Pathological Studies on the Effects of Some Locust Insecticides on Rabbits

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ABSTRACT

Forty-five, New Zealand, rabbits of two months age were used to study the pathological effects of some locust insecticides (chloropyrifos and diflubenzuron) to evaluate the less toxic one. The rabbits were divided into 5equal groups. Gp.1 was kept as untreated control. Gp. 2 treated with chloropyrifos(48%) in a dose of 750 ppm of the diet. Gp.3 treated with chloropyrifos (48%) in a dose of 1500 ppm of the diet Gp. 4 treated with diflubenzuron in a dose of 8000 ppm of the diet. Gp. 5 treated with 16000 ppm diflubenzuron of the diet. Three rabbits from each group were necropsied 15, 30 and 45 days post-administration (PA). Specimens were collected from liver and kidneys and fixed in 10% neutral buffered formalin. Paraffin sections, 5μ thick, were prepared and stained with hematoxylin and eosin and examined microscopically.

No clinical signs were recorded among rabbits of gps 2 and 4, Meanwhile rabbits of gps 3and 5 showed reduction in body weight 15 and 30 days PA besides salivation and lacrimation among rabbits of gp3 at 45 days PA . No mortalities were recorded in all groups throughout the experimental period.

Grossly, the liver and kidneys at gps. 2, 3, 4 and 5 were large and dark red in color 15 and 30 days PA .The rabbits of gps. 3 and 5 showed enlarged and pale-yellow liver, 45 days PA. The liver was firm in some cases. The kidneys were enlarged and showed few grayish white foci, 1 mm in diameter on its surface.

Microscopically, the liver and kidneys gp.1 were normal. The liver at gps.2 and 4 showed congestion of the hepatic blood vessels and sinusoids. The hepatocytes showed cloudy swelling. The portal areas were infiltrated with lymphocytes particularly around the bile ductules 15 and 30 days PA. Focal coagulative necrosis of the hepatic parenchyma with lymphocytic infiltration could be seen 45 days PA. Moreover, the bile ductules showed hyperplasia of its epithelial lining besides periductal fibrosis. The kidneys showed cloudy swelling and congestion of the renal blood vessels 15 days PA. hypercellularity of some glomeruli was noticed 30 days PA. Periglomerular lymphocytic infiltration and fibrous connective tissue proliferation could be seen 45 days PA. The liver at gp.3 showed severe lesions represented by vacuolation of hepatocytes mainly steatosis or focal coagulative necrosis of the hepatic parenchyma with mononuclear leukocytic infiltration 15 and 30 days PA. The portal areas were severely distended with hyperplastic bile ductules and fibrous connective tissue proliferation 45 days PA. The renal parenchyma showed numerous mononuclear leukocytic infiltration and cystic dilatation of some renal tubules 15 and 30 days PA. Interstitial fibrous connective tissue proliferation was noticed in the renal cortex 45 days PA. The liver at gp.5 showed congestion, perivascular hemosiderosis and hypertrophy of the kupffer cells 15 days PA. Congestion of hepatic sinusoids with pressure atrophy of the adjacent hepatic cords, besides focal coagulative necrosis of the hepatic parenchyma and hyperplastic bile ductules were noticed at 30 and 45 days PA,. The kidneys showed congestion and vacuolation of the epithelial lining of some renal tubules 15 days PA, Subcapsular cystic dilatation of some renal tubules was noticed 30 days PA. Focal replacement of the renal parenchyma with mononuclear leukocytes mainly lymphocytes could be seen 45 days PA.

Finally, it could be concluded that administration of chlorpyrifos (48%) and diflubenzuron induced some pathological changes in liver and kidneys of rabbits. The severity of the lesions depends upon the dose and duration of administration. This study declared that diflubenzuron was less toxic for rabbits than chlorpyrifos.

INTRODUCTION

The desert locust (Schistocerca gregaria, Forscal) has threatened agricultural crops in the desertic and semi-desertic zones of northern Africa, the near east and south-west of Asia for thousands of years. Despite the development of improved monitoring and control technologies; this threat continues to the present day *(1)*.

The control of the desert locust with increasing human population is considered as an international problem. Many problems have been encountered as a result of excessive use of synthetic pesticides. Increasing problems concerning the application of pesticides included pest resistance, residue contamination of human food, mammalian toxicity and pollution of the environments *(2)*.

Most of the locust control carried out in the last 40 years has used conventional chemical insecticides (organochlorines, organophosphates, carbamaties and pyrethroides). They work either by direct contact action (droplet land on the locusts) or by secondary contact action (locusts touch the droplets on the vegetation) or by stomach action (locusts eat the sprayed vegetation). The insecticides are usually neurotoxic and their mode of action by interfering with its nervous system. There are some new products of both chemical and biological nature with many advantage such as diflubenzuron fipronil botanicals and metarhizium species *(3)*.

