Sahel J. Vet. Sci. Vol. 5, No. 1, pp. 13 - 19 (2006)

1605-8954/06/$25.00 + 00

Sahel Journal of Veterinary Science

Toxicity Studies and Effects of *Momordica balsamina* (Balsam Apple) Aqueous Extract on Serum Electrolytes and Plasma Trace Elements

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ABSTRACT

Graded doses of *Momordica balsamina* aqueous leaf extract were administered orally and intraperitoneally (i.p) to separate groups of rats to determine the acute toxicities. The effects of the prolonged (3 weeks) oral administration of *M. balsamina* aqueous extract on serum electrolytes and plasma trace elements were also tested. No toxic clinical signs were observed in the animals given the extract orally. Sixteen grams per kilogram body weight (16 g/kg) was the maximum amount that was physically administered. The i.p LD_{50} with confidence interval of 95% was estimated to be 3750 mg/kg. Clinical signs observed before mortality included weakness, sleepiness and depression, dilation of the pupils, urination and death within 24 hours. The extract did not produce any statistically significant change in serum electrolytes (Na^+, K^+, HCO_3^+ and Cl^-) after prolonged oral administration. However, the effects of *M. balsamina* extract on plasma Zn and Mn were significantly increased ($p<0.001$), while the plasma Cu and Cd were not significantly increased ($p>0.01$). It was, therefore, concluded that *M. balsamina* at low dosages is safe (not toxic).

Key words: Acute toxicity, *M. balsamina,* electrolytes, trace elements

INTRODUCTION

Balsam apple (*Momordica balsamina*) is a tropical plant occurs mainly in Africa (Upholf 1958) and belongs to Cucurbitaceae family. Various parts of the plant are used as food and popular folk remedy for ailments (Akinniyi *et al.,* 1983) in many African cultures.

There are reports (Watt and Breyer 1962; Akinniyi *et al.,* 1983) that amongst the Hausa and Kanuri tribes of Nigeria, aqueous extract of the leaf of balsam apple are given to children as worm expellant and to lactating mothers, apparently to enhance lactogenesis. The Shangaans use the leaves in tea form as blood purifier and treatment of liver diseases. The Portuguese are known to have used the leaves for digestive disorders, fever, ulcer and mild form of malaria "paludismo" (Bionatural Kekanah hemme page 2002). It is especially sought after as a detoxifier. A culinary specialty recommends the leaves, ground peanut and honey, mixed together as sauce in chicken and meat dishes (Bionatural Kekanah hemme page 2002). The young leaf and tendrils are used by the Pedi as a pot herb and as an anti emetic (Bionatural Kekanah Hemme, 2002). Kekanah is name given to the traditional medicine made from the leaves of *M. balsamina* in South Africa. Kekanah medicinal properties changes according to the environment in which it is found. Climate and soil play an important role in the concentration of the active ingredients and medicinal properties (Bionatural Kekanah Hemme, 2002). Where kekanah is consumed regularly, there is very low occurrence of osteoporosis. It is also known that kekanah consumers show an increased strength in their nails and hair. Kekanah is rich vitamins A, C, calcium, iron and phosphorus in their natural forms which are easily absorbed into the system (Bionatural Kekanah Hemme, 2002). Herbalist in certain parts of Nigeria are of the opinion that the leaf preparation or its aqueous extract acts as an anti emetic compound probably due to the ability of components of balsam apple to stimulate or promote hematopoiesis(Karumi *et al.,* 1998).

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The aim of present study was therefore, to investigate the acute toxicity of the balsam apple leaf extract in rats and the effect of the extract on serum electrolytes and plasma trace elements following prolonged oral administration.

