

Sub-chronic Toxicity Studies of the Aqueous Extract of *Boerhavia diffusa* Leaves

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(Received April 29, 2003; Accepted August 15, 2003)

The acute and subchronic toxicity studies of *Boerhavia diffusa* (*B. diffusa*) leaves in albino mice and rats were investigated. Phytochemical analysis was also carried out. 500, 1000 and 2000 mg/kg of the aqueous leaf extract were administered orally to the test groups while distilled water was given to the control group. The parameters measured include food and fluid intake, body weight, absolute and relative weight of various organs, haematological parameters [total white blood cell (WBC) and packed cell volume (PCV)], and tests for liver function: glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase and total bilirubin. The lethal dose (LD₅₀) was found to be greater than 2000 mg/kg (*p.o.*) in both mice and rats. Rats treated with the extract had progressive increase in body weight, which was significantly ($p < 0.05$) different from control. The aqueous extract of *B. diffusa* leaves increased both food and fluid intake. There were no significant changes in both the absolute and relative organ weights between the control and the test groups. The liver enzymes and haematological parameters were statistically equal in all the groups. *B. diffusa* aqueous leaf extract is non toxic in albino rats.

Key words — *Boerhavia diffusa*, subchronic toxicity, liver function, packed cell volume, absolute weight, relative weight

INTRODUCTION

Boerhavia diffusa (*B. diffusa*) (family Nyctaginaceae) is a tropical plant commonly found in swampy areas in Nigeria, India and other parts of the world in both dry and rainy seasons. The local population use extracts of the crushed soaked leaves in the management of diabetes.

B. diffusa aqueous root extract has been reported to show marked protection against thioacetamide-induced hepatic injury maintaining the various liver enzymes and serum bilirubin in rats.¹⁾ The aqueous extract of the thinner roots of *B. diffusa* is used in traditional medicine for inflammatory disorders, bacterial infections and heart diseases.²⁾ It is also used in the treatment of elephantiasis, night blindness, corneal ulcers, various hepatic disorders and as an antiviral agent.^{1,3)} It has been found to be devoid of any teratogenic effects, as it has been administered

to pregnant rats during the entire period of gestation.⁴⁾

Although *B. diffusa* is widely used in folklore medicine, there is a dearth of information on the toxicity of the aqueous extract even as the local population administers it. The present study was undertaken to investigate the acute and sub-chronic toxicity studies of the aqueous leaf extract of *B. diffusa* in rats.

MATERIALS AND METHODS

Collection of Plant Material — The leaf sample of *B. diffusa* was obtained from the surrounding of College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Nnewi, Nigeria. The plant was identified by Mr. A. Ozioko of the Botany Department, University of Nigeria, Nsukka, Nigeria. A voucher specimen was deposited at the herbarium of University of Nigeria.

Preparation of Extract — The plant material was dried, pulverized, finely sieved and soaked in water for 24 hr, after which it was filtered. The filtrate was freeze-dried and the percent yield gave

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Table 1. Effect of *B. diffusa* Aqueous Extract on Weekly Food (g) and Fluid^{a)} (ml) Intake in Rats (*n* = 6)

Treatment	Week		
	1	2	3
Control	301.0 ± 10.0 (830.0 ± 25.8)	310.0 ± 10.7 (815.0 ± 25.0)	315.0 ± 12.0 (845.0 ± 30.3)
500 mg/kg	441.0 ± 12.9 (880.0 ± 35.6)	392.0 ± 11.2 (850.0 ± 33.4)	406.0 ± 12.1 (1085.0 ± 45.0)
1000 mg/kg	455.0 ± 13.1 (1210.0 ± 48.5)	393.0 ± 11.9 (1040.0 ± 40.6)	395.0 ± 12.0 (1185.0 ± 48.0)
2000 mg/kg	469.0 ± 14.5 (1529.0 ± 52.5)	413.0 ± 13.5 (1350.0 ± 50.4)	378.0 ± 12.8 (1190.0 ± 45.8)

a) Values in parenthesis indicate volume of fluid ingested. Values are expressed as mean ± S.D.

11.67.

Phytochemical Screening — Tests for alkaloids, flavonoids, glycosides, tannins, saponins and proteins were carried out using the methods of Trease and Evans⁵⁾ and Odebiyi and Sofowara.⁶⁾

Experimental Animals — Sexually mature male Wistar albino rats weighing 104–214 g and sexually mature albino mice weighing 18.2–24.5 g were obtained from the Animal Facility Center, National Institute for Pharmaceutical Research and Development Idu, Abuja, Nigeria. The animals were kept in a well-ventilated room of 12 hr light and 12 hr darkness. All the animals were fed with standard rat/mouse cubes from Pfizer Pharmaceutical Plc, Ikeja, Nigeria, while water was provided *ad libitum*. The principles of laboratory animal care were followed, while the department's ethical committee approved the use of the animals and the study design.

