Acute toxicity of aqueous and petroleum ether extracts of *Datura innoxia* leaves in mice

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Abstract

The present study aimed to evaluate and compare the acute toxicity of two extracts (aqueous and petroleum ether) of *Datura innoxia* leaves. Determination of LD_{50} was carried out by using forty eight mice for petroleum ether extract that divided into six equal groups (eight mice in each group), and 40 mice for aqueous extract that divided into five equal groups (eight mice in each group). Doses of LD_{50} of petroleum ether extract were (400, 500, 600, 700, 800) mg/kg B.w., and those for aqueous extract were (2000, 2250, 2500, 2750, 3000) mg/kg B.w. The calculated median lethal dose (LD_{50}) was (500) mg/kg B.w. for petroleum ether extract, and (2400) mg/kg B.w. for aqueous extract of *D. innoxia*. By comparing the values of LD_{50} of both extracts, it showed that the toxicity of petroleum ether extract was nearly five times more potent than the aqueous extract. Both extracts had the same efficacy. This difference in LD_{50} of two extracts may be due to the presence of materials like flavenoids, glycosides and essential oil that are soluble in petroleum ether but insoluble in water.

السمية الحادة للمستخلصين المائي والإيثري البترولي لأوراق نبات الداتورا إنوكسيا في الفئران

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الخلاصة

استهدفت الدراسة تقييم ومقارنة السمية الحادة لكل من المستخلصين الإيثري البترولي والمائي لأوراق نبات الداتورا إنوكسيا. أستخدم ثمانية وأربعين 48 فأر ذكر لدراسة الجرعة السمية المميتة الوسطية للمستخلص الإيثري حيث قسمت الفئران إلى ست مجاميع متساوية (8 فأر لكل مجموعة) واربعين فأر ذكر لدراسة الجرعة السمية الوسطية المميتة للمستخلص المائي وقسمت الى خمس مجموعات متساوية (8 فأر لك مجموعة). جرعت فمويا مجموعات المستخلص الإيثري (700،600،500،400،800) ملغم/ كغم من وزن الجسم، في حين جرعت مجموعات المستخلص المائي (3000،2500،2250،2000) ملغم/ كغم من وزن الجسم. أظهرت النتائج أن الجرعة المميتة الوسطية كانت 500 ملغم/ كغم من وزن الجسم للمستخلص الإيثري بينما كانت 2400 ملغم/ كغم من وزن الجسم مستخلص المائي، أظهرت المقارنة بين الجرعتين المميتتين الوسطية لكلا المستخلصين (الإيثري والمائي) أن الجرعة المميتة الوسطية للمستخلص الإيثري كانت اشد بخمس مرات من قوة الجرعة المميتة الوسطية للمستخلص المائي، بينما كانت الفعالية متساوية لكلتا الجرعتين، من الممكن أن يعزى الاختلاف بين قوة الجرعتين المميتتين الوسطية للمستخلصين الى أن بعض المواد الفعالة مثل الفلافينويدات والكلايكوسيدات والزيوت الأساسية لها قابلية الذوبان في الإيثر فقط ولا تذوب بالماء.

Introduction

One of the most important medicinal plants is *Datura innoxia Mill* (Thorn apple). It is an annual herb belongs to the family solanaceae (1). *Daturainnoxia* (solanaceae) is a plant distributed throughout most parts of the world (2) and is a rich source for numerous medicinal substances (3). *Datura* plant is a very important medicinal plant as it is a well-known source of the tropane alkaloids; hyoscyamine, atropine and scopolamine (hyoscine). The total alkaloid yield has been estimated to be between 0.06 and 0.50%. The young leaves contain mainly scopolamine (hyoscine), whereas hyoscyamine is the major constituent of the mature leaves. Hyoscine has anticholinergic, antiasthmatic and antispasmodic effects. Hyoscyamine has similar chemical structure to hyoscine, but it is smaller only by a single molecule of oxygen. Hyoscyamine has the same pharmacological effects as hyoscine. Atropine is the racemic form of (-) hyoscyamine which effects the nervous system. Atropine is used in eye drop preparation for dilation iris. It is also used to treat nerve gas poisoning, Parkinson's disease, peptic ulcers, diarrhea and bronchial asthma. In addition to these alkaloids, the plant contains other minor tropane derivatives, as well as chlorogenic acid and lectins. Furthermore the seeds of *Datura innoxia* contain up to 30% fixed oil and about 0.2% alkaloids (4). *Datura innoxia* plant is abundant in Iraq, but information about its tropane alkaloids content according to species and geographical area cultivation still not satisfying taking in consideration its possible therapeutic use and toxic effect hazard. So this work conducted to determine the acute toxicity of leaves extracts of local plant *Datura innoxia* in mice.

