Acute toxicity study of three type of *Nerium.oleander* leaves of hexane extract in mice

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Summary

Three types of *Nerium.oleander* leaves of hexane extract were prepared according the color of their flower, red flower group (RFG), pink flower group (PFG) and white flower group (WFG).After drying in the sunlight, grinding by electrical grinder. Extraction with hexane was done by a soxhlet apparatus for each type the extract of each type was dissolved in propylene glycol which was used to dissolve the extract with the aid of magnetic stirrer mixer for ten minutes. The median lethal dose (LD**50)** experiment for each type, fifty adult mice of mixed sex were used. They were divided into 5 equal number groups and were given different oral doses as following:-The red flower groups (RFG) received oral doses ranging from 225-425mg⁄Kg ,the pink flower group(PFG) received oral doses ranging from 200-400 mg ⁄Kg while the white flower groups (WFG) received doses ranging from 250- 450 mg⁄ Kg of body weight. The LD_{50} was calculated by employing probit method and found to be 325mg/kg for the RFG, 300mg/kg for the PFG and 350mg/kg for the WFG respectively. These results indicate that the toxic constituents of the leaves nearly same since there were no significance differences between LD_{50} of the three types.

Key word: Toxicity, Oleander, LD_{50}

دراسة السمية الحادة لثلاث أنواع من مستخلصات الهكسان لأوراق الدفلة في الفئران

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الخلاصة

استهدفت هذه الدر اسه مقارنة ألسميه إلحاده لثلاث مستحضرات لنبات الدفلة المحلية تبعا للون أزهارها،مجموعة الأزهار الحمراء (RFG)ومجموعة الأزهار الوردية (PFG)ومجموعة الأزهار البيضاء(WFG) وبعد تجفيفها تحت ضوء الشمس تم سحنها بالمسحنة الكهربائية وتم استخلاص موادها الفعالة بالمذيب العضوي الهكسان وبواسطة جهاز الاستخلاص لكل نوع.والمستخلص لكل نوع يذوب في مادة البروبنيل كلايكول بعد مزجه بواسطة المازج المغناطيسي .استخدم خمسون فارا بالغا من كلا الجنسين لتجربة تقدير الجرعة القاتلة الوسطية (LD**50**) حيث قسمت إلى خمس مجاميع متساوية العدد وأعطيت كل مجموعه جرعه مختلفة وعلى النحو التالي :- مجموعة الأزهار الحمراء (RFG) أعطيت جرع تتراوح من225-425 ملغم/كغم ومجموعة الأزهار الوردية (PFG) اعطيت200 جرع تتراوح من 200-400 ملغم/كغم ومجموعة الأزهار البيضاء (WFG)250- 450ملغم/كغم من وزن الجسم .بلغت قيم (LD**50**) للمجاميع الثلاث باستخدام طريقة بروبت 325 ملغم/كغم لمجموعة RFG و300ملغم/كغم لمجموعة PFG و350 ملغم/كغم لمجموعة WFG . اظهر التحليل الإحصائي للاختلافات بين المجاميع الثلاث هي غير معنوية إحصائيا مما يدل على إن مكوناته متشابهة.

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Introduction

Oleander is the botanical name of the genus ***Nerium.oleander*** which is a part of the Pocynaceae Family (1).The plant found almost all around of the world but mostly in the area of moderate weather like Mediterranean countries. They are usually found in the cities, towns and country sides along streets, roads, high ways or garden for decoration. The toxic material is believed to be due to the presence of the numbers of glycosides which have a digitalis-like action, the most important glycosides is oleandrin (2).In Iraq the plant is found mostly in the middle area especially in Baghdad province. The color red, pink and white flowers are known for the Iraqi variety. The potency of this plant as a toxic material may be attributed to the variety, the age of the plant, the season and the place or the soil (3).Aim of the study to estimate the acute toxicity of hexane extract of leaves of three types of Iraqi ***N. oleander*** in mice according to the color of their flowers.

