

STUDY OF ACUTE TOXICITY OF DIFFERENT PREPARATION OF OLEANDER LEAVES IN MICE

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ABSTRACT

Three preparations of dried oleander leaves were prepared. The first one was by extraction with hexane; the second by extraction with 70% ethanol while the third was suspended as a whole ground leaves in distilled water. Different concentration of each form was prepared and the range of lethal dose in mice had estimated by pilot studies. The acute toxicity study was carried out by determine the median lethal dose (LD₅₀) after administration of each preparation using 5 subgroups (10 mice) each receive dose range from 60-64 mg/kg, 504-536 mg/kg and 1100-1300 mg/kg for the hexane extract, ethanol extract and leave suspension respectively.

The lethal dose calculated by employing probit method and found to be; 62.6 mg/kg for hexane extract, 521.0 mg/kg for ethanol extract and 1164.8 for leave suspension.

This result indicates that the toxic constituents of the leaves are mostly non-polar, also that the potency of toxicity is far less than that mention in some literature.

INTRODUCTION

Nerium oleander or aldefla, as it is call locally, is permanent green shrubs, which carry flowers of different colors. It is very suitable to planting in gardens and along high ways in many cities and villages.

All part is report to be toxic when consumed, the reason of toxicity is mainly due to the presence of cardiac glycoside especially oleandrin (1) which has a digitalis-like action. Several attempts have made to use it as cardiac tonic but not succeeded due to narrow therapeutic index. Other toxic constitute of this plant is niriine and cardinolides which are glycosides (2) while the bark of the plant contain rosagenin which has a strychnine like action (3).

The symptoms of toxicity and the reason of death is the same as known for other cardiac glycosides like digoxin (2).

The aim of the present study is to estimate the toxic potency of different preparations and compare them with that mentioned in the literature and the possibility of employing some of these constituent as pesticides.

MATERIAL AND METHOD

Preparation of powdered oleander leaves:

Fresh leaves of local plated pink flowers oleander collected from Baghdad area, identified botanically, dried at room temperature and ground by electrical grinder.

Extraction of hexane:

Fifty grams of the ground leaves put in a thimble and placed in a soxhlet apparatus according to (4). Percolation at 70°C continued for 4 hours, the percolate evaporated as before and kept at -20°C.

Extraction with 70% ethanol:

One hundred grams of ground leaves was mixed with 500 ml of 70% ethanol using magnetic stirrer at 30-40 °C (4). After filtration, the process was repeated three times. The filtrate evaporated by rotary evaporator under negative pressure at 39°C. The extract was weighed and kept at -20 °C.

Whole leaves suspension:

One hundred grams of ground leaves was directly suspended with 10 ml distilled water and kept at -20 °C.

Preparation of solutions for LD₅₀ study:

Those solutions were prepared on bases of pilot studies, which determined the range of lethal dose of each preparation as followed.

Hexane Extract (HE):

A stock solution of 10 mg/ml was prepared in propynl glycol then a dilution of 3.20, 3.15, 3.10, 3.05 and 3.00 mg/ml to achieve a dose of 64, 63, 62, 61 and 60 mg/kg when give as above.

Ethanol Extract (EE):

A stock solution of 100mg/ml in distilled water was prepared and dilution of 28.8, 26.4, 26.0, 25.6 and 25.2 mg/ml to achieve a dose of 536, 528, 520, 512 and 504 mg/kg when given as above.

Powdered Leaves Suspension (LS):

A stock solution of 100 mg/ml was prepared in distilled water then a series of dilutions (65.0, 62.5, 60.0, 57.5and 55.0 mg/ml) to achieve a dose 1300, 1250, 1200, 1150 and 1100 mg/kg respectively when given at a rate 0.5 ml/25g mouse.

Median Lethal dose (LD₅₀):

Fifty albino male mice with close ages and weights used to estimate the LD₅₀ of each preparation when given orally. They divided in to 5 subgroups (10mice) and given an increasing dose that mentioned before for each preparation. The clinical symptoms before death and the number of dead mice within 24 hours of dosing recorded. Probit number calculated from the percentage of death, a plot of probit number constructed against logarithmic dose, and the curve had calculated by least square method, the LD₅₀ values also calculated.