Materials and Methods

Animals: Eighty eight male albino Swiss mice were obtained from the Iraqi National Centre for Drugs Safety and Evaluation, with age ranged 8-10 weeks and weight (25-30) g. The animals were kept for two weeks for acclimatization before experiment start in 25°C air-conditioned room with 14 Light: 10 Dark cycle. Feed and water were given *ad libitum*.

Plant: Fresh *Datura innoxia* leaves were collected from a local garden in South of Hilla. They classified and identified by Ministry of Agriculture/ State Board for Seeds Testing and Certification S.B.S.T.C. in Abu Graib/ Baghdad number 142 in 24/12/2008. The leaves dried at room temperature then grounded to fine powder.

Preparation of aqueous and petroleum ether extract of *Datura innoxia:* The aqueous extract of *Datura innoxia* leaves is prepared according to (5) by mixing 60g of dried powder of *Datura innoxia* leaves with distilled water 200 ml by using hot plate magnetic stirrer at 40° C temperature for 24 hours then filtered to get rid of residue and placed in incubator at 40C° to obtain dried extract and kept in -20°C till use. The Organic solvent extraction of the *Datura innoxia* leaves was carried out by using petroleum ether according to the method described by (5) by using Soxhlet apparatus. Thirty grams of leaves powder of *Datura innoxia* plant were put in the thimble and 150 ml of petroleum ether were added into the extraction flask.

Preparation of Stock Solution of Petroleum ether and Aqueous *Datura innoxia* Extracts: Preparation of the stock solution of petroleum ether extract was done by dissolving 2g of the extract in ethyl alcohol 99.8% by completing the volume to 5 ml. One ml of this solution was taken for dilution by completing the volume to 20 ml of distilled water to btain 20 mg/ml used for oral dosing of animals in both pilot and acute toxicity studies. The dosing volume of *DI* petroleum ether extract was 0.2, 0.25, 0.3, 0.35, 0.4 ml/10g body weight which are equivalent to oral doses of (400, 500, 600, 700, 800) mg/kg

B.w. The stock solution of the aqueous extract was prepared by dissolving the amount of crude extract of (2000, 2250, 2500, 2750, 3000) mg in distilled water by completing the volume to 20 ml for preparation the following corresponding concentrations of (100, 112.5, 125, 137.5, 150) mg/ml used for oral dosing at dose volume of 0.2 ml/10 g. animal body weight.

Median Lethal Dose (LD_{50}): LD_{50} values and their toxic potency and efficacy of aqueous and petroleum ether plant leaves extract were estimated by using probit method (6). The base dose and roughly the ranges of toxic doses were estimated by primary study (Pilot). Forty mice were used for the assessment of $LD_{50}$of aqueous extract. The animals were divided equally to five groups and given doses of (2000, 2250, 2500, 2750, 3000) mg/kg. BW of the aqueous extract. Forty eight mice were used for the assessment of LD_{50} values of petroleum ether extract. They were divided equally into six groups, the first five of them dosed with (400, 500, 600, 700, 800) mg/Kg. BW of petroleum ether extract orally respectively, The sixth group (8 mice) was dosed with 5% ethanol to exclude any toxicity due to the solvent. The lethality percentage of animals was recorded during 24 hrs following treatment then converted to probit number. A plot of logarithm doses was constructed against obtained probit numbers. The values of LD_{50} and toxic potency of the extract were estimated from the curve.

Clinical Toxicity Signs: During the 24 hours after treatment the animals were observed for development of toxicity signs Time of appearance and disappearance of toxic symptoms, severity and lethality due to each dose used were recorded.

