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THE HISTOPATHOLOGICAL CHANGES OF DIGOXIN ON MICE VITAL ORGANS

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ABSTRACT : The aim of this study was investigating to detriment the toxic effects of digoxin on the vital organs of mice like heart, Brain, liver, and kidneys. The experimental animals were categorized into 3 groups (T1, T2& C) each consist of 10 mice divided according to daily treatment with digoxin. T1, T2 groups representing dosing orally with 5, 10 mcg/kg respectively. C group acts as a control and treated with distilled water for 21 days. After a study period, animals were sacrificed. Histological samples were prepared by using hematoxylin and eosin stain. The study results of the T1 group show that different effects in liver tissue: degeneration signs, necrosis, low to severe periportal fibrosis and appear septal fibrosis. In Brain samples, perineural oedema with mild ganglion cell degeneration was clear. Kidney slices show enlargement of Bowman space with shrinkage of glomerular tuft and vacuolation of epithelia of lining urinary tubules. The T2 study group: brain slices present perineural and vascular edema, severe ganglion cell degeneration and necrosis. The renal tissue appears few infiltrations of inflammatory cells and vacuolation of urinary tubules. The main histological changes of Heart slices in both groups (T1, T2) were hemorrhage with oedema but the severity of lesion in T2 was higher characterized by hemorrhage with oedema and hyalinization of the myocardium. In conclusion, this study revealed that the digoxin of both doses: 5 mcg/kg and10 mcg/kg has histopathological effects in different vital organs of mice like (liver, brain, kidney and heart tissue), but these effects were severe in T2 group.

Key words : Histological effect, mice, vital organs, Digoxin.

INTRODUCTION

Histopathological changes regard one of the best methods to study the effects of the drug in experimental animals(Brundha and Nallaswamy, 2019). Digoxin is used in the treatment of several cardiovascular diseases (Virgadamo *et al,* 2015) extracted from digitalis lanata leaves with more effective compound(s) such as glycosides (cardiae glycol-steles) (Roberts *et al,*2015). Molecular weight 780.95. Digoxin is known from the 13th century as the name of digitalis puerperal and in 1776, its active ingredient is known as digoxin (Aronson, 1986). In ancient medical treatments, it was used as diuretic but not as atonic to treat muscle, while it has been recently recorded as atonic for heart muscle by incusing cardiac muscle contraction and increasing cardiac output as well. Digoxin absorption (60-70) on oral usage could produce adverse effect more rapidly (Schoner and Scheiner, 2007). Digoxin is considered as a congestive heart failure, in which heart is unable to pump the blood into the body, leading to deficiency of oxygen of nutrient supplied to the tissue. The risk of heart failure(s) has been raised by hypertension, diabetes, and sexual hormones disturbance that affect the cardiac cells, accordingly, around 60% of patient with congestive heart disease die after 5 years even with treatment (Andersson and Leppert, 2001). Drug usage for a long period has caused a problem like accumulating in the body which is led to appear its toxic effect as appetite, vomiting, and nausea. Additionally, 25% of patients treated by digoxin has been suffered from other symptoms like vertigo and headache, because this is pharma-kinetic occurred by hepatocytes with low therapeutic range (Ehle *et al,* 2011). Therefore, the aim of this study is to evaluate the toxic impact of medical dose and double dose usage on different vital organs of mice.

MATERIALS AND METHODS

Totally, 30 albinomice(adult) have been used in this study with a mean weight of 25 ±30 mg. All arerandomly divided into three groups,including 10 mice as first groupor control group C. Second group or T1 has 10 mice and received Digoxin of 5micro g/kg as oral treatment. Third group or T2 has 10 mice, which isorally treatedby Digoxin

