Journal of Applied Sciences Research, 3(5): 377-386, 2007

Comparative Histopathological and Histochemical Studies on IGR, Lufenuron and Profenofos Insecticide Albino Rats

[1]Abdel Razik H. Farrag and [2]Shehata E.M. Shalby

[1]Department of Pathology, National Research Centre, Dokki, Cairo, Egypt
[2]Pests and Plant Protection Department, National Research Centre, Dokki, Cairo, Egypt

Abstract: The present study was planned to compare the histopathological and histochemical effects of the insect growth regulator, lufenuron and the organophosphorus insecticide, profenofos, on the liver and kidney of rats. Administration of both compounds to rats with one-tenth of their median lethal doses for two months (day by day), the toxicants were withdrawn for 30 days to allow recovery from toxicity. The histopathological investigation indicated that $^{1}/_{10}$ LD_{50} caused venous congestion in the liver, focal necrosis of hepatocytes in the portal and periportal areas. Many of the hepatocytes are pale-stained and a few exhibit early vacuolation. Also, several cells show histological features of necrosis. Kidney exhibited inflammatory cell infiltration, congestion and hypercellularity of the glomeruli. The histochemical examination indicated depletion of the polysaccharides in liver and kidney. This study showed that both of lufenuron and profenofos caused histopathological and histochemical effects in liver and kidney tissues even after the recovery period. The effects of Lufenuron are more strength than profenofos.

Key words: IGR's, lufenuron, profenofos, histology, histochemistry, toxicity - rats.

INTRODUCTION

Pesticides are widely used throughout the world in agriculture to protect crops and in public health to control diseases transmitted by vectors or intermediate hosts.

Insect growth regulators (IGRs) are third-generation insecticides less toxic and compatible with insect pest management that were developed to reduce the pollution of food and environment. These compounds have a specific mode of action on insects and a lower toxicity against vertebrates than conventional insecticides[11]. IGRs include compounds that affect moulting and metamorphosis by mimicking juvenile hormone (JH agonists) or usually antagonizing JH activity (ecdysteroid agonists) or by interfering with cuticle formation (chitin synthesis inhibitors)[33, 22, 23].

During application of IGR on plants, part of the agent usually falls on the soil surface. Its subsequent penetration into the subsurface environment can cause pollution of soil, sediment and ground water. Evaluation of the corresponding ecotoxicity of IGR should take into consideration, in addition to the actual agent used, also its degradation products arising for the most part as metabolites of soil aerobic microorganisms[35].

IGR's have a large potential for becoming an environmentally and economically important group of chemicals, however, no or very few toxicological studies have been carried out to evaluate the acute and chronic toxicity effects of lufenuron on the laboratory animals. The researchers assented the obvious residues on fruit and vegetables during food processing, especially in acidic food becoming more persistence and less decayed even with used high temperature[1].

Lufenuron (antimoulting compound) is one of the most newly introduced synthetic insect growth regulators. It is used for control of Lepidoptera and Coleoptera larvae on cotton, maize and vegetables; and citrus white fly and rust mites on citrus fruits[2].

Organophosphorus insecticides are among the most frequently used pesticides. They are used in agriculture, forestry, horticulture, public health (i.e., hospitals) and the house[37, 38]. Organophosphates are well resorbed after uptake via the oral, dermal or inhalation route[8, 7] and are rapidly metabolized in the human body[9]. In general, 90% of the compounds are excreted within 6–24 h after oral uptake[8, 10, 34, 17, 18].

Profenofos is a widely organophosphorus insecticide used in Egypt for the control of various caterpillars, white fly and mites on cotton and vegetable crops[5, 2].

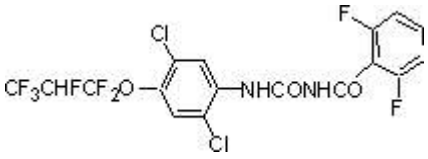
Therefore, the present study aimed to compare the histopathological and histochemical effects of profenofos and lufenuron on liver and kidney of the albino rats.

Corresponding Author: Shehata E. M. Shalby, Pests and Plant Protection Department, National Research Centre, Dokki, Cairo, Egypt. Email: sh_shalbynrc@yhoo.com

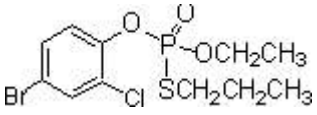
MATERIAL AND METHODS

Chemicals:

Lufenuron: (Match 5 % E.C). (RS)-1-[2, 5-dichloro-4-(1, 1, 2, 3, 3, 3-hexafluoropropoxy)-phenyl]-3-(2, 6-difluorobenzoyl) urea Empirical Formula: $C_{17}H_8Cl_2F_8N_2O_3$ Activity: insecticides (chitin synthesis inhibitors) and structural formula:



Profenofos: (Selecron 72 % E.C), O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate. Empirical Formula: $C_{11}H_{15}BrClO_3PS$, Activity: insect pests and mites on cotton and vegetables (Organophosphorus insecticides) and structural formula:



Animals: Male albino rats (80-100 g) were obtained from the Animals Laboratory of Helwan Farm, Ministry of Health and Populations, Egypt. The animals were housed in standard environmental conditions and had free access to tap water and food throughout the experiment period. The animals were divided into five groups of 10 animals each. Control (1), given corn oil. Lufenuron (2) or profenofos (3) groups was given the $^1/_{10}$ LD_{50} of the compounds orally day after day for two months. Lufenuron (4) or profenofos (5) groups was treated as in the later groups and stayed one month without treatment. The LD_{50} of profenofos is 380 mg/kg b.w.; while for lufenuron is 2000 mg/kg b.w.[2].