Chloropyrifos is non systemic organophosphorus insecticidesactive. The mode of action through contact and stomach poisoning. It is concentrated insecticide for ULV application to control locusts and grasshoppers that infest the agricultural crops. It is harmful to man and animals if swallowed or inhaled or via eye and skin contact *(4)*.

Diflubenzuron is a synthetic compound used in agriculture forestry and pubic health programme to control locust. Different formulation of diflubenzuron are available for these uses. There is no relevant information on human exposure to diflubenzuron *(5)*.

The aim of the present study was to evaluate toxicity and lesions of chloropyrifos and diflubenzuron on liver and kidneys of rabbits.

MATERIAL AND METHODS

I) Experimental animals

Forty-five New Zeeland rabbits, 2 months age were divided into 5 equal groups, one group was kept as control for both insecticides and two groups were used of each of them and kept under hygienic measures in battery systems and feed a bellet ration for 45 days.

II) Tested locust insecticides

(1) Chlorpyrifos (Chlozane 48% purity)

Molecular formula: $C_9H_{11}C_{13}NO_3P_5$.

Toxicity: rabbit oral LD_{50} 3 gm/kg ration according to MOBED, Arab pesticide industries company.

(2) Diflubenzuron (Dimilin)

Molecular formula: (N-[4-chlorophenyl] amino carbonyl-2,6-difluorobenzamide.

$C_{14}H_9ClF_2N_2O_2$.

Toxicity: rabbit oral LD_{50} 4640-5000 mg/kg b.wt according to *(6)*.

Three rabbits from each group were necropsied 15, 30 and 45days PA and specimens from liver and kidneys were collected and fixed in 10% neutral buffered formalin solution. Paraffin sections of 5 μ thick were prepared and stained with Hematoxylin and Eosin for histopathological examination *(7)*.

Table 1. The experimental design included experimental groups, number of rabbits/group, type of insecticide and it's dose and of sacrificed period.

Group	No. of rabbits/group	Type and dose of insecticide		Numbers of sacrificed rabbits among different experimental periods (day)		
		Chlorpyrifos	Diflubenzuron	15	30	45
1	9	-	-	3	3	3
2	9	750 ppm	-	3	3	3
3	9	1500 ppm	-	3	3	3
4	9	-	8000 ppm	3	3	3
5	9	-	16000 ppm	3	3	3

RESULTS

Clinical signs

The observed clinical signs varied according to the pesticide concentration and time of exposure. They were unclear in gps. 2 and 4 at 15 and 30 days PA, while it appeared mild 45 days PA, Slight reduction in the body weight was detected in rabbits of gp. 3 at 15 and 30 days PA, beside lacrimation, salivation and muscle tremor in some cases at 45 days PA, In gp 5 the only marked signs were reduction in both food consumption and body weight particularly at 45 days PA.

Gross lesions

The gross lesions observed at 15 and 30 days PA, among rabbits of gp 2 were mild and represented by congestion of both hepatic and renal tissue later on 45 days PA, liver appeared firm, dark in color with grayish necrotic foci, distended gall bladder. Kidneys of some rabbits were firm or enlarged. Rabbits of gp 3 showed moderate to severe congestion of both liver and kidneys 15 and 30 days PA, On 45 PA, the liver became more dark, firmer with irregular hemorrhagic areas. Other cases showed pale, yellow enlarged liver and firm kidneys. Rabbits of gp 4 showed no lesions 15 and 30 days PA, slight congestion of hepatic and renal tissue was detected 45 days PA, in some cases. Rabbits of gp5 showed enlargement and congestion in both liver and kidneys 15 and 30 days PA, While liver became firm and small in size with grayish white foci scattered on both hepatic and renal surfaces 45 days PA,

The microscopical findings

Rabbits of gp 2 at 15 days PA, the hepatic tissue showed congestion of central vein and cloudy swelling represented by swollen hepatocytes with granular cytoplasm Fig. 1 and congestion of the portal blood vessels. There were lymphocytic infiltration around bile ductule Fig. 2. Hypertophy of the Kupffer cells, congested portal blood vessels with periductolar fibrous connective tissue proliferation could be seen at 30 days PA, Fig. 3. The liver at 45 days PA, showed scattered apoptotic bodies in the periphery lobular areas, hyperplasia of epithelial lining of bile ductules with periductolar edematous fibrous tissue infiltrated with leukocytes Fig.4. Kidneys, at 15 days PA, showed cystic dilatation of some renal tubules which contained casts with interstitial lymphocytic infiltration Fig. 5. Hypercellularity of some glomeruli was detected at 30 days PA. Fig. 6. and at45 days PA, periglomerular lymphocytic infiltration and fibroblast proliferation were noticed Fig. 7.