Results and Discussion

The yield of Datura innoxia leaves extract was 15% and 4.86% in aqueous and organic solvent (petroleum ether) respectively. This may be due to the difference in the extraction methods. The results of pilot study for over viewing acute toxicity of aqueous extract in mice caused by different doses are listed in Table (1).

Table (1) The outcome of pilot study of different doses of *DI* leaves aqueous extract on mice

Group No.	Dose mg/kg B.W.	Total mice	Dead mice	Survived mice	Mortality%
G1	2000	2	0	2	0
G2	2250	2	0	2	0
G3	2500	2	1	1	50
G4	2750	2	2	0	100

According to outcome of mortality percent of pilot study, the dose 2500 mg/kg was chosen as a base dose for acute toxicity study for aqueous extract.

The (LD_{50}) of aqueous extract: The LD_{50} of this extract in mice was calculated according to probit method as listed in Table (2) and Fig. (1).

Table (2) Acute toxicity of different doses of aqueous extract of *Datura innoxia* leaves (orally) in mice

Group No.	Dose mg/kg B.W.	Log dose No.	Total no. of animal	Dead animal	Mortality percent	Probit no.
G1	2000	3.30	8	1	12.5	3.82
G2	2250	3.35	8	2	25	4.33
G3	2500	3.39	8	4	50	5.00
G4	2750	3.43	8	6	75	5.76
G5	3000	3.47	8	8	100	7.33

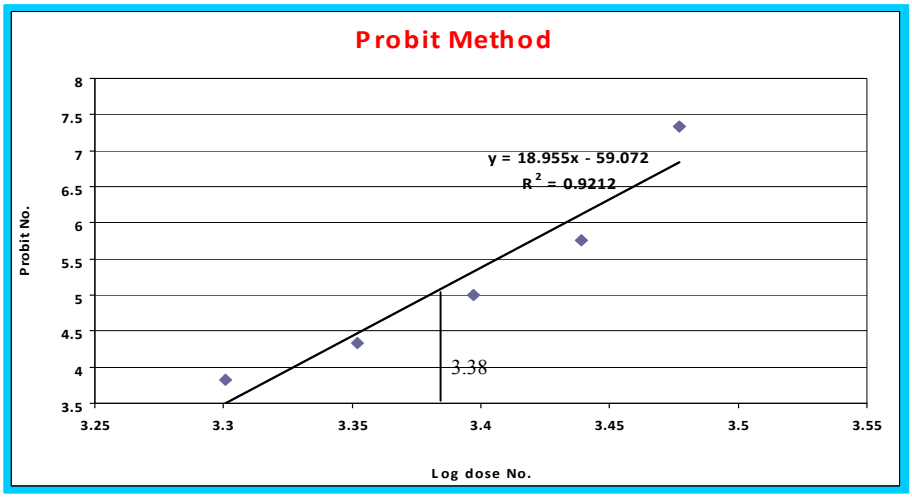

Fig. (1) The LD_{50} of aqueous extract of *Datura innoxia* leaves according to probit method (6)

The log dose which is compatible with probit 5 is equal to 3.38, so the LD_{50} of aqueous extract was 2400 mg/kg B.W. It seems that the value aqueous extract LD_{50} ranked (3) due to the toxicity categorization table and as considered moderately toxic compound (7). Results of the pilot study to over view the acute toxic effect of petroleum ether extract of *Datura innoxia* observed in mice after administration of different chosen doses are listed in Table (3).

Table (3) Mortality of mice due to different doses of petroleum ether extract of *Datura innoxia* leaves (pilot study)

Group No.	Dose mg/kg B.W.	Total mice	Dead mice	Survived mice	Mortality %
G1	400	2	0	2	0
G2	500	2	1	1	50
G3	600	2	2	0	100

According to mortality results of pilot study, the dose 500mg/kg caused 50% mortality. This dose was chosen to be the base of doses for acute toxicity study of petroleum ether extract. The LD_{50} of petroleum ether extract of Datura innoxia leaves in mice was calculated using probit method as listed in Table (4) and Fig. (2).

