Plant Archives Volume 20 No. 2, 2020 pp. 7840-7846 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ACUTE TOXICITY OF LEAVES ETHANOLIC EXTRACT OF *NERIUM OLEANDER* (ALDEFLA) IN LOCAL IRAQI RABBITS (*LEPUS CUNICULUS*)

Duaa F. Hassan* and Falah M. Kadhim

Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad, Baghdad. Iraq

Abstract

This study was conducted to evaluate the acute toxicity of the one of worldwide botanical *Nerium oleander*. First of all, the leaves of plant had collected from *Nerium oleander* tree which grew up in Alneel distinct, Babil province, Iraq during August 2019. Leaves powder have been extracted by cold method of extraction by soaking in 95% ethanol. Median lethal dose (LD_{50}) of plant extract was performed in seven local healthy male Iraqi rabbits (*Lepus cuniculus*), up and down method have been used for deriving LD_{50} after oral administration. Animals were observed during 24 hr of extract administration for any signs of toxicity in all subjected animals, while necropsy finding and histopathology for several internal organs like lung, stomach and heart have been studied in mortal animals only. The results showed, the yield extract of plant is 9.02%, while the LD_{50} of leaves ethanolic extract is 232.23 mg/Kg.BW orally after 24 hours. Various clinical signs of toxicity were observed, briefly, salivation, dyspenea, piloeraction, hind limbs extension, depression, convulsion, gasping etc. Necropsy finding included emphysema, pulmonary congestion and bronchopneumonia, mild endocarditis, fibril layer on pericardial infiltrate and inflammatory cells filaments, in stomach specifically, in glandular region there were severe erosion, lysis of mucosa extend to submucosa, most glands in submucosa show severe necrosis.Various histopathological changes were observed such as pulmonary congestion, thickening of alveolar walls, dilated pulmonary blood vessels, severe degeneration of bronchial epithelial mucosa and necrosis, also there were pleuritis and pulmonary emphysema.Heart showed, infiltration and extension of inflammatory cells to myocardium and acute pericarditis, thickened inflammatory layer replaced the flattened epicardium .whereas in stomach there were severe atrophic necrosis and sloughing of mucosal epithelium, the sub mucosal glands severely degenerated and necrotic.In conclusion: The ethanolic cold extraction is one of the useful method for obtaining significant yield extract of *N. Oleander* leaves. The *N. Oleander* leaves ethanolic extract is considered severely toxic in rabbits after oral administration.

Key words: *Nerium*, *oleander*, lethal dose, ethanolic extract, rabbit, histopathology.

Introduction

Nerium oleander known by local Iraqi name Aldefla, is a toxic plant of the *Apocynaceae* family to humans, animals and insects (Khordadmehr and Nazifi, 2018). It is a small tree evergreen with a wide distribution in world. Its flowers grow in clusters in incurable branches and show various colors including yellow, pink, red, peach and white. All parts of the plant can be very toxic to insects, several of animals and humans(Farkhondeh *et al*., 2020; Ghurghure *et al*., 2019). Children are very susceptible to the *N. oleander* toxicity. Unintentional ingestion of plant in children and use of this plant for suicide are two main lead to *N. oleander* toxic in the world. The important clinical finding consists of vomiting, nausea, diarrhea, abdominal pain, arrhythmias and hyperkalemia. The significant toxic impact of *Nerium oleander* poisoning is cardiotoxicity (ventricular arrhythmia, bradycardia, tachycardia (Farkhondeh *et al.,* 2020). *Nerium Oleander* poisoning is not uncommon in domestic animals and man and there have been some cases of dangerous toxicosis in children and adults. *Nerium oleander* is a plant that is often grown in gardens and public areas. Various Pharmacological effects of *Nerium oleander* including antinociceptive, anti-inflammatory and anticancer activity were reported (Salih 2017). The extracts of many parts of *Nerium oleander* Leaves are used as antidiabetic remedy in the traditional medicinal systems of many parts of the world (Dey *et al*., 2019). *Nerium oleander* is used to produce cardiotonic medicine, this plant used as an anti-cancer

Author for correspondence : E-mail: dr.duaa.pharma@gmail.com

medicine and alternative medicine for many causes (Azzalini *et al*., 2019), but the potential toxic effects of all parts of the shrub either fresh or dried on animal and human body were documented (Serra, 2016). It contains numerous toxic compounds. The major toxic components found are the cardiac glycosides oleandrin and neriin (Abdou, Basha and Khalil, 2019).