Rabbit of gp 3 at 15 days PA, the liver showed focal necrosis of hepatocytes, which replaced with lymphocytes and macrophages Fig. 8. Numerous leukocytic infiltrtation could be seen in the portal area. The rabbits necropsied 30 days PA, had numerous mononuclear leukocytic infiltration in the portal area which extended to the interlobular area Fig. 9. On 45 days PA, vacuolations of hepatocytes mainly fatty change were seen Fig. 10. Distended portal areas with proliferated bile ductules also seen. Kidneys, 15 days PA, showed congestion of corticomedullary junction with shrinkage of

glomeruli. Intense leukocytic infiltration in the renal medulla with cystic dilatation of some renal tubules could be seen 30 days PA, Fig. 11. On 45 days PA, numerous leukocytic infiltrations in the renal cortex and medulla were seen Fig. 12. Mild interstitial fibrous connective tissue proliferation was observed in the renal cortex Fig. 13.

Rabbits of gp 4 at 15 and 30 days PA, liver showed congestion of central vein and hepatic sinusoids beside hypertrophy of Kupffer cells with granular hepatocytes cytoplasm Fig. 14. On 45 days PA, swollen hepatocytes with granular cytoplasm with pyknosis and karyolysis of their nuclei were seen Fig. 15. Focal coagulative necrosis and mononuclear cell infiltration, congested portal blood vessels, proliferation of bile ductules with edema and mild periductolar fibrosis were common Fig. 16. Kidneys 15 days PA showed mild congestion. Cloudy swelling of some renal tubules was detected 30 days PA,Fig. 17. On 45 days PA, congestion of intertubular capillaries in the renal cortex and nephrosis of some tubular epithelium were seen Fig. 18.

Rabbits of gp (5), liver 15 days PA, showed severe congestion and perivascular hemosiderosis Fig. 19. Hypertrophy of the Kupffer cells and distended portal area with edema, congestion and proliferation of bile ductule could be seen also Fig. 20. Congestion of hepatic sinusoids with pressure atrophy of the hepatic cords were noticed 30 days PA, Other cases showed focal coagulative necrosis and mononuclear cell infiltrations Fig. 21. Later on, 45 days PA, periductolar fibrosis was seen Fig. 22. Kidneys at 15 days PA, showed congestion and vacuolation of epithelial lining of some renal tubules. Subcapsular cystic dilatation of some renal tubules with flat nuclei was common 30 days PA, On 45 days PA, severe congestion with degenerative changes of some tubular epithelium were noticed Fig. 23. Moreover, focal replacement of necrotic renal tubules with mononuclear cells mainly lymphocytes were seen Fig. 24.

