Research Article

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Experimental *Nerium oleander* poisoning in Balb/c mice and Wistar rat: comparative hepatotoxicity and nephrotoxicity effects based on biochemical and pathological studies

[Balb/c Fare ve Wistar Sıçanlarında Deneysel Nerium Zakkum Zehirlenmeleri: Biyokimyasal ve Patolojik Çalışmalara Dayalı Karşılaştırmalı Hepatotoksisite ve Nefrotoksisite Etkileri]

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Abstract

Objective: *Nerium oleander* is a member of the Apocynaceae family. All parts of the plant are considered toxic and can poison livestock and humans.

Method: The present paper was carried out to compare the toxic effect of oral administration of *N. oleander* extract at single doses of 10, 12.5, 15 and 25 mg/kg body weight in Balb/c mice and Wistar rat. The toxicity of this plant was determined by measuring serum levels of ALT, AST, total protein, albumin, BUN and creatinine. Histopathological examination was performed on the liver and kidney.

Results: Significant differences were observed in the level of the ALT, AST, BUN and creatinine. Interestingly, the biochemical changes were more severe in AST in rats compared with mice (15–16 and 4–5 times compared to control, respectively). In comparison, the values of BUN in rats were higher compared with mice (2–2.5 and 1–1.5 times, respectively). In mice and in rat more severe toxic lesions were observed in the liver and kidney, respectively.

Conclusion: In conclusion, the biochemical and pathological results of the current study suggested that mice have more susceptibility to hepatotoxicity of *N. oleander* intoxication. But, rats show more susceptibility to nephrotoxicity of *N. oleander* poisoning.

Keywords: *Nerium oleander*; Experimental poisoning; Biochemical parameters; Histopathological examination; Hepatotoxicity; Nephrotoxicity.

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Amaç: Nerium zakkum Apocynaceae ailesinin bir üyesidir. Bitkinin tüm parçaları toksik olarak kabul edilir ve hayvanlar ile insanlar zehirlenebilir.

Metot: Bu çalışma Balb/c farelerinde ve Wistar sıçanlarında 10, 12.5, 15 ve 25 mg/kg vücut ağırlığındaki tek doz *N. oleander* ekstraktının oral yoldan verilmesinin toksik etkisini karşılaştıracak şekilde gerçekleştirildi. Bu bitkinin toksisitesi serum ALT, AST, toplam protein, albumin, BUN ve kreatinin düzeyleri ölçülenerek belirlendi.

Bulgular: Histopatolojik inceleme karaciğer ve böbrek üzerinde yapıldı. ALT, AST, BUN ve kreatinin düzeyinde
belirgin farklılıklar gözlenmiştir. İlginçtir ki, sıçanlarda AST’de biyokimyasal değişiklikler farelere kıyasla daha şiddetlidir (srasıyla kontrol grubuna kıyasla 15-16 ve 4-5 kat). Buna karşılık, sıçanlarda BUN değerleri farelere karşılaştırıldığında daha yüksektir (srasıyla, 2-2.5 ve 1-1.5 kez). Farelerde ve sıçanda srasıyla karaciğer ve böbrekte daha şiddetli toksik lezyonlar gözlemlenmiştir.

Sonuç: Sonuç olarak, mevcut çalışmanın biyokimyasal ve patolojik sonuçları, farelerin N. oleander zehirlenmesinin hepatotoksisitesine daha yakın olduğunu düşündürmektedir. Fakat sıçanlar N. oleander zehirlenmesinin nefrotoksisitesine karşı daha yatkınlık göstermektedir.

