

Possible involvement of *Dioscorea* species in human poisoning at Bwakila Juu in Morogoro Rural District, Tanzania

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SUMMARY

For many years' wild plants are known to be used as a source of food, medicines, poisons and for ornamental purposes. *Dioscorea* are reported to be poisonous but if well processed the local people in places where they are found eat them as food especially during hunger. The purpose of this study was to assess the toxicity of *Dioscorea* spp. after it was reported that the plant was involved in deaths of two humans in Bwakila Juu in Morogoro Rural District. Two types of *Dioscorea* bulbils were collected, one being aerial (D1) and the second was tuber (D2), they were sliced, dried under the sun and grounded to make coarse powder. Toxicity test of *Dioscorea* spp. was done using 4 weeks 60 cockerels which were allocated into 6 groups each consisting of 10 birds. Cockerels in groups I – III were fed with chick starter mash which was mixed with D1 *Dioscorea* bulbils coarse powder at a concentration of 0%, 10% and 20%; group IV - VI were fed D2 bulbils' coarse powder mixed with chick starter mash at a concentration of 0%, 10% and 20%. The feeding experiment was done for four days and any changes in clinical signs and deaths were observed for 7 days. In addition, blood samples were collected from all the birds on day 7 of the experiment to measure for aspartate aminotransferase enzyme (AST), albumin and total protein. All the cockerels in group III fed on 20% of D1 started developing clinical signs of inappetence, ruffled feathers, severe diarrhea, convulsion and torticollis. On day 1 of feeding, one bird died and the other two deaths were recorded on day 2. Postmortem results indicated generalized hemorrhages in the lungs and atria, hyperemia of the atria, congestion of kidneys, mucohaemorrhagic intestinal contents and generalized enteritis. The birds in the other groups appeared normal throughout the 7 days of observation but were all sacrificed for postmortem examination which again did not show significant lesions. The mean AST concentration in the plasma ($16.9 \pm 0.2 \mu\text{g/l}$) of the treated birds was significantly higher ($p < 0.05$) compared to the birds in the control groups which is an indication of the liver damage. The total protein and albumin in plasma of all the birds was within normal ranges. Almost six incidences of *Dioscorea* spp. poisoning in humans in which lead to death were reported by the local people in Bwakila Juu during different periods normally occurred during shortage of food. These preliminary results suggest that *Dioscorea* spp. may be involved in human poisoning. More studies are recommended before concluding with certainty the involvement of *Dioscorea* spp. in human poisoning.

Keywords: wild plants, *Dioscorea*, poisoning, cockerels

INTRODUCTION

For many years wild plants have been used as a source of food (Ruffo *et al.*, 2002), medicines (John, 1984; Sezik and Yesilada, 1992; De Foe and Senatore, 1993; Pamplona Roger, 2007), poisons (Thabet *et al.*, 1999; Gidado *et al.*, 2007) and for ornamental purposes (Ruffo *et al.*, 2002). Plants belonging to the Genus *Dioscorea* are reported to be poisonous (Watt and Breyer-Brandwijk, 1962), but at the same time, if well processed, are used as food especially in times of food shortage. The genus contains about 600 species, which are distributed worldwide (Huber, 1998). In Tanzania, there are 14 *Dioscorea* species distributed in different regions /areas of the country; some of the species are found in the wild and some are cultivated as a food crop (Ruffo *et al.*, 2002). The plants produce underground as well as aerial tubers (bulbils) which contain large amounts of starch. As such the tubers are consumed particularly at times of food shortage,

eaten fresh as roasted or cooked. Tubers are collected, peeled, cut into small pieces and soaked in water overnight to remove toxic substance before being cooked (Ruffo *et al.*, 2002). Alternatively, tubers have to be peeled then soaked in water for several days, washed, sliced into small pieces and dried under the sun. Dried slices may be pounded into flour and used for porridge and for stiff porridge (*Bondei* and *Zaramo* ethnic groups) in Tanzania (Ruffo *et al.*, 2002).

