Sci., J.Fac., Sci., Minufiya Univ., 2005 Vol. XIX, 111 - 124

INSECTICIDES – INDUCED HISTOPATHOLOGICAL CHANGES IN THE LIVER AND TESTIS OF THE HEDGEHOG

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ABSTRACT

The present work deals with the effect of organophosphate insecticide "diazinon" and pyrethroid insecticide "cypermethrin" on the liver and testis of hedgehog. Animals exposed to both insecticides showed destruction of the liver architecture, cytoplasmic vacuolation of the hepatocytes, leucocytic infiltration, congestion of blood vessels and focal necrosis. Moreover, liver fibrosis and fatty infiltration spread over several liver areas were recorded in the exposed animals. Histological examination of the testis showed that the seminiferous tubules were elongated and contained reduced spermatogenic cells. The sperm bundles were completely absent. Marked cytoplasmic vacuolation of the spermatocytes and vesiculation of the nuclei of most cells were observed together with clear signs of pyknosis and karyolysis.The diameter of the seminiferous tubules and the germinal epithelial height were significantly reduced. The obtained results collectively indicated that diazinon and cypermethrin insecticides induced liver injury and strongly decreased the spermatogenesis in the testis of hedgehog.

INTRODUCTION

A number of pesticides are of interest because of their toxicity. Some organophosphorus pesticides are very toxic and were found to cause abdominal cramps, nausea, vomiting, diarrhea, urinary incontinence, weakness and respiratory disturbances in man. Also, they affect the central nervous system causing restlessness, dizziness and confusion coma. Repeated exposure may have a cumulative effect (Reynolds, 1994). While pyrethroids possess potent insecticidal activity with low mammalian toxicity, exposure to these compounds may lead to local irritation in man (Foye, 1975; Reynolds, 1994).

The toxicity of insecticides to mammalian animals has received much attention in the recent years. Animals exposed to these insecticides exhibited changes in the physiological activities and histological structures of many organs (Ahmed *et al.*,1986; Tasheva and Hristiva, 1993; Saleh, 1996; Lukkowicz-Ratojczak *et al.*, 1998; Sakr *et al.*,2000). Concerning the histological effect of insecticides, many investigators reported histopathological changes in the liver of different animals exposed to vaious insecticides (Datta and Dikshith, 1973; Hurket, 1978; Mikhail *et al.*,1979; Sakr and Gabr, 1992; Saleh, 1996; Sakr, 1999; Saleh *et al.*,1999) of different animals exposed to various insecticides.

Insecticides were also found to induce many histopathological changes in male reproductive system of many animals (Dikshith and Datta, 1972; Eroschenko and Wilson, 1975; Sakr and Salib, 1987 and Saleh, 1996). Moreover, insecticides have effects on reproductive function through changes in hormonal balance. Smith *et al.* (1972) reported that DDT may adversely affect male reproductive function in mice by changing both accessory gland metabolism and binding of steroids as indicated by decreased prostate uptake of testosterone. Saleh (1996) reported that lannate insecticide reduced the level of testosterone in mice resulting in inhibition of spermatogenesis.

Most of the available reports were focussed on the toxic effects of insecticides on mammals as mouse or rat and rabbit as key animal model, and, to our knowledge, no work has been done on hedgehog, the common mammalian animal in Saudi Arabia. This stimulates us to study the histopathological effects of two-insecticide diazinon and cypermethrin widely used in pest control by Ministry of Agriculture of Saudi on the liver and testis of *Parechinus hypomelus*.

MATERIAL AND METHODS

Experimental animals:

Five Saudi Arabian hedgehogs (*Parechinus hypomelus*) at Al-Khansaa area in Makka City were collected from botanical garden, where the insecticides, diazinon and cypermethrin were used in insect control. Some of these animals were moribund. They were brought to the laboratory and immediately dissected out and their organs were examined macroscopically. Their livers and testes appeared abnormal and therefore prepared for histological examination. No parasitic infections were observed. Healthy animals from the same species were obtained from animal house of Faculty of Applied Sciences, Umm Al-Qura University, Makkah.