Anahtar Kelimeler: Nerium zakkum; Deneysel zehirlenme; Biyokimyasal parametreler; Histopatolojik inceleme; Hepatotoksisite; Nefrotoksisite

Introduction

Nerium oleander is a member of Apocynaceae family (Dogbane family). It is an evergreen perennial shrub originating from the Mediterranean and is widely cultivated in tropical and subtropical regions as an ornamental plant [1]. This extremely toxic plant can poison livestock and humans, all parts of the plant, both green and dry are considered toxic at any time of the year [2]. The toxic components are the two potent cardiac glycosides which can be isolated from all parts of the plant, both are very similar to the toxin of Foxglove [3]. Oleander poisoning is not infrequent in man and domestic animals. Cases of accidental toxicosis have been reported in adults and children [4]. Most symptoms from oleander poisoning are cardiac and gastrointestinal in nature and appear 4 h after the ingestion [5]. Accidental and/or experimental oleander toxicosis has been described in cattle [6, 7], horses [8], sheep [9], goats [10], donkeys [11], rats [12, 13], mice [14], rabbits [15, 16] and chickens [17]. There are records stating that the plant can be used as a rodenticide, insecticide and for digestion, fever, ringworm, leprosy, venereal diseases [18], also as cardiac drugs [19] and antidiabetic agent [20]. It has also been used as a bioindicator of lead and other heavy metals pollution in the Mediterranean environments [21].

Previously, experimental poisoning due to this plant has also been induced in various animals, but the data on gross and histopathological changes in animal models or human patients are rare. With this information in mind, the present study was performed on mice and rat to evaluate the toxic effect of Nerium oleander’s aqueous leaf and flower extracts by studying clinical signs, mortality rate, biochemical parameters and, also, pathological changes. Thus, the main aim of this experiment was to compare the hepatotoxicity and nephrotoxicity of this plant in mice and rat in the same condition.

Materials and methods

Preparation of flower extract

The leaves and flowers of Nerium oleander were collected from the plants growing in the central part of Iran (Yazd province). The plant was properly identified. Fresh plant flowers and leaves were washed with distilled water and then air dried at room temperature to a constant weight and ground to a coarse powder which was dissolved in phosphate buffer saline (1 : 3 g/mL) and then extracted in water bath at 100°C for 15 min. The extract was filtered and subjected to rotary evaporator at 40°C under reduced pressure to remove the solvent according to Coles [22] with some modifications. The extract was dried by lyophilizing (Zibrus Technology Vaco 5, Germany) and stored at –20°C until used.
10% buffered formalin, embedded in paraffin, sectioned at about 5 μm, stained with hematoxylin and eosin and studied microscopically with a light microscope.

**Animal ethics**

The experiment was performed under the approval of the state committee on animal ethics, Shiraz University, Shiraz, Iran (IACUC no: 4687/63). Also, the recommendations of European Council Directive (86/609/EC) of November 24, 1986, regarding the protection of animals used for experimental purposes were considered.

**Measurement of serum biochemical parameters**

The activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using a commercial kit (Pars Azmoon Diagnostics, Tehran, Iran) by modified method of Reitman-Frankel. The measurement of serum total protein, albumin, blood urea nitrogen (BUN) and creatinine was accomplished based on the methods of biuret, bromocresyl green, diacetyl monoxime and Jaffe, respectively by commercial kits (Pars Azmoon Diagnostics, Tehran, Iran). All measurements were done using the Hitachi 912 clinical chemistry automatic analyzer (Roche Diagnostic GmbH, Mannheim, Germany).

**Statistical analysis**

Analysis was performed using SPSS packages (SPSS 22 for Windows, SPSS Inc, Chicago, IL, USA). All data were checked for normality with the Kolmogorov-Smirnov Z-test before analysis. Kruskal-Wallis H and Mann-Whitney U-tests were used for non-parametric data and analysis of variance (ANOVA) was performed for parametric data. Mean comparisons were done with the Tukey’s multiple range tests. p-Values < 0.05 were considered statistically significant.

**Results**

**Clinical and pathological findings**

The clinical signs of acute toxicities in both mice and rats appeared in the 12 h after the exposure to the extract, which were more severe in higher doses and were represented by anorexia, nervous signs, depression, restlessness, crying, ataxia, pawing of the ground, convulsion, falling and turning of the head backward. However, there was no mortality in different experimental groups of mice and rat. The animals in control were clinically normal. Gross examination of euthanized and necropsied animals with various doses of extract did not show any lesions in the livers and kidneys except mild hypertrophy associated with mild congestion.

