Histopathological and Physiological Study of *Nerium oleander* and Lead on the Liver in the Male Albino Mice.

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**Abstract:** The current study prepared to evaluate histopathological and some physiological parameters changes after treated with *Nerium oleander* L and lead. Thus study concentrated on some tissue structures in the liver after treated with the aqueous extract of *Nerium oleander* and lead solution. Its caused intense flaws in tissue structures of liver of treated albino mice. After the expiration of the experimental time, the blood specimens collecting to evaluate the value of Aspartate Transaminase (AST), Alanine Transaminase (ALT), urea (BUN) and creatinine (CR-S) in blood of treated animals, then animals sacrificed for histopathological study. The results of present study noted remarkable tissue changes in liver parenchyma, these changes included acute cellular destruction after treated with Nerium oleander and acute blood hemorrhage. The effects of lead showed clear space between hepatocyte of liver tissue, no prominent hepatic cords, most hepatocyte didn’t have normal nuclei. The Physiological alterations observed significantly increased in the enzymes value (Aspartate Transaminase, Alanine Transaminase, (BUN) and (CR-S) ) when exposure to Oleander and lead compared with control group.

**Keywords:** *Nerium oleander* L, lead, liver, Physiological results.

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1. **Introduction**

*Nerium oleander* is a long green shrub high up four meters, and classified within Apocynaceae family, distributed in tropical Asia. Oleander is implanted the whole world as decorative plant, it is widely spread in Mediterranean region, and set in Southwest Asia and South Europe, it grows very easily in many areas [1]. Leaves of *Nerium oleander* elongated from ten to twenty cm, acute at the apex, with a coriaceous dusky green blade and cramped, dusky short stalk and ashen-green color.

Many varieties categories have yellow or white splotch leaves, leave have a distinguished midial ribs, it is emerge in three groups from the stems, the flower are pink or white grows as terminal heads, the diameter of the flowers is about five cm in and 5 petalled, many farmers have or own an oleander that contains double flower, Nerium branches are flexible.
smooth, green phloem ultimately turns dusky ashen green in the adult plant[s] [2].

All plant parts contain toxic milky white sap that give rise to an passive react in case of intake by living organisms, oleander contains much poisonous ingredients, the main venomous one is the cardiac glycosides nerin and oleandrin [3]. Every part of the oleander has a high toxicity which contain different non-digitalis cardiac glycosides, like andolinerin, nerin and digitoxigenin, they are all referred cardenolides, due to the high percentage of fat, which leads to rapid and wide absorption of the digestive system and a slow urine excretion rate, the most active ingredient is oleandrin [4].

The effect of Nerium oleander on liver was mononuclear cell infiltration in portal spaces. Also cause congestion and hemorrhage and individual cell necrosis with dilation of sinusoidal spaces [5]. The study of Al-Hakak et al., (2019) [6] showed that section of treated liver mice with alcoholic extract of Nerium oleander caused programmed death in hepatocyte, cell membrane thickness, enlargement of the germs, emergence of hemosiderin and accumulation of fats.

Lead is conceded as a bluish-gray, heavy solid metal, low melting degree, with density at twenty °C is 11.34 g/cm³, chemical formula is Pb “comes from the Latin word "Plumbum”, the boiling point is 1,740 °C”, while the melting point 327.4°C”, its insoluble in water, and the molecular weight is 207.20 [7].

Lead is the main toxic of heavy metals, it is among two hundred seventy-five dangerous substances by (EPA) Environmental Protection Agency’s Priority List of Hazardous Substances formulated by (ATSDR) Agency for Toxic Substances and Disease Registry [8]. Pb is non-biodegradable, present in the environment, and became a provenance of contamination and dangerous for life organisms [9]. Lead considered as chronic and acute intoxication for animals and human, it used in industrial activities, exposure to lead by water or food and air pollution in some countries become a universal problem, pollution caused from industrial emission and combustion of lead like gasoline [10].

Lead transported to the liver, it may cause damage and defect in functions, liver deterioration could be advanced by histopathological results, and most accompanied with increase blood enzyme rates; reducing protein industry, and toxic effectiveness on kidney by the damage in kidney and abnormal within excretory function [11].

Abdou and Hassan, (2014) [11] studied lead acetate by injection wistar albino female rats, the study showed that dilatation of blood sinusoids, degenerated
hepatocytes and pyknotic nuclei, lymphocytes aggregation inside hepatic tissues and loss cellular architecture and vacuolated cytoplasm.

1.1 Aims of study: To evaluate the histopathological and physiological influences of *Nerium oleander* and lead on the liver in albino mice.

2. Materials and methods

2.1 Experimental Animals: The experimental study was conducted 60 male albino mice with weight about (twenty five to thirty five) g, Animals obtained from Drug and Health center in Baghdad province. The experimental animals living in laboratory plastic cages, all cages put in the animal house of the college of science at Al Muthanna University and the mice were left for at least two weeks for adaptation. living in a lab house under controlling temperature 25 to 28ºC with feeding by using standard pellets.

2.2 Plant: Oleander leaves were collected from the gardens of Al-Samawa city. Leaves were washed, cleaned and drying up at 25 ºC then shatter by a blender in the same day of preparation of the extract.