Acute Toxicity Test — LD₅₀ was determined in mice and rats by the method of Lorke.⁷⁾

Sub-chronic Toxicity Study — A total of twenty-four mature albino rats were used in this study. These were divided into four groups of six rats each. Three of the groups were given 500, 1000 and 2000 mg/kg body weight of the aqueous extract (*p.o.*), respectively, while the control group received distilled water only. Food and water intake were monitored daily. After 30 days of exposure, blood was collected from the animals, by cardiac puncture, for haematological and biochemical assays. Thereafter, the animals were sacrificed and the following organs isolated and weighed: kidney, liver, heart, testis, lungs, spleen and pancreas. Relative weight of the respective organs was calculated from each organ's wet weight and the animal's body weight.

Effect of Extract on Liver Function — About 5 ml of whole blood collected into a plain tube was

centrifuged at 3500 rpm for 5 min using table centrifuge (Beckman, England) and the serum separated and analyzed for the liver enzymes. Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were assayed using the methods of Reitman and Frankel, alkaline phosphatase (ALP) was analysed by the method of King and Armstrong, while total bilirubin level was determined by the method of Malloy and Evelyn. All assay methods employed were as reported by Varley *et al.*⁸⁾

Haematological Assay — EDTA-anticoagulated tubes were used to collect whole blood for these investigations. Packed cell volume (PCV) was determined by the microhaematocrit method, while total WBC was determined by visual method.⁹⁾

Statistical Analyses — Data were analysed using Student's *t*-test and Chi Square.

RESULTS AND DISCUSSION

The acute and sub-chronic toxicity studies of the aqueous leaf extract of *B. diffusa* were carried out. Phytochemical tests indicate that the aqueous extract contains flavonoids, tannins, proteins, glycosides and saponins in the ratio of 3 : 3 : 3 : 2 : 1.

The LD₅₀ (*p.o.*) of aqueous leaf extract of *B. diffusa* was found to be greater than 2000 mg/kg body weight in both mice and rats. It could be due to the wide margin of safety that *B. diffusa* is used as a non-conventional food consumed in various parts of Indian sub-continent.¹⁰⁾

Table 1 shows the effect of various doses of *B. diffusa* aqueous extract on weekly food and fluid intake. The extract increased the food intake of the animal compared to control at *p* < 0.05 throughout

the three weeks of exposure. There was however, a slight reduction in the quantity of food intake in all the extract-treated groups at the second and third weeks compared to the first week. The results also showed significant increases ($p < 0.05$) in water intake among the test groups compared to the control throughout the exposure period. In all, the effect of the extract on food and fluid intake were dose dependent (Table 1).

Rats treated with the various doses of the extract (500, 1000 and 2000 mg/kg) had progressive increase in body weight, which was significantly ($p < 0.05$) different from control (Fig. 1), apart from those in the 1000 mg/kg group, which had a slight decrease in weight at the second week. The progres-

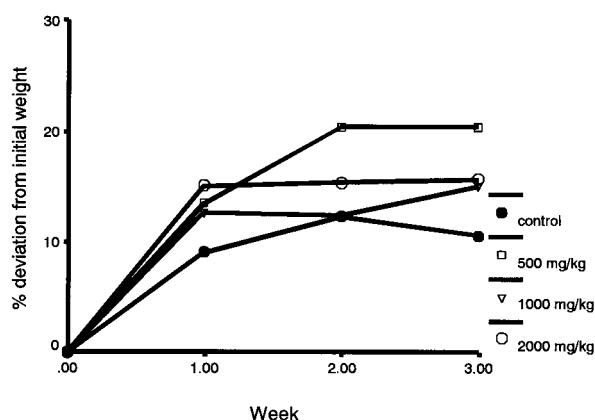


Fig. 1. Effects of Various Doses of *B. diffusa* Aqueous Extract on Weekly Weight of Rats

sive increases in body weight shown in all the animal groups indicate growth response, while the animals treated with the plant extract showed more increase in body weight. This could be as a result of increased food and water intake by these animals. The fact that *B. diffusa* extracts increase body weights of experimental animals has been reported.¹¹⁾

No statistically significant differences existed in the absolute and relative weights of all the isolated organs between the treated and the control rats (Table 2). Kluwe,¹²⁾ documented that the absolute organ weight has been observed to be a relative sensitive indicator of nephrotoxicity for known nephrotoxics, and thereafter defined nephrotoxicity as increased kidney weight (either absolute or relative). The aqueous leaf extract of *B. diffusa* did not induce any toxic effect on the kidneys and the other organs going by this indicator, since the absolute and relative weights of the organs were not significantly different from control values. The hepatoprotective function of 2 mg/kg aqueous root extract of *B. diffusa* against thioacetamide-induced hepatotoxicity in rats has been earlier reported.¹⁾

