African Journal of Pharmacy and Pharmacology Vol. 3(7). pp. 354-358, July, 2009
Available online http://www.academicjournals.org/ajpp
ISSN 1996-0816 © 2009 Academic Journals

Full Length Research Paper

Acute and oral subacute toxicity of methanolic extract of *Bauhinia monandra* leaf in rats

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Accepted 2 July, 2009

In this study, the acute and subacute toxicity of *Bauhinia monandra* methanolic leaf extract were investigated in rats. Acute administration of the extract up to a dose of 8 g/kg body weight to the animals elicited no deaths or treatment related signs of toxicity. Oral subacute administration of the extract (2.0 and 4.0 g/kg body weight) did not show any macroscopic changes in the key organs investigated in the rats. Histopathological examination revealed no significant adverse effects on the liver, spleen, testes and kidneys except for focal expansion of the interstitial stroma and lymphoid follicles in the lungs. Biochemical investigations revealed no significant ($p>0.05$) alterations in the total cholesterol, total protein and lactate dehydrogenase (LDH) activity in the serum. However, there was a significant ($p<0.05$) increase in the serum triglyceride concentration at 4.0 g/kg, which represented 33% of the control values. The extract produced no significant changes in the total protein concentration and LDH activity in the liver of the treated groups when compared with the control. The results suggest that the acute administration of methanolic extract of *B. monandra* may possess relatively low toxicity but caution has to be exhibited when used subacutely as anti-diabetic remedy.

Key words: Toxicity, *Bauhinia monandra*, rat, acute, subacute.

INTRODUCTION

The genus *Bauhinia* (Caesalpnaceae) consists of about 600 species, which include trees, vines and shrubs frequently, planted for its showy flowers and ornament foliage (Miyake et al., 1986). In Nigeria, *Bauhinia monandra* is used in the treatment of diabetes by traditional health practitioners and also in Brazil (Abo and Jimoh, 2004; Argolo et al., 2004). Furthermore, *Bauhinia forticata* that is a related species is one of the most frequently used anti-diabetic herbal remedies in Brazil (Pepato et al., 2004). It has also been shown that *B. monandra* has antioxidant properties (Aderogba et al., 2006), factors and haemagglutinating activity (Abreu et al., 1988, 1990).

Studies on the chemical composition of the leaves have led to the isolation of Quercetrin-3-O-rutinoside and Quercetin (Aderogba et al., *2006*), ß-Carotene (Essien and Fetuga, 1989) and lecitin (Coelho and Silva, 2000). Although phytoconstituents with pharmacological properties play a significant role in the health-economy of man, often times, not much information is known about their possible toxic side-effects. Thus, studies on the toxicity of such phytoconstituents become imperative in order to appraise adequately their suitability for use by man (Calixto, 2000; Matos, 2000; Lapa et al., 2002). The purpose of this study therefore is to evaluate the potential toxicity of the methanolic extract of *B. monandra* considering its widespread ethnomedical use in the management of diabetes.

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MATERIALS AND METHODS

Plant collection and extraction

The leaves of *B. monandra* were collected at Obafemi Awolowo University (O.A.U.), Ile-Ife, Nigeria and identity of the plant was confirmed by Mr. Oladele of the Department of Pharmacognosy, Faculty of Pharmacy, O.A.U., Ile-Ife.

A voucher specimen (FHI106762) was subsequently deposited at the Herbarium of Forestry Research Institute of Nigeria, Ibadan, Ni

geria. The fresh leaves were dried in an Oven at 40°C and the dried leaves then milled to a fine powder. The powdered plant material (4.15 kg) was then extracted at room temperature with methanol (3x 1000 mls). The extract obtained was concentrated in vacuo to give a residue (yield, 9.4% w/w).

Animals

Forty inbred young wistar rats of both sexes (125.3 ± 25.1 g), 8 weeks old at the start of the experiment were obtained from the Animal House, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. They were acclimatized to the standard laboratory conditions (12 h day/night cycle) for 2 weeks prior to initiation of the study. Standard laboratory feeds (Ladokun feeds Ltd., Ibadan, Nigeria) and water was supplied *ad libitum*. The animal experiments were performed according to the approved guidelines of the Obafemi Awolowo University research ethics committee.

Chemicals

Assay kits for the estimation of serum triglyceride, cholesterol and lactate dehydrogenase (LDH) were purchased from Randox Laboratories Limited, U.K. All other chemicals were of analytical grade.

Acute toxicity testing

The possible acute toxicity of the test extract of *B. monandra* was tested by using adult rats of either sex. Twenty five animals were randomly divided into five groups with 5 animals each. Group I served as normal control and was administered with distilled water p.o. Groups II – V were administered (p.o.) with extract, in distilled water, using a canular at a dose of 1.0, 2.0, 4.0 and 8.0 g/kg respectively (Waynforth, 1969; Cheng et al, 1992).