RESULTS

Extraction result:

Extraction with 70% ethanol of powdered leaves gave a bright green color with high viscosity extract, which is soluble in water; the yield of extraction was 30% of the original weight, while extraction with hexane gave a powder, green in color and insoluble in water. The yield was 29.5% of original weight.

Clinical symptoms:

The clinical symptoms of mice received the hexane extract were more sever and characterized by cholinergic symptoms like vomiting, diarrhea, hyper salivation and colic and also central nervous system symptoms like ataxia, drowsiness, seizure and comma before death.

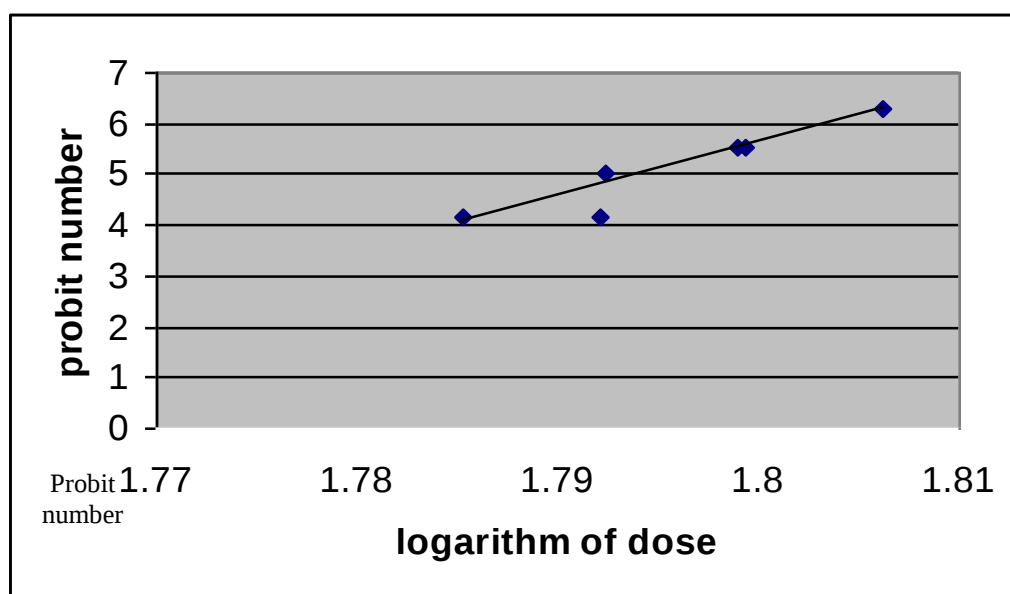
While the symptoms of ethanol extract and leave suspension was less, sever characterized by neuromuscular symptoms, muscle tremor and cholinergic symptoms.

Median lethal dose (LD₅₀):

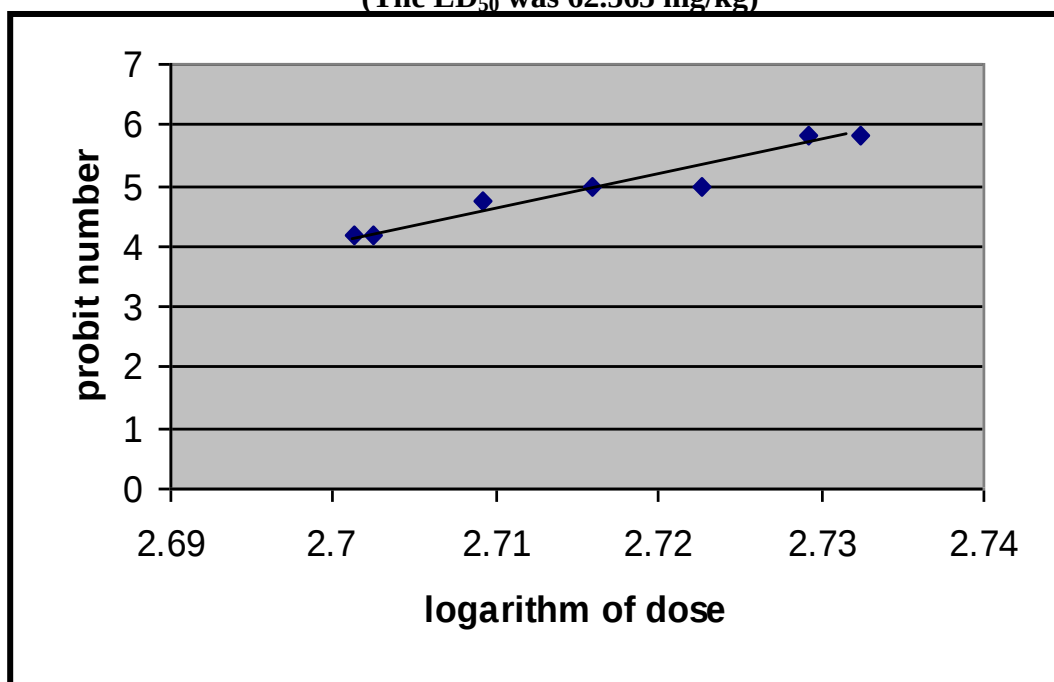
The percentages of death of each of the three preparations listed in table (1).The probit number obtained and a plot of log dose verses probit number was constructed as shown in figure 1, 2 and 3 for HE, EE and LS respectively.

