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Toxicity of the Aqueous Extract of Colocynth *Citrullus colocynthis* (*L.*) Schrad on laboratory rat *Rattus norvigicus*

Ehab E. M. Alias*[1], Yousif O. H. Assad[1], Elameen M. A. Eltoum[1] and Mohamed H. Zeinelabdin[2]

1. Department of Pesticides and Toxicology, Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan.
2. Department of Crop Protection, Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan.

*Corresponding Author: Email: eihabelias1@gmail.com

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Abstract

This study aimed at investigating the acute toxicity of the aqueous-extract of colocynth (Hanzal) *Citrullus colocynthis* (L.) Schrad, using albino laboratory rats *Rattus norvigicus* as laboratory experimental animals. Five dosage levels were selected according to preliminary screening tests. These were 90, 180, 225, 270 and 315 mg. They were administered orally through intubation to each group of albino rats. The treated rats were observed for 24 hr for acute toxicity symptoms. Death almost occurred between 1 – 18 hrs after the treatment. Corrected mortalities were 0, 33.3, 50, 66.7 and 100 corresponding to the above mentioned dosage levels respectively. The mean oral LD_{50} was determined through probit analysis, it was found to be 2402 mg/kg body weight. Symptoms of toxicity were severe anorexia, diarrhoea, difficulty in breathing, inability to stand upright, collapse, muscles atrophy and death.

Abbreviations: Hf.Aq.Extr. : Hanzal fruit Aqeous extract

Key words: Colocynth (*Citrullus colocynthis*), albino rats, intubation, probit analysis, anaroxia, acute toxicity.

Introduction

Plants develop a number of novel secondary metabolic pathways to produce several noxious compounds to withstand and to protect themselves from herbivores attack. These compounds represent an enormous diversity of biodegradable biologically active ones.

Citrullus colocynthis (L.) Schrad., commonly called colocynth, bitter apple or bitter cucumber and locally known as "*Hanzal*", is one of many species of the family Cucurbitaceae. It is a wild plant; it is an ancestral type of watermelon, widely distributed in Northern and Central Sudan (Abd Elgadir, 1995). Colocynth is irritant and cathartic; it acts powerfully, producing copious watery evacuation. It causes inflammation of the mucous membrane of the intestines, vomiting and bloody stools with violent griping. Death has resulted from a dose 1½ teaspoonful of the powder (Anonymous, 2003).

The objectives of this study were to:

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1) report on the acute Toxicity of Hanzal Fruit Aqueous Extract in the adult albino Norway rats
2) calculate the LD50 of Hnzal.
3) determine the clinical signs and symptoms of Hanzal toxicity on the adult albino Norway rats.

Materials and Methods

Site of the experiment

The experiment was carried out in the Biology Laboratory of the Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan (14° 24´N 33° 29´E, 408 m above sea level)

Test animals

Stock parents of albino rat *R. norvegicus* reared in the laboratory, were housed together in a communal wire-bottom cage with wire bar lids used to hold water bottles. A feed composed of sorghum grains *Sorghum bicolor*, Lucerne leave, *Medicago sativa* was delivered daily. A mincemeat source of animal protein was delivered every two weeks .Water was supplied through drinking bottles held in the cage. The second generation was taken for testing after reaching maturity stage. six animals (3 males and 3 females) 115 days old at an average weight of 89.1 g were used for each concentration..

Preparation of Hanzal fruit aqueous extract

Yellow mature *Hanzal* fruits were collected from Atra village East of Wad Medani, Gezira State. The collected *Hanzal* fruits (all fruit contents viz. pulp, seeds and rind) were dried under shade and were mechanically milled by using electric blender to a powder form. Three hundred grams of *Hanzal* Fruit Powder (HFP) were dissolved in 2L of distilled water. The mixture was stirred for about 10 minutes and allowed to stand overnight. It was then reagitated for few minutes and filtered to remove solid residues through fine gauze. The resulting filtrate was poured in glass cylinder and left to stand overnight for further separation of solid residue through sedimentation. The crude aqueous extract was separated by using a pipette prepare to the final stock solution and stored in a refrigerator for investigation. The remaining residue was dried, weighed (209.37g) then subtracted from three hundred grams which added to the 2L of distilled water to calculate the concentration of the final solution the difference was the amount extracted in 2L of distilled water. The calculation procedure was as follows:

- Actual amount extracted by 2L equal 300g (the amount added) -209.37g (the amount remained after extraction) = 90.63g (the amount extracted in 2 L).

- Actual amount dissolved in 1L equal 90.63/2 = 45.3g. The concentration of the stock solution was 4.53 % one ml in this case contain 0.045g (45mg/ml).

