Regular Article

Hepatoprotective and antioxidant efficacy of aqueous stem bark extracts of *Balanites aegyptiaca* (Linn.) Del. against acetaminophen induced liver injury in rats

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ABSTRACT

Traditional use of various extracts of *Balanites aegyptiaca* in the management of many diseases has been previously reported. Though oxidative stress is known to contribute to the development of many of the disease conditions on which extracts of *B. aegytiaca* have been found efficacious, effects of these extracts on free radical-induced lipid peroxidation is not well understood. This study investigated the protective effects of stem bark extracts of *B. aegyptiaca* on acetaminophen-induced lipid peroxidation in rats. Phytochemical analysis of the plant extract used was conducted. Following a 10 day pre-treatment with extracts of *B. aegyptiaca*, serum levels of thiobarbituric reactive substances (TBARS), vitamin C, cholesterol/phospholipids, catalase (CAT) activity as well as markers enzymes of hepatotoxicity were measured in rats administered with acetaminophen (2 mg/kg body weight). Results obtained indicated preponderance of alkaloids, flavonoids, glycosides, phenols, saponins, and tannins. Pre-treatment with extracts of *B. aegyptiaca* protected against acetaminophen-induced elevation of TBARS and marker enzymes of hepatotoxicity as well as inhibited the depletion of serum levels of vitamin C, CAT activity and phospholipids in a dose-dependent manner. Serum levels of cholesterol were not affected by acetaminophen intoxication or treatment with the plant extract. These results indicated that therapeutic actions previously linked with extracts of *B. aegyptiaca* might be a consequence of its antioxidative and hepatoprotective effects.

KEY WORDS: Acetaminophen, antioxidative effects, *Balanites aegyptiaca*, hepatotoxicity lipid peroxidation, thiobarbituric reactive substances

INTRODUCTION

The use of plant-based materials in the treatment of human diseases and infections has become a common practice in many parts of the world, including Asia and Africa. The use of plant extracts in disease management has been linked to traditional knowledge of their efficacy. However, scientific investigations targeted at the characterization of phytochemical constituents of many medicinal plant species as well as their biological actions are increasing. In addition, increasing drug discovery efforts are targeted at identifying novel plant materials with medicinal effects or novel therapeutic applications of previously identified plants. As part of a program of investigation targeted at the characterization of biological effects of extracts of selected Nigerian plants commonly used in African trado-medicinal practices, we have reported hepatoprotective and antioxidant effects of *Cymbropogon citratus, Azadirachta indica, Tamarindus indica, Eucalyptus camadulensis, Khaya senegalensis,* and *Camelia sinensis* (Ojo and Ladeji, 2005; Ojo *et al.*, 2005; Ojo *et al.*, 2006). Our preliminary studies also revealed that extracts of *Psidium guajava, Anarcadium occidentale,* and *Moringa oleifera* exhibited significant antidiabetic effects which are consistent with their traditional use in the management of diabetes (Omijeh *et al.*, 2008, Tella *et al.*, 2008, Olayaki *et al.*, 2015).

Balanites aegyptiaca Del. (family Zygophyllaceae) is commonly known as desert date and is commonly found in Africa and South Asia (Hall and Waljer, 1991; Hall, 1992). Traditionally, extracts of the plant is used in the treatment of various

ailments including jaundice, intestinal worm infection, wounds, malaria, syphilis, epilepsy, dysentery, constipation, diarrhoea, haemorrhoid, stomach aches, asthma, and fever (Watt and Breyer-Brandwijk, 1962; Beentje, 1994; Jagtap *et al.*, 2009). Extracts of the plant have also been reported to exhibit spermicidal, antibacterial, antifungal, cardioprotective, anthelmintic, antitumor, antidiabetic and anti-inflammatory actions (Reviewed in Chothani and Vaghasiya, 2011). Phytochemical constituents previously isolated from various extracts of *B. aegyptiaca* include compounds belonging to saponin, furanocoumarin and flavonoids (Table 1). The role of oxidative stress in the development of many of the ailments against which extracts of *B. aegyptiaca* is active are well established (Madamanchi *et al.*, 2005; Adly, 2010). However, it is not yet clear if *B. aegyptiaca* extracts exert its pharmacological effects by protecting against oxidative stress *in vivo*. Therefore in this study, we examined protective effects of aqueous extracts of *B. aegyptiaca* in acetaminophen-induced oxidative stress and lipid peroxidation in rats.