Table (1) show the acute toxicity of HE, EE and LS when given at increasing dose in mice

Type	Subgroup	Dose mg/kg	Dead	Mortality rate%	Probit number
Hexane Extract (HE)	1	60	0	0	----
	2	61	2	20	4.16
	3	62	5	50	5.00
	4	63	7	70	5.52
	5	64	9	90	6.28
Ethanol Extract (EE)	1	504	2	20	4.16
	2	512	4	40	4.75
	3	520	5	50	5.00
	4	528	5	50	5.00
	5	536	8	80	5.84
Leave Suspension (LS)	1	1100	3	30	4.48
	2	1150	5	50	5.00
	3	1200	6	60	5.25
	4	1250	7	70	5.52
	5	1300	8	80	5.84



**Figure (1) Log-dose probit curve of hexane extract (HE) of oleander leaves
(The LD₅₀ was 62.565 mg/kg)**



**Figure (2) log-dose probit curve of ethanol extract (EE) of oleander leaves
(The LD₅₀ was 520.987 mg/kg)**

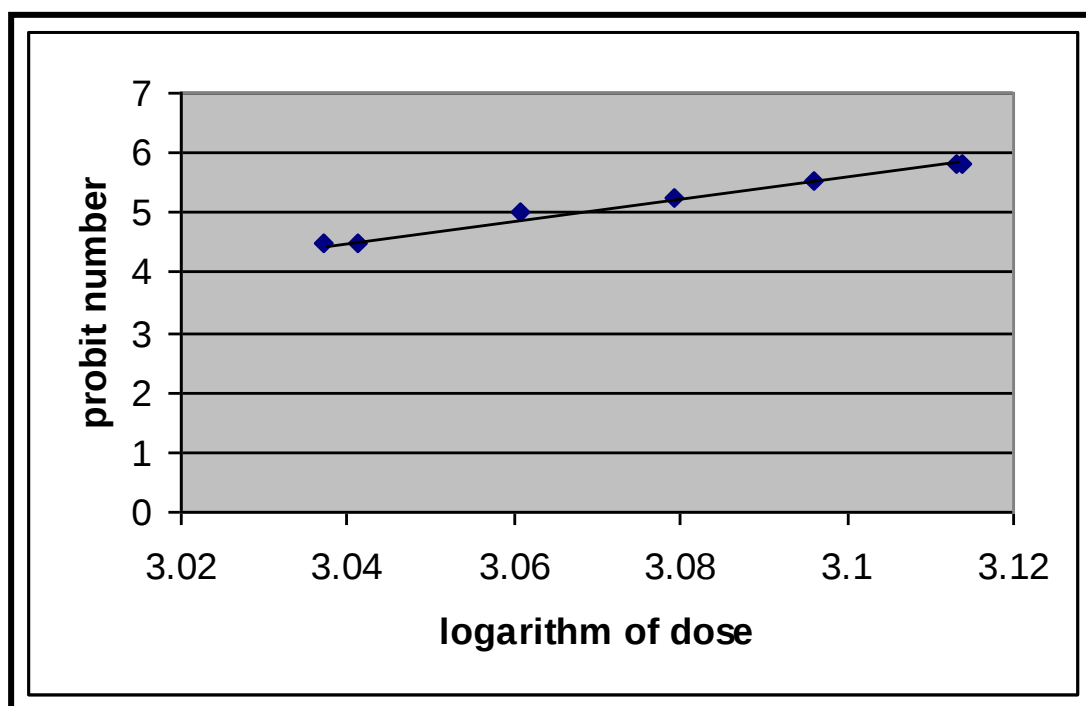


Figure (3) log-dose probit curve of leave suspension (LS) of oleander leaves (The LD₅₀ was 1164.793 mg/kg)

DISCUSSION

The three forms tested in this study proved to be fatal when given orally but the hexane extract was more potent than the ethanol extract and as expected, both were more potent than the leave suspension.

The reason of the toxic effect is mostly due to the presence of different glycosides. Oleandrine is the most important (5,6). Other glycoside like rosagenin, which has strychnine like action, and hydrocyanic acid (3), may add to the toxic effect. These materials are mostly non-polar and accordingly most of the toxic constituents had found in the hexane extract.

The clinical symptoms reflect the potency of each preparation. With the hexane extract, the mice showed more sever central nervous system symptoms like drowsiness, ataxia and seizure before death, while with the other two preparations the symptoms were mostly cholinergic like colic, vomiting, hyper salivation and diarrhea and neuromuscular symptoms like muscle tremor. Similar finding were report by (2), the central nervous system symptoms may be attributed to hypoxia resulted from bradycardia caused by cardiac glycosides or cyanogenic glycosides. While the gastrointestinal symptoms may be due to cholinergic action of some constituents specially those concentrate in the two extracts.

