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Effects of *Balanites aegyptiaca* seed oil extract on doxorubicin-induced cardiotoxicity in albino rats

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Abstract

Doxorubicin is a chemotherapeutic agent used to treat cancer, but its use is associated with cardiotoxicity. This study evaluated the effects of *Balanites aegyptiaca* seed oil extract on doxorubicin-induced cardiotoxicity in rats. Extraction of the *B. aegyptiaca* seed oil was done by cold maceration, using n-hexane. Twenty-one male albino rats were used for the study. They were randomly assigned to three groups (A, B and C) of seven rats each. Baseline (day 0) parameters were assayed before groups B and C were given doxorubicin at 2.5 mg/kg body weight, by intraperitoneal injection every other day for five days to induce cardiotoxic damage. Group C rats were further treated daily with *B. aegyptiaca* oil extract by oral gavage at 2 ml/kg for seven days. Group A rats served as the normal control. Serum activities of superoxide dismutase (SOD), lactate dehydrogenase (LDH) and creatine kinase (CK), as well as malondialdehyde (MDA) and calcium levels were further assayed on days 7, 14 and 21 of the experiment. Three rats from each group were euthanized and the heart tissues were dissected and processed for histopathology. Results showed that doxorubicin administration led to significantly ($p < 0.05$) higher serum MDA levels and lower serum SOD activity on days 7, 14 and 21 in Group B rats and on days 7 and 14 in Group C rats. LDH and CK activities were significantly ($p < 0.05$) higher on day 14 in Groups B and C rats, and on day 21 in Group B rats only. Serum calcium levels was significantly ($p < 0.05$) higher on day 21 in Group C rats. There was severe coagulative necrosis, petechial haemorrhages and mild infiltration of mononuclear inflammatory cells in the interstitial and perivascular spaces of the heart tissue of the Group B rats. The heart tissue of Group C rats showed no evidence of myocardial necrosis. It was concluded that *B. aegyptiaca* seed oil extract ameliorated doxorubicin-induced cardiotoxicity, and may serve as a useful adjunct to doxorubicin chemotherapy.

Keywords: Doxorubicin; Cardiotoxicity; *Balanites aegyptiaca* seed oil extract*;* Histopathology, Oxidative stress.

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Introduction

As animals are increasingly being spared from physical injuries and infectious diseases, they are living longer and are increasingly prone to age-related diseases such as cancers. Cancers are one of the major causes of death in dogs, accounting for 27% of deaths in purebred dogs in the UK (Dobson, 2013). Cancers accounts for approximately half of the deaths in dogs over the age of ten and, approximately one in four dogs would develop cancer during their life (Dams *et al.*, 2010).

Chemotherapy is the most effective and widely used treatment for most types of malignancies (Chabner and Roberts, 2005). Cancer chemotherapy has been associated with some serious side effects which include neutropenia, severe gastroenteritis, cardiotoxicity and sepsis (Hammer *et al*., 1991). A total of 132 cancer chemotherapy drugs were approved by the US Food and Drug Administration [USFAD], and 56 out of the 132 have been reported to cause oxidative stress (Chen *et al*., 2007). One of the main disadvantage of cancer chemotherapy drugs is that they produce free radicals, which do not specifically damage the cancer cells only, but also damage the normal cells (Conklin, 2004).

Doxorubicin (DOX) belongs to the anthracycline family of drugs isolated from a pigment of *Streptomyces peucetius* and it was introduced in 1969 for cancer treatment (Johnson-Arbor and Dubey, 2021). Since then, DOX has remained one of the most effective and widely used anti-tumour drug ever developed, with high anti-neoplastic activity against breast cancer, aggressive lymphomas, solid tumours, and soft tissue sarcomas (Volkova and Russell, 2011). However, DOX use in chemotherapy has been limited due to its diverse toxicities, including stomatitis (IARC, 1979), nephropathy (Carvalho *et al.*, 2009), gastrointestinal disturbances, neurologic disturbances (Liu *et al*., 2007), tissue vesication (Dhaliwal and Kitchell, 2003), bone marrow aplasia (IARC, 1979), and its most feared side effect which is cumulative cardiotoxicity (Maluf and Spriggs, 2002).

