IJPAS ONLINE
ISSN:1597-9261
FACULTY OF SCIENCE
BAYERO UNIVERSITY,
KANO - NIGERIA
INTERNATIONAL JOURNAL OF PURE AND APPLIED SCIENCES

FULL LENGTH RESEARCH PAPER

Evaluation of Acute Toxicity of *Momordica charantia* Extract Using Wistar Rats To Determine Safety Levels and Usefulness of The Plant In Ethnochemotherapy

[*1]Abalaka, M. E., [2]Olonitola, O.S., [3]Onaolapo, J.A. and [2]Inabo, H.I.

[1]Department of Microbiology, Federal University of Technology, Minna.
[2]Department of Microbiology, Ahmadu Bello University, Zaria.
[3]Department of Pharmacognosy, Ahmadu Bello University, Zaria.

*** Corresponding Author email:** modorc2005@yahoo.com

Received: August 2009 *Accepted: September 2009*

ABSTRACT

Safety levels and chemotherapeutic usefulness of Momordica charantia *were determined by evaluating the acute and subacute toxicity of the plant using thirty (30) wistar rats. The rats were grouped into six (6) groups A, B, C, D, E and F with groups B- F administered with 100, 500, 800, 1,200 and 1,500 mg/kg of body weight daily respectively for two weeks. The ones in group A served as control. The animals in group F died after a couple of days indicating none tolerance of the extract at that dose. The animals in group D showed 50% mortality at the end of the two weeks which indicates* LD_{50} *of the extract as 1,200 mg/kg-*^{1} *body weight. Macroscopic examination of some visceral organs revealed changes in sizes, coloration as well as some pathological differences when compared with those of the control. The acute treatment with 100, 500, 800 mg/kg-*^{1} *per day did not produce any symptom of toxicities or mortality. The haemato-biochemical parameters show no significant differences (P<0.05). Administration of* Momordica charantia *extract up to 800mg/kg-*^{1} *body weight is safe (P>5mg/kg) and tolerated by the body.* Momordica charantia *is therefore safe to use as Ethnochemotherapeutic agent.*

Keywords: *Toxicity*, Momordica charantia, *Visceral organs, Ethno-chemotherapy*

INTRODUCTION

Herbal medicine is still the most abundance, affordable, reliable, trusted and well understood by locals in virtually all African villages. Before the coming of the colonial masters and the consequent advent of orthodox medicine, our people have relied on herbs growing in and around them to take care of their health problems. Plants growing around them were for both food and medicine. Kidney problems, Liver diseases, enteric fevers, complications due to childbirth, shortage of blood, leukemia e.t.c were not their problems as some of these diseases were even unknown. Unbelievably, life expectancy was very high as most of them lived well over hundred years.

The coming of orthodox medicine seemed to have relegated our herbal health care system but development of resistance against orthodox medicine by pathogens and high costs as well as non availability of some of these drugs have

made man to return to native for help (Ogbunugafor *et al*., 2008; Lee, 2006; Lam, 2007).
Herbal drugs are often bulky, doses not quantified and most importantly toxicity of these plants' drugs are largely unknown (Galati and O! Brien, 2004; Sa'ad *et al.*, 2006).

Although a number of scientific researches have revealed activities of so many African plants not many people venture into studying toxicity of these plant materials. Some of such researches were those of Sibanda and Okoh 2008; Darwish *et al*., 2002; Tamboura *et al*., 2005; Gulcin *et al*., 2003.

Momordica charantia has been shown to have activity against so many organisms and it has also been shown to have other therapeutic values like hypoglycermic. The present work is to ascertain the toxicity and safety levels for administration of *M. charantia*.

MATERIALS AND METHODS

Sample collection: Plant materials, (*Mormodica charantia*) were collected from Bida, Niger State. Identification was carried out by local people and confirmed by a Botanist and Taxonomist (Dr. O.A. Falusi, Dept. of Biological Sciences, Federal University of Technology, Minna, Niger State).

Extraction and preparation of plants' materials: Ethanol was used as solvent for the extraction of the plant materials. The method of Silva *et al* (1997) was adopted. Twenty (20) grammes of ground sample was suspended in 100ml of 95% ethanol for a period of about 120 hours. The extract was decanted and filtered and the filtrate evaporated in vacuo at 45^0C. The residue was reconstituted in 95% ethanol and reserved as stock concentration then stored.

Phytochemicals analysis
Presence of phytochemicals was determined using the methods of Trease and Evans (1989) and Sofowora. (1993).