MATERIALS AND METHODS

Collections, identification and preparation of the plant material

Fresh leaves of Balsam apple (*Momordica balsamina)* were collected in the month of September 2002 from Alau, Konduga local Government Area situated in the eastern part of Borno State of Nigeria. The plant was identified by Dr. S. S. Sanusi of the Department of Biological Sciences, University of Maiduguri, Maiduguri, Nigeria, and voucher specimen was deposited in the Department of Biochemistry laboratory. The sample was air-dried in the laboratory and subsequently pulverized into fine powder. The extraction was by standard method of Mittal *et al.* (1981) and WHO (1992). Two hundred grams of the powder was mixed with one liter of distilled water and boiled for two (2) minutes, then allowed to cool to 40 °C and there after filtered using glass wool. The filtrate was collected in a beaker and evaporated until the volume was reduced to 100 ml so that 1 ml of the extract represents 200 mg of the dried weight.

Animals

Albino rats of both sexes weighing between 150 and 200 g were obtained from animal unit of the Department of Biochemistry, University of Maiduguri, Maiduguri, Nigeria. They were acclimatized for two weeks in Biochemistry laboratory and maintained on standard diet (Pfizer Animal Feed, Lagos, Nigeria) containing 48% carbohydrate, 29% protein, 16% fibre, 3% fat, 2% mineral and 1% vitamins supplement. Drinking water was given *ad libitum*.

Acute toxicity testing

Oral route

Six group of five rats each (weighing between 150 - 200 g) were fasted overnight and used for this experiment. Groups 1, 2, 3, 4 and 5 were given orally graded doses 1, 2, 4, 8 and 16 g/kg bw of the extract respectively. Group 6 was used as control and was given physiological saline of corresponding volume with the largest volume of extract administered. The rats were observed for acute toxicity signs for 24 hrs.

Intraperitoneal route

Fifty-six albino rats of both sexes weighing between 130 and 190 were used. They were separated into seven groups, each containing 8 rats. Groups 1-6 were treated intraperitoneally with graded doses (200, 400, 800, 1600, 3200, and 6400 mg/kg bw) of the extract respectively. Group 7 (control) was given sterile normal saline of corresponding volume with the largest volume of extract administered. The rats were fed and allowed access to clean fresh water ad-libitum. They were also observed for acute toxicity signs like behavioral changes and death for 24 hours. The arithmetic method of Kaber as modified by Aliu and Nwude (1982) was used to calculate the LD_{50} with confidence interval of 95%.

Effects of the extract on blood electrolytes and trace elements

A set of 20 white Wister strain rats weighing between 160 and 200 g were obtained from the animal house unit of Department of Biochemistry, University of Maiduguri, Maiduguri, Nigeria. These were divided into four groups of five rats each. Group 1, which served as control group, received only distilled water orally, while groups 2, 3 and 4 were treated orally with plant extract at the following doses 100, 200 and 400 mg/kg bw, respectively. The animals were kept under good laboratory conditions, fed on standard diet (growers mach ECWA feeds Nig. Ltd Jos Nigeria) and were allowed tap water at *ad libitum.* Then at the end of every week blood was collected from the animals for three weeks into containers, one heparinized and the other without heparin. The samples were then centrifuged at 3000 rpm in bench top centrifuge machine. Plasma and sera were harvested for the analysis of serum electrolytes (Na^+, K^+, HCO_3- and $Cl^{-)}$ and plasma trace elements (Cu, Zn, Cd and Mn) respectively.

Determination of serum electrolytes

Serum sodium ($Na^{+)}$ and potassium (K^+) were determined by flame photometry as described by Kolthoff and Elving (1976). Serum bicarbonate when treated with acid produces carbon dioxide which is estimated either monometrically or volumetrically using the method of Van Slyke and Aullen (1977). Chloride was determined by

titrimetric method of Schales and Schales, (1971) which is based on principles of precipitation with silver chloride.

Determination of plasma elemental content

Plasma Copper, Zinc, cadmium and manganese concentration were measured directly using the Sp-9 single beam atomic absorption spectrophotometer (Phillips/Pye Unicam Ltd, England). Plasma sample were aspirated in to the atomic absorption flame. The trace element concentrations were determined by comparing the signal from the plasma with the signal from the aqueous standards. One in five (1:5) and one in two (1:2) dilutions were made for zinc and copper determinations respectively, using deionized water. The aqueous standards were prepared in a diluted glycerol matrix (5 ml/dl) to simulate the viscosity of the diluted plasma. Cadmium and manganese only were estimated by aspirating the plasma neat (Sunderman 1973).

