MEDICINAL PROPERTY AND TOXICOLOGICAL ASSESSMENT OF THE AQUEOUS EXTRACT *BALANITES AEGYPTIACA* MESOCARP.

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ABSTRACT

The medicinal property and toxicological effect of *Balanites aegyptiaca* mesocarp aqueous extract were evaluated in rats. Subchronic oral administration of 0.5, 1.0 and 1.5g/kg body weight of mesocarp aqueous extract for 21 days to rats did not result in significant difference in the activities of liver enzymes (alanine aminotransferase{ALT}, aspartate aminotransferase{AST}, alkaline phosphatase {ALP}) when compared with the control. The ability of partially purified anthocyanin from the extract to cure carbon tetrachloride (CCl_4) induced lipid peroxidation was investigated using plasma melondialdehyde (PMDA) as an index of this process. Administration of 0.5mg/kg anthocyanin to rats for 4 and 7 days respectively after CCl_4 administration did not show any significant ($P>0.05$) decrease in PMDA concentration when compared with CCl_4 treated rats. This shows that 0.5mg/kg anthocyanin did not cure the lipid peroxidative effect within 4 and 7 days. The administration of 1mg/kg anthocyanin for 4 and 7 days after CCl_4 administration showed significant decrease in PMDA concentration compared with CCl_4 treated rats. The result suggests possible cure of the peroxidative effect of CCl_4.

Keywords: Liver enzymes, *Balanites aegyptiaca*, Anthocyanin, Carbon tetrachloride.

INTRODUCTION

Balanites aegyptiaca (L.) Delile (Balanitaceae), popularly known as Desert date, is a spiny, evergreen tree commonly grown in the arid regions of Africa, the Middle East and Southern Asia (Yadav and Panghal, 2010). It is a multi-branched, spiny shrub or tree which grows up to 10 m in height (Yadav and Panghal, 2010; Chothani and Vaghasiya, 2011). Almost all the parts of *B. aegyptiaca* plant are traditionally used in several folk medicines. In the Sahara region of Africa, the fruits are used as oral hypoglycemic drug (Kamel, 1998) while the stem, root and leaf extracts of *B. aegyptiaca* have commonly been used as various traditional folk medicines especially in Africa and southern Asia. Anthocyanins have long been known to be involved in plant pigmentation. They are responsible for brilliant orange pink, scarlet, red, and blue color of flower petals and fruits of higher plants (Harborne, 1967). The roles of anthocyanin pigments as medicinal agents have been a well-accepted dogma in folk medicine throughout the world, and in fact, these pigments are linked to an amazinghy broad-based range of health benefits (Lila, 2004). For example, anthocyanins from Hibiscus sp. have historically been used in remedies for liver dysfunction and hypertension, bilberry (Vaccinium) anthocyanins have an anecdotal history of use for vision disorders, microbial infections, diarrhea and other heath disorders (Wang *et al.,* 2000; Smith, *et al.* 2000).

Carbon tetrachloride induces lipid peroxidation and liver damage. The peroxidative effect is associated with increased production of malondialdehyde, diene conjugates (Recknagel and Glende, 1973) as well as 4-hydroxynonenal (Pryor and Porter, 1990; Esterbauer *et al.,* 1991) which are degradation products of polyunsaturated fatty acid hydroperoxides. The lipid peroxidation and liver damage happens when trichloromethyl radical ($^{*}CCl_3$) obtained from CCl_4 reacts with molecular oxygen forming trichloromethylperoxy radical ($CCl_3 - 0 - 0^{*}$) (Mico and Pohl, 1983). Its effect on the liver provides a model with which natural plant products have been assessed for antioxidant and/or hepatoprotective functions (Hase *et al.,* 1996). The present investigation was aimed at evaluating the sub-chronic toxicity of aqueous mesocarp extract and hepatocurative effect of partially-purified anthocyanin from *B. aegyptiaca* by measuring liver enzyme activities and plasma malondiadehyde.

MATERIALS AND METHODS

Plant materials and Experimental Animals.

