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EVALUATION OF TOXICITY OF DATURA INNOXIA SEEDS AND LEAVES

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ABSTRACT
The study aimed to evaluate the toxicity of Datura innoxia seeds and leaves. The seeds and leaves were collected from an east police station in El-Obeid city, North Kordofan State. The plant leaves and seeds were cleaned, shade-dried and grounded and then extracted by maceration method using distilled water and by soxhelt using methanol. Sixty five male rats were used in the experiment divided into thirteen groups. Six groups for aqueous extracts, six groups for methanol extracts and one group served as a control. The rats groups treated orally for thirty days with 40, 60 and 80 mg extract/kg of rat body weight. Five animals for each dose levels. The control group consisted of five rats to which distilled water mixed with ethanol with the same percentage as that used for dissolving the extracts was administered. Phytochemical screening of Datura innoxia seeds and leaves extracts revealed the presence of saponin, cumarin, alkaloids, flavonoids, tannins, steroids and triterpene with different concentrations. There is Toxicity symptoms (depression, restlessness, ataxia and paralysis) were observed in the extracts treated groups compared to the control group. 100% mortality of the rats occurred with the highest dosage 80 mg/kg. At 60mg/kg dosage 60% mortality was occurred and no mortality was observed for 40mg/kg. The results indicated that both aqueous and methanol extracts have nearly the same toxicity, this due to the presence of the alkaloid compounds in Datura innoxia seeds and leaves.

KEYWORDS: Datura innoxia, seeds and leaves, toxicity on rats, alkaloid.

INTRODUCTION
The uses of traditional medicines and medicinal plants in most developing countries for the treatment of various diseases have been widely observed.[1] According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs.[2] The genus name *Datura* is from the Bengali name 'dhatura'. *Datura innoxia* (Family: Solanaceae, locally known as Elsakran) is used for many medicinal purposes.[3] Datura natural distribution is uncertain, owing to its extensive cultivation and naturalization throughout the temperate and tropical regions of the globe. In Sudan *Datura innoxia* is widely spread in AL Jazeera State, Khartoum, Kordofan and other States. It is native to Central and South America, and introduced in Africa, Asia, Australia and Europe.[3] It contains atropine alkaloids, flavonoids, cardiacs glycosides, essential oils, saponins and phenols.[4]

Figuer (1): View of Datura innoxia flowers and leaves.
Source: from near east police station Elobied North Kordofan state - 27/10/2016.

Figuer (2): View of Datura innoxia seeds after cutting fruits.
Source: from near east police station Elobied North Kordofan 27/10/2016.

MATERIALS AND METHODS

Plant material (seeds and Leaves)

D. innoxia (Figure 1 and Figure 2) were collected from near eastern police station Elobied, North Kordofan state, Sudan in October, 2016. The plant was authenticated by a plant taxonomist at the Department of Botany Faculty of Science University of Kordofan to be Datura innoxia. The plant leaves and seeds were cleaned, shade-dried and grinded by a mechanical grinder.

Animals (rats): Sixty five male rats, three months old and with an average body weight ranged (110-120g), were used in the present study. The rats were clinically healthy and housed within the premises of the Faculty of Science and Technology, Sudan University, Khartoum.

Animal housed under standard husbandry conditions (30°C ± 2°C, 60–70% relative humidity and 12hour day-night cycle) and fed on the rat diet (flour 55.6%, meat 35%, edible oil 7.5%, sodium chloride 1.2% and vitamins and minerals 0.7) and water provider. Animal experiments were designed and conducted in accordance with the guidelines of institutional animal ethical committee.

Methods

Preparation of Extracts

Preparation of methanol extract of seeds and leaves

Extraction was carried out according to method described by.[5] 600 g of each sample was coarsely powdered using mortar and pestle. Coarsely sample was successively extracted with 1200 ml petroleum ether and 1200 ml methanol using soxhelt extractor apparatus. Extraction carried out for about four hours for petroleum ether and eight hours for methanol till the colour of solvents at the last siphoning time returned colorless. Solvents were evaporated under reduced pressure using rotary evaporator apparatus. Finally the extracts allowed to air till complete dryness.