Statistical analysis

The data collected were summarized as mean ± SD and subjected to analysis of variance (ANOVA). A $p \leq 0.5$ value was considered significant (Armitage, 1980).

RESULTS

Acute toxicity

No clinical signs of toxicity were observed in the animals given the extract orally. Sixteen grams per kilogram body weight was the maximum dose that could be physically administered. The clinical signs observed following the intraperitoneal administration were weakness, depression, dilation of the pupils, urination, defecation with blood tinged faeces and death within 24 hours. These clinical signs started to manifest within 30 minutes of administration of the extract in the group that received the highest dose. The observed clinical signs appears be dose-dependent. Mortality in groups 1 - 4 was zero, in group 5, it was 12.5%, and while in group 6 it was 37.5% and 100% in group 7. These deaths occurred within 24 hours of administration of extract (Table 1). LD_{50} was found to be 3750 mg/kg within confidence interval of 95%.

Serum electrolytes and plasma trace elements

Table 2 shows the effect of different doses of the aqueous leaf extract of *M. balsamina* on serum electrolytes after prolonged oral administration to rats. The serum electrolytes (Na^+, K^+, HCO_3^- and Cl^-) showed no statistically significant variation in the treated animal when compared with control ($p>0.01$).

Figures 1 to 4 show the effect of *M. balsamina* aqueous leaf extract on plasma trace elements following prolong oral administration of different doses. The result indicated plasma Zn and Mn were significantly increased ($p<0.01$) by the treatment but the increases were not dose or time dependent There was no difference in plasma Cu and Cd between the treated groups.

Table 1. Mortality rate in rats given *Momordica balsamina* leaf extract at different doses

Group (n = 8)	Extract dose (mg/kg)	Number of death	% mortality
1	0	0	0.00
2	200	0	0.00
3	400	0	0.00
4	800	0	0.00
5	1600	1	12.50
6	3200	3	37.50
7	6400	8	100.00

Plant extract was given intraperitoneally

DISCUSSION

The oral administration of the extract of *M. balsamina* to rats at various doses (1, 2, 4, 8 and 16 g/kg) did not produce death in the animals hence the LD_{50} was not determined. The intraperitoneal administration of the extract resulted in 12.5 and 100% mortality in rats given 1600 and 6400 mg/kg of extract respectively. The intraperitoneal

Table 2. Effect of different doses of the aqueous leaf extract of *Momordica balsamina* on mean serum electrolytes following prolonged (21 days) administration to rats

Parameters	Time (wks)	Extract dose (mg/kg)			
		Control	200	400	800
Na^+ (mm/l)	1	135.33 ± 0.63	132.00 ± 0.49[a]	136.00 ± 0.48[a]	135.67 ± 0.65[a]
	2	135.33 ± 0.03	132.50 ± 0.55[a]	132.50 ± 0.37[a]	134.50 ± 0.59[a]
	3	135.02 ± 0.91	134.50 ± 1.59[a]	132.00 ± 0.45[a]	132.00 ± 0.49[a]
K^+ (mm/l)	1	5.20 ± 0.43	5.03 ± 0.24[a]	5.03 ± 0.41[a]	5.06 ± 0.40[a]
	2	5.03 ± 4.44	5.08 ± 0.854[a]	4.65 ± 2.20[a]	4.20 ± 2.05[a]
	3	5.02 ± 0.22	4.20 ± 2.05[a]	5.15 ± 0.17[a]	5.15 ± 2.17[a]
HCO_3^- (mm/l)	1	22.67 ± 0.76	24.00 ± 4.91[a]	26.00 ± 4.05[a]	23.67 ± 0.87[a]
	2	22.67 ± 0.47	19.00 ± 4.40[a]	19.00 ± 4.40[a]	29.50 ± 0.05[a]
	3	22.67 ± 0.47	26.00 ± 0.09[a]	24.00 ± 0.09[a]	25.25 ± 0.05[a]
Cl^- (mm/l)	1	103.33 ± 0.17	102.00 ± 1.01[a]	95.00 ± 0.55[a]	100.00 ± 0.10[a]
	2	102.27 ± 0.23	97.00 ± 0.85[a]	94.00 ± 0.70[a]	95.00 ± 0.55[a]
	3	99.02 ± 1.17	107.50 ± 0.37[a]	95.00 ± 0.75[a]	97.50 ± 0.87[a]

