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Hepatotoxicity and Nephrotoxicity Evaluation after Repeated Dose of Acetamiprid in Albino Rats

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Abstract

Acetamiprid (ACP) is neonicotinoid insecticide and it is the most highly effective insecticides worldwide for crop protection and control fleas infesting in livestock and pet animals. It has potential toxicity to mammals and there is no clear data regards to hepatotoxicity, nephrotoxicity and oxidative stress at low doses. The present study aimed to investigate the acute toxicity of ACP as formulation toward male adult albino rats and evaluate the impact effect of repeated sub-lethal dose of ACP. The high dose equal 1/10 of estimated LD50 and the low dose approximately equivalent 1.5X of No Observed Adversed Effect Level (NOAEL) dose. The study concluded the examination of hepatotoxicity, nephrotoxicity and some parameters that covered oxidative stress. The results revealed that, ACP may not have such extensive effects as inducer to oxidative stress but may be induce synergism of the defense system to increase the antioxidant self-system. Moreover, the histopathological investigation revealed that the exposure to high dose of ACP led to destructive and severe effects on the kidney tissue but may be has moderate effects on the liver tissue. The low dose which equal approximately 1.5X of NOAEL led to slight effects on the liver tissue while the kidney tissue was still more affected by this insecticide. Finally, It seems that the ACP induces the adverse effect at the high dose level but the low dose level and short term exposure may not make adverse effect. So, it can be recommend to use this insecticide as long as using the recommended dose.

Key Words: Acetamiprid, hepatotoxicity, nephrotoxicity, oxidative stress, histopathology.

Introduction

Acetamiprid is a member of the neonicotinoid group of insecticides and it is the most highly effective and largest selling group of insecticides worldwide for crop protection. Also it used along to control fleas infesting in livestock and pet animals **(Singh et al., 2012)**. In addition, acetamiprid is being highly water soluble indicates a high potential for the compound to leach in soil or to run off in surface water **(Zhang et al., 2012)**. And, it may be reach to human or non-target organisms via the pesticide residues in air, water, and food chain **(Ford and Casida, 2006)**. So, more research about the side effect of acetamiprid need to be available. On the other hand, the histopathology is a critical part of the toxicologic and risk assessment of foods, drugs, chemicals, biologics, and medical devices. It

is important that the approach to histopathologic examination satisfy regulatory demands for unbiased observations while facilitating the sensitive and efficient evaluation of large amounts of microscopic information **(Crissman et al., 2004).** Therefore, determination of the histopathologic effects of any toxic chemical by using different organisms is noteworthy in environmental studies. Investigation of histopathological changes in animal tissues is a sensitive and rapid method, commonly used to detect effects of pesticides on various tissues and organs and to evaluate toxic potential and risk assessment of chemicals in the environment **(Travlos et al., 1996; Al-Sharqi et al., 2012; Al-Qudsi, and Linjawi, 2012).** Because there are a few studies on the histological effects of Acetamiprid **(Păunescu et al., 2011; Zhang et al., 2011, 2012).** And because there is no clear data regards to whether acetamiprid induces hepatotoxicity, nephrotoxicity and oxidative stress at low doses or not. Therefore, the present study was designed to provide more information about the effect of the high dose of acetamiprid which equal 1/10 of estimated LD50 and the low dose that approximately equivalent the 1.5X of No Observed adversed Effect Level (NOAEL) of acetamiprid through examination of hepatotoxicity, nephrotoxicity and some parameters that covered oxidative stress on albino rats.

Materials and Methods

2.1 The tested insecticide (ACP)

The tested insecticide used in this study was a commercial insecticide with active ingredients of acetamiprid 20%, the IUPAC name: (E)-N1-[(6-chloro-3-pyridyl) methyl]-N2-cyano-N1-methylacetamidine. The formulation was supplied as an soluble powder (SP) by the Mammalian Toxicology Department, Central Agricultural Pesticides Lab, Dokki, Egypt.

2.2 Animals

A total of 40 apparently healthy male white rats (Rattus rattus) of Wistar strain weighing 120g+10% were used throughout the whole study. The animals were obtained from the laboratory animal house of the Modern Veterinary Office, Giza, Egypt. Animals were kept under full hygienic conditions, had free access to fresh water and fresh well-balanced diet, and kept under supervision for two weeks before commencing the experimental work. The animals were housed in all groups of five rats per cage.

