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Original Research Article

Toxicity evaluation of *Abrus precatorius* seeds collected from Bunda District, Tanzania

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Abstract: The plant *Abrus precatorius* is found in tropical countries and widely used as an abortifacient, oral contraceptive and anti-nausea. Seeds are the most poisonous part of the plant due to a toxic poison - abrin. Ingested seeds can affect the gastrointestinal tract, the liver, spleen, kidney, and the lymphatic system. This work aimed at establishing the toxicity/safety status of *A. precatorius* seeds collected from Bunda District, Mara Region Powdered seeds were extracted with methanol using a Soxhlet apparatus. Cytotoxicity and Acute Oral Toxicity tests employed *Artemia salina* and albino mice respectively. Brine Shrimps Test gave the LC_{50} value of 169.36µg/ml regarded as non-toxic. Acute Oral Toxicity test showed no mortality up to the highest tested dose of 5000mg/kgBWT thus classified as practically non-toxic. However, after dissection, the morphological observation revealed gross pathological changes of lungs, small intestines and liver. Histological results indicated hepatotoxicity and nephrotoxicity. This study has confirmed that, *A. precatorius* seeds from Bunda, Tanzania are also not safe for medicinal use. Therefore, the public have to be made aware about their toxicity through the media and other means including various bodies dealing with traditional medicine and complementary/alternative medicines such as a regulatory body; the '*Traditional and Alternative Health Practice Council*' of Tanzania. A team work with clinicians is recommended to associate the use of *A. precatorius* seeds and development of liver and kidney diseases among the patients suffering from such problems.
Keywords: *Abrus precatorius* seeds, cytotoxicity, Acute toxicity, *Artemia salina*, Mice, Bunda District. Tanzania.

INTRODUCTION

Abrus precatorius belongs to the family Fabaceae. It is known by different names all over the world with various common names e.g. Crab's eye, Jequirity beans, Rosary pea, Indian bead etc. It was reported to originate from East Asia but also grows in other countries located in the tropical and subtropical regions [1]. Figure 1 is the photograph showing seeds and some plant parts.

The most poisonous part of the plant is the seed which contains the toxic poison abrin responsible for the inhibition of cell protein synthesis. It is one of the most potent plant toxins with estimated human fatal dose of 0.1-1 µg/kg, and has caused death after accidental and intentional poisoning. It is classified as potential chemical warfare agent [3]. Its toxicological profile is similar to that of Ricin from *Ricinus communis* [4]. Ingested seeds can affect the gastrointestinal tract, the liver, spleen, kidney, and the lymphatic system. Infusion of seed extracts can cause eye damage after contact [5].

Fig-1: *Abrus precatorius* [2]

Although abrin is poorly absorbed, poisoning can be associated with severe gastrointestinal toxicity with large exposure. Typical gastrointestinal symptoms include *nausea, vomiting, and diarrhea*, resulting in

more serious poisonings with severe dehydration and death. Death has been reported with twenty seeds blended with water. The symptoms included vomiting of blood, severe pain in the eyes and burning of ears. Death ensued in two days [6]. Ingestion of one or two seeds by children can be fatal [7]. There is no specific antidote for abrin poisoning, and treatment is mainly supportive with intravenous fluids and correction of electrolyte abnormalities.

Different parts of *A. precatorius* are used for various medicinal purposes [8, 9]. Seeds in particular are taken orally as aphrodisiac in Afghanistan and Egypt, against intestinal worms and as contraceptive in Central African tribes, infusion with hot water is taken orally against malaria in Cambodia, abortifacient and contraceptive agent in India, the water extract is applied against eye infections and taken for tuberculosis and painful swellings [7].

Studies using seed extracts of *A. precatorius* has demonstrated several biological effects such as; antihelminthic activity on *Caenorhabditis elegans* [10], antidiarrheal activity against castor oil-induced diarrhea [11], abortifacient effect from water extract administered intragastrically to pregnant rats at a dose of 125.0 mg/kg bwt [12] and, antifertility effect in both female [13,14] and male rats [15,16] using various extracts and routes of administration.