Animals
Thirty (30) wistar rats of both sexes at the weights ranging between 80-110g were purchased from the National veterinary Research Institute (NVRI) Vom, Jos Plateau State of Nigeria. They were split into groups of six (6). The weights of rats in each group were matched and animals acclimatized to their new environment for two (2) weeks before further analysis were carried out. Rats had free access to drinking water and food *ad libitum*. Commercial pelleted food was given and net weight gain or otherwise recorded and animals were maintained in six separate standard rat cages.

Treatment
Before administration of the extract, the rats were starved for 12 hours (Anosike *et al*., 2008). The six groups were designated A, B, C, D, E and F with each of the groups of rats administered with different concentration of extract through the oral route using a gavage needle once daily (Okoko and Oruambo, 2008) for two (2) weeks. Groups B-F were given 100, 500,800 1,200 and 1,500mg/kg of body weight for two weeks. Group A rats which served as control were given 0 dose of the extract. The LD_{50} and LD_{100} of extract were monitored and documented and acute toxicity noted (Lorke 1983).

At the end of the two week period all group E animals are dead. Two animals each from the remaining groups were sacrificed and macroscopic examination of all organs was carried out. Organs like liver, kidney and the heart were placed in 10% formalin to prepare histological slides. The slides were stained by haematoxylin-eosin and observed.

STATISTICS
Results were analyzed as the mean ± standard deviation. The student's t-test was used to evaluate the statistical significance of the differences ($P < 0.05$).

RESULTS

Table 1. initial weights of wistar rats before treatment

	A	B	C	D	E
	85.75	91.24	101.50	82.56	86.77
	86.07	93.86	102.85	82.72	87.88
	84.04	93.36	101.59	83.10	88.96
	86.25	93.56	103.65	82.23	86.84
	85.22	94.31	101.72	82.42	86.86
Mean	85.47	93.27	102.26	82.61	87.46

Table 2. weights of wistar rats after two weeks treatment with extract

	A	B	C	D	E
	210.36	225.54	284.21	206.39	232.84
	215.47	236.51	273.25	211.87	226.23
	208.96	242.89	282.53	215.81	dead
	206.35	232.59	276.24	207.60	dead
	198.35	242.09	288.73	209.08	dead
Mean	207.90	235.92	281.00	210.15	229.54

No dose-related differences in body weight gain were found.

Acute toxicity

After 14 days of administration of extract to the rats at the various concentrations the LD_{50} was calculated and found to be 1,200 mg/kg-^{1} body weight of the Wister rats.

Representatives of each group were slaughtered but visceral organs showed no changes when examined macroscopically and compared with the control.

Representative from group E showed swollen kidney, liver, patches on the heart and other features signifying toxic effects of the dose of 1200mg/kg of extract.

Biochemical & Haematological Assessments

Tables 3 and 4 indicate the Biochemical and Haematological parameters. There was no dose-related responsiveness in body weights of rats given doses up to 800mg/kg-^{1} body weight. Some changes were observed in parameters of rats given 1,200mg/kg-^{1} body weight.

The rats given 1,500mg/kg all died after a couple of days which indicates the concentration of 1,500mg/kg-^{1} body weight as the LD_{100}.

Biochemistry

Table 3. Results of Biochemical tests on the rats after two weeks.

Dose mg/kg/day	Group	GLU (mmol/l)	BUN (mmol/l)	CRE (Umol/l)	AST (U/l)	ALT (U/l)	ALP (U/l	Na^+ (mmol/l)	K^+ (mmol/l)	Ca^{2+} (mmol/l)	Cl^{2-} (mmol/l)
0	A	16.3±1.4	5.8±0.1	56.2±2.3	152.2±16.2	75.3±11.2	328.1±14.2	144.6±0.7	8.9±0.6	3.4±0.4	97.0±0.1
100	B	16.9±1.3	5.2±0.2	57.3±2.4	156.6±11.1	83.4±20.2	382.2±21.4	143.8±0.6	8.2±0.5	3.3±0.1	96.0±0.3
500	C	15.8±1.5	6.6±0.5	59.4±2.5	158.7±1.5	85.6±16.3	402.4±32.3	143.2±0.2	9.6±0.1	3.1±0.2	94.8±0.4
800	D	16.4±1.2	6.2±0.1	47.6±3.1	142.3±2.3	98.2±12.1	426.7±41.2	145.1±0.3	9.4±1.3	3.5±0.3	94.6±0.0
1200	E	15.6±1.6	5.5±0.3	48.9±3.6	150.5±17.1	68.4±10.4	574.2±62.0	143.3±0.4	8.4±0.4	3.4±0.5	95.2±1.0

Results are mean±S.E.M., n=5, significantly different from control: P< 0.05
Key: GLU=glucose, **BU**N=urea, **CRE**=creatinine, **AST**=asparate aminotransferase, **ALT**=alanine aminotransferase, **ALP**=alkaline phosphatase, **Na^+**=sodium ion, **K^+**=potassium ion, **Ca^{2+}**=calcium ion, **Cl^-**=chloride ion.