Aldefla is toxic when consumed, the cause of toxicity is mostly due to the presence of cardiac glycoside particularly oleandrin, firstly it has a digitalis action. Many attempts are made to use it as cardiac tonic but not be successful due to narrow therapeutic index. Other toxic constituents of this plant is cardinolides and niriine which are glycosides, second the bark of the plant have rosagenin which has a strychnine action, three the signs of toxicity and the causes of death is the similar for other cardiac glycosides as digoxin (Li *et al*., 2020).

The toxic effects of plant or their active alkaloids lead to infiltration of cells with hemorrhage and severe negative variations in the lung, induce lesions and infiltration of inflammatory cells into the portal places with scattered necrosis of hepatocytes in the liver, heart toxicity of the plant in the cardiac were included, induced multiple degrees of hemorrhage, myocardial degeneration and necrosis. It also lead to arrhythmia, sinus bradycardia and in ECG records prolonged P-R interval. The toxic effects of *N. oleander* are typically related to its inhibitory effects on the *Na-K ATPase* pump in the cellular membrane (Karthik *et al*., 2020).

Due to wildly distribution of this plant which is may contribute into various toxicosis in animals which may grazing and in human regarding un-intentional ingestion. We are planned to assessment its acute toxicity which is considered the important tool for assessment the risk of any chemical, biological and botanicals.

Fig. 1: *Nerium Oleander* (Aldefla) from AL Neel district, Babel, Iraq.

Materials and Methods

Leaves of plant *Nerium oleander* are collected from Babil province, A.L. Neel at 2019 August (Fig. 1). The plant is 3 meters in long with dark-green leathery lanceolate leaves and has white flowers. It is dried at room temperature with opened windows and door for one week, then grind by using electrical grinder and kept in the tightly stopped glass bottle and kept in the refrigerator under 4°C till used. The powder of *Nerium oleander* leaves is extracted by ethanol (Chem. Lab NV, Belgium). Fifty g of plant dried powder have been soaked into 500 ml of 95% ethanol with continuous stirring for two hours by using magnetic stirrer machine and kept in conical flask in ice bath for 24 hours in refrigerator, then filtered by using piece of gauze, after that the filtrate filtered one more by using apparatus of Buchner through 0.4 millipore filter under negative pressure (27). The yield extract was calculated by applying the following equation:

$$\text{Yield extract} = \frac{\text{weight of extract (gm)}}{\text{weight of } N \textit{ oleander} \text{ crude powder (gm)}} \times 100$$

(Banso and Adeyemo, 2006).

• **Animals:** A total of seven male local Iraqi rabbits (*Lepus cuniculus*) purchased from the animal house of college of veterinary medicine, university of Baghdad, their weight range is 1000-1700 gm and age 6-12 month, the animals raised in the animal house of college of veterinary medicine, university of Baghdad for four weeks to acclimatization under suitable condition of temperature 25±1°C and dark/light cycle 12/12 hours, the animals fed *ad labium* with standard pellet, green grass and water in air-conditioned room.

Assessment of Median Lethal Dose (LD_{50})

The median lethal dose of leaves ethanolic extract of *N. oleander* is assessed by up and down method (Dixon, 1965) briefly, *N. oleander* leaves extract was given orally by gastric gavag to the animals at range of

Fig. 2: Ehanolic extract of *N. oleander* leaves extract.

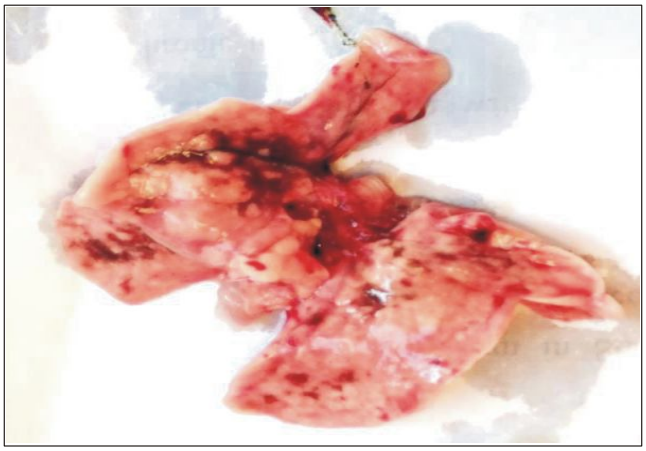

Fig. 3: Lung of rabbit after administration of *Nerium oleander* leaves by ethanolic extract at dose 240 mg/kg Bw.

doses between 150-240 mg/kg.BW and the difference in doses was 30mg/kg.BW. LD_{50} was calculated after 24 hours observation of dead and life animal by applying the following equation:

LD_{50}= xf + kd

Xf = last dose administrated,

K= constant, d= difference between dose

• **Pathology:** Each dead rabbit has been dissected as soon as for necropsy finding and microscopic lesion of several organs like heart, lung and stomach. The animal is examined grossly after 10 minute from death. The organs preserved in 10% formalin buffer for fixation before embedding the tissues in paraffin block, 3-10 micron thickness of histological sections were prepared by microtome. All histological sections have been stained by hematoxylin and eosin stain (H&E) (AL-Naqeeb and Yousif, 2011).