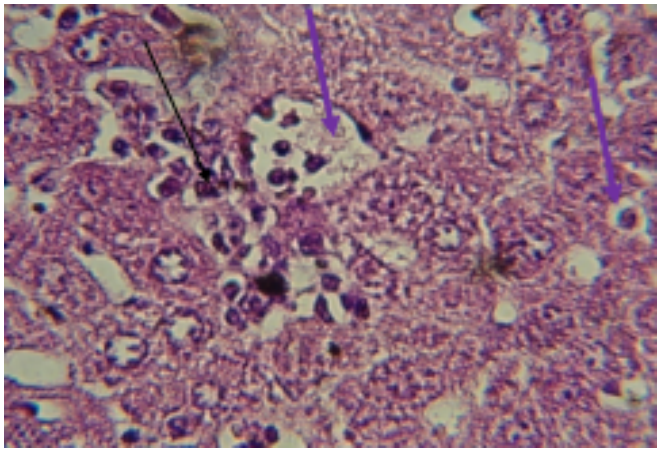

Figure 1 : Histopathological section of treated liver withT1, showing infiltration of inflammatory cells (mainly neutrophyl), dilatation of central vein and enlarged vaculative hepatocytes. (H&E X40)

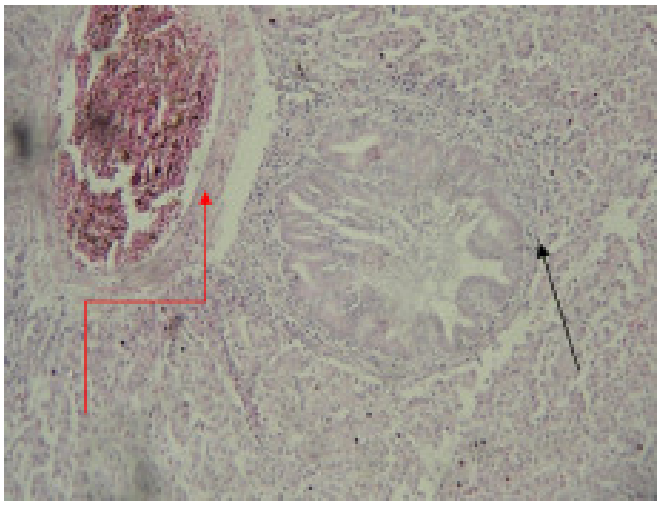

Figure 2 : Histopathological section of treated of liver withT2, showing severe infiltration of inflammatory cells and fibrosis perivascular and perihyperplastic bile duct (H&E X20).

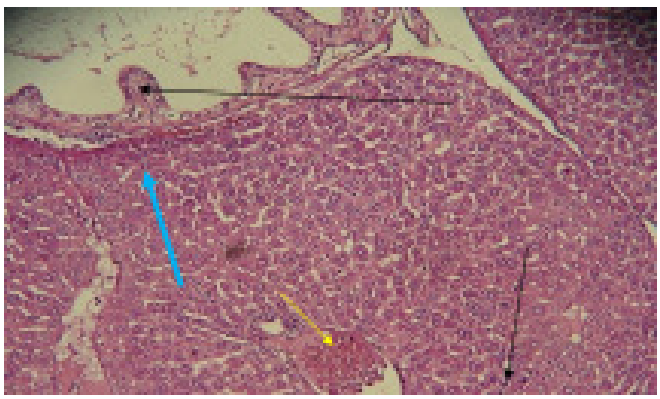

Figure 3 : Histopathological section of treated of liver withT2, showing hyperplasia of bile duct and congested of blood vessels observing of few infiltration of inflammatory cells (H&E X20).

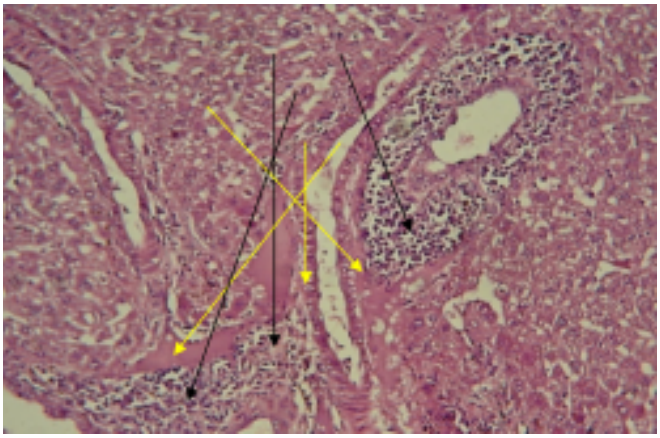

Figure 4 : Histopathological section of treated liver, withT2, showing thickening of blood vessels wall with infiltration of inflammatory cells.

