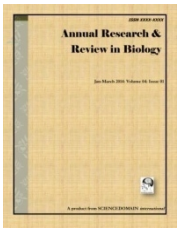

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Histopathological Changes Associated with Exposure of Male Mice to Profenofos and Chlorpyrifos

H. M. El-bendary[1*], M. H. Shaker[2], A. A. Saleh[3], S. E. Negm[3], M. E. Khadey[3] and F. A. Hosam Eldeen[3]

[1]Plant Protection Department, Faculty of Agriculture, Fayoum University, Egypt.
[2]Histopathology Department, Institute of animal health, Ministry of Agriculture, Egypt.
[3]Pesticides Department, Faculty of Agriculture, Mansoura University, Egypt.

Authors' contributions

Author HME designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MHS, AAS, SEN, MEK and FAHE managed the analyses of the study. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The histopathological effects of Profenofos, and Chlorpyrifos, as synthetic organophosphorus pesticides, on the liver, kidney, brain and spleen tissues in mice (*Mus musculus*) were determined by light microscopy. Recently the toxic effects of pesticides have been of public interest. The usage of pesticides is still the most effective and accepted means to protect plants from the pests and to increases productivity. The misuse of pesticides is connected with serious problems of pollution and health hazards. Profenofos and Chlorpyrifos is used widely in Egypt and they play a vital role in controlling Lepidopteron pests of cotton and vegetables [1].
Study Design: Mice were treated with Profenofos, and Chlorpyrifos sub-lethal concentrations (1/10, 1/40 and ADI LD_{50}) orally to twice a week for 30, 60, and 90 consecutive days.
Place and Duration of Study: Department of chemistry Faculty of Agriculture, Cairo University, Egypt, between June 2012 and January 2013.
Results: Histopathological examination revealed various abnormalities in liver tissues, such as congestion of blood vessels, vacuolar degeneration of hepatic cells, focal

**Corresponding author: Email: bendary005@gmail.com;*

infiltration and mononuclear cells, Moreover, all central veins and other hepatic blood vessels were dilated, some hepatic cells showed necrosis, disorganization with the formation of a denoid structure and some areas showed hepatocytomegaly with the increase of the number of cells showing double nuclei. Pathological finding in kidney showed perivascular edema with congestion of renal blood vessels, infiltration of mononuclear cells and around some of glomerular tubules, edema of Bowman's capsule and some renal tubules showed coagulation necrosis. Pathological finding in spleen showed disorganization of lymphocytes in lymphoid follicles and in white pulp, depletion of lymphocyts with sub capsular edema, and other cases showed increasing the number of megaterocytes with hemorrhages and haemosiderosis. Pathological finding in Brain showed menengial hemorrhages and congestion of blood vessels, with neuronophagia and satelletosis and sub meningial encephalomalacia, with neuronal degeneration of purkinjie cells were noticed and lesions, there was lyses of some neurons with demylenation of nerve fibers and privascular and pricellular edema. This investigation proves the toxic effects of Profenofos, and Chlorpyrifos at organ level.

Conclusion: The histopathological data showed that profenofos exhibited histopathological changes in liver, kidney, spleen and brain. Liver showed hepatic cell damage with degenerative changes. Kidney showed heamorrhages, edema, necrosis and glomeruli shrinkage. The spleen showed slight deplesion of the lymphocytes of the white pulp. The brain showed interstitial edema and severe necrosis. From these results we concluded that liver is the most sensitive organ and profenofos damage the structure of liver cells more severely than chlorpyrifos on albino mice.

Keywords: Profenofos; chlorpyrifos; male mice (Mus musculus); histopathology; hepatotoxicity; tumors; liver; kidney; brain; spleen.

