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Histological Study of *Nerium Oleander* on the Kidney, Small Intestine and Brain in the Male White Mice

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Abstract

The present study designed to evaluated the important histological and some biochemical parameters changes after treated with main compassions of Siqua. The *Nerium oleander* is one of the main structure of siqua, the study focused on the tissue structures of kidney, small intestine and brain after treated with the watery extract of *Nerium oleander*. Its caused acute defects in tissue structures of kidney, small intestine and brain of treated white mail mice.After the end of experimental period, the blood samples collecting to evaluation the level of AST, ALT, urea and creatinine in treated animals, then the animals sacrificed for histological study. The current result showed prominent tissue destruction in the cortical region of the kidney in treated animals, the renal corpuscle have significant increase in diameter when compared with control group, wide spaces filled with blood between the proximal and distal convoluted tubules, necrosis lesions in the cortical region and renal tubules wall. The biochemical changes noted significant increase in the enzymes level (AST, ALT, urea and creatinine) of treated group compared with control group.
Keyword :Nerium oleander, kidney, small intestine, brain, biochemical result.

Introduction

Nerium oleander L. is an evergreen shrub elongated up to four meters in height. and belongs to the family – Apocynaceae, spread in tropical Asia *Nerium oleander* L. is cultivated worldwide as an ornamental plant, it is native to the Mediterranean region, also found in Southern Europe and Southwest Asia, but is naturalize very easily and in many areas the plant is sub-spontaneous (WCSP, 2014).

Leaves are narrow, elongated from 10 to 20 cm, acute in the apex, shortly petiolate, with a coriaceus dark green blade narrow, short-stalked and dark or grey- green in color and un toothed. Some cultivars have leaves variegated with white or yellow patches, all leaves have a prominent mid rib, are "leathery" in texture and usually arise in groups of three from the stem, the plant have

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terminal flower heads, usually pink or white, each flower is about 5cm in diameter and five petalled although some cultivators have double flowers, Oleander branches characterized with flexibility with green, smooth bark eventually tending to dark grey in mature plant (Diane *et al*., 1999).

All plant parts, including the milky white sap are toxic and can cause an adverse reaction, when uptake by living organisms, plant has numerous toxic compounds, the major toxic components are the cardiac glycosides neriin and oleandrin (Abdou*et al*., 2019).All parts of the plant are highly toxic as they contain several non-digitalis cardiac glycosides,including oleandrin, nerin, digitoxigenin, andolinerin, collectively referred to as cardenolides,due to a relatively highlipophilicity resulting in a rapid and extensive gastrointestinal absorption and a slow urinary excretion rate, the most active molecule is oleandrin(Praveen *et al*., 2012).

When tissues are exposed to damaging conditions, intracellular enzymes leak from injured cells into the systemic blood circulation or may be found in the urine. In general toxicology studies, changes in specific enzyme levels are one of the most common markers of target organ toxicity. The most common measured enzymes arealanine aminotransferase, aspartate aminotransferase, creatinine, ureachanges in oleander toxicity (Khordadmehr*et al*., 2017).

The pathological effects of oleander toxicity on the tissue included cell necrosis with hyperaemia and haemorrhage were observed (Omidi*et al*., 2011) in the brain, scattered necrosis of surface enterocytes areprobably direct effects of the toxins on the vascular endothelial bed (Aslani*et al*., 2007), vacuolation and necrosis in the liver (Mohammed and Abdullah., 2002) and damage in kidney tissues was necrosis of tubular epithelium (Barbosa*et al*., 2008).

Aim of study : To evaluated the histological and biochemical effects of *Nerium oleander on* the organs in white mice.

Materials and methods

1. Experimental Animals: This study was carried out 40 male white mice with weighing was 30 gm, The animals were housed under standard laboratory conditions of light, temperature (25-28ºC) and relative humidity (40 to 45%), the animals were obtained from Drug and Health center in Baghdad province. Animals living in laboratory plastic cages, all cages put in animal house of the college of science in AlMuthanna University, the animals were adapted for 2 weeks feeding with pellets.

2. Plant:*Nerium oleander* leaves collected from markets in Al- Samawa city. The leaves

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cleaned and dried at room temperature then crushed by a blender at the same day of preparation of the extract.

3. Experimental design: Forty male mice were divided into two groups, as a control and treatment groups.20 mice in first group were as control group, 20 mice in second group were as treated group gives orally administration with watery extract of 0.75ml *Nerium oleander* for 30 days.

4. Preparation of histological slide

The tissue samples collected from sacrificed animals which included kidney, small intestine and brain, the samples were collected carefully from the mice organs by standard procedure. The kidney, small intestine and brain samples were washed with normal saline to removed blood droplet, the tissue samples were passed through the histological technique which including many stages fixation, washing, dehydration, clearing, embedding, cutting, staining (Luna,1968). histological changes were observed under a light microscope and snaps were taken.

Result and Discussion:

1. Effects of *Nreium Oleander* on kidney:

Thehistological result of control group showed the renal corpuscle (Fig.1),(Table1).The result noted the diameter of renal corpuscle was (16.35±0.350µm), proximal convoluted tubules was (2.54±0.114µm), distal convoluted tubules was (5.09±0.129µm). The wall of proximal convoluted tubule have limited cells that composed of the proximal convoluted tubules. The cell have oval or elongated nuclei centrally location.

