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Full Length Research Paper

Protection of rats by extracts of some common Nigerian trees against acetaminophen-induced hepatotoxicity

Ojo, O. O.[1], Nadro, M. S.[2] and Tella, I. O.[3]

[1]Department of Integrated Science, Federal College of Education, Yola, Nigeria.
[2]Department of Biochemistry, Federal University of Technology, Yola, Nigeria.
[3]Department of Forestry and Wildlife Management, Federal University of Technology, Yola, Nigeria.

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Extracts of *Balanites aegyptiaca, Khaya senegalensis, Prosopis africana* and *Vitellaria paradoxa* were screened for their phytochemical constituents. Stem barks of the species were also investigated for hepatoprotective effects in Wistar albino rats. Different groups of animals were pre- treated with 100 mg/kg body weight of plant extracts for 10 days and administered with paracetamol (2 g/kg) on the 10th day. The effect of the extracts on serum transaminase and alkaline phosphatase (ALP) were measured in the rats. The leaf, stem and root extracts of all the plants except *Vitellaria paradoxa* showed preponderance of saponin and tannin. Phlobatannin, cardiac glycosides and anthraquinones were observed in concentrations ranging between 10 to 100 mg/kg plant material. Stem bark extracts of the four plants produced significant ($P < 0.05$) hepatoprotective effects by decreasing the activity of serum enzymes. Values recorded for AST, ALT and ALP were significantly lower compared to those recorded for control rats. A higher inhibition of serum level elevation of ALP was observed with the four extracts. From these results, it was suggested that the extracts could protect the liver cells from paracetamol-induced liver damages perhaps by eliminating the deleterious effects of toxic metabolites from the drug.

Key words: *Balanites aegyptiaca, Khaya senegalensis*, *Prosopis africana*, *Vitellaria paradoxa*, hepatoprotective effect, paracetamol, transaminases, alkaline phosphatase.

INTRODUCTION

Many research efforts have been directed towards the provision of empirical proof to back up the use of many tropical plants in trado-medicinal practices (Ojo et al., 2005; Tella and Ojo, 2005; Baladran et al., 1985; Naranjo, 1995; Maiti et al., 2004; Madusolomuo and Okoye, 1995) Atawodi (2005) reported the antioxidant potentials of many african plants. However, there still exist a vast number of tropical trees with tremendous medicinal potential but with no empirical proof to support claims of efficacy. It is known that phytochemicals confer pharmacological relevance on plants generally (Ojo et al., 2005) and the growing interest in herbal medicine (Atawodi, 2005) demands information on various plant preparations used in the treatment of diseases (Sofowora, 1991). Scientific evaluation of medicinal plants is important to the discovery of novel drugs and also helps to assess toxicity risks associated with the use of either herbal preparations or conventional drugs of plant origin.

Balanites aegyptiaca (L.) Del. is a savannah tree characterised by long straight green spines arranged spirally along the branches. Each spine has a two leaflet compound leaf below it. All the branches (whether bearing flower or not) are armed with spines which are usually simple and practically straight. Various parts of the tree is traditionally used for pharmaceutical purposes (Table 1). *Khaya senegalensis* (Desr.) *A. Juss* is widely distributed in the savannah regions of Nigeria. The species is recognized by its round evergreen crown made up of dark shinning pinnate leaves and characteristic

*Corresponding authors E-mail: cecejyde@yahoo.com, Tel: +2348053372266.