The fruit samples were collected from *B. aegyptiaca* trees within Bayero University Kano old campus and taken to Biological Sciences Department of the University for authentication. The mesocarp was removed from the fruits by soaking overnight in water after which it was washed away from the hard-pointed wood endocarp that surrounds the kernel seeds. The viscous liquid obtained was allowed to dry in the Laboratory to obtain the solid brown mesocarp. A known weight (50g) of the dried mesocarp was dissolved in 100cm^3 of distilled water and poured into air-tight amber-bottle kept in the refrigerator. Partially purified anthocyanin was obtained from the mesocarp by the method of Takeda *et al.* (1994) for administration to the rats.

White albino rats of wistar strain weight 180 – 220 g were used for this study. They were obtained from the animal house of Biological Sciences Department of Bayero University Kano old campus. The rats were allowed to adjust to the Laboratory condition for a period of two weeks before commencement of the experiments. Food (Vital feeds, Jos) and water were provided ad libitum during the stabilization period.

Experimental Design

For the sub-chronic toxicity study, twenty rats were divided into four groups of five rats each housed in standard cages. The medicinal property of *B. aegyptiaca* aqueous mesocarp was evaluated using thirty rats which were divided into six groups of five rats each. In the four groups of rats for the subchronic toxicity study, group I was the control while groups II to IV were orally administratered with 0.5, 1.0 and 1.5 g/kg mesocarp aqueous extract respectively for 21 days after which they were sacrificed and blood samples collected.

The blood samples collected were allowed to clot, centrifuged and serum samples collected for Liver function tests. Alanine aminotransaminase (ALT), Aspartate aminotransaminase (AST) and Alkaline phosphatase (ALP) activities and serum bilirubin concentrations were determined. For the curative effect of CCl_4 induced liver damage by the partially purified anthocyanin from the *B. aegyptiaca* mesocarp, the rats in group I (normal control) were not treated with either anthocyanin or CCl_4 but were given 5% aqueous ethanol which was used in the extraction procedure, as a vehicle for anthocyanin administration. Group II rats (test control) were administered with CCl_4 alone at a single dose of 120 mg/kg of CCl_4 in vegetable oil subcutaneously. Groups III and IV were administered with 0.5 mg/kg anthocyanin daily for 4 days and 7 days respectively after 48 hrs of CCl_4 administration. Groups V and VI were administered with 1mg/kg anthocyanin daily for 4 days and 7 days respectively after 48 hrs of CCl_4 administration.

The rats in groups I and II were sacrificed 48 hrs after CCl_4 administration to confirm the inducement of liver damage. Those in groups III to VI were sacrificed after 4 days and 7 days respectively and blood samples were collected and centrifuged at 5000rpm for 5 minutes to separate the plasma for Malondialdehyde determination.

Biochemical Analysis

Alanine aminotransferase and aspartate aminotransferase activities were determined using the method of Reitman and Frankel (1957)., alkaline phosphatase by the method of Kind and King (1954); serum bilirubin by the method of Kingsley *et al.* (1953); and plasma malondialdehyde by Hunter *et al.* (1963) modified by Gutteridge and Wilkins (1982).

Statistical Analysis

Significant difference ($P<0.05$) between mean values of the parameters assessed was calculated using students't' test (Muktar, 2003).

RESULTS

Subchronic toxicity study (Table I) showed no significant differences ($P>0.05$) in ALT and AST activities after 21 days treatment with various doses of the aqueous mesocarp extract. However signifant ($P<0.05$) increases in ALP activities were obtained when compared with the control. Also no significant difference in unconjugated bilirubin levels in the rats treated with the extract when compared with control. Results of plasma malondialdehyde (PMDA) concentration in rats subcutaneously administered with 120 mg/kg

CCl_4 before anthocyanin administration for 4 days and 7 days are shown in Table 2. The result showed significant increase ($P<0.05$) in PMDA concentration in the CCl_4 administered group compared with the normal control group and significant decreases were obtained in groups administered with anthocyanin1mg/kg for 4 days and 7 days respectively when compared with CCl_4 alone treated group.

Table 1: Liver enzymes activities and unconjugated Bilirubin concentrations in rats Administered with three different doses of B. aegyptiaca Mesocarp Aqueous Extract for 21 days.