Haematology

Table 4 Results of haematological screening of rats' blood after two weeks treatment.

Dose mg/kg/day	Group	WBC ($x10^3/\mu l$)	RBC ($x10^6/\mu l$)	HGB (g/dl)	HCT (%)	MCV (fi)	MCH (pg)	MCHC (g/dl)	RDW (%)	PLT ($x10^3/\mu l$)	MPV (fi)
0	A	11.2±0.4	8.2±0.2	15.1±0.3	46.2±1.2	58.3±1.2	18.6±0.3	34.2±0.2	15.1±0.2	765±49.1	9.0±0.3
100	B	14.1±0.1	7.6±0.3	16.2±0.2	47.8±1.4	57.5±0.8	18.3±0.3	33.3±0.1	15.4±0.3	723±72.1	8.7±0.1
500	C	12.5±1.0	8.5±0.4	15.4±0.1	46.8±2.3	55.8±1.6	18.9±0.1	34.7±0.3	15.7±0.2	692±64.0	8.8±0.3
800	D	15.3±0.3	8.7±0.2	17.1±0.3	45.6±1.7	58.0±1.8	19.2±0.4	35.1±0.2	15.9±0.4	816±72.3	9.1±0.2
1200	E	13.2±0.4	7.8±0.5	15.7±04	46.7±2.4	56.6±1.4	18.4±0.2	34.2±0.4	15.6±0.3	812±56.1	9.2±0.3

Results are mean±S.E.M., n=5, significantly different from control: P< 0.05
Key: WBC=white blood cells, **RBC**=red blood cells, **HGB**=haemoglobin, **HCT**=hematocrit, **MCV**=mean cell volume, **MCH**=mean cell haemoglobin, **MCHC**=mean cell haemoglobin concentration, **RDW**=red cell distribution width, **PLT**=platelets, **MPV**=mean platelet volume.

DISCUSSION

The results of mean body weight are contained in tables 1 and 2. There are no significant differences in the body weights of the animals administered with various concentrations of the extracts compared with the control. This suggests that there are no dose related increases in body weights of the rats. Rats in group F given daily dose of 1,500mg/kg body weight per day died in a couple of days. This is an indication that the dose is highly intolerable and was toxic to the animals. The death of all the rats indicates LD_{100} of the extract concentration (1,500mg/kg). The LD_{50} was calculated as 1,200mg/kg^{-1} since about half of the animals was lost within the 2 weeks of this experiment. The macroscopic examination of the visceral organs such as kidney, liver, heart e.t.c shows some physical changes compared with the control in those rats given 1.200mg/kg^{-1} but not in those given lower doses. Obici *et al*.,2008; Mukinda and Syce 2007 had similar result in their experiments. It is important that a chemotherapeutic agent be selectively toxic. This means that the dose tolerated by the animal cells be higher than dose cidal to the offending organisms, usually pathogenic microorganisms. These results confirm that the extracts from *Momordica charantia* are selectively toxic. Selective toxicity is the cardinal point in antimicrobial chemotherapy.

The haematological and biochemical parameters (tables 3 and 4) show that there are no significant clinical changes ($P<0.05$) between the experimental rats and the control. In essence there are no doses related changes in the haematological and biochemical parameters except that there is a slight increase in the mean counts of white blood cells (WBC) table 4. This is a very useful finding with respect to the mode of action of the plant extract as it may help to boost the immune system because white blood cells are important in fighting disease through phargocytosis (Ramirez *et al.*, 2007).

CONCLUSION

One major problem of herbal medicine with relation to acceptability is the fear of toxic side effects. Since herbal drugs are administered in bulk and usually doses are not quantified there is fear of accumulation of drugs materials in organs of the body thereby leading to more serious problems other than those being treated. Fears abound that drugs may accumulate in the liver, spleen, heart, kidney e.t.c causing dysfunction of these organs which may prove fatal. This fears is a justifies these studies as the studies have shown that lower doses of this plant's extract are safe thereby confirming the usefulness of these plant in ethnochemotherapy. It is important to note that 800mg/kg^{-1} body weight is high enough dose and this levels showing safety is good news in the use of *Momordica charantia* for curative purposes.