Table 1. Traditional Medicinal Uses of Plant Species

Plant/Part	Traditional Medicinal Uses
Balanites aegyptiaca	
Root + Bark	Laxative, diarrhoea, haemorrhoid.
Bark	Vermifuge, stomach aches, sterility, jaundice, yellow fever, syphilis, coughs, epilepsy and anxiety
Root	Snake bites, anthrax
Thorns	Leprosy
Leaves	Weakness, anthrax
Fruit	Rheumatism
Seed	Ointments, balms, magico-religious uses
Khaya senegalensis	
Roots	diarrhoea, jaundice, headaches
Bark	Vermifuge, abortifacient, malaria fever, jaundice, colic, phagedenic ulcers, dysmenorrhoea, blennorrhoea, condermatitis and urticaria, anaemia, sickle cell diseases.
Leaves	Malaria, headaches, fever, amenorrrhoea, smallpox, diarrhoea, jaundice, lumbago, rheumatism.
Flowers	Gastritis, syphilis,
Seeds	Fever
Gum	Sedative for coughs
Prosopis africana	
Root	Tooth decay, bronchitis, dysentery, blenorrhoea
Root + Leaves	Fatigue,
Bark	Vermifuge, wound and skin diseases, ophthamia, tooth decay
Bark + Leaves	Tooth decay, rheumatism
Leaves	Tranquilizer, diaphoretic, vertigo, otitis, headaches, migraine, childhood fever, dysentery, haemorrhoid
Vitellaria paradoxa	
Root	Gastritis, liver cancer, female sterility, ascites
Bark	Dysentery, haemorrhoids, schistosomiasis, coughs, Jaundices, nausea, diarrhoea, constipation, headaches, fever
Leaves	Conjunctivitis and trachoma, convulsion
Seeds	Antivenom, diarrhoea

Source: Arbonnier (2002).

round capsules. It is often planted by roadsides for shade. Arbonnier (2002) stated the various uses of the plant (Table 1). *Prosopis africana* (Guill. and Perr.) Taub. is native to Africa, occurring from Senegal to Ethiopia throughout the Sudanian and Guinean ecozones, reaching the border of the Sahelian ecozone to the north of Africa. Table 1 presents the various trado-medicinal uses of the plant. *Vitellaria paradoxa* (Gaertn. f.) species is locally abundant in the derived savannah and Guinea zones, especially near towns and villages. In appearance, it is very similar to *Lophira lanceolata* with which it often grow, but it is distinguished by its very long leaf stalks, more widely spaved nerves and abundant white latex in the slash and petioles. The economic function of this species is considerable in Nigeria, due to the vegetable fat extracted from the fermented fruit stone (shea butter) (Arbonnier, 2002). The shea butter is frequently used in the making of ointments and poultices for emollients and healing (Table 1).

The vast traditional therapeutic applications of these plants suggest that they possess hepatoprotective effects. However, empirical work is lacking to back this assertion. Also, literature on the side effects of the usage if these plants is lacking. Moreover, empirical work to identify basic components of these trees that confer pharmacological advantages on them is equally not available. This paper presents the report of an attempt to find out the basic phytochemicals present in these plants and to examine and the abilities of the plants extracts to protect the liver against chemical-induced hepatotoxicity.

Paracetamol (acetaminophen), a widely used anti-pyretic and analgesic, produces acute liver damage if an overdose is consumed (Savides and Oehme, 1983). Paracetamol is mainly metabolized in liver to excretable

glucuronide and sulphate conjugates (Jollow et al., 1974; Wong et al., 1981). However, the hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites when a part of paracetamol is activated by hepatic cytochrome P-450 (Savides and Oehme, 1983) to a highly reactive metabolite N-acetyl-P-benzoquinoneimine (NAPQI) (Vermeulen et al., 1992). NAPQI is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid (Moore et al., 1985). However, when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or -SH group of protein and alters the homeostasis of calcium after depleting GSH.

MATERIALS AND METHODS

Preparation of plant material

Fresh parts of *Balanites aegyptiaca*, *Khaya senegalensis, Prosopis africana,* and *Vitellaria paradoxa* were collected in Sangere Village near the Federal University of Technology, Yola. Taxonomy of the species was determined at the Herbarium of the department of Forestry and Wildlife Management of the University. For phytochemical screening, an extract of each plant part was prepared as described by Ojo et al. (2005).