Tanzanian traditional healers and users are unaware of the toxicity and health risks associated with the consumption of *A. precatorius* seeds. However, geographical location and other factors may affect the synthesis/proportions of phytoconstituents. Toxicity evaluation of the *A. precatorius* seeds was essential for establishment of their safety status as evidence generated from local studies to enlightening users on risks of chronic health problems.

MATERIAL AND METHODS

Study design

This study involved plant identification, collection of seeds, extraction, cytoxicity testing, albino mice animal procurement, oral toxicity testing and acute toxicity testing for clinical signs, macroscopic and histo-pathological evaluation.

Plant identification

The plant was prepared and identification done by the botanist Mr. Haji Selemani in Botany Department - University of Dar es Salaam.

MATERIALS

Collectionof *Abrus precatorius* seeds

A. precatorius seeds were obtained from Kung'ombe village, Bunda district in May, 2014, sun dried for seven days then kept in an airtight container and stored in a refrigerator at -4°C.

Extraction

Dry seeds were powdered with a mechanical grinder and stored in an airtight container. 25 g of the powder was extracted with 100 ml of methanol for 18 hours at 60°C using a Soxhlet apparatus. The extract was filtered using a filter paper and dried on a vacuum rotary evaporator to yield the crude methanolic extract. The extract was stored at -4°C in amber glass container before toxicity testing.

Cytotoxicity testing

Larvae production: Brine shrimp eggs were hatched in a rectangular dish filled with artificial sea water prepared by dissolving 3.8g of sea salt into 1litre of distilled water. A plastic divider with several 2mm holes was clamped in the dish to make two unequal compartments. The eggs were sprinkled into the darkened large compartment, while the smaller compartment was illuminated. After 48 hours the phototropic larvae were collected by a pipette from the illuminated side.

Bioassay procedure: Brine shrimp lethality test was done according to the method previously described by Meyer et al., [17]. Stock solution (1 mg/ml) of the methanol extract was prepared. Various of concentrations (1000, 640, 320, 160, 80, 40, 20 and 10µg/ml) were prepared by drawing different volumes from the stock solutions and then added into the Petri dishes each containing ten brine shrimps larvae. The negative control contained brine shrimp larvae, and artificial sea water. The Petri dishes were incubated under light, the number of survivors in each concentration after 24 and 48 hours were counted and the percentage mortality was determined. LC_{50} and 95% confidence interval was determined from the 48 hour counts using the Probit analysis method.

Determination of acute oral toxicity

Ethical considerations

The protocol of the study was approved by the MUHAS Ethics Committee while the use of mice in acute toxicity study followed guidelines for use of animals in experiments as adopted from internationally accepted principles for laboratory animal use and care stipulated in EEC Directive of 1986; 86/609/EEC [18].

Procurement of mice, Pretesting handling and Drug administration

Fourteen albino mice of both sexes were obtained from the MUHAS animalia. They were then kept in well ventilated cages and given standard animal feed and water ad libitum and allowed to acclimatize with the environment at ambient temperature under natural day light/night conditions for five days before the beginning of the experiments. Animals were food fasted (but not water) for 2 hours before treatment. The

extract was administered in a single dose by gavage using a special designed mice oral needle. The administration volume was 2ml/100g body weight of the animal. Based on the animal body weight on the day of treatment, the quantity of *Abrus precatorius* extract was calculated.

Acute toxicity testing:

Acute toxicity was performed according to Lorke's Method - a new approach to practical acute toxicity testing [19] having an advantage of employing a few animals. This method consists of two phases as briefly described below:

Phase I

Three groups of three mice each were administered with the extract oral doses of 10mg, 100mg, and 1000mg per kg body weight. Food was with held for 3 hours prior to the administration and one hour post administration. After administration of the extract, observation was made for 1 hour and thereafter at regular intervals to check for the onset of adverse effect; which include but not limited to convulsion, paw-licking, salivation, stretching, and rubbing of nose on the floor and wall of cage or time to death. Observation period was 24 hours.