REFERENCES

Anosike, C.A., Ugwu, U.B. and Nwakama, O. (2008). Effect of ethanol extract of *Pyrenacantha staudtii* on carbontetrachloride induced hepatotoxicity in rats. *Biokemistri.* 20(**1**):17-22.

Darwish, R.M., Aburjai, T., Al-khalil, S., mahafzah, A. (2002). Screening of antibiotic resistant inhibitors from local plant materials against two different starins of *Staphylococcus aureus*. *Journal of Ethnopharmacology*. **79**:359-364.

Galati, G. and O'brien, P.J. (2004). Potential toxicity of flavonoids and other dietary phenolics: significance for their chemoprotective and anticancer properties. *Free radicals Biology Med*. **37**:287-303.

Gulcin, I., Oktay, M., Kirecci, E. and Kufrevioglu, I.O. (2003). Screening of antioxidant and antimicrobial activities of *Anise* (*Pimpinella anisum L.*) seed extracts. *Food chem*.. **83**:371-382.

Lam, K.S. (2007). New aspects of natural products in drug discovery. *Trends Microbiology*. 15(**6**):279-289.

Lee, C.P. (2006). Who's in the business of saving lives? *J. Med. Philos.* **31**:465-482.

Lorke, D. (1983). A new approach to practical acute toxicity testing. iArch. Toxicity. **54**:275-287.

Mukinda, J.T. and Syce, J.A. (2007). Acute and chronic toxicity of the aqueous extract of *Artemisia afra* in rodents. *J. of Ethnopharmacology*. **112**:138-144.

Obici, S., Otobone, F.J., Silvasela, V.R., Ishida, K., Silva, J.C., Nakamura, C.V., Cortez, D.A.G. and Audi, E.A. (2008). Preliminary toxicity study of dichloromethane extract of iKielmeyer coriacea stems in mice and rats. *J. of Ethnopharma*. **115**: 131-139.

Ogbunugafor, H.A., Okochi, V.I., Okpuzor, J. and Emeka, P. (2008). Tolerance and antiplasmodial screening of *Ritchea longipedicellata* in *Plasmodium berghei*. *Biokemistri*. 20(**1**):23-27.

Okoko, T. and Oruambo, I. F. (2008). The effects of *Hibiscus sabdariffa* calyx on cisplatin-induced tissues damaged in rats. *Biokemistri.* 20(**2**):47-52.

Ramirez, J.H., Papacies, M., Tomayo, O., Jaramillo, R. and Gutierrez, O. (2007). Acute and sub-acute toxicity of *Salvia scuttlelarioides* in mice and rats. *J. of Ethnopharma*. 19, 109(**2**):348-353.

Sa'ad, B., Azaizeh, H., Abu-Hijleh, G. and Said, O. (2006). Safety of traditional Arab herbal medicine. Evid based complement Alternative medicine. 3(**4**):433-439.

Sibanda, T. and Okoh, A.L. (2008). *In vitro* evaluation of the interactions between acetone extracts of *Garcinia kola* seeds and some antibiotics. *African Journal of Biotechnology*. **7**:1672-1678.

Silva, O., Daurk, A., Pimentel, M., Viegas, S., Barroso, H., Machado, J., Pires, I., Carbrita, J. and Gomes, E. (1997). Antimicrobial Activity of *Terminilia macroptera* root. *Journal of Ethnopharmacology*. **57**:203-207.

Sofowora, A., (1993) Medicinal Plant and Traditional Medicine in Africa, 2nd Edition, Spectrum Books Ltd. Ibadan, Nigeria. 50 – 58.

Tamboura, H.H., Bayala, B., Lompo, M., Guissou, I.P. and Sawadogo, L. (2005). Ecological distribution, mophorlogical characteristics and acute toxicity of aqueous extracts of *Holarrhena floribunda* (G. Don), Durand and Schinz, *Leptadenia hastata* (Pers.), Decne and *Cassia sieberiana* (dc) used by veterinary healers in Burkina faso. *African Journal of Trad. Cam.* 2(**1**):13-24.

Trease, G.E. and Evans, W.C. (1989). A text book of *Pharmacognosy, I* 13th edition, Bailliere Tinall Ltd., London. Pp. 101-104.