1. INTRODUCTION

Around the world, approximately three million acute poisoning and 220000 deaths from pesticide exposure have been reported annually. In addition, farmers with prolonged exposure, such as, neurobehavioral abnormalities and increased cancer incidence, e. g., leukemia, nonhodgkin, Lymphoma and multiple myeloma. The potential utility of biomarkers for monitoring both environmental quality and the health of organism inhabiting in the polluted ecosystems has received increasing attention during the last years [2,3,4,5]. Toxicities of pesticides cause adverse effects on many organs. Other systems that could be affected by organophosphorus (OP) intoxicant are immune system [6,7]; urinary system [8]; reproductive system [9]; pancreases [10]; and homological and biochemical changes [11]. Pesticides affect mitochondrial membrane transportation in mice liver [12]. Furthermore, it disturbs cytochrome P450 system in human liver [13,14]. Meanwhile, (OPs) causes toxic effects on other organisms [15]. Many insecticides are hydrophobic molecules which bind extensively to biological membranes, especially to the phospholipids baitlayers [16]. The majority of research done with pesticides is based on their lethal effects. Diagnosis and predication of physiological consequences of sub lethal contamination can be obtained thought histopathology [17,18,19,20,21]. Retention of (OPs) in the liver for days or months after intoxication opposes the usual opinion that such pesticides are quickly degraded in nature [22,23]. This work is important due to the use of pesticide as well as the use of any potentially injurious chemical substance must be taking into consideration the balance of the benefits that may be expected versus the possible risk of injury to human health or degeneration of environmental quality [24]. The previous issue may be explore an help in

establishing the no observed adverse effect levels (NOAEL) and the application of a safety factors, there by arriving at an acceptable daily intake (ADI).

2. EXPERIMENTAL DETAILS

2.1 Animals

180 male albino mice were used in this investigation, aged 4-5 weeks and of mean weight 20 gram. The animals were randomly housed in appropriate stainless cages in group of 20 animals/cage. The animals were also monitored daily for abnormal symptom and weight change was recorded weekly

2.2 Chemicals

Profenofos and Chlorpyrifos are an organophosphorus insecticides which introduced by Giba-Geigy AG (Novartis). Commercially were kindly provided from Central Agricultural Pesticide Laboratory (Dokki, Giza, Egypt) and all compounds were of 99 % purity.

2.3 Animal Treatment Schedule

Randomized groups of mice housed in cages containing saw dust as bedding and were allocated into 6 groups, each group contained 15 males, the first, second, and third, group were treated with Profenofos at doses 1/10 LD_{50}, 1/40 LD_{50} and daily acceptable intake (ADI) via oral administration for 30, 60 and 90 days respectively. But the other (4 - 5 - 6) groups were treated with Chlorpyrifos as a previously as mentioned in Table (1).

Table 1. Treatment schedule and design

Treatment	Group No.	Doses mg/kg./b.wt.	Period	Dose/week
Profenofos	Group (1)	1/10 LD_{50} = 35	30, 60, and	two doses
	Group (2)	1/40 LD_{50} = 8.95	90 days	
	Group (3)	(ADI) = 0.01		
Chlorpyrifos	Group (4)	1/10 LD_{50} = 15	30, 60, and	two doses
	Group (5)	1/40 LD_{50} = 3.75	90 days	
	Group (6)	(ADI) = 0.01		

2.4 Sampling

After completion of the treatment period each group were sacrificed by cervical dislocation, mice were decapitated and liver, kidney, brain, and spleen were removed immediately, washed with sodium phosphate buffer (pH 7.4). Histopathological samples were fixed in 10% neutral buffered formalin and stored at 4°C for histopathological examination.

2.5 Histopathological Studies

The samples were removed and placed in fresh fixative, were washed in a running tap water overnight, dehydrated in ascending grades of alcohol, cleared in xylol, fixed tissue samples were processed routinely by paraffin embedding technique. Liquefied Para film, (melting point between 55°C and 60°C) for one and a half hours. After solidification of Para film, wax

blocks were cut at section of 5.5 um in thickness were trimmed with rotary microtome at 200 um intervals, and every eight section thought the tissue was collected on the Super Frost Plus slides and stained with haematoxylin and eosin.

2.5.1 Staining method

Haematoxylin and eosin [25]. The section were placed in descending grades of alcohol and rinsed in distilled water. The sections were stained in haematoxylin for 1/2 minutes and then placed in tap water for 3-5 minutes. Counter staining was done in 1 % solution of eosin for one minute followed by washing in distilled water. The sections were dehydrated, cleared in xylol and mounted in Canada balsam, (the nuclei will stain and the cytoplasm will take red color). The resulting sections covered with cover slides to be ready for microscopically examinations.