The tissue section of kidney after treated with 0.75 ml of *Nerium oleander*showed the diameter of renal corpuscle was (13.05±0.320µm),(Table1)which have significant decreased compared with control group.The descending branch of Henle loop have narrow lumen with (2.75±0.102µm)in diameter (Table1). The result showed the ascending branch of Henle loop was wider lumen than the lumen of descending branch with (5.23±0.091µm) in diameter (Table1).

The tissue section of kidney after treated with *Nerium oleander* showed the diameter of proximal convoluted tubules (P.C.T) was (4.73±0.127µm) which have significant increased compared with control group (Table1). While the diameter of distal convoluted tubules (D.C.T) was (11.00±0.327µm) showed in the (Table1) which have significant increase compared with control group, the histological result showed wide lumen in the proximal convoluted tubules anddistal convoluted tubules, also noted very thin bowman's space and wide cystic dilation

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(Fig.2). These change may be due to due to the toxin caused increased infiltration and increase pressure on tubules, were these result confirmed with (Taub*et al*., 2011) which noted the high toxicity caused degeneration in the parenchyma of kidney.

The descending branch of Henle loop was (3.89±0.093µm)in diameter (Table1) and ascending branched was (9.18±0.288µm). Both diameters of descending and ascending branches of the Henley loop have significant increased compared with the control group.

The tissue section noted inflammatory cells aggregation in different locations of kidney, so, the results appeared many necrosis lesions in the cortical region of treated kidney (Fig.3), this result may be due to the oleandrin toxicity which causedaccumulation of large amounts of toxin lead to severe destruction inproximal convoluted tubule and distal convoluted tubules and blood congestion was result agreement with (Ni *et al*., 2002) which showed the kidney of goats after exposure to *Nerium Oleander* caused degeneration in the kidney tissue and tubules.

Effects of *Nerium Oleander* on small intestine

The current study showed the histological structures of small intestine in control group which appeared the wall of small intestine, the wall of small intestine composed of four layers which included the first internal layer called the mucosa that composed from sub layers, the outer layer was simple columnar epithelial that rest on the basal lamina. The second layer called submucosa contain blood vessels, the third layer called muscularis is a region of muscle adjacent to the submucosa membrane and last layer called serosa (outermost layer) of the intestine (Fig.4).

The other tissue section of the terminal portion of small intestine showed epithelial cells hypertrophy and the epithelial cells have large vacuoles in their cytoplasm, the outer surface of villi appeared smoothly (Fig.5). These histological changes of small intestine may be due to toxic agent have direct effects on smooth muscles of intestine, these results were confirmed with (Jubb*et al*., 1995) which noted the *Nerium* toxicity caused hypertrophy in intestine and degeneration observed in the organs.

The tissue section noted abnormal structures of submucosal layer under the mucosal layer, prominent space belong the internal core and villi, most tissue section showed separated the base of villi from the under muscular layer in the wall of small intestine, so, showed blood congestions between the basal portions of villi (Fig.6)these results may be due to oleandrin caused separated the villi and hemorrhage, these histological change agreement with (Akhtar*et al*., 2014) which noted the *Nerium oleander* effect on intestine caused congestion and hemorrhage.

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3. Effects of *Nreium Oleander* on brain

The tissue section of brain in control group noted the nerve cells have normal distribution in the cortical regions of brain. The supportive cells also have normal distribution between the nerve cells (Fig.7).

The tissue section of brain have prominent blood hemorrhage and blood congestion in different location of brain, abnormal aggregation of abnormal cells around the hemorrhage, the section showed cellular proliferation beside the infected regions in brain (Fig.8). This result in the tissue section of brain may be because high toxicity of *Nerium Oleander* that lead to cellular destruction of these results similar to (Khordadmehr and Nazifi, 2018) which noted the brain of mice when treated with *Nerium Oleander* caused hemorrhage in brain. So agreement with (Bassim Abdullah J. and Duaa J.M 2020) which noted abnormal cellular destruction and necrosis lesions in deferent locations of brain after treated with halothane in white mice.

The tissue sections of brain showed prominent histological destruction and necrosis in the brain tissue, so, noted nodular formation in irregular shape in different locations of brain, the tissue section showed abnormal cells have oval or spherical nuclei nearly from the nodular lesions, the other cells have large spherical nuclei with granular chromatic material (Fig.9). These histological changes may be because of the *Nerium Oleander* contain oleandrin which penetrated the blood brain barrier which lead to cellular destruction and changes shape these result agreement with (Ni *et al*., 2002) which said oleandrin caused damage in nodular formation and degenerative in nuclei cells.

4. Effects of *Nreium Oleander* on biochemical results

The level of ALT in serum control group was (42.00±1.891) in (Table2). While the level of ALT after orally administered with *Nerium oleander* which have significant increase compared with control group (84.70±7.142) (Table2). This change in value of ALT after treated with *Nerium oleander*agreement with (Al-Farwachi*et al*., 2008) which noted significant increased ALT after treated with *Nerium oleander*.