Histological and histochemical Studies: Twenty four hours after the last treatment, animals were anesthetized with ether and livers and kidneys were isolated and fixed in 10 % buffered formalin, sectioned, stained for histological examination (Haematoxylin and eosin)[6] and for histochemical estimation (Periodic acid Schiff method)[19]. Leica Qwin 500 image analyzer computer system (England) was used for detection polysaccharides. The optical density of PAS reaction was measured in the cytoplasm of liver and kidney cells, using the grey image menu in ten small measuring frames in each specimen using an objective lens of magnification 40 i.e. at a total magnification of 400. The image was transformed into a grey image [a grid of pixels each representing the intensity or brightness at that point by a range of numbers, typically from 0 (black) to 255 (white)]. The parameter chosen was the grey level [the brightness of each point of image by numerical scale, typically from 0 (black) to 255 (white)].

Statistical Analysis: Data are expressed as mean±standard error. The effects of lufenuron and profenofos on polysaccharides of liver and kidneys were analyzed with ANOVA and student's t- test. Statistical significance was accepted at $P<0:05$.

RESULTS AND DISCUSSIONS

A-The histopathological effects:

I-Liver: The hepatic lobules are the structural units of the liver; each is formed of cords of hepatocytes and blood sinusoids in between (Figure 1).

Administration of oral doses equivalent to $^1/_{10}$ LD_{50} of lufenuron day after day for two months caused venous congestion in the liver, focal necrosis of hepatocytes in the portal and periportal areas. Many of the hepatocytes are pale-stained and a few exhibit early vacuolation. Also, several cells show histological features of necrosis. The dead cells become intensely eosinophilic stain and stand out from the other cells. Compared to living cells, the nuclei of each necrotic cell is smaller, condensed and intensely stained with haematoxylin (pyknotic) and in several cells the nuclei became fragmented into several particles (karyorrhexis) (Figure 2-A).

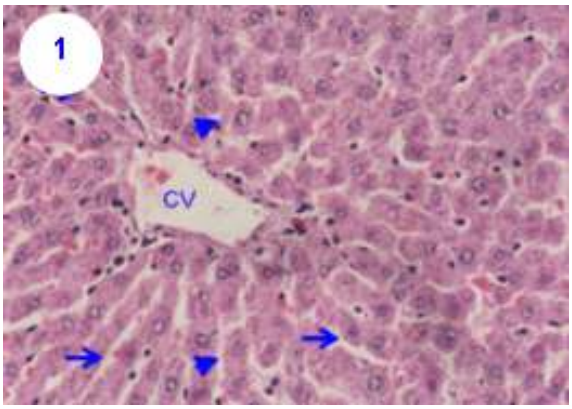


Fig. 1: Liver of control rat showing the architecture of a hepatic lobule. The central vein (CV) surrounded by the hepatocytes (arrowheads), between the hepatocytes, the hepatic sinusoids (arrows) are shown. (H and E stain-X 300)

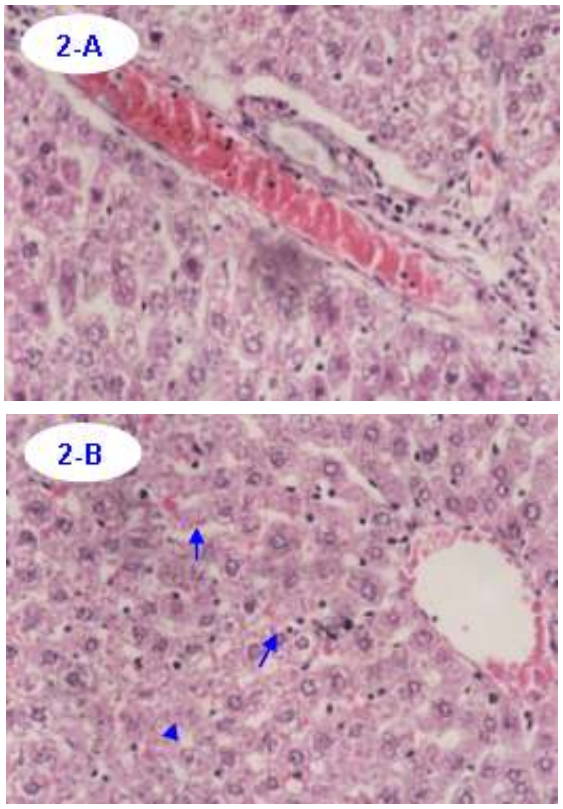


Fig. 2: Liver of rat daily given an oral dose equivalent to $^{1}/_{10}$ LD_{50} day after day for two months A) In case of lufenuron showing congested and dilated vein in the portal area. Many of the hepatocytes are pale-stained and a few exhibit early vacuolation. Several cells also show histological features of necrosis. The dead cells stain a bright pink and stand out from the other cells. The nuclei the necrotic cells become pyknotic or karyorrhexis form and B) In case of profenofos showing cell necrosis (arrow head), lymphocyte infiltration (long arrow) and dilated blood sinusoids (short arrow). (H and E stain-X 300)