3. RESULTS AND DISCUSSION

3.1 Pathological Finding in Liver

The liver of mice which sacrified after one month (30 days) which treated with profenofos at 1/10 LD_{50} showed congestion, blood vessels and vacuolar degeneration of hepatocytes, with focal infilration and mononuclear cells Fig. (1). While mice which treated with chlorpyrifos at 1/10 LD_{50} for two months (60 days) showed nearly all central viens and other hepatic blood vessels were dilated Fig. (2). But the liver of mice which sacrified after three month (90 days) which treated with profenofos at 1/10 LD_{50}, revealed different abnormalities such as hyperplastic proleferation of bile ducts were prononced with newly formed bile ductus Fig. (3). While when treated with profenofos at 1/40 LD_{50} showed some hepatic cells necrosis and disorganization with the formation of a denoid structure Fig. (4). On the other hand (ADI) profenofos treatment showed slightand very rear hepatocytomegally Fig. (5). Mice exposed to chlorpyrifos at 1/10 LD_{50} for 90 days showed condensation of chromatin Fig. (6). While mice which trated with chlorpyrifos at 1/40 LD_{50} for 90 days showed hepatic cells under the hepatic capsule were swallen with different types of degeneration Fig. (7). We can say that profenofos and chlorpyrifos as a toxic materail reached to the liver via the gastro intestinal tract blood supply, therefore, the necrosed aresa mainly appeared around portal tract. Also, inflammatory cells were aggregated in portal tracts and present as differential foci in the liver parenchyma. They act as a defence mechanism due to irritation of toxic material and necrosed tisse for the same reson the kupfer cells were activated [26]. In high dose of pesticides subcapsular haemorrhage was observed in the liver of the treated albino mice. This ocurred due to damge of endotheliallining of blood vessels by the tested insecticides. Liver lession were observed by many investigator [27]. Liver suffered from severe lesions after treating the experimental animals with tested pesticides. Moreover, haemorrhage was evident intertubular or subcapsular. This happened as a sequale of liver lessions which leading to lack of clotting factors. Also, observed severs toxicicty led to necrosis of renal tubules which were replaced with inflammatory cells. This findings were confirmed with results of [28] and [29].