To test for the effect(s) of the plants on acetaminophen- induced liver damage, stem bark of plants were collected and dried at room temperature. Dried stem barks were ground to powder and a 10% aqueous suspension of the ground sample was prepared by mixing 10 g of the powdered stem barks with 100 ml of distilled water. The mixture was left for 12 h and then filtered with Whatman's No 1 filter paper. The filtrate was stored in refrigerator until used.

Chemicals

Assay kits for the estimation of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were purchased from Randox, UK. Commercially available paracetamol produced by Dana Pharmaceutical (Nig.) was used to induce liver damage. Other chemicals used were of analytical grade.

Administration of experimental treatments

Matured, male albino rats of Winstar strain of average weight of 130 g were used for this study. They were purchased from the animal house of the University of Jos, Nigeria. Experimental animals were kept under standard laboratory condition and were allowed feed of commercial rat pellets and water *ad libitum*. Using a completely randomized design (CRD) with five replicates, 25 rats were divided randomly into groups namely normal (no treatment), control (paracetamol alone) and test (extract of plants + paracetamol). Rats in normal group received an oral dose of 1 ml/rat of sucrose on the 10^{th} day of the experiment. Control rats were administered with hepatotoxic dose (2 g/kg body weight) of paracetamol (Tella and Ojo, 2005) on the 10^{th} day of the experiment orally and rats in test groups received a daily oral dose of 100 mg/kg body weight of the extract corresponding to their group for 10 days and a single dose of 2 g/kg body weight of paracetamol on the 10^{th} day. All rats were killed 12 h after administration of paracetamol under mild ether anaesthesia. Blood were collected and serum separated immediately and stored in the refrigerator. All serum samples were used 1 h after collection.

Phytochemical screening

Phytochemical screening for tannin, phlobatannin and anthraquinone was carried out by the method of Trease and Evan (1978). Cardiac glycoside and saponin were determined by the method described by Sofowora (1991). Assay for alanine transaminase (ALT) and aspartate transaminase (AST) were done by the method described by Reitman and Frankel (1957) and serum levels of alkaline phosphatase was measured by the method of Wright et al. (1970).

Statistical analysis

Results were presented as Mean ± S.E.M for five determinations. Data collected were further analyzed by ANOVA. Mean separation was done by computing the least significant difference (LSD). Values of $P<0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Phytochemical composition of plants extracts

Table 1 shows the result of phytochemical screening for the four plant species considered in this study. Saponin was present in a concentration greater than 100 mg/kg of plant part in both the leaf, stem bark and root extracts of *B. aegyptiaca*. Also, the concentration of saponin was in the range of 10 to 50 mg/kg in the leaf, 50 to 100 mg/kg in the stem bark and greater than 100 mg/kg in the root of *K. senegalensis* (Table 2). Leaves of *P. africana* contained saponin in a concentration less than 10 mg/kg of plant part while other parts exhibited values in the range of 10 to 50 mg/kg. The concentration of saponin in *V. paradoxa* was not significant (less than 10 mg/kg) in all the extracts of the plant examined in this study.

The quantity of tannin was significant observed in the leaf, stem, and root extracts of *K. senegalensis*, *P. africana* and *V. paradoxa* was greater than 100 mg/kg plant part. However, lower concentrations were observed in the root (50 to 100 mg/kg), leaf and stem (10 to 50 mg/kg) of *B. aegyptiaca* (Table 2). The concentrations of cardiac glycosides and phlobatannin recorded for the four plants ranged between 10 and 50 mg/kg plant part. Similarly, cardiac glycosides was not significantly present in the stem and root of *B. aegyptiaca* and leaf of *P. africana* (Table 2). Anthraquinone occurred in a very high concentration (> 100 mg/kg) in the leaf extract of *K. senegalensis*, moderately high in (50 to 100 mg/kg) in stem and root of *K. senegalensis*, and very low (< 10 mg/kg) in the leaves of *B. aegyptiaca*, *V. paradoxa* and *P. africana*.