Pathological changes in the kidney of rats were moderate in group 1 (with 10 mg/kg dose of *N. oleander* extract) and severe in groups 2, 3, 4 (Figure 1A) (with 12.5, 15 and 20 mg/kg dose of *N. oleander* extract) including hyperemia and hemorrhage, coagulative necrosis and interstitial nephritis associated with mononuclear inflammatory cell infiltration (it means that group 1 in comparison with groups 2, 3, 4 is significantly different; p<0.05). While, all of the pathological lesions in mice in groups 1, 2, 3 were mild and were moderate only in group 4 (it means that groups 1, 2, 3 in comparison with group
4 are significantly different) (Figure 1B). Interestingly, in the liver of mice, pathological changes were moderate in group 1 and severe in groups 2, 3, 4 (Figure 2A) including hyperemia and hemorrhage, coagulative necrosis (single diffuse hepatocyte necrosis) and periacinar hepatitis associated with mononuclear cell infiltration (it means that group 1 in comparison with groups 2, 3, 4 is significantly different; \( p < 0.05 \)). While, all of the pathological lesions in the liver of rats in groups 1, 2, 3 were mild and were slightly moderate only in group 4 (Figure 2B) (it means that groups 1, 2, 3 in comparison with group 4 are significantly different) which were coagulative necrosis (focal necrosis), perportal hepatitis and cholangiohepatitis associated with mononuclear cell infiltration and mild bile duct hyperplasia.

### Biochemical findings

The results of the biochemical tests at different days of the study (first, second, third and fourth days) are shown in Tables 1–4. The biochemical changes showed a

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**Figure 2:** Experimental toxicity with *Neurium oleander* extract; liver. Histologic hepatic lesions indicated severe toxicosis including hemorrhage, periacinar hepatitis (arrow) and also, single diffuse hepatocyte necrosis (arrow heads) in mice (A). While in rat, microscopic examination of the livers revealed moderate toxicosis including congestion, periportal hepatitis associated with mononuclear cell infiltration (arrow heads) and mild bile duct hyperplasia (B). H&E.

**Table 1:** Mean \( \pm \) SD of biochemical parameters after the exposure of animals with 10 mg/kg dose of the *Nerium oleander* extract on different days post exposure.