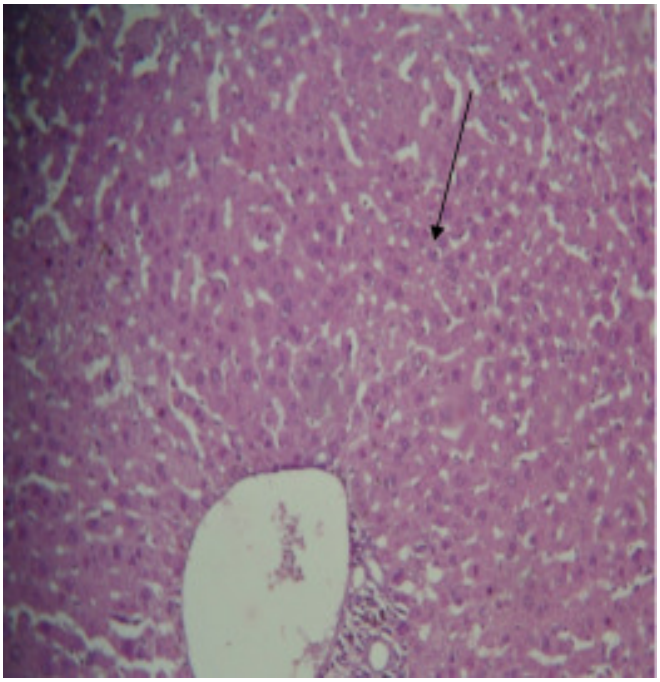

Figure 5 : Histopathological section of treated liver withT1, showing few infiltration the inflammatory cells (H&E x20).

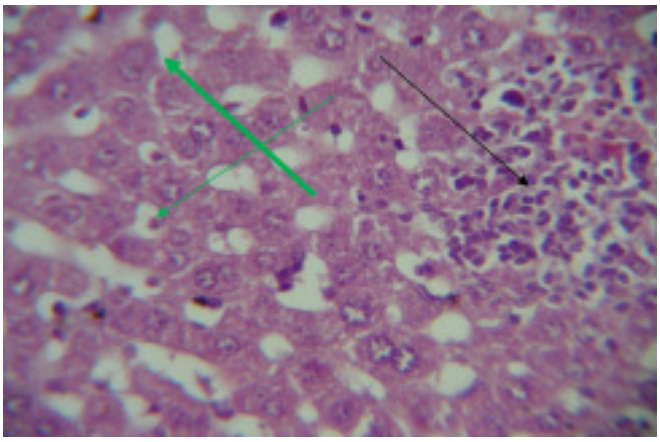

Figure 6 : Histopathological section of treated liver with T1, showing aggregation of inflammatory cells with vacuolation of hepatocye. (H&E x40)