Table (2) showed the level of AST enzyme in serum of control group was (365.15±12.295). The level of AST after exposure to *Nerium Oleander*(481.55±26.238) wasnon-significant increased compared with control group. This result may be due to oleandrin toxicity caused damage in liver tissue. This result not similar to (Altaee, 2011) which noted significant increased AST and ALT enzyme after oral administration by *Nerium Oleander*.

The present study showed the level of urea in table (4-4) **,** the control group was

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(53.15±1.052), The level of urea after treated with *Nerium Oleander* was non significantincreased compared with control group (77.50±13.098). This result may be due to damage in kidney or inflammation in kidney tissue lead to enzyme leak to blood circulation. This physiologic change non confirmed with (Radostits and Gay, 2007) which noted increased the level of serum urea after treated with *Nerium Oleander*.

The biochemical result noted the level of creatinine enzyme in control group was (0.417±0.0046)(Table2), also the level of creatinine after treated with *Nerium oleander*was (0.569±0.0427) which have significant increased compared with control group showed in (Table2). These change confirmed with (Rahymah*etal*., 2011) which showed the increase the level of creatinine after exposure to *Nerium oleander*.

Table (1) : Statistical information of the kidney parts(µm)

Treatment		Control	*Nerium Oleander*
Diameter	**Renal corpuscle**	16.35±0.350^{a}	13.05±0.320^{b}
	D.C.T	5.09±0.129^{d}	11.00±0.327^{c}
	P.C.T	2.54±0.114^{c}	4.73±0.127^{b}
	A.B	5.23±0.091^{d}	9.18±0.288^{a}
	D.B	2.75±0.102^{d}	3.89±0.093^{b}
	C.D	7.43±0.122^{c}	11.41±0.163^{b}

* Different letters vertically indicate the existence of significant differences between the averages at the possibility of (0.01)

Table (2): The level of ALT, AST, urea and creatinine

Parameters	Control	*Nerium oleander*
ALT	42.00±1.891^{d}	84.70±7.142^{c}
AST	365.15±12.295^{b}	481.55±26.238^{ab}
Urea	53.15±1.052^{bc}	77.50±13.098^{ab}
Creatinine	0.417±0.0046^{b}	0.569±0.0427^{a}

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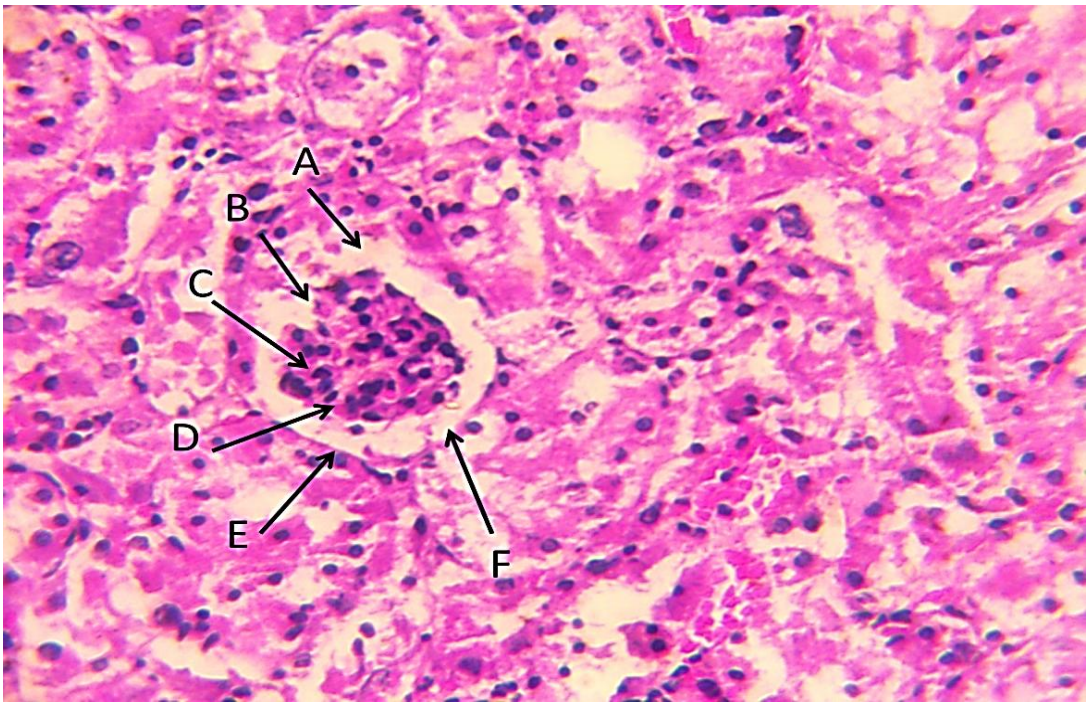

Fig (1): Transverse section of kidney in control group which showed A- Bowman space, B- Mesangial cell, C- Glomerular capillary , D- Visceral layer, E- Inter lobular Bowman capsule, F- Parietal layer. H&E stain 40X.