Phase II

The three mice each was administered with the extract orally at higher doses of 1600, 2900 and 5000 mg/kg body weight respectively. Toxic symptoms were observed for 24 hours as well as delayed toxic symptoms for 7 days.

For each phase, one mouse was untreated and taken as negative control. Number of deaths in each group within 24 hours was recorded and the final LD_{50} value was calculated as the geometric mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where deaths occurred). Then the LD_{50} is calculated by the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where;

D_0 = Highest dose that gave no mortality and,

D_{100} = Lowest dose that produced mortality.

Toxicity signs

Observed clinical/behavior changes were; paw-licking, dullness, convulsions, salivation, rubbing the nose on the floor and wall of cage, tremors, gasping, vocalization, constipation, diarrhea, piloerection, paralysis, eating/drinking or death.

Macroscopic observation and histopathological studies: At the end of observational period, the control and surviving mice were humanely sacrificed for macroscopic observation and removal of internal organs (the heart, liver and kidney) later stored in formalin for histopathological studies.

Histopathological sample preparation: The liver, kidney and heart of all the animals were fixed in 10% well-buffered formalin in labeled bottles, and processed routinely for histopathological examination as previously described [20]. Tissues embedded in paraffin wax were sectioned 5 µm thick, stained with haematoxylin and eosin, mounted on glass slides and then examined under a standard light microscope as previously described by the same teams.

Microscopic observation: Specimens of heart, liver and kidney on various doses were observed at low and high power magnification objectives lenses of a routine microscopy coupled to a digital camera. All features were compared between both treated and control groups and done according to the previously described method [20].

Tissue processing, histological staining, microscopic evaluation and photomicrography: These were done according to the method previously described [20] Briefly, after the macroscopic evaluation (grossing), tissues from the mice internal organs were fixed for 24 hours in neutral well-buffered (10%) formalin, embedded in paraffin and sections (5 µm) mounted on SuperFrost_ slides (Menzel GmbH & CoKG, Braunschweig, Germany). These were then deparaffinized, rehydrated and stained with haematoxylin and eosin (H&E). Histological evaluation and photomicrography was performed by the Histopathologist using an Olympus (CX31RBSF Model) light microscope equipped with a digital camera (Olympus Corporation, Tokyo, Japan) on 7 low-power fields (x10 magnification) as well as on their high-power fields (x40 magnification) while taking pictures. Picture processing and printing was performed using Adobe Photoshop 7.0 (Adobe Systems Incorporated, San Jose, CA, USA) and Microsoft-Power Point 2003 (Microsoft Corporation, Redmond, WA, USA).

RESULTS

Cytotoxicity results

Brine shrimp lethality of methanol extract is shown in the Table 1.

Table-1: Brine shrimp lethality test data Abrus precatorius seed methanolic extract

Concentration [µg/ml]	Number of Shrimps	After 24 hours		After 48 hours		LC_{50} after 48 hrs
		Mortality	% Mortality	Mortality	% Mortality	
10	10	0	0%	1	10%	169.36 µg/ml (95% Confidence interval = 156.59 -182.14)
20	10	0	0%	2	20%	
40	10	0	0%	2	20%	
80	10	0	0%	3	30%	
160	10	0	0%	5	50%	
320	10	10	100%	10	100%	
640	10	10	100%	10	100%	
1000	10	10	100%	10	100%	
Control	10	0	0%	0	0%	

Acute toxicity

Toxicity signs of the treated mice are summarized on Tables 2 & 3. Gross morphological changes of internal organs are shown in Figure 1 and the summarized description on Table 4 whereas the histopathological features are presented in Figure 2.