2.3 Lead: Lead was provided from a laboratory in Baghdad.

2.4 Experimental Design: Sixty albino mice were divided to three group, as a control group and treatment groups. Twenty mice as control group, twenty mice as gives orally aqueous extract of 0.75 ml Oleander for 30 days and 20 mice were a third group gives orally administration with lead solution 0.3 ml for 30 days.

2.5 Preparation of aqueous extract of *Nerium oleander*

According to [12] mode (10 gm) of plant powder added to (200ml) of filtered water in a disinfected glass beaker and left 24 hours with continued shake, then passed on the layers of disinfected smooth rag for its filtered and then separation of the filtrate by using a centrifuge (3000 rpm) and then dried the liquid in an electric oven degree (40 c) for drying the extract. Then collected and placed in a sterile bottle and preserved in refrigerator to until use.

2.6 Preparation of Histological Slides

Liver specimens collected from mice, the specimens collected neatly from mice by principle procedures. The liver samples washed with normal saline for removing any contest of blood, the liver specimens passed through histological technique which including several phases
(washing, fixation, dehydration, clearing; embedding, cutting and staining) [13].

Histopathological alterations observed by using light microscope and snaps were taken.

3. Results and Discussion:

3.1 The effects of Oleander on the Liver:

The histological results of liver in control group showed that diameter of hepatocyte nuclei was \(9.18 \pm 0.208 \mu m\), parenchyma of liver composed of hepatic cords which consist from hepatocytes that arranged as long cordal structures between hepatic cords showed the prominent sinusoids. The distribution of kupffer cells were normal through hepatic parenchyma. The liver was surrounding by thin connective tissue capsule (Fig. 1).

The current results showed that hepatocyte have spherical pigments, dark nuclei with acidophilic cytoplasm, while results of liver tissue in control group showed normal distributed of kupffer cells between hepatic cords, the histological results of liver in the control group appear normal blood sinusoid that line by normal endothelial layer (Fig. 2). Most tissue sections of liver after treated with Nerium oleander for thirty days showed prominent tissue changes in liver parenchyma, these changes included acute cellular damaging, acute blood hemorrhage, blood congestions, while the histological results of liver after treated showed wide aggregation of inflammatory cells located around the region of hemorrhage (Fig.3), irregular spaces showed in the tissue sections may be due to acute hepatocyte destruction, and these space filled with blood. Those results may be due to that liver consider as first sensitive organ to the poisons of Nerium oleander. The results were similar to [14] who noted that liver after exposure to Nerium oleander led to degenerative in its tissue, blood congestion and inflammatory proliferation.

The histological results of liver when treated with Nerium oleander had significant decreased in the diameter of hepatocyte nuclei \((7.82 \pm 0.181 \mu m)\), (Table 1) when compared with control group. Inflammatory cells among the hepatocyte were showed in tissue section of liver, most hepatocyte had clear cytoplasmic vacuoles (Fig.4). The results confirmed with [15] which explained that a cross section of liver showed infiltration of inflammatory cells and damage in the cytoplasmic hepatocyte.

3.2 The effects of Lead on Liver:

The histological results of liver after exposure to lead solution showed no significantly increased in the diameter of hepatocyte nuclei
(9.92± 0.312µm), (Table 1) compared with control group. The results agreed with [17] who noted that after liver exposure to lead caused prominent necrosis and inflammation.

The histological results of liver after treated with lead solution for thirty days of experimental period showed that liver parenchyma had blood hemorrhages in liver tissue with prominent degeneration; the tissue section showed clear space between hepatocyte, no prominent hepatic cords while most of hepatocyte didn’t have normal nuclei, the hepatocytes had abnormal distribution in parenchyma of liver (Fig. 5). Those results may be due to accumulation of lead solution in liver tissue caused injury and disorder in liver tissue. The results agreed with [16] who noted degeneration in hepatocyte, severe hemorrhage in liver and injury in the liver tissue.

3.3 The effects of Oleander on physiological results

Levels of Alanine Transaminase in control group in (Table 2) was (42.00±1.891). But the levels of Alanine Transaminase after treated with Oleander which had significantly increased compared with the control group (84.70±7.142) (Table 2). The changes in level of Alanine Transaminase when giving Oleander agreed with [17] who noted significant increasing in Alanine Transaminase.

The levels of Aspartate Transaminase in (Table 2) of control group was (365.15±12.295). The levels of Aspartate Transaminase when treated with Oleander (481.55±26.238) there was no significantly increasing compared with control group. The result may be porbability to nerin toxic which caused defect in liver parenchyma. The results disagree with [19] who noted significant increasing in Aspartate Transaminase and Alanine Transaminase when exposure to Oleander.

The current experimental noted the levels of BUN in (Table 2) was (53.15±1.052) in control group, while the levels of urea in treated group showed no significant increasing compared with control group (77.50±13.098). The results prospect to disorder in kidney cause toxic in the body.
these biochemical changes not coincided with [20] who noted increasing in the levels of serum urea after in treated group.