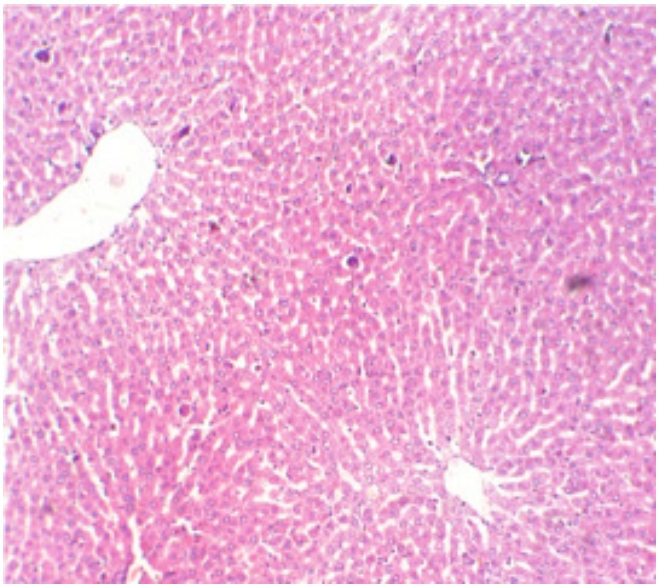

Figure 7 : Histopathological section the control liver, showing no abnormal lesion (H&E X10).

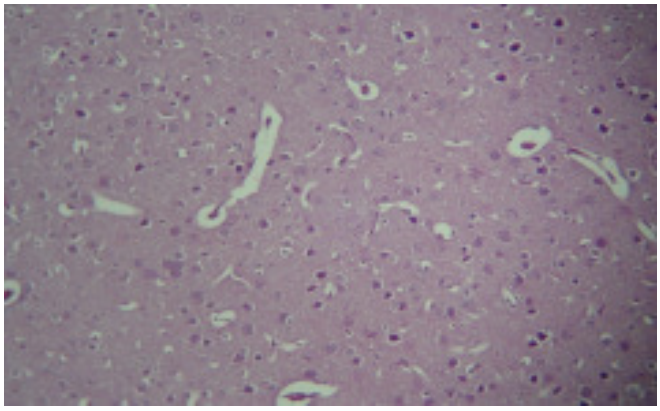

Figure 8 : Histopathological section ofcontrol brain, showing normal tissue (H&E x10).

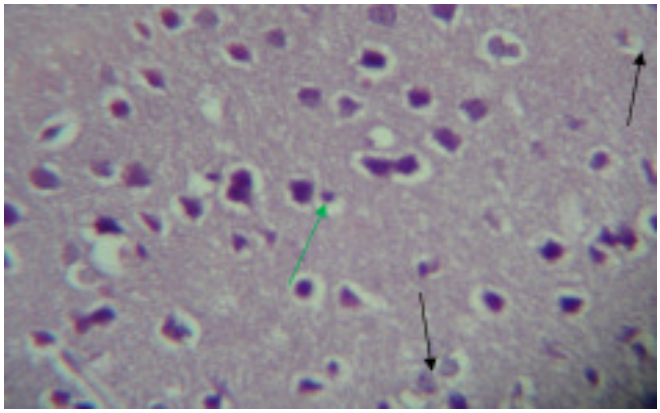

Figure 9 : Histopathological section of treated brain withT1, showing perineural oedema with mild ganglion cell degeneration (H&E x40).

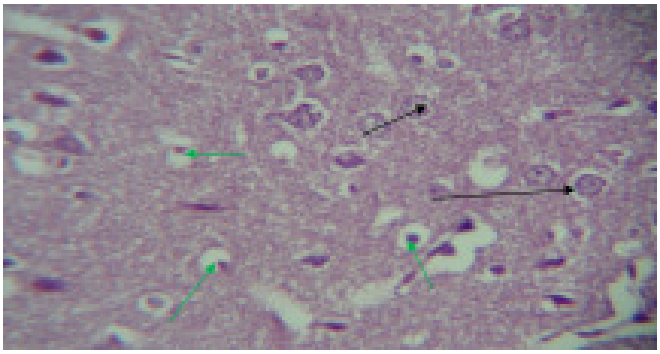

Figure 10 : Histopathological section of treated brain with T2, showing perineural and vascular oedema with severe ganglion cell degeneration and necrosis (H&Ex40).