Materials and Methods:-

Fresh leaves of three types ***N.oleander*** (Red, pink and white flowers) were collected from Baghdad, province then the leaves were cleaned up from dust, dried by sun light in open air, ground by an electrical grinder, and then kept in glass containers which were closed to be used in different parts of this experiment.

Hexane Extract: Fifty grams of the dried powdered plant leaves was put in a thimble where placed in the soxhlet apparatus and according to (4), about 200ml of hexane was place in the flask. Percolation at 70C˚ started after 15-30 minutes of operation. Percolation was allowed to be repeated 8-12 times. The extract was evaporated by leaving it in an incubator at a temperature of 45C˚ for 24 hours. The extract was weighed and kept at -20C˚ in sterile and dark glass containers.

Preparation of the stock solution of extracts: Stock solution of each extract was prepared by weighing 1gram of hexane extracts and dissolved it in propylene glycol then the volume was completed to 20ml so the final concentration is 50mg/ml as in table (1).

Median lethal dose (LD_{50}) experiment:A total of 150 mice were used, they were divided in to 3 groups (50 mice). Each group was divided in to 5 equal sub- groups (10 mice).The range of doses of each type were detected by doing pilot study, LD_{50} depend on percentage of dead animals during 24 hours, then converted to probit number. A plot of logarithm dose was constructed against obtained probit number (5).

Results and Discussion

The ratio of the extract weight of the ***N.oleander*** leaves by hexane to the original weight of the dry leaves powder was 27.3% for RFG, 28.1% for PFG and 26.5% for WFG , The extract form was a paste, green in color, insoluble in water, but dissolved in propnyl glycol after shaking for ten minutes. The clinical signs showed during 24hours after administration like ataxia, drowsiness, seizures, dullness, , rapid respiration ,convulsion of the abdominal cavity ,paralysis of the hind limbs , tremors or shaking of the muscles, but after 4hrs the animal was unable to walk ,the animal lose righting reflex (correct position) and some animals (about10%) show hemorrhage spot on the nose and leg, coma, which led to death, all these symptoms appeared in all groups treated with ***N.oleander*** .

Median lethal dose (LD_{50}) results:

The calculated value of LD_{50} of the types RFG, PFG and WFG were estimated by probit method results which were obtained from plotting the curve of logarithm of doses of different extracts verses the probit number of death according to(5) and were 325,300 and 350mg\kg respectively (tables 2, 3 and 4 and figures 1,2 and 3). Comparison of the values of LD_{50}, the slopes and the results of statistical analysis which indicate no significant differences are shown in table (5).

The present study was conducted to evaluate acute toxicity of the leaves of three preparation of Iraqi *N. oleander* in mice. Each type of the local plant was chosen according to the color of its flower (Red, Pink and white). Hexane extract was used by (6) had shown that this form is more toxic than alcoholic extract, which indicate that most of toxic ingredients are chemically non polar and highly lipid soluble. The main reason of acute toxicity is the presence of different types of cardiac glycosides especially oleandrin (7) which act in a way resemble that of the digitalis glycosides, that is cardiac blocking action through inhibition of Na^+-K^+ ATPase enzyme which is necessary for contractile calcium due to bradycardia . Acute toxicity study was carried out by measuring the median lethal dose (LD_{50}) by using probit method described by (5).This method although is less time consuming and require large number of laboratory animals but give more accurate result with least degree error(5). Median lethal dose values of the three types were calculated and were 325 mg/kg for RFG, 300 mg/kg for PFG and 350 mg/kg for WFG these values can be attributed to the differences in season, soil, method used to dry out the plant, as all these factors are considered important in determines the degree of toxicity(3). The reason for the differences in the values of lethal or median lethal dose among different species of animals may be due to species variation (8, 9, 10), mice is considered among the tolerate species as reported by (11).The main clinical symptoms which precede death or with there survive the median lethal dose were different in intensity but depend on the dose, There were no significant differences in this respect between the three preparations used in this experiment. A central nervous system symptoms like convulsion and paralysis, this can be attributed to the hypoxia that result from the bradycardia which is known to be the action of cardiac glycosides due to stimulation of vagus nerve which consider the cause of death by cardiac block, rapid respiration which is an indication of oxygen deficiency which also due to bradycardia (12). Blood spots were seen in the nose indicated hemorrhage .No such result has been reported with this plant in other species.