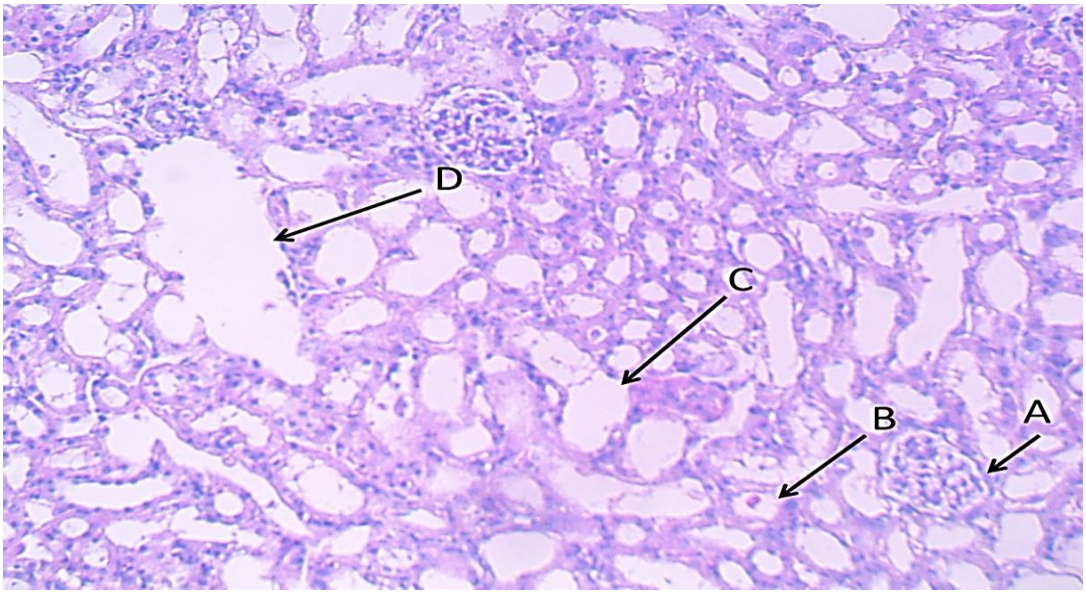

Fig (2): Transverse section of kidney in treated group with *Nerium oleander* which showed A- Thin Bowman space , B- Wide P.C.T lumen, C- Wide D.C.T lumen, D- Wide cystic dilation. H&E stain 40X.

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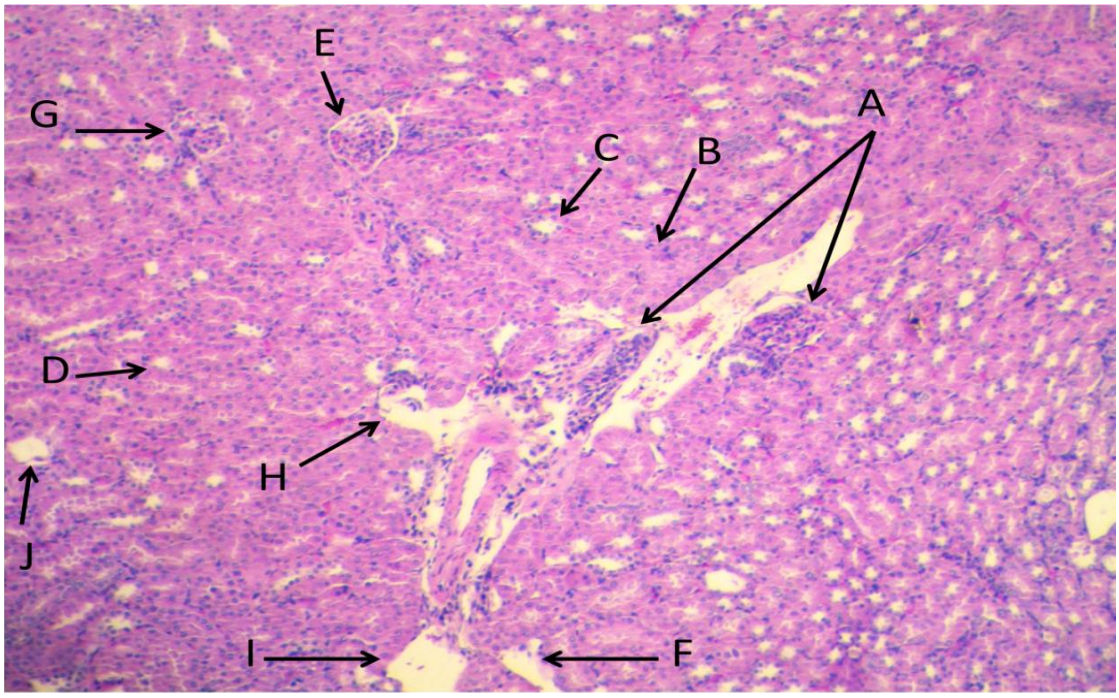

Fig (3): Transvers section of kidney in treated group with *Nerium oleander* which showed A- Abnormal cellular proliferation, B- Inflammatory cells, C- D.C.T, D- P.C.T, E- Abnormal Bowman's space, F- Necrosis, G- Progressive renal corpuscle, H- Necrosis, I- Cystic dilation, J- Necrosi. H&E stain 20X.

