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Acute and Subchronic Toxicity Studies of the Aqueous Extract of *Mezoneuronbenthamianum*Baill(Caesalpiniaceae)

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Abstract: *Mezoneuronbenthamianum (MB) is used traditionally in Nigeria for the treatment of peptic ulcer, diarrhea, and pain. Acute and sub-chronic toxicity studies of the aqueous mixture of the root and leaves of MB were carried out in rodents to evaluate the safety profile. In the acute studies, 7 groups of mice (10/group) were administered with 5-20g/kg of MB orally and 250-2000mg/kg intra-peritoneally (ip). The treated animals were examined for toxic signs and compared with the controls. In the chronic study, MB was administered orally to 20 rats and 20 mice at a daily dose of 320mg/kg. Control animals received 10ml/kg/day of distilled water. During the treatment, animals were observed for signs of toxicity. In addition, feeding habit and mortalities among others were recorded. At the end of treatment, haematological and biochemical parameters were determined, semen and spermatozoa morphology were assessed. In the acute studies, MB produced no toxicities or mortality with oral dose up to 20g/kg. The LD50 for ipadministration was 1021.31mg/kg. The control animals for both oral and ip groups showed no ill effects. Except for increases in ALT and AST, other parameters showed no significant differences between control and treated animals in the chronic studies. Semen and spermatozoa morphology, however showed very significant ($p<0.05$) abnormalities when compared with control animals. These results show that when given orally, and for a short period, MB is non-toxic. Long term use also does not result in serious derangement of the vital organs except the male reproductive cells which were severly damaged.*

Keywords:*Mezoneuronbenthamianum, acute toxicity, sub-chronic toxicity, LD50, oral, intra-peritoneal.*

I. Introduction

*Mezoneuronbenthamianum*Baill (Caesalpiniaceae) is a slender climbing plant widely used in folk medicine. The plant is synonymous with *Caesalpiniabenthamianum* Bail. I t belongs to the family Fabaceae and is commonly called "Tiger claw" due to its numerous prickly thorns. It is known as "*Mbougo*" in South East and "*Jenifinran*" in South West Nigeria, where traditional medicinepractitioners claim that it is effective in the treatment of suchconditionsasdiarrhea, peptic ulcer and general malaise [1, 2]. It has also been claimed to be used traditionally in the treatment of erectile dysfunction and urethral discharge [3]. In Ghana the plant is used for the treatment of skin infections and in promoting wound healing [4].The leaf extract has also been found to possess anti-microbial activity [5]. In his review on the Ethnopharmacological properties of the plant,Osho[6] concluded that the plant has a huge biological property that could prove to be of immense benefit clinically that might lead to developing novel compounds for the management of various disorders.

II. Materials and Methods

2.1 Plant Materials

Whole plant materials were collected from a farmland in Busogboro village, Ibadan, Oyo State of Nigeria. Taxonomic identification was made by Mr. T.K. Odewo of the Forestry Research Institute of Nigeria (FRIN), Ibadan and confirmed by Professor J.D. Olowokudejo of the Department of Botany and Microbiology, Faculty of Science, University of Lagos, Nigeria. A voucher specimen (FHI No. 106493) is preserved at FRIN for future reference.

1.2. Extract Preparation

A coarsely powdered, air-dried plant material (210g) was boiled in 2L of distilled water for 30 minutes, decanted and filtered. The filtrate was evaporated to dryness in an oven. The dried extract was weighed (yield, 4.91%w/w) and reconstituted to a concentration of 100mg/ml.

1.3. Animals

Albino Wistar rats (150-165g) and Swiss mice (25-30g) of either sex obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Nigeria were used. The animals were maintained under standard environmental conditions of 23 $\pm$1oC temperature and a 12/12h light/dark cycle [7], had free access to standard diet obtained from Ladokun Feeds Plc., Ibadan, Nigeria and water *ad libitum*. Animals were acclimatized for at least 14 days before each of the experiments.

2.4. **Acute toxicity studies**

Group of mice (10 per group) were fasted for 12h and administered with *Mezoneuronbenthamianum*(MB)up to 20g/kg orally. In the same manner, MB (250-2000mg/kg) was administered to another set of mice intraperitoneally. The control mice were givendistilled water (10ml/kg orally). The general symptoms of toxicity and mortality in each group, within 24 hours, were recorded. The median lethal dose (LD50) was estimated by log dose probit analysis [8]. Animals that survived after 24 hours were further observed for 14 days for signs of delayed toxicity.