| Parameters | Day post exposure | AST (\( \mu \)L) | ALT (\( \mu \)L) | Protein total (g/dL) | Albumin (g/dL) | BUN (mg/dL) | Creatinine (mg/dL) |
|------------|-------------------|-----------------|-----------------|---------------------|----------------|-------------|-------------------|
| 1st Day    | Balb/C            | 183.3 ± 31.4\*a| 79.4 ± 18.7\*e | 6.39 ± 0.23         | 33.2 ± 4.9     | 1.67 ± 0.24a| 3.39 ± 0.11     |
|            | Control           | 37.3 ± 3.4\*a  | 19.2 ± 2.3      | 6.20 ± 0.21         | 20.7 ± 5.1     | 0.84 ± 0.19a| 3.44 ± 0.13\*f |
|            | Rat               | 586.4 ± 69.2\*b| 132.1 ± 12.4\*f| 7.64 ± 0.25         | 30.5 ± 3.9     | 2.89 ± 0.58b| 4.11 ± 0.08h    |
|            | Control           | 42.9 ± 10.1\*b | 35.1 ± 13.3\*f | 7.52 ± 0.27         | 16.9 ± 2.1     | 1.59 ± 0.79b| 4.17 ± 0.21h    |
| 2nd Day    | Balb/C            | 164.3 ± 32.1\*a| 80.1 ± 16.7\*e | 6.41 ± 0.21         | 35.3 ± 5.3     | 1.79 ± 0.26\*a| 3.47 ± 0.13     |
|            | Control           | 38.3 ± 3.8\*a  | 20.23 ± 2.54\*a| 6.26 ± 0.31         | 21.2 ± 6.2     | 0.91 ± 0.24\*a| 3.52 ± 0.21\*f |
|            | Rat               | 609.8 ± 71.7\*b| 117.4 ± 15.7\*f| 7.77 ± 0.29         | 37.4 ± 3.6     | 3.36 ± 0.52\*b| 3.87 ± 0.07h    |
|            | Control           | 44.1 ± 11.4\*b | 36.91 ± 14.32\*f| 7.69 ± 0.32         | 17.3 ± 2.9     | 1.64 ± 0.82\*a| 4.31 ± 0.28h    |
| 3rd Day    | Balb/C            | 171.7 ± 39.3\*a| 76.3 ± 15.3\*e | 6.51 ± 0.29         | 36.4 ± 5.5     | 1.71 ± 0.21\*a| 3.52 ± 0.14     |
|            | Control           | 38.0 ± 3.2\*a  | 19.0 ± 2.6      | 6.29 ± 0.32         | 21.1 ± 5.4     | 0.82 ± 0.24\*a| 3.60 ± 0.21\*f |
|            | Rat               | 607.4 ± 54.6\*b| 128.3 ± 12.6\*f| 7.56 ± 0.27         | 32.7 ± 3.7     | 2.99 ± 0.62\*b| 3.71 ± 0.28h    |
|            | Control           | 43.7 ± 10.8\*b | 36.7 ± 13.9\*f | 7.71 ± 0.41         | 16.4 ± 2.6     | 1.53 ± 0.83\*a| 4.28 ± 0.26h    |
| 4th Day    | Balb/C            | 239.7 ± 38.5\*a| 78.9 ± 17.7\*e | 6.46 ± 0.25         | 35.6 ± 5.3     | 1.78 ± 0.25\*a| 3.59 ± 0.19     |
|            | Control           | 38.0 ± 3.6\*a  | 20.0 ± 2.9      | 6.22 ± 0.31         | 21.7 ± 5.9     | 0.88 ± 0.29\*a| 3.71 ± 0.29\*f |
|            | Rat               | 719.6 ± 92.3\*b| 138.8 ± 13.5\*f| 7.62 ± 0.29         | 33.1 ± 4.1     | 3.09 ± 0.66\*b| 3.66 ± 0.27h    |
|            | Control           | 45.2 ± 12.7\*b | 37.1 ± 14.7\*e | 7.75 ± 0.45         | 17.6 ± 3.1     | 1.57 ± 0.87\*a| 4.35 ± 0.31h    |

Different letters in the same column are significantly different (\( p < 0.05 \)).
Table 2: Mean ± SD of biochemical parameters after the exposure of animals with 12.5 mg/kg dose of the *Nerium oleander* extract on different days post exposure.

| Parameters | Day post exposure |
|------------|-------------------|
|            | 1st Day | 2nd Day | 3rd Day | 4th Day |
|            | 6.63 | 6.20 | 6.26 | 6.22 |
|            | 6.67 | 6.20 | 6.25 | 6.26 |
|            | 34.7 | 20.7 | 21.2 | 21.7 |
|            | 1.72 | 0.84 | 0.91 | 0.98 |
|            | 3.28 | 3.44 | 3.52 | 3.71 |
|            | 3.36 | 3.36 | 3.49 | 3.55 |
|            | 3.87 | 3.87 | 3.91 | 3.50 |
|            | 4.17 | 4.26 | 4.26 | 4.31 |

Different letters in the same column are significantly different (p < 0.05).