2.3 experimental design

Rats were randomly divided into two main groups (a and b) which were further subdivided into subgroups. Group (a) consists of 5 subgroups were used to determine the oral LD_{50} of the ACP. Group (b) consists of 3 subgroups were used in repeated treatments respectively. The first subgroup was kept as control. The

two subgroups were treated orally by gavage for 35 days. The exposure period was increased one week than 28 days of protocol **(OECD, 2008)** because there were two days in the week without treatment. The high dose 1/10 of estimated LD_{50} and low dose that approximately equivalent 1.5X No Observed adversed Effect Level dose (NOAEL) of ACP 12.4 mg/kg/daily/male rat **(EPA, 2002).**

2.4 Determination of the median lethal dose, LD50

Estimation of the oral LD_{50} (median lethal dose) of the tested insecticide was carried out according to the method described by **(Weil, 1952)** This method also reduces the time of calculations of the LD50 and its confidence interval to a minimum without the sacrifice of accuracy. When the mortality data (r-values) and the f-values were obtained from the tables. The LD50 can be calculated from the following equation:

log m = log D + d (f + 1), Where: **log m** = the logarithm of the LD50, **log D** = the logarithm of the lowest dose used, **d** = the logarithm of the constant ratio (=1.46) between dosage levels.

2.5 Blood collection and tissue preparation

Rats were sacrificed at the end of treatments through cutting of their neck veins after they were slightly anaesthetized by diethyl ether. Blood samples were and placed immediately on ice. Heparin was used as an anticoagulant for plasma samples while other part (serum samples) were left to clot 30 min. Then all samples were centrifuged at 1,100 rpm for 20 min and stored at −20◦C. Plasma and serum were analyzed for oxidative stress and kidney function parameters. Liver and kidney were immediately removed and washed using chilled saline solution then were preserved in formalin (10%, w/v) for histological parameter.

2.6 Oxidative stress parameters

2.6.1. Total antioxidant capacity

The antioxidant capacity which represents the sum of all antioxidant enzymes can be measured by the reaction of antioxidants in the sample with a defined amount of hydrogen peroxide (H_2O_2). The antioxidants in the sample eliminate a certain amount of (H_2O_2) and the residual is determined colorimetrically by an enzymatic reaction **(Koracevic et al., 2001).**

2.6.2. Catalase (CAT)

The activity of Catalase (CAT) which is one of the antioxidant enzymes was estimated by the method based on the CAT reacts with a known quantity of (H_2O_2) and the reaction is stopped after exactly one minute with catalase inhibitor. Remaining (H_2O_2) react with 3,5-dichloro-2-hydroxybenzene sulfonic acid

(DHBS) and 4-aminophenazone to form chromophore with a color intensity inversely proportional to the amount of catalase in the sample **(Aebi H.,1984).**

2.6.3. Lipid peroxidation

The end-products of lipid peroxidation, is malondialdehyde (MDA) which reacts with thiobarbituric acid reactive substances (TBARS) in acidic medium at temperature of 95°C for 30min to form thiobarbituric acid reactive product which has a pink color and can be measured at 534 nm **(Okhawa et al., 1979).**

2.6.4. Glutathione-S-transferase

The enzyme protects cells against toxicants by conjugating them to glutathione. The glutathione conjugates are metabolized further to mercapturic acid then excreted. The method based on measuring the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione. The conjugation is accompanied by an increase in absorbance at 340 nm. The rate of increase is directly proportional to GST activity in the sample **(Habig et al., 1974).**

2.7 kidney function parameters

2.7.1. Uric acid

Uric acid is the waste product produced from the degradation of purines. In healthy human, uric acid is filtered and removed from the blood by the kidneys and excreted into urine. Because a number of kidney diseases are known to affect uric acid levels, uric acid determination is thus important and useful in diagnosing and evaluating kidney diseases. Increased levels of uric acid are also known to be associated with uremia, leukemia and pneumonia. The method based on measuring the conjugation 3,5-dichloro-2-hydroxybenzene sulfonate and uricase to form chromophore with a color intensity inversely proportional to the amount of uric acid in the sample **(Sanders et al., 1980).**