Balanites aegyptiaca, family Zygophyllaceae, also known as 'desert date,' is a spiny shrub or tree up to l0 metres tall, widely distributed in dry land areas of Africa and South Asia (Chothani and Vaghasiya, 2011). The tree is native to much of Africa and parts of the Middle East. In India, it is particularly found in Rajasthan, Gujarat, Madhya Pradesh, and Deccan. It is one of the most common trees in Senegal (Ndoye *et al*., 2004). It is also commonly found in Jigawa, Yobe and Borno States of Nigeria (Isa *et al*., 2023).

Some of the well documented pharmacological activities of *Balanites aegyptiaca* extracts include cardio-protective cum antioxidant, anthelminthic, anti-venom, anti-bacterial, analgesic, anti-inflammatory, hepato-protective, anti-diabetic, anti-viral, *in vitro* antioxidant, xanthine oxidase and acetyl cholinesterase inhibitory, wound healing, hypocholesterolemic, mosquito larvicidal, and diuretic activities (Chothani and Vaghasiya, 2011). The seed oil of *Balanites aegyptiaca* is reported to be rich in saturated fatty acids (Hall and Walker, 1991). Reports on studies of *B. aegyptiaca* seed oil indicate that it consists of four major fatty acids: linoleic, oleic, stearic, and palmitic acid (Mohamed *et al*., 2002). The level of unsaturated fats (65%) was also higher than that of saturated fats (34.4%) in *B. aegyptiaca* seed oil (Abu-Al-Futuh, 1989). Unsaturated fatty acids had been reported to have anti-cancer and anti-mutagenic activity (O'Hagan and Menzel, 2003).

The ability to administer an effective therapeutic dose of doxorubicin in human clinical/veterinary practice is limited by the occurrence of dose-dependent cardiotoxicity. The search for agents that can ameliorate chemotherapy-induced cardiotoxicity is on, and there is little or no information in available literature on the effects of *B.*

aegyptiaca seed oil on doxorubicin-induced cardiotoxicity. The present study evaluated the effects of *B. aegyptiaca* seed oil on doxorubicin-induced cadiotoxicity in albino rats.

Materials and Methods

Experimental Animals: Twenty-one male albino rats were used for the study. They were housed in neat metal cages and acclimatized for two weeks, within which they were screened for helminths and haemoparasites by faecal floatation test and buffy-coat smears, respectively. Commercially available pelleted feed (Chikun Feeds, Kaduna, Nigeria) and water were provided for the rats *ad libitum*.

Ethical Statement: The institutional and international guidelines for the ethical care and use of animals were fully complied with during the study. The animals were humanely handled throughout the experiment.

Extraction of *Balanites aegyptiaca* seed oil: The seeds of *Balanites aegyptiaca* were retrieved from the fruit and grounded to a powder using an industrial blender. The grounded seeds (400 g) were suspended in half a litre of n-hexane and placed in an air-tight container (to reduce evaporation) and was gently but thoroughly rocked every 2 hours for 72 hours. The n-hexane extract was then carefully decanted into a graduated cylinder where it was allowed to evaporate at room temperature (25^{o}C), and the seed oil extract of about 15ml, which was left, was used for the study.

Study Design: The rats were randomly assigned to three groups (A, B and C) of seven rats each. Group A rats served as the normal control and were given water placebo. Group B rats were given intra-peritoneal injections of doxorubicin hydrochloride (Celon Laboratories, Talangana India), at the dose of 2.5mg/kg body weight every other day (days 1, 3, 5, 7, and 9) for 5 days. Group C rats were also injected intra-peritoneally with doxorubicin hydrochloride like the Group B rats, but were further treated daily with *Balanites aegyptiaca* seed oil extract at 2ml/kg body weight, by oral gavage, for 7 days. The 2.5 mg/kg body weight dose of DOX used in the study was based on earlier reports of usage for experiments in rats by Podyacheva *et al*. (2021), while the 2ml/kg body weight of B. aegyptiaca seed oil extract was also based on earlier reports of use by Ali *et al*. (2017). The study lasted for 21 days.