Table 3: Mean ± SD of biochemical parameters after the exposure of animals with 15 mg/kg dose of the *Nerium oleander* extract on different days post exposure.

| Parameters | Day post exposure |
|------------|-------------------|
|            | 1st Day | 2nd Day | 3rd Day | 4th Day |
|            | 6.63 | 6.26 | 6.25 | 6.22 |
|            | 34.7 | 20.7 | 21.2 | 21.7 |
|            | 1.72 | 0.84 | 0.91 | 0.98 |
|            | 3.28 | 3.44 | 3.52 | 3.71 |
|            | 3.36 | 3.36 | 3.49 | 3.55 |
|            | 3.87 | 3.87 | 3.91 | 3.50 |
|            | 4.17 | 4.26 | 4.26 | 4.31 |

Different letters in the same column are significantly different (p < 0.05).
Table 4: Mean ± SD of biochemical parameters after the exposure of animals with 20 mg/kg dose of the Nerium oleander extract on different days post exposure.

| Parameters | Day post exposure |
|------------|-------------------|
|            | AST (μ/L)         | ALT (μ/L) | Protein total (g/dL) | Albumin (g/dL) | BUN (mg/dL) | Creatinine (mg/dL) |
| 1st Day    |                   |           |                     |               |            |                   |
| Balb/C     | 234.8 ± 39.2      | 101.5 ± 19.7 | 6.59 ± 0.22         | 35.1 ± 4.8    | 1.78 ± 0.27 | 3.16 ± 0.13       |
| Control    | 37.3 ± 3.4        | 19.2 ± 2.3  | 6.20 ± 0.21         | 20.7 ± 5.1    | 0.84 ± 0.19 | 3.44 ± 0.13       |
| Rat        | 643.6 ± 72.3      | 139.8 ± 14.6 | 7.81 ± 0.23         | 46.8 ± 4.3    | 3.73 ± 0.62 | 3.55 ± 0.07       |
| Control    | 42.9 ± 10.1       | 35.1 ± 13.3 | 7.52 ± 0.27         | 16.9 ± 2.1    | 1.59 ± 0.79 | 4.17 ± 0.21       |
| 2nd Day    |                   |           |                     |               |            |                   |
| Balb/C     | 169.4 ± 34.8      | 96.8 ± 19.2 | 6.62 ± 0.24         | 33.6 ± 5.1    | 1.85 ± 0.23 | 3.28 ± 0.11       |
| Control    | 38.3 ± 3.8        | 20.23 ± 2.54 | 6.26 ± 0.31          | 21.2 ± 6.2    | 0.91 ± 0.24 | 3.52 ± 0.21       |
| Rat        | 694.7 ± 71.2      | 151.2 ± 12.8 | 7.61 ± 0.27         | 48.6 ± 4.4    | 3.89 ± 0.71 | 3.43 ± 0.27       |
| Control    | 44.1 ± 11.4       | 36.91 ± 14.32 | 7.69 ± 0.32        | 17.3 ± 2.9    | 1.64 ± 0.82 | 4.31 ± 0.28       |
| 3rd Day    |                   |           |                     |               |            |                   |
| Balb/C     | 211.4 ± 36.5      | 95.1 ± 18.9 | 6.73 ± 0.22         | 33.5 ± 4.8    | 1.81 ± 0.25 | 3.31 ± 0.13       |
| Control    | 38.0 ± 3.2        | 19.0 ± 2.6  | 6.29 ± 0.32         | 21.1 ± 5.4    | 0.82 ± 0.24 | 3.60 ± 0.21       |
| Rat        | 677.8 ± 76.9      | 174.2 ± 13.9 | 7.94 ± 0.21         | 49.5 ± 4.7    | 3.98 ± 0.77 | 3.41 ± 0.29       |
| Control    | 43.7 ± 10.8       | 36.71 ± 13.4 | 7.71 ± 0.41        | 16.4 ± 2.6    | 1.53 ± 0.83 | 4.28 ± 0.26       |
| 4th Day    |                   |           |                     |               |            |                   |
| Balb/C     | 266.7 ± 30.9      | 97.2 ± 18.8 | 6.72 ± 0.24         | 31.7 ± 5.2    | 1.87 ± 0.23 | 3.35 ± 0.16       |
| Control    | 38.0 ± 3.6        | 20.0 ± 2.9  | 6.22 ± 0.31         | 21.7 ± 5.9    | 0.88 ± 0.29 | 3.71 ± 0.29       |
| Rat        | 768.7 ± 89.4      | 198.2 ± 14.6 | 7.83 ± 0.26         | 49.8 ± 4.9    | 4.09 ± 0.79 | 3.38 ± 0.28       |
| Control    | 45.2 ± 12.7       | 37.11 ± 14.7 | 7.75 ± 0.45        | 17.6 ± 3.1    | 1.57 ± 0.87 | 4.35 ± 0.31       |