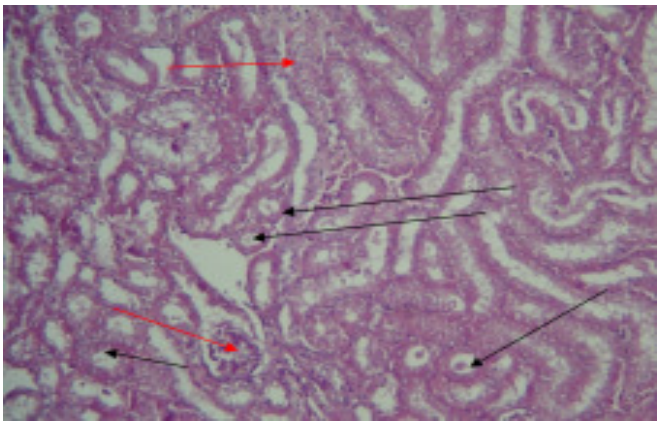

Figure 11 : Histopathological section the treated kidney with T2, showing few infiltration of inflammatory cells andvaculation of urinary tubules (H&E X20).

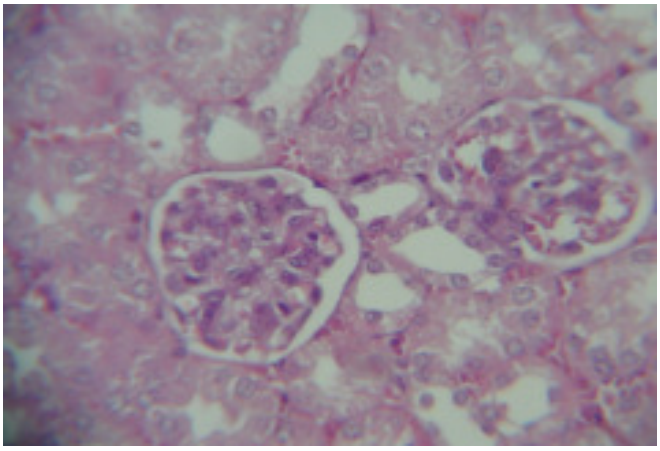

Figure 12 : Histopathological section of control kidney, showing no lesion (H&E X 20)

of 10microg/kg. All tests have been performed at College of Veterinary Medicine, University of Baghdad/Iraq. All mice have free access to water andfood, while they all are left for 2 weeks in order to make adaptation to the experimental conditions.The ethical issue for this study has been confirmed by the animal ethics committee members in the College of Veterinary Medicine.After study period, experimental animals were sacrificed by cervical

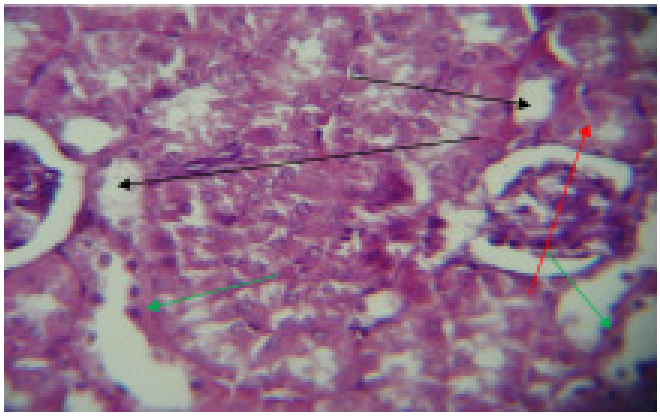

Figure 13 : Histopathological section of treated kidney withT1, showing enlargement of bowman space with shrinkage of glomerulartaft and vacuolation of epithelia lining urinary tubules. (H&E X20)