The rats were fasted for 16 hours before and 3 h after administration of the extract as described by Bidhe and Ghosh (2004). The animals were then examined at 10, 30, 60 and 120 min and at 4, 6 and 24 h for gross behavioural changes and mortality.

Subacute toxicity testing

In a 28-days subacute toxicity study, fifteen male rats were divided into three groups of 5 rats each. Group I that served as normal control was administered with distilled water (p.o.) while groups II and III were administered daily with the extract (p.o.) for 28 days at a dose of 2.0 and 4.0 g/kg respectively.

The animals were then observed daily for gross behavioural changes and any other signs of subacute toxicity. The weight of each rat was recorded on day 0 and weekly throughout the course of the study, food and water consumption per rat was calculated. At the end of the 28 days they were fasted overnight, each animal was anaesthetized with diethylether, following which they were then dissected and blood samples were obtained by cardiac puncture into heparinised tubes. The Spleen, testes, lung, liver and kidney tissues were excised and preserved in 10% neutral formalin for histopathological assessment. Portions of the liver samples were also excised and washed immediately with ice cold 0.25 M sucrose after which a 10% (w/v) homogenate was prepared in ice cold 0.25 M sucrose solution using a motor driven glass-Teflon potter-Elvejhem (TRI-STR-R K43) homogenizer running at 1000 rev/min.

The resulting crude homogenates were put in pre cooled centrifuge tubes and centrifuged at 3000 X g at 4°C for 5 minutes. The supernatant was assayed for LDH and protein. The blood sample collected from each rat was centrifuged with 3000 X g at4°C for 10 min to separate the serum and used for the biochemical assays.

Biochemical analysis

Rat serum levels of triglyceride and cholesterol were determined by the enzymatic colorimetric methods of Wahlefeld and Bergmeyer (1974), Siedel et al. (1981) and Tietz (1990) using commercially available kits (Randox, U.K), (Cat. No. TR 210 for triglyceride and Cat. No. CH 200 for cholesterol) while the enzymatic activities of LDH in blood and liver were determined also using a commercially available kit (Randox, U.K), (Cat. No. LD401).

The Protein concentration of the serum and supernatant fraction of the liver was determined by the method of Lowry et al. (1951) as described in Plummer (1988) using Bovine serum albumin as a protein standard.

Histopathology

Immediately after dissection, the sections of the spleens, testes, lungs, livers and kidneys were placed in a tissue cassette and fixed in 10% buffered formalin for 24 h after which they were processed using standard histopathological methods. The processed tissues were then embedded in paraffin. Sections of 6 µm thickness were cut on a rotary microtone and stained with haematoxylin and eosin (Drury and Wallington, 1973) for microscopic assessment.

Statistical analysis

Values were represented as mean ± SD. Data were analysed using one-way analysis of variance (ANOVA) and group means were compared using the Tukey-Kramer Multiple Comparisms Test using a GraphPad Instat® software. P values < 0.05 were considered significant.

RESULTS

During the subacute toxicity tests, the results obtained on the average daily water, food intake and weekly weight gain are presented in Table 1. The eating and drinking habit and behavior of all the animals used were normal in both vehicle-treated and *B. monandra* extracts- treated animals.

The results obtained on the biochemical parameters of rats fed with methanolic extract of *B. monandra* for 28 days are presented in Table 2. Plates (1,2,3,4 and 5) show the photomicrography of the kidney, lung, liver, spleen and testes isolated from animal used as controls (As) and methanolic leaf extract of *B. monandra* (2.0 g/kg (Bs), 4.0 g/kg (Cs)) during the 28 days administration respectively.

The results revealed that other essential organs such as the liver, kidney, spleen and testes were not adversely affected during the subchronic administration. Acute and subacute oral administration of methanolic leaf extract of *B. monandra* did not cause any significant changes in gross behavioural effects in rat.

DISCUSSION

In the acute toxicity study, it was observed that all animals showed no overt signs of distress, neither was there any observable symptom of toxicity. Furthermore all the animals survived the duration of the acute toxicity test

Table 1. Feeding pattern in the subchronic study of *Bauhinia monandra.*

Parameters	Control	2 g/kg	4 g/kg
Average water intake (ml/day)	43.20 ± 4.00	40.80 ± 0.80	41.40 ± 0.60
Average feed intake per (g/day)	19.00 ± 0.70	19.70 ± 0.20	19.60 ± 0.40
Average weekly weight gain (g)	2.30 ± 0.04	2.30 ± 0.04	2.20 ± 0.03
Average feed conversion efficiency (%)*	10.60 ± 2.00	11.50 ± 010	10.20 ± 0.20

*Feed conversion efficiency (%) = weekly body weight gain (g) / weekly food consumption X 100.
Each value represents the mean ± standard deviation (n = 5).