Table-2: Toxicity signs of *Abrus precatorius* seed methanolic extract - Phase I

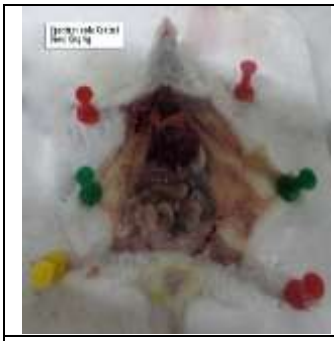
Treated mice	**Mice Weight [gm]**	**Dose [mg]**	**Mortality**	**Toxicity signs**
Dose: 10mg/kg body weight				
P1:AI	29.6	0.296	0	apparently normal
P1:AII	27.0	0.270	0	apparently normal
P1:AIII	30.0	0.300	0	apparently normal
Dose: 100mg/kg body weight				
P1:BI	21.0	2.1	0	Paw licking 1 hour post administration
P1:BII	30.0	3.0	0	Paw licking 1 hour post administration
P1:BIII	22.5	2.25	0	Paw licking 1 hour post administration
Dose: 1000mg /kg body weight				
P1:CI	23.7	23.7	0	Paw licking 13 minutes post administration
P1:CII	26.7	26.7	0	Paw licking 30 minutes post administration
P1:CIII	29.2	29.2	0	Paw licking 24 minutes post administration
				Average paw licking 22 ± 0.33 minutes post administration is

Table-3: Toxicity signs of *Abrus precatorius* seed methanolic extract - Phase II

Treated mice	**Administered Dose [mg/kg body weight]**	**Mortality**	**Toxicity signs**
P2-0I	1600	0	Paw licking 10 minutes post administration and dullness
P2-0II	2900	0	Paw licking 7 minutes post administration and dullness
P2-0III	5000	0	Paw licking 5 minutes post administration and dullness

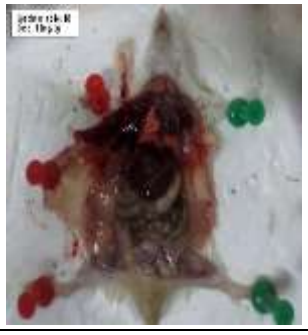
Table-4: Morphological Changes on Internal Organs of Treated Mice

Organ	Dosage (mg/kg body weight)	Observation
Small intestine	10, 100 and 1000	A decrease in the thickness compared with that of control mouse. This could be associated with loss appetite due to the gastrointestinal disturbances
	2900 and 5000	Turned brownish.
Liver	5000	Liver surface was rough
Lungs	All tested dosages	Lungs color change from pinkish to reddish in a dose dependant manner.



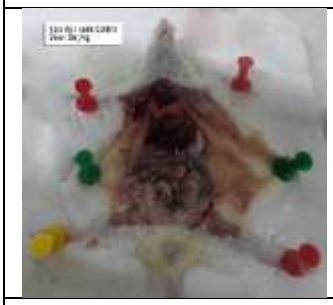
Negative control

Dose 10mg/ kg body weight



Dose 100mg/kg body weight

Dose 1000mg/kg body weight



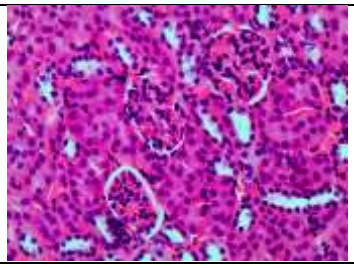
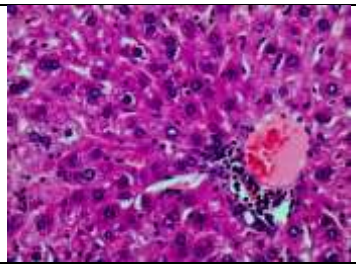
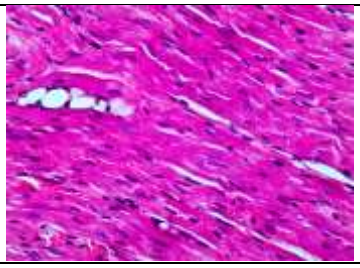
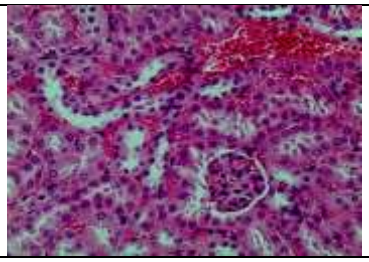
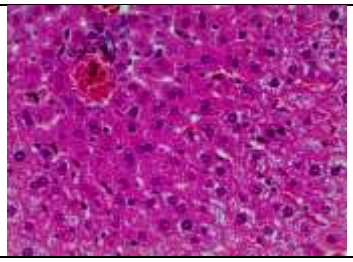
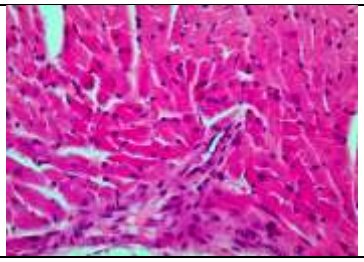
Negative control

