Acute toxicity of saponins from the fruit of bitter apple *Citrullus colocynthis* (L.) Schrad,on the Norway rat, *Rattus norvegicus* (Berkenhout)

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ABSTRACT

Citrullus colocynthis (L.) Schrad (CCT) is an important medicinal plant belonging to the family Cucurbitaceae. It is a well-recognized plant in the traditional medicine and used in rural areas as a purgative, ant diabetic and insecticide. The objective of this study was to evaluate saponins acute toxicity. Crude saponins were extracted from the rind andtheir acute toxicity was determined on Norway rat *Rattus norvegicus* (Berkenhout). Five dosage levels (60, 70, 80, 90 and 100 mgs saponins/rat) were selected following a pilot study and administered intraperitoneally (I.P.) to each batch of albino rats (4 rats/ batch). The treated rats were observed for 96 hr for acute toxicity symptoms. Death occurred between 1–4 days post- treatment. The corrected mortalities were 0, 25,50,75 and 100%, respectively. The LC_{50} was 79.43 mg / rat. Symptoms and behavioural changes during the observation period were anorexia, abnormal gait, twitches, blepharoptosis, reduced activity and bleeding. Severe diarrhoea was the most serious symptom.The study has identified CCT, *viz*. its saponins content, as a promising plant with acute and broad rodenticidal activity.

INTRODUCTION

The Sudan is a vast country with a total area of about 1.882 million km². It is inhabited by a population of approximately 30.5 million. Eighty percent of the work force is engaged in agricultural activities. Synthetic pesticides are needed to keep crops and their products free from pests and diseases. These arsenals of chemicals are not without shortcomings for the environment and consumer's health. Moreover, they are very expensive and exhaustive to the limited returns of the farmers.

Around 2000 plant species or more have been examined worldwide for their biological activity against hazardous pests and diseases. The most important products from these plants are pyrethrum, rotenone, nicotine, sabadilla, limonene and azadirachtin. Several studies were carried out in the Sudan on the pesticidal activities of some indigenous plant species such as, milkweed (*Calotropisprocera*Ait.), hoary basil (*Ocimumcanum*Sim),neem (*Azadirachtaindica* A.juss) and several others.

The management of rodent pests in Sudanese agriculture, for crop protection in the field and stores, and for disease prevention is a time-consuming, expensive and difficult task. Currently used rodenticides are extremely toxic to man and other non-target organisms.

Colocynth (*Citrullus colocynthis*) is a well-known medicinal plant that grows naturally in many tropical and subtropical countries (Wasfi,1994);and its fruit are recommended for indigestion and diabetic people in traditional medicine(Wasfi,1994). Cases of acute toxic colitis after ingestion of colocynth were reported (Goldfain *et al*.,1989;Al-Faraj,1995). Toxicity studies on small ruminants suggest that the fruit causes organ damage in the liver, kidney and the gastrointestinal tract (Barri,1983;Elawad*et al*.,1984) .According to Wasfi (1994), a dose of 800 mg/kg of the ethanolic extract of the leaves killed 60% of the treated rats. Pharmacological examination of the surviving animals and histopathological observations suggested hepatorenal damage.

A previous study (Alias, 2004) on the potential use of indigenous Sudanese botanicals has identified *C. colocynthis*as a promising plant with broad rodenticidal activity, resulting in significantly high levels of mortality. Moreover, the plant is naturally available, cheap and easy to collect and prepare the fruit for use as rodenticides by local farming communities. The present study is a continuation of the previous work, and

designed to investigate aspects pertaining to *C. colocynthis* fruit contents as botanical rodenticides, *viz*. saponins, aiming to understand the mode and site of action of this chemical group and to provide additional base- line information in the area of chemical ecology by using indigenous plants in the field of crop protection. The objective of this study was to evaluate saponins acute toxicity, which might lead to elucidation of their site of action

MATERIALS AND METHODS

Bioassay

Sites of the experiments

The experiments were carried out in July 2011 in the Biology Laboratory of the Faculty of Agricultural Sciences, University of Gezira, (FAS, Uof G) Wad Medani, Sudan. Crude saponins

were extracted in the Food Science Laboratory, Faculty of Engineering and Technology (FE and T), U of G, Wad Medani, Sudan.