The serum biochemical parameters (creatinine kinase (CK) activity, malondialdehyde (MDA) level, lactate dehydrogenase (LDH) activity, calcium level and superoxide dismutase (SOD) activity) were evaluated in all the rats prior to treatment of the rats (day 0) and on days 7, 14 and 21 of the experiment.

Blood sample collection and centrifugation to obtain serum: Blood samples for the biochemical assays were obtained from the retrobulbar plexus in clean glass test tubes, and allowed to clot for 45 minutes before being centrifuged at 3000 rpm for 10 minutes to obtain clear serum samples, which were then split into two aliquots and refrigerated at 4^{o}C.

Biochemical Analyses: Serum activities of creatine kinase (CK) and lactate dehydrogenase (LDH), and serum levels of calcium were measured by the colorimetric method using commercial test kits obtained from Quimica Clinica Applicada, Spain and Elabscience Biotechnology Co. Ltd., USA, following the manufacturers' instructions. Serum malondialdehyde (MDA) level was measured by the modified thiobarbituric acid method (Placer *et al*., 1966; Draper and Hadley 1990). Superoxide dismutase (SOD) activity in the serum was determined according to the method developed by Misra and Fridovich (1972), based on the ability of SOD to inhibit the autoxidation of epinephrine

to adrenochrome at pH 10.2. Both assays were performed using the Elabscience SOD and MDA Assay Kits (Elabscience Biotechnology Co. Ltd., USA). The biochemical analyses were completed within 48 hours of blood sample collection.

Histopathology: On day 21 of the experiment, three rats from each of the groups were sacrificed by cervical dislocation and dissected. Tissue sections from the heart were fixed in 10% neutral-buffered formalin, routinely processed, and sectioned at 5 µm thickness and stained with haematoxylin and eosin (H & E). They were viewed for presence of lesions under a light microscope, and photographed at × 100 magnification.

Data Analysis: Quantitative data generated from the study were subjected to one-way analyses of variance (ANOVA) using SPSS software package version 21. Variant means were separated post hoc using the least significant difference (LSD) method. Probability less than 0.05 was considered statistically significant.

Results

Serum Biochemistry: The serum MDA levels of Groups B and C rats were significantly ($p < 0.05$) higher than that of the Control (Group A) on days 7, 12 and 21 of the experiment (Figure 1). The MDA levels of Group C rats were, however, significantly lower than that of Group B rats on day 21 of the experiment (Figure 1). Groups B and C rats had a significantly ($p < 0.05$) lower SOD activity in serum when compared to Group A on days 7, 14 and 21 of the experiment (Figure 2). However, on day 21, the serum SOD activity of the Group C rats was significantly ($p < 0.05$) higher than that of the Group B rats (Figure 2). Serum CK and LDH activities were significantly higher ($p < 0.05$) in Groups B and C rats when compared to Group A on day 14 of the experiment (Figures 3 and 4). By day 21, however, the serum CK and LDH activities of Group B rats were significantly higher ($p < 0.05$) than those of the Group A and C rats (Figures 3 and 4). There were no significant ($p > 0.05$) variations between the groups in their serum calcium levels on days 0 and 7 of the experiment, but on day 14 of the experiment, serum calcium level of Group B rats was significantly ($p < 0.05$) lower compared to those of Groups A and C rats (Figure 5). On day 21 of the experiment, serum calcium level of Group C rats was significantly ($p < 0.05$) higher than those of Groups A and B rats (Figure 5).