MATERIALS AND METHODS

Plant Materials

Fresh stem barks of *B. aegyptiaca* were collected in Sangere Village near Federal University of Technology, Yola. After botanical identification, a voucher specimen (No: BR2014-17) was deposited in the herbarium maintained at Bioscience Research Education and Advisory Centre. Stem barks were dried under room temperature and pulverized using mortar and pestle into fine powder. Powdered plant material (10 g) was soaked in 100 ml distilled water for 12 h and filtered using a Whatman's No. 1 filter paper. The filtrate was concentrated using a vacuum concentrator and the residue was re-dissolved in distilled water to make a stock solution of 100 mg/ml which was stored at $-20°C$ until used.

Phytochemical Screening

The aqueous extracts of *B. aegytica* stem bark was screened for the presence of saponins, tannins, triterpines, alkaloids, and flavonoids were carried out as previously described (Saeed *et al.*, 2012).

Measurement of Total Phenolic and Total Flavonoid Contents

Total phenolic and phenolic contents of the aqueous stem bark extracts of *B. aegyptiaca* were estimated as previously described (Olayaki *et al.*, 2015). Briefly, total phenolic content was estimated by Folin-Ciocalteu's method following the protocol described by Kim *et al.* (2003)

Table 1: List of active constituents isolated from extracts of *Balanites aegytiaca*

Phytochemical constituent	Plant part	References
3-rutinoside	Fruit	Salwa and Hadidi (1988)
3-rhamnogalactoside	Fruit	Salwa and Hadidi (1988)
Diosgenin	Fruit	Khare (2007)
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	Fruit kernel	Staerk *et al.* (2007)
Balanitoside (furostanol glycoside)	Fruit kernel	Hosny *et al.* (1992)
6-methyldiosgenin	Fruit mesocarp	Hosny *et al.* (1992)
Balanitin-3 (spirostanol glycoside)	Fruit mesocarp	Kamel (1998)
Pregn-5-ene-3β,16β,20(R)-triol 3-0-(2,6-di-0-α-l-rhamnopyranosyl)-β-d-glucopyranoside	Fruit mesocarp	Gnoula *et al.* (2008), Pettit *et al.* (1991)
Pregn-5-ene-3β,16β,20(R)-triol 3-0-β-d-glucopyranoside	Fruit mesocarp	Kamel and Koskinen (1995)
25D-spirosta-3, 5-diene and 3β-chloro-25D-spirost-5-ene	Leaves	Saeed *et al.* (1995)
3β,12α,14β,16β)-12-hydroxycholest-5-ene-3,16-diyl bis(β-D-glucopyranoside	Root	Hardman and Sofowora, (1970); Saharan *et al.*, (2008)
3β,20S,22R,25R)-, and (3β,20S,22R,25S)-26-(β-D-glucopyranosyloxy)-22-methoxyfurost-5-en-3-yl-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)[α-L rhamnopyranosyl-(1→2)]-β-D-glucopyranoside	Root	Hardman and Sofowora, (1970); Saharan *et al.*, (2008)
3β,20S,22R,25R)- spirost-5-en-3-yl β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4) [α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside	Root	Farid *et al.*, (2002)
(3β,20S,22R,25S)-spirost-5-en-3-yl β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4) [α-L-rhamnopyranosyl-(1→2)]-β-D-Glucopyranoside	Root	Farid *et al.*, (2002)
Furanocoumarin bergapten	Bark	Seida *et al.*, (1981)
Dihydrofuranocumarin D- marmesin	Bark	Seida *et al.*, (1981)
N-trans-feruloyltyramine	Bark	Sarker *et al.*, (2000)
N-cis-feruloyltyramine	Bark	Sarker *et al.*, (2000)
Vanillic acid	Bark	Sarker *et al.*, (2000)
Syringic acid	Bark	Sarker *et al.*, (2000)
3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone	Bark	Sarker *et al.*, (2000)
10-methyl-n-heptacosane	Bark	Ansari *et al.*, (2006)
Diglucosyldirhamnoside	Bark	Ansari *et al.*, (2006)