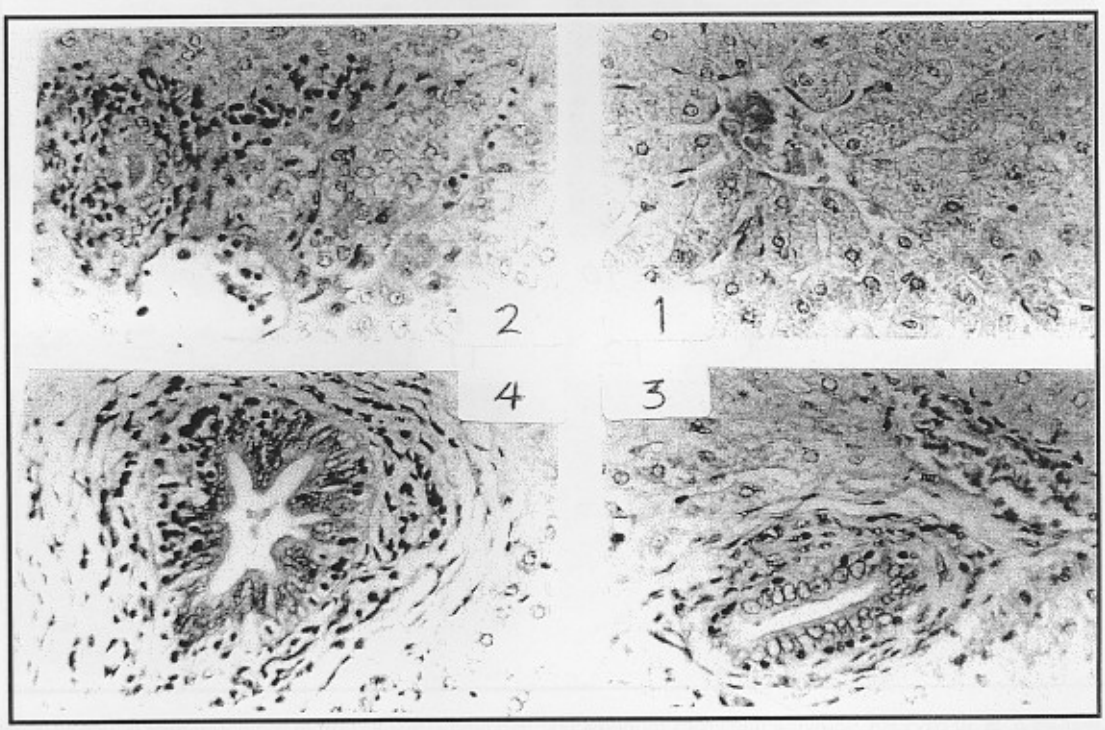

Fig. 1. Liver **gp2**, 15 days PA, showing congestion of central vein and cloudy swelling of the hepatocytes, H&E x 520.

Fig. 2. Liver gp 2, at 15 days PA, showing lymphocytic infiltration around the bile ductule, H&E x 520.

Fig. 3. Liver gp 2, at 30 days PA, showing congestion of portal blood vessels and periductolar fibrous connective tissue proliferation, H&E x 520.

Fig.4. Liver gp 2, at 45 days PA, showing hyperplasia of epithelial lining of bile ductule with periductolar edematous fibrous tissue infiltrated with leukocytes, H&E x 520

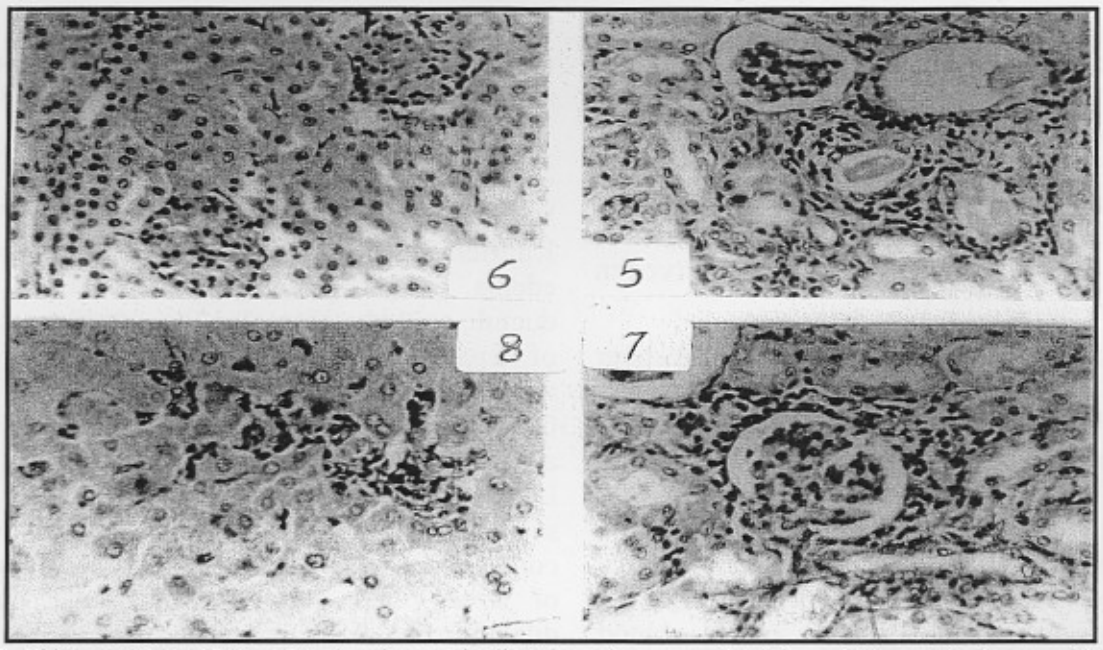

Fig. 5. Kidney gp 2, at 15 days PA, showing cystic dilatation of some renal tubules which contained casts with interstitial lymphocytic infiltration, H&E x 520.

Fig. 6. Kidney gp 2, at 30 days PA, showing hypercellularity of some glomeruli, H&E x 520.

Fig. 7. Kidney gp 2, at 45 days PA, showing periglomerular lymphocytic infiltration and fibroblast proliferation, H&E x 520.

Fig. 8. Liver **gp 3**, at 15 days PA, showing focal necrosis which replaced with lymphocytes and macrophages, H&E x 520.