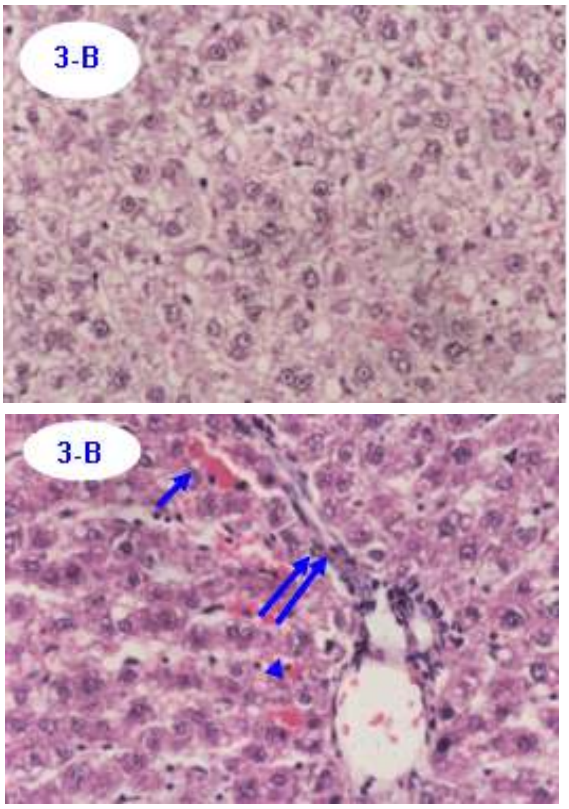


Fig. 3: A photomicrograph of section of liver of rat given day after day oral doses equivalent to $^{1}/_{10}$ LD_{50} of for two months then stayed without treatment for another month (A) In case of lufenuron showing disruption of liver structure that associated with focal necrosis and developed vacuoles and (B) in case of profenofos showing dilated sinusoids (arrows). Notice necrosis of some hepatocytes (short arrow), presence of cell debris in the blood sinusoids (arrow head) and pyknosis of some nuclei (long arrow) (H and E stain- X 150).

Examination of liver sections of rats received an oral dose equal to the $^{1}/_{10}$ LD_{50} of profenophos day after day for two months showed cell necrosis, lymphocyte infiltration and dilation of blood sinusoids (Figure 2-B).

Examination of liver sections of treated rats by $^{1}/_{10}$ LD_{50} of lufenuron for two months then stayed without treatment for another month demonstrated mild lymphocyte infiltration with dilated and congested veins together with dilated sinusoids (Figure 3-A).

Examination of liver sections of rats received to $^{1}/_{10}$ LD_{50} of profenofos showed periportal necrosis of the hepatocytes near the portal areas. The specimens also, showed dilated and congested portal vessels as well as mild areas of inflammatory cell infiltration especially in the vicinity of the portal veins and near the bile ductules. Some cells exhibited necrosis together with pyknosis of some nuclei. Slight haemorrhage was also noticed (Figure 3-B).

II- Kidney: Kidney of control rat showed normal structure of the renal corpuscle (glomerulus and urinary space) and renal tubules (proximal convoluted tubules and distal convoluted tubules) (Figure 4).

Examination of kidney of treated rats by $^{1}/_{10}$ LD_{50} of lufenuron day after day for two months showed

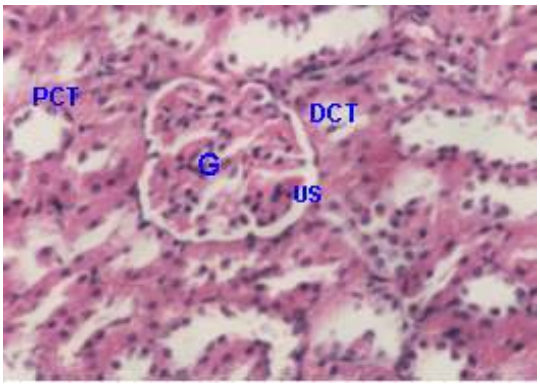


Fig. 4: Kidney of control rat showing renal corpuscle, glomerulus (G) and urinary space (US) and renal tubules, proximal convoluted tubules (PCT) and distal convoluted tubules (DCT). (H and E X 300).

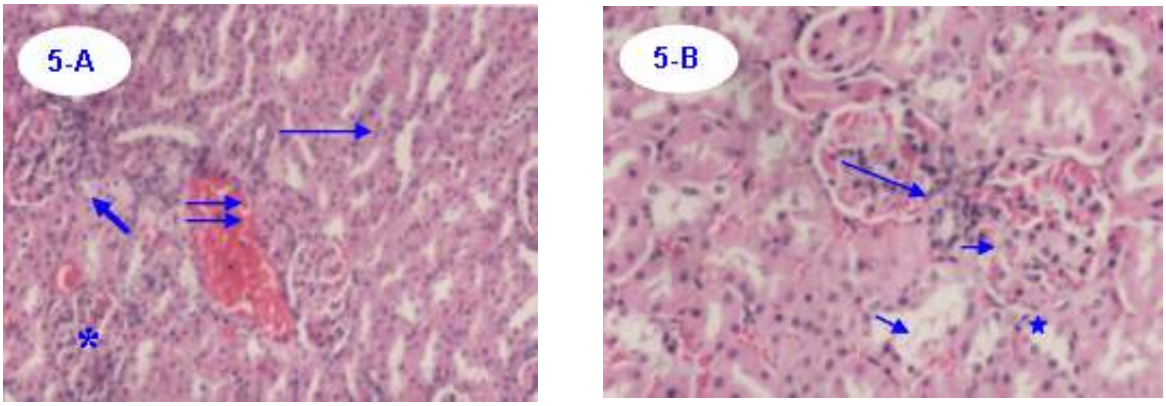


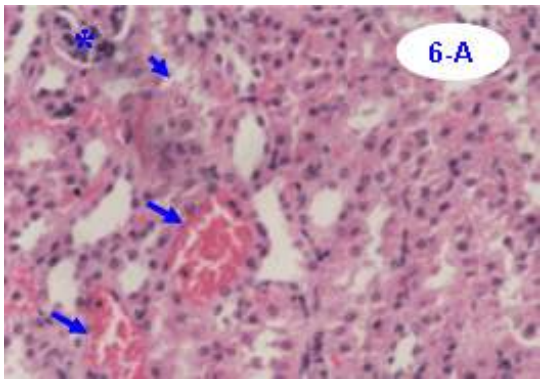
Fig. 5: Kidney of rat daily treated with oral dose equivalent to $^{1}/_{10}$ LD_{50} of day after day for two months (A) In case of lufenuron showing inflammatory cell infiltration associated with congested glomerulus (arrow). The glomeruli show hypercellularity (asterisk) with narrow urinary spaces (arrow head). The renal tubules exhibit almost normal structure (arrow). Haemorrhagic areas in the interstitium (arrows) also seen. (H and E X 150) (B) In case of profenofos showing inflammatory infiltration in the interstitial spaces associated with the glomeruli (arrow). The renal corpuscles show congestion and hypercellularity and wide urinary space (arrow head). Notice the haemorrhagic areas in the interstitium and the glomeruli (arrows). Some tubules show desquamation of its epithelial cells (arrow head) (H and E X 300)