Results

Extraction yield of *N. oleander* leaves ethanolic extract is dark green in color, amorphous crystals (Fig. 2), it is yield about 54.12g from 600 g crud powder which is representing 9.02%.

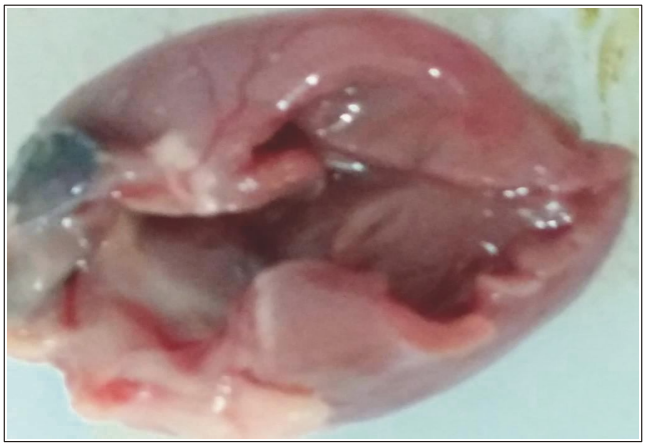

Fig. 4: Heart of rabbit after administration of *Nerium oleander* leaves by ethanolic extract at dose 240 mg/kg Bw.

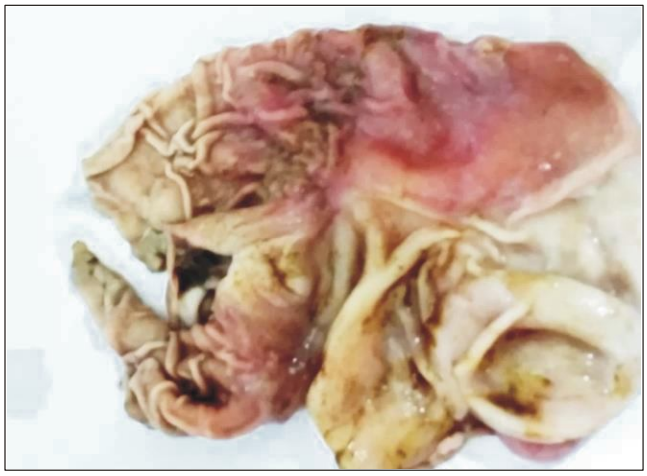

Fig. 5: Stomach of rabbit after administration of *Nerium oleander* leaves by ethanolic extract at dose 240 mg/kg Bw.

The oral median lethal dose (LD_{50}) of *N. oleander* ethanolic extract that found by up and down method is 232.23 in local male Iraqi rabbits (*Lepus cuniculus*) for 24 hr (Table 1).

LD_{50} = xf +kd

LD50 = 210 +(0.741*30)

LD_{50} = 232.23 mg /kg.BW orally in rabbit after 24 hours.

Kd = 0.741

• **Clinical findings:** The morbid rabbits showed various signs of poisoning like depression, pawing the ground, anorexia, piloeraction, dyspeniea, slightly watery salivation, extended of hind limb while the fore limb is unaffected, tachypeniea, froth in the mouth. Table 2, shows the most important symptoms with the timing of their emergence and disappearance within 24 hours after the dose with the leaves ethanolic extract of *N. oleander*

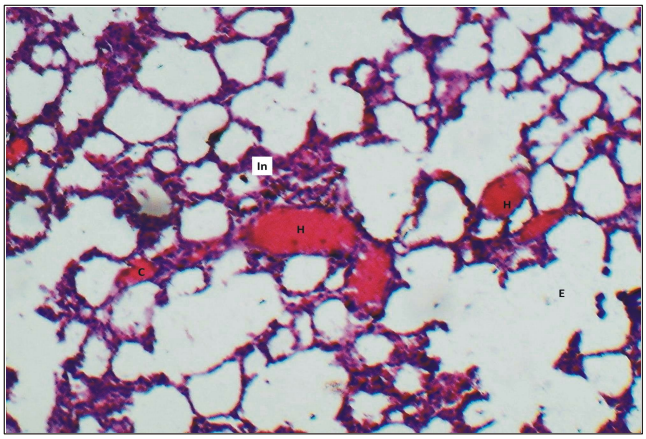

Fig. 6: Section of lung shows: mild interstitial pneumonia (In), pulmonary emphysema (E) & congestion (C). H&E stain. 40x

Table 1: The outcome of median lethal dose (LD50) of orally administration of ethanolic extract of *Nerium oleander* leaves in Local Iraqi rabbit (*Lepus cuniculus*) calculated by up and down method.