and was expressed as gallic equivalent per gram of sample (GE/g). Similarly, estimation of total flavonoid content expressed as quercetin equivalents per gram sample (QE/g) was conducted as described by Park *et al.* (2008).

Experimental Animals

Male Wistar albino rats (average weight = 130 g) purchased from the animal house of University of Jos, Nigeria, were used for the study. Animals were maintained in an air-conditioned room (22 ± 2°C) with regulated light:dark cycle with food and water provided ad libitum. All animal experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 and EU Directive 2010/63EU for animal experiments.

In vivo Studies

Groups of rats ($n = 6$) were treated with once daily oral administration of either saline or graded doses of aqueous stem bark extracts of *B. aegyptiaca* (50-200 mg/kg body weight) for 10 days prior to a single oral overdose with acetaminophen (2 g/kg body weight). Another group of rats, treated with saline for 10 days and administered with saline instead of acetaminophen, was used as control. All animals were sacrificed within 12 h of acetaminophen administration under mild anesthesia. Serum was separated from terminal blood collected by heart puncture into heparinized bottles and stored at −20°C until used for biochemical analysis.

Determination of Lipid Peroxidation

Plasma concentrations of thiobarbituric reactive substances (TBARS) were measured as described by Buege and Aust (1978) to estimate the effect of the plant extract on lipid peroxidation experimental animals. Briefly, aliquots of plasma samples (0.8 ml) were mixed with 1.2 ml of TBA-reagent and heated in a boiling water bath for 10 min. Tubes were allowed to cool down and were centrifuged at 300 rpm after the addition of 0.2 N NaOH (2 ml). Absorbance of the pink colored adduct formed was measured at 535 nm, and results were expressed as mmol/ml.

Assessment of Catalase (CAT) Activity

CAT activity in the erythrocyte lysates of treated and untreated animals was measured as described by Sinha (1972). Hydrogen peroxide (0.2 M) was used as a substrate and the decrease in the concentration of H_2O_2 at 22°C in phosphate buffer (0.05 M, pH 7.0) was measured by spectrophotometer at 240 nm. Enzyme activities were presented as units per gram hemoglobin (U/gHb).

Measurement of Biochemical Parameters

Serum levels of alanine transaminase (ALT), aspartic transaminase (AST) and alkaline phosphatise (ALP) were estimated to assess the effect of the plant extract on liver function in treated rats. Commercially, available kits (Randox Laboratories, UK) were used following the manufacturer's recommended protocol. Similarly, serum cholesterol and phospholipids were estimated as previously described by Searcy *et al.* (1960) and Kobayashi *et al.*, (2001) respectively. Serum ascorbic acid levels were measured by 2,4-dinitrophenlyhydrazine (DNPH) method through which vitamin C is oxidized to diketogluconic acid which reacts with DNPH to form diphenylhydrazone. The hydrazone dissolves in strong acid solution to form orange-red colored complex. The absorbance was read at 520 nm.

Statistical Analysis

Results are expressed as mean ± standard error of the mean. Values were compared using one-way ANOVA followed by Student–Newman-Keuls *post-hoc* test. $P < 0.05$ was considered statistically significant.