2.7.2. Creatinine

Creatine is a substance found in the muscles, and when creatine is broken down, creatinine is formed. Creatinine is eliminated from the body in urine, through the kidneys. High levels of creatinine in serum or plasma may indicate impairment of functions of the kidneys. The purpose of this test, therefore, is determination of proper functioning of the kidneys. The method based on the creatinine in alkaline solution reacts with picric acid to form a colored complex. The amount of the complex formed is directly proportional to the creatinine concentration **(Bartels et al., 1972).**

2.8. Histological section preparation

Liver specimens were obtained from rats, and immediately fixed in 10% formalin for 24 hrs. and decalcification was occurred on formic acid then washed in tap water. Serial dilutions of alcohol (absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in hot air oven for 24 hr. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by Hematoxylin & Eosin stain then examination was done through the light microscope **(Banchroft et al., 1996).**

2.9 Statistical analysis

The data obtained in this study were calculated and statistically analyzed, according to Studen's t-test **(Venables and Ripley, 2002)**, using SigmaPlot statistics software, Ver. 11. Data were reported as mean ± SE.

Results and Discussion

3.1. Median lethal dose and repeated dose toxicity

The test of estimated oral LD_{50} should be performed to provide preliminary information on the toxic nature of the tested compound for which no other toxicology information is available for this formulation. The mortality data of treated rats were recorded during the observation period 14 days after oral administration of ACP, as shown in Table (1) it was observed that ACP exhibited moderate toxicity toward rats. The symptoms of toxicity manifested by the animals were appeared two hours after the administration. The animals showed slightly tremors and convulsions of the whole body particularly the head movements, these movements increased rapidly after five hours, finally followed by death after 24hrs. No mortality occurred in the animals which were survived after 24hrs and during the observation period. The result of estimated LD50 is illustrated in table (1) and the result is complementary with report of **(Anonymous, 2002)** which reported that the acute oral LD_{50} of acetamiprid for rat was 330 mg/kg bwt and has moderately acute toxicity. On the other hand, there is no signs of toxicity observed after repeated doses of both treatments. In addition, there is no different in body weight of treated rats during 35 days of exposure (Fig 1). in comparison to the control group. This result is apparently in agreement with **(Singh et al., 2012)** who found that ACP induced decrease in body weights and clinical toxicity symptoms of mice received dose of 40 mg/kg body weight (1/5 of LD50) for a period 28 days but did not produce significant changes in body weights and biochemical values of dose 10 mg/kg (1/20 of LD50). Also **(Arfat et al., 2012)** demonstrate that imidacloprid which is from neonicotinoids group induced significant effects on body weight, liver and kidney functions in male

albino mice at a dose of 15 mg/kg body weight but 5 and 10 mg/kg/day may be considered have adverse effect for mice. These variations in response to toxic agents may be due to the different between the species in toxicity tolerance.

3.2. Effects of ACP on oxidative stress parameters

Oxidative stress induction involves an excessive production of reactive oxygen species (ROS) resulting from impaired balance between the ROS generation and antioxidant defense capability. Induction of oxidative stress is one of the main mechanisms of the action of many pesticides. The damage of membrane lipids is one of the endpoint of oxidative stress-inducing by the pesticides **(Tuzmen et al., 2008).** Although many reports of acute toxicity of neonicotinoids on mamanalian are available **(PAN, 2006),** the information on oxidative stress of these compounds after short term exposure is still publically unavailable **(EL-Gendy et al., 2010)**. Previous findings reported that the effects of ACP may be mediated by increasing oxidative stress, as it increased malondialdehyde and nitric oxide NO in the testes, and reduced the activity of catalase, but the vitamin E significantly ameliorated the effects of ACP **(Mondal et al., 2009).** In our study which summarized in Table (2) indicated that the ACP did not only induce oxidative stress but also induced synergist effect of antioxidant system that obvious by increase the level of catalase (CAT) activity as an antioxidant enzyme at high dose 42.72 mg/kg (1/10 oral LD50) but low dose did not induced significant variation. In addition, the level of total antioxidant capacity (TCA) did not change at high and low doses in comparison with control. Also the activity of glutathione-s-transferase (GST) which is a multifunctional enzyme and plays a key role in cellular detoxification did not affected by the treatments. In addition, the lipid peroxidation (MDA) was slight increased at high dose but the low dose was similar to the value of control group. On the other hand, if the quantity of dose take in consideration these findings are apparently agreement with **(Keshta et al., 2016)** who revealed that ACP could stimulate oxidative toxicity and reproductive toxicity in male rats administrated orally 100 mg/Kg body weight (1/10 oral LD50) daily for 30 days. It is obvious the different of the quantity of dose that rat received. Also if we take in consideration the different in tolerance between the species **(Singh et al., 2012)** found that ACP administered 40 mg/kg body weight (1/5th of LD50 value) per day for 28 days induces prominent toxic symptoms with hematological and biochemical effect in mice but 10 mg/kg body weight (1/20 of LD50 value) proved to be non-toxic for the experimental animals. More addition **(Mondal et al., 2014)** reported that the ACP were administered orally to rats at (25, 100 and 200 mg/kg of body weight) for a period of 28 days, the high dose made extensive neurotoxic effects but low dose did not induced such effects. So, It seems that the ACP made the adverse effect according to the quantity of dose given and the kind of species.