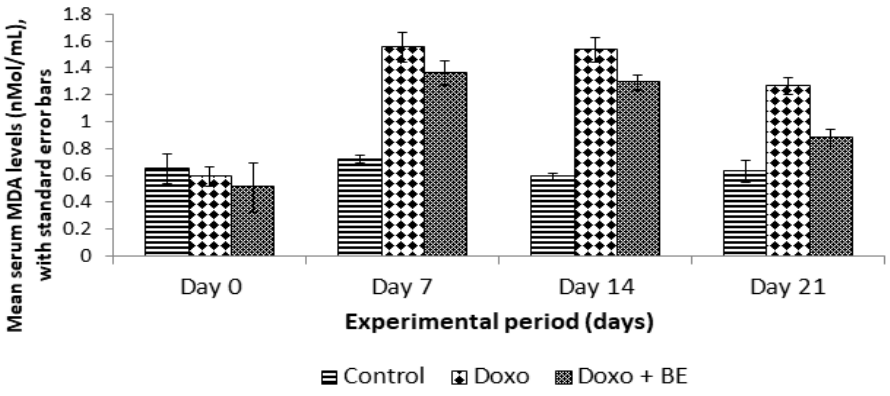

Figure 1: Mean serum malondialdehyde (MDA) levels (nMol/mL) of rats groups intoxicated with doxorubicin (Doxo) and treated with *Balanites aegyptiaca* seed oil extract, compared with normal control. [Control – Normal control not given doxorubicin and not treated with *B. aegyptiaca*; Doxo – Group given with doxorubicin only; Doxo + BE – Group given doxorubicin and treated with *B. aegyptiaca* seed oil extract].

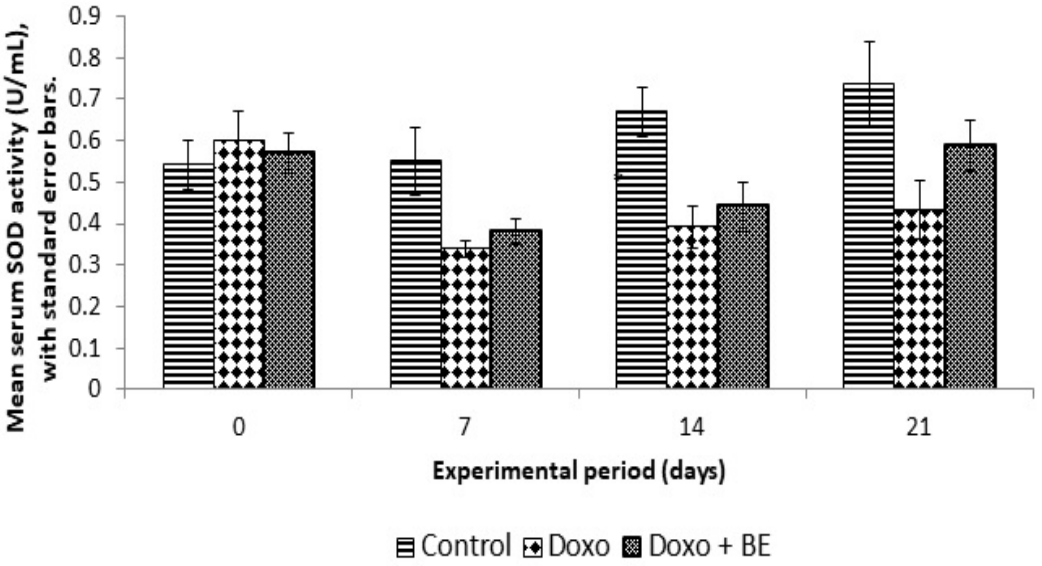

Figure 2: Mean serum superoxide dismutase (SOD) activity (IU/ml) of rat groups given doxorubicin (Doxo) and treated with *Balanites aegyptiaca* seed oil extract, compared with normal control. [Control – Normal control not given doxorubicin and not treated with *B. aegyptiaca*; Doxo – Group given with doxorubicin only; Doxo + BE – Group given doxorubicin and treated with *B. aegyptiaca* seed oil extract].

