Study of cytotoxic Effects Alcoholic *Nerium Oleander* L. Extract on female Albino mice

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Received 28, September, 2009
Accepted 20, June, 2010

Abstract:

This study involved the evaluation of Alcoholic extract of *Nerium Oleander L*. plant that have a promising anticancer cell. This extract was compared to the well known anticancer drug Cis – Platinum by utilizing an *in vivo* system in female Albino mice. The first direction was cytogenetically using the mitotic Index of bone marrow cells as a parameter for the cytotoxic effect of this extract. The second direction was enzymatical using a widely distributed enzyme GOT in the different organs of mice: Liver , kidney , spleen and lung . Animals were treated with three doses of Cis-platin , 50 , 200 and 350 Mg/mouse for three days . The same doses were used for the other extract . This study showed that the extract have a promising anticancer cell as could be seen from these effect on mitotic Index (MI) of mice bone marrow , (MI) decreased in animals treated with different doses of extract , mitotic index was reduced to 78% on day three in animals treated with 350 µg/mouse . These effects were similar to the effect of Cis-platin at the same doses. Comparing the effect of this extract on GOT enzyme showed that Cis-platin was more effective on activating the spleen GOT enzyme of about 95% than the extract while the extract is more effective in Lung , The extract activated GOT enzyme activity in the all organs.

Key words: Nerium oleander ; Cisplatin ; GOT ; Cytotoxicity

Introduction:

The importance, necessity and potentiality of medicinal plants in practice of medicine today is well established and cannot be over looked [1]. *Nerium Oleander Linn*. Belongs to family Apocynaceae commonly known as Gandeera ,is large glabrous evergreen shrub with milky juice .Leaves in threes , shortly stalked. Flowers are rose-colored or white, fragrant. The *Oleander* is an attractive and hardly shrub that thrives in tropical and subtropical regions. The common Pink *Oleander, Nerium Oleander* and Yellow *Oleander*, Thevetia Peruviana are the principle *Oleander* representatives of the same family [2]. *Oleander* is one of the most poisonous plants and contains numerous toxic compounds. The most significant of these toxins are oleandrin and Neriine which are cardiac glycosides (cardenolides) they are present in all parts of the plant, but are most concentrated in the Sap. Many of *Oleander*`s relatives have similar leaves and contain toxic compounds. It's thought that oleander may contain many other unknown or un-researchable compounds that may have dangerous effects. *Oleander* bark contains rosagenin which is known for it's strychnine –like effects. The entire plant including the milky white sap is toxic and any part can cause an adverse reaction. *Oleander* is also known to hold it's toxicity even after drying [3].Cases of poisoning by *Oleander* were observed in several species then evaluate the Pathological effects of in

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goats [4]. Hussain and Gorsi [5] studied the *in vitro* antimicrobial activity of *Nerium Oleander* roots , bark and leaf extracts against *Bacillus pumilus ,Bacillus , subtilis ,staphylococcus aurous , Escherichia Coli* and *Aspergillus niger* and they showed the *Nerium oleander* whose a high activity against all their tested. Adam *et al* [6] studied the Susceptibility of sheep to oral administration of citrullus colocynthis fruits , Nerium oleander or their mixture and they observed the Effects were correlated changes in the activities of serum lactic dehydrogenises (LDH) and aspartatetransaminase (AST) and concentrations of cholesterol , bilirubin ,total protein , albumin , globulin and urea and hematological parameters . The objective of this study was to determine the efficacy of Ethanol curde extract from *Nerium oleander* plant on the *in vivo* system in Albino mice to determine the cytotoxic effects of this extract on bone marrow cells and the other hand , investigation the activity of GOT in different organs , liver , kidney , spleen and lung , these results were compared with the anti tumor compound Cis-$[Pt(NH_3)_2Cl_2]$ (Cis platin) exert there cytotoxic effect by inducing DNA damage and activating programmed cell death (apoptosis)[7].

Materials and Methods:

1. The Animals

Female (8–12 weeks old) Albino mice were used supplied by Science College for Women. They were transferred to the chemistry department in controlled condition of temperature (23+2C˚) humidity (50+5%), and light (10 and 14hr .of light and dark respectively). The Animals were fed on special formula food pellets and supplied with water ad libitume. Throughout the experiment ; 5–6 animals were housed in stainless steel cages containing hard – wood chip as bedding .

