Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 6 (4): 418-426, 2011
ISSN 1816-496X / DOI: 10.3923/jpt.2011.418.426

Evaluation of the Toxicity of *Manihot esculenta* on Wistar Rats after Traditional Sudanese Processing

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ABSTRACT

In Sudan and other countries, *Manihot esculenta* roots (cassava) consumed mainly as flour after a traditional processing which we are suggesting that it's not enough to eliminate all cyanogenic glycoside (toxic compound). This study was carried out to evaluate the aqueous and methanolic extracts toxicity of *Manihot esculenta* (cassava) tubercular roots on Wistar rats after traditional processing in two weeks consumption. The plant was peeled and dried after that it was extracted using methanol and water. Wistar rats were allotted at random to five groups, each of 6 rats; four groups were given their designated dose of the extracts of both aqueous and methanol in two different dose orally which was 75 and 300 mg kg^{-1}, the fifth group was control. The mortality and weight gain, serobiochemical and hematological parameters were recorded in addition to pathological changes. Demonstration of *M. esculenta* extracts orally result in alteration in (aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) activities, changes in the concentration of urea, cholesterol and other serobichemical parameters, pathological changes in fatal organs, including Necrosis and shrinkage of the glomeruli and aggregates of lymphocytes in the renal cortex was observed, this changes were accompanied with cytoplasmic fatty vacuolation of entrilobula hepatocytes and cerebral neurons. We conclude that *Manihot esculenta* is toxic causing alteration on various serobiochemical and hematological parameters, this toxicity was correlated to dysfunction of vital organs and we retained all consequence of toxicity to the presence of cyanoginc glycoside; linamarin and lotaustralin.

Key words: *Manihot esculenta*, cassava, cyanoginc glycoside, sudanese processing, pathological changes

INTRODUCTION

Plants are used since ever for several purposes, as foods for both human and livestock, also as source of fuel; now the ultimate use is as source of biofuel, plants through ages was used as remedy for several diseases and it was the bases of pharmaceuticals drugs. Treatment of skin rashes, boils, skin irritations, wounds, dermatitis and pyoderma with plant extracts is a common practice in Russia and Central Asia (Mamedov *et al*., 2005). Nevertheless of the benefit of plants, some are containing toxic compounds that can affect the health of consumers in different ways. As example *Aleurites moluccana* oil (kukui oil or lumbang oil) is not suitable for cooking but is used in

cosmetics, industrially in paints, for illumination and as medicine. The raw seeds are toxic and have a strong purgative effect, despite these some varieties are not toxic at all (Walter and Sam, 2002).

M. esculenta a member of family Euphorbiaceae and locally known as cassava is glabrous laxly branched shrub up to 4 m high with thick tuberous roots, *M. esculenta* is distributed in many part of world, in Sudan mainly found in southern region (Sousa *et al*., 2002).

Many of the Euphorbiaceae, it is toxic and can cause skin eczema in some people. It is also toxic if eaten, though in small quantities (Ogunwenmo *et al*., 2007).

Analysis carried out on roots reveled that they are very rich in starch and contain significant amounts of calcium, phosphorus and Vitamin C. *M. esculenta* leaves are a good source of acids (Popoola *et al*., 2007). Also oil from parts of plants has been shown to exhibit antifungal activity against a wide range of pathogenic fungi and bacteria species (Okeke *et al*., 2001; Pawar and Thaker, 2006). It is a major source for ethanol production (Linley *et al*., 2002). Also it contains a cyanogenic glucosides, linamarin and lotaustralin (White *et al*., 1998).

It has been reported that linamarin and lotaustralin were glucosides of acetone cyanohydrin and ethyl-methyl-ketone-cyanohydrin, respectively. Linamarase has optimum pH at 5.5 to 6.0. In general, animals have a detoxification mechanism which can avoid death when cyanide release is slow (Cereda and Mattos, 1996).

Linamarin is the most representative glucoside accounting for about 80% of the total cassava glucoside (Nwabueze and Odunsi, 2007). The structure of linamarin is shown in Fig. 1.

In the processing of *M. esculenta* roots, linamarase-the hydrolytic enzyme-remains active and catalyzes the reaction which releases one molecule of glucose, acetone and hydrocyanic acid (O'Brien *et al*., 1991).

The consumption of large amounts of the bitter variety (containing higher cyanogenic glucosides) can cause severe toxicity resulting in Konzo, a paralytic disorder or even death. Routine ingestion of low levels of cyanide leads to chronic toxicity possibly developing into goiter (enlargement of the thyroid gland) or tropical ataxic neuropathy (a disorder involving the nervous system). Women and young children are often involved in the processing of *M. esculenta*, placing them at a higher risk for inhalation and ingestion of cyanide. Those who consume a low-protein diet at the same time as consuming *M. esculenta* are more susceptible to cyanide poisoning. Proteins have the ability to reduce some of the toxic effects of cyanide and it is, therefore, recommended to consume fish or other meat products along with cassava (Linley *et al*., 2002).

Thus the objective of present study was to evaluate the toxicity of *M. esculenta* (cassava) after traditional sudanese processing.

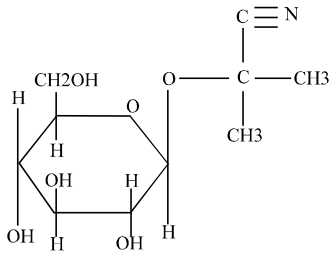

Fig. 1: Linamarin structure

MATERIALS AND METHODS

Plants material: *M. esculenta* roots purchased from a local market in Khartoum (March, 2010), Sudan. The plant roots were peeled and cut into pieces and shade-dried mimicking the traditional method.

Study design: Five hundred grams of the powdered bulb were subjected to extraction by methanol then with water using maceration method and then it was filtered through No.1 Whatman paper. The filtrate for methanolic extract was separated using Rotary evaporator while filtrate for aqueous extract was separated using Freeze-drying. For preparation of doses distilled water were used as solvent.

Thirty 2-weeks old male Wistar rats were housed within the premises of the Faculty of Science and Technology, El-Neelain University, Khartoum, with feed and water provided *ad libitum*. The rats were allotted at random to five groups, each of 6 rats. Group 1 receives no dose from extract and served as control. Groups 2 and 3 were given methanolic extract of *M. esculenta* roots of at 75 and 300 mg/kg/day via oral route, respectively. The rats in group 4 and 5 were given aqueous extract at 75 and 300 mg/kg/day via oral route, respectively. All rats were dosed their designated experimental oral dose for 2 weeks.

Lots of 3 rats from each group were anaesthetized with diethyl ether and dissected at one and two weeks. Average, body weight and weight gain for each group were recorded weekly.

Hematological methods: These techniques were performed according to Automated Hematology Analyzer (Sysmex KX-21, Japan, 1999). The parameters measured were Hemoglobin Concentration (Hb), Packed Cell Volume (PCV), Red Blood Cells (RBCs), White Blood Cells (WBCs), differential WBCs counts and erythrocytes indices; Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC).

Serobiochemical methods: Blood samples were collected and sera were separated by centrifugation at 350 rpm for 5 min. The methods for enzyme activity of control and tested rats were performed according to the instructions in the manual of Roche Diagnostic Hitachi 902 Analyzer (Germany, 1996). Here we measured Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), total protein, albumin, globulin, cholesterol and urea.

Pathological methods: Necropsy was conducted to identify gross lesion, after anesthetizing, the rats were dissected. Specimens of the liver, kidneys, brain, spleen and intestines were collected and immediately fixed in 10% neutral buffered formalin.

The organs were embedded in paraffin wax, sectioned at 5mm diameter and stained routinely with hematoxylin and eosin (H and E) (Andrew *et al*., 2008).

Statistical analysis: The significance of difference between means was compared at each time point using Duncan's multiple range tests after ANOVA for one-way classified data (Snedecor and Cochran, 1989).