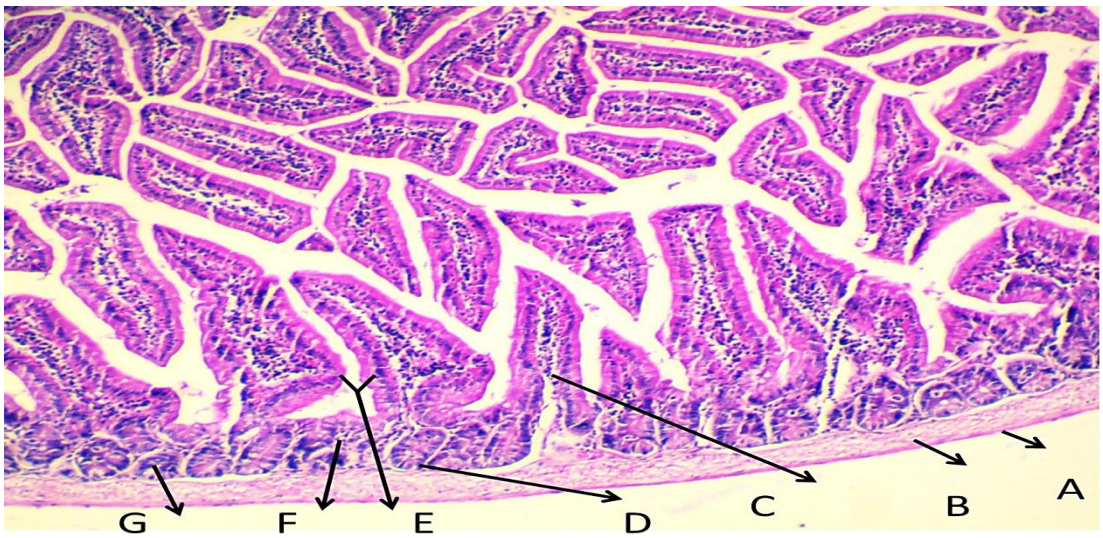

Fig (4): Transverse section of small intestine in control group which showed A-Serosa , B-Muscularis, C-Simple columnar, D-Sub mucosa, E-Villi, F-Blood vessel, G-Intestinal gland. H&E stain 20X.

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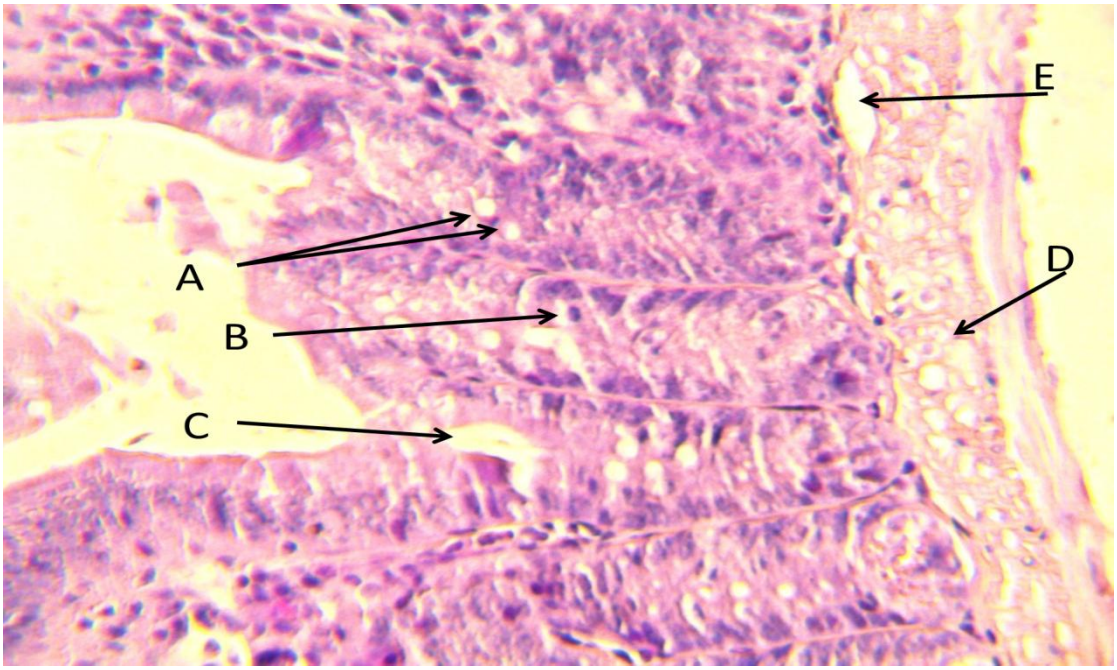

Fig (5): Transverse section of small intestine in treated group with *Nerium Oleander* which showed A-Empty vacuole, B-Cell have dark nuclei, C- Epithelia destruction, D-Weakness of muscle layer, E- Wide space. H&E stain 40X.