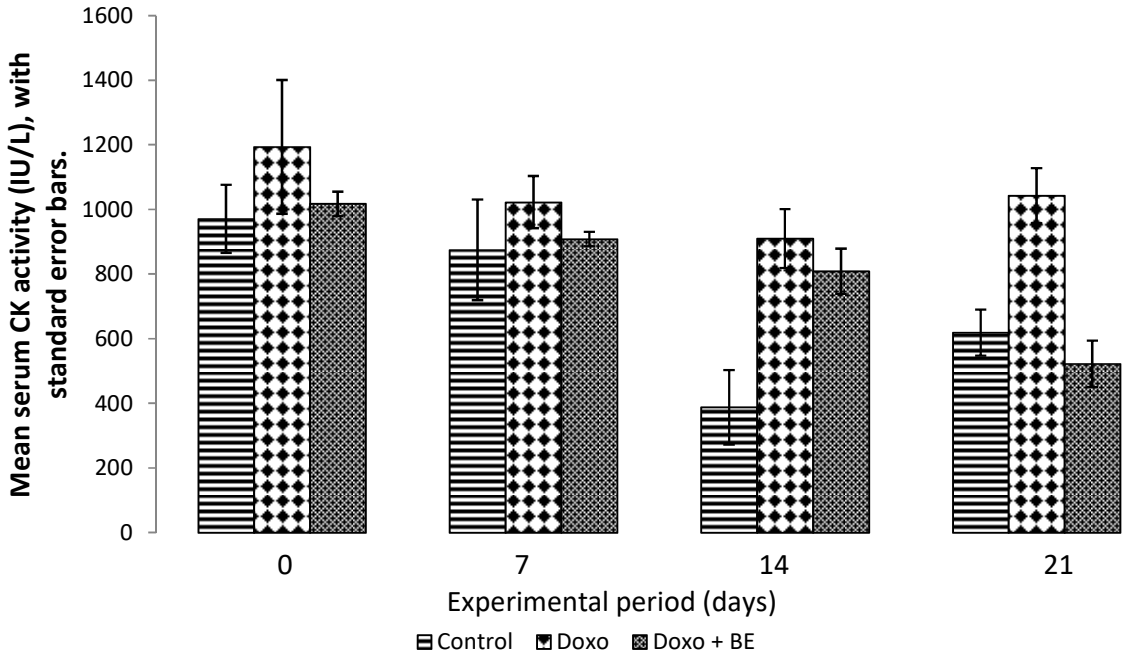

Figure 3: Mean serum creatine kinase (CK) activity (IU/L) of rat groups given doxorubicin and treated with *Balanites aegyptiaca* seed oil extract, compared with normal control. [Control – Normal control not given doxorubicin and not treated with *B. aegyptiaca*; Doxo – Group given with doxorubicin only; Doxo + BE – Group given doxorubicin and treated with *B. aegyptiaca* seed oil extract].