infiltration of the inflammatory cell that associated with the congested glomeruli. The glomeruli exhibited hypercellularity with wide urinary spaces. The renal tubules exhibited almost normal structure. Haemorrhagic areas in the interstitium and desquamation in the epithelial cells of some tubules are also seen (Figure 5-A). Kidney of rat given oral doses equivalent to $^{1}/_{10}$ LD_{50} of profenofos day after day for two months exhibited inflammatory infiltration in the interstitial spaces. The renal corpuscles showed congestion and hypercellularity and wide urinary space. The haemorrhagic areas in the interstitium and the glomeruli were noticed (arrows). Some tubules show desquamation of its epithelial cells (Figure 5-B).

Kidney of treated rats by $^{1}/_{10}$ LD_{50} of lufenuron for two months then stayed without treatment for another month showed almost normal structure glomeruli and renal tubules. In same cases, desquamation of the epithelial cells of tubules and haemorrhagic areas in the interstitium was noticed (Figure (6-A). On the other hand, examination of kidneys in case of profenofos showed inflammatory infiltration and haemorrhagic areas present in the interstitium. Necrosis of some cells of the proximal convoluted tubules was noticed. The nuclei of these cells are pyknotic (Figure 6-B).

B- Histochemical Effects:

I- Liver: Examination of liver sections of control rats stained with Periodic acid Schiff's (PAS) showed the



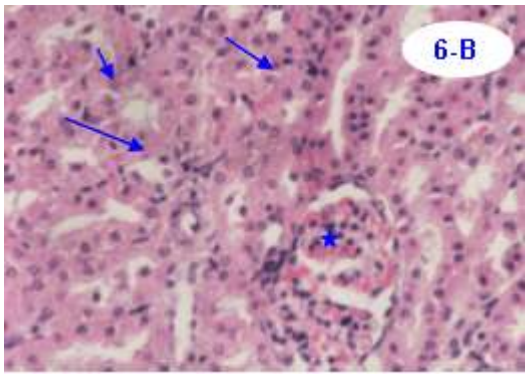


Fig. 6: Kidney of rat daily given day after day oral doses equivalent to $^{1}/_{10}$ LD_{50} of for two months then stayed without treatment for another month (A): In case of lufenuron showing the normal structure of glomeruli (asterisk). The renal tubules exhibit almost normal structure. Notice the haemorrhagic areas in the interstitium (arrows). Some tubules show desquamation of its epithelial cells (arrow head) and (B): In case of profenofos showing the inflammatory infiltration (arrow) and haemorrhagic areas present in the interstitium. Notice necrosis of some cells of the proximal convoluted tubules (arrow head). The nuclei of these cells are pyknotic (long arrow). Notice the congested glomeulus (asterisk) (H and E stain-X 150)

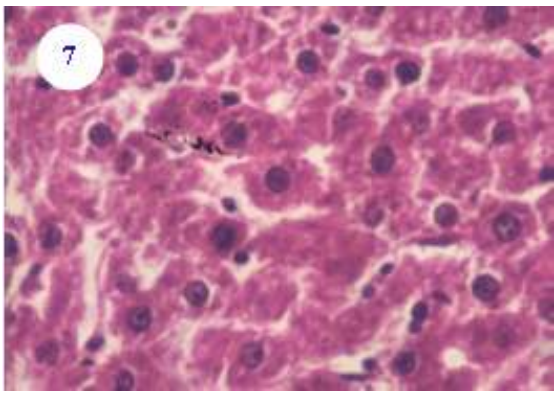


Fig. 7: Liver of a control rat showing the polysaccharides particles accumulated at the cytoplasm of hepatocytes. (PAS. X 300)

distribution of the polysaccharides inclusions in the form of purple granules and particles in the cytoplasm of the hepatocytes (Figure 7).

A repeated oral administration of $^{1}/_{10}$ LD_{50} doses of lufenuron or profenofos for two months showed a severe depletion in the polysaccharides content of the hepatocytes (Figure 8-A and B). At the $P<0.05$ level, the mean values of the gray level of polysaccharides content in liver treated with $^{1}/_{10}$ LD_{50} of lufenuron or profenofos for two months are significantly decrease as compared to control (Table 1).

Regarding to oral administration of $^{1}/_{10}$ LD_{50} doses of lufenuron or profenofos for two months and stayed one month without treatment, liver showed a mild depletion in the polysaccharides contents of the hepatocytes (Figure 9- A and B). At the $P<0.05$ level, the mean values of the gray levels of polysaccharides content in liver of the rats receiving $^{1}/_{10}$ LD_{50} dose of lufenuron or profenofos for two months then stayed for one months without treatment are significantly increase compared with the treated rats by both lufenuron or profenofos for two months (Table 1). These results demonstrate a trend of decrease of polysaccharides in case of lufenuron treated rats as compared with the profenofos treated rats.

Table 1: The mean values of the gray level of polysaccharides in liver of rats treated with lufenuron and profenofos.