Table 1: Doses and dilutions of *N.oleander* of hexane extract.

Types	Hexane extract dose(mg/kg)	Solution concentration mg/ml*	Volume taken from stock solution (ml)**	Final volume (ml)
RFG	225	11.25	1.125	5
	275	13.75	1.375	5
	325	16.25	1.625	5
	375	18.75	1.875	5
	425	21.25	2.125	5
PFG	200	10.0	1.00	5
	250	12.5	1.25	5
	300	15.0	1.50	5
	350	17.5	1.75	5
	400	20.0	2.00	5
WFG	250	12.5	1.25	5
	300	15.o	1.50	5
	350	17.5	1.75	5
	400	20.0	2.00	5
	450	22.5	2.25	5

***0.5 ml/25 gram of mouse.**
**stock solution is 50mg/ml.

Table 2:- Acute toxicity effect of different doses of hexane extract of RFG of ***N.oleander*** leaves in mice.

Sub-group	Dose mg/kg	Log of dose	Total mice	Dead mice	Survived mice	Mortality %	Probit No.
1	225	2.352	10	1	9	10	3.72
2	275	2.439	10	3	7	30	4.48
3	325	2.511	10	5	5	50	5.00
4	375	2.574	10	8	2	80	5.84
5	425	2.628	10	10	0	100	8.00

Table 3:- Acute toxicity effect of different doses of hexane extract of PFG of ***N.oleander*** leaves in mice.

Sub-group	Dose mg/kg	Log of dose	Total mice	Dead mice	Survived mice	Mortality %	Probit No.
1	200	2.301	10	2	8	20	4.16
2	250	2.397	10	3	7	30	4.48
3	300	2.477	10	5	5	50	5.00
4	350	2.544	10	7	3	70	5.52
5	400	2.602	10	9	1	90	6.28

Table 4:- Acute toxicity effect of different doses of hexane extract of WFG of ***N.oleander*** leaves in mice.

Sub-group	Dose mg/kg	Log of dose	Total mice	Dead mice	Survived mice	Mortality %	Probit No.
1	250	2.397	10	2	8	20	4.16
2	300	2.477	10	3	7	30	4.48
3	350	2.544	10	5	5	50	5.00
4	400	2.602	10	6	4	60	5.25
5	450	2.653	10	10	0	100	8.00