Isolation of crude saponins

The saponins were isolated according to a method described by Feroz *et al*.(1993), and Otsuka *et al*.(1997). In brief, 30g of the rind (epicarp and outermost layer of mesocarp) of the fruit was sun-dried, powdered and defatted in a Soxhlet with petroleum ether at 40-60°C for 16 hr. The residue was added to absolute methanol (Me.OH) and left overnight under reflux at 70°C. It was then filtered, and the filtrate was evaporated to dryness. The yield was dissolved in distilled water(D.W) extracted in a separatory funnel with 1-butanol (three times), and dried by evaporation. Finally, the extract was dissolved in absolute Me. OH and saponins were precipitated by adding diethyl ether. A yellowish-brown dry powder of pure saponins was collected (5g in four intervals) and identified by their frothing and formation of the honey comb.

Test animals

Adult Sprague Dawely rats (3 months old), of both sexes and weighing 130 -140gwere used in the study. Rats were supplied by the Animal House, National Institute for Medicinal and Aromatic Plants, The National Center for Research (NCR), Khartoum, Sudan. The rats were housed in plastic cages with bar lids used to hold water bottles in accordance with the protocol set by Institutional Animal Care and Use Committee (IACUC)(Rand,2001).These rats were kept under controlled conditions of temperature (20-30°C), R.H. (50%) and L:D (Ligt-Dark) periods (12:12-hrs). Rats were fed with meals composed of minced meat, flour and drinking water. All rats were apparently healthy. An acclimatization period of 7 days was allowed before experimentation.

Intraperitoneal (I.P.) administration of saponins

Twenty four rats were used in this experiment, divided into 6 groups of 4 animalseach, 2 males and 2 females/ group. Groups 1-5 received 1ml of saponins solution at a dosage level of 60, 70, 80, 90, and 100 mg/rat, respectively**.** Group 6 was used as a control and received 1 ml of saline solution /rat.

The animals were kept under observation for 96 hr. LD_{50} values were calculated by using Probit analysis (Finney, 1980).Corrected mortality% was calculated according to Abbott's formula:

$$\text{Corrected mortality}(\%) = \frac{\text{Mortality (\%) in treated} - \text{Mortality (\%)in control}}{100 - \text{Mortality (\%)in control}} \times 100$$

Signs of pain and distress were visually evaluated. Symptoms of morbidity and moribund condition were observed in accordance to the protocol set by Rand (2001). (Tables1 and 2).

Table 1. Typical signs of pain and distress in laboratory animals reported by Rand (2001).

Mild to moderate pain	Severe or chronic pain/distress
1. Eyelids partially closed	1. Eyes closed
2. Porphyrin staining around eyes	2. Weight loss.
3. Increased aggression (towards humans and cage mates) - licking - biting or scratching – guarding.	3. Depressed unresponsive animal
4. Reduced exploratory behaviour.	4. Muscle wasting along back- dehydration- incontinence.
5. Aggressive vocalization when handled	5. Aggressive vocalization when handled.
6 Hunched posture.	6. Decrease vocalization and hypothermia.
7. Sudden running movement.	7. Recumbent position with head tucked into abdomen.
8. Change in respiration.	
9. Rough hair coat and hair loss.	

Table 2. Signs and symptoms for judging morbidity and moribund condition reported by Rand (2001).

Mild to moderate pain	Severe or chronic pain/distress
1. Rapid breathing rate.	1. Impaired ambulation (unable to reach food and water).
2. Hunched posture.	2. Weight loss.
3. Anorexia (loss of appetite).	3. Signs of lethargy drowsiness - aversion to activity - lack of physical or mental
4. Diarrhoea or constipation.	4. Difficulty in breathing.
5. Hypo- or hyperthermia.	5. Inability to remain upright.
6 Rapid weight loss.	6. Emaciation (body weight is not always appropriate)..
7. Breathing rate very slow, shallow, and laboured.	7. Central nervous system disturbance bleeding- Chronic diarrhoea.
	8. Prolonged anorexia

RESULTS AND DISCUSSION

Bioassay

As mentioned earlier, treated rats were observed for 96hr for acute toxicity symptoms. Death occurred within 24-96 hr, as saponins showed delayed effect in causing death. One (25%), two (50%), three (75%) and four (100%) of the rats died after treatments corresponding to dosage levels of 70, 80,90 and 100 mg/rat, respectively (Table 3). No death cases were recorded at 60 mg/rat, and the control treatments after 96 hr. Dosing schedule, average weights and corrected mortalities are given in Table3. Mortality following administration of different dosages of saponins was plotted *vs.* Probit values (Fig.1). The values obtained proved that saponins killing effect is dosage-dependent, especially when the readings were taken after 24 hr. The dosage 60 mg/rat did not kill the rats within 24 hr, whereas the three next dosages were able to kill as little as 25% of the treated rats within the same period. The highest dosage (100 mg/rat) killed 50% of the treated rats within 24 hr. Moreover, none of 60 and 70 mg/rat treated animals died within the next three days, which may indicate that the lethal dose was not attained. The effect of the dosages started to show up at 80 and 90 mg/rat in the third day where 50% of the rats died and, in the 100 mg /rat where 75% died. The effect continued in the fourth day for 80 and90 and 100 mg/rat; they killed50%., 75% and 100% of the treated rats, respectively. These results indicated that saponins effects must be evaluated after 96 hr, and the tabular LC50 (Table3) is 80 mg/rat, whereas the graphical (Fig.1) was 79.43. Moreover, the LC90 proved to be 98.97 mg/rat, whereas the log dose – line slope was 13.4. This steep slope indicates that the response of the tested rats to the extract was homogeneous.