Table 2. Biochemical parameters of rats fed with *Bauhinia monandra* for 28 days.

Parameters	Control	2 g/kg	4 g/kg
Triglyceride concentration (mmol/l)	0.31 ± 0.09	0.31 ± 0.02	0.40 ± 0.10*
Cholesterol concentration (mmol/l)	1.30 ± 0.18	1.40 ± 0.29	1.30 ± 0.36
Serum protein (mg/ml)	9.30 ± 1.62	6.90 ± 2.09	7.20 ± 1.79
Liver protein (mg/ml)	165.20 ± 7.93	167.50 ± 8.69	180.30 ± 12.70
Serum lactate dehydrogenase activity (nM/min/ml)	0.70 ± 0.08	0.80 ± 0.04	0.80 ± 0.07
Liver lactate dehydrogenase activity (nM/min/ml)	1.20 ± 0.19	1.50 ± 0.48	1.08 ± 0.28

Each value represents the mean ± standard deviation (n = 5), *statistically different from control ($p < 0.05$) one-way ANOVA + Tukey –Kramer Multiple Comparisms Test.

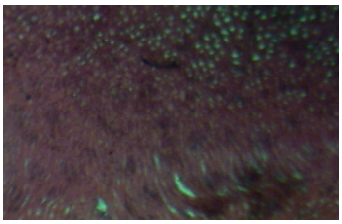

Plate A

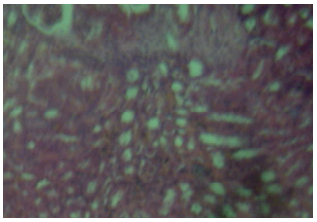

Plate 1B

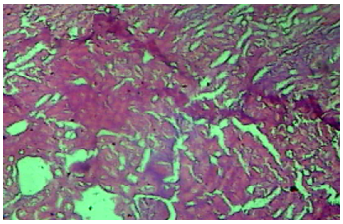

Plate 1C

Plate 1 (A, B and C). Photomicrograph of the Kidney of Rat (A, Control), B, 2 g/kg; C, 4 g/kg) *B. monandra* leaf methanolic extract. A is essentially normal while B has focal proximal tubular epithelial necrosis and scanty inflammatory infiltrate of the interstition compared to the control which is normal. X50

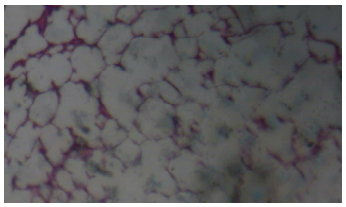

Plate 2A

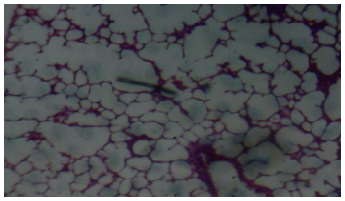

Plate 2B

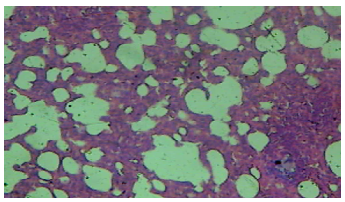

Plate 2C

Plates 2 (A, B and C). Photomicrograph of the Lungs of Rat (A, Control), B, 2 g/kg; C, 4 g/kg) *B. monandra* leaf methanolic extract. C has dense infiltration of the stroma, focal expansion of interstitial stroma, vascular channel congestion and compressed alveolar spaces, likewise B but not as intense and these features are consistent with interstitial pneumonitis when compared to the normal control. X50.

(24 h). Similarly, in the subacute study, no signs and symptoms of toxicity were observed. The average water and food intake in the control group was not significantly ($p>0.05$) different from that of the test groups (Table 1). The feed conversion efficiency followed the same pattern, thus indicating a normal metabolism of the animals. The changes in the serum cholesterol, triglyceride, liver protein, serum protein concentrations and the activity of lactate dehydrogenase (LDH) of the liver and serum following the administration of 2.0 and 4.0 g/kg of *B. monandra*

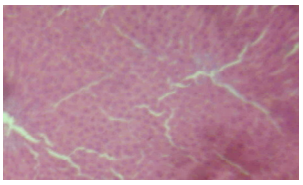

Plate 3A

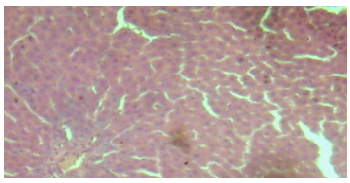

Plate 3B

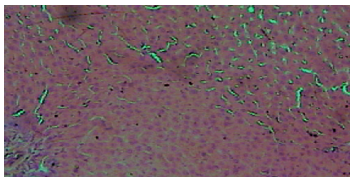

Plate 3C

Plate 3(A, B and C). Photomicrograph of the Liver of Rat (A, Control), B, 2 g/kg; C, 4 g/kg) *B.monandra* leaf methanolic extract. B has preserved lobular architecture, normal sinusoids, normal portal area and preserved limiting plate hepatocytes as the control while all these were the same for C except for the presence of focal hepatocyte proliferation and the inflammatory infiltration of the portal areas. X50.