RESULTS

Phytochemical Constituents of Aqueous Stem Bark Extracts of *B. aegyptiaca*

Phytochemical screening of aqueous stem bark extracts of *B. aegyptiaca* indicated the preponderance of alkaloids, flavonoids, glycosides, phenols, saponins, and tannins. In addition, total flavonoids and phenolic contents of the extract were estimated as 67.14 ± 0.72 mg QE/g and 44.12 ± 0.39 mg GE/g, respectively. Values were interpolated using standard curves with $R^2 = 0.962$ or 0.945 for flavonoids or phenolic compounds, respectively.

Effects of Aqueous Stem Bark Extracts of *B. aegyptiaca* on Lipid Peroxidation, CAT Activity and Plasma Vitamin C Level

Basal plasma TBARS level was observed as 10.24 ± 2.20 nmol/l in saline treated rats. This was increased by 4.6-fold ($P < 0.001$, Figure 1a) in rats administered as single dose of paracetamol (2 g/kg body weight). However, elevated plasma TBARS concentrations caused by paracetamol intoxication was inhibited in a dose-dependent manner in rats pre-treated with aqueous stem bark extracts of *B. aegyptiaca* (23-60%, $P < 0.01$). CAT activity in rats administered acetaminophen was reduced by 75% ($P < 0.001$) compared with rats treated with saline (Figure 1b). Pre-treatment with aqueous

stem bark extracts of *B. aegyptiaca* resulted in increased CAT activities in rats intoxicated with acetaminophen. CAT activities increased by 1.4-fold in rats treated with 50 mg/kg body weight plant material compared with 2.8- and 3.1-fold ($P < 0.001$) observed in rats treated with high concentrations of the aqueous stem bark extracts. Moreover, treatment with acetaminophen decreased plasma vitamin C concentration by 54%. However, a concentration-dependent increase in plasma vitamin C concentration was observed in rats treated with aqueous stem bark extracts of *B. aegyptiaca*. Plasma vitamin C concentration increased by 1.3-fold ($P < 0.05$), 1.5-fold ($P < 0.001$), and 1.7-fold ($P < 0.001$) in rats treated with 50, 100, and 200 mg/kg body weight of aqueous stem bark extracts of *B. aegyptiaca*, respectively, compared with saline-treated rats (Figure 1c).

Effects of Aqueous Stem Bark Extracts of *B. aegyptiaca* on Plasma Cholesterol and Phospholipid Concentrations in Acetaminophen Treated Rats

Administration of acetaminophen did not affect plasma cholesterol levels (Figure 2a) but resulted in significantly reduced plasma phospholipid concentration (39%, $P < 0.01$, Figure 2b), leading to significantly higher cholesterol-phospholipid ratio (1.8-fold, $P < 0.01$) in Wistar albino rats. However, treatment with aqueous stem bark extracts of *B. aegyptiaca* inhibited the reduction in plasma phospholipid levels in acetaminophen treated rats

(Figure 2c). Reduction in plasma phospholipid reduced (10-31%, $P < 0.01$-0.05) in rats treated with aqueous stem bark extracts of *B. aegyptiaca* translated to 1.1-1.4-fold increase in cholesterol phospholipid ratio in that order (Figure 2).

Effects of Aqueous Stem Bark Extracts of *B. aegyptiaca* Extracts on Liver Function

As expected, elevated plasma levels of AST (5.9-fold, $P < 0.001$), ALT (6.6-fold, $P < 0.001$) and ALP (4.2-fold, $P < 0.001$) were observed in acetaminophen-intoxicated rats (Figure 3). Treatment with aqueous stem bark extracts of *B. aegyptiaca* significantly inhibited elevation of plasma levels of these enzymes in a dose-dependent manner. In rats treated with 50 mg/kg body weight, plasma AST, ALT, and ALP were reduced by 35.5% ($P < 0.01$), 41.4% ($P < 0.001$), and 25.8% ($P < 0.05$), respectively, compared to saline-treated rats. The highest concentration of the aqueous stem bark extracts of *B. aegyptiaca* tested produced 63% ($P < 0.001$), 72% ($P < 0.001$), and 54% ($P < 0.01$) reduction in plasma AST, ALT, and ALP in acetaminophen-treated rats.