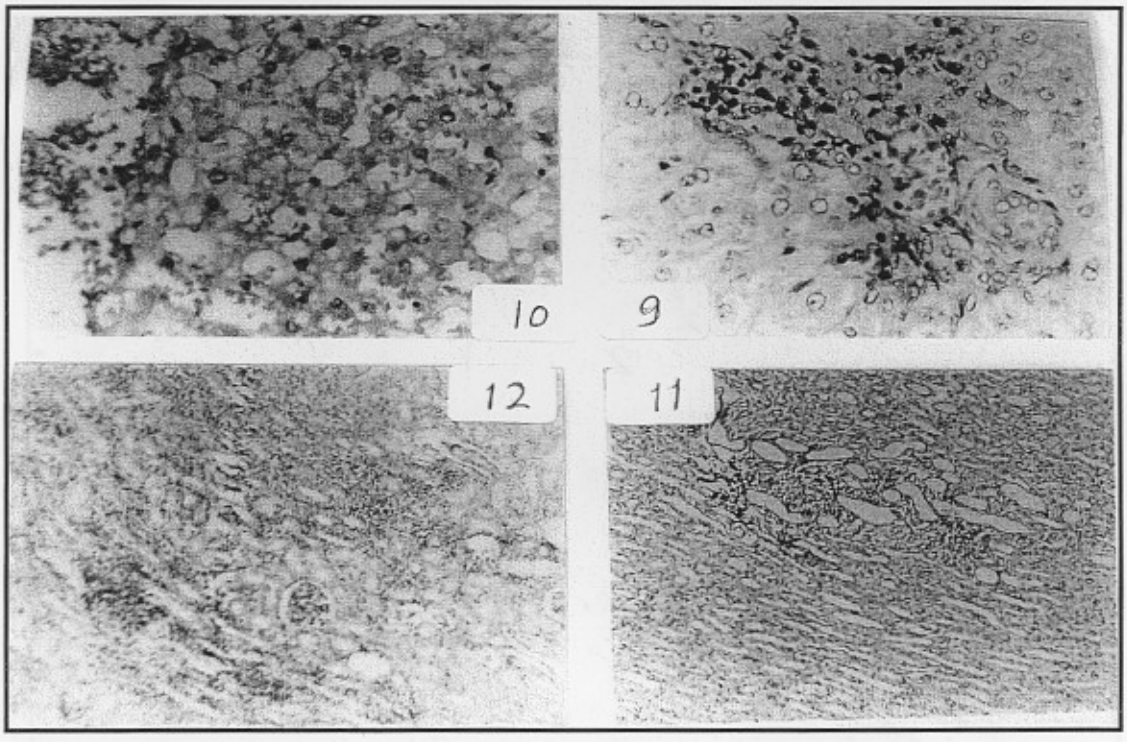

Fig. 9. Liver gp 3, at 30 days PA, showing numerous mononuclear leukocytic infiltration in the portal area which extended to the interlobular area, H&E x 520.

Fig. 10. Liver gp (3), at 45 days PA, showing congestion and vacuolation of hepatocytes of mainly fatty change, H&E x 520.

Fig. 11. Kidney gp 3, at 30 days PA, showing numerous leukocytic infiltration in the renal medulla, the adjacent renal tubule showing cystic dilatation, H&E x 520.

Fig.12. Kidney gp 3, at 45 days PA, showing numerous leukocytic infiltration in the renal medulla, H&E x 130.