Initial dose mg/kg .BW	Different between doses mg /kg .Bw	Last dose	Outcomes*	LD_{50} orally mg/kg .BW after 24 hr
150	30	210	OOOXOXO	232.23
* X= Dead animal; O= alive animal				

for different doses.

• **Necropsy Finding:** The necropsy finding of dead animals showed, there were severe emphysema, pulmonary congestion and bronchopneumonia (Fig. 3), while in heart we are observed was clots, fibrinous exudate, mild endocarditis, fibril layer on pericardial infiltrate and inflammatory cells filaments, (Fig. 4) and, in stomach specifically, in glandular region there were severe erosion, lysis of mucosa extend to submucosa most glands in submucosa show severe necrosis (homogenous and structure less) (Fig. 5).

Histopathological finding

The histopathological finding of organs rabbit after administration of ethanolic extract of *Nerium Oleander* leaves at dose 240 mg/kg Bw. orally lungs in rabbit which have been observed are pulmonary congestion, thickening of alveolar walls, dilated pulmonary blood vessels filled with blood and few inflammatory cells, severe degeneration of bronchial epithelial mucosa and necrosis, also there were pleuritis and pulmonary emphysema (Fig. 6). Heart showed, infiltration and extension of inflammatory cells to myocardium and acute pericarditis, thickened inflammatory layer replaced the flattened epicardium (Fig. 7). Whereas the histopathological finding in stomach included severe atrophic necrosis of mucosal epithelium, complete sloughing in other section, the sub mucosal glands severely degenerated and necrotic of stomach) and showed the submucosal glands severely degenerated and necrotic (Fig. 8).

Discussion

Acute toxicity defines the adverse effects of a substance that outcome either from a single exposure or from many exposures in a short period of time (typically less than 24 hours). To be pronounced as acute toxicity, the adverse effects should happen within 14 days of the administration of the substance, also refer to poisonous state (Hatif, Kafi and Alkhayyat, 2010). *Nerium Oleander* leaves decoction give to male rat injected intramuscularly in both hind limbs in two groups,group one injected 5 ml/kg and group two injected 10 ml/kg. The results refer to both doses can induce acute phase condition (Abbasi *et al*., 2017; Klaassen and toxicology, 1986).

Median lethal dose (LD_{50}) is the dose essential to kill half the members of a examined population after a specified test duration. It is a tool to measure the acute toxicity of a chemical active ingredient. Also use to detect the toxicity of plant, to ass therapeutic dose of plant from through test LD_{50} (Saliem, 2010). The estimated LD_{50} of ethanolic extract of *Nerium Oleander* leaves in current study is 232.23 mg /kg.Bw orally in rabbit could be scheduled in the grade 4 severely toxic agent according to classification dependent by (Hayes and Loomis, 1996). The clinical signs that have been observed on exposed rabbit included depression, anorexia, piloeraction, dyspeniea, slightly watery salivation, extended of hind limb while the fore limb is unaffected and tachypeniea. These clinical signs are in agreement with those observed after one hour in mice exposed ethanolic extract of *Nerium oleander* leaves at of 520 mg/kg body weight

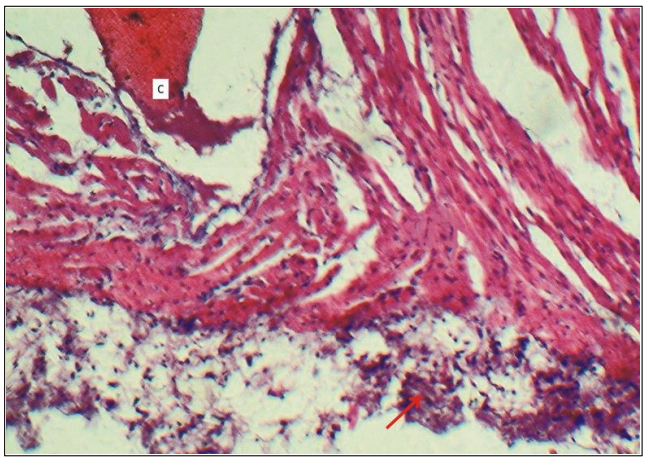

Fig. 7: Section of heart shows: mild endocarditis with clot (c), fibrin exudate and inflammatory cells (red arrow). H&E stain. 40x.