Preparation of aqueous extract of seeds and leaves of D. innoxia

For aqueous extract, 600 g of coarsely powdered plant (seeds and leaves) were extracted with 3000ml of distilled water, heat to (60-70°C) for three hours, filtered through whatman filter paper (No.1) and dried further by freeze drier.

Preparation of stock solution of aqueous and methanol extracts of Datura innoxia seeds and leaves

The preparation of stock solution was done by dissolving 5 g of each extract in 12.5 ml of 99.9% ethyl alcohol and completed to 250 ml with distilled water and then 2ml, 3ml and 4ml (40 mg, 60 mg and 80 mg) were taken from the stock solution and used as doses for rats orally.

Experimental Design

A total of sixty five male rats divided in to thirteen groups, each group containing five rats. Group (1) served as control, received pure water mixed with 99.9% ethyl alcohol with the same percentage as that used for preparation of the stock solution. Groups 2, 3 and 4 received doses of 40, 60 and 80 mg of methanol seeds extract/kg rat body weight/day respectively and Groups 5, 6 and 7received doses of 40, 60 and 80 mg methanol leaves extract /kg rat body weight /day respectively. Groups 8, 9 and 10 received doses of 40, 60 and 80 mg of aqueous seeds extract /kg rat body weigh/day respectively and Groups 11, 12 and 13 received doses of 40, 60 and 80 mg of aqueous leaves extract /kg rat body weight/ day respectively. Body weight changes and daily feed intake are monitored in the rats until termination of the experiment for thirty days. The extract administered to the rats interagastrically using cathidel tube.

Phytochemical screening of Datura innoxia seeds and leaves

Phytochemical screening for the active constituents was carried out using the methods described by Martinez and Valencia (1999[6], Sofowora (1993)[7], Harborne (1984)[8], Harborne (1998)[9] and Wall et.al., (1952).[10]

RESULTS AND DISCUSSION

The result of phytochemical screening of leaves and seeds extracts was shown in table 1. The results showed high concentration of alkaloids, flavonoids and triterpens in methanol seed extract. Aqueous seed extract showed

high concentration of alkaloids. Methanol leaves extract showed high concentration of tannins and steroids.

Table (1): Phytochemical screening of leaves and seeds extracts.

Sample	Saponin	Cumarin	Alkaloids	Flavonoids	Tannins	Steroids	Triterpens	Anthraquinone	Cyanogenic
Aqueous Leaves extract	++	+	++	++	++	-	-	-	-
Aqueous seeds extract	+	+	+++	+	+	-	-	-	-
Methanol leaves extract	++	+	++	++	+++	+++	++	-	-
Methanol seeds extract	+	+	+++	+++	++	+	+++	-	-

(+++): High concentration
(++): medium concentration
(+): low concentration
(-): Not detected

Toxic symptoms of Datura innoxia methanol and aqueous seeds and leaves extracts

The toxic symptoms and number of rats dead at different dosages after oral administration of seeds and leaves extracts were given in tables 2, 3, 4, 5, 6 and 7. All rats treated with 40mg extract/kg rat body weight were alive during the thirty days of treatment. The animals treated with 40mg/kg showed toxic symptoms but disappeared this suggested that the LD_{50} of the extracts were higher than 40mg/kg. The clinical symptoms appeared on the administered rats due to alkaloids in Datura innoxia leaves and seeds extracts especially which have anticholinergic effects such as (atropine, scopolamine and Hyoscyamine). These agree with the results of Mohamed, R. A.[11], in acute toxicity of aqueous and petroleum ether extacts of Datura innoxia leaves in mice, Shama, I. Y. *et.al.*,[12] in Biochemical and Histopathological study of aqueous and methanol extracts of Datura innoxia leaves and seeds on wistar rats and Navaratanrojah, K. *et. al*.,[13], in evaluation of analgesic effect of Datura stramonium leaves in hot plate and formalin test on male rats.

Table (2): Toxic clinical symptoms in rats treated orally with (40mg\kg) doses of Datura innoxia seeds and leaves methanol extracts.