Different letters in the same column are significantly different (p < 0.05).

significant increase (p < 0.05) in both AST and ALT activities. The highest values of both AST and ALT in rats and AST in mice were seen on the fourth day post treatment in group 4 (20 mg/kg dose) in comparison with control groups. However, the highest value of ALT in mice was recorded on the first day of study in group 4. Interestingly, the values of the AST in the mice showed a 4–5-fold increase. While, in the rats a 15–16 times increase was seen in this enzyme. There were slight changes in the values of the albumin and total protein which did not show any significant differences (p > 0.05) in both rats and mice. Generally, the albumin showed more changes in the rats as compared with affected mice. Also, the biochemical results showed a significant difference (p < 0.05) in BUN and creatinine values in different groups of rat and mice. In mice the highest values of the BUN (1–1.5 times in comparison with control group) and creatinine (2 times in comparison with control group) were seen on the third day in group 3 and on the fourth day in group 4. While in rat the highest values of both BUN (2–2.5 times in comparison with control group) and creatinine (2 times in comparison with control group) were seen on the fourth day in group 4.

Discussion

In recent years, experimental oleander toxicosis has been conducted in rats [12, 13], mice [14], rabbits [15, 16] and chickens [17]. Some reports showed that the plant can be used as a rodenticide and insecticide [18]. For example, the tribes used oleander (Nerium indicum) plant parts as rat poison in Maharashtra [13]. It seems that different species of animals have different susceptibility to the poisoning with N. oleander, all parts of which, either fresh or dried, are toxic. For this reason, in the present study, comparative susceptibility of mice and rats was studied.

Oleander is originally a Mediterranean and Asian plant and is widely distributed in the world, especially in tropical and subtropical regions. Apparently, in some eras, different parts of the plant have been used as rat poison [13]. But few experimental studies have been conducted in this field in rats and mice [12–14]. Which have not studied the various aspects of this intoxication. For this reason, in the present study, biochemical parameters and histopathological features of comparative oleander intoxication in mice and rats were performed experimentally.
In most literature, the same clinical symptoms with different intensity have been reported which include mainly nervous and gastrointestinal signs such as anorexia, restlessness, crying, ataxia, pawing of the ground, convulsion, falling and turning of the head backwards, paralysis, sluggishness, feeble or no muscular movement and abdominal contractions. Similar signs were also observed in the present study. Recently, biochemical examination of mice after oral administration of *N. oleander* for 2 and 4 weeks (chronic toxicity) in comparison with control, showed significant increase in the AST and ALT activities which, in both enzymes were 2–3 times higher compared with control [23]. In addition, those animals did not have any mortality with experimental chronic toxicity. In an experimental study that had been done on chronic toxicity of *N. oleander* in rabbits, 20% mortality was reported to be associated with nervous signs. Moreover, these researchers had recorded significant differences in the values of total protein and albumin on 30, 60, 90 and 120 days after treatment. In another study, in an experimental acute toxicity of *N. oleander* in rabbits, significant increase in the AST and ALT activities has been reported [15]. Similar signs have been reported in acute toxicity of bandicoot rat [13] which showed 100% mortality with the dosage of 12.5 mL/kg of crude extract of *N. indicum* and 10, 25 and 50% mortality have been recorded with 5, 7.5 and 10 mL/kg. In the present study, there was not any mortality with different dosages in both mice and rats. Also, severe increase (15 times) in the values of AST was observed in rats compared with rat control group. While, mice showed a 4–5 time increase (as compared with mice control group). Also, in the present study, the results of the biochemical tests showed slight changes in values of the total protein (increased) and albumin (decreased) which were not significant between different groups. Hyperproteinemia and hypoalbuminemia have also been recorded in chronic oleander toxicity of rabbits [13]. The hyperproteinemia was usually observed in the dehydrated animals and also in animals that were suffering from anorexia and their livers were not efficiently synthesizing protein, thus total protein values were usually observed with liver diseases. Hypoalbuminemia may be attributed to inhibition of its synthesis, its rapid breakdown and its losses [24–26].