DISCUSSION

Oxidative stress reflects an imbalance between the productions of reactive oxygen species (ROS), arising from effects of ionizing radiation, free radicals and other systemic sources, and the ability of a biological system to detoxify the reactive intermediates or repair damage caused
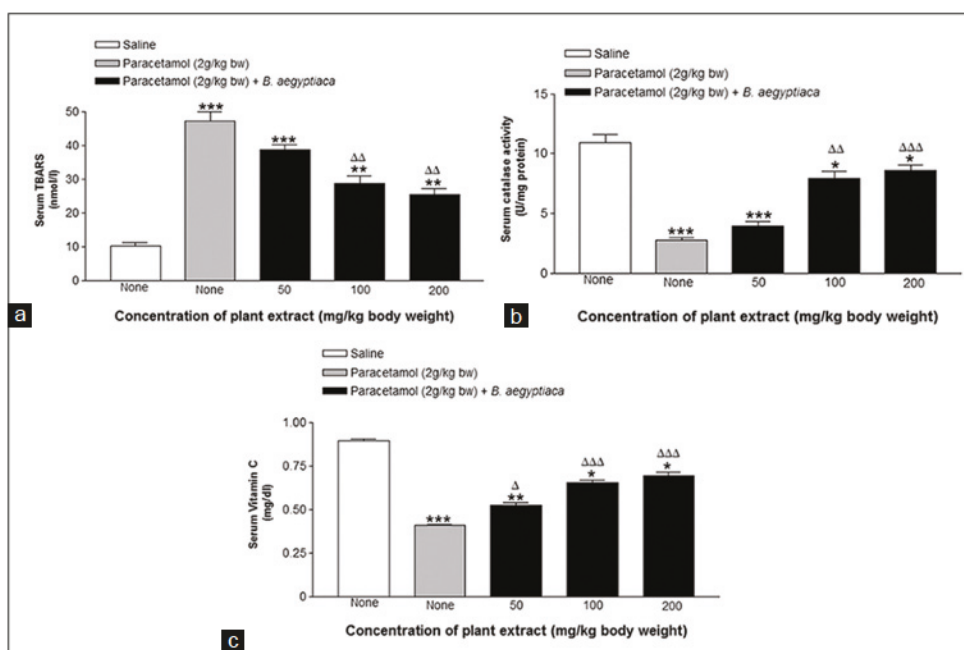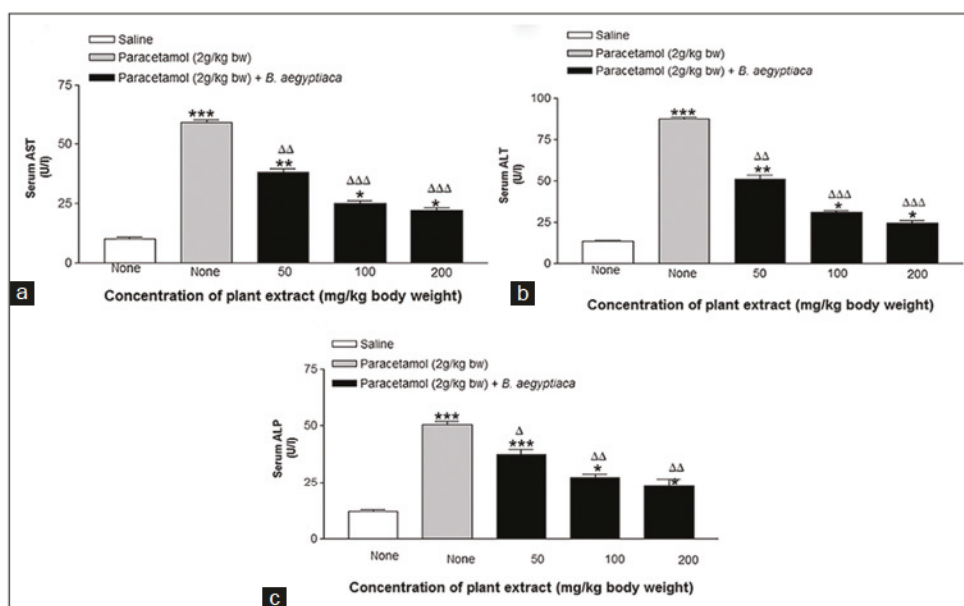