3.3. Effects of ACP on the Liver tissues

Liver is a target organ for detoxification and is prone to various disorders as a consequence of exposure to environmental pollutants. Histopathological alterations in tissue may be used as a rapid method to evaluate the toxic effects of chemicals in different tissues and organs **(Bernet et al.,1999).** Histopathological examination of liver specimens taken from control group showed normal histological structure of the central vein and surrounding hepatocytes in the parenchyma Fig.2(1-A). The histopathological examination of the low dose group (18 mg/kg) indicated few inflammatory cells between the hepatocytes (1-B) but the high dose group (42.72 mg/kg) showing degeneration and necrosis of hepatocytes with hyperplasia associated with inflammatory cells infiltration (1-C & 1-D). So, the dose response relationship was cleared in this investigation. The present study are complementary with **(Mondal et al., 2014)** Who reported that the Mild degenerative changes were observed in liver of rats received 25 mg/kg of ACP, but the group of rats administrated 100 and 200 mg/kg respectively showed severe liver fatty changes and necrosis. On the other hand, several mechanisms have been identified on liver cell damage **(Grattagliano et al., 2002; Lee, 2003)**. One of them is mitochondrial dysfunction. It may occur in three different ways, Inhibition of fatty acid beta-oxidation, inhibition of respiratory enzymes or by a direct effect on mitochondrial DNA. Some chemicals like pesticides inhibit both beta-oxidation and the functions of respiratory enzymes. Consequently, free fatty acids are not metabolized and lead to accumulation of lactate and reactive oxygen species. After that these radicals will damage the mitochondrial DNA., and also when free fatty acids are not metabolized lead to accumulation in the liver that increase delivery and uptake of free fatty acids leading to induced fatty liver. More addition, some chemicals may cause hepatocellular necrosis, rapid disorganization of the hepatic architecture, breakdown of sinusoidal structures and pooling of blood in the liver through these mechanisms **(Rasgele et al., 2015)**.

3.4. Effects of ACP on the kidney tissues

The kidney is the excretory organ shared the liver the main role of detoxification of the toxic chemicals. Both of them suffered from the stress of these injury compounds in the body. There are a few studies on the histological effects of ACP on different tissues and organs in the different organisms **(Rasgele et al., 2015)**. In the present study the histopathological examination of kidney specimens of control group showed normal histological structure of the glomeruli and tubules at the cortex Fig 3(2-A). The low dose group showed focal inflammatory cells infiltration and necrosed tubules with hyperemia of the glomerular tufts at the cortex (2-B). But the high dose exposure induced large effect on the kidney tissue than were happened in the liver tissue. This obvious by forming dysplasia with

irregular histological structure (2-C). And sever congestion necrosis in the lining epithelium in the most tubules (2-D). In previous studies **Păunescu et al., (2011)** found that there were dilation of the marginal channel, hyperplasia of the epithelial cells and lifting of the lamellar epithelium in Prussian carp fish exposed to ACP. More addition **(Zhang et al., 2011)** reported that ACP damaged seminiferous tubules and Leydig cells of mice; and also **(Zhang et al., 2012)** reported that ACP damaged renal corpuscles and tubules in kidney of mice. In the present study, the low dose of ACP induced moderate histopathological lesions but the high dose made severe congestion in the glomerular tufts associated with coagulative necrosis in the tubular lining epithelium at the cortex of the kidney. The above findings suggested that the liver is not alone being the major detoxifying organ suffered from the toxic agents while the kidney may be affected than the liver.