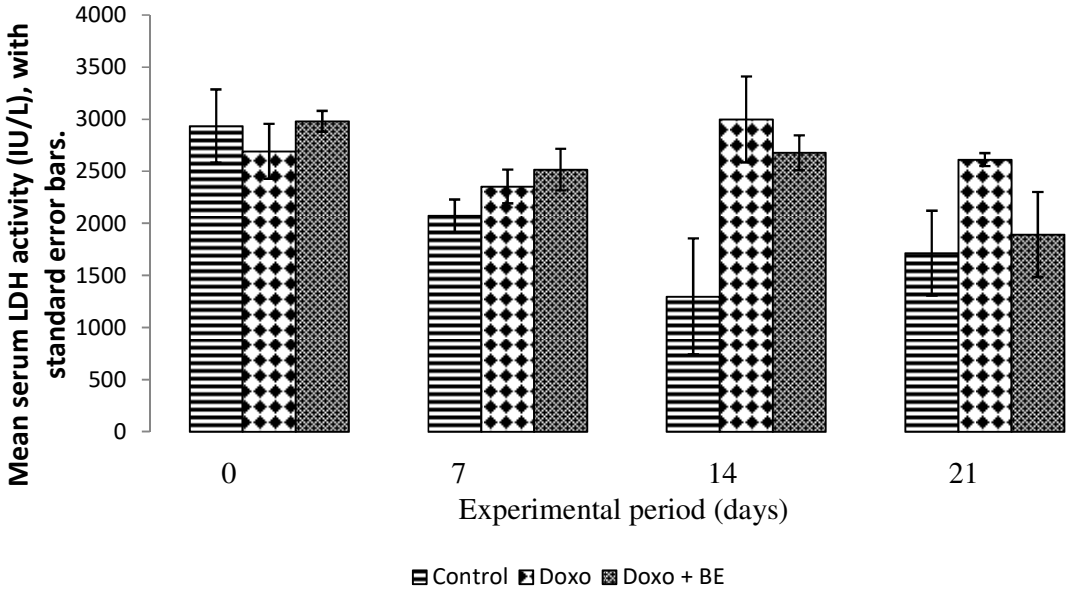

Figure 4: Mean serum lactate dehydrogenase (LDH) activity (IU/L) of rat groups given doxorubicin and treated with *Balanites aegyptiaca* seed oil extract, compared with normal control. [Control – Normal control not given doxorubicin and not treated with *B. aegyptiaca*; Doxo – Group given with doxorubicin only; Doxo + BE – Group given doxorubicin and treated with *B. aegyptiaca* seed oil extract].

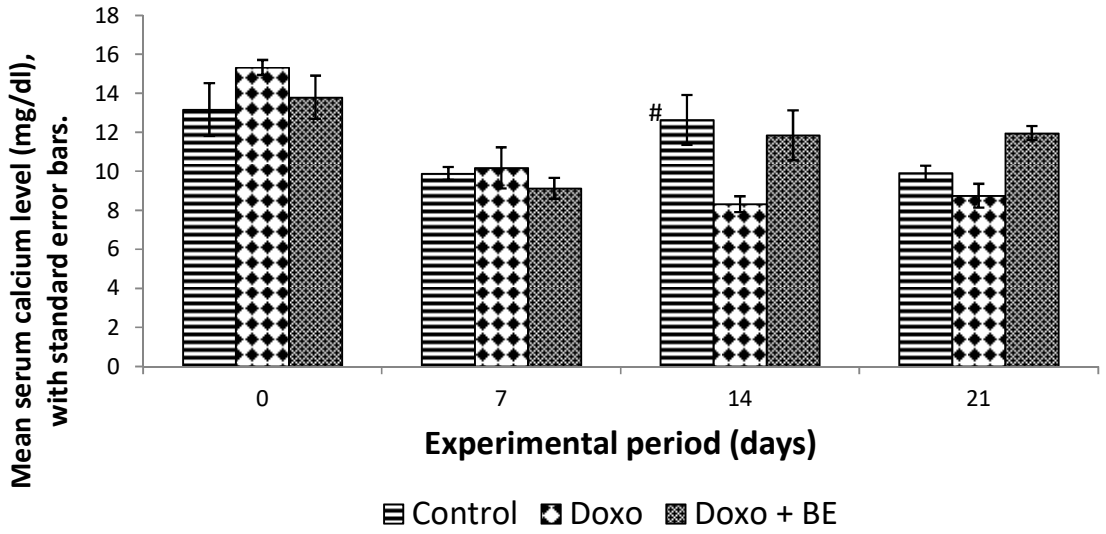

Figure 5: Mean serum calcium levels (mg/dl) of rat groups given doxorubicin and treated with *Balanites aegyptiaca* seed oil extract, compared with normal control. [Control – Normal control not given doxorubicin and not treated with *B. aegyptiaca*; Doxo – Group given with doxorubicin only; Doxo + BE – Group given doxorubicin and treated with *B. aegyptiaca* seed oil extract].

