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. aegyptiaca* 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 *Cymbopogon 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*, *Anarcardium occidentale*, and *Moringa oleifera* exhibited significant anti-diabetic 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...
aliments 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. aegyptiaca* 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>
<thead>
<tr>
<th>Phytochemical constituent</th>
<th>Plant part</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>3-rutinoside</td>
<td>Fruit</td>
<td>Salwa and Hadidi (1988)</td>
</tr>
<tr>
<td>3-rhamnosylactoside</td>
<td>Fruit</td>
<td>Salwa and Hadidi (1988)</td>
</tr>
<tr>
<td>Diosgenin</td>
<td>Fruit</td>
<td>Khare (2007)</td>
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<tr>
<td>26-(β-D-glucopyranosyl)-3-β-[4-O-(β-D-glucopyranosyl)]-2-O-(α-L-ramnopyranosyl)]-β-D-glucopyranosyloxy)-22,26-dihydroxyfurost-5-ene</td>
<td>Fruit Kernel</td>
<td>Staerk et al. (2007)</td>
</tr>
<tr>
<td>6-methyldiosgenin</td>
<td>Fruit Kernel</td>
<td>Hosny et al. (1992)</td>
</tr>
<tr>
<td>Balanitin-3 (spirostanol glycoside)</td>
<td>Fruit Mesocarp</td>
<td>Hosny et al. (1992)</td>
</tr>
<tr>
<td>3β-[12α,14β,16β]-12-hydroxycholest-5-ene-3,16-diyli bis(β-D-glucopyranoside)</td>
<td>Fruit Mesocarp</td>
<td>Gnoula et al. (2008), Pettit et al. (1991)</td>
</tr>
<tr>
<td>3β-[20S,22(R),25S]-26-([β-D-glucopyranosyl]+[4-O-([α-L-rhamnopyranosyl]-[1→4]-[β-D-glucopyranosyl]+[1→2])-β-D-glucopyranosyl]-[1→4]-[α-L-rhamnopyranosyl]-[1→2])-β-D-glucopyranosyl</td>
<td>Root</td>
<td>Farid et al., (2002)</td>
</tr>
<tr>
<td>Furanocoumarin bergapten</td>
<td>Bark</td>
<td>Seida et al., (1981)</td>
</tr>
<tr>
<td>Dihydrofuranocoumarin D- marmesin</td>
<td>Bark</td>
<td>Seida et al., (1981)</td>
</tr>
<tr>
<td>N-trans-feruloyltarymine</td>
<td>Bark</td>
<td>Sarker et al., (2000)</td>
</tr>
<tr>
<td>N-cis-feruloyltarymine</td>
<td>Bark</td>
<td>Sarker et al., (2000)</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>Bark</td>
<td>Sarker et al., (2000)</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>Bark</td>
<td>Sarker et al., (2000)</td>
</tr>
<tr>
<td>3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone</td>
<td>Bark</td>
<td>Ansari et al., (2006)</td>
</tr>
<tr>
<td>10-methyl-n-heptacosane</td>
<td>Bark</td>
<td>Ansari et al., (2006)</td>
</tr>
<tr>
<td>Diglucosyldihamnoside</td>
<td>Bark</td>
<td>Ansari et al., (2006)</td>
</tr>
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Measurement of Biochemical Parameters

Serum levels of alanine transaminase (ALT), aspartic transaminase (AST) and alkaline phosphatase (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-dinitrophenylhydrazine (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 \( (P < 0.001, \text{Figure } 1a) \) in rats administered as single dose of paracetamol (2 g/kg body weight). However, elevated plasmaTBARS 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 aceterminophen 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...
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 sulphhydryl 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)

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.

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. ∆P < 0.05, ∆∆P < 0.01 and ∆∆∆P < 0.001 compared with acetaminophen-treated control rats.
with aqueous stem bark extracts of vitamin C concentration were observed in rats treated by significantly elevated 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 activity 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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