Data are mean ±SD based on 5 observation; normal physiological serum Na^+ ranges between 135 - 145 mm/l; normal physiological serum K^+ ranges between 3.5 - 5.2 mm/l; normal physiological serum HCO_3- ranges between 20 - 30 mm/l ; normal physiological serum Cl^- ranges between 95 - 110 mm/l ; [a] = values are statistically the same ($p>0.01$)

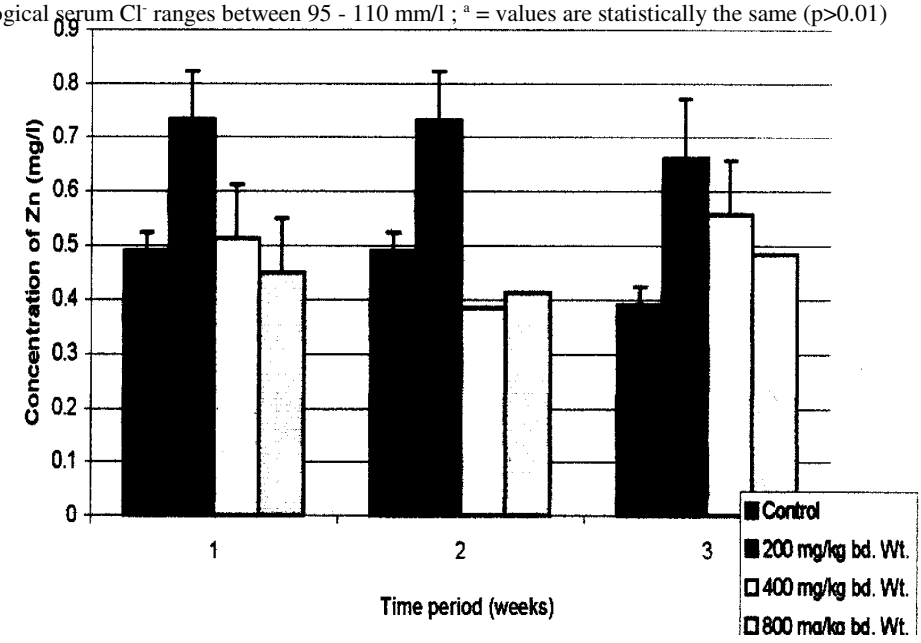

Fig. 1. Effect of *Momordica balsamina* extract on plasma zinc following oral administration of different doses to rats for three weeks ($p<0.01$)

LD_{50} of the aqueous extract of *M. balsamina* was calculated to be 3750 mg/kg. This may be an indication of very low toxicity. Clarke and Clarke (1977) reported that any substance whose intraperitoneal (ip) LD_{50} in rats fall between 50 - 500 mg/kg is regarded as highly toxic while LD_{50} above 500 mg/kg but below 1000 mg/kg are classified as moderately toxic. Onyeyilli *et al.* (2000) showed that 1440 mg/kg intraperitoneal LD_{50} of *Ficus thoningi* in rats is an indication of low toxicity. The signs observed before death following *M. balsamina*

administration in this study included weakness, depression, urination, blood tinged feaces and dilation of the pupils.