Testing procedure

Five dosage levels were selected according to preliminary screening tests. These were 90, 180, 225, 270 and 315 mg, corresponding volumes taken from the stock solution which were 2, 4, 5, 6 and 7 ml respectively. Distilled water was used for the control. Each of the selected dosage levels were administrated orally through intubations for each group of rats. The treated rats were observed for 24 hrs for acute toxicity symptoms, and percent mortality rate were obtained. LD_{50} values were calculated by using probit analysis (Finney, 1990).Visual evaluation of signs of pain and distress and symptoms of morbidity and moribund condition was done in accordance with the protocol set by Rand (2001) (Table 1 and 2)

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Table 1: Species 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) Eye closed.
2) Porphyrin staining around eyes	2) Weight loss
3) Increase aggression towards cage mates liking - biting – scratching – guardining.	3) Depressed unresponsive animal.
4) Reduced exploratory behavior.	4) Sunken or distended abdomen
5) Hunched posture.	5) recumbent position with head tucked into abdomen
6) Sudden running movement.	
7) Change in respiration.	
8) Rough hair coat and hair loss	

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

Morbidity (disease/illness)	Moribund condition (state of dying)
1) Rapid breathing rate.	1) Impaired ambulation (unable to reach food and water).
2) Hunched posture.	2) Muscle atrophy.
3) Anorexia (loss of appetite)	3) Signs of lethargy drowsiness - aversion to activity - lack of physical or mental alertness.
4) Diarrhea.	4) Difficulty breathing.
	5) Inability to remain upright.
	6) Emaciation (body weight is not always appropriate).
	7) Prolonged anorexia.

Results and Discussion

Acute Toxicity of Hanzal Fruit Aqueous Extract

Treated albino rats were observed for 24 hrs for acute toxicity symptoms. No death cases were recorded at 90 mg dosage level and the control treatment after 24 hr. Death occurred from 1-18 hrs. 2, 3, 4 and 6 albino rats were killed after treatment corresponding dosage levels of 180, 225, 270 and 315 mg respectively. Dosing schedule, average weight and corrected mortalities are given in Table (3).

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Table 3: The acute toxicity of Hanzal fruit aqueous extract to albino laboratory rats Rattus norvegicus

Albino rats		Crude extract			Mortality
No. and sex	Average weight in grams	Concentration mg/ml	Amount administered		
			Dosage mg/rat	Dose quantity mg /kg bw	
(3M,3F)	85.0	45	315	3500	100
(3M,3F)	88.7	45	270	3000	66.7
(3M,3F)	91.4	45	225	2500	50.0
(3M,3F)	90.7	45	180	2000	33.3
(3M,3F)	89.4	45	90	1000	0.0
(3M,3F)	89.3	0.0 (control)	0.0 (control)	0.0 (control)	0.0

M = Male
F = Female
Bw = body weight

Calculated LD_{50} and LD_{90}, regression line equation and fiducial limits are given in Table (4). Dosage/ mortality line was fitted by eye (Figure 1).

Table 4: The LD50 and LD90 values (mg/kg) of Hanzal fruits aqueous extract to albino laboratory rats Rattus norvegicus

Lethal dose	Values(mg/kg orally)	Fiducial limits at 95% (mg/kg)	Equation of regression line
LD 50	2402	1953-2929	
LD 90	3804	2817-5623	Y = – 9.48+6.22 X

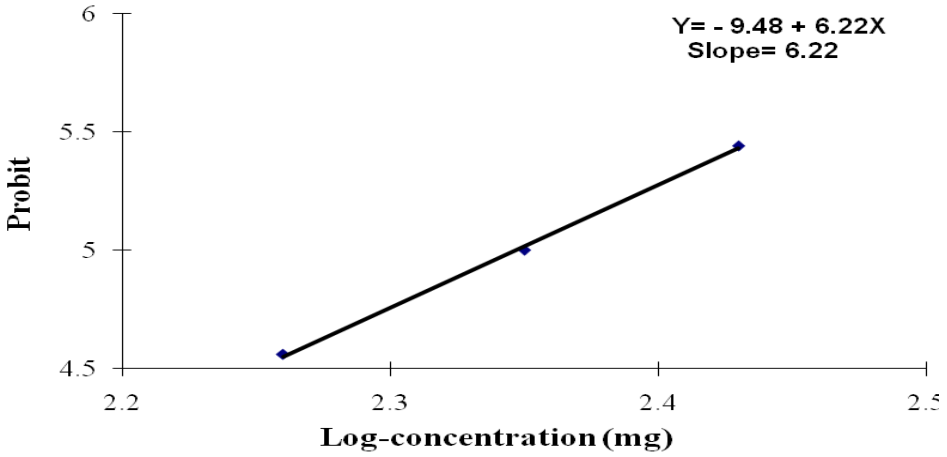

Figure 1: Dosage / mortality response of albino laboratory rats Rattus norvegicus to Hanzal fruits aqueous extract

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The previous results demonstrated a high mortality of rats, this goes with the findings reported by Kingsbury (1980) that at toxic levels, cardioactive glycosides produce cardiac irregularities and heart block. Furthermore, in many lethal poisonings, heart failure can be brought about by malfunction of innervation or of the heart's conductive tissues, or it may be a result of a more direct effect on the heart musculature.