Table (4) Acute toxic effect of different doses of petroleum ether extract of *Datura innoxia* leaves given orally to mice

Group No.	Dose mg/kg B.W.	Log dose No.	Total No. of animal	Dead animal	Mortality percent	Probit No.
G1	400	2.60	8	2	25	4.33
G2	500	2.69	8	4	50	5.00
G3	600	2.77	8	6	75	5.76
G4	700	2.84	8	7	87.5	6.13
G5	800	2.90	8	8	100	7.33
G6 (control)	0.2ml 5% ethanol/10gm BW	-	8	0	0	-

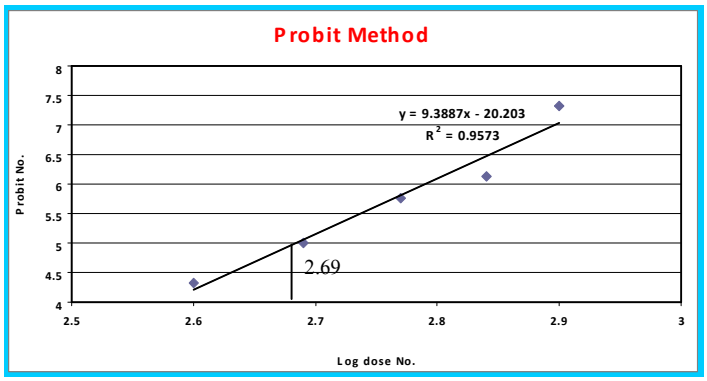

Fig (2) Estimation LD_{50} of *Datura innoxia* leaves petroleum ether extract by probit method (6)

From the Fig. (2) the probit 5 value is equal to log dose 2.69 so the calculated LD_{50} is equal to 500 mg/kg.B.w. This LD_{50} value could be classified among toxicity rank (4) that considered as very toxic compound according to the toxicity rank table of drug and xenobiotics (7).

Clinical symptoms of acute toxicity of *Datura innoxia* extracts: The clinical symptoms appeared on the animals may be due to the toxicity of active ingredients present in the plant which have anticholinergic effects (atropine and scopolamine). The results revealed positive proportionality between severity of symptoms and mortality with given dose. This is depicted by short time of appearance and longer time of recovery of symptoms for survivals and increased mortality percent with increasing doses in different groups. The main clinical symptoms could be summarized by shallow respiration, depression, ataxia and paralysis. The symptoms were recorded during 24 hours after administration of both extracts. There were differences in severity of symptoms according to the type of extract in which petroleum ether extract was more potent causing severe symptoms with the development of convulsion at higher doses. The animals of group 6 that dosed with the vehicle 5% ethanol showed no toxicity symptoms or lethality during observation period 24 hrs.. This excludes the possibility of any toxicity due to vehicle. Details of symptoms are summarized in Table (5) and (6).

Table (5) Acute clinical symptoms in mice treated orally with different doses of *Datura innoxia* leaves aqueous extract

Group	Dose mg/kg	Toxic symptoms	Appearance time	Disappearance time	Dead animal	Death time
G1	2000	Depression Shallow respiration Ataxia Paralysis(1)*	30 minute 1 hrs. 3 hrs. (5-7) hrs.	3 hrs. 4 hrs. 6 hrs. 24 hrs.	1	(5-7) hrs.
G2	2250	Depression Shallow respiration Ataxia Paralysis (2)*	5 minute 50 minute 2.5 hrs. (1-15) hrs.	5 hrs. 7 hrs. 5 hrs. 24 hrs.	2	(1-15) hrs.
G3	2500	Depression Shallow respiration ataxia Paralysis (4)*	Immediately 1 hrs. 2 hrs. (1-8) hrs.	6 hrs. 7.5 hrs. 12 hrs. 24 hrs.	4	(1-8) hrs.
G4	2750	Depression Shallow respiration Ataxia Paralysis(7)*	Immediately 1 hrs. 3 hrs. (1-7) hrs.	7 hrs. 6 hrs. 8 hrs. 24 hrs.	7	(1-7) hrs.
G5	3000	Depression Shallow respiration Ataxia Paralysis(8)*	Immediately 30 minute 6 hrs. (3-17) hrs.	-	8	(3-17) hrs.