	Liver	Kidney
Groups	Means ± SE	Means ± SE
Control group	142.38 ± 2. 08	109.86 ± 1.92
Lufenuron group (2 month)	220.14 ± 1.24 *	172.13 ± 2.47 *
Lufenuron group (2 month) and 1 month	164.11 ± 3.57 * ˙	143.46 ± 1.95 * ˙
Profenofos group (2 months)	190.12 ± 2.12 *	161.63 ± 2.10 *
Profenofos group (2 months) and 1 month	151.11 ± 3.17 * ˙	123.76 ± 2.15 * ˙

* Significant decrease at the $P < 0.05$ as compared with the control group.

˙ Significant increase at the $P < 0.05$, the means are compared the recovery with treated only.

II- Kidney: Examination of kidneys of control rats showed the presence of polysaccharides in the Bowman's capsule, capillaries of the glomeruli, the basement membranes of the proximal and distal convoluted tubules and the brush border of the proximal convoluted tubules (Figure 10).

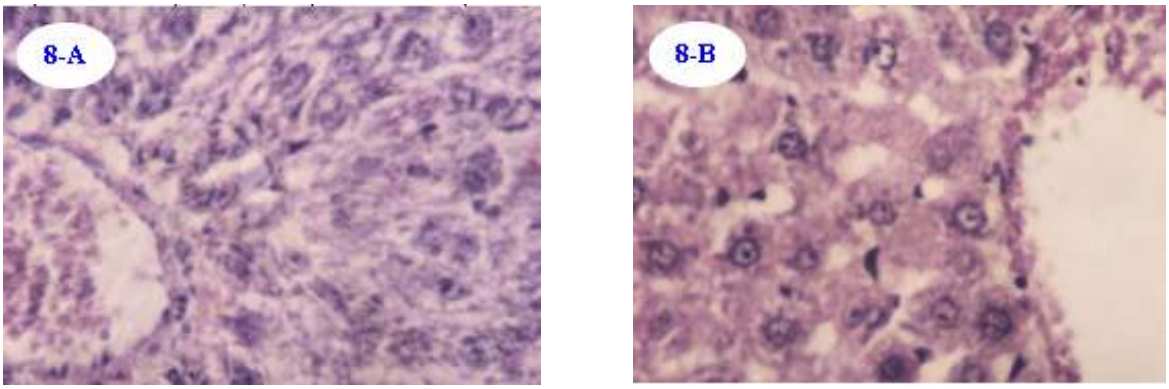


Fig. 8: Liver of a rat received 1/10 LD_{50} of lufenuron (A) or profenofos (B) day after day for two months showing a severe depletion of the positive PAS materials of the hepatocytes. (PAS X 300)

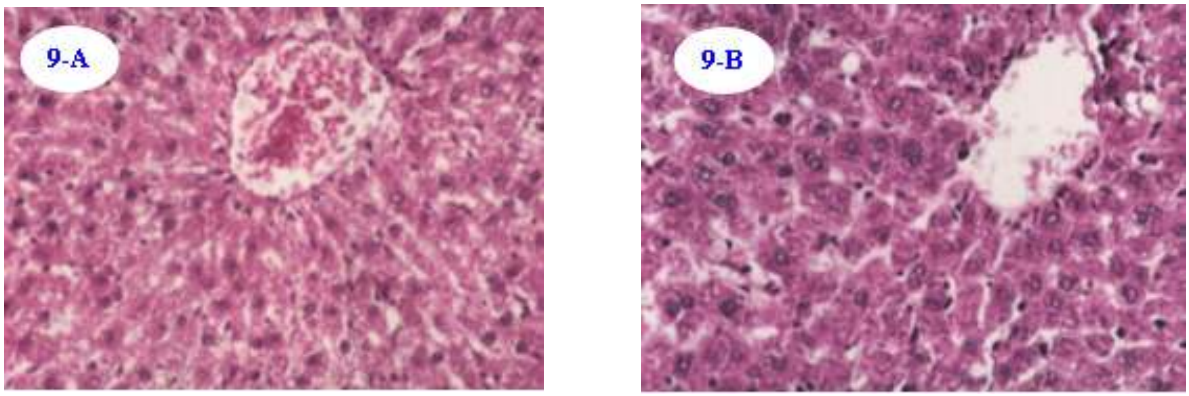


Fig. 9: Liver of a rat received $^{1}/_{10}$ LD_{50} of lufenuron (A) or profenofos (B) day after day for 2 months and stayed another month without treatment showing reduction of the polysaccharides content. (PAS X 300).

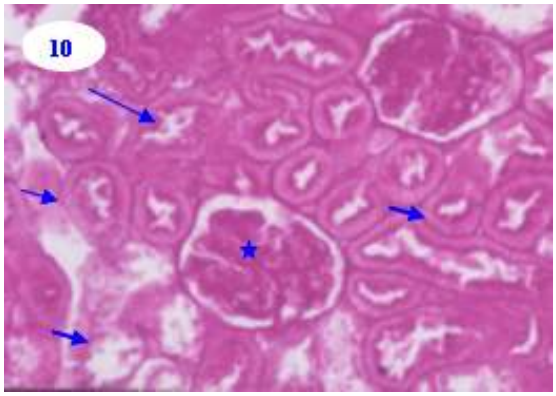


Fig. 10: Kidney of a control rat showing the polysaccharides content. The positive reaction in the renal corpuscles (asterisk), the basement membrane of proximal and distal convoluted tubules (arrowhead) and the brush border of the proximal convoluted tubules (arrow). (PAS X 300).