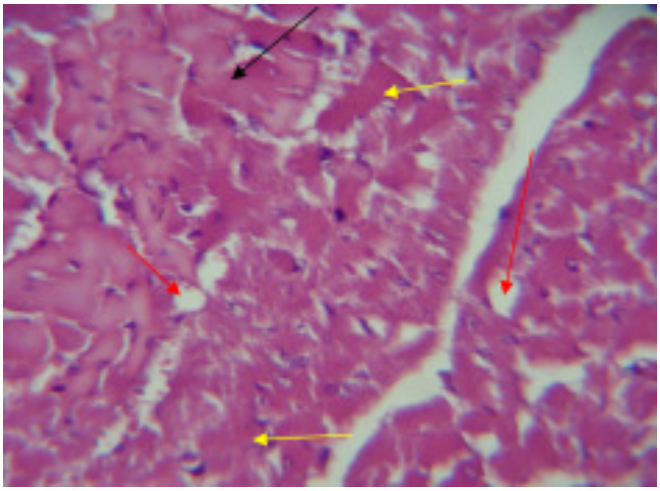

Figure 15 : Histopathological section of treated heart withT2, showing haemorrhage with oedema and hyalinization of myocardium (H&E X20

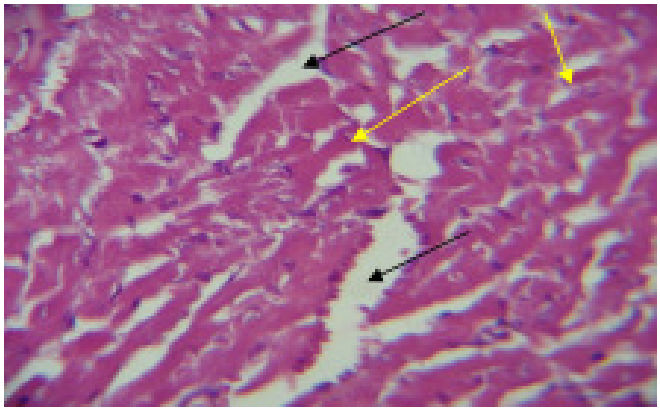

Figure 14 : Histopathological section of treated heart withT1, showing hemorrhage with oedema (H&E X20).

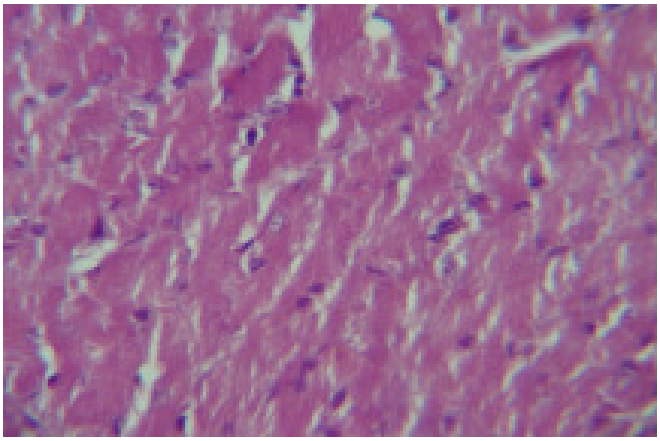

Figure 16 : Histopathological section of control heart showing no abnormal lesion (H&E X20).

dislocation. Subsequently, heart, brain, liver, and kidney of mice have been precisely taken out and preserved for the histological examination. In the following, the histological results oftestshave beenperformed ashistological scoring after hematoxylin and Eosin (H & E) (Luna, 1968). Afterward, the histopathological sections were examined by a light microscope to assess the histological suspected changes.

RESULTS AND DISCUSSION

Toxicopathological changes of liver have shown different severity levels according to the treatment, which mild changes are started at T1 and characterized by congestion of central vein (cv) and infiltration with inflammatory cells that is mainly neutrophils (*figure* 1). Then the severity of lesion is increased in other treatment T2 to attain the fibrosis periportal level and septal fibrosis (*figure* 2). In addition to vacuolation of hepatocyte, sinusoids absence (*figure* 3) with the diffuse and focal aggregation of mononuclear cells (*figure* 4), and in the presence of hyperplasia of bile duct (*figure* 5 – 6) the liver of control group has shown no lesion appearing (*figure* 7).

Brain

The histopathological changesof control brain have shown normal tissue with no abnormal lesion (*figure* 8), while the treated brain withT1 has shown perineural oedema with mild ganglion cell degeneration (*figure*9). Some sections of treated brain with T2 have indicated perineural and vascular oedema with severe ganglion cell degeneration and necrosis(fig. 10).