***Number of animals showed such symptoms.**

Table (6) Acute clinical symptoms in mice after oral treatment with different doses of *Datura innoxia* leaves petroleum ether extract

Group	Dose mg/kg	Toxic symptoms	Appearance time	Disappearance time	Dead animal	Death time
G1	400	Depression Shallow respiration Restlessness Ataxia Paralysis(2)*	Immediately 45 minute 1.5 hrs. 3 hrs. (5-7) hrs.	5 hrs. 7 hrs. 8 hrs. 12 hrs. 24 hrs.	2	(5-7) hrs.
G2	500	Depression Shallow respiration Ataxia Restlessness Paralysis (4)*	Immediately 30 minute 1 hrs. 4 hrs. (5-7) hrs.	7 hrs. 10 hrs. 15 hrs. 14 hrs. 24 hrs.	4	(5-7) hrs.
G3	600	Depression Shallow respiration Convulsion Paralysis (6)*	Immediately 15 minute 40 minute Before death	12 hrs. 8 hrs. 12 hrs. 24 hrs.	6	(2-4) hrs.
G4	700	Depression Convulsion Paralysis (7)*	Immediately 1.5 hrs. Before death	12 hrs. 13 hrs. 24 hrs.	7	(1-3) hrs.
G5	800	Depression Convulsions Paralysis(8)*	Immediately 1 hrs. Before death	-	8	(1-1.5) hrs.
G 6	5% ethanol	No toxicity symptoms	Zero	Zero	Zero	Zero

***Number of animals showed such symptoms.**

By comparing the acute toxicity slopes for both extracts (petroleum ether and aqueous) in accordance with their probit method results as in Fig. (3).

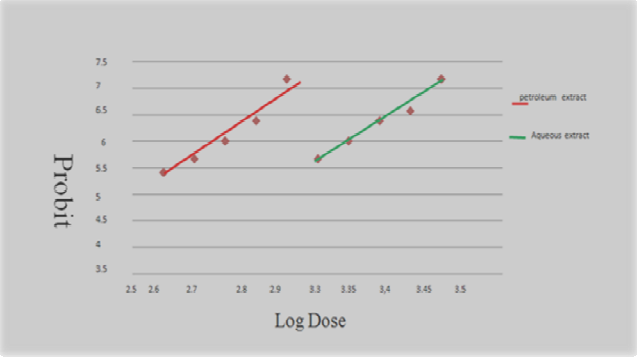

Fig. (3) Comparison of acute toxicity effects of Datura innoxia extracts (petroleum ether and aqueous) according to probit method

The results showed that both extracts have different LD_{50} value. The LD_{50} of petroleum ether extract was nearly five times lower (more toxic) than that of the aqueous extract. The slopes of mortality of both extracts however, were nearly the same and parallel that means they have the same efficacy this may denote that the same active ingredients (tropane alkaloids) were mainly responsible for the development of toxicity and mortality in mice for both extracts of *Datura innoxia* leaves. These results were confirmed by appearance nearly same toxicity symptoms developed by both extracts in mice. Since petroleum ether extract was nearly five times more potent than aqueous extract, this may be because of the

presence of other active and toxic materials like flavinoids, glycosides and essential oils dissolved in petroleum ether but not in distilled water and may be little more tropane alkaloids. These agree with the results of (8) who reported that petroleum ether extract was more toxic than other plant extracts. Wannang *et al.* (9) and Navaratnarojah *et al.* (10) who reported the presence of cardiac glycoside, tannins and flavenoids in petroleum ether extract of the plant. The LD_{50} of aqueous extract nearly resembles that reported by other investigators such as (11) who reported that LD_{50} of aqueous extract of Datura innoxia leaves was 3200 mg/kg. B.W. given orally in mice. It also agrees with results of (12) and with (13). This was considered as an important result because toxicity rank for aqueous extract was (3) (moderately toxic), so it could be used for therapeutic purposes since it may contain enough tropane alkaloids that give very good antispasmodic effect with high safety margin .This was confirmed by the in vitro study of the effect of different concentrations of aqueous extract of Datura innoxia on isolated rabbit duodenum motility which showed very good relaxant effect and complete blockade of muscarinic receptors caused by all used extract concentrations in a way comparable to atropine drug standard especially by higher aqueous extract concentrations.

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