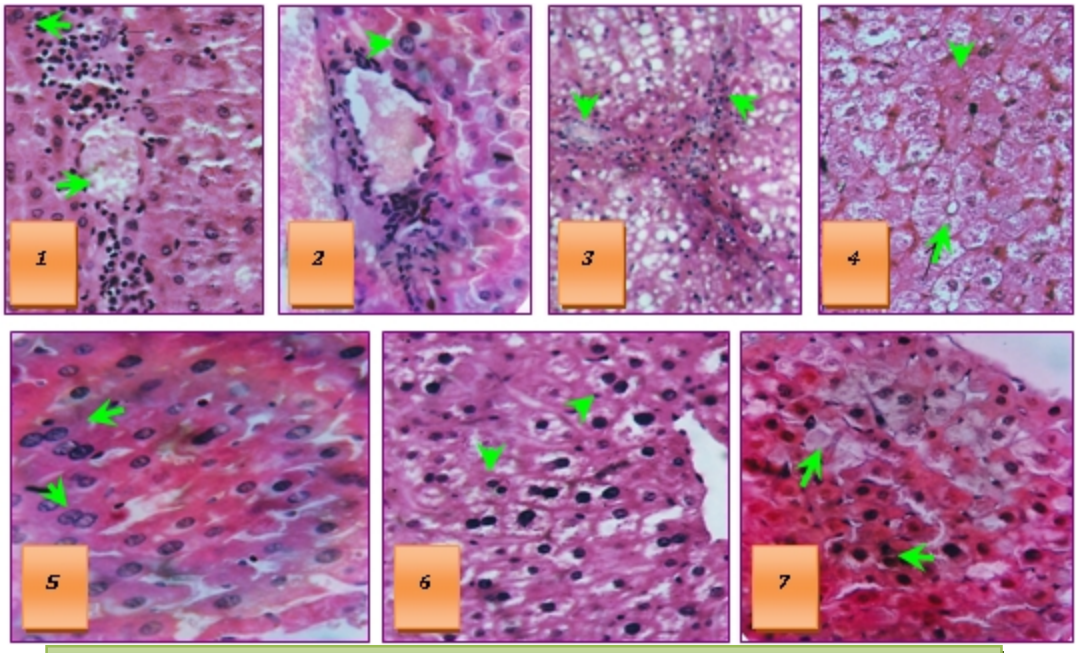


Fig. (1) congestion blood vessels and vacuolar degeneration of hepatocytes
Fig. (2) hepatic blood vessels were dilated
Fig. (3) hyperplastic proleferation of bile ducts were prononced
Fig. (4) necrosis and disorganization with the formation of a denoid structure.
Fig. (5) slight hepatocytomegally
Fig. (6) condensation of chromatin
Fig. (7) different types of degeneration

From these results we concluded that toxicity assessment revealed that liver is the most sensitive biomarker, and profenofos can be rated as highly toxic to mice in comparison with chlorpyrifos. Generaly, Chlorpyrifos and Profenofos showed histopathological alterations in liver of male mice like showing double nuclei, condensation of chromatin, degeneration, necrosis, and odema were noted at 1/10 LD_{50}, where minimal histological evidence of damage was observed with low dose administration 1/40 LD_{50} that is agree with [30].

Major damages caused by profenofos toxicity were diffuse necrosis, cordal disarrangement, individualization of hepatocytes, etc.; significant changes induced by chlorpyrifos were hyperplasia, disintegration of hepatic mass, focal coagulative necrosis, etc [31]. In both cases, damages were dose-dependent, with profenofos exhibiting more sensitivity than chlorpyrifos.

Finally the results show that profenofos and chlorpyrifos exposure causes renal lesions in mice liver. The frequency of liver lesions (steatosis, intravascular granulocyte accumulations, interstitial cell infiltrations, lipid granulomas, portal fibrosis and bile duct hyperplasia) were also highest in the exposed group to 1/10 LD_{50} profenofos more than mice group which treated with 1/10 LD_{50} chlorpyrifos. The livers of both treated groups showed an abnormal size and shape of hepatic cells. [32]. These results suggest that the effects of profenofos are dose

dependent. Histopathological changes in liver and kidney were observed only in 1/10 LD_{50} chlorpyrifos given group [33]. We suggest that mice exposed to profenofos and chlorpyrifos are at risk for developing chronic liver damage.

3.2 Pathological Finding in Kidney

The kideny of sacrificed mice after one month (30 days) and two month (60 days) which treated with chlorpyrifos at 1/10 LD_{50} showed perivioscular edema with congestion Fig. (8). While treatment with profenofos at ADI for 90 days refered to Infeltration of mononuclear cells and arround some of glomeruli Fig. (9), but with profenofos at 1/40 LD_{50} for two months (60 days) refered to edema of Bowman's capsule Fig. (10), on the other hand profenofos at1/10 LD_{50} for 60 days showed cytic dilalation of some renal tubules, also some renal tubules showed coagulation necrosis Fig.(11). After three month (90 gays) when trated wirh profenofos at 1/10 LD_{50} in addition the previous mentioned lesions, hemolysis and heamorrphages were noticed in between renal tubules Fig. (12), renal casts with different origens were clearly noticed Fig. (13), large number of renal tubules showed cystic dilatation and glomeular lipopathy Fig. (14). Shrinkage of large number of glomeruli with edema Fig. (15), and heamorthages were observed. One slides after three month (90 gays) when trated wirh profenofos at 1/40 LD_{50} showed sever cystic dilation with renal casts and infeltration with mononuclear cells and necrosis of renal tubules Fig. (16,17). The glomerular tubules of the kidney were vaculated due to edema, with excessive toxicity concentration and destruction of the glomerular tubules occurred which may be due degenerative changes. Degeneration of renal tubules resulted from collection of albuminous material lining during its excretion in the urine [27,34].

Necrosis of tubular epithelium, cloudy swelling of epithelial cells of renal tubules, narrowing of the tubular lumen, contraction of the glomerulus and expansion of space inside the Bowman's capsule were observed in the kidney tissues of fish after exposure [35]. Profenofos and chlorpyrifos caused degenerative changes in the kidney of mice. Changes were more intense in mice which were treated with 1/10 LD_{50} profenofos than in mice treated with 1/40 LD_{50} profenofos [36]. Finally the kidneys of treated mice showed tubular vascular degeneration and lumen dilatation in both groups.