The kidneys of rats treated with the $^{1}/_{10}$ LD_{50} of lufenuron or profenofos for two months (Figure 11- A and B) and that treated with of lufenuron or profenofos for two months then stayed without treatment another one month (Figure 11- A and B) exhibited a heterogeneous stainability, where the cells of degenerating tubules were weakly stained, the healthy tubules were stained more or less like normal one. The glomeruli displayed a weak PAS reaction

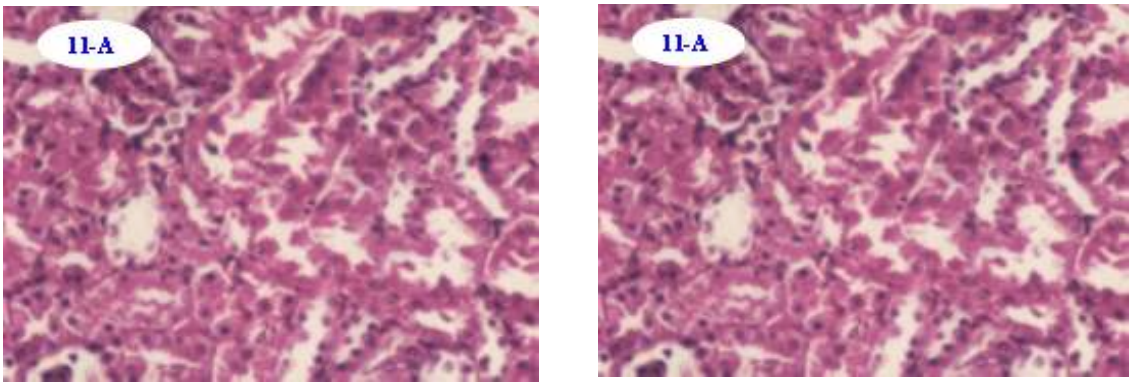


Fig. 11: Kidney of a rat treated with the $^{1}/_{10}$ LD_{50} of lufenuron (A) or profenofos (B) day after day for two months showing the heterogeneous stainability, where the degenerated tubular cells are weakly stained and the healthy tubules are normally stained. (PAS X 300).

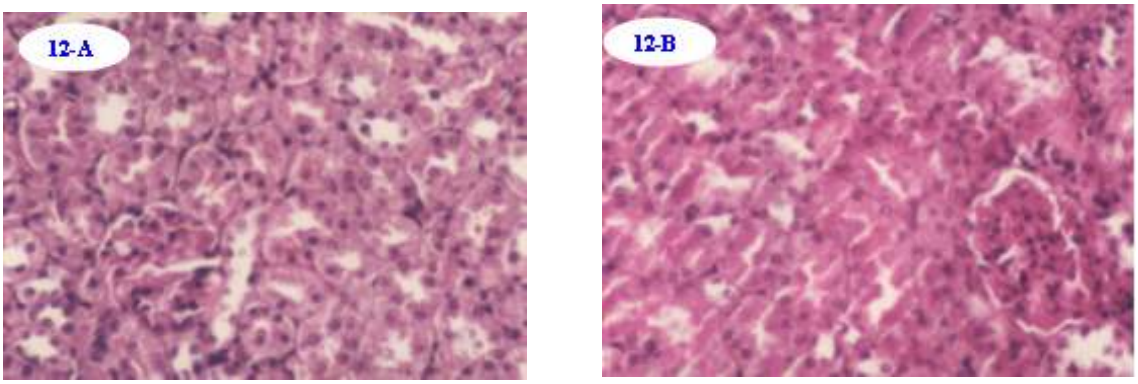


Fig. 12: Kidney of a rat treated with the $^{1}/_{10}$ LD_{50} of lufenuron (A) or profenofos (B) day after day for two months then stayed without treatment another one month showing the heterogeneous stainability, where the degenerated tubular cells are weakly stained and the healthy tubules are normally stained. (PAS X 300)

The mean values of the gray level of polysaccharides content in kidney of control rats and those given lufenuron or profenofos ($^{1}/_{10}$ LD_{50}) for two months and those given lufenuron or profenofos ($^{1}/_{10}$ LD_{50}) for two months and stayed without treatment for another month are presented in Table (1).

These data in table 1 indicated that the means of the gray level of polysaccharides content in kidney of rats received lufenuron or profenofos ($^{1}/_{10}$ LD_{50}) for two months and that given lufenuron or profenofos ($^{1}/_{10}$ LD_{50}) for two month and stayed without treatment for another month showed significant decrease (P<0.05) as compared to control. On the other hand, in rats treated with $^{1}/_{10}$ LD_{50} of lufenuron or profenofos for two months and stayed without treatment for another month showed significant increase (P<0.05) in the polysaccharides content as compared with that given lufenuron or profenofos for two month.

These data demonstrate a trend of decrease of polysaccharides contents in kidneys of rats treated with lufenuron as compared with those treated with profenofos.

Discussion: Pesticides are used extensively in agriculture and their residues have affected the environment adversely. The use of such biologically active compounds poses potential problems of toxicity among those who manufacture, formulate, or use these compounds. Pesticides are also used directly in aquaculture to control the ectoparasites and insects in nursery and grow-out systems.

In the present investigation, it has been observed that both of the IGR, lufenuron and the organophosphorus insecticide, profenofos, caused histopathological and histochemical changes in the liver and kidney of the albino rats.

The liver is the centre for detoxifying any foreign compounds entring the body. So, it uniquely exposed to a wide variety of exogenous and endogenous products. These include environmental toxins and chemicals present in food or drinking[36].

The present study revealed that oral treatment of rats with lufenuron or profenofos showed different pathological lesions in the liver tissue. Nevertheless, it is clear that liver tissues are markedly responded to the

adverse effect of the insecticide; it displayed marked histological changes with the lufenuron.