1.4. Sub-chronic Toxicity Studies

A total of 80 animals (40 mice and 40 rats) of either sex were randomly allotted to the control and extract treated groups. In the treated groups, the extract was administered daily through gastric gavage, throughout the 90 day period. The dose selected was 320mg/kg/day which is1/5 of the highest pharmacologically active dose [9,10]. The animals were observed for all external symptoms of toxicity, body weight changes, feeding and drinking habits and mortality. At the end of the 90-day period, blood and semen were withdrawn from the animals before they were sacrificed. Vital organ weights, haematological, biochemical, seminal and spermatogenic characteristics were determined as appropriate.

2.5.1. Effects on weight of vital organs

The vital organs including brain, heart, lungs, liver, kidneys, spleen, stomach, testes and ovaries were excised, dried with blotting paper, weighed and thereafter standardized for 100g body weight of each animal [11].

2.5.2. Haematological analysis

Blood samples were collected from the heart by direct needle puncture following ether anaesthesia. The samples (collected in heparinized sample bottles) were analyzed for red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), white blood cells (WBC) and WBC differentials (neutrophils, eosinophils, lymphocytes and monocytes) (Feldman *et al*[12].

2.5.3. Biochemical analysis

The blood samples were allowed to clot and then centrifuged at 5000 rpm and clear sera were obtained for the following investigations: Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline phosphatase (ALP) according to the method of Reitman & Frankel [13]; Total serum bilirubin according to the method of Doumas*et al* [14], serum total proteins according to the method of Lubran [15], serum Albumins according to the method of Doumas& Peters [16], serum Globulin (calculated by subtracting the quantity of albumins from that of total proteins), serum total cholesterol according to the method of Allain*et al* [17], serum creatinine according to the method of Blass*et al* [18], serum urea according to the methods of Fawcett & Scott [19] and Searcy*et al* [20] as well as glucose, according to the method of Trinder [21].

2.5.4. Semen collection and microscopy Semen samples were collected from the epididymis through a 1.0cm incision made with a scalpel blade. Sperm cells were sucked into Pasteur pipette from the caudal epididymis. One half of the semen sample was stained using Wells and Awa stain for morphological studies and eosin and nigrosin stain for live-dead ratio. The second half was mixed with 0.5 ml of 2.9% sodium citrate to study the progressive motility while undiluted samples were used to study the mass activity [22].

2.6. Statistical analysis

Data were expressed as mean $\pm$ SEM and significant differences of means were determined by Student's t-test. A probability level less than 5% was considered significant.

III. Results

3.1 Acute toxicity

In the oral acute toxicity test, MB treated animals showed no signs of toxicity and no mortality was observed up to 20g/kg dose level within 24 h and for additional 14 days. Acute intraperitoneal toxicity test, however, produced a dose dependent mortality with LD50 of 1021.31mg/kg.

3.2 Sub-chronic Toxicity:

3.2.1. Mortality and Body weight

There were no significant differences in the body weights of the control and MB treated groups. In the rat, a total of four (4) animals representing 20% died in the extract treated group while two (2) representing 10% died in the control group. MB treatment in mice also resulted in four (4) deaths while three (3) representing 15% died in the control group (Table 1).

3.2.2 Vital organ weights

Except in mice where the spleen and stomach showed significant ($p<0.05$) increases in organ weights, there were no significant differences in weights of other organs in MB treated and control groups (Table 2).

3.2.3. Haematological parameters

The effects of 90-day administration of 320mg/kg/day of MB on haematological parameters are shown in tables 3 and 4. The PCV and RBC of mice treated with MB were significantly different ($p< 0.05$). Also, when compared to the control mice, WBC differentials showed significant neutrophilia and lymphopaenia. All the other parameters exhibited no significant differences between the treatment and control groups.

3.2.4. Biochemical Parameters

There were significant ($p<0.05$) increases in ALT and AST in MB-treated mice. However, treatment with MB did not show any significant increase ($p> 0.05$) in these parameters in rats. All other parameters did not differ significantly in the extract treated and control groups (Table 5).

3.2.5. Semen characteristics and Spermatozoa Morphology

There were significant ($p<0.05$) differences in the sperm count, motility and live/dead ratio in MB treated animals compared to control. Similarly, several abnormalities including headless tail, tailless head, bent mid-piece and curved tails were observed in MB-treated group. The control group showed no such abnormalities (Tables 6 and 7).