The effect of aqueous extract of *B. diffusa* on liver enzymes and bilirubin is shown on Table 3. The levels of bilirubin and the liver enzymes: GOT, GPT and alkaline phosphatase were not significantly affected by the extract. Certain drugs and other substances are known to affect and influence circulating bilirubin levels,¹³⁾ and elevation in bilirubin levels suggests increase in haemolysis. The aqueous leaf

Table 2. Effect of Various Doses of Aqueous Leaf Extract of *B. diffusa* on the Relative (%) and Absolute^{a)} (g) Weights of Organs ($n = 6$)

Organ	Treatment			
	Control	500 mg/kg	1000 mg/kg	2000 mg/kg
Lung	0.87 ± 0.10 (1.70 ± 0.26)	0.68 ± 0.07 (1.47 ± 0.15)	0.73 ± 0.10 (1.37 ± 0.21)	0.90 ± 0.15 (1.48 ± 0.26)
Testes	2.23 ± 0.15 (4.37 ± 0.25)	2.19 ± 0.20 (4.67 ± 0.40)	2.18 ± 0.21 (4.10 ± 0.43)	1.83 ± 0.52 (3.00 ± 0.91)
Liver	3.60 ± 0.21 (7.17 ± 0.41)	4.03 ± 0.30 (8.63 ± 0.60)	4.36 ± 0.40 (8.20 ± 0.81)	4.38 ± 0.48 (7.18 ± 0.85)
Pancreas	0.26 ± 0.05 (0.50 ± 0.12)	0.33 ± 0.03 (0.70 ± 0.09)	0.26 ± 0.04 (0.48 ± 0.07)	0.25 ± 0.01 (0.40 ± 0.10)
Kidney	0.73 ± 0.01 (1.43 ± 0.05)	0.73 ± 0.02 (1.55 ± 0.05)	0.79 ± 0.06 (1.48 ± 0.12)	0.92 ± 0.11 (1.45 ± 0.20)
Heart	0.39 ± 0.01 (0.77 ± 0.05)	0.39 ± 0.06 (0.83 ± 0.12)	0.41 ± 0.02 (0.77 ± 0.05)	0.38 ± 0.03 (0.63 ± 0.05)
Spleen	0.41 ± 0.07 (0.80 ± 0.17)	0.51 ± 0.02 (1.10 ± 0.12)	0.43 ± 0.07 (0.80 ± 0.17)	0.69 ± 0.11 (1.13 ± 0.20)

a) Values in parenthesis indicate absolute weight. Values are expressed as mean ± S.D.

Table 3. Dose Effect Relationship of Aqueous Leaf Extract of *B. diffusa* on the Liver Function of Rats ($n = 6$)

Treatment	Analyte			
	GOT (iu/l)	GPT (iu/l)	ALP (iu/l)	Total Bilirubin (mg/dl)
Control	56.83 ± 2.11	22.50 ± 1.95	178.89 ± 13.91	0.07 ± 0.007
500 mg/kg	55.50 ± 1.64	22.90 ± 1.44	178.97 ± 10.09	0.09 ± 0.003
1000 mg/kg	53.67 ± 1.44	23.00 ± 1.28	179.00 ± 18.48	0.08 ± 0.008
2000 mg/kg	59.00 ± 1.45	23.20 ± 1.28	179.10 ± 10.79	0.07 ± 0.008

Values are expressed as mean ± S.D. GOT = glutamic oxaloacetic transaminase, GPT = glutamic pyruvic transaminase, ALP = alkaline phosphatase.

Table 4. Dose Effect Relationship of Aqueous Leaf Extract of *B. diffusa* on the Haematological Parameters of Rats ($n = 6$)

Treatment	PCV (%)	WBC (cells/mm ³)
Control	59.67 ± 2.50	5966.50 ± 86.64
500 mg/kg	60.30 ± 2.86	6283.50 ± 90.05
1000 mg/kg	60.34 ± 2.90	6383.50 ± 92.30
2000 mg/kg	60.67 ± 3.00	6533.50 ± 95.00

Values are expressed as mean ± S.D.

extract of *B. diffusa* however, did not alter significantly, the bilirubin levels of the exposed rats, as well as other liver enzymes compared to the control.

According to Onyenyili and co-workers, anemia following administration of an agent can be as a result of lysis of blood cells and/or inhibition of blood cell synthesis by the active constituents of the extract, and decrease in hematological parameters in experimental animals has been associated with anemia.¹⁴ There was no reduction of haematological parameters in the extract-treated animals compared to the control (Table 4), which indicates that there is no lysis of blood cells and/or inhibition in blood cells synthesis by the active constituents of *B. diffusa* extract.

From the foregoing, the aqueous leaf extract of *B. diffusa* seems to be atoxic in rats.

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