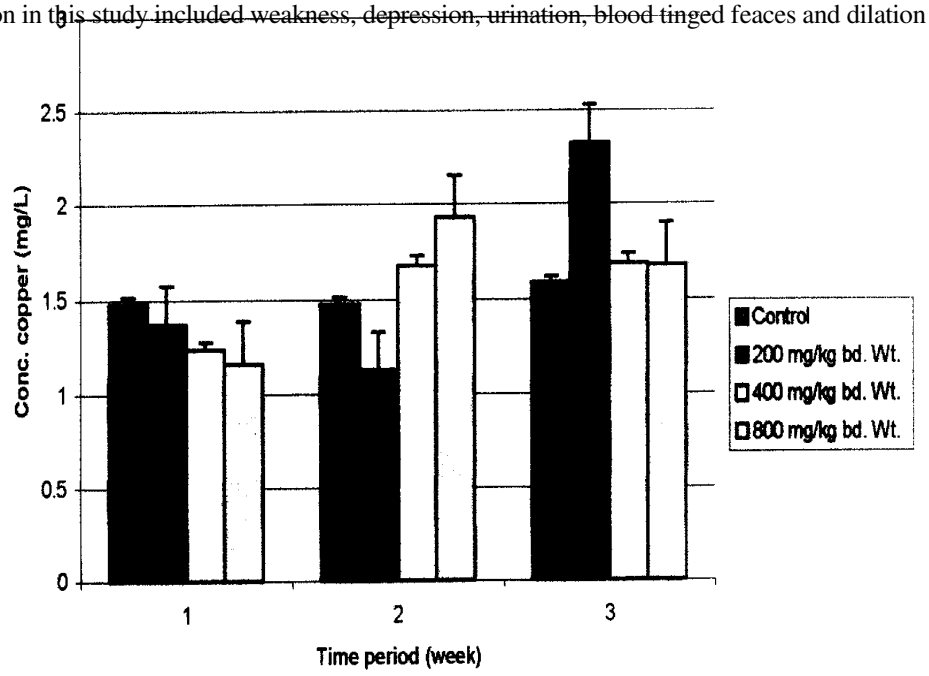

Fig. 2. Effect of different doses of *Momordica balsamina* extract on plasma copper following prolonged 3 weeks oral adminis-tration to rats($p>0.05$)

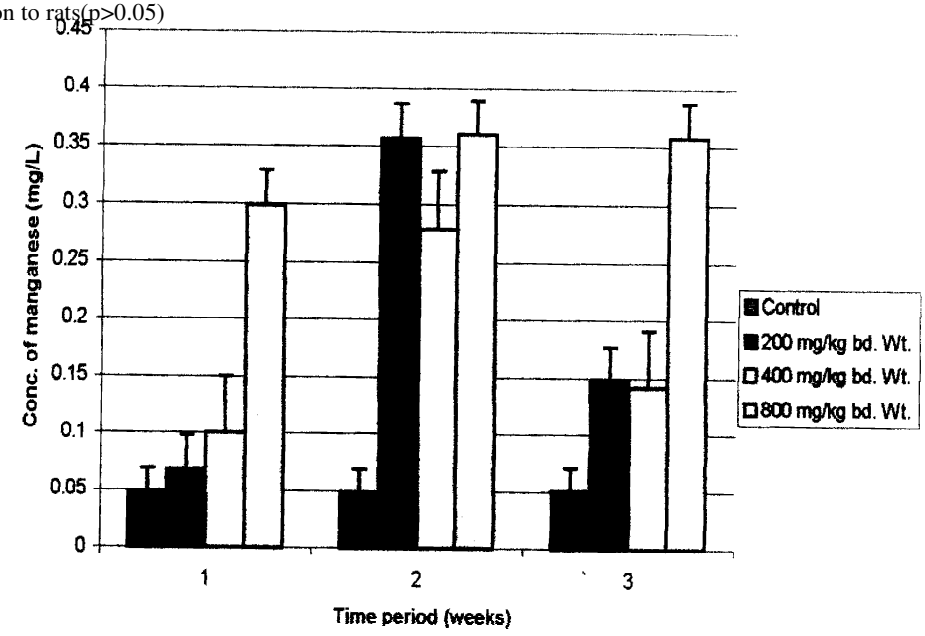

Fig. 3. Effect of different doses of *Momordica balsamina* leaf extract on plasma manganese following prolonged 3 weeks oral administration to rats($p<0.05$)