The median lethal dose, which obtained from the probit curve of each preparation show difference in potency and efficacy and the median lethal dose calculated from the curve, was about 62 mg/kg that is about ten times less than EE indicate more potency while with LS it was about 2-3 times less potent than the EE that reflect the amount of yield (30%) of the total weight.

This study suggests that the toxicity of this plant varies according to species, as mice could be consider more tolerant than large animals or human. The literatures indicate that consumption of few leaves of this plant could be fatal to animals or man (7,8). Also the type of the plant and season may add to this variation, therefore a more detail study is necessary to evaluate this plant and the possibility of using it therapeutically or as toxic material to be employ as pesticide.

دراسة السمية الحادة لثلاث تحاضير من أوراق الدفلة في الفئران

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الخلاصة

تم عمل ثلاث مستحضرات من أوراق الدفلة المجففة ، كانت الأولى عبارة عن مستخلص الاوراق في الهكسان والثانية مستخلصها في 70 % ايثانول والثالثة عالق مسحوق الأوراق في الماء. وقد حضرت تراكيز مختلفة من كل من هذه المستحضرات وأختبرت في تجربة أولية لمعرفة مدى الجرعة القاتلة في الفئران عند اعطائها عن طريق الفم . قدرت الجرعة القاتلة الوسطية (LD_{50}) لكل مستحضر باستخدام خمس مجاميع (10 فئران لكل مجموعة) ، اذ أعطيت مجاميع مستخلص الهكسان ومستخلص الايثانول وعالق الاوراق جرعة تراوحت بين 60 - 64 ملغم / كغم و 504 - 536 ملغم / كغم و 1100 - 1300 ملغم / كغم على التوالي . تم حساب ال LD_{50} بواسطة طريقة البروبت ووجدت بأنها 62.6 ملغم / كغم لمستخلص الهكسان و 521 ملغم / كغم لمستخلص الايثانول و 1164.8 ملغم / كغم للعالق المائي للاوراق. تدل النتائج ان المحتويات الاكثر سمية هي المتمركزة في المستخلص العضوي أي الهكسان كونها غير مستقطبة ، كذلك تشير النتائج ان شدة السمية لهذا النبات هي أقل مما ذكرته بعض المراجع .

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