The results provide an empirical basis for the use of these plants in traditional medicinal practices. Phytochemicals have been reported to have medicinal uses (Godwin and Mercer, 1993; Tella and Ojo, 2005).

Table 2. Phytochemical Compositions of *Balanites aegyptiaca*, *Khaya senegalensis, Prosopis africana,* and *Vitellaria paradoxa.*

Species/Plant part	Saponin	Tannin	Phlobatannin	Cardiac glycosides	Anthaquinones
Balanites aegyptiaca					
Leaf	+++	+	-	+	+
Stem	+++	+	+	-	-
Root	+++	++	+	-	-
Khaya senegalensis					
Leaf	+	++	++	+	+++
Stem	++	+++	+	+	++
Root	+++	+++	+	+	++
Prosopis africana					
Leaf	-	+++	+	-	+
Stem	+++	+++	+	++	+
Root	+++	+++	-	+	++
Vitellaria paradoxa					
Leaf	-	+++	+	+	+
Stem	-	+++	+	+	++
Root	-	+++	+	+	++

Note: +++ = Very high concentration (> 100 mg/kg), ++ = Moderately high concentration (50 mg/kg < x < 100 mg/kg), + = Low concentration (10 mg/kg < x < 50 mg/kg) and - = Not significantly present (< 10 mg/kg).

Specifically, saponin has been reported to have antimicrobial effects (Mahato et al., 1988, 1992) and could serve as precursors of steroidal substances with a wide range of physiological activities (Madusolomuo et al., 1999). The preponderance of saponin in the extracts of *B. aegyptiaca*, *K. senegalensis* and *P. africana* could then justify the use of these plants in the treatment of some microbial infections mentioned earlier. However, the observation that *V. paradoxa* does not contain saponin in a significant concentration but possesses remarkable physiologic activities suggested that the activities observed may not be due to the presence of saponin only, but that other phytochemical components may be physiologically active as well. High concentration of tannin in *V. paradoxa* could be related to the observed hepatoprotective potentials of the plant extract.

Hepatoprotective effects of plants extracts

In the assessment of liver damage by paracetamol or any other hepatotoxin, the determination of enzyme levels such as ALT and AST is largely used (Dobbs et al., 2003; Zu et al., 2002). Necrosis or membrane damage releases the enzyme into circulation; therefore, it can be measured in serum. High levels of AST indicate liver damage, such as that due to viral hepatitis, cardiac infarction and muscle injury. ALT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, ALT is more specific to the liver, and is thus a better parameter for detecting liver injury (Williamson et al., 1996). Serum ALP level on the other hand, is related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis of the enzyme, in presence of increasing biliary pressure (Moss and Butterworth, 1974). Table 3 shows that paracetamol alone caused a significant elevation ($P<0.05$) of serum levels of AST, ALT and ALP in the experimental animals. Administration of paracetamol to experimental animals increased the serum levels of AST, ALT and ALP.

Our results using the model of paracetamol-induced hepatotoxicity in rats demonstrated that the different plant extracts caused significant inhibition of elevated serum AST, ALT and ALP levels (Table 3). Effective control of alkaline phosphatase activity points towards an early improvement in the secretory mechanism of the hepatic cell. Rats treated with all the plant extracts and paracetamol significantly ($p<0.05$) less values for AST, ALT and ALP compared to control rats (Table 3) . The general trend observed indicated that these plants could protect the liver against acetaminophen induced hepatotoxicity.