Elawad, *et al.*, (1984) also reported that as time after ingestion goes on, the saponin isolated from the rind has enough time to exert its 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, since hypoglycaemia was confirmed in these animals by blood test in present study.

Symptomatic Signs of Toxicity

Treated albino rats with the Hf. Aq. Extr. extracted showed increased aggression, porphryrin staining around eyes, reduced exploratory behavior, sunken abdomen, eye lids partially closed; rough hair coats and recumbent position with head tucked into abdomen were observed.

Symptoms occurred directly before death were, rapid breathing rate, hunched posture, anorexia diarrhoea, impaired ambulation, muscle atrophy, lethargy and inability to remain upright. These signs are similar to those reported by Rand (2001).

However taking into consideration the symptoms of toxicity appeared in table (5) with the results of the bioassay to explain or elucidate the reason for or behind death.

Table 5: Symptomatic signs of toxicity on rats treated with different dosages of saponins

Symptoms	No. of treated rats	Dosage (mg/rat)					
		Control	90	180	225	270	315
Difficulty breathing	6	0	6	6	6	6	6
Anorexia	6	0	5	5	5	6	6
Twitches	6	0	0	0	3	3	3
Impairedambulation	6	0	1	5	5	5	5
Reduced activity	6	0	6	6	6	6	6
Diarrhoea	6	0	0	0	3	4	5
Bleeding	6	0	0	1	2	4	5

All treated animals showed toxicity symptoms. However, some of them (the lower concentrations) were able to recover or tolerate the dosage applied. This could be attributed to either quick elimination or detoxication of the administered dose.

The afore-mentioned symptoms were also typical to those mentioned by Diwan *et al.* (2000) .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 has been used as purgative acting directly on the GIT.

Batanouny (1999) revealed that the Et.OH-extract of the colocynth fruit produced stimulation, accompanied by increased motor activity, tremors, convulsions, diarrhoea and rapid irregular respiration preceeding death in

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mice. All these symptoms are cholinergic symptoms. The same extract demonstrated cytotoxic, as well as mutagenic effects.

Conclusions

It is well–known that rodenticides in general are very expensive particularly in devolving countries. It is therefore possible to use crude extracts directly against pests as the quantity of active constituents is sufficient and can be prepared easily by the ordinary farmers. This study has identified *Citrullus colocynthis* (L.) Schrad., a promising plant with acute and broad rodenticidal activity resulting in significantly high levels of mortality. The plant is available in almost all parts and regions of the Sudan, cheap and easy to collect, store, prepare and use as a rodenticide by local farming communities.

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سمية المستخلص المائي لثمار نبات الحنظل على جرذ المعامل النرويجي

إيهاب السر محمد الياس[1] و محمد حمزة زين العابدين[2] و نبيل حامد حسن بشير[3] و يوسف عثمان حسين أسد[1]

[1]قسم المبيدات والسميات، كلية العلوم الزراعية ، جامعة الجزيرة، واد مدني، السودان
[2]قسم وقاية المحاصيل كلية العلوم الزراعية ، جامعة الجزيرة، واد مدني، السودان
[3]معهد النيل الأزرق للأمراض السارية– جامعة الجزيرة، واد مدني، السودان

المستخلص:

هدفت هذه الدراسة إلي اختبار السمية الحادة عن طريق الفم للمستخلص المائي لثمار نبات الحنظل *Citrullus colocynthis* (L.) Schard علي فئران المعامل النرويجية البيضاء *Rattus norvegicus* تم اختيار خمسة جرعات بناءً علي اختبارات تمهيدية مسبقة. حيث كانت الجرعات 90 ، 180، 225، 270 و 315 ميلجرام . تم تطبيق هذه الجرعات بواسطة أنبوب عن طريق الفم لأي مجموعة من مجموعات الفئران . تمت مراقبة الفئران المعاملة لمدة (24) ساعة لتحديد علامات السمية الحادة للمستخلص . حدوث الموت كان بين 1 – 18 ساعة من المعاملة وكانت نسب الموت المصححة صفر ، 33.3 ، 50 ، 66.7 و 100 للجرعات المذكورة أعلاه بالترتيب . تم تحديد الجرعة القاتلة النصفية عن طريق تحليل الـــ (Probit) وكانت حوالي 2402 ملجم / كجم من وزن الجسم. علامات السمية الحادة كانت فقدان كامل للشهية ، الإسهال ، صعوبة التنفس ، عدم المقدرة علي الوقوف ، الانهيار التام والموت .

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