Dioscorea species, both wild and some of the cultivated varieties, contain a toxic principle dioscorine, which may cause death when tubers are consumed fresh uncooked or partially cooked (Watts and Breyer-Brandwijk, 1962). Poisoning ability of *Dioscorea* species occurs after eating either aerial or underground tubers. It has further been reported that wild species are more poisonous than the cultivated ones (Watt and Breyer-Brandwijk, 1962). The genus *Dioscorea* consists

of plants which are twining dioecious pubescent (hairy) or glabrous (hairless) herbs with annual stems arising from tubers; leaves alternate or opposite, heart shaped, long and broad with prominent veins in some of the species, usually entire, occasionally compound with 3-7 leaflets. Aerial tubers of *Dioscorea* sometimes occur and arise in leaf-axils, and may be irregularly roundish. Inflorescence spicate, and may be pendulous. The capsules are rigid either deeply 3-lobed or triangular-ellipsoid, dehiscing into three valves while the seeds are variously winged or rarely wingless (Milne-Redhead, 1975).

In Tanzania, few cases of *Dioscorea* poisoning have been reported to occur, and are through hearsay. Nevertheless, *Dioscorea* spp are commonly eaten by the local community especially in areas where the plants are common. The purpose of this study was to assess the toxicity of *Dioscorea* spp. after the report that the plant was involved in deaths of two humans in Bwakila Juu in Morogoro Rural District. It appears that *Dioscorea* as a crop has not featured very much in recent years, notwithstanding its important role in times of food shortage.

MATERIALS AND METHODS

Study area and experimental area

The *Dioscorea* spp bulbils (Figure 1&2) were collected Bwakila juu village in Morogoro rural District and transported to Sokoine University of Agriculture (SUA) where the toxicity experiment was undertaken in cockerels. The brooding rooms in the Poultry Unit at SUA were cleaned thoroughly and well disinfected using glutaraldehyde/quaternary ammonium compound (V-RID®). Brooders were constructed in circular form using hard boards measuring 60 cm wide and

240 cm long. Electrical system was connected and fitted with several 200 watts bulbs, to generate light and heat (Figure 3).



Figure 1. *Dioscorea sansibarensi* aerial bulbis (regarded as more poisonous) Source: <https://garden.org/plants/photo/347911/>

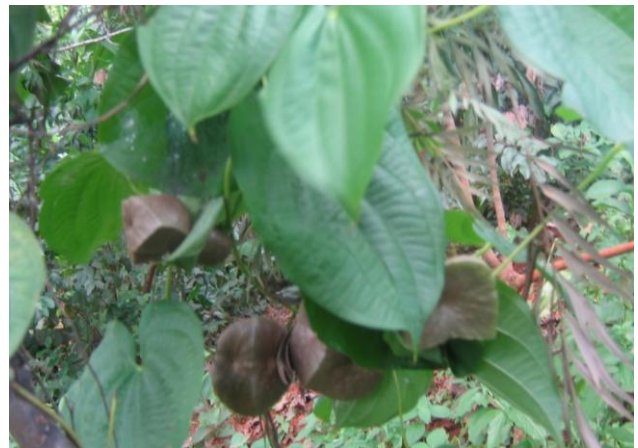


Figure 2. *Dioscorea bulbifera* aerial bulbis (regarded as less poisonous types). Sources: <https://garden.org/plants/photo/347911/>



Figure 3. Brooder facility constructed of hard boards, showing cockerel chicks being provided light, heat, feed and water

Study design and animals

An experimental study design was conducted where by 250 day old cockerels were purchased from Ideal Chick Farm, Dar es Salaam, and reared for 4 weeks in the brooding facility within the Poultry Unit at Sokoine University of Agriculture (SUA) Farm (Figure 3). Brooder was constructed using two hard boards joined in a round form, attached together by means of bent nails. The smooth concrete floor was covered by spreading newspapers to protect chicks from cold. Three bulbs were used to generate heat and to supply light in the brooder. Water was provided using plastic water drinkers which were placed at the centre of the brooder. The chicks were fed chick starter mash put in plates, *adlibitum* and heat and light were provided for 24 hours during the first 4 weeks.

Chicks were vaccinated against Newcastle Disease on Day 3 and Day 21 of age, using live attenuated La-sota strain, orally in water. Vaccination against Infectious Bursal Disease (Gumboro) was done on the 13th day of age. The route of administration was oral via drinking water. Before administration of the vaccines cockerels were denied water for 2 hours to make them thirsty.