The hepatic tissues exhibited dilated and congested portal vessels with perivascular mild lymphocyte infiltration in cases of treatment of lufenuron or profenofos orally for two months or after treatment for two months and stayed for one month without treatment. Rather similar results were obtained by Hurket[16] in rats treated with dursban, by Gupta *et al*.,[12] in buffalo calves received a single dose of malathion and by Hanafy *et al*.,[15], in rats given tamaron.

The present experimental material has also revealed the development of focal necrosis in the liver under the effect of profenofos. The basis of the focal necrosis is poorly understood[29, 27]. However it consists of discrete areas of hepatocytic necrosis that can be found at any location within the hepatic lobule with small number of mononuclear inflammatory cells frequently found in the lesions.

In the present investigation the liver blood sinusoids became dilated under the effect of profenophos. This observation was also recorded in the liver of rats under the influence of dursban intoxication[21]. Such lesion was also reported by Guzelian *et al*.,[14] in human poisoned with chlordecone (kepone) and in human and experimental animals poisoned by chlorodecone[13].

The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are liver specific enzymes and they are more sensitive measure of hepatotoxicity and histopathological changes and can be assessed within a shorter time[3]. Elevation in the transaminases indicates the utilization of amino acids for the oxidation or for glucogenesis[26] and is used to determine liver damage. These results support the findings in our study that indicate liver damage. Also it is supported by the finding of[24, 25, 40, 28].

Our results are also in accordance with Shalby[30], who reported that treated rats by $^{1}/_{10}$ LD50 of lufenuron and profenofos (day after day for two month) caused significant changes on blood contents and some chemical parameters (ALT, AST, urea and creatinine activities) of treated rats without return to normal levels at the end of recovery period (30 days).

In the present study, the histochemical investigation of polysaccharides in liver and kidneys of lufenuron or profenofos-treated rats showed weak and heterogeneous stainability which indicate depletion of these inclusions. The use of lufenuron led to more decrease in the polysaccharide inclusions in both liver and kidneys than that in case of profenofos.

Rao,[28] reported that the depletion of glycogen in the tissues is an indication of typical stress response in fish challenged with pesticides. Glycogen depletion in liver and muscle after toxic stress has been reported in several studies with aquatic animals[31, 4].

In general, the reduced carbohydrate components under the effect of pesticide could be due to the release of hydrolytic enzymes from the ruptured lysosomes under the toxic effect of toxic agents[32, 29].

In conclusion, the IGR, Lufenuron and the insecticide, profenofos caused histopathological and histochemical changes that not retained to normal then after one month of recovery. It was found that lufenuron is more effective than profenofos.

REFERENCES

1. Abdel-Mageed, M.I., U.M. Radwan, A.Z. Hindy and A. Zrook, 2001. Liver functions under stress of certain common pesticides residue used on fruits and vegetables orally administrated. Annals Agric. Sci., Ain Shams Univ., Cairo, Egypt, 46: 383-404.
2. Anonymous, 2004. The Pesticide Manual, version 3.1, 2004-05, Thirteen Ed. Editor: C D S Tomlin.
3. Balint, T., J. Ferenczy, F. Katai, I. Kiss, L. Kraczer, O. Kufcsak, G. Lang, C. Polyhos, I. Szab, T. Szegletes and J. Nemcsok, 1997. Similarities and differences between the massive eel (*Anguilla anguilla* L.) devastations that occurred in lake Balaton in 1991 and 1995, Ecotoxicol. Environ. Saf., 37: 17-23.
4. Bhavan, P.S. and P. Geraldine, 1997. Alterations in concentrations of protein, carbohydrate, glycogen, free sugar and lipid in the prawn *Macrobrachium malcolmsonii* on exposure to sublethal concentrations of endosulfan, Pestic. Biochem. Physiol., 58: 89–101.
5. British Crop Protection Council, 1991. Pesticide Manual pp: 705. London.
6. Drury, R.A.B. and E.A. Wallington, 1980. Carleton's histological technique- 4th Ed- oxford, Oxford University press.
7. Feldmann, R.J. and H.I. Maibach, 1974. Percutaneous penetration of some pesticides and herbicides in man. Toxicol. Appl. Pharmacol., 28: 126–132.
8. Fisher, H.L., B. Most and L.L. Hall, 1985. Dermal absorption of pesticides calculated by deconvolution. J. Appl. Toxicol., 5: 163–177.
9. Gallo, M.A. and N.J. Lawryk, 1991. Orgaophosphorus pesticides. In: Hayes, Jr., W.J., Laws, Jr., E.R. (Eds.), Handbook of Pesticide Toxicology, Classes of Pesticides, vol. 2. Academic Press, San Diego (Chapter 16).
10. Gompertz, D., 1996. Organophosphorus pesticides. In: World Health Organization (Ed.), Biological Monitoring of Chemical Exposure in the Workplace, Guidelines, vol. 1. Geneva.