Histopathology: The heart tissue of Group A rats showed no lesions and had normal cardiac myofibres (Figure 6A), but the heart tissue of group B rats showed severe coagulative necrosis of cardiac myocytes, with highly eosinophilic cytoplasm, and there were petechial haemorrhages in the interfibrillar spaces (Figure 6Bi). Mild infiltration of mononuclear inflammatory cells was also observed in the interstitium and perivascular spaces of the heart tissue of Group B rats (Figure 6Bii). The heart tissues of Group C rats showed only a focal haemorrhagic area in the perivascular spaces, with no other obvious lesions (Figure 6C).

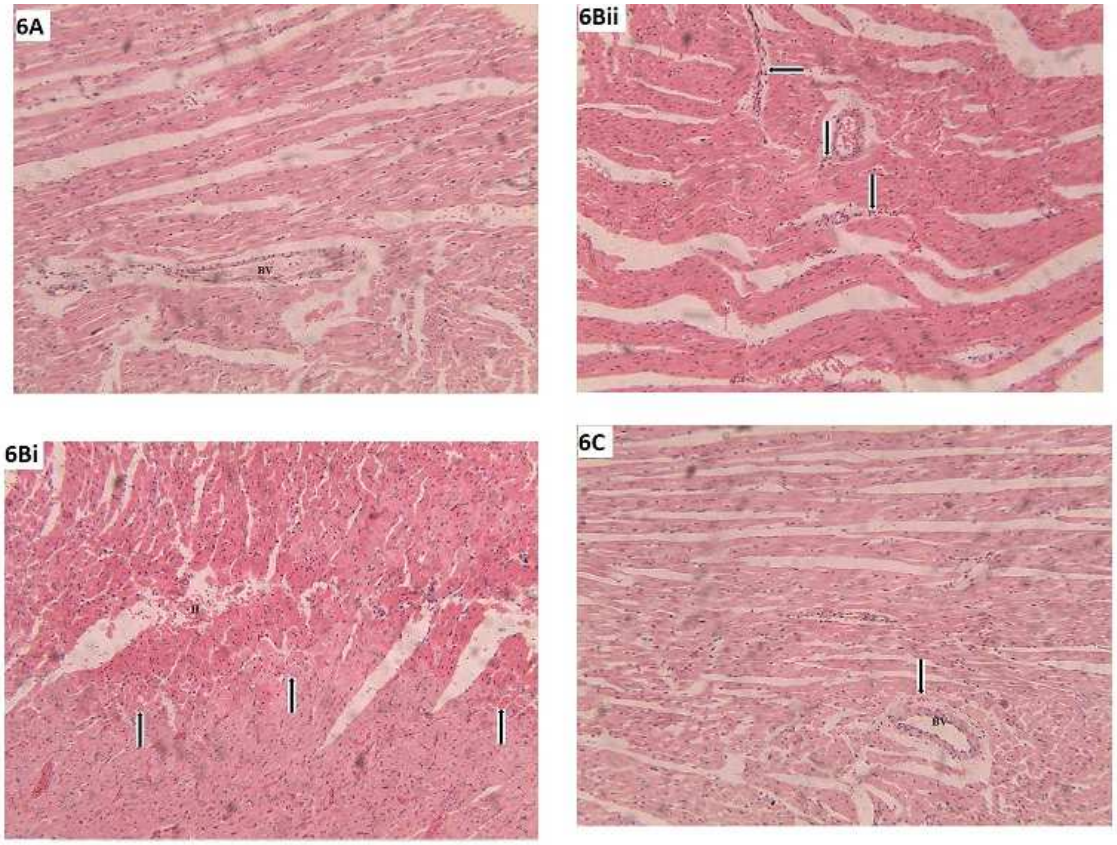

Figure 6. Histopathology of heart tissue of albino rats given doxorubicin and treated with *Balanites aegyptiaca* seed oil extract, compared with untreated controls: **6A –** Heart tissue of Group A normal control rats (not treated with doxorubicin and not treated with *B. aegyptiaca* seed oil extract) showing normal cardiac myofibres. Note the blood vessel (BV); **6Bi –** Heart tissue of Group B rats, given doxorubicin only, showing severe coagulative necrosis of cardiac myocytes (arrows) with haemorrhage (H); **6Bii –** Heart tissue of Group B rats, given doxorubicin, further showing mild infiltration of mononuclear inflammatory cells in the perivascular spaces; **6C –** Heart tissue of Group C rats given doxorubicin and further treated with *B. aegyptiaca* seed oil extract, showing normal cardiac myofibres with mild haemorrhage in the perivascular space (arrow). Note the blood vessel (BV). [H & E Stain, × 100].

Discussion

The significantly higher levels of serum MDA coupled with a significantly lower serum SOD activity in the DOX treated rats is indicative of high levels of oxidative stress. SOD is an enzyme that decreases reactive oxygen species generation and oxidative stress (Seguí *et al*., 2004). Reactive oxygen species (ROS) degrade polyunsaturated fatty acids to form malondialdehyde (Pryor and Stanley, 1975). The production of this aldehyde is used as a biomarker to measure the level of oxidative stress (Moore & Roberts, 1998; Del-Rio *et al*., 2005). The lower levels of serum MDA in the Group C rats on day 21 of the experiment may be attributed to the ameliorative effect of *B. aegyptiaca* seed oil on lipid peroxidation, and such effects had earlier been reported by Al-Harthi *et al*. (2014) in rats given doxorubicin and treated with resveratrol.