3.5. Effects of ACP on the kidney function

Serum creatinine and uric acid determine the glomerular filtration rate. Creatinine is derived mainly from the catabolism of creatine found in muscle tissue and its catabolism to creatinine. Severe kidney damage will lead to increased creatinine levels. In the present study, serum creatinine and uric acid showed normal values except that the high dose group induced significant increase in the level of uric acid ($p>0.01$) in comparison with the control group. Uric acid can not only play a preventive anti-oxidation function through combination with iron and copper ions but also remove single oxygen and hydroxyl radicals directly. Recent studies have found that uric acid was the highest content of antioxidants in the body and the content of uric acid in serum is an important parameter which represents the anti-oxidation capacity in body **(Pasalic et al., 2012).** Our study found that ACP increased the level of uric acid and although, the old theory about uric acid is recognized as a marker of oxidative stress because the production of the uric acid includes enzyme xanthine oxidase which is involved in producing of radical-oxigen species (ROS). As by-products ROS have a significant role in the increased vascular oxidative stress **(Higgins et al., 2011).** Uric acid may play a predictive role as an antioxidant by scavenger a free radical and a chelator of transitional metal ions which are converted to poorly reactive forms **(Settle and Klandorf, 2014)**. This may explain why this insecticide decrease or prevent the effect of oxidative stress in the serum or plasma. On the other hand increase the levels of uric acid could lead to increase the amount of nitric oxide (NO) synthesis **(Robinson et al., 2011).** Since the NO synthesis initiated the cascade of reactions, eventually leading to the destruction of tubular epithelial cells, and development of acute renal failure **(Goligorsky et al., 2002; Ruan et al., 2015).**

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Conclusion

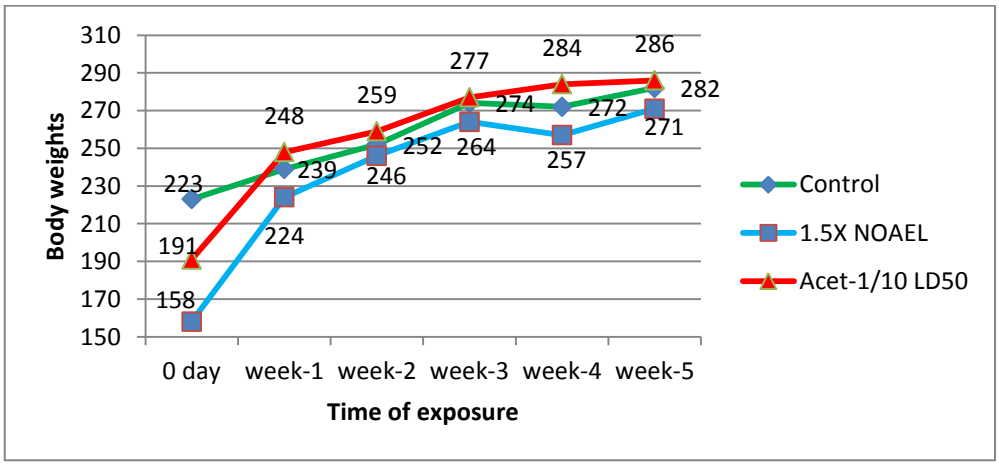
It seems that the ACP at the dose levels tested in the present study for a period of 35 days may not have such extensive effects as inducer to oxidative stress. According to the histopathological findings in this study, exposure to high dose led to destructive and severe effects on the kidney tissue but may be has moderate effects on the liver tissue. The low dose which equal approximately 1.5X of NOAEL led to slight effects on the liver organ and while the kidney organ still was the main organ affected by this insecticide. Finally, It seems that the ACP induces the adverse effect at the high dose level but the low dose level and short term exposure may not make adverse effect. So, it can be recommend to use this insecticide as long as using the recommended dose.

Table(1): Represents the acute toxicity treatment groups, the mortality of the rats, the value of estimated oral LD50 of ACP and the high and low doses used in repeated treatments.