Processing of aerial tubers

Aerial tubers of two *Dioscorea* spp. collected from Bwakila Juu, and identified as D1 and D2, one of them being the poisonous type which was suspected to had caused death of the two people were separately sliced into very thin slices and dried in the sun for two days. The sun-dried slices were ground into coarse powder using an electric driven grinder. D1 and D2 bulbils were very similar in appearance although they belong to two different species, and on drying and grinding formed powders which differ in color, milky white and brown/chocolate, respectively.

Feeding trials and observations of changes

Cockerels in groups I – III were fed with chick starter mash mixed with D1 coarse powder at a concentration of 0%, 10% and 20%; group IV - VI were fed D2 bulbils' coarse powder mixed with chick starter mash at a concentration of 0%, 10% and 20%. The feeding experiment was done for four days and any changes in clinical signs and deaths were observed for 7 days. Blood samples were collected from all the birds on day 7 of the experiment to measure for aspartate aminotransferase enzyme (AST), albumin and total proteins.

Experimental birds were examined at interval of 2 hrs during the first 24 hours and subsequently at intervals of 6 hours and changes in behavior, clinical manifestations and any other abnormalities were recorded. Mortalities were recorded and a post-mortem examination was later conducted on all dead and sacrificed cockerels.

Blood samples collection, handling and processing

Blood samples were collected from the wing vein of cockerels using 23 gauge needles, and transferred to vacutainer tubes containing ethylenediamine tetraacetic acid (EDTA) as an in vitro anticoagulant. Blood was centrifuged at 3000 rpm for ten minutes

Data analysis

The toxicity test data from the experiment were calculated using Microsoft Excel and subjected to analysis of variance (ANOVA) and Chi-square tests at a critical probability of $P < 0.05$.

RESULTS

The feed intake in the cockerels in group I - III had poor feed intake as the consumption rates was lower compared to the control group which were being fed on D2. All the 10 cockerels in group III fed on 20% of D1 developed clinical signs of inappetence, ruffled feathers, severe diarrhea, convulsion and torticollis (Figure 4). On day 1 of feeding, one bird in group III fed on 20% of D1 died and the other two deaths were recorded on day 2. Postmortem examination indicated generalized hemorrhages in the lungs and atria, congestion of kidneys, mucohaemorrhagic intestinal contents and generalized enteritis. The mean AST concentration in the plasma ($16.9 \pm 0.2 \mu\text{g/l}$) of the treated birds was significantly higher ($p < 0.05$) compared to the birds in the control groups which is an indication of the liver damage (Table 1). However, the total protein and albumin in plasma of all the birds was within normal ranges.

The birds in the other groups appeared normal throughout the 7 days of observation but were all sacrificed for postmortem examination which again did not show significant lesions.

to isolate plasma which was used to determine concentrations of AST enzyme, albumin and total protein. The absorbance of the standard and test samples was read at wavelength 630 nm in spectrophotometer.

The formula that was used in calculation of concentration of AST enzyme, albumin and total protein was:

$$\text{Concentration of test sample} = \frac{\text{Absorbance of test sample} \times \text{Concentration of standard}}{\text{Absorbance of standard}}$$



Figure 4. Dead cockerel with chalky faecal materials on the floor

Table 1. The mean plasma concentration of albumin, total protein and AST enzyme in cockerels

Groups	Albumin $\mu\text{g/l}$	Total protein $\mu\text{g/l}$	AST $\mu\text{g/l}$
i - D1 0%	16.69 ± 2.05	7.37 ± 0.65	7.76 ± 1.34
ii - D1 10%	16.24 ± 0.96	7.41 ± 0.38	10.7 ± 1.62
iii - D1 20%	16.71 ± 0.18	7.45 ± 0.48	16.85 ± 0.21
iv - D2 0%	16.71 ± 0.18	7.41 ± 0.66	7.62 ± 1.24
v - D2 10%	16.67 ± 0.19	7.36 ± 0.59	7.66 ± 1.15
vi - D2 20%	16.69 ± 2.01	7.41 ± 0.71	7.69 ± 1.51