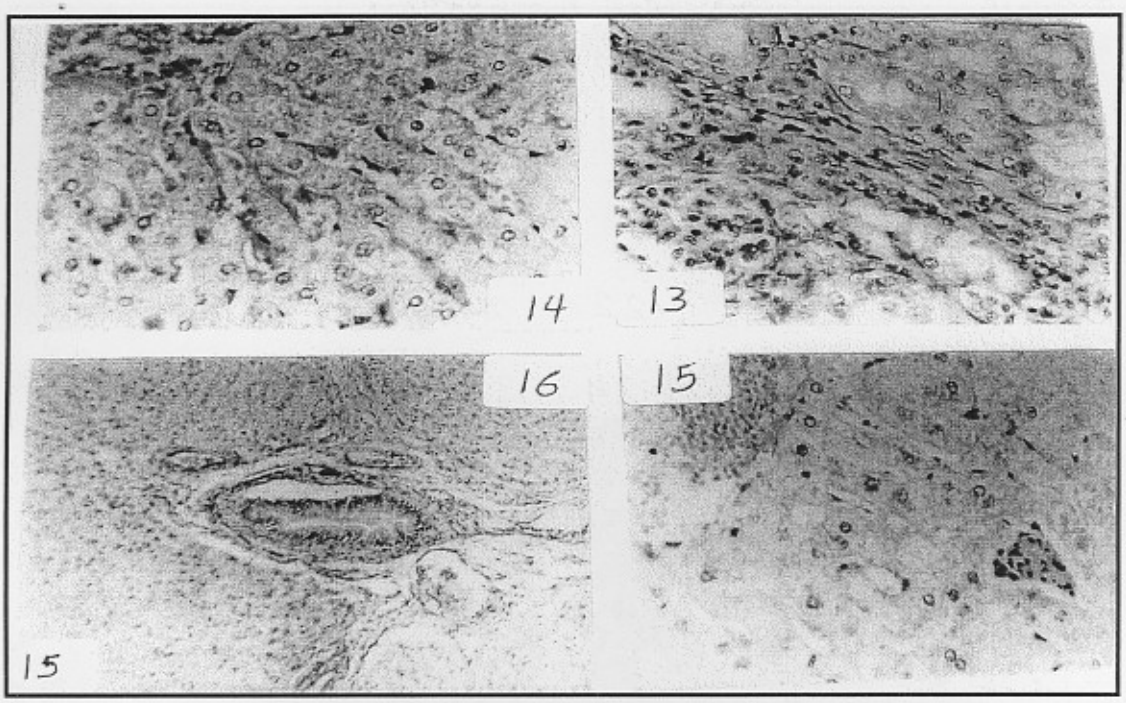

Fig.13. Kidney gp 3, at 45 days PA, showing mild interstitial fibrous connective tissue proliferation in renal cortex, H&E x 520.

Fig. 14. Liver gp 4, at 15 days PA, showing congestion of central vein and hepatic sinusoids beside hypertophy of Kupffer's cells. The cytoplasma of hepatocytes is granular, H&E x 520.

Fig. 15. Liver **gp 4**, at 45 days PA, showing focal coagulative necrosis and mononuclear cell infiltration, H&E x 520.

Fig. 16. Liver gp 4, at 45 days PA, showing congested portal blood vessels, proliferation of bile ductules, edema and mild periductolar fibrosis, H&E x 520.

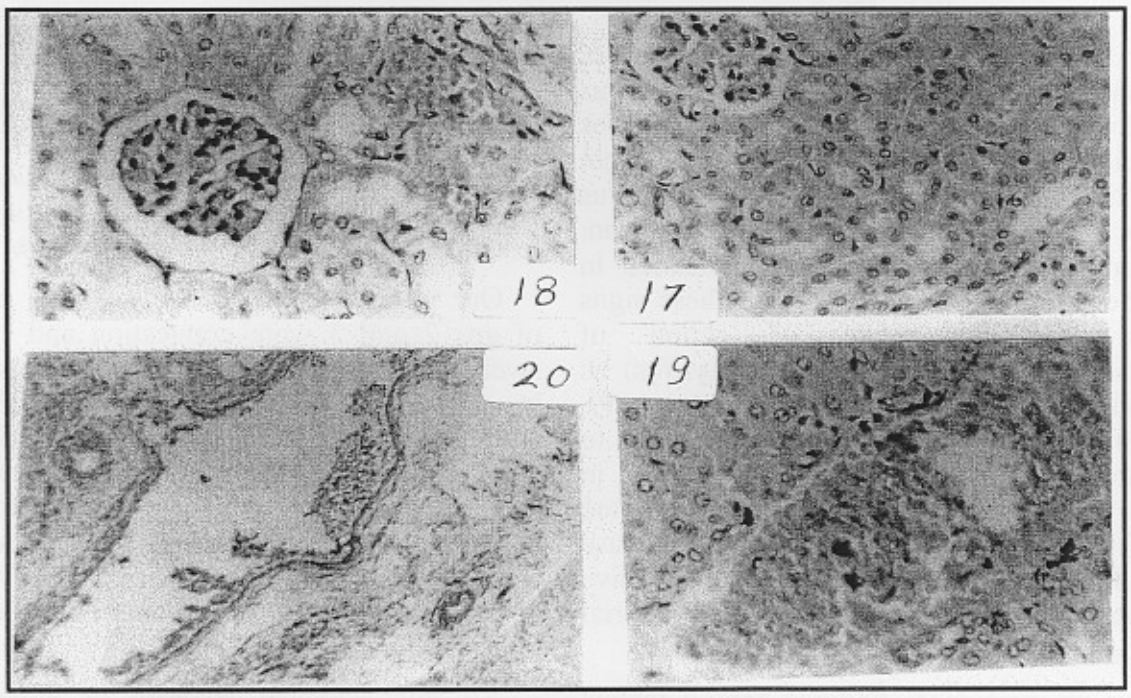

Fig. 17. Kidney gp 4 at 30 days PA, showing cloudy swelling of some renal tubules, H&E x 520.