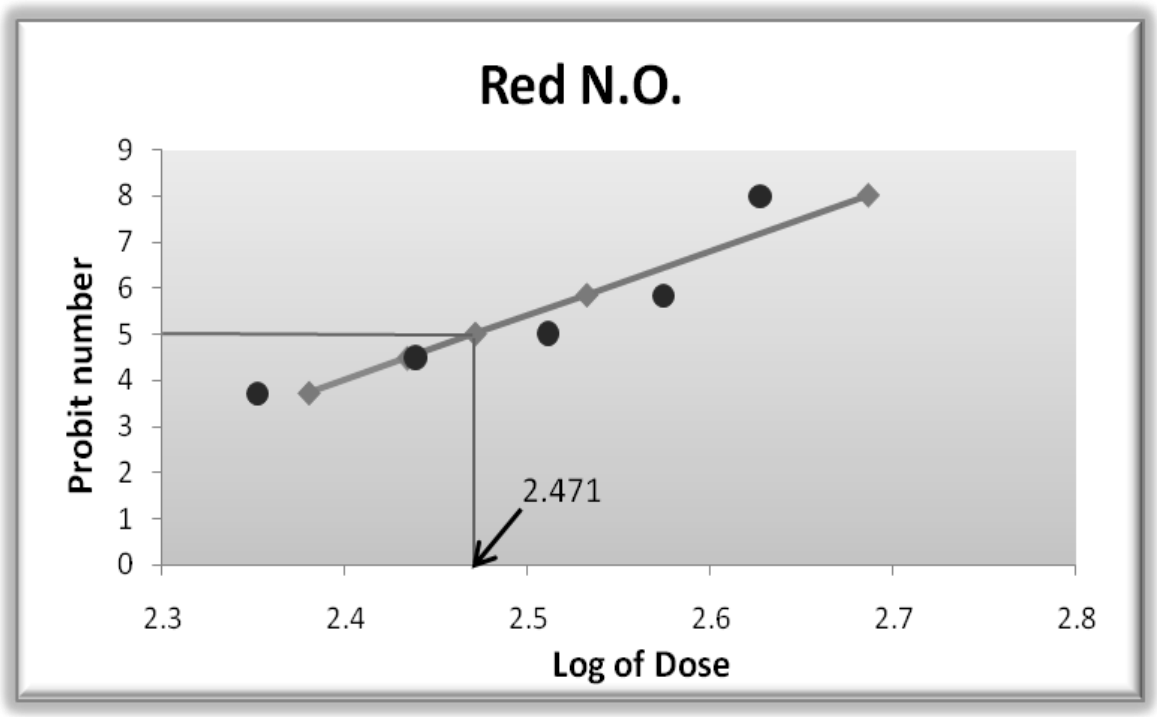

Fig 1:- Estimation log dose-probit curve for hexane extract of the RFG of *N.oleander* after fitting LD_{50} was 296.465 mg/kg of body weight.