11. Grenier, S. and A.M. Grenier, 1993. Fenoxycarb, a fairly new insect growth regulator: a review of its effects on insects. Ann. Appl. Biol., 122: 369–403.
12. Gupta, R.C., N. Singh, B.S. Paul and M.S. Kwatra, 1981. Role of residual estimation and clinico-biochemical and pathological changes in diagnosis of toxicity in Bulbalus bubalis caused by malathion. Indian Anim. Sci., 51: 616-622.
13. Guzelian, P.S., 1982. Comparative toxicology of chlordecone in human and experimental animals. Pharmacol. Toxicol., 22: 89-113.
14. Guzelian, P.S., G. Vranian, J.J. Boylan, W.J. Cohn and R.V. Blanke, 1980. Liver structure and function in patients posioned with chlordecone (Kepone). Gastroenterology, 78: 206-213.
15. Hanafy, M.S.M., M.S. Arbid and M.M.H. Afify, 1991. Biochemical and histopathological effects of the organophosphorous insecticide (Tamaron) in rats Indian J. Anim, Sci., 61: 43-47.
16. Hurket, P.O., 1978. Some histological and histochemical studies in rabbits during of subchronic administration of Dieldrin (HEOD). Ind. Exp. Biol., 16: 716.
17. Leng, G., K.H. Kuhn and H. Idel, 1997a. Biological monitoring of pyrethroids in blood and pyrethroid metabolites in urine: applications and limitations. Sci. Total Environ., 199: 173-181.
18. Leng, G., A. Leng, K.H. Kuhn, J. Lewalter and J. Pauluhn, 1997b. Human dose-excretion studies with the pyrethroid insecticide cyfluthrin: urinary metabolite profile following inhalation. Xenobiotica, 27: 1272–1283.
19. McManus, J.F.A., 1946. Histological demonstration of mucin after periodic acid. Nature, London: Cited from "Theory and practice of Histological Techniques"
20. Bancroft, J.D. and A.S. Mbbs, eds, 1977. pp: 148. Edinburgh, Churchill Livingstone.
21. Mikhail, T.H., N. Aggour, R. Awadallah, M.N. Boulos, E.A. El-Dessoukey and A.I. Karima, 1979. Acute toxicity of organophosphorous and organochlorine insecticides in laboratory animals. Z. Ernahrungswiss., 18: 258-268.
22. Oberlander, H., D.L. Silhacek, E. Leach, I. Ishaaya and E. Shaaya, 1991. Benzoylphenyl urea inhibits chitin synthesis without interfering with amino sugar uptake in imaginal wing discs of Plodia interpuntella, Arch. Insect Biochem. Physiol., 18: 219–227.
23. Oberlander, H., D.L. Silhacek, E. Shaaya and I. Ishaaya, 1997. Current status and future perspectives of the use of insect growth regulators for the control of stored product insects, J. Stored Prod. Res., 33: 1–6.
24. Oluah, N.S., 1998. Effect of sublethal copper (II) ions on the serum transaminase activity in catfish *Clarias albopunctatus*, J. Aquat. Sci., 13: 45–47.
25. Oluah, N.S., 1999. Plasma aspartate aminotransferase activity in the catfish *Clarias albopunctatus* exposed to sublethal zinc and mercurys, Bull. Environ. Contam. Toxicol., 63: 343–349.
26. Philip, G.H., P.M. Reddy and G. Sridevi, 1995. Cypermethrin induced in vivo alterations in the carbohydrate metabolism of freshwater Wsh *Labeo rohita*, Ecotoxicol. Environ. Saf., 31: 173–178.
27. Popp, J.A. and R.C. Cattley, 1991. Hepatobillary system. In: Handbook of Toxicologic pathology (Haschek, W.M. and Rousseaux, C.G. eds) pp: 279-314. San Diego, Academic Press Inc.
28. Rao, J.V., 2006. Toxic effects of novel organophosphorus insecticide (RPR-V) on certain biochemical parameters of euryhaline Wsh, *Oreochromis mossambicus* enkateswara. Pesticide Biochemistry and Physiology, ARTICLE IN PRESS.
29. Shalaby, A.A., 1985. Effect of cyolane on the cytology and histochemistry of the ileum of *Clarias lazera*. M. Sc. Thesis, Faculty of Science, Zagazig University.
30. Shalby, Sh.E.M., 2006. Comparative Haematological and hepatorenal toxicity of IGR, Lufenuron and profenofos insecticide on albino rats. J. Egypt. Soc. Toxicol., 34: 85-98
31. Shobha, R.J.V., P.V. Venkateswarlu and C. Janaiah, 1989. Changes in carbohydrate metabolism of *Clarias batrachus* (Linn.) when exposed to two organophosphorus insecticides, J. Environ. Biol., 10: 197-204.
32. Sivaprasada, K., K.R.S. Sambasiva and K.V. Ramana, 1983. Effect of parathion on tissue ionic changes in fish *Channa punctatus*. Geobios (JODHPUR)., 10: 60-62.
33. Smet, H., M. Rans and A. De Loof, 1990. Comparative effectiveness of insect growth regulators with juvenile hormone, anti-juvenile hormone and chitin synthesis inhibiting activity against several stored food insect pests, In: Fleurat-Lessard, F., Ducon, P. (Eds.). Proceedings 5th International Working Conference on Stored Product Protection, France, pp: 649–657.
34. Vasilic, Z., V. Drevenkar, B. Stengl, Z. Frobe and V. Rumenjak, 1993. Diethylphosphorus metabolites in serum and urine of persons poisoned by phosalone. Chem. Biol. Interact., 87: 305–313.
35. Vining, L.C., 1980. in: A.H. Rose (Ed.), Economic Microbiology, vol. 5, Academic Press, London, pp: 523.

36. Wight, D.G.D., 1982. Atlas of Liver pathology. Lancaster, MTP Press Ltd.
37. World Health Organisation, 1986. Environmental Health Criteria 63: Organophosphorous Insecticides: a General Introduction, WHO, Geneva.
38. World Health Organisation, 1990. Environmental Health Criteria 94. Permethrin. WHO, Geneva.
39. Zemmerman, H.J., 1978. Hepatotoxicity: The adverse effects of drugs and other chemicals on the liver. pp: 49-56. New York, Appletonenury - Crofts.
40. Zikic, R.V., A.S. Stajn, S.Z. Pavlovic, B.I. Ognjanovic and Z.S. Saicic, 2001. Activities of superoxide dismutase and catalase in erythrocytes and plasma transaminase of gold Wsh (*Carassius auratus gibelio* Bloch.) exposed to cadmium, Physiol. Res., 50: 105–111.