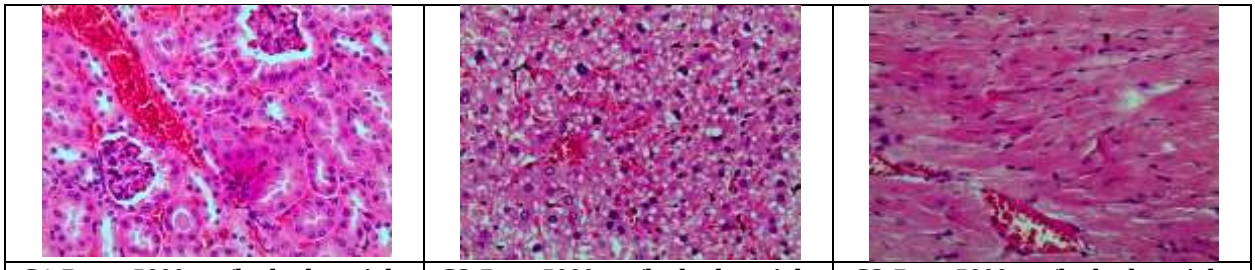
Dose 1600mg/kg body weight

Dose 2900mg/kg body weight

Dose 5000mg/kg body weight

Fig-1: Photographs showing gross pathological changes observed macroscopically

KIDNEY	LIVER	HEART
		
A1-Control	**A2-Control**	**A3-Control**
		
B1-Dose: 1000 mg/kg body weight	**B2-Dose 1000 mg/kg body weight**	**B3-Dose 1000 mg/kg body weight**



C1-Dose: 5000 mg/kg body weight	C2-Dose 5000 mg/kg body weight	C3-Dose 5000 mg/kg body weight

Fig-2: Photomicrographs of Kidney, Liver and Heart.

A1 A2 and A3: Normal. B1: Moderate congestive nephropathy, B2: Congestive hepatopathy and mild balloon degeneration and necrosis of hepatocytes, B3: Slight myocardial degeneration and, C1: Severe congestive nephropathy, glomerular changes (including shrinking, congestion and haemorrhage), C2: Severe congestive hepatopathy as well as severe degeneration (including balloon and severe necrosis), C3: Vasocongestion in the myocardium as well as mild degeneration.

DISCUSSION

Cytotoxicity: After 24 hours no mortality was observed with tested extract concentrations of ≤ 160µg/ml but, 100% mortality was observed with higher tested concentrations i.e. 320, 640 and 1000 µg/ml. After 48 hours the brine shrimp lethality showed a dose dependant manner starting from the lowest tested concentration i.e 10 - 160µg/ml and 100% mortality at higher doses (320, 640 and 1000 µg/ml). Observed mortality with ≤ 160µg/ml concentrations after 48 hours suggests that, the toxicity of the extract on brine shrimps larvae depends on exposure duration /time. The obtained LC_{50} of 169.36µg/ml was ten times less cytotoxic than Cyclophosphamide (LC_{50} = 16.3µg/ml) [21] thus considered non toxic.