Kidney

Histopathological section of control kidney has shown no lesion (fig.12), while a section of the treated kidney with T1 has shown enlargement of Bowman's space with shrinkage of glomerular tuft and vacuolation of the urinary

tubules epithelia (*figure* 13). Histopathological section of the treated kidney with T2 has shown few infiltrations of inflammatory cells and vacuolation of urinary tubules (*figure* 11).

Heart

The main histopathological changes of treated heart withT1 has shown haemorrhage with oedema (fig. 14) and the severity of lesion has been increased at T2 that the lesion hasshown haemorrhage with oedema and hyalinization of myocardium (*figure* 15) of control group has shown no abnormal lesion (*figure* 16).

The present study has shown several histopathological changes in vital organsof mice, therefore, the histopathological observationof liver has revealed the sever periportal fibrosis, bile duct proliferation and aggregation of mononuclear cells especially with high dose group. In the following, serum AST & ALT are the highest concentration relevant high group compared to other study groups (Khadhair and Majeed, 2017; Farrah, 2018; Saliem, 2010). High dose T2 has the most severe damage, also toxic effects of digoxin in liver has histologically presented hepatic necrosis, sever periportal and septal fibrosis on the accumulation of mononuclear cells compared to other study groups. Align with this observation, Majeed (2012) and Aslani *et al,* (2004) have stated that the damage isremarkably massive in high dosage, however, damageis less in other groups compared tothe control group. Alternatively, align with Maiali *et al* (2010), the study has shown that the toxicity effects of digoxin in the liver cells are presented as 1) degeneration, 2) necrosis, 3) low to sever periportal fibrosis and 4) septal fibrosis (Figures. 2-3).Regarding the brain, the histopathological examination of rat brain'sdigoxin has caused degeneration changes a sorally treated, effecting theneurotransmitter level.

Farrah (2017)has revealed that "digoxinof 5mcg/kg and10 mcg/kg thatcause abnormalneuro behaviorhas affected thepropotinalin the periodand dosage of exposure, even ifthe therapeutic dosagehas indicated the precaution of using these drugs". Theovercome of kidney group (T2) has shown haemorrhage and infiltration of inflammatory cells (mnc)and vaculation of urinary tubules. Inadditionto the presence of pretentious material inside tubules, due to digoxin, the resultshave been affected by over blood flowing and over glomerular filtrationrate (Lees, 1994). Yangp *et al,* Have stated thatthe kinetic evidence of digoxin is uptake transporter in kidney, while other studies have proved active sodium-dependentdigoxin uptake in human embryonic kidney cell line (Taub *et al,* 2011).

This drug belongs to the groups of cardio tonic steroidswith a steroid nucleus of 5- or 6-member lactone ring at C (Hollman, 1996; Müller,2011).This drug has a narrow therapeutic range with toxic effects at (Digoxin e" 2ng/ml) of plasma (Huang *et al,*1999). The cardiotonic action of digoxin has increased the force of the cardiac contraction and all Na+pump(Hollman, 1996). The digoxin withanti-hypertension effect has been confirmed as salt - sensitive model of hypertension (Huang *et al,*1999).

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REFERENCES

Abbas A, Khadhair and Saleh Majeed K (2017) Study the toxicity of digoxin on liver and their transaminase enzymes level in *Journal University of kufa medical sciences.* **8**(2), 73-80

Al-Maiali H K, Al-Hmedawi H A, Al-Lebawi Z I (2010) The effect of alcoholic extract of Nerium oleander leafs on some biochemical parameters and function of liver and kidney in white rats. *Journal of wassit for science and medicine*. **3**(2), 1

Andersson P and Leppert J (2001) Men of low Socio- economic andeducational Level possess pronounced deficient knowledge about therisk factors related of CHD. *J-cardiovas- Risk.* **8**(6), 371-7

Aronson J K (1986) An Account of the foxglove and its medical uses, 1785-1985. oxford university press.