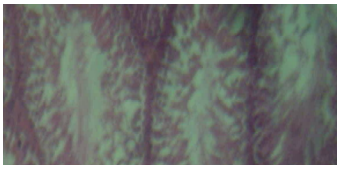

Plate 4A

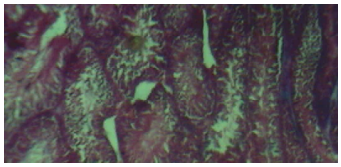

Plate 4B

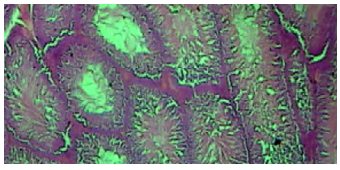

Plate 4C

Plate 4(A, B and C). Photomicrograph of the Testes of Rat (A, Control), B, 2 g/kg; C, 4 g/kg) *B. monandra* leaf methanolic extract. B and C are essentially normal compared to the control. X50.

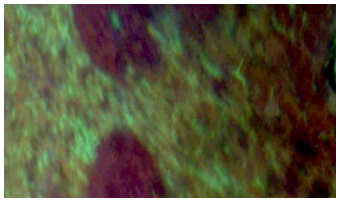

Plate 5A

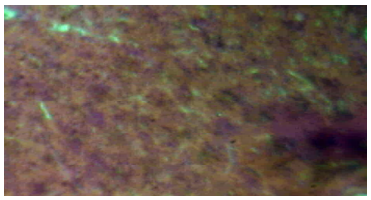

Plate 5B

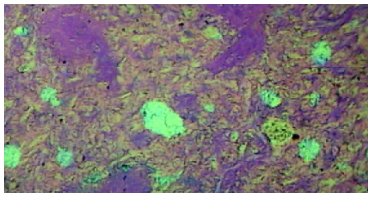

Plate 5C

Plate 5(A, B and C). Photomicrograph of the Spleen of Rat (A, Control), B, 2 g/kg; C, 4 g/kg) *B. monandra* leaf methanolic extract. B and C are essentially normal compared to the control .X50.

methanolic extract are shown in Table 2. There were no significant alterations in the serum cholesterol, liver protein, LDH activity of the liver and serum as well as serum protein concentration when compared to the controls. On the other hand, there was a significant increase ($p<0.05$) in the triglyceride concentration only at 4.0 g/kg, this increase represented 33% of the control values. This may be an indication of lipid lipoprotein metabolism disorder particularly hyperlipidaemia (Pagana and Pagana, 2002). Macroscopically, the liver, spleen, lung, testis and the kidney showed no discolouration and the textures were consistent when compared with the control groups.

Histopathological examination revealed that the spleens, livers, testes and the kidneys of rats administered with *B. monandra* methanolic extract showed no differences relative to those of the control group (Plates 1, 3, 4 and 5) at the two dose levels, though there was focal proximal tubular epithelial necrosis in the kidney at 4.0 g/kg (Plate 1C) while there was a variation in the lung between the test doses and the control groups with a sign of Pneumonitis (Plates 2B and C). These results indicate that extract of *B. monandra* at 4.0 g/kg body weight is not toxic to the liver, spleen and testes of rat but has a minor effect on the lungs and kidney. It is well known that the cytotoxicity of a xenobiotic can be evaluated by the alteration in the levels of some key enzymes or metabolites in the serum. One of such enzyme is LDH, a cytoso-lic enzyme that, though distributed throughout the body, possesses an isoenzyme recognized as markers for liver and muscle injury (Pagana and Pagana, 2002). It is also well established that changes in the lipid profile and total protein of serum could be indicative of perturbations in the liver or kidney following toxic injury (Pagana and Pagana, 2002). Thus, since there was no any significant changes in either LDH or serum levels of choleste-rol, triglycerides and protein concentration following a 28 days treatment of the leaf extract of *B. monandra,* it may indicate therefore, that it is not toxic to the animal. Our findings also corroborated an earlier study by Pepato et al. (2004) using the leaf decoction of *B. forficata* in normal and streptozotocin induced diabetic rats in which no significant changes was observed in some key enzymes and metabolites following 4 weeks of treatment.

In conclusion, the present results show that *B. monandra* leaf possesses very low toxicity as indicated in our rat model. No deaths or signs of toxicity were observed in the rats that received the extract up to an oral acute dose of 8 g/kg thus establishing its safety in use.

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