A comparative analysis of the inhibitive potentials of the four plant extracts is presented in Figure 1. The figure showed that the plants extracts generally produced a greater inhibition of serum level of ALP with *K. senegalensis* producing the highest % inhibition. *V. paradoxa* produced the best inhibition of paracetamol-induced serum elevation of ALT. Similarly, *P. africana* exhibited the greatest potential for the protection against serum elevation of AST by acetaminophen administ-ration. Our result suggested that the plants studied could

Table 3. Effects of Extracts of *Balanites aegyptiaca*, *Khaya senegalensis, Prosopis africana, and Vitellaria paradoxa* on serum levels of AST, ALT, and ALP.

Groups	AST (IU)	ALT (IU)	ALP (KA/Unit)
Normal (No treatment)	10.10 ± 1.63^{a}	13.24 ± 1.25^{a}	12.05 ± 1.02^{a}
Control (Paracetamol alone)	59.12 ± 3.19^{b}	87.17 ± 3.15^{b}	150.62 ± 9.62^{b}
Test 1 (*B. aegyptiaca* + Paracetamol)	25.10 ± 2.15^{c}	31.00 ± 2.45^{c}	87.22 ± 5.85^{c}
Test 2 (*K. senegalensis* + Paracetamol)	25.25 ± 0.57^{c}	37.75 ± 2.46^{d}	98.56 ± 7.24^{d}
Test 3 (*P. africana* + Paracetamol)	35.23 ± 1.54^{d}	31.10 ± 2.35^{c}	87.59 ± 3.56^{c}
Test 4 (*V. paradoxa* + Paracetamol)	31.48 ± 2.53^{e}	48.23 ± 2.35^{e}	89.26 ± 5.42^{c}

Values are mean ± S.E.M for five determinations. Values in the same column having different superscript are significantly.

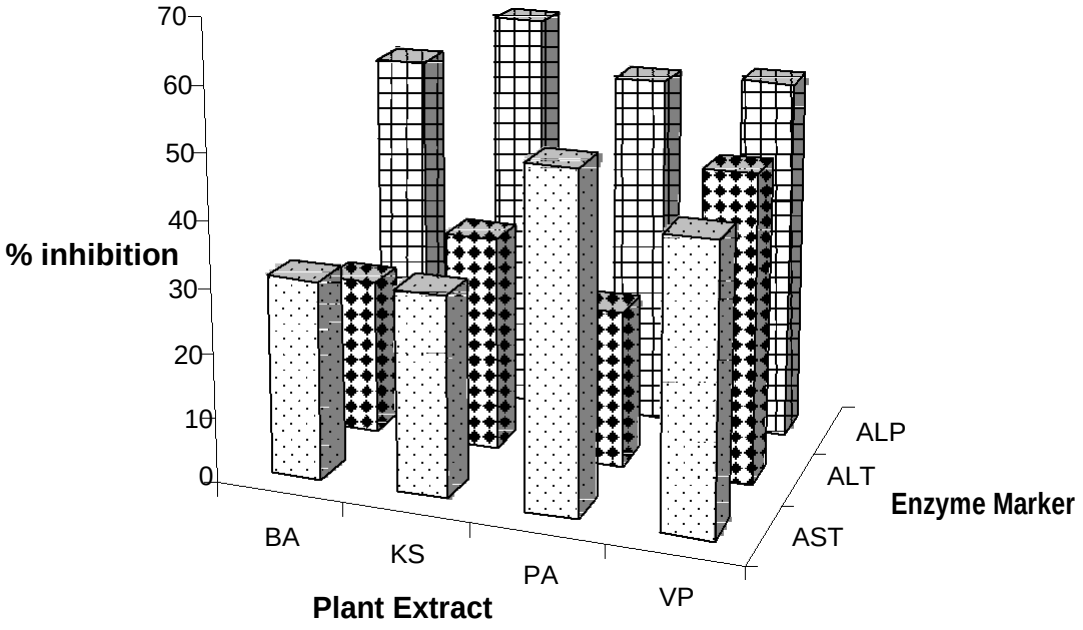

Figure 1. % Inhibition of Liver Damage by plant Extracts.

offer protection against acetaminophen-induced hepatic injury.

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