Table 3. Acute toxicity of saponins administered in traperitoneally(I.P.) to rats

Dosage (mg/rat)	No. and sex	Body weight (g)	No. of dead rats				Total	Corrected mortality after 96 hr (%)
			4 r	8 r	2 r	6 r		
Control	2M, 2 F	140					0	0
60	2M, 2 F	133					0	0
70	2M, 2 F	140					1	25
80	2M, 2 F	141					2	50
90	2M, 2 F	131					3	75
100	2M, 2 F	133					4	100

M=Male F=Female

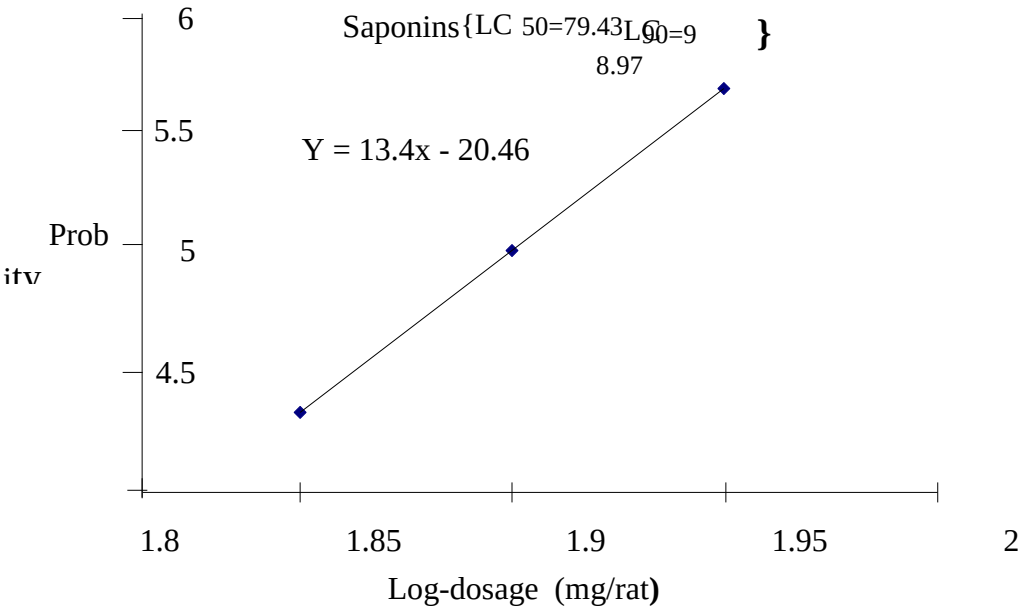

Figure 1. Dosage / mortality line (L-dP-Line) of albino laboratory rats treated with different dosages of saponins after 96 hr.

It should be taken into consideration that the symptoms of toxicity shown in Table4 with the results of the bioassay are needed to explain or elucidate the cause of death. All treated animals showed toxicity symptoms as shown in Table 3. However, some of them (the low concentrations) were able to recover or tolerate the dosage applied. This could be attributed to either elimination or detoxication of the saponins.

Reduced activity and severe diarrhoea were the most serious symptoms. Death was the ultimate fate for all rats that developed diarrhoea. The animals that survived showed some symptoms, including mild diarrhoea, but finally were able to recover. There were no differences between the males and females regarding the symptoms and mortality.

Batanouny(1999) revealed that the ethanolic-extract of the colocynth fruit produced stimulation, accompanied by increased motor activity, tremors, convulsions, diarrhoea and rapid irregular respiration preceeding death in mice. All these symptoms are cholinergic symptoms. The same extract demonstrated cytotoxic, as well as mutagenic effects.

Diwan *et al*. (2000) reported that heart failure, acute hypoglycaemia and hepatorenal damage are the main causes of death after oral administration of saponins. The immediate cause of death of animals that died early was probably heart failure. As time after ingestion goes on, the saponins have enough time to exert their action, and death is likely to be, due to acute hypoglycaemia rather than heart failure. This probably explains the death of the animals that died later (48-96 hr), since hypoglycaemia was confirmed in these animals by blood test.