Figure 1: Effects of *Balanites aegyptiaca* extracts on serum levels of (a) thiobarbituric reactive substances, (b) catalase activity and (c) vitamin C in acetaminophen-treated rats. Values are mean ± standard error of the mean with $n = 6$. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared with saline treated rats. ΔΔ$P < 0.01$ and ΔΔΔ$P < 0.001$, compared with acetaminophen-treated control rats, Δ: ???

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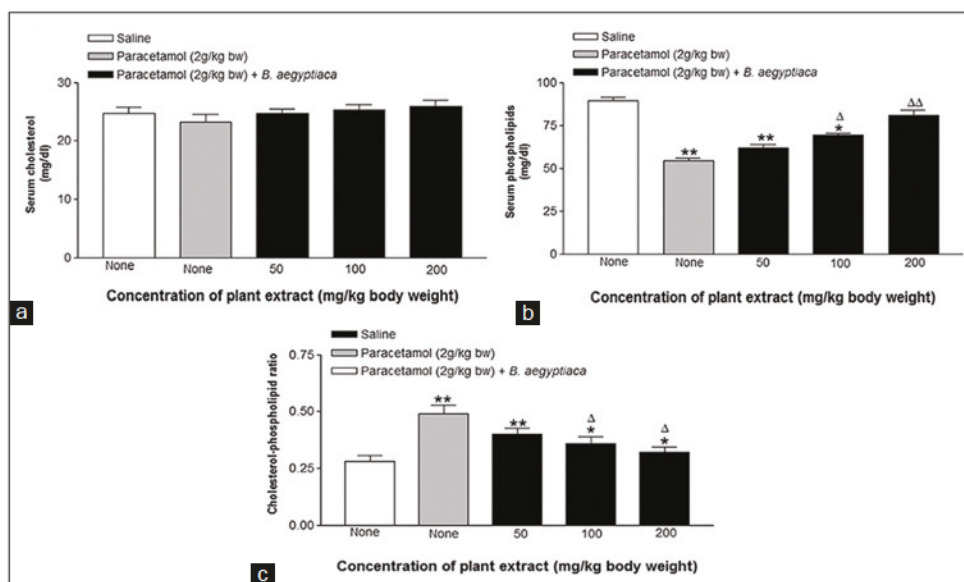
Figure 2: Effects of *Balanites aegyptiaca* extracts on serum levels of (a) cholesterol, (b) phospholipids and (c) cholesterol-phospholipids ratio in acetaminophen-treated rats. Values are mean ± standard error of mean with $n = 6$. $*P < 0.05$ and $**P < 0.01$ compared with saline treated rats. $^{\Delta}P < 0.05$ and $^{\Delta\Delta}P < 0.01$ compared with acetaminophen-treated control rats

Figure 3: Effects of *Balanites aegyptiaca* extracts on serum levels of (a) aspartic transaminase, (b) alanine transaminase and (c) alkaline phosphatise in acetaminophen-treated rats. Values are mean ± standard error of the mean with $n = 6$. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ compared with saline treated rats. $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$ and $^{\Delta\Delta\Delta}P < 0.001$ compared with acetaminophen-treated control rats