DISCUSSION

The purpose of the current study was to assess the toxicity of *Dioscorea* spp. after it was reported that the plant was involved in deaths of two humans in Bwakila Juu in Morogoro Rural District. The results indicated that all the cockerels in fed on 20% of D1 were affected by the *Dioscorea* spp. since all the birds showed some clinical signs suggestive of poisoning and some deaths were experienced in the group. The mean AST concentration in the plasma was high ($16.9 \pm 0.2 \mu\text{g/l}$) further giving evidences of liver damage. Postmortem results indicated generalized hemorrhages in different visceral organs

and generalized enteritis which further supported possibilities for poisoning. With the results observed in toxicity trials in cockerels, it is imperative to state that *Dioscorea* spp. may have been involved with the poisoning of humans in Bwakila Juu in Morogoro Rural District. Nevertheless, studies show that if the *Dioscorea* bulbils are well processed by peeling, soaking in water for several days, sliced, dried under and pounded into flour can be used as food with minimal or no poisoning (Watt and Breyer-Brandwijk, 1962; Ruffo et al., 2002).

The current study established that *D. sansibarensis* (*Dendego*) was given in-feed to cockerels and caused deaths in two days of feeding indicating that the species is poisonous. The other species *D. Bulbifera* (*Libika*) given in-feed caused no harm to cockerels, an indication that they are harmless. The distribution of *D. sansibarensis* in Tanzania is wide; it has some features which resemble the less poisonous species. The people who consume the tubers of *D. sansibarensis* were poisoned as compared to those who took *D. Bulbifera*. *Dioscorea* are known to contain a toxic principle called dioscorine which causes various toxic effects in mammals that are related to liver damages. To date, almost six incidences of *Dioscorea* spp. poisoning in humans has been reported and two individuals in Bwakila Juu after they consumed the plant during shortage of food. These preliminary results suggest that *Dioscorea* spp. may be involved in human poisoning.

The clinical signs of severe diarrhoea, torticollis, and narcosis that were observed in cockerels after being fed on bulbils of *D. sansibarensis* have also been previously reported by other scholars (Broadbent and Schnieden, 1958; Bhandari and Kawabata, 2005; Azanza and Patricia, 2006). The active ingredient *Dioscorin* is a neurotoxin that acts by blocking the nicotinic acetylcholine receptor. It has an LD50 of 60 mg/kg in mice through an intra peritoneal route of administration. Saponin has a wide range of effects; it cause haemolysis of red blood cells, vacuolization of the cytoplasm of hepatocytes, canalicular cholestasis and cause liver necrosis (Bhandari and Kawabata, 2005; Azanza and Patricia, 2006). The post-mortem lesions of generalized haemorrhages in the lungs and atria, congestion of kidneys, mucohaemorrhagic intestinal contents and generalized enteritis suggests an acute poisoning. This further show that *D. sansibarensis* if not well prepared has a potential of toxicity effects to whoever consuming the plant.

Experimental cockerels in the present study had low feed intake and did not readily accept the feed containing *Dioscorea* powder. This again may be due to presence of bitter alkaloids dioscorine, furanoid norditerpenes and saponins in *Dioscorea* plant (Broadbent and Schnieden 1958; Bhandari and Kawabata, 2005).

The analysis of the total protein and albumin in plasma of the control group and the experimental cockerels showed no variations between the groups. The difference was not observed probably because of the short duration of the experiment, therefore there was no much damage to the liver. Damage to the liver usually interferes with protein synthesis which results into decreased plasma protein concentration (Maxine, 1979). The high mean ($16.85 \pm 0.21 \mu\text{g/l}$) AST concentrations in the plasma of the treated birds than that measured in the control groups of birds suggestive of some extent of liver damage, specifically liver cells (hepatocytes) (Maxine, 1979).

From the results obtained from the toxicity study of *D. sansibarensis* cockerels show that the plant is poisonous. It caused severe illness to the exposed cockerels and may be responsible for deaths in humans in Morogoro at Bwakila Juu. Therefore, more studies are recommended before concluding with certainty the involvement of *Dioscorea* spp. in human poisoning. These preliminary results should build the foundation to further explore the toxicity of the plant and possibly identify the toxic ingredients of *Dioscorea* plants of Tanzania. Local people in the locality can be used as the starting point towards bioprospecting of the importance of *Dioscorea* plants. Nevertheless, education to the people in localities where the *Dioscorea* are found should be educated on poisonous and non poisonous species and where they are in acute shortage of food, better methods of *Dioscorea* preparations should be practiced.

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