Insecticides used:

a- Diazinon: it is an organophosphate insecticide widely used for controlling resistant soil insects, such as root warms, wireworms; also effective against many insect pests of fruits and vegetables; controls cockroachesand and other household insects. It contains 60% diazinon diluted to 1: 50.

b- Cypermethrin: it is a pyrethroid insecticide used in control of beetles, cabbageworms, and leafhoppers; also used for controlling insect parasites, such as cattle grubs, lice, fleas and ticks. It contains 10% cypermethrin diluted to 1: 100. These two insecticides were used 3 times / week for pest control.

Histological study:

Control and insecticides exposed animals were sacrificed by decapitation and their livers and testes were removed and fixed in Bouin's fluid for 24 hours. After fixation, the tissues were dehydrated through ascending grads of ethanol, cleared in xylene and embedded in paraffin wax. Specimens were sectioned at 5 μm and the sections were mounted on clean slides and stained with haematoxylin and eosin.

Seminiferous tubule diameter and epithelial height were measured with an ocular micrometer. For statistical analysis of the data, the Student "t" test was used.

RESULTS

Livers:

Control animals:

Control liver is formed of hepatic lobules, each lobule is made up of radiating strands of cells forming a network around a central vein (Fig. 1). The liver strands are alternating with narrow sinusoids. These sinusoids have irregular boundaries composed of only a single layer of fenestrated endothelial cells and large irregularly phagocytic cells, which are known as Kupffer cells. The hepatocytes are polyhedral in shape with relatively large sizes and granular cytoplasm. Each cell has a centrally located nucleus and sometimes, binucleate hepatocytes were observed. Outside the hepatic lobule at certain angles, lie the portal areas of connective tissue each including a hepatic portal vein, a branch of hepatic artery and a bile ductule.

Insecticides-exposed animals:

Liver of exposed animals showed general impairment of the normal structural organization of hepatic lobules in many areas. Cord-like arrangement of the normal hepatocytes not well distinct. Severe fatty infiltration was observed in the liver sections of all exposed animals. Most of the hepatocytes manifested clear necrotic signs (Fig. 2). Marked cytoplasmic vacuolation of the hepatocytes and some cells appeared to be suffering from certain degree of cloudily swelling. The nuclei of most cells revealed clear signs of pyknosis and karyolysis. The sinusoidal spaces were somewhat dilated and filled with red blood cells. Kupffer cells appeared swollen and some of them were detached from the underlying basement membrane. (Fig. 3).

Inflammatory cellular infiltration was found around the blood vessels (Fig. 4). The central veins distended and engorged with blood (Fig. 5). The portal veins were also congested (Fig. 6). Such congested blood vessels indicate clear phenomena of hemorrhage.

There was mild fibrosis near the portal blood vessels which appeared congested and fatty infiltration was also observed (Fig. 6).

Testes:

Control animals:

Histological examination of the testes of control hedgehogs showed that the seminiferous tubules were rounded and embedded in tightly packed connective tissue stroma (Fig. 7). The germinal epithelia were formed of normal spermatogenic layers (spermatogonia, primary and secondary spermatocytes, spermatids and sperms). The lumen of the tubules was occupied by clumps of tightly packed sperms, which were oriented with their heds towards the germinal epithelium. Sertoli cells were rested on the basement membrane of the tubules (Fig. 8).

Exposed animals:

Table (1) shows changes in the diameter and epithelial height of seminiferous tubules in the exposed hedgehogs to the insecticides, diazinon and cypermethrin. It can be seen that insecticides induced a significant decrease in diameter, of seminiferous tubules ($P < 0.05$). There was likewise significant decrease in the epithelial height of the seminiferous tubules in the exposed hedgehogs.

Testes of hedgehogs exposed to diazinon and cypermethrin showed that, the seminiferous tubules appeared elongated and irregularly shaped with clear wide spaces between them (Fig.9). The connective tissue stroma appeared loosely packed around the seminiferous tubules. Many vacuoles appeared in the seminiferous tubules opposite to the boundary tissue and the spermatogenic cells itself showed marked cytoplasmic vacuolation (Fig. 10). The number of spermatogenic cells was significantly reduced and the sperm bundles were completely absent (Fig. 11). Marked cytoplasmic vacuolation of the spermatocytes and vesiculation of the nuclei of most cells were recorded with clear signs of pyknosis and karyolysis (Fig. 12).