The inability of the extract to produce death in rats when administered orally may be an indication that the extract possesses low toxicity. The fact that high LD_{50} was obtained following intraperitoneal administration is an indication that the extract could be administered with some degree of safety, especially when administered through the oral route where absorption might not be complete due to inherent factors limiting absorption in the gastrointestinal tract (Dennis, 1984).The result shown that the different doses of aqueous extract of *M. balsamina* does not have adverse effects on the serum electrolytes after prolong (21 days) oral administration to rats. The plant was observed to have effect on the plasma trace elements Zn, Mn and Cd after prolong (21 days) oral administration of the different doses. The plasma trace elements (Zn and Mn) were significantly increased ($p<0.01$), plasma Cu

was not significantly changed (p>0.01), while plasma Cd was decreased insignificantly (p>1.01). These changes were observed to be dose and time independent. This is in agreement with the findings of Schroeder (1965) which shows that high plasma Zn will prevent the accumulation of Cd thereby preventing the deleterious effect of high tissue Cd, which include hypertension with large heart and changes in blood vessels of the kidney.

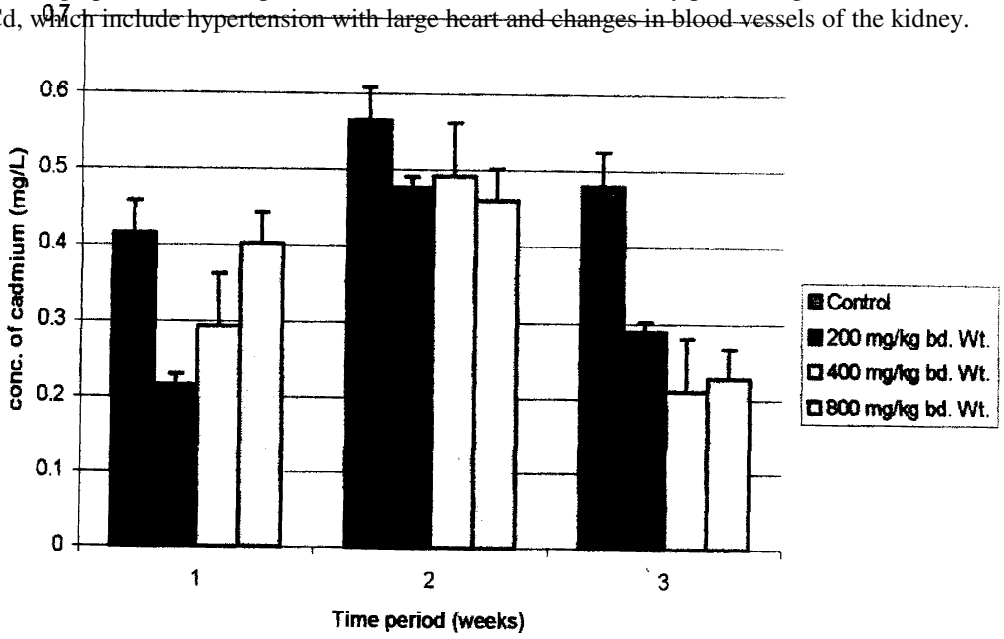

Fig. 4. Effect of different doses of *Momordica balsamina* leaf extract on plasma cadmium following prolonged 3 weeks oral administration to rats

High plasma Mn prevents the changes in plasma Cu, plays a role in the utilization of insulin and helps maintains metabolism of fat as well (Schroeder 1974). Thus, the presence of Mn in high concentration in the plant may be a contributory factor for the anti diabetic properties. It was, therefore, concluded that *M. balsamina* at low dosages is safe and useful for the management of diabetes

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