3.3 Pathological Finding in Spleen

The spleen of sacrificed mice tratment with chlorpyrifos at 1/10 LD_{50} after one and two months showed disorganization of lymphocytes in lymphaid follicles Fig. (18), and in white pulb it self Fig. (19), some slides showed absens of lymphaid follicels and lymphocytes which spreaded all over the spleen when exposed to profenofos at 1/10 LD_{50} for 60 days Fig. (20), while after three months (90 days) spleen showed depletion of lymphocyts in every where of the spleen with sub capsular edema Fig. (21), sometimes extended to the red pulb of the spleen with increase number of reticulo endothelial cells, mainly macrophages, Fig. (22), other cases showed increasing the number of megaterrocytes with heamorrhages and heamosideerosis Fig. (23). The toxic effect of profenofos and chlorpyrifos on hepatic lession leading to congestion and hemorrhages of spleen. Also lymphocytes occurred, which many be affected on the immunity. This findings were confirmed with results of [37].

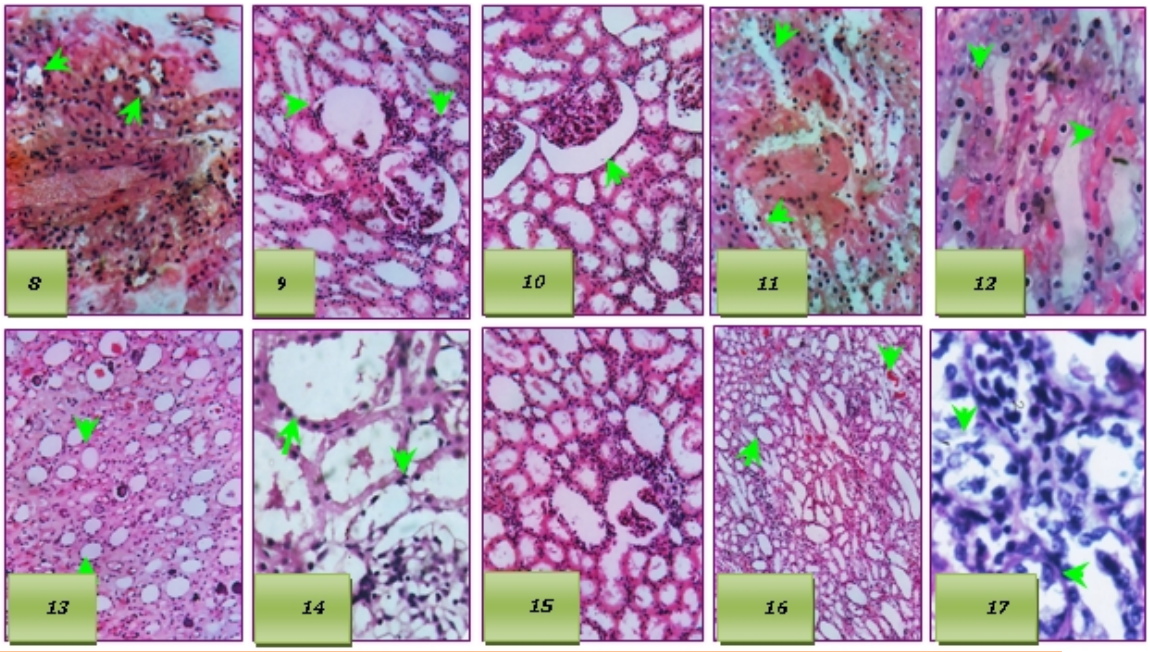
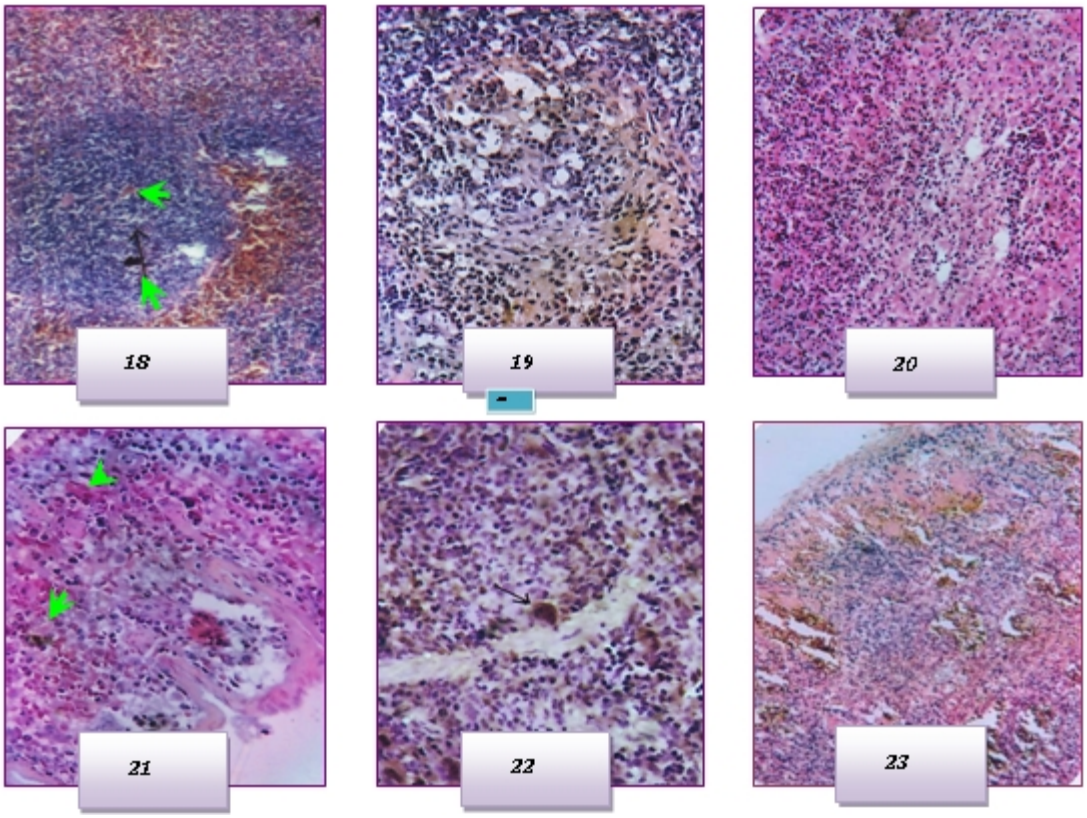