Aslani M R, Movassaghi A R, Mohri M, Abbasian A and Zarehpour M (2004) Clinical and pathological aspects of experimental oleander (Nerium oleander) toxicosis in sheep. *Vet. Res. Commun*. **28**(7), 609-616.

Blanco G, Mercer R W (1998) Isozymes of the Na-K-ATPase: heterogeneity in structure, diversity in function. *Am J Physiol Renal Physiol.* **275**, F633–F650.

Brundha M P and Nallaswamy D (2019) Hide and seek in pathology-A research on game-based histopathology learning. *International Journal of Research in Pharmaceutical Sc*. **10**(2), 1410-1414.

Ehle M, Patel C, Giugliano R P (2011) Digoxin: clinical highlights: a review of digoxin and its use in contemporary medicine. *Crit Pathw Cardiol.* **10**(2), 93-8.

Farrah R, Jaafar (2017) Study on Neurotoxic effects of Digoxin different doses in mice. *Journal University of kufamedical sciences*. **8**(2), 187-195

Farrah R, Jaafar (2018) Some toxic Impact of Digoxin inmice *International Conference on Materials Engineering and Science IOP Publishing*. **454**(1), 012114.

Hollman A (1996) Digoxin comes from Digitalis lanata. *British Medical Journal*. **312**, 7035-912

Huang B S, Kudlac M, Kumarathasan R, Leenen F H (1999) Digoxin prevents ouabain and high salt intake-induced hypertension in rats with sinoaortic denervation. *Hypertension.* **34**, 733–738.

Lees G J (1994) Inhibition of Na+_KA+ PTaseapotentially ubiquitous mechanism contributing to cns neuropathology brain, *Res Rev.* **16**, 283

Luna L G (1968) Manual of histology staining. Methods of armed forces. Institute of Pathology. 3rd edition. McGraw-Hill Book Company, New York and London.

Majeed L J (2012) Histopathological effects of aqueous extract of oleander (*Nerium oleander*) flower in albino male mice. *Al-Mustansiriya J. Sci.* **23**(1), 29-38.-9.

Müller F, Fromm M F (2011) Transporter-mediated drug-drug interactions. *Pharmacogenomics*. **12**, 1017–1037.

Roberts D M, Gallapatthy G, Dunuwille A and Chan B S (2015) Pharmacological treatment of cardiac glycoside poisoning. *British Journal of Clinical Pharmacology*, **81**(3), 488–495.

Saliem A H (2010) Effect of the median lethal dose of the ethanolic extract of (Nerium Oleander) leaves on the histopathology features of the vital organs in mice. *Iraqi journal of Vet. Medicine*, **34**(2), 103-109.

Schoner W and Scheiner B G (2007) Endogenous and exogenous cardiac glycosides: Their roles in Hypertension, Salt Metabolism, and Cell Growth. *Am. J. Physiol. Cell Physiol*. **293**, 509-536.

Taub K, Sane R S, Watson C A, Chen L, Ellens H, Hirakawa B, Reyner E L, Jani M, Lee C A (2011) Digoxin is not a substrate for organic anion-transporting polypeptide transporters OATP1A2, OATP1B1, OATP1B3, and OATP2B1 but is a substrate for a sodium-dependent transporter expressed in HEK293 cells. *Drug Metab Dispos*. **39**, 2093–210.

Virgadamo S, Charnigo R, Darrat Y, Morales G and Elayi C S (2015) Digoxin: A systematic review in atrial fibrillation, congestive heart failure and post myocardial infarction. *World J Cardiol.* **7**(11), 808-816.

Yangp, Menter D G, Cartwright C, Chan, Dixon S, Suraokar M, Mendoza G and Llandsa N (2009) Oleandrin-mediated inhibition of human tumer cell proliferation: Importance of Na+_KA+ PTase sub units as drug targets". *Molecular cancer Theraputic.* **8**(8), 2319-2328.