Table 1: Mortality and body weight during 90-day sub-chronic treatment in rodents

Week	1	2	3	4	5	6	7	8	9	10	11	12
RATS:												
Control	153.00±1.25 (20)	154.00±1.09 (20)	152.37±1.21 (19)	153.63±1.74 (19)	154.58±1.15 (19)	161.63±0.95 (19)	156.00±0.78 (19)	157.00±0.93 (18)	158.00±0.57 (18)	159.00±0.69 (18)	158.00±0.65 (18)	159.00±0.89 (18)
MB	151.00±1.17 (20)	152.00±1.18 (20)	151.21±1.19 (19)	151.79±1.14 (19)	152.37±1.13 (19)	152.95±1.09 (19)	153.00±1.16 (17)	154.00±1.13 (17)	154.00±1.01 (17)	155.00±0.98 (17)	156.00±1.08 (16)	156.00±0.91 (16)
MICE:												
Control	22.68±0.56 (20)	22.98±0.21 (20)	24.03±0.27 (20)	26.84±0.98 (20)	24.48±0.34 (18)	25.30±0.79 (18)	25.14±0.33 (18)	25.25±0.32 (18)	25.79±0.24 (18)	25.78±0.15 (18)	26.40±0.19 (17)	26.09±0.25 (17)
MB	22.54±0.42 (20)	22.77±0.35 (20)	24.37±0.25 (19)	24.28±0.23 (18)	24.69±0.25 (18)	24.85±0.24 (17)	24.49±0.33 (17)	24.76±0.39 (17)	25.58±0.26 (17)	25.68±0.13 (17)	26.28±0.23 (16)	25.98±0.25 (16)

Values are mean ± SEM. Figures in parenthesis indicate number of surviving animals at that period.

Table 2: Effect of MB on vital organ weights (per 100g body weight) after sub-chronic treatment in rodents

	Treatment	Brain	Heart	Lung	Liver	Kidney	Spleen	Stomach	Testes	Ovaries
RATS:	Control	1.03±0.33	0.39±0.12	0.99±0.31	3.19±1.01	0.63±0.19	0.56±0.18	1.87±0.59	1.52±0.84	0.15±0.58
	MB	1.05±0.33	0.38±0.12	0.91±0.29	2.96±0.94	0.67±0.21	0.49±0.16	1.84±0.58	1.43±0.46	0.13±0.00
MICE:	Control	1.44±0.46	0.44±0.14	0.69±0.22	3.85±1.22	1.26±0.39	0.37±0.12	2.75±0.87	2.04±0.91	0.24±0.11
	MB	1.42±0.43	0.47±0.15	0.59±0.19	4.27±1.35	1.55±0.49	0.77±0.24*	4.03±1.28*	1.96±0.88	0.16±0.07

Values are Mean ± SEM, n=10; *: significantly different when compared to the control group ($P<0.05$)

Table 3: Effect of MB on haematological parameters after sub-chronic treatment in rodents

	Treatment	PCV (%)	Hb (g/dl)	RBC (x100)	WBC (x10³)	MCV (fl)	MCH (%)	MCHC (%)
RATS:	Control	35.40±5.74	12.20±1.98	7.25±1.18	6.67±1.08	48.99±1.59	49.20±7.98	34.70±5.63
	MB	38.50+6.25	12.35±2.00	7.59±1.23	4.74±7.69*	50.59±0.93	50.80±8.24	33.20±5.23
MICE:	Control	44.40±14.04	14.39±4.55	7.48±2.36	9.68±3.06	59.39±0.49	59.30±18.75	30.50±10.28
	MB	50.80±16.04*	14.94±4.72	14.94±4.72*	8.96±2.83	59.46±1.39	58.00±18.48	29.60±9.36

Values are Mean ± SEM, n=10; *: significantly different when compared to the control group ($P<0.05$)

Table 4: Effect of MB on WBC differentials after sub-chronic treatment in rodents

	Treatment	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
RATS:	Control	28.30±8.95	69.60±22.01	0.30±0.09	1.30±0.41
	MB	38.20±12.08*	60.60±19.16	0.60±0.19	0.60±0.19
MICE:	Control	39.60±12.52	55.90±17.68	1.40±0.44	2.50±0.79
	MB	49.20±15.56*	47.00±14.86*	0.60±0.19	2.50±0.79

Values are Mean ± SEM, n=10; *: significantly different when compared to the control group (P<0.05)

Table 5: Effect of MB on blood chemistry after sub-chronic treatment in rodents

	Treatment	ALT (u/l)	AST (u/l)	ALP (u/l)	Urea (mmol/l)	Total cholesterol (mmol/l)	Creatinine (mmol/l)	Total proteins (mmol/l)	Albumins (mg/l)	Glucose (mmol/l)
RATS:	Control	76.53±27.06	43.00±15.20	91.63±32.39	5.29±1.87	3.33±1.18	31.13±11.00	189.25±66.91	33.10±11.70	7.46±2.64
	MB	79.50±28.11	55.38±19.58	110.63±39.11	5.33±1.88	2.54±0.89	36.88±13.04	130.00±45.96	28.41±10.05	5.73±2.02
MICE:	Control	67.25±23.28	29.00±10.25	25.25±8.93	5.78±2.04	3.51±1.24	36.13±12.77	78.50±27.75	42.25±14.94	7.66±2.71
	MB	117.13±41.41*	37.00±13.08*	11.25±3.98*	5.41±1.91	3.49±1.24	39.10±13.83	78.25±27.67	41.38±14.63	7.19±2.54