RESULTS

Growth changes: The effect on the body weight and body weight gain of rats given daily oral doses of *M. esculenta* roots methanolic and aqueous extracts are presented in Table 1. All groups

Table 1: Body weight and body weight gain in rats orally given *M. esculenta* roots extracts

	Body weight gain (g)		
Treatment groups	0 week	1 week	2 weeks
Control (normal diet)	95.7±2.2	20.6±5.1	16.3±5.0
Methanolic extracts (75 mg/kg/day)	95.3±2.4	13.7±4.5*	6.6±7.1*
Methanolic extracts (300 mg/kg/day)	95.3±2.2	15.0±3.5*	15.3±6.1^{NS}
Aqueous extracts (75 mg/kg/day)	95.3±2.2	10.0±2.8*	11.4±0.7*
Aqueous extracts (300 mg/kg/day)	95.7±2.0	18.7±2.7^{NS}	15.0±1.9^{NS}

Values are expressed as Mean±SE. NS = Not significant; *Significant = ($p<0.05$)

Table 2: Hematological analysis of rats given *M. esculenta* methanolic and aqueous extracts orally for one and two weeks

		Methanolic extracts		Aqueous extracts	
Parameters	Control (normal diet)	*M. esculenta* (75 mg/kg/day)	*M. esculenta* (300 mg/kg/day)	*M. esculenta* (75 mg/kg/day)	*M. esculenta* (300 mg/kg/day)
One week					
Hb (g dL^{-1})	13.2±0.6	12.2±0.3^{NS}	12.5±0.6^{NS}	12.6±0.3^{NS}	12.5±0.3^{NS}
RBC ($\times 10^6 mm^3$)	7.4±0.2	7.1±0.5^{NS}	7.3±0.5^{NS}	6.8±0.3^{NS}	7.2±0.2^{NS}
PCV (%)	42.5±1.1	40.4±0.9^{NS}	41.2±2.4^{NS}	38.9±0.6*	41.2±1.1^{NS}
MCV (fl)	57.4±0.0	57.0±2.9^{NS}	56.8±1.0^{NS}	59.1±1.3^{NS}	56.8±1.0^{NS}
MCH (pg)	17.8±0.3	17.3±0.9^{NS}	17.3±0.0^{NS}	18.6±0.4^{NS}	17.3±0.4^{NS}
MCHC (%)	31.1±0.5	30.3±0.2^{NS}	30.5±0.6^{NS}	31.5±0.3^{NS}	30.5±0.2^{NS}
WBC ($\times 10^3 mm^3$)	11.6±2.8	6.6±0.9*	7.0±0.3*	3.9±0.6*	8.1±2.7*
Lymphocytes (%)	70.2±5.4	64.1±6.2*	77.6±8.7*	74.9±6.5*	77.6±6.9*
Neutrophils (%)	29.8±5.4	33.0±6.2*	22.4±4.7*	25.1±6.5*	22.4±5.0*
Two weeks					
Hb (g dL^{-1})	13.5±0.9	14.4±0.4*	13.3±0.4^{NS}	13.2±0.4^{NS}	(-)
RBC ($\times 10^6 mm^3$)	7.7±0.7	6.5±0.7^{NS}	7.5±0.7^{NS}	7.4±0.1^{NS}	(-)
PCV(fl)	44.9±2.7	38.8±3.0*	43.7±1.3^{NS}	42.2±0.0^{NS}	(-)
MCV (m^3)	58.3±0.8	59.9±1.7^{NS}	58.3±0.7^{NS}	56.8±0.4^{NS}	(-)
MCH (pg)	17.7±0.4	22.7±2.6*	17.7±0.2^{NS}	17.8±0.7^{NS}	(-)
MCHC (%)	30.0±0.5	37.6±3.0*	30.4±1.3*	31.3±0.2^{NS}	(-)
WBC ($\times 10^3 mm^3$)	14.7±2.8	8.6±0.9*	10.2±1.0*	8.6±0.7*	(-)
Lymphocytes (%)	52.5±6.6	67.6±8.8*	79.9±9.6*	65.2±0.0*	(-)
Neutrophils (%)	47.5±6.6	32.4±8.8*	20.1±4.4*	34.8±4.4*	(-)

Values are expressed as Mean±SE. NS: Not significant, *Significant ($p<0.05$), (-): Not available

showed significant decrease in week one ($p<0.05$) except (Group 5) which showed no significant change compared to control (Group 1). Rats after two weeks; only (Group 2) and (Group 4) showed significant changes having low ($p<0.05$) compared to control (Group 1) while other groups 3 and 5 showed no significant changes.

Hematological changes: Hematological changes for rats given daily oral doses of *M. esculenta* roots methanolic extracts at 75 mg kg^{-1} (Group 2), 300 mg kg^{-1} (Group 3) and aqueous extracts at 75 mg kg^{-1} (Group 4), 300 mg kg^{-1} (Group 5) for one and two weeks are presented in Table 2. After one week of treatment, WBCs and lymphocytes in all group were lower ($p<0.05$) than control (Group 1).