Fig. 18. Kidney gp 4, at 45 days PA, showing congestion of intertubular capillaries in the renal cortex and nephrosis of some tubular epithelium, H&E x 520.

Fig. 19. Liver **gp 5** at 15 days PA, showing severe congestion and perivascular hemosiderosis, H&E x 520.

Fig. 20. Liver gp 5, at 15 days PA, showing distended portal area with edema, congested blood vessels and proliferated bile ductules, H&E x 130.

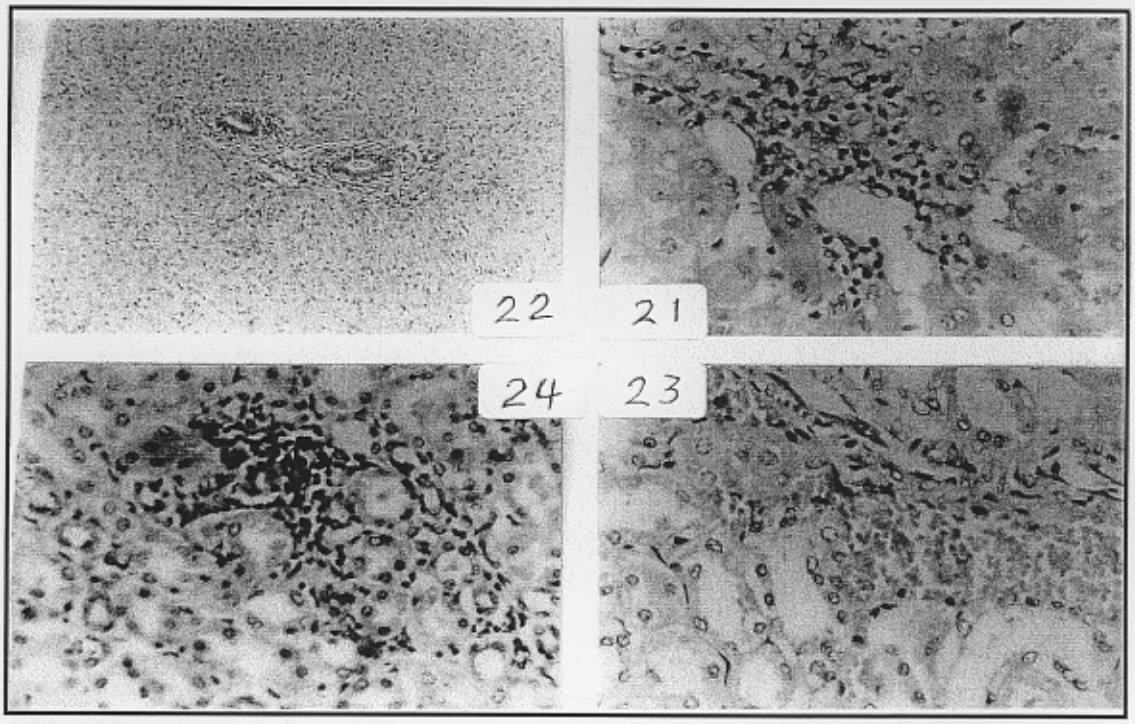

Fig.21. Liver gp 5, at 30 days PA, showing congestion of hepatic sinusoids, hepatocellular necrosis and mononuclear cell infiltration, H&E x 520.
Fig. 22. Liver gp 5, at 45 days PA, showing periductolar fibrosis, H&E x 130.
Fig. 23. Kidney gp 5 at 45 days PA, showing severe congestion and degenerative changes of some tubular epithelium, H&E x 520.
Fig. 24. Kidney gp 5 at 45 days PA, showing focal replacement of necrotic renal tubules with mononuclear cells mainly lymphocytes, H&E x 520.

DISCUSSION

As a member of organophosphorus pesticides, the clinical signs of chlorpyrifos toxicity among rabbits of gps 2 and 3 were similar to the other organophosphorus pesticides which represented by salivation, lacrimation, muscle tremor and reduction in the body weight. Similar to ***(8)***. These signs occur due to mode of action of organophosphorus pesticide (chlorpyrifos) as acetyl cholinesterase inhibitors ***(9)***. Meanwhile, the clinical signs observed due to diflubenzuron toxicity were only reduction in body weight and food consumption as reported by**(5)** due to its cytotoxic effect which increase with time of exposure. In addition to extensive metabolization with rat liver submitochondrial fraction gave ride to its metabolites ***(10)***.