Values are Mean ± SEM, n=10; *: significantly different when compared to the control group (P<0.05)

Table 6: Effect of MB on semen after sub-chronic treatment in rodents

	Treatment	Volume (ml)	Count (x 10^6)	Motility (%)	Live/Dead
RATS:	Control	5.18±2.32	71.20±31.84	89.00±39.80	96.80±43.29
	MB	5.14±2.30	52.60±23.52*	60.00±26.83*	78.00±34.88*
MICE:	Control	5.10±2.55	67.50±2.08	90.00±5.00	94.50±47.25
	MB	5.13±2.56	54.75±27.38*	55.00±27.05*	72.50±36.25*

Values are Mean ± SEM, n=10; *: significantly different when compared to the control group (P<0.05)

Table 7: Effect of MB on spermatozoa morphology after sub-chronic treatment in rodents

	Treatment	Tailess Head	Headless tail	Bent tail	Bent mid-piece	Curved mid-piece	Total
RATS:	Control	3.80±1.69	3.00±1.34	5.00±0.71	6.00±12.68	5.80±2.59	400
	MB	5.40±2.41*	5.00±2.24	7.40±3.31*	7.60±3.39*	8.20±3.67*	400
MICE:	Control	5.75±2.86	3.00 ±0.82	3.25±1.63	2.50±1.25	1.00±1.15	200
	MB	6.50±3.25	4.25±2.25*	5.00±2.50*	5.00±2.50*	3.25±1.63*	200

Values are Mean ± SEM, n=10; *: significantly different when compared to the control group (P<0.05).

IV. Discussion

In this experiment, acute oral toxicity test shows that *Mezoneuronbenthamianum*, MB was tolerated up to 20g/kg. In the toxicity rating by Gosselin*et al* [23], substances with oral LD50 more than 15g/kg are classified as *practically nontoxic*. It implies therefore that MB, when administered orally is very safe. Intra-peritoneal administration, however, produced a dose dependent mortality with LD50 of 1021.31 mg/kg. Substances with LD50 of 0.5-5g/kg in toxicity rating are classified as *slightly toxic*. The aqueous extract of the whole plant has been reported to contain flavonoids, phenols, anthraquinones, reducing sugars, tannins and saponins[24].The slight intra-peritoneal toxicity of MB could be due to the saponins present in the extract since saponins are known to cause irritation in tissues and haemolysis [25]. Since the route of administration by the users is oral, even the slight intra-peritoneal toxicity would hardly be encountered.In the sub-chronic study, feeding habit, water intake, body weight and mortality record did not differ significantly in both the control and extract-treated groups. Generally, reduction in these parameters is a simple sensitive index of toxicity [26, 27]. In the present study, MB did not produce any statistically significant differences between control and extract-treated groups in these parameters, thereby attesting to its safety. Also, except in mice where the spleen and stomach showed significant increases in weights, there were no significant differences in weights of other vital organs between MB- treated and control groups. The haematological parameters for the extract treated groups showed no signs of toxic effects. All values lay within the limits considered normal for these species of animals [28-29].Serum analyses revealed significant increases in AST and ALT in the MB- treated group in mice compared to control. Increases in these parameters were insignificant in the rat group treated with MB. AST is found in the liver, cardiac muscles, skeletal muscles and pancreas. ALT concentration is highest in the liver and therefore is a more sensitive test for hepatocellular damage than AST (Rees and Spector, 1961)[30]. Leakage of large quantities of enzymes into the blood stream is often associated with massive necrosis of the liver [26]. The fact that not very high levels of these enzymes were recorded across the two species of rodents indicate that MB's chronic administration had no deleterious effects on both the liver enzymes and other biochemical parameters which were within normal limits [29].Seminal analyses revealed very significant differences in all parameters assessed in the extract-treated groups. Spermatozoa morphology also revealed various head and tail abnormalities. The fact that seminal depression and spermatozoa abnormalities occurred in the testes suggest that these abnormalities are secondary in nature [22].

V. Conclusion

In conclusion, the acute toxicity studies of *Mezoneuronbenthamianum*in rodents did not produce any visible toxicities or mortality with oral doses up to 20g/kg. Hence, when given orally and for a short period, MB is non- toxic. Long term use also does not result in serious derangement of the vital organs except the male reproductive cells which were severely damaged.

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