Significantly higher CK and LDH activities were recorded in Groups B rats on day 14 of the experiment; this is an indication of damage to muscle tissues rich in these enzymes, as had earlier been reported in conditions of rhabdomyolysis, myocardial infarction, myositis, myocarditis and DOX cardiotoxicity (Hekimsoy and Oktem, 2005). CK and LDH are usually released from the muscle cells when they are injured, and the magnitude of CK and LDH activities in the blood following myocardial injury usually reflect the extent of damage to the muscles (Preus *et al*., 1988). The CK activity of Group C rats was lower when compared to that of Group B rats; this probably indicated less severe damage to the cardiac muscle tissue of Group C rats treated with *B. aegyptiaca*.

The serum levels of calcium in the Group B rats was lower than those of Groups A and C rats on days 14 and 21 of the experiment; this can be explained as the manifestation of the biphasic effects of doxorubicin on cardiac calcium ion channels; initially, DOX activates the channel, but after a mean of 8 minutes, the channel becomes irreversibly inhibited (Ondrias *et al*., 1990) leading to an increase in intracellular calcium levels (Octavia *et al*., 2012) and consequently a decrease in serum calcium levels, which was recorded in the present study. The higher serum levels of calcium in Group C rats on day 21 of the experiment implies that *B. aegyptiaca* might be involved in mechanisms that either directly increase serum levels of calcium or lead to the activation/unblocking of calcium ion channels.

The histopathological changes in the cardiac tissue of the Group B rats that were given doxorubicin only is consistent with previous reports of severe interstitial inflammatory cellular infiltration, eosinophilia of the cytoplasm of the cardiac myocytes, interfibrillar haemorrhage and degeneration of the myocardium in cases of DOX toxicity (Al-Majed *et al*., 2002; Osman *et al*., 2013). Coagulative necrosis had been reported to be the default pattern of necrosis associated with ischemia or hypoxia and can be caused by lipid peroxidation in the heart (Wang *et al*., 2019). A cardinal sign of coagulative necrosis is the eosinophilia of surrounding tissue with maintenance of normal architecture of necrotic tissue for several days after cell death as well as preserved cell outlines, which lack nuclei (Adigun *et al*., 2020).

Conclusion: The results of this study suggest that *Balanites aegyptiaca* seed oil, as used in the study, had an ameliorative effect on DOX-induced oxidative stress and cardiotoxicity in the albino rat model, and may thus be beneficial in doxorubicin chemotherapy.

Conflict of interest

The authors declare no conflict of interest, with regards to this study and publication.

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