2.

a. Experiment No. one (test standardization with Cis – platin)

b. Experiment No. two (test treated with extract)

The animals in each experiment were divided into two groups: Group 1:treated with different doses of Cis–platin (Exp.one) or extract (Exp.Two) 0.05, 0.2 and 0.35mg/mouse) for three days .The single dose was injected in intraperitonealy (I.P) at 8 mg / kgm from body weight [8] .

Group 2 : untreated (Controls)

The animals sacrificing were carried out at three – days post – treated , using four animals for each dose and control , two animals were used for estimating Glutamate Oxaloacetate Transaminase activity in Liver , kidney , spleen and Lung cells . The other two animals were used for bone marrow cells harvest .

3. Cis – platin drug .

The anticancer drug were provided by Ebew (Austria) (10 mg / 20 ml).

4. Preparation of Alcoholic Extract of *Nerium oleander* plant .

According to the method of Osman Goktas *et al*, [3], the oleander's leaves and flowers used in this study were collected from the Gardens – University of Baghdad – Jadryah in August. The collected samples were washed and air dried. The oleander's leaves and flowers were ground into particles with 1 – 2 mm, blended with 100 ml ethyl Alcohol then for each trial and placed into the alcohol bath at 50 C˚ for 5 hours . The resultant extract solution was filtered through a wool filter and then rinsed with a small quantity (about 30ml) of 95% ethyl alcohol. The filtrate was evaporated under pressure at 40C˚ ,to give a total weight of Nerium oleander powder .

The Nerium oleander powder was dissolved 10mg in 20ml of normal saline (the stock concentration) and the solution to make concentration 0.05 , 0.2 and 0.35 mg .

5. Cytogenetic Analysis in vivo .

Cytogenetic analysis of bone marrow cells was studied *in vivo* according to Allen *et al* [9] .

- Bone marrow cells Harvest

Albino – mice used for analysis of cellular division formation in bone marrow cells. Two hours prior to sacrifice, 0.3 ml (300 mg) of colchicine was injected intraperitonealy to arrest cells in the metaphase by inhibiting the operation of spindle mechanism. The animals were killed by cervical dislocation. Bone marrow cells were collected from femurs after removed both epicondyl tips with scissors. The cells were collected using a syringe with PBS into the centrifuge tube and centrifuged at 2000 rpm for 10 min. The cells were then treated with 5 ml of warm hypotonic solution (0.075 M, KCl) for breaking down RBC'S and the Suspension mixed well. The tubes were incubated in a water bath at 37C˚ for 1 hour and then centrifuge at 2000 rpm for 10 min , the supernatant discarded , freshly prepared fixative methanol : acetic acid (3 : 1) was added drop – wise , with initial mixing , to give a total volume of 5 ml . The purpose of fixation is to kill the tissue without causing any distortion of components to be studied .Three other washes with fixative solution were made. 1 ml of the fixative was added to the cells after the last wash, cells ready now for microscopic examination .

- Slide preparation and staining

The cells were resuspended and then dropped from a height of about 1-meter ,using a pasteur pipette on to cleaned microscopic Slides that had been washed in methanol then distilled water . Slider were then on a 50C˚ hot plate to estimate the mitotic index , Slider were stained with Giemsa stain for 10 min. , washed with distilled water and examined microscopically under light microscopic – (Olympus – BH2)[9].

- Mitotic Index (MI) Analysis

The MI was determined as a ratio of mitotic cells to interphase nuclei in 1000 cells [9].

6. Tissues collection (Liver , Kidney , lung and spleen)

The sample was collected from sacrificed animal using an Eppendrof tubes containing phosphate buffer saline (PBS). two treated animals and two non treated (controls) were used for this purpose and the samples ,were stored at – 70C˚ until processing .

- Tissue Homogenization and sample preparation

After the organs of animals were collection, the samples were prepared according to the method of Jennan [9] , then 80% was extracted from the total activity of enzyme . The method summarized in the sand were riddled by the sieve to remove the blemishes and take the soft sand from it and their wash by diluted acetic acid (5%) , washed with distilled water. We mixed the dry sand with prepared tissues for extraction. Each tissue (Liver , Kidney , Lung and Spleen) was homogenized in glass homogenizer with equal quantity of dry sand and mixed well until homogeneous solution , then added the buffer solution (PH=7.4) 2ml for each 1ml of tissue (weight) and mixed well until homogeneous solution . After that added Butanol : tissue (1:1) with mixing for 15 min. . The tubes were incubated in a water bath at 37C˚. The tubes were centrifuged at 3700 rpm for 10 min. and take the supernatant which contain the Enzymatic extract.

- Glutamate Oxaloacetate Transaminase Activity Assay

The activity of Glutamate oxaloacetate transaminase (GOT) was determined in Liver , Kidney , Spleen and Lung cells according to the method of Jennan [9] .

- Protein Determination

The protein content in the samples was determined according to the method of Naeem [10] . Using 0.5 gm / 100ml Humain Serum albumin (Bio test – Germany) as a standard .

- Statistical Analysis

Data were analyzed by analysis of Variance ANOVA. Investigations of variability between controls and the relation with other groups by towards using the statistical program (SPSS) [11].

Results and Discussion:

- Cytogenetic Analysis of Mouse Somatic stem cells

The mitotic Index (MI%) of bone marrow cells in four study groups and their controls Varied with concentration of two materials Cis – platin and Nerium oleander extract .

The results presents in figure 1 (A , B) the mean value of MI in each treatment group A ; with Nerium oleander Extract and B ., with Cis – platin at day three which is found to be high significantly ($P < 0.05$) than that for the control at each three doses (0.05 , 0.2 and 0.35 mg / mouse) while , there was not significant differences between the treatment with Cis – Platin and Nerium oleander Extract at the same doses .

This could be attributed to the ability of Nerium oleander extract cytoxic effects in living cells with approximate percent to produce damage or defect of spindle fibers structure during the mitotic process [9] .

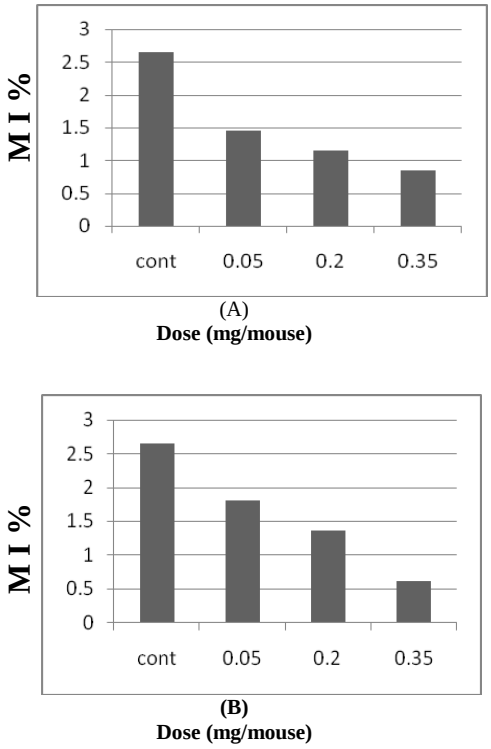

Fig. (1): mitotic Index of A: Bone marrow cells treated with difference doses of *Nerium oleander* extract. B: With different doses of Cis – platin compared to the non – treated control

Glutamate Oxaloacetate Transaminase (GOT) Activity of tissues

Liver

The mean value of GOT Specific activity of liver from mice after treatment with *Nerium Oleander* extract and their Cis – platin comparbly with controls and presented in table (1) . The results present an evidence that treatment with *Nerium Oleander* extract showed increases in enzymatic activity at two doses 0.05 mg / mouse and 0.2 mg / mouse with highly significant ($p < 0.05$) about 58.42%, 90.55%, while the inhibition of activity reached of 10.09% at 0.35 mg / mouse only .

The results also presents the mean value of GOT Specific activity after treatment with Cis – platin which was found to be highly significant ($P < 0.05$) reduction from the mean value

of treatment with *Nerium Oleander* extract . The result an evidence that liver GOT was not significantly (P < 0.05) from each three doses after treatment of *Nerium Oleander* Extract .

Glutamate oxaloacetate transaminase enzyme is the transferase enzyme which catalysis Transaminase of L -Asparate to ketoglutarate on the contrary.

The increased in level GOT enzyme of leukemia serum patients were attributed to liberated this enzyme from cancer cells which cause their damage[8].

Table (1) : Glutamate oxaloacetate transaminase specific activity of liver of Female mice treatment with different doses of *Nerium Oleander* extract compared to the Cis – platin and control .

Doses / Treatment	Glutamate Oxaloacetate Transaminase Specific activity (unit/mg protein) Standard error ± average		
	0.05 mg / mouse	0.2 mg / mouse	0.35 mg / mouse
Control	A , a 0.47 ± 4.45	A , a 0.58 ± 4.66	A , a 0.78 ± 5.15
Cis – platin	A , a 0.91 ± 1.99	A , a 0.50 ± 1.78	B , a 0.66 ± 1.77
Nerium oleander extract	B , a 3. 91 ± 7.05	B , a 2.65 ± 8.88	A , a 2.59 ± 4.63

- Different letters (A,B) significant differences (P < 0.05) as comparable between Rows and (a , b) between column .

- Kidney

As shown in table (2) the kidney Glutamate oxaloacetate transaminase enzyme showed a relative activation of about 47.23% , 141.43% and 59.56% respectively on day three comparable with controls obtained from *Nerium Oleander* extract treated mice at three doses . As with Kidney GOT , showed the same pattern of activation from treated with Cis – platin at 0.05 mg/mouse only.

Also table -2- showed the combined effect of Cis – platin and *Nerium Oleander* extract was found that the mean value of GOT specific activity in each treated was to be not significant difference between them at 0.05 and 0.35 mg / mouse .

Table (2): Glutamate oxaloacetate transaminase specific activity of Kidney of female mice treated with different doses of *Nerium Oleander* extract compared to the Cis – platin and controls .

Doses / Treatment	Glutamate Oxaloacetate Transaminase Specific activity (unit/mg protein) Standard error ± average		
	0.05 mg / mouse	0.2 mg / mouse	0.35 mg / mouse
Control	A , a 0.39 ± 2.71	A , a 1.12 ± 2.51	A , a 0.36 ± 2.77
Cis – platin	B , a 0.06 ± 3.81	A , b 0.19 ± 0.82	A , c 0.40 ± 2.60
Nerium oleander extract	B , a 0.56 ± 3.99	B , a 1.04 ± 6.06	A , a 3.09 ± 4.42

- Different letters (A,B) significant differences (P < 0.05) as comparable between Rows and (a , b) between column .

- Spleen

The data of GOT – specific activity of Spleen from mice treated with Nerium oleander extract and their treated with Cis – platin comparable with controls are summarized in table (3). The results an evidence that was significantly (P < 0.05) enhanced from day three between Cis – platin and *Nerium Oleander* extract treated at each three doses , but it was not significant differences after extract treated comparable with controls , While there was not significant when treated with extract compared to controls .

Table (3) : Glutamate oxaloacetate transaminase specific activity of Spleen of female mice treated with different doses of *Nerium Oleander* extract compared to the Cis – platin and controls .

Doses / Treatment	Glutamate Oxaloacetate Transaminase Specific activity (unit/mg protein) Standard error ± average		
	0.05 mg / mouse	0.2 mg / mouse	0.35 mg / mouse
Control	A , a 0.20 ± 1.72	A , a 0.35 ± 1.53	A , a 0.28 ± 1.93
Cis – platin	B , a 11.47 ± 83.15	B , b 8.50 ± 36.89	B , a 10.46 ± 63.77
Nerium oleander extract	A , a 0.45 ± 2.22	A , B 2.14 ± 5.74	A , a 0.05 ± 2.14

- Different letters (A,B) significant differences (P < 0.05) as comparable between Rows and (a , b) between column .

- Lung

The mean value of GOT – specific activity of lung from mice experimentally divided in four – study group and their controls varied with different concentrations and different treatment (Cis – Platin and *Nerium Oleander* extract) are presented in table (4) . It showed the combined effect of Cis – platin and *Nerium Oleander* extract at 0.2 and 0.35 mg/mouse. It was found not significant, while there was significant when treated with *Nerium Oleander* Extract compared to controls at three doses .

Table (4) : Glutamate oxaloacetate transaminase specific activity of Lung of female mice treated with different doses of *Nerium Oleander* extract compared to the Cis – platin and controls .

Doses / Treatment	Glutamate Oxaloacetate Transaminase Specific activity (unit/mg protein) Standard error ± average		
	0.05 mg / mouse	**0.2** mg / mouse	**0.35** mg / mouse
Control	**A , a** **0.05 ± 0.83**	**A , a** **0.09 ± 0.84**	**A , a** **0.09 ± 0.86**
Cis – platin	**A , a b** **1.24 ± 3.97**	**B , a** **0.40 ± 5.82**	**B , b** **0.34 ± 3.47**
Nerium oleander extract	**B , a** **2.94 ± 15.29**	**B , b** **4.02 ± 5.04**	**B , b** **1.49 ± 2.72**

- Different letters (A,B) significant differences (P < 0.05) as comparble between Rows and (a , b) between column .

The stimulated of GOT activity at most doses could be attributed to difference of parts of plant in chemical compounds ,then the different concentrations were used and some inhibitors chemical were change to non – toxic forms to cause type of difference effect when the extract used. The differences of extraction methods to cause break up or crowd for molecules were effective also in activity of extraction [12].

According to the our results give the higher inhibition percent in Liver GOT at higher doses compared to Cis – platin which recorded the higher inhibition at the same dose, could be attributed to Gurde extract was mixture of many chemical compounds may be their to overlap cause to inhibition one of these compounds, Miyoshi et al ., [13] were explain sugar finding lead to change to lactine structure cause to inhibit of stimulated act [14] **.**

Conclusion:

Alcoholic extract of *Nerium oleander* L. Showed to have a cytotoxic effects by reducing the mitotic index at different concentration these effects were similar to the effect of Cis – platin at the same doses. *Nerium oleander* extract was more effective on the Lung , Spleen , Kidney and Liver GOT enzyme than Cis – platin .

Acknowledgments:

This work was supported by Chemistry Department College of Science for Women , University of Baghdad . I would like to thank, D. Saadia Al Dulami and D. Hasium Esmail in University of Al Nahrrin for their helpful advice.

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دراسة التأثيرات السمية الخلوية لمستخلص الدفلة الكحولي على اناث الفئران نوع Albino

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الخلاصة :

تم في هذه الدراسة تقييم تأثير ألمستخلص الكحولي لنبات الدفلة المتوقع أن يكون مضاد لنمو الخلايا السرطانية للسرطان وتمت مقارنته مع العقار المضاد للسرطان "السزبلاتين" من خلال توظيف نظاماً للفحص داخل الجسم *in vivo* في إناث الفئران نوع Albeno . *أولاً* : عن طريق تطبيق احدى التحليلات الوراثية الخلوية في خلايا نقي العظم والمتضمنة معامل الانقسام الخلوي . *ثانياً* : تقدير فعالية انزيم GOT في أعضاء الكبد والكلية والطحال والرئة للفئران . تمت معاملة الحيوانات بالجرعة العلاجية البشرية للسزبلاتين بعد ان تم احتسابها للفئران ليصبح عدد الجرع ثلاثاً ,الواطئة 50 و 200 والعالية 350 مايكروغرام / للفار وقد استخدمت الجرع نفسها للمستخلص الكحولي وأستمر طور المعاملة مدة ثلاثة ايام , وقد تبين أنه من المتوقع ان يصبح المستخلص الكحولي لنبات الدفلة مضاداً للخلايا السرطانية من خلال تناقص معامل الانقسام الخلوي (MI) لخلايا نقي العظم عند جرع مختلفة من المستخلص والذي تقارب مع تأثير السزبلاتين حيث انخفض معامل الانقسام الخلوي بمقدار 78% لدى الحيوانات المعاملة بالجرعة 350 مايكروغرام/للفأر، وعند مقارنة فعالية انزيم GOT في أنسجة الاعضاء المختلفة للفئران وجد أن المعاملة بالسزبلاتين كانت أكثر فعالية لتحفيز الانزيم في الطحال بمقدار 95% من المعاملة بالمستخلص والتي حصل فيها التحفيز في جميع الأعضاء بعد ثلاثة أيام من المعاملة .