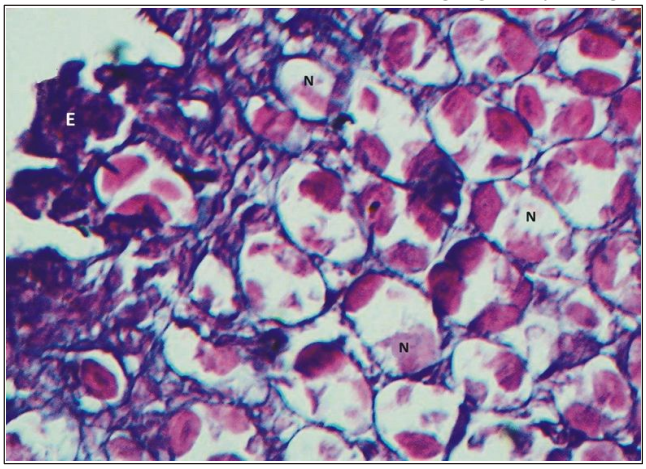

Fig. 8: Section of stomach shows: (E) erosion and, (n) sever degeneration and necrosis of gastric glands (Arrows). H&E stain. 40x.

Table 2: Signs of poisoning and mortality outcome that developed according to different doses of *N. Oleander* leaves Ethanolic Extract in Local Male Iraqi Rabbits **(***Lepus cuniculus*) according to Up and Down method.

Dose mg/kg BW	Clinical signs during 24 hrs	Time of signs appeared	Results (O, X)
150 mg	depression, anorexia, piloeraction, dyspeniea, slightly watery salivation, extended of hind limb while the fore limb is unaffected, tachypeniea.	After five hours from administration	O
180 mg	depression, anorexia, piloeraction, dyspeniea, slightly watery salivation, extended of hind limb while the fore limb is unaffected, tachypeniea.	After three hours from administration	O
210 mg	depression, anorexia, piloeraction, dyspeniea, slightly watery salivation, dragging of hind limb while the fore limb is unaffected, tachypeniea.	After three hours from administration	O
240 mg	sever convulsion, gasping,slightly watery salivation, piloeraction,extruded of eye ball, head down, incordination, circuling around itself .	After 15 minute from administration	X
210 mg	depression, anorexia, piloeraction, dyspeniea, slightly watery salivation, dragging of hind limb while the fore limb is unaffected, tachypeniea.	After two hours from administration	O
210 mg	depression, anorexia, piloeraction, dyspeniea, slightly watery salivation, dragging of hind limb while the fore limb is unaffected, tachypeniea.	After two hours from administration	O

was administered orally, the signs of toxicosis have appeared, abdominal pain, frequent urination, weakness, diarrhea, depression, convulsive movement and death (Saliem, 2010). While clinical signs of aqueous extract of *Nerium oleander* in female goat that appeared after 1 hr from receiving the extract were abdominal pain, convulsion, ruminalatony, depressin, convulsion and death (Aslani *et al*., 2007). The clinical signs that observed on rabbits of our study were not fully agree with a study conducted in dogs have been administered orally 0.25g.kg^{-1} of fresh ground leaves of *Nerium oleander*, when several dogs died after the ingestion, but all showed signs of poisoning such as vomiting, diarrhea, nausea, apathy, conjunctiva congestion, dehydration, abdominal pain, tremors, diarrhea, loss of appetite and tenesmus. Loss of appetite was prominent in 30% of the dogs even after the treatment. The first obtainable clinical sign was vomiting, within 27- 75 minutes of administration of ground green leaves. Second presented sign was loss of appetite, after the intoxication and after the treatment, with 40% of the animals having these signs for as long as 12 hours after giving (Camplesi *et al*., 2017). The clinical signs appeared in sheep after 30 minute from take dried *Nerium Oleander* leaves were decrease heart rate (cardiac pauses), tachyarrthemia, mild to moderate tympany, abdominal pain, polyuria and pollakiuria (Aslani *et al*., 2004).