In two weeks treatment, in Group 2 Hb and PCV has the lowest ($p<0.05$). WBCs and Neutrophils in all groups were lower than control (Group1) ($p<0.05$).

Serobiochemical changes: Serobiochemical changes for rats are presented in Table 3. One week after treatment, the activity of ALP, ALT and AST in all groups were lower ($p<0.05$) compared with control (Group1). Only (Group 4) had the lower ($p<0.05$) total protein, albumin and globulin than control. All groups show significant changes in urea compared to control (Group1). Only Groups 3 and 5 had the lower ($p<0.05$) than control (Group 1).

At two weeks treatment, ALP and AST activities had the lower ($p<0.05$) in all groups compared to control (Group 1). ALT activity in all groups except (Group 3) show significant decrease in ($p<0.05$) compared to control (Group 1). In total protein concentration only (Group 5) had the significant change ($p<0.05$) lower than control. Group 3, 4 and 5 show significant changes in urea and cholesterol compared to the control (Group1).

Pathological changes: After 2 weeks of treatment by oral doses of *M. esculenta* roots daily, Necrosis, segmentation and shrinkage of the glomeruli and aggregates of lymphocytes in the renal cortex (Fig. 2a), cytoplasmic fatty change in medulla (Fig. 2b), Packing and hemorrhage of the renal tubules, epithelial cell degeneration or necrosis of the renal convoluted tubules in cortex, cytoplasmic fatty vacuolation of entrilobula hepatocytes (Fig. 3), infiltration of lymphocytes in the intestinal lamina propria (Fig. 4), vacuolation of the cerebral neurons were detected in rats orally given *M. esculenta* roots methanolic and water extracts 75 and 300 mg kg^{-1} (Group 2-5), respectively for 2 weeks. No lesions were observed in the spleen of any of the extracts dosed rats. The tissue obtained from control Group 1 show no lesion throughout the two weeks.

Table 3: Serobiochemical analysis of rats given *M. esculenta* methanolic and aqueous extracts for one and two weeks

		Methanolic extracts		Aqueous extracts	
Parameters	Control (normal diet)	*M. esculenta* (75 mg/kg/day)	*M. esculenta* (300 mg/kg/day)	*M. esculenta* (75 mg/kg/day)	*M. esculenta* (300 mg/kg/day)
One week					
ALP (IU)	210.0±4.9	272.0±6.6*	341.7±8.0*	387.0±4.2*	511.0±5.3*
ALT (IU)	37.7±9.6	63.0±8.4*	67.7±1.0*	76.3±3.7*	78.0±2.0*
AST (IU)	143.6±8.6	379.2±4.7*	265.6±8.0*	501.8±8.1*	296.1±5.6*
Total protein (g dL^{-1})	7.8±0.2	8.3±0.0^{NS}	7.6±0.3^{NS}	8.5±0.1*	7.5±0.4^{NS}
Albumin (g dL^{-1})	3.3±0.1	4.2±0.3^{NS}	3.8±0.2^{NS}	5.2±0.4*	3.6±0.5^{NS}
Globulin (g dL^{-1})	4.5±0.3	4.1±0.3^{NS}	3.8±0.2^{NS}	3.3±0.4*	3.9±0.2^{NS}
Bilirubin (mg dL^{-1})	0.2±0.0	0.2±3.2^{NS}	0.2±0.0^{NS}	0.2±0.0*	0.2±0.0^{NS}
Urea (mg dL^{-1})	96.3±5.2	96.3±2.8*	84.7±8.3*	136.0±7.0*	88.7±0.7*
Cholesterol (mg dL^{-1})	98.5±5.3	95.7±5.0^{NS}	95.7±6.0*	95.7±5.0^{NS}	104.3±5.0*
Two weeks					
ALP (IU)	237.0±0.6	295.0±3.2*	370.0±0.6*	366.3±4.7*	381.0±4.0*
ALT (IU)	59.0±1.0	65.2±7.6*	61.9±0.0^{NS}	85.3±0.2*	78.7±1.0*
AST (IU)	173.7±0.8	210.2±5.1*	193.7±3.2*	292.5±8.3*	247.8±9.6*
Total protein (g dL^{-1})	7.9±0.1	7.3±0.3^{NS}	7.7±0.4^{NS}	8.2±0.6^{NS}	6.7±0.1*
Albumin (g dL^{-1})	3.7±0.0	3.1±0.2^{NS}	3.6±0.0^{NS}	4.0±0.2^{NS}	3.2±0.0^{NS}
Globulin (g dL^{-1})	4.3±0.0	3.9±0.3^{NS}	3.6±0.4^{NS}	4.2±0.4^{NS}	3.6±0.2^{NS}
Bilirubin (mg dL^{-1})	0.2±0.0	0.2±0.0^{NS}	0.2±0.0^{NS}	0.2±0.0^{NS}	0.2±0.0^{NS}
Urea (mg dL^{-1})	66.0±0.6	67.0±2.1^{NS}	81.0±0.6*	79.7±5.3*	81.5±4.5*
Cholesterol (mg dL^{-1})	84.1±7.6	81.2±6.5^{NS}	113.1±5.0*	130.4±6.3*	126.1±6.0*