by ROS (Imlay, 2003). This process has been implicated in the etiology of many human disease conditions, including inflammation, reperfusion injury, diabetes, cancer, myocardial infarction, and Alzheimer's disease (Singh *et al.*, 1995; Nunomura *et al.*, 2005; Halliwell, 2007; Ramond *et al.*, 2011; Pohanka, 2013). Lipid peroxidation is a biologically-important consequence of increased production of ROS and has been recognized as a biomarker for oxidative stress (Niki, 2008). Acetaminophen is widely used as analgesic and antipyretic and is considered safe at therapeutic doses. However, at doses above 2 g/kg body weight, acetaminophen consumption has been linked with hepatic centrilobular necrosis and lipid peroxidation in experimental animals (Rowden *et al.*, 2006). It has been suggested that acetaminophen's metabolite, N-acetyl-P benzoquinone imine, binds to sulphydryl groups of protein in the liver to induce cell necrosis and lipid peroxidation (Boyd and Bereczky, 1966; Hinson *et al.*, 2010). This has also been linked with increased circulating concentrations of alanine and aspartate transaminases (ALT and AST)

(Drotman and Lawhorn, 1978, Aleksunes *et al.*, 2006), ALP and γ-glutamyl transpeptidase (Muriel *et al.*, 1992).

Consistent with these observations, rats intoxicated with acetaminophen in the present study exhibited increased lipid peroxidation, indicated by elevated serum TBARS and significantly reduced serum CAT activity and vitamin C contents, compared with saline treated controls. However, reduced serum TBARS accompanied by significantly elevated serum CAT activity and vitamin C concentration were observed in rats treated with aqueous stem bark extracts of *B. aegyptiaca*, indicating the ability of the plant extract to protect rats from the deleterious effects of acetaminophen intoxication. TBARS are degradation products of lipids which accumulates in the serum in the event of lipid peroxidation (Janero, 1990) and CAT catalyzes the decomposition of hydrogen peroxide to oxygen and water, thereby protecting the cell from ROS-induced oxidative damage (Chelikani *et al.*, 2004). Therefore, the reduced serum TBARS level together with the increased CAT activity observed in aqueous stem bark extracts of *B. aegyptiaca* treated rats is indicative of the ability of the plant extract to protect against lipid peroxidation / oxidative damage arising from acetaminophen intoxication. Moreover, the increased serum vitamin C concentration in rats treated with the plant extract is also an indication of a good antioxidant status (Padayatty *et al.*, 2003). Al-Ghannam *et al.* (2013) reported that balanitoside; a diosgenyl saponin isolated from the *B. aegyptiaca* fruit, exhibited antitumor effects and decreased the formation of malondialdehyde in Ehrlich ascites carcinoma bearing Swiss albino mice. Consistent with our observation, increased CAT activities and other parameters indicating reduced lipid peroxidation were also observed in balanitoside-treated mice. These observations suggest that balanitoside may be the active compound responsible for the effects observed in this study. However, further structural characterization studies are needed to confirm this speculation.

Cholesterol-phospholipid ratio was measured to investigate the effect of acetaminophen intoxication on cell membrane integrity in rats (Fajardo *et al.*, 2011). Consistent with our previous observations (Ojo *et al.*, 2006), elevated cholesterol-phospholipid ratio was observed in saline treated rats intoxicated with acetaminophen. This effect was however countered in rats treated with the aqueous stem bark extracts of *B. aegyptiaca*. This observation indicates the ability of the plant extract to protect against the alteration of lipid membranes in acetaminophen intoxication. Impaired liver function following acetaminophen intoxication

has been reported widely (Olayaki *et al.*, 2015). However, elevated serum levels of alanine and aspartate transaminases caused by acetaminophen intoxication was inhibited in rats treated with aqueous stem bark extracts of *B. aegyptiaca*, indicating the hepatoprotective actions of the plant extract. Though the molecular mechanism underlying the hepatoprotective actions of the plant extract is not yet known, inhibition of hepatic damage has been reported for many plant species commonly used in African traditional medicinal practices.

CONCLUSION

Observations reported in this study together with previously reported pharmacological actions strongly necessitate the investigations of therapeutic application of compounds derived from aqueous stem bark extracts of *B. aegyptiaca* as agents with multiple actions. For instance, the reported antimicrobial (Jahan *et al.*, 2013) and antitumor (Al-Ghannam *et al.*, 2013) activities reported for balanitoside represents a combination of effects that could be beneficial in treating infections in cancer patients.

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