The estimated LD_{50} of ethanolic extract of *Nerium oleander* leaves in current study is (232.23 mg /kg.Bw orally in rabbit is more than the LD_{50} s/c injected aqueous leaf extract *Nerium oleander* in rabbit which was 157.37 mg / kg B. wt, but fall into the same grade of toxicity (Al-Badrani, Rhaymah and Al-Farwachi, 2008). While the median lethal dose of leaves *Nerium oleander* ethanolic extract is 520 mg/kg body weight were giving orally to male mice (Saliem, 2010). Whereas several studies found the orally LD_{50} in mice of hexane leaf extracts of *N oleander* has red, pink and white flowers was 325, 300 and 350mg/kg respectively (6). The oral LD_{50} of aqueous extract of *Nerium oleander* female goat is 110mg/kg BW (12). The LD_{50} that has been found in rabbits in current study is disagree with the LD_{50} of *N. oleander* leaves collected from Amiriya /Baghdad-IRAQ in summer and winter, which were extracted by hexane and derived by "up and down" method were 94.36, 79.75 mg/kg BW respectively (Salih and Alkhayyat, 2016), on the other hand the oral LD_{50} of dried *Nerium Oleander* leaves was 110 mg /kg.BW in sheep which possessed clinical signs of poisoning appeared too early after 30 minutes of administration (Aslani *et al*., 2004), whereas the oral LD_{50} of *N. oleander* leaves after a simple extraction from biological samples, for example- in cattle is 50 mg/ kg.BW. The leaf fragments found in the rumen contents were small and not simply isolatable from the rest of the contents. Though, there were leaves in the forage that were clearly distinct by the direct examination. Oleander poisoning due to active components contained in the plant are cardiac glycosides belonging to the cardenolides that are very toxic to many species, from human to insects (Rubini *et al*., 2019).

The toxicity of *Nerium oleander* due to the present of the highest concentrations of cardiac glycosides are in the seeds and roots of the common pink *Nerium oleander*, while the highest component of oleandrin are found in the leaves of the plant. Furthermore, *N. oleander* with red to pink flowers has higher concentrations of cardiac glycosides than *Nerium oleanders* with white flowers (Khordadmehr and Nazifi, 2018). So the thought of decrease in LD_{50} rate resulted from administration *N. oleander* leave ethanolic extract to rabbits in current study comparable to that found in different literature may be due less abundant of oleanrinin leaves of plant with white flower which has been used. *Nerium oleander* Leaves have high amount of cardiac glycoside and flavonoids compounds from methanolic extract of flower (Vu, Bui and Nguyen, 2016). Oleander toxicity is a common problem in several parts of the world. Oleander poising due to oleandrin (the chief cardiac glycoside of oleander) and neriin, which lead damage by inhibiting the plasmalemmal Na^+/K^+ ATPase (Abdou, Basha and Khalil, 2019). Oleander contains cardiac glycosides called cardinolide. Cardiac glycoside may cause hyperkalemia because of ability to inhibit the Na+K+-ATPase pump, leading to increased intracellular sodium and calcium and decreased intracellular potassium (Salih, 2017). The concentration of oleandrin in *N. oleander* tissues is about 0.08% (Salama *et al*., 2019). *Nerium oleander* is cardiotoxic plant and Its toxicity is due to the content of cardioactive glycosides, specially oleandrin, found throughout the plant (Botelho *et al*., 2018). The ethanol extracts of *Nerium oleander* leaves which have the highest amount of entirephenolics and flavonoids, appeared the highest antioxidant and cause toxicity of plant (Einali *et al*., 2018). Oleander poisoning due to active components contained in the plant are cardiac glycosides belonging to the cardenolides that are very toxic to many species, from human to insects (Rubini *et al*., 2019).

Extraction yield is mass of extract or mass of dry matter is used as indicator of the extraction condition and it is the amount of component of interest obtained from extractions output. The effect of extraction environments on yield. There are several steps to get the phytochemicals from plant, for example milling, grinding, homogenization and extraction. The extraction method is the important step to minimize interfering from compounds that may co-extract with the board compounds. It is supposed that bioactive compounds from plant foods may have health beneficial effects and reduce the risk of diseases (Dhanani *et al.,* 2017). The yield of *Nerium oleander* leaves extraction has been extracted by 95% ethanol is 9.02%. This is closely with ethanol extract of *Nerium oleander* leaves was (8.46%) extracted with absolute ethanol 100 ml for 24 h. at room temperature, were collected in late May 2014 from University of Sistan, Baluchestanin southeast of Iran (Einali *et al*., 2018) and the our result better than this studies by the non-polar solvents via petroleum ether (2.87%) was less as compared to the yield obtained from the polar solvents by ethanol and water 5.7% and 6.8% respectively. The fraction yield obtained from the successive extraction using the solvents petroleum ether, ethanol and aqueous extract obtained by maceration, from root of Nerium Oleander (Kawalekar *et al*., 2012), while disagree with the yield of extract 26%, 18% by Soxhlet method by the two hydro-methanoic and hydro-ethanolic extracts respectively of *Nerium oleander* leaves 20g of fresh leaves extract by ether petroleum and then dry for 10 minutes at room temperature, after that solute is placed in Soxhlet (Malika, Badiaa and Farida, 2016). The other studies showed extraction yields for every kilogram of leaves *Nerium oleander* by ethanolic extract was 1.03% (de Melo *et al.,* 2020).