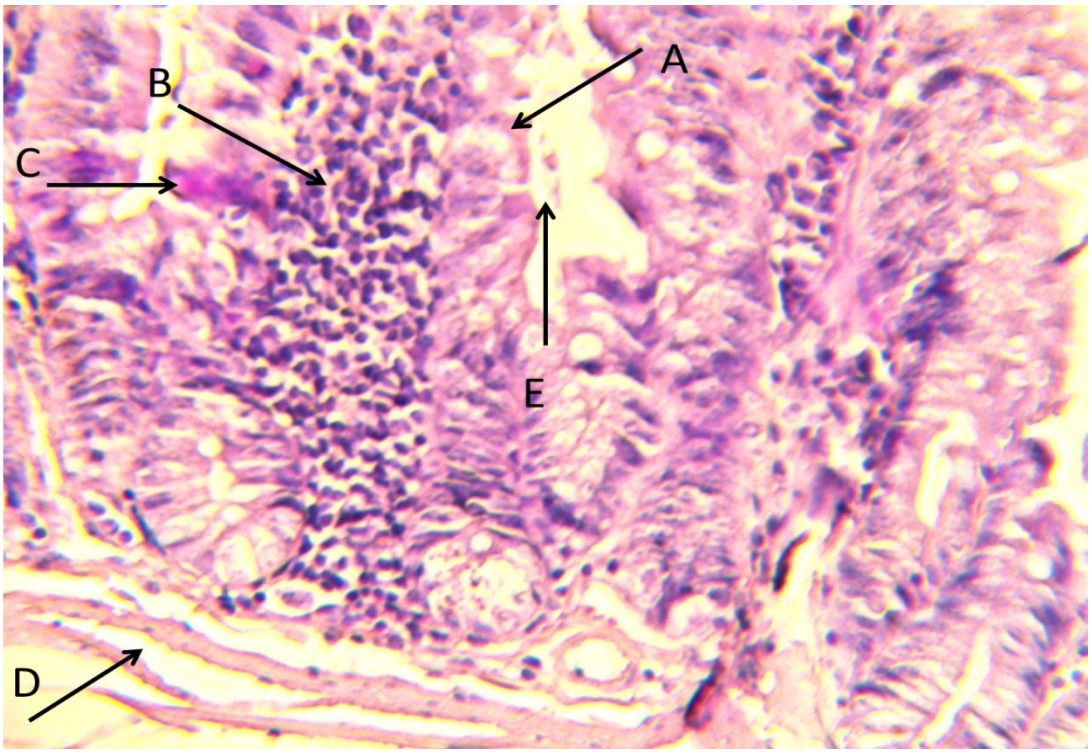

Fig (6): Transverse section of small intestine in treated group with *Nerium Oleander* which showed A-Empty vacuole, B-Cell have dark nuclei, C- Epithelia destruction, D-Weakness of muscle layer, E- Wide space. H&E stain 40X.

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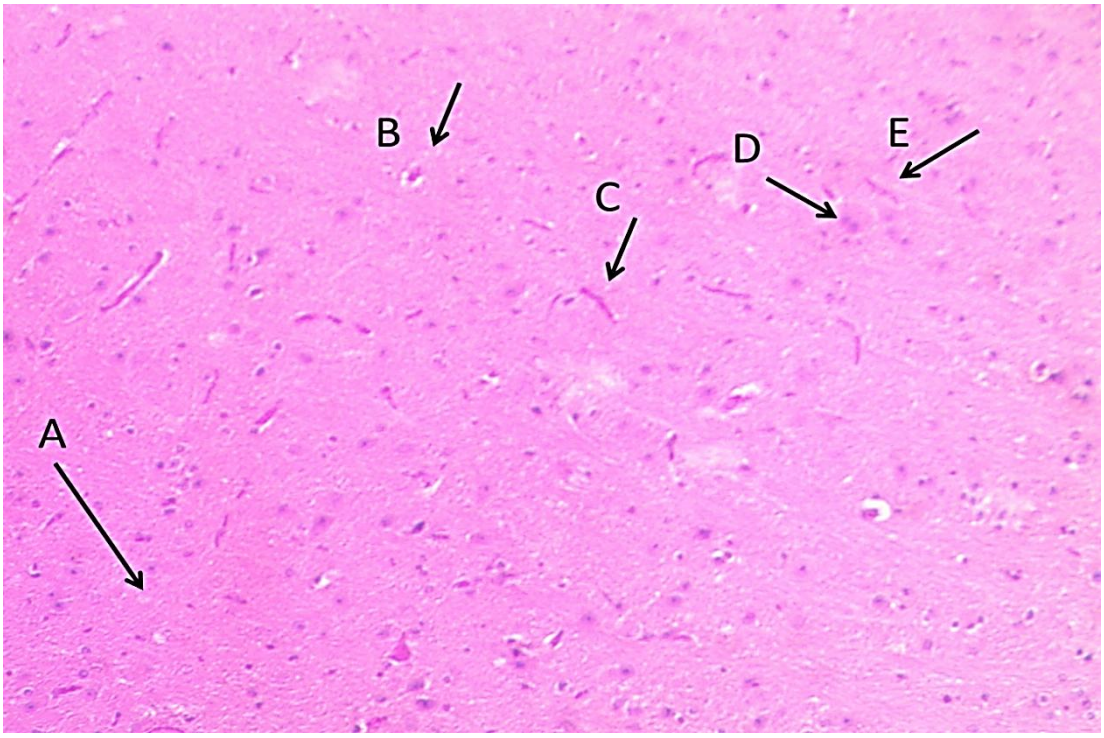