Acute Oral Toxicity: Paw-licking and dullness were the only observed toxicity signs while others remained normal even at the highest dosage of 5000mg/kg body weight. Paw licking was observed in all doses except with the dosage of 10mg/kg body weight. Dullness was observed during phase II of the study among all treated mice. The onset of both paw-licking and dullness were inversely proportional to the administered dosages.

No death occurred within 24 hours post administration implying that, the median lethal dose (LD_{50}) is greater than 5000mg/kg body weight. Based on the toxicity classes of Hodge and Sterner [22] any compound with oral LD_{50} (rat) of 5000mg/kg body weight or more should be considered as practically non-toxic. A study conducted in Nigeria using aqueous extract of *A. precatorius* seeds in mice gave similar results [23]. These findings contradict reported toxicity of *A. precatorius* seeds from America and India e.g. twenty seeds blended with water gave severe symptoms including; vomiting of blood, severe pain in the eyes and burning of ears and death after two days [6] while the ingestion of one or two seeds by children can be fatal [7]. To the contrary here in Tanzania, children while grazing their livestock are said to take up to ten or more seeds at once and no death has been reported! Similarly, women in Iringa region regularly swallow the seeds for contraception and alleviation of persisting nausea and vomiting during pregnancy. Such experiences mask the toxicity effects.

Based on toxicity signs our results remained misleading. It is from the histopathological evaluation that revealed the toxicity including; (i) degeneration and necrosis of liver cells (i.e. hepatotoxicity) in a dose dependent manner, (ii) shrinkage of the glomerulus, congestion and hemorrhage due to damage of the nephron-a functional unit of the kidneys and, (iii) slight vasocongestion and myocardial degeneration seen at the highest dose of 5000mg/kg body weight that may explain why immediate death was not observed in the mice due to mild and/or delayed myocardial toxicity. The damage of organs noted in this current study is internal and thus the clinical sequelae may not manifest early enough to allow for complete reversal of the progression of the toxicity.

Our results are in agreement with the fact that, *A. precatorius* seeds are not safe for medicinal purposes due to the presence of abrin. Despite the common use of these seeds in Tanzania it obvious that, repeated oral consumption may cause chronic toxicity leading to liver, kidney and heart diseases or organs failure. At this juncture it is important to establish *A. precatorius* seeds contribution to the occurrence of kidney, liver and cardiac problems in areas where seeds are highly consumed. This should involve a multidisciplinary team consisting of both researchers and clinicians to investigate on relevant biomedical parameters among the population in those areas. Another approach is to interview hospital attending patients suffering from such diseases e.g. kidney failure which is on the rise in Tanzania [24] to check if they have consumed the seeds.

On the other hand, since abrus seeds had shown some interesting results including antifertility activity [11,12], anticancer activity of hydro-alcoholic and petroleum ether extracts [25] and antimicrobial activity against clinically important pathogens [5] etc, scientists may find ways of getting rid abrin and other poisonous substances (if any) to facilitate their medicinal applications. Such efforts have been done in India where abrus seeds are common herbal medicine by the detoxification of the chloroform/water extract

leading to no toxicity in mice at the dosing of 2000 - 5000g/kg body weight [26].

CONCLUSION

Comprehensive safety evaluation requires multiple approaches (tests) to avoid misleading conclusion as found with Brine Shrimp Lethality and Acute Oral Toxicity tests (by observation of toxicity signs) that matched very well and suggested no toxicity from *A. precatorius* seeds in this study. Histopathological evaluation revealed acute organs' toxicity that usually does not clinically manifest early. Latent inherent toxicity is a reason of why people continue using *A. precatorius* seeds as they neither have witnessed instant death nor able to associate chronic diseases with previously consumed seeds. Since toxicity from local *A. precatorius* seeds is evident from this study, Tanzanian traditional healers, users and the public at large should be informed in order to avoid their consumption.

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