Groups	Dose of extract (g/kg)	AST (U/L)	ALT (U/L)	ALP (U/L)	Unconjugated bilirubin (mg/dl)
Control	-	91.68±31.51	89.65±10.50	227.71±20.10	4.20±2.82
I	0.5	93.36±30.15	91.50±11.20	*275.16±23.00	4.40±2.40
II	1.0	92.45±28.70	90.80±10.40	*250.60±20.45	4.80±2.65
III	1.5	94.20±32.76	92.50±11.50	*280.94±21.07	4.00±2.66

n= 5 Rats per group
Values with asterisk are significantly different at $P<0.05$ when compared with control rats.

Table 2: Results of Plasma Malondialdehyde (PMDA) Concentration in Rats Administered with CCl_4 before anthocyanin Administration for four and seven days.

Groups	Treatment	PMDA (Umol/L)
I	Normal control (no CCl_4 and anthocyanin administered)	1.00±0.32
II	Test control(120mg/kg CCl_4 administered no anthocyanin)	3.87±1.78[a]
III	0.5mg/kg anthocyanin administered for 4 days after CCl_4 administration.	4.20±1.80
IV	0.5mg/kg anthocyanin administered for 7 days after CCl_4 administration	4.02±1.60
V	1mg/kg anthocyanin administered for 4 days after CCl_4 administration	1.51±0.31[b]
VI	1mg/kg anthocyanin administered for 7 days after CCl_4 administration	0.75±0.12[b]

DISCUSSION

The measurement of hepatic enzymes released into the blood as a consequence of injury has proven to be a sensitive indicator of hepatoticity. The activities of hepatic enzymes in the serum play a significant role in the assessment of drugs or plant extracts for safety or toxicity risk. The enzymes considered in this study (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase) are useful marker enzymes of the liver cells (Schmidt and Schmidt, 1979).

The subchronic toxicity study results of liver enzyme activities as well as unconjugated bilirubin concentrations in control rats and those administered with the mesocarp aqueous extract for 21 days are shown in Table 1. Oral administration of the aqueous mesocarp extract for 21 days did not result in significant difference in the activities of AST and ALT. However, significant increases in ALP activities were obtained after 21 days of the aqueous extract administration. Since higher ALT and ASP activities are indicative parameters of liver damage or disorders (Kaplan *et al.,* 1995; Annino and Giese, 1976), the mesocarp may thus be said to have no hepatotoxic effect at the given doses. The increase in ALP activities obtained in extract-treated groups would have come from the liver only if other enzyme activities (ie. AST, or more specifically ALT) are also high. The increase in ALP could come from its other sources such as the bones, kidney and the intestine. The oral administration of the extract at the specified doses showed no significant difference in unconjugated serum bilirubin levels.

The medicinal effect of partially-purified anthocyanin extract obtained from the aqueous mesocarp extract of *B. aegyptiaca* was studied to determine its curative effect against CCl_4 induced-lipid peroxidation in rats. The result in Table 2 showed that CCl_4 caused elevation in PMDA concentration in group II (CCl_4 alone treated) which is indicative of lipid peroxidation. This is in conformity with the result of previous study (Obi and Uneh, 2003). It showed a significant increase in PMDA ($P<0.05$) concentration when compared with normal control group. administration of 0.5 mg/kg anthocyanin for 4 and 7 days respectively (Groups III and IV) after CCl_4 administration did not show any significant decrease in PMDA concentration. This shows that 0.5mg/kg anthocyanin did not cure the peroxidative effect within 4 and 7 days or that the dose is not effective enough for the cure at the stated durations. Administration of 1mg/kg anthocyanin for 4 and 7 days respectively (groups V and VI) after CCl_4 administration showed significant decrease ($P<0.05$) in PMDA concentrations compared with group II (CCl_4 alone treated group).

This result suggests possible cure of the peroxidative effect of CCl_4. The curative effect can be said to be dose-dependent as 0.5 mg/kg anthocyanin could not cure this effect and the 7 days 1mg/kg anthocyanin administration gave a much lower PMDA concentration than the 4 days administration of the same dose.

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