In the previous studies nephrotoxicity of *N. oleander* in different animals was demonstrated. For example, in chronic toxicity of rabbits by *N. oleander* extract significant increase in the blood urea nitrogen and creatinine levels was reported [16]. Also, the main lesions in sheep treated with daily oral doses of *N. oleander* included nephropathy and gelatinization of the renal pelvis and were accompanied by significant increases in serum bilirubin and urea concentration [9]. The results of the present study correspond with their findings which resulted in renal impairment. It seems that because of the severe damage to the glomeruli and renal tubules and reduced renal perfusion, creatinine and BUN levels changed, especially in rat. These findings showed progressive damage to the kidney of rat which showed the most changes on the fourth day. When a large number of nephrons are disabled, increased levels of these two enzymes are observed. Even a slight change in the values of these two factors should be considered [22, 26]. According to the results of the present study it seems that rats have higher susceptibility to nephrotoxicity effects of the *N. oleander* toxicity rather than mice in the same condition.

As already mentioned, experimental intoxication due to this plant has also been induced in various animal models (rabbit, mice, rat), but the data on gross and histopathological changes in animals or human patients is rare. Recently, in the liver of broiler chickens with experimental oleander (*N. oleander*) intoxication showed coagulative necrosis of hepatocytes with hyperemia and hemorrhage [17]. Also, in cattle with experimental oleander (*N. oleander*) poisoning multifocal degenerative and necrotic changes with inflammatory cell infiltration in the liver parenchyma were reported [7]. These researchers also reported more severe pathological lesions in higher dosages. Histopathological examination of the present study also revealed multifocal degenerative and coagulative necrosis of hepatocytes with hyperemia and hemorrhage associated with mononuclear inflammatory cell infiltration which were dose dependent and more severe in Balb/c mice. According to the statistical analysis of histopathological lesions, in higher dosages of intoxication (15, 20 mg/kg) significant differences were observed between necrosis, hemorrhage and bile duct hyperplasia in mice compared with rats. Necrosis and hemorrhage did not have any significant differences in different groups of rat and all dosages showed mild lesions. Generally, in histopathological lesions in different groups of mice and also rat, there were significant differences between lower dosages (10, 12.5 mg/kg) and higher dosages (15, 20 mg/kg).

In conclusion, in this study, changes in levels of AST in rats were much more severe than in mice. However, in histopathological examination of the liver in rats, compared with mice, moderate lesions were observed. While the liver of the mice showed severe pathological lesions. But, the biochemical changes of AST in rats were milder than mice. On the other hand, the values of BUN in rats show much more severe changes rather than mice and
more severe pathological lesions were observed in histo-pathological studies which indicate that small changes in BUN levels can be due to severe pathological lesions in the kidney tissue. Therefore, according to the biochemical and pathological results of the present study, it seems that rats have more susceptibility to the nephrotoxicity effect of *N. oleander* poisoning (in low dose) and in mice, there is more susceptibility to the hepatotoxicity of this poisoning (in low dose). These findings can be considered in cases of medical use of the plant as mentioned previously [18, 19] such as indigestion, fever, ringworm, leprosy, venereal diseases, also as cardiac drugs and antidiabetic agent.

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