Fig (7): Transverse section of brain in control group which showed the A- Gray mater, B- Nerve cell body, C- Nerve cell, D- Axon, E-Nerve cell . H&E stain 10X

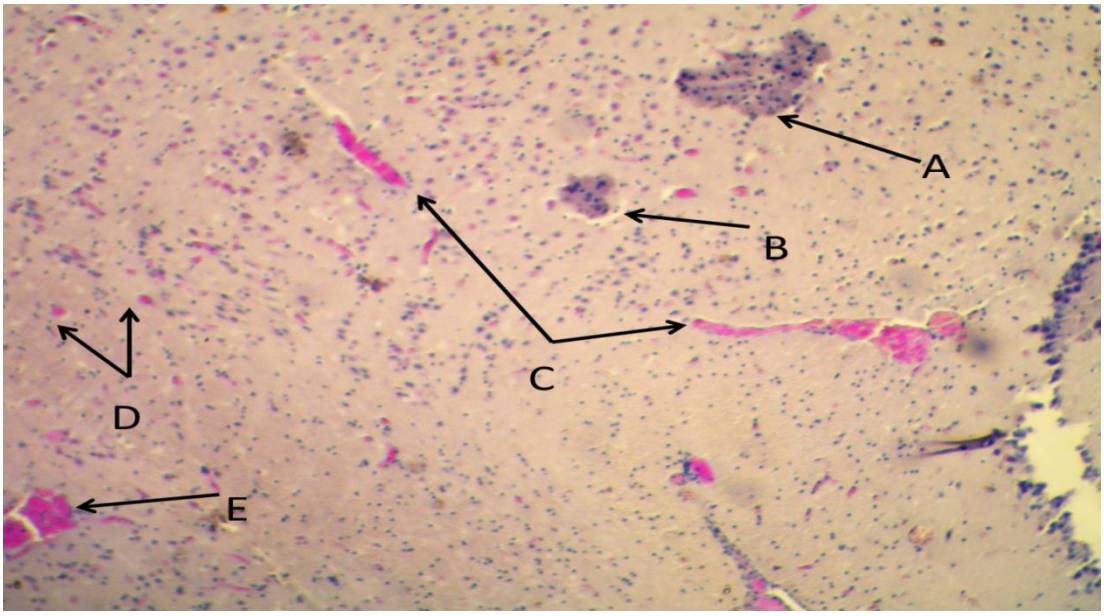

Fig (8): Transverse section of brain in treated group with *Nerium Oleander* which showed A-Cellular proliferation , B- Apoptosis, C-Blood vessel congestion, D- Blood congestion E- Cystic dilation filled with blood. H&E stain 10X.

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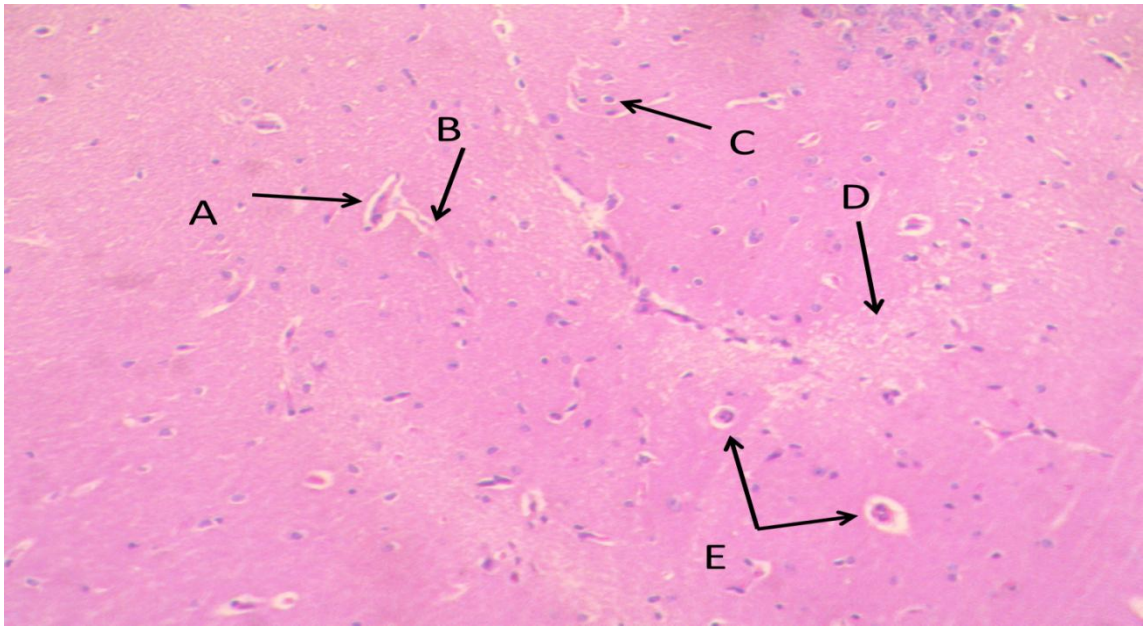

Fig (9): Transverse section of brain in treated group with *Nerium Oleander* which showed A- Nerve cell body, B- Dendrite , C- Nerve cell have oval nuclei , D- Necrosis, E- Supprative cell. H&E stain 10X.