Table (1): Effect of insecticides on the diameter and epithelial height of the seminiferous tubules.

Experimental animals	Seminiferous tubules diameter (Mean µm ± S.D)	Epithelial height (Mean µm ± S.D)
Control	2.2 ± 0.3	0.9 ± 0.2
exposed	1.1 ± 0.4*	0.3 ± 0.3*

(*): Significant at $P < 0.05$ in comparison with control.

DISCUSSION

The widespread use of pesticides in agriculture and forestry conservation programs has prompted the need for evaluation of the hazards of such materials to wild life. Many reports have emphasized the probability of exists within the indoor living space, as well as in the agricultural and industrial workplace (Russel and Overstreet, 1987). In this concern, Reinert (1984) suggested that the indoor use of pesticides might create a different and more direct exposure situation. Several organophosphorus (Levin *et al.*,1976; Duffy *et al.*,1979; Abou-Zaid and El-Balshy, 1995) and pyrethroid insecticides (Crofton and Reiter, 1984; Saleh *et al.*,1999) have been shown to have long term as well as behavioral effects. The present results showed that animals exposed to diazinon and cypermethrin revealed many histopathological changes in liver. The most prevalent morphologic features of hepatic tissue impairment were destruction of liver archetecture, cytoplasmic vacuolation of the hepatocytes and remarkable abundance of leucocytic infiltration. These alterations seemed to follow almost the same pattern recently reported in mammalian liver after exposure to organophosphate insecticide "sumithion" (Saleh, 1999) and pyrethroid "tetramethrin" (Sakr, 1999).

In agreement with Saleh *et al.* (1999), the present results showed clear necrotic signs and dilated sinusoidal spaces filled with red blood cells in all exposed liver sections and congestion of blood vessels. Such congested blood vessels indicate clear phenomena of hemorrhage. Abou-Zaid and El-Balshy (1995) observed focal liver cell necrosis, blood vessel congestion

and leucocytic infiltration in the liver of newly born mice that inhaled "Ezalo", a commercial formulation of synthetic pyrethroid, for 15 days. In experimental animals exposed to pyrethrins, the lungs and liver showed considerable congestion and leuccocytic infiltration (El-Dessouky *et al.*,1986). Abu El-Zhab *et al.* (1993) reported that pyrethroid inhalation induced focal necrosis, fatty metamorphossis and cellular infiltration in the liver of rats.

According to Datta and Dikshith (1973) infection of rats with a mixture of ethyl parathion or methyl parathion with DDT injected into rats produced hepatic damages, including sinusoidal congestion, cytoplasmic vacuolation and necrosis.In the present study ,the results showed that animals exposed to the mixture of organophosphate insecticide "diazinon" and pyrethroid "cypermethrin" induced an injury in the liver of hedgehog, being obvious in severe fatty infiltration and liver necrosis, which were remarkable signs for liver damage (Waschek, and Roussenaux, 1991).

In the present study, it was found that the measurements of exposed testis showed significant reduction in the diameter of the seminiferous tubules as well as the germinal epithelial height. Similarly, Saleh (1996) found that oral treatment of mice with lannate induced significant reduction in the diameter of seminiferous tubules and germinal epithelial height and also induced histopathological and histochemical alterations in the ovary of rats (Saleh and Sakr ,1996) and (Sakr *et al.* 2000).

The histological evidence showed that spermatogenesis was also decreased, as indicated by the reduction of spermatozoa in the lumen of most of the tubules. Moreover, testicular androgenic activity was significantly decreased, as seen from the degenerated interstitial tissue. These results concur with those of Krause and Homola (1974),who concluded that oral treatment of mice with dichlorvos induced significant testicular histopathological changes such as degeneration of seminiferous tubules and disappearance of spermatozoa and spermatides. Feeding mature male quial with the chlorinated insecticide, kepone, had produced oedematous testes with highly dilated seminiferous tubules and reduced the germinal epithelium (Eroschenko and Wilson, 1975). El- Sammannody *et al.* (1986) reported that injecting mice with kepone had resulted in decreased spermatogenic layers in the seminiferous tubules. Moussa and Abdel-Hafez (1982) reported that feeding guinea pