxorubicin cardiotoxicity in rats. Anatol J Cardiol 2016; 16:234–241.

58 Gao Y, Yang H, Fan Y, Li L, Fang J, Yang W. Hydrogen-rich saline attenuates cardiacand hepatic injury in doxorubicin rat model by inhibiting inflammation and apoptosis. Mediators Inflamm 2016; 1320365:10.

59 Montasser AOS, Saleh H, Ahmed-Farid OA, Saad A, Marie MAS. Protective effects of *Balanites aegyptiaca* extract, melatonin and ursodeoxycholic acid against hepatotoxicity induced by Methotrexate in male rats Asian Pacific. J Trop Med 2017; 10:557–565.

60 Yang F, Teves SS, Kemp CJ, Henikoff S. Doxorubicin, DNA torsion, and chromatin dynamics. Biochim Biophys Acta 2014; 1845:84–89.

61 Sarker SD, Bartholomew B, Nash RJ. Alkaloids from *Balanite aegyptiaca*. Fitoterapia 2000; 71:328–330.

62 Zhang J, Wu Q, Songetal S. Effect of hydrogen-richwater on acute peritonitis of rat models. Int Immunopharmacol 2014; 21:94–101.

63 Fukuda R, Zhang H, Kim JW, Shimoda L, Dang CV, Semenza GL. HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. Cell 2007; 129:111–122.