Toxicity symptoms

Symptoms and behavioural changes during the observation period (96 hr) were anorexia (in all animals and the five dosages), abnormal gait (in 75% of the animals in the first three dosages and 100% in the others), twitches (started to appear at 80 mg/rat and the rest of the concentration in 50% of the treated rats), blepharoptosis(drooping of the upper eyelid in all treatments: 25% in 60 mg, 75% in 70, 80 and 90 mg/rat, and 50% in100 mg/rat), reduced activity 100% in all tested dosages (Table 4). Severe diarrhoea was the most serious symptom. Diarrhoea started when the rats were dosed with 80 mg and above: 25%, 50% and 75% for 80, 90 and 100 mg/ rat, respectively. After developing diarrhoea, none of the treated animals

survived. The animals that survived showed some symptoms, including mild diarrhoea, but were able to recover within one week. Bleeding proved to be concentration-dependent. Bleeding started at 70 mg/rat in 25% of the treated rats, increased to 50% in 80 mg/ rat, 75% in 90 mg/rat, and 100% in 100 mg/rat.

There were significant differences when comparing the standard deviations of some toxicity symptoms, with the untreated control. This could be done by subtracting (zero) from the number of animals, which exhibited symptoms in different dosages levels, as the result, in most dosages was larger than the standard deviation (Table 4). The afore-mentioned symptoms were typical to those mentioned by Diwan *et al.*(2000) and Rand (2001).Symptoms, such as diarrhoea, may indicate that colocynth acts on the digestive system. Elawad *et al.* (1984) reported that the powder generated from the ripped fruit pulp was used as purgative acting directly on the gastrointestinal tract**.**

Table 4. Symptomatic signs of toxicity on rats treated with different dosages of saponins.

Symptoms	No. of treated rats	Dosage (mg/rat)						S.D.
		Control	60	70	80	90	100	
Anorexia	4	0	4	4	4	4	4	1.63
Abnormal gait	4	0	3	3	3	4	4	1.47
Twitches	4	0	0	0	2	2	2	1.10
Prepharpotosis	4	0	1	3	3	3	2	1.26
Reduced activity	4	0	4	4	4	4	4	1.63
Diarrhoea	4	0	0	0	1	2	3	1.26
Bleeding	4	0	0	1	2	3	4	1.63

S.D= Standard Deviation

CONCLUSION

The study has identified CCT, *viz*. its saponins content, as a promising plant with acute and broad rodenticidal activity resulting in significantly high levels of mortality.

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السمية الحادة لصابونينات ثمار نبات الحنظل على الجرذ النرويجي

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الخلاصة

الحنظل *Citrullus colocynthis* (L.) Schrad من النباتات الطبية المهمة المنتمية للعائلة القرعية، وهو معروف في الطب الشعبي في الأرياف بوصفه مادة مسهلة وفي علاج داء السكري ومبيداً حشرياً. هدفت هذه الدراسة إلي تقويم سمية صابونينات ثمار هذا النبات. تم اختبار السمية الحادة للصابونينات المستخلصة من قشور ثمار نبات الحنظل عليطريق الحقن عبر الغشاء البريتوني للجرذان النرويجية البهقاء *Rattu norvegicus* (Berkenhout). بناءً على تجارب أولية تم اختبار خمس جرعات وهى 60 ، 70، 80، 90 و 100 مجم صابونين خام / جرذ .خضعت الجرذان المعاملة والشاهد للملاحظة لمدة (96) ساعة لتحديد علامات (أعراض) السمية الحادة للمستخلص ماتت الجرذان خلال 1– 4 أيام من المعاملة. وكانت النسبة المئوية المصححة للموت كالآتي: صفر، 25، 50 ، 75 و100 للجرعات المذكورة أعلاه بالترتيب. التركيز القاتل النصفي LC_{50} عن طريق تحليل الاحتمالات (البروبيت) كان حوالي 43 .79مجم /جرذ. تمثلت علامات السمية الحادة في الفقدان الكامل للشهية ، الارتجاف ، صعوبة التنفس ، عدم المقدرة علي الوقوف ، انسدال جفون العين مع بعض الإفرازات ، النزيف وأخطرها كان الإسهال الحاد ثم الانهيار التام والموت. اثبتت هذه الدراسة أن الحنظل ممثلاً في الصابونينات المستخلصة منه نباتاً واعدا يمكن استخدامه في مكافحة القوارض.