Conclusion

The ethanolic cold extraction is one of the useful method for obtaining significant yield extract of *N. Oleander* leaves. The *N. Oleander* leaves ethanolic extract is considered severely toxic in rabbits after oral administration.

Conflict of interest

The authors declare that they have no conflict of interest

Acknowledgments

The authors are highly appreciable to department of physiology, biochemistry and pharmacology, College of Veterinary medicine University of Baghdad for providing equipments.

References

Abbasi, M.H., S. Fatima, M.B. Khawar, N. Naz, K.A. Mujeeb and T. Akhtar (2017). Dose-dependent acute phase response of aqueous leaf decoction of Nerium oleander in Wistar rats. *Journal of biological regulators Sheikh and homeostatic agents,* **31**: 985-89.

Abdou, Rania H, Walaa A Basha and Waleed F %J Toxicological research Khalil. 2019. 'Subacute Toxicity of Nerium oleander Ethanolic Extract in Mice', 35: 233.

Al-Badrani, BA, MS Rhaymah and MI %J Iraqi Journal of Veterinary Sciences Al-Farwachi. 2008. 'Acute toxicity of Nerium oleander aqueous leaf extract in rabbits', 22: 1-4.

AL-Naqeeb, MMN and AA %J Iraqi Journal of Veterinary Sciences Yousif. 2011. 'Electron microscopic study of ileum of mice infected experimentally with Salmonella hadar', 25: 55-60.

Aslani, Mohammad Reza, AR MOVASAGHI, PIROUZ H JANATI and M Karazma. 2007. 'Experimental oleander

(Nerium oleander) poisoning in goats: a clinical and pathological study'.

Aslani, Mohammad Reza, Ahmad Reza Movassaghi, Mehrdad Mohri, A Abbasian and M %J Veterinary research communications Zarehpour. 2004. 'Clinical and pathological aspects of experimental oleander (Nerium oleander) toxicosis in sheep', 28: 609-16.

Azzalini, Elena, Marzia Bernini, Sara Vezzoli, Anna Antonietti andrea %J Journal of forensic Verzeletti and legal medicine. 2019. 'A fatal case of self-poisoning through the ingestion of oleander leaves', 65: 133-36.

Botelho, Ana FM, Fabiano AS Oliveira, Aparecida TL Fiúza, Heloísa P Pedroza, Stephanie EMT Branco, Felipe Pierezan, Marília M Melo and Benito %J Pesquisa Veterinária Brasileira Soto-Blanco. 2018. 'Improved method for diagnosis of Nerium oleander poisoning in necropsy tissues', 38: 967-72.

Camplesi, Annelise Carla, Carolina Bellodi, José Javier Mesa Socha, Mário Roberto Hatayde, Márcia Ferreira da Rosa Sobreira, Gustavo Henrique Marques Araujo and Carla Fredrichsen Moya %J Ciência Rural Araujo. 2017. 'Dogs poisoned with Nerium oleander fresh leaves: clinical and electrocardiographic findings', 47.

de Melo, Bruna Silva, Brenda Pinto de Morais, Vaniza Sheila de Souza Ferreira Sá, Filipe Dantas Lourinho, Ingrid Perpétuo Socorro Pinheiro Toda, José Luíz Martins do Nascimento, Dienifer Negrão Marques, Márcia Cristina Freitas da Silva, Giuliana Thaissa Modesto Cardoso and Luis André Luz %J Neurotoxicology Barbas. 2020. 'Behavioural, electrocorticographic and electromyographic alterations induced by Nerium oleander ethanolic extract: Anticonvulsant therapeutics assessment', 78: 21-28.

Dey, Priyankar, Manas Ranjan Saha, Sumedha Roy Choudhuri, Indrani Sarkar, Biswajit Halder, Mousumi Poddar-Sarkar, Arnab Sen, Tapas Kumar %J Advances in Pharmacological Chaudhuri and Pharmaceutical Sciences. 2019. 'Oleander Stem and Root Standardized Extracts Mitigate Acute Hyperglycaemia by Limiting Systemic Oxidative Stress Response in Diabetic Mice', 2019.

Dhanani, Tushar, Sonal Shah, NA Gajbhiye and Satyanshu %J Arabian Journal of Chemistry Kumar. 2017. 'Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of Withania somnifera', 10: S1193-S99.

Dixon, WJ %J Journal of the American Statistical Association. 1965. 'The up-and-down method for small samples', 60: 967-78.