Acute toxicity treatment Groups*	Doses (mg/kg)	Mortality during 14 days	Estimated LD50 (mg/kg)	High dose =(1/10 LD50) (mg/kg)	Low dose ≅(1.5X NOAEL) (mg/kg)
I	261.24	0/5	427.2	42.72	18.03
II	381.40	2/5			
III	556.85	4/5			
IV	813.00	5/5			

*Each group was consisted of five rats

Fig (1). The correlation between the time of exposure of ACP and the body weights of control and treatment groups



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Table(2): The levels of CAT, MDA, GST and TAC in the plasma of rats treated with the ACP

Parameters	Control group	high dose (1/10 of LD50)	low dose (1.5x NOAEL)
TAC (mM/L)	1.79 ± 0.21	1.69 ± 0.11$^{(N.S)}$	1.50 ± 0.14$^{(N.S)}$
CAT (U/L)	60.26 ± 2.82	80.00 ± 5.57$^{(\uparrow)*}$	72.10 ± 2.37$^{(N.S)}$
MDA (mg/g.tissue)	2.81 ± 0.36	3.61 ± 0.07$^{(N.S)}$	2.32 ± 0.12$^{(N.S)}$
GST (U/L)	47.13 ± 5.85	62.13 ± 5.61$^{(N.S)}$	70.19 ± 7.79$^{(N.S)}$

Values are from five replicates in each group and expressed as mean ± SE of each group. P: Significant difference when compared to negative control group.
(N.S), Non-significant; $(\uparrow)^*$, Significant increase; $(\downarrow)^*$, Significant decrease at ($P < 0.05$)

Table(3): The levels of uric acid and Creatinine level in the plasma of rats treated with the ACP

Parameters	Control group	high dose (1/10 of LD50)	low dose (1.5x NOAEL)
Uric acid(mg/dl)	1.89 ± 0.19	3.18 ± 0.144$^{(\uparrow)*}$	1.91 ± 0.18$^{(N.S)}$
Creatinine(mg/dl)	1.19 ± 0.12	1.49 ± 0.06$^{(N.S)}$	1.19 ± 0.07$^{(N.S)}$

Values are from five replicates in each group and expressed as mean ± SE of each group. P: Significant difference when compared to negative control group.
(N.S), Non-significant; $(\uparrow)^*$, Significant increase at ($P < 0.01$)