Our macroscopical findings among rabbits of gp 2 and 3 at 15, 30 and 45 days PA were similar to those noticed in other organophosphorus pesticides as those noticed by ***(11&12)*** and this could attributed to the toxic effect of chlorpyrifos and it's metabolites on the hepatic and renal tissue ***(13)***. Meanwhile our macroscopical findings among rabbits of gps 4 and 5 at 15, 30 and 45 days PA were mild due to it's low acute toxicity by any route of exposure as a product unlikely to resent an acute hazard in normal use ***(5).***

Our microscopical findings among rabbits of gps 2 and 3 were congestion and cloudy swelling of hepatic tissue. These findings were in agreement with ***(14)***. Hyperplasia of epithelial lining with perivascualr, fibrous tissue infiltrated with leukocytes were similar to ***(15)***. Hypertrophy of the central lobular heptocytes and kupffer's cells as in agreement with ***(16)***, hepatocyte vacuolation mainly fatty change as similar to that mentioned by several investigations ***(17&18)***.

All microscopical changes in kidney as similar to those observed by ***(19)*** and as other organophosphorus pesticide lesions as those mentioned previously by ***(11&12)***. Meanwhile our microscopical findings among rabbits of

gps 4 and 5 were mild due to the less toxic effect and relative safty *(10)*. These lesions suggested to be due to derivatives of hydroxylated metabolite *(20)*. Perivascular hemosiderosis was the marked microscoopical findings which similar to that reported by several cited investhgations *(21,22&23)*. It could be attributed to the stimulation of diflubenzuran to hematopoiesis *(20)* or may be due to excess the hemolysis of erythrocytes.

It could be concluded that both chlorpyrifos and diflubenzuran had pathological effect on liver and kidneys of rabbit through 45 days with a different doses. Meanwhile, diflubenzuran had a minimal side effect so it was more safe to use, while chlorpyrifos was more toxic.

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الملخص العربى

دراسات باثولوجية على تاثيرات بعض مبيدات الجراد على الارانب

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كلية الطب البيطرى – جامعة الزقازيق

معهد بحوث صحة الحيوان (فرع الزقازيق)

تمت هذه الدراسة على عدد ٤٥ أرنب نيوزلندى عمر شهرين وقد قسمت هذه الأرانب الى خمسة مجموعات متساوية حيث تركت المجموعة الأولى كضابط للتجربة بينما تعرضت كل من المجموعتين الثانية والثالثة الى ٧٥٠ و ١٥٠٠ جزء فى المليون من مبيد الكلوروبيرفوس (٤٨٪) بالترتيب على العليقة لمدة ٤٥ يوماً بينما تعرضت أرانب المجموعتين الرابعة والخامسة الى ٨٠٠٠ و ١٦٠٠٠ جزء فى المليون من مبيد الدايفلوبنزيرون بالترتيب على العليقة لنفس المدة وكان ذلك لدراسة الأعراض الإكلينيكية والتغيرات المرضية ولمقارنة الاكثر سمية للتقليل من استخدامه ولذلك تم ذبح ثلاث أرانب من كل مجموعة فى الأيام ١٥ ، ٣٠ ، ٤٥ من بداية التجربة حيث لوحظ بعض الأعراض المتشابهة من الأعراض الناتجة عن باقى المبيدات المبيدات الفوسفورية فى المجموعتين الثانية والثالثة وأعراض بسيطة جداً فى المجموعتين الرابعة والخامسة وهى نقص فى الوزن كما تم فحص العينات بالعين المجردة حيث لوحظ احتقان فى الكبد والكلى فى المجموعتين الثانية والثالثة بدرجات متفاوتة تفوق تلك التى فى المجموعتين الرابعة والخامسة بالاضافة الى زيادة حجم الكبد مع لون داكن وبعض البؤر النكرزية بينما اظهرت حالات أخرى زيادة حجم الكبد مع اصفرار اللون وكان للفحص المجهرى أكبر الأثر فى إظهار التأثير المرضى الأكبر فى المجموعتين الثانية والثالثة مقارنة بالمجموعتين الرابعة والخامسة حيث لوحظ احتقان الكبد وتحلل خلاياه ووجود خلايا وحيدة النواة مع ظهور تنكرز تخثرى لخلايا الكبد كما لوحظ إحتقان الكلية مع بعض التغيرات الإنتكاسية للخلايا الطلائية المبطنة للانابيب الكلوية.وعلى ذلك فان مركب الدايفلوبنزيرون يعد اقل سمية واكثر امنا عن مركب الكلوربيريفوس.