Einali, Alireza, Omid Azizian-Shermeh, Ali %J Journal of Food Measurement Ghasemi and Characterization. 2018. 'Phytochemical screening and antimicrobial activities of Periploca aphylla Decne, Persian walnut (Juglans regia L.) and oleander (Nerium indicum Mill.) Leaf extracts', 12: 1350-59.

Farkhondeh, T, M Kianmehr, T Kazemi, S Samarghandian, MR %J Human Khazdair and Experimental Toxicology. 2020. 'Toxicity effects of Nerium oleander, basic and clinical evidence: A comprehensive review': 0960327120901571.

Ghurghure, SM, AA Dhange, NR Kamalapure, SN Kate, A RKatkar, AV Katta, CU Kattimani, SG %J Research Journal of Topical Kattimani and Cosmetic Sciences. 2019. 'Formulation and Evaluation of Herbal Hand Wash Gel using Nerium oleander', 10: 1-6.

Hatif, Zena Ahmed, Lubna Ahmed Kafi and Ali Aziz %J The Iraqi Journal of Veterinary Medicine Alkhayyat. 2010. 'Acute toxicity study of three type of Nerium. oleander leaves of hexane extract in mice', 34: 194-201.

Hayes, A Wallace and Ted A Loomis. 1996. *Loomis's essentials of toxicology* (Elsevier).

Karthik, Gunasekaran, Ramya Iyadurai, Ravikar Ralph, Vijay Prakash, KP Prabhakar Abhilash, Sowmya Sathyendra, OC Abraham, Catherine Truman, Alex %J Journal of family medicine Reginald and primary care. 2020. 'Acute oleander poisoning: A study of clinical profile from a tertiary care center in South India', 9: 136.

Kawalekar, Jyoti S, P Varsha, N %J International Journal of Pharmacognosy Vijayalakshmi and Phytochemical Research. 2012. 'Preliminary Phytochemical Investigations on Roots of Nerium Oleander, Linn', 4: 134-38.

Khordadmehr, Monireh and Saeed %J Journal of veterinary research Nazifi. 2018. 'Study of troponin, creatine kinase biomarkers and histopathological lesions in experimental Nerium oleander toxicity in rats and mice', 62: 97-102.

Klaassen, Curtis D %J Casarett and Doull's toxicology. 1986. 'Principles of toxicology': 11-32.

Li, Xiao-xi, Da-qing Wang, Cheng-guang Sui, Fan-dong Meng, Shu-lan Sun, Jian Zheng, You-hong %J Biomedicine Jiang and Pharmacotherapy. 2020. 'Oleandrin induces apoptosis via activating endoplasmic reticulum stress in breast cancer cells', 124: 109852.

Malika, Boualem, Osmane Badiaa and Saïah %J VII International Scientific Agriculture Farida. 2016. 'Study of the larvicidal activity of hydro-alcoholic extracts Nerium oleander L. and Ricinus communis L. on Tuta absoluta M".

Rubini, Silva, Sabina Strano Rossi, Serena Mestria, Sara Odoardi, Sara Chendi andrea Poli, Giuseppe Merialdi, Giuseppina Andreoli, Paolo Frisoni and Rosa Maria %J Toxins Gaudio. 2019. 'A Probable Fatal Case of Oleander (Nerium oleander) Poisoning on a Cattle Farm: A New Method of Detection and Quantification of the Oleandrin Toxin in Rumen', 11: 442.

Salama, Islam, Abd El-Megid, HM %J Egyptian Journal of Radiation Sciences Mohamed and Applications. 2019. 'Reflex of Oleandrin Production and Molecular Changes on the Nerium oleander Cell Suspension Culture under UV-A Radiation Stress Effect', 32: 117-24.

Saliem, Ali Hussien %J The Iraqi Journal of Veterinary Medicine. 2010. 'Effect of the median lethal dose of the ethanolic extract of (NeriumOleander) leaves on the histopathology features of the vital organs in mice', 34: 103-09.

Salih, RA and AA %J Al-Anbar Journal of Veterinary Sciences Alkhayyat. 2016. 'Toxic Effect of Nerium Oleander Leaves Extract on Biochemical Parameters in Rabbits Serum', 9: 1-8.

Salih, Rana Abdulla %J The Iraqi Journal of Veterinary Medicine. 2017. 'Toxic effects of subacute exposure to Nerium oleander leaves hexane extract on the heart of rabbits', 41: 54-60.

Serra, Vital Garcia-Espana. 2016. "Nerium plant named 'Juwel'." In.: Google Patents.

Vu, Duc Loi, Thanh Tung Bui and Tien Vung Nguyen. 2016. 'Isolated Compounds and Cardiotonic Effect on the Isolated Rabbit Heart of Methanolic Flower Extract of Nerium oleander L'.