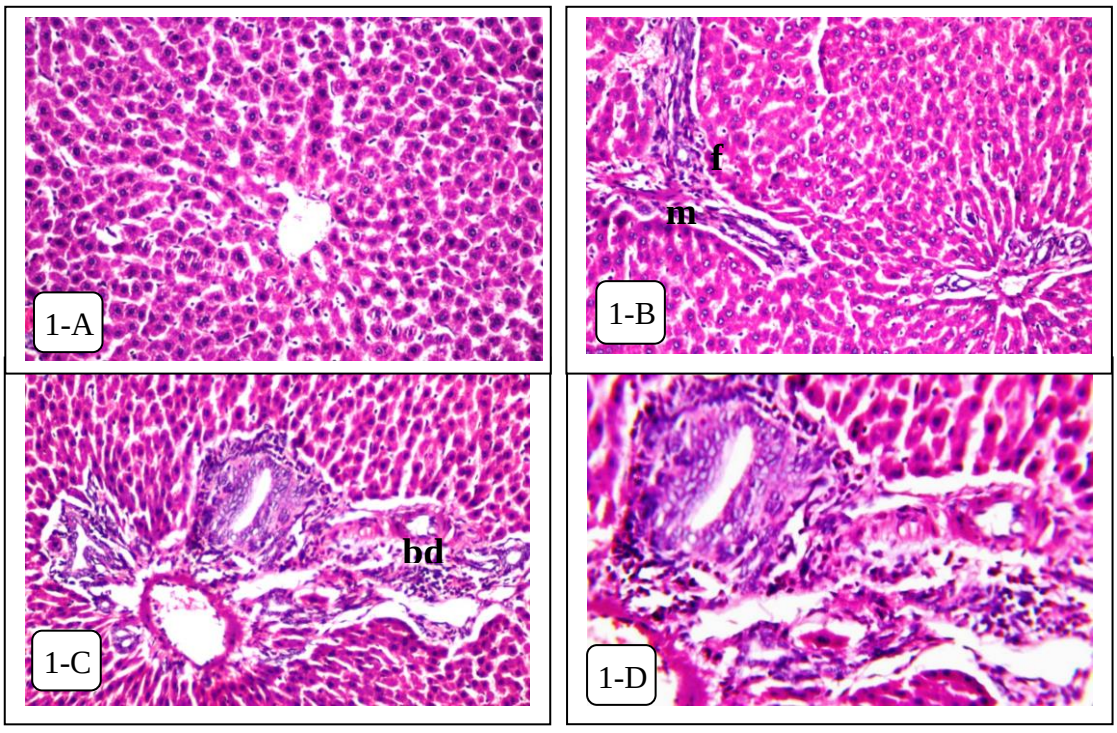

Fig (2) . Light micrographs of the rats liver tissue show as follow: (1-A), control group showing normal histological structure of hepatocytes; (1-B), low dose group showing fibrosis(f) with few inflammatory cells(m) between the hepatocytes; (1-C), high dose group showing newly formed bile ductules(bd) with hyperplasia in the bile ducts of the portal area; (1-D), showing the magnification of (1-C).

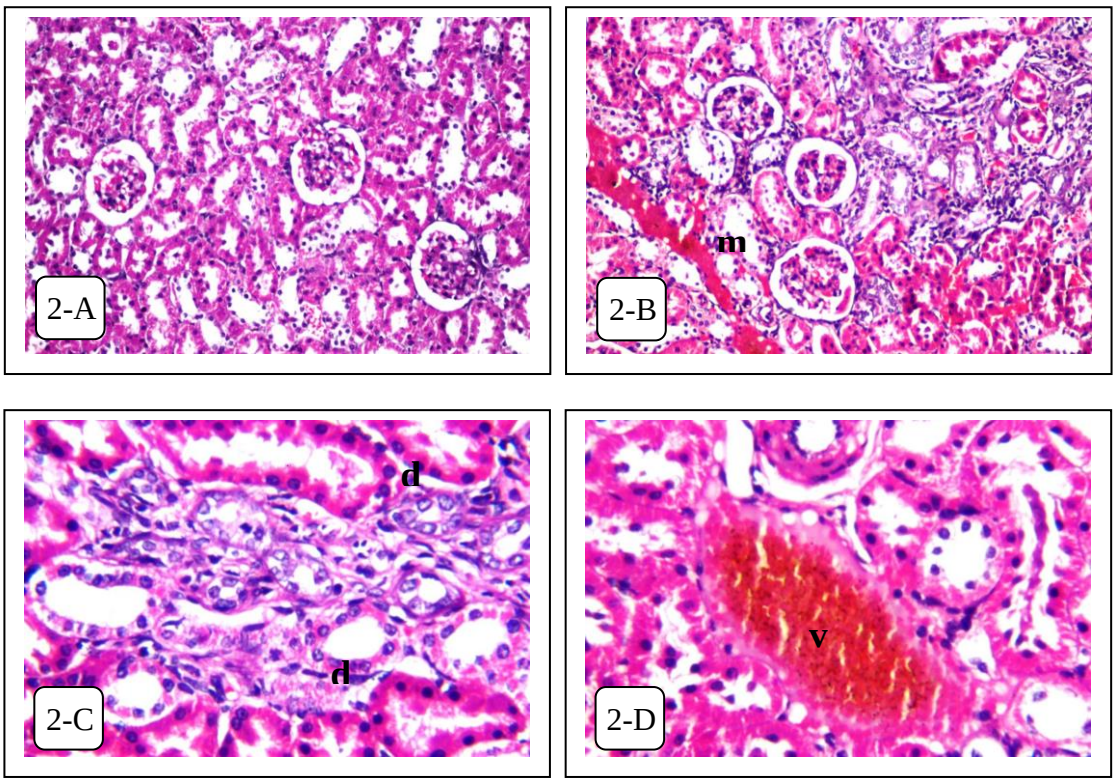

Fig (3) . Light micrographs of the rats kidney tissue show as follow: (2-A), control group showing normal histological structure of glomeruli and tubules at the cortex; (2-B), low dose group showing focal inflammatory cells infiltration(m); (2-C), high dose group showing dysplasia(d) with irregular histological structure ; (2-D), high dose group showing also Sever congestion in the cortical blood vessels(v) and coagulative necrosis in the lining epithelium in most tubules.

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