Fig. (8) periviouscular edema with congestion.
Fig. (9) Infeltration of mononuclear cells and arround some of glomeruli
Fig. (10) edema of Bowman's capsule
Fig. (11) cytic dilalation of some renal tubules and coagulation necrosis
Fig. (12) lesions, hemolysis and heamorrphages noticed in between renal tubules
Fig. (13) renal casts with different origens
Fig. (14) large number of renal tubules and glomeular lipopathy
Fig. (15) Shrinkage of large number of glomeruli with edema
Fig. (16-17) dilation with renal casts and infeltration with mononuclear cells

3.4 Pathological Finding in Brain

The brain of mice sacrificed after one month showed menengial heamorrhages Fig. (24) with profenofos at 1/10 LD_{50}, and cengestion of blood vessels Fig. (25), with neuronophagia and satelletosis Fig. (26), after two months, sub meningial encephalomalacia Fig. (27), with neuronal degeneration of purkinjie cells were noticed Fig. (28), but after three months, in addetion to the previously mentioned lesions, there was lysis of some neurons with demylenation of nerve fibers and privascular and pricellular edema Fig. (29). Some slides revealed satillilosis, neuronophagia, focal gliosis and encephalomalacia with demyleration of nerve fibers with chlorpyrifos treatment at 1/10 LD_{50} for 90 days Fig. (30). Histopathological examination revealed congestion of blood vessels and vacuolar degeneration of hepatic cells, necrosis and hepatocytomegaly with the increase of the number of cells showing double nuclei. In kidney showed edema with congestion of renal blood vessels, edema of Bowman's capsule and coagulation necrosis. In spleen showed edema, and increasing the number of megaterocytes with hemorrhages and haemosiderosis. In brain showed hemorrhages, congestion of blood vessels and edema. The authors finding proves the toxic potential in terms of the damages induced by Profenofos, and Chlorpyrifos at organ level. This findings were confirmed with results of [38,39,40].