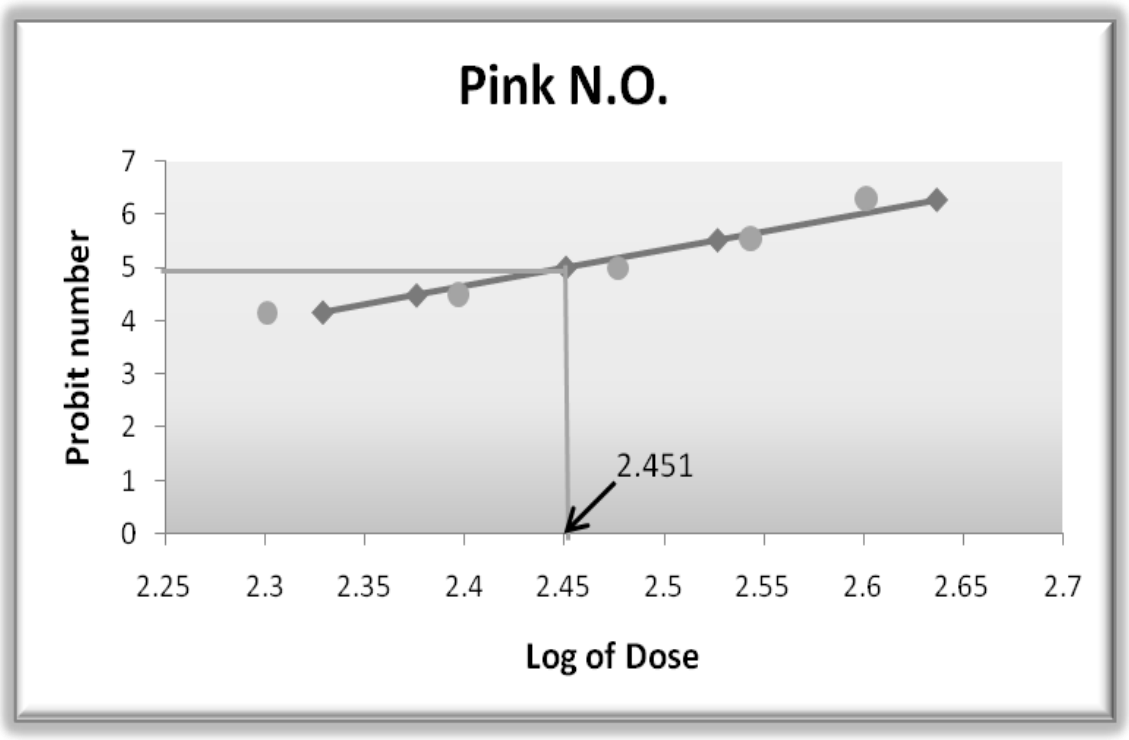

Fig 2:-Estimation log dose-probit curve for hexane extract of the PFG of *N.oleander* after fitting LD_{50} was 282.918 mg/kg of body weight.

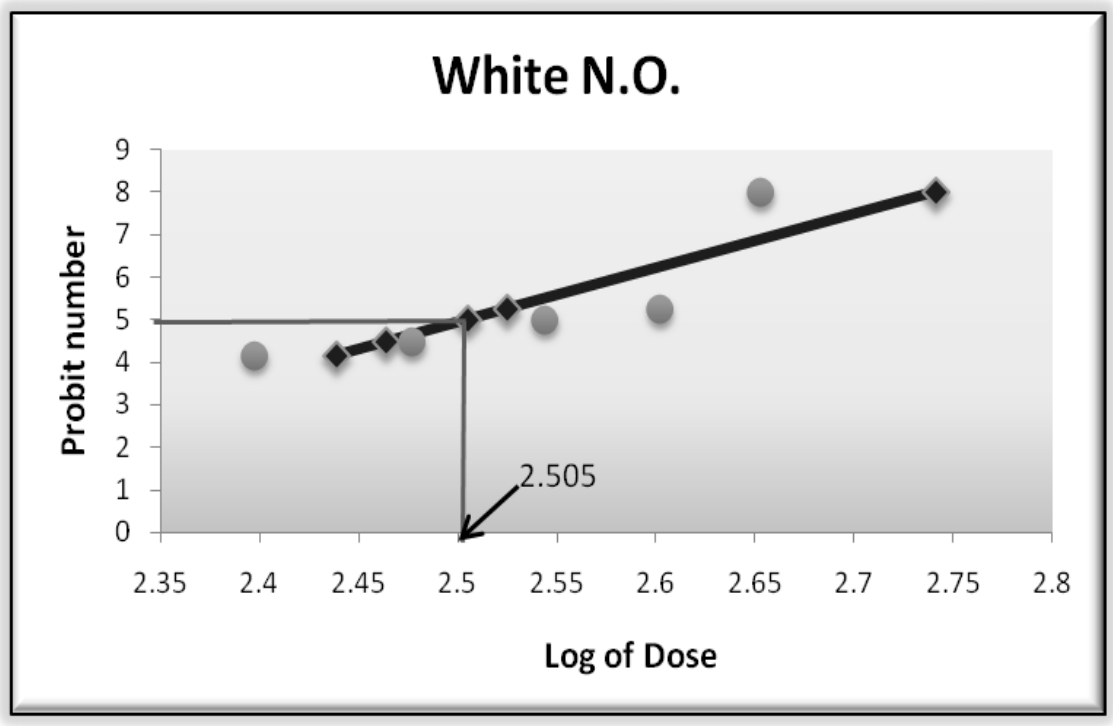

Fig 8:-Estimation log dose-probit curve for hexane extract of the WFG of *N.oleander* after fitting LD_{50} was 319.979 mg/kg of body weight.

Table 5:- values of LD_{50} and slope of the three preparations

●Type	*LD_{50} ± S.E.	**Slope ±S.E.
RFG	296.46 ± 28.37 A	6.07 ± 1.45 A
PFG	282.91 ± 18.07 A	2.45 ± 1.25 A
WFG	319.97 ± 45.99 A	5.51 ± 1.66 A

***LD_{50} was compared with the two others.**
**** Slope was compared with the two others.**

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