Group	Dose mg\kg	Toxic symptoms	Appearance time	Disappearance time	Dead rats	Death time
G2 leaves extract.	40	Depression. Shallow. Restlessness. Ataxia. Paralysis	Immediately. 1. hour. 1.5 Hours. 3.5 hours. -	2 hours. 2 hours. 1 hours. 4 hours. -	-	-
G3 seeds extract.	40	Depression. Shallow. Restlessness. Ataxia. Paralysis	Immediately. 1.hour. 1.5 Hours. 2 hours. -	2 hours. 2 hours. 1 hours. 4 hours. -	-	-

Table (3): Toxic clinical symptoms in rats treated orally with (40mg\kg) doses of Datura innoxia seeds and leaves aqueous extract.

Group	Dose mg\kg	Toxic symptoms	Appearance time	Disappearance time	Dead rats	Death time
G4 leaves extract.	40	Depression. Shallow. Restlessness. Ataxia. Paralysis	Immediately. 1.hour. 1.5 Hours. 3.5 hours. -	2 hours. 2 hours. 1 hours. 4 hours. -	-	-
G5 seeds exract.	40	Depression. Shallow. Restlessness. Ataxia. Paralysis	Immediately. 1.hour. 1.5 Hours. 2 hours. -	2 hours. 2 hours. 1 hours. 4 hours. -	-	-

Table (4): Toxic clinical symptoms in rats treated orally with (60mg\kg) doses of Datura innoxia seeds and leaves methanol extracts.

Group	Dose mg\kg	Toxic symptoms	Appearance time	Disappearance time	Dead rats	Death time
G6 Leaves extract.	60	Depression. Shallow respiration. Convulsion. Paralysis(3)*.	Immediately. 15 minute. 30 minute. Before death	6 hours. 3 hours. - 6 hours. 9 hours	3	14-30 days.
G7 seeds extract.	60	Depression. Shallow respiration. Convulsion. Paralysis (3)*.	Immediately. 15 minute. 30 minute. Before death	6 hours. 3 hours. - 6 hours. 9 hours	3	14-30 days.

Table (5): Toxic clinical symptoms in rats treated orally with (60mg\kg) doses of Datura innoxia seeds and leaves aqueous extracts.

Group	Dose mg\kg	Toxic symptoms	Appearance time	Disappearance time	Dead rats	Death time
G8 Leaves extract	60	Depression. Shallow respiration. Convulsion. Paralysis(3)*.	Immediately. 15 minute. 30 minute. Before death	6 hours. 3 hours. - 6 hours. 9 hours	3	14-30 days.
G9 seeds extract	60	Depression. Shallow respiration. Convulsion. Paralysis (3)*.	Immediately. 15 minute. 30 minute. Before death	6 hours. 3 hours. - 6 hours. 9 hours	3	14-30 days.

Table (6): Toxic clinical symptoms in rats treated orally with (80mg\kg) doses of Datura innoxia leaves and seeds methanol extracts.

Group	Dose mg\kg	Toxic symptoms	Appearance time	Disappearance time	Dead rats	Death time
G10 Leaves extract	80	Depression Convulsion Paralysis(5)*	Immediately1 hour. Before death.	-	5	3-28 days.
G11 seeds extract.	80	Depression. Convulsion. Paralysis(5)*	Immediately1 hour. Before death.	-	5	3-29 days

Table (7): Toxic clinical symptoms in rats treated orally with (80mg\kg) doses of Datura innoxia leaves and seeds aqueous extracts.

Group	Dose mg\kg	Toxic symptoms	Appearance time	Disappearance time	Dead rats	Death time
G12 Leaves extract	80	Depression. Convulsion. Paralysis(5)*	Immediately one hour Before death.	- - -	5	3-28 days.
G13 seeds extract	80	Depression Convulsion Paralysis(5) *	Immediately one hour Before death.	-	5	4-29 days.

* Means the number of died rats which had paralysis symptoms before death.

CONCLUSION

There is toxicity symptoms observed in all the extract treated groups compared to the control group. The use of Dature innoxia leaves and seeds are common in Sudan for treatment of bacteria diseases and also used by some as drugs. The results indicate that the oral administration of seeds and leaves extracts had poisonous effect in the experimental animals. This explains that the alkaloids present in seeds and leaves are highly toxic.

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