Values are expressed as Mean±SE. NS: Not significant, *Significant ($p<0.05$), NS: Not significant

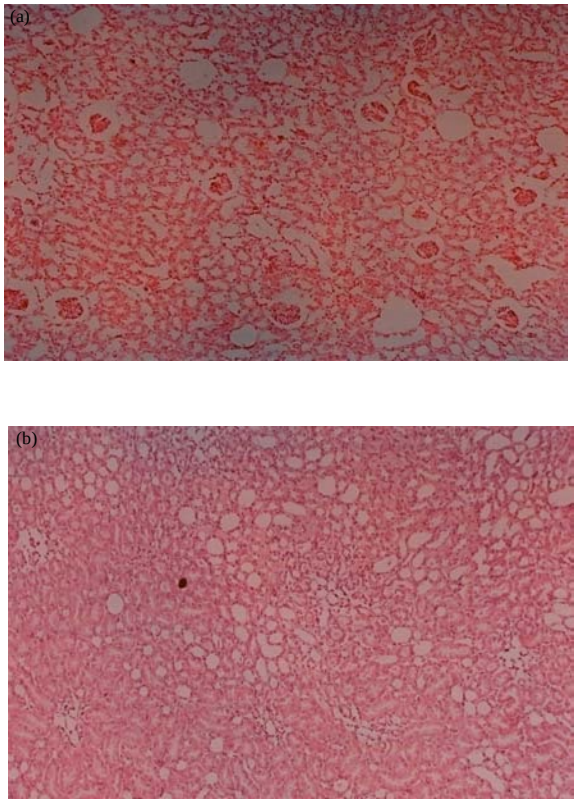

Fig. 2: kidney of rats receiving daily oral doses of *M. esculenta* roots methanol extract (a) 75 mg kg^{-1} and (b) 300 mg kg^{-1} for 2 weeks showing glomerular alteration, necrosis, segmentation and packing of glomulartubules H and E X10

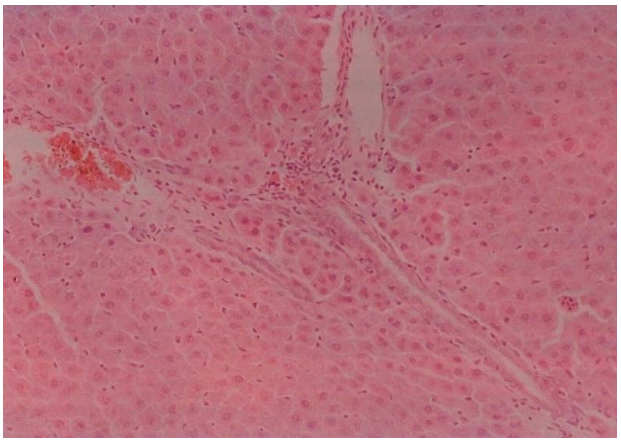

Fig. 3: Liver of a rat receiving daily oral doses of *M. esculenta* roots aqueous extract at 300 mg kg^{-1} for 2 weeks, showing, fatty cytoplasmic vacuolation of the interlobular hepatocytes, lymphocytic infiltration and hemorrhage H and E X 100

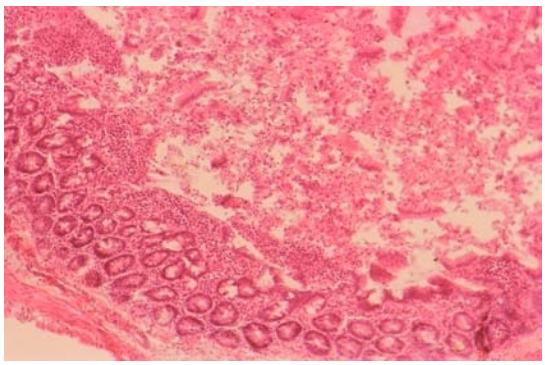

Fig. 4: Intestine of a rat receiving daily oral doses of *M. esculenta* roots aqueous extract at 75 mg kg^{-1} for 2 weeks, showing vacuolation and desquamation of the intestinal epithelium in the lumen, H and E X 10

DISCUSSION

Despite the toxicity of *M. esculenta* roots, it was used as consumable food after traditional processing and in traditional medicine in several countries in Africa and Asia for treatment of various diseases.

Dry fermentation used me be the cause of inability of linamarase to release all cyanide responsible of toxicity which approved by Darman Roger *et al.* (2007).

The result of the present investigation indicated that *M. esculenta* roots were toxic but not fatal to rats in daily oral doses of (75 and 300 mg kg^{-1}) for two weeks, mimicking result in other study (Lu, 1996; Barlow *et al.*, 2002). The liver, kidney and lungs are the primary organs affected by metabolic reaction caused by toxicants. The liver is the most affected organ because it is the site of detoxification in the body (Dybing *et al.*, 2002). In the rats, damage to the liver and kidneys could explain the gradual loss of reflexes. The liver showed fatty vacuolation of hepatocytes, kidneys had an altered glomeruli and degeneration or necrosis of the convoluted tubules, main cause of this pathological changes is the presence of linamarin and consequently hydrogen cyanide, prolonged oral exposure to cyanide promote hepatotoxicity and nephrotoxicity (Sousa *et al.*, 2002). The changes in liver and kidneys probably contributed to the increase in the activity of serum AST, ALT, ALP and urea. Also the infiltration of lymphocytes in the intestinal lamina propria might be contributed to hypoproteinaemia. It has been shown that hepatic, renal and intestinal damage can contribute to hypoproteinaemia in sheep and rats fed *Cuminum cyminum* (Haroun *et al.*, 2002).

The mechanism whereby the plant constituent (s) injured body tissue cannot be tasted from the present study. The toxicity of plant came from its content of a cyanogenic glucosides, linamarin and lotaustralin (White *et al.*, 1998). Also slightly elevated content of toxic metal (Pb and Cd) may be concerned (Arinola *et al.*, 2008).

Increased urea may be due to one of two reasons either kidney dysfunction or increased protein catabolism. Bilirubin concentration was not changed, similar result observed in rats which had been fed *Artemisia abyssinica* (Adam *et al.*, 2000).

An increase in cholesterol was observed which may be due to inability of (apo100) a ligand to be recognized by target cell (due to decrease in protein synthesis), this dysfunction is reflected on total protein concentration and albumin which are decreased due to liver damage which is the

factory of synthesis. The globulin increase as it present immunoglobulin which defend antigens (toxin), this is clearly obvious when any antigen is present result in increase in antibody concentration Infection and Immunity (Qamar *et al*., 2001).

There is no observed effect on blood RBCs, consequently no effects were detected in Hb and PCV while total WBCs count decrease.

CONCLUSION

We concluded that the Sudanese processing of *M. esculenta* is not enough to eliminate all cyanogenic glycosids which is the main toxic component founded in it. The toxicity for Wister rats from *M. esculenta* tubular methanolic and aqueous extracts at concentration of (75 and 300 mg/kg/day) administrated orally, as a result of consumption, alteration in serobiochemicl and hematological parameters occurs, damage of vital organs may occur exemplified by inflammation, necrosis, fatty changes and hemorrhage. Also the doses from both extracts are not fatal.

We suggest that further researches might be carried out to determine the most appropriate methods of detoxification to be used traditionally to be save for human and an animal consumption. Further phytochemical investigations, toxicokinetic and antimycotic activity of active constituents presents in alcohol extract and possibly other fractions are required especially concentration of cyanide.

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