References:

1. **Abdou**, Rania H., Walaa A. Basha, and Waleed F. Khalil. "Subacute toxicity of Nerium oleander ethanolic extract in mice."*Toxicological research* 35.3 (2019): 233-239.
2. **Akhtar**, T.; Sheikh, N.; Abbasi, M.H. Clinical and pathological features of Nerium oleander extract toxicosis inwistar rats. BMC Res. Notes 2014, 7, 947. [CrossRef]
3. **Al-Farwachi**, M. I.; Rhaymah, M.S. and AlBadarani, B.A. (2008). Acute toxicity of Nerium oleander aqueous leaf extract in rabbits. Iraqi J. Vet. Sci., 22(1):1-4.
4. **Altaee** MF. In vivo toxicity study of Nerium oleander's leaves and flowers aqueous extracts in mice (Cytogenetic, biochemical and hematological study). Baghdad Sci J 2011;8:366–72.
5. **Aslani**, M.R.; Movassaghi, A.R.; Janati-Pirouz, H.; Karazma, M. Experimental oleander (Nerium oleander)poisoning in goats: A clinical and pathological study. Iran. J. Vet. Res. 2007, 8, 58–63.
6. **Barbosa**, R.R.; Fontenele-Neto, J.D. and Soto-Blanco, B.Toxicity in goats caused by oleander (Nerium oleander). Res. Vet. Sci.,85(2): 279-281.(2008).
7. **Bassim** Abdullah J. and Duaa J. M.: Histological study of general anesthesia on the brain in female white mice.Biochem. Cell. Arch**.** Vol. 02, No. (Issue1), May 2020.
8. **Diane**, C.; Hegewald, N. &Dandamudi, J. Asuicide Attempt with An Oleander Cocktail-Abstruct. Chest., 1999, 116(4): 405-406.
9. **Hernandez**, M. ; Lopez , R. ; Abanas , R. M. ; Paris , V. and Arias , A. (1994) . Antimicrobial

activity of Visneamocanera Leaf extracts . J. Ethnopharmacology ,41 ; 115-119).

10. **Jubb**, K.V.F., Kennedy, P.C. and Palmer, N. 1995. Pathology of Domestic Animals. 3rdedition, Academic Press Inc., New York.
11. **Khordadmehr** M., Nazifi S., Mansourian M., Basiri S. Kolahian S.: Experimental Nerium Oleander poisoning in Balb/c mice and wistar rat: comparative hepatotoxicity and nephrotoxicity effects based on biochemical and pathological studies. Turk J Biochem 2017, 42, 427–434.
12. **Khordadmehr**, M., &Nazifi, S. (2018). Study of troponin, creatine kinase biomarkers, and histopathological lesions in experimental Nerium oleander toxicity in rats and mice. *Journal of veterinary research*, *62*(1), 97-102.
13. **Luna**, G. (1968): Manual of histological staining methods of the armed forced institute of pathology. 3rd.MCRW hill book Co. New York.
14. **Mohammed**, Abdullah Ibrahim Toxicology Foundations and Concepts, First Edition. National Library of Books, University of Garyounis - Benghazi: 2002,pp. 217-357.
15. **Ni**, D., Madden, T.L., Johansen, M., Felix, E., Ho, D.H., Newman, R.A.,2002. Murine pharmacokinetics and metabolism of oleandrin, a cytotoxic component of Nerium oleander. Journal of Experimental Therapeutics and Oncology 2, 278–285.
16. **Omidi** A, Razavizadeh A, Movassaghi A, Aslani M. Experimental oleander intoxication in broiler chickens. Hum ExpToxicol 2011;31:853–8.
17. **Praveen**, U.; Gowtham, M.; Yogaraje-Gowda, C.; Nayak, V.G.; Mohan, B.M. Detection of residues ofcardenolides of Nerium oleander by high-performance thin-layer chromatography in autopsy samples. Int. J.Med. Toxicol. Forensic Med. 2012, 2, 135–142.
18. **Radostits** O.M., Gay C.C., Blood D.C., Hinchcliff K.W.: Poisoning. In: Veterinary Medicine: A textbook of the diseases of cattle, sheep, pigs, goats, and horses. Philadelphia. W.B. Saunders Comp., 2007, pp. 100–198.
19. **Rahymah** MS, Al-Farwachi MI, Al-Badrani BA. Chronic toxicity of Nerium oleanderaqueous leaf extract in rabbits. Al-Anbar J Vet Sci 2011;4:88–93.
20. **Taub** K, Sane R S, Watson C A, Chen L, Ellens H, HirakawaB,Reyner E L, Jani M and Lee C A(201 1) Digoxin is not asubstrate for organic anion-transporting polypeptide transporters OATP1A2, OA TP1B1, OA TP1B3 and OA TP2B1 but is a substrate for a sodium-dependent transporter expressed in HEK293 cells. Drug MetabDispos. 39, 2093–210.
21. **WCSPF**, "World Checklist of Selected Plant Families, entry for Nerium oleander"(http://apps.kew.org/wcsp/synonomy.do?name_id=135196). Retrieved May 18,2014.