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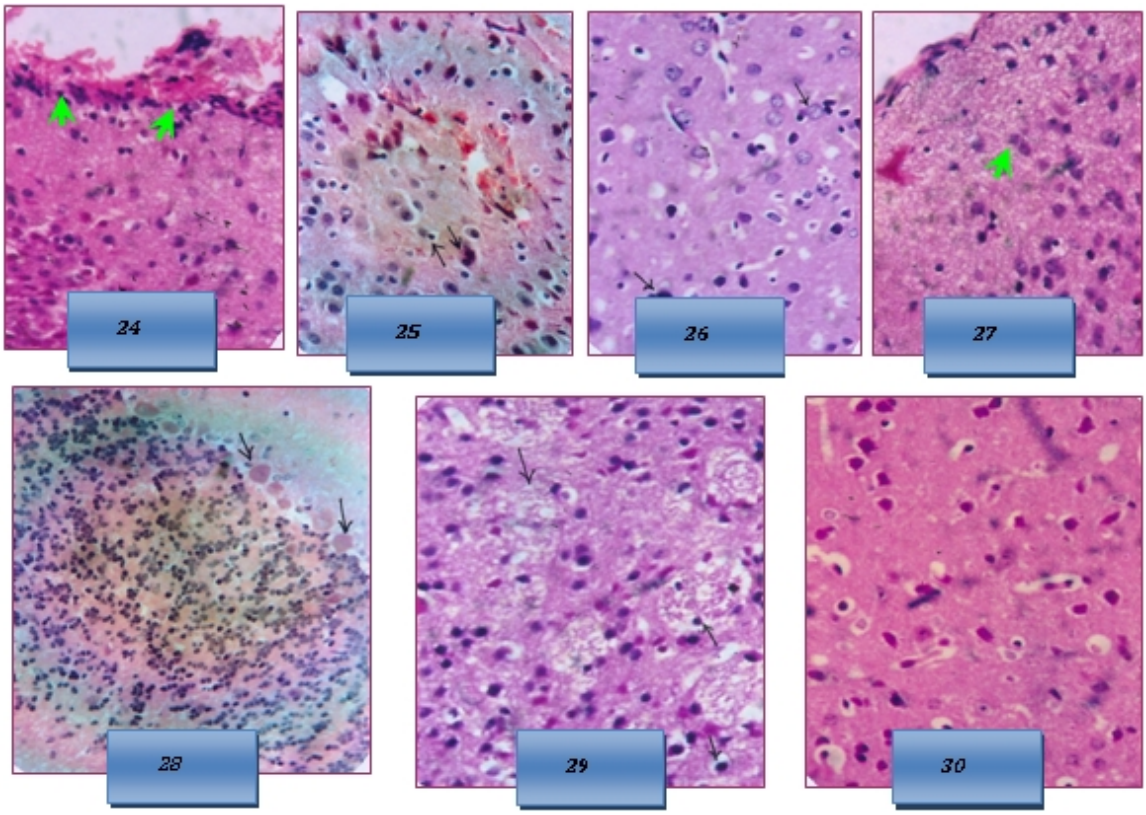

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Fig. (27) sub meningial encephalomalacia
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Fig. (29) lysis of some neurons with demylenation of nerve fibers and privascular and pricellular edema
Fig. (30) satillilosis, neuronophagia, focal gliosis and encephalomalacia with demyleration of nerve fibers

4. CONCLUSION

The histopathological data showed that profenofos and chlorpyrifos exhibited histopathological changes in liver, kidney, spleen and brain. Liver showed hepatic cell damage with degenerative changes. The kidney showed heamorrhages, edema, necrosis and glomeruli shrinkage. The spleen showed slight deplesion of the lymphocytes of the white pulp. The brain showed interstitial edema and severe necrosis. From these results we concluded that toxicity assessment revealed that liver is the most sensitive biomarker, and profenofos the most exert histopathological effects on albino mice comparison with chlorpyrifos. On the other hand, Chlorpyrifos and Profenofos showed histopathological alterations in liver of male mice like showing double nuclei, condensation of chromatin,

degeneration, necrosis, and odema were noted at 1/10 LD_{50}, where minimal histological evidence of damage was observed with low dose administration 1/40 LD_{50} .

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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