The creatinine level when exposure to Oleander in Table (2) was (0.569±0.0427 mg/dl) which have significantaly increasing compared with control group. This physiological change may because of acute damage in glomeruli and renal tubules lead to increase in creatinine excretion in kidney. This result was constant with [21] which noted the value creatinine increase after treated with Oleander toxic.

3.4 The effects of Lead on Physiological Results

Table (2) showed the levels of Alanine Transaminase enzyme of control group in (Table 2) were (42.00±1.891), but levels of Alanine Transaminase when exposure to lead had significant increasing compared with control group (143.35±10.957 U/L) (Table 2). The results may be due to accumulation of lead in liver that caused damage in cytoplasm of hepatocyte. These results agreed with [22] who showed significant increasing in the ALT levels in mice after treated with lead.

The current experimental noted levels of AST in (Table 2) in control group were (365.15±12.295 U/L) while after treated with lead was (609.20±14.746 U/L) and had significant increasing compared with control group. The results may because destruction of hepatocyte that led to those changes, the destruction of hepatocytes led to release high levels of liver enzyme from destroyed cells. The results confirmed with [23] which showed significant increasing in levels of AST after treated with lead. This result may be due to accumulation of the lead solution in the liver tissue caused disorder and injury in liver tissue.

The results in table (2) showed the levels of urea in control group which was (53.15±1.052 mg/dl) ) while after treated with lead was (30.00±1.309 mg/dl) and didn’t have significantaly increasing compared with control group, the results the urea after exposure to lead were disagreed with [24] who noted that levels of urea and creatinine increased in the serum after lead administration in rats.

The normal value creatinine of white mice in control group was (0.417±0.0046 mg/dl) (Table 2), while the CR-S when exposure to lead were (0.297±0.0090 mg/dl) which have significantaly decreased compared with control group, the results were disagreement with [25] which observed the accumulation of lead in kidney tissue caused damage and dysfunction of kidney lead to increase the levels of creatinine in serum. This physiological change may be due to increase the rate of execration or elimination the toxic material through the
kidney which caused this effect on creatinine level.

4. Conclusion

The effect of *Nerium oleander* on the liver lead to acute cellular damaging, acute blood hemorrhage, while the effect of lead on the liver caused clear space between hepatocyte, no prominent hepatic cords while most of hepatocyte didn’t have normal nuclei. The physiological results of liver after treated with *Nerium oleander* and lead showed significant differences in the value of Aspartate Transaminase, Alanine Transaminase, BUN and CR-S.

(Table 1) : Measurement Hepatocyte Nuclei in Study Group in Mice M± S.D

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Lead</th>
<th><em>Nerium oleander</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter</td>
<td>9.18 ± 0.208&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.92 ± 0.312&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.82 ± 0.181&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Sympoles reference significantly differentes among the mean at the probable of (0.01)

(Table 2) : Value the physiological parameters in serum of treated group M±S.D

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST U/L</th>
<th>ALT U/L</th>
<th>Urea mg/dl</th>
<th>Creatinine mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>365.15±12.295&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.00±1.891&lt;sup&gt;d&lt;/sup&gt;</td>
<td>53.15±1.052&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.417±0.0046&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lead</td>
<td>609.20±14.746&lt;sup&gt;c&lt;/sup&gt;</td>
<td>143.35±10.957&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.00±1.309&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.297±0.0090&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Nerium oleander</em></td>
<td>481.55±26.238&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>84.70±7.142&lt;sup&gt;c&lt;/sup&gt;</td>
<td>77.50±13.098&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.569±0.0427&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Sympoles reference significantly differentes among the mean at the probable of (0.01)
(Figure 1): Cross section the liver in the control group which showed A- Hepatic cord, B- Central vein, C- Sinoid, D- Hepatocyte, E- Kupffer cell. 

Hematoxyline&Eosine Stain 40X.

(Figure 2): Cross section the liver in the control group which showed A- Sinusoid, B- Central vein, C- Blood vessel, D- Sinusoid. Hematoxyline&Eosine Stain 20X.
(Figure.3): Cross section the liver when exposure to Oleander which showed A- Hepatocyte destruction, B- Blood congestion, C- Inflammatory cells, D- Cellular destruction. **Hematoxyline&Eosine** Stain 40X.

(Figure.4): Cross section the liver when exposure to Oleander which showed A- Necrosis, B- Hepatocyte, C- Inflammatory cells, D- Necrosis, E- Inflammatory cell, F- Central vein with congestion. **Hematoxyline&Eosine** Stain 40X.
Acknowledgment

We thank the advanced researches Laboratory of science college, Department of Biology for allowing use of their equipment to complete current study.

(Figure.5): Cross section the liver when exposure to lead which showed A- Prominent degeneration, B- Hepatocyte with abnormal nuclei, C- Degeneration in hepatocyte, D- Central vein filled with blood. Hematoxyline&Eosine Stain 40X.

(Figure.6): Cross section the liver when exposure to lead which showed A- Inflammatory cells, B- Congestion, C- Necrosis, D- Hypertrophy in hepatocyte, E- Necrosis. Hematoxyline&Eosine Stain 40X.
References:


