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Assessment of acute toxicity and LD_{50} of *Moringa oleifera* ethanolic leave extract in albino rats and rabbits

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ABSTRACT

Leaves of *Moringa oleifera* extracted in 70% ethanol were assessed experimentally for acute toxicity in two groups each of six, one of albino rats and the other of rabbits. Each animal of the two groups was injected via intraperitoneal route with 150 mg/ml every five minutes till death occurred. The lethal dose of acute toxicity was found to be 6616.67mg/kg body weight (BW) for rats and 26043.67 mg/kg BW for rabbits. Injection with 14ml of concentrated form of 450mg/ml over 10 minutes showed no death among albino rats. The results of histopathology confirmed that the death of both animals was due to excessive fluid injected for the acute toxicity test. Based on these results it can be concluded that the plant had low toxicity effect when given in concentrated doses for short period of time.

Key word: *Moringa oleifera*, acute toxicity, LD_{50}, albino rats, rabbits, medicinal plant.

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INTRODUCTION

The plant *M. oleifera* is widely used as food product and in the treatment of various diseases. Its different parts whether in powdered form, aqueous or ethanolic extract were used to treat cancer (Krishnamurthy et al., 2015), ulcers (Pal and Sahib, 1995), hypertension, diarrhea and inflammation (Minaiyan et al., 2014). They were also used against intestinal worms, as skin antiseptic, antihyperlipidemic (Souravh et al., 2014), antimicrobial (Caceres et al., 1992), antidiabetic as well as antiduretic agent (Jaiswal et al., 2009; Al-Malki and El Rabey, 2015). Moreover, it was used to promote the immune system against various infections (Jaiswal et al., 2009). In India, it was used by men and women as a sexual virility drug and for prolonging sexual activity (Oluduro et al., 2120).
As becoming increasingly used in treatment of diseases, various studies were conducted to investigate its degree of safety and so far no adverse effects were reported in association with the human studies (Stohs and Hartman, 2015). In animals, an oral dose of aqueous leaf extract of up to 1000mg/kg in rats was reported to be safe (Adedapo et al., 2009). In a different study, the lethal dose of 50% orally administered ethanolic extract of *M.*

oleifera in mice was reported greater than 6.4g/kg (Bakre et al., 2013). On the other hand, a single oral dose of 5000mg/kg of an aqueous *M. oleifera* extract as well as oral doses of up to 1000mg/kg of the same extract for 14 days on rats were noted to have neither overt adverse reactions nor histopthalogical effects (Asiedu-Gyekye et al., 2014). In these animals, signs of acute toxicity of methanolic seed extract of *M. oleifera* was observed at extract dose of 4000mg/kg, mortality was recorded at 5000mg/kg while adverse effect at concentrations lower than 3000mg/kg (Ajibade et al., 2013).

However, despite the growing number of research studies involving assessment of acute toxicity of *M. oleifera* primarily on rodents, the lethal doses reported so far were differing from one another. The aim of this study is to assess the toxicity of ethanolic leaf extract of this plant in albino rat and rabbits.

MATERIALS AND METHODS

Extract preparation

The leaves of *M. oleifera* were harvested from different trees cultivated in central Sudan. The leaves were first rinsed with distilled water, dried in shade and were completely extracted with ethanol (70%) using Soxhlet apparatus for 3 days. The percolated extract was then dried in Rotary Evaporator apparatus, weighed and dissolved in distilled water to give the final concentration of 150 mg /ml for acute lethal test and 450 mg/ml for median lethal dose (LD_{50}).

Experimental animals

Two groups of animals were used; white albino rats (*n* = 6, average BW = 250 g) and rabbit of local breed (*n* = 6, average BW = 960 g). The experimental animals were housed in animal cages, under environmentally controlled condition at animal house of the Faculty of Pharmacy, University of Khartoum. The rats were fed on the normal diet concentrate (dried meat, milk powder, oil mixed in flour plus some water) while rabbits on clover leaves and water *ad libitum,* and were acclimated for a week prior commencement of experiments. The procedures of animal care and handling were approved by Research Guide of Animal's Ethics of University of Khartoum.

Acute toxicity and animal responses

For determination of acute toxicity, each animal was carefully given intra-peritoneal dose of *M. oleifera* extract (150 mg/ml) every 5 Min. carefully until death occurred (Ezike et al., 2010).

For animal responses, each animal was monitored during the course of injection. The responses observed, included those related to vision, body temperature, animal activity and consciousness. The effect on vision would be ensured if the animal failed to avoid a pointer brought closer to its eyes, on its body temperature by direct contact when it was held for injection and through visible behavior for the activity and consciousness.

The median lethal dose (LD_{50}) of *M. oleifera*

For determination of median lethal dose (LD_{50}), 10 groups each of 10 albino rats were used. The accumulative acute lethal dose already determined was used to determine the median lethal dose (LD_{50}). For this purpose, the accumulative acute lethal dose was converted into a concentrated lethal dose with minimum volume of distilled water. Then, each group was exposed to graded percent of single concentrated lethal dose up to 50%. The percentages received by rats of each groups were: 100 %, 95.6 %, 91.2 %, 89.7 %, 88 %, 84.4 %, 76.6 %, 68.7 %, 59.4 % and 50 % and for each percentage the required fluid volume of distilled water calculated and used to give the respective percentage of dose. During exposure, rats were monitored for 48 hours to record any signs of abnormal behavior or mortality.

Histopathology

After death, only rats were dissected to prepare slides for histopathology examination, and the dissected organs were the heart, liver and kidneys. The tissues of each organ were placed in formalin buffer 10%, embedded in paraffin wax, routinely processed and sections of 5µ thickness were prepared, stained with haematoxylin and eosin and examined under the light microscope (Olympus-CH-20). These preparations and their indicative results were carried out by a histopathologist at the veterinary research institute, Khartoum, Sudan. The slides photos were taken by Grand vision digital camera (Digital lens f = 7.58 mm).

Statistical analysis

The values obtained were expressed as means (± SD). Simple regression analysis using Excel program was conducted to find out the correlation of data.

RESULTS

Acute toxicity

The results of lethal doses of *M. oleifera* for rats and rabbits are shown in Table 1 and Figure 1 and 2.

Table 1. Mean values of body weights and lethal doses in rats and rabbits.

Groups	Weight (mean ± SD)	Lethal dose (mean ± SD)	P value
Rats	249.17±17.73	6616.67±160.21	0.025*
Rabbits	960.5±11.64	26043.67±830.54	0.004**

Values are means ± SD, n= 6, * = *P*≤0.05, ** = *P*≤0.01 versus.

Table 2. Responses of experimental animals during the course period (early, mid and late) of injections of acute dose.

Animal response	Rats			Rabbits		
	Early	Mid	Late	Early	Mid	late
Vision (blurred)	*	**	***	*	**	***
Temp. (dropped)		*	***		*	***
Activity (ceased)		*	***		*	***
Consciousness (lost)	*	***	***		*	***
Death (occurred)			***			***

*= slight, **= moderate and ***= severe.

Table 3. The percentage of death among group of rats of ten provided with concentrated lethal dose i.e. (with minimum water: 7.3 -14.7 ml).

Groups	Percent (%) of lethal dose	Dose per mg	Dose (450 mg/ml)	Death in rats (%)
1	100%	6616.7	14.7 ml	0 %
2	95.6%	6325.9	14 ml	0%
3	91.2%	6035.1	13.4 ml	0 %
4	89.7%	5938.2	13.2 ml	0 %
5	88%	5841.3	12.9ml	0 %
6	84.4%	5582.8	12.4 ml	0 %
7	76.6%	5065.8	11.3 ml	0 %
8	68.7%	4548.9	10.1 ml	0 %
9	59.4%	3928.7	8.7 ml	0 %
10	50%	3308.4	7.3 ml	0 %

10 rats/group, the doses received orally by gavage.

For the rats the lethal dose was found to be 6616.67mg/kg for rats and 26043.67mg/kg for rabbits. The data for two groups showed linear relationship between lethal doses and animal body weights. The accuracy of relationship was higher and significant (P< 0.05) where determination coefficient (R^2) was 0.754 and 0.900 for rats and rabbits, respectively.

Animals' responses

The responses of experimental animals during the injection with acute lethal dose are shown in Table 2.
Animal vision started to blur slightly at the beginning of injection and continued until became prominent late in the course of injection for both animals. This was the same for the animal consciousness of rats which appeared to be slightly lost at the beginning and became prominent by the end of the course. For the temperature and activity were both started to decrease gradually as from the mid course and became obvious in the two groups by its end. This was the same for the consciousness of rabbits.

Median lethal dose (LD_{50})

The results showed that the LD_{50} of *M. oleifera* is more than 6616.7 as all doses below this incurred zero percentage of death among all groups of rats (Table 3).

Histopathology of rats exposed to *M. oleifera*

The results of histopathological changes in heart, kidneys

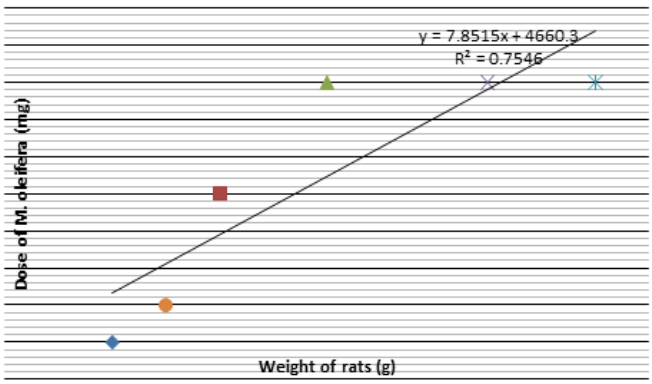

Figure 1. Lethal doses of *M. oleifera* in albino rats (6 rats/group).

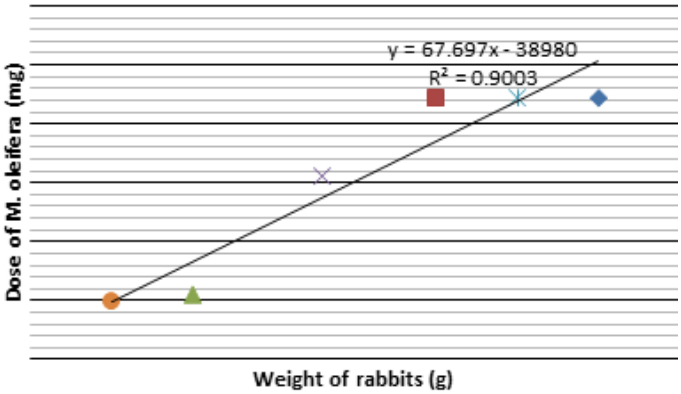

Figure 2. Lethal doses of *M. oleifera* in rabbits (6 rabbits/group).

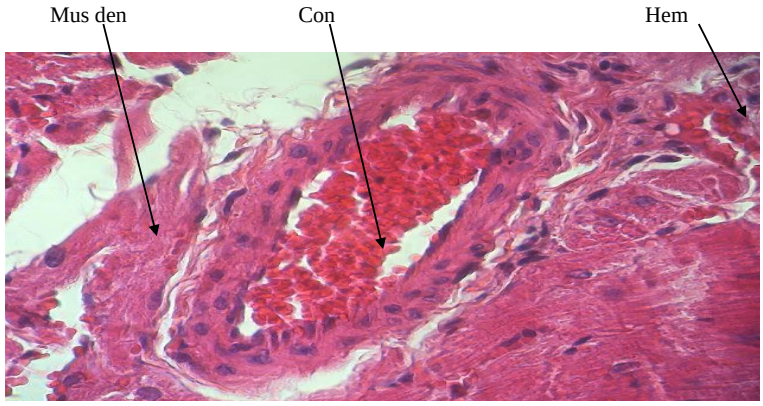

Plate 1. Heart showed cardiac muscle degeneration and hemorrhage and congestion (stain H & E x 400).

and liver of dead rats are shown in plates 1-3. The results revealed that in the kidneys there were hemorrhage, necrosis and degeneration of epithelial renal tubules. Also cardiac muscle degeneration and hemorrhage occurred in the heart. On the other hand, in the liver hepatic cells hemorrhage, necrosis, cytoplasmic vacuoles and degeneration had occurred.

DISCUSSION

In the present study, the lethal dose of *M. oleifera* administered to rats was 6616.67mg/kg BW and for rabbits was 26043.67mg/kg BW. It was evident that death occurred among animals when injected with doses of leaves extract exceeding LD_{50}. The death was probably

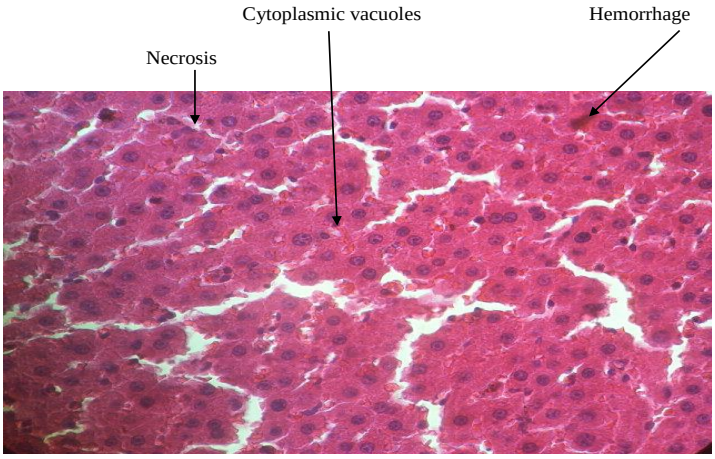

Plate 2. Liver showed hemorrhage, some hepatic cells showed necrosis and cytoplasmic vacuoles (stain H & E x 400).

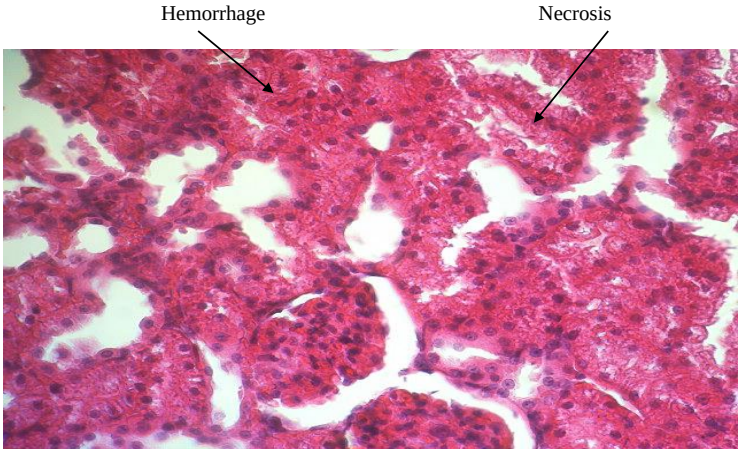

Plate 3. Kidney showed hemorrhage and necrosis of epithelial renal tubules (stain H & E x 400).

caused by accumulation of excessive fluid rather than due to toxicological effect of the plant. This claim was strengthened when no death occurred following injecting of animals with single concentrated doses of 450mg/kg BW being given in volumes of distilled water ranging between 7.3ml for 50% and 14.7ml for 100% of the dose, while accumulative doses which injected to the rat in the lethal dose was found more than 40ml of distilled water.

In this context, water intoxication was reported to occur with consumption of a high quantity of water without giving the body the proper nutrients it needs to be healthy (Thevenon et al., 2013). At the onset of this condition, fluid outside the cells becomes excessively low in amount of solutes such as sodium and other electrolytes in comparison to that inside the cells causing the fluid to shift through into the cells to balance its concentration. This eventually leads to swelling and perhaps rupturing of the cells. This phenomenon perhaps was further confirmed in this study as damage in tissues of selected body organs which is more likely to be due to hemorrhage revealed by histopathological examinations (plates 1, 2 and 3).

However, lack of toxicity of *M. oleifera* being given in concentrated dose of 450mg with minimum water agreed to some extent with the previous reports on the same plant which concluded that it had low toxic effect on experimental laboratory animals (Berger et al., 1984; Grabow et al., 1985; Ali et al., 2004, Ferreira et al., 2007). Recently, a study to investigate the potential toxicological effects of oral doses of an aqueous *M. oleifera* extract in rats revealed that a dose of 5000mg/kg had no adverse reactions and did not show histopathological changes (Asiedu-Gyekye et al., 2014). However, only small but statistically significant dose-dependent increases in some liver enzymes were reported in their study.

However, the exceedingly high lethal dose of rabbits

(26043.67) in this study can be attributed to the fact that the body weight of this animal (960g) was nearly four times that of albino rats (249g) which in this sense brings their lethal dose almost equal to four times of the lethal dose (6616.67mg/kg BW) of the same animals. Accordingly, the lethal doses of ethanolic leaf extract of *M. oleifera* for the two groups of animals of the present study was nearly equal to that reported elsewhere for the rats which was found to be greater than 6.4g/kg BW (Bakre et al., 2013).

On the other hand, abnormal responses shown by experimental animals such as blurred vision, drop of temperature, and cease of activity and apparent loss consciousness may be due to painful sensitization of injections as well as losing blood at cellular level and having damage in their body cells causing the animal to find difficulty in receiving adequate supply of oxygen and nutrients.

From this study, it can be concluded that the ethanolic leaf extract of *M. oleifera* was not harmful to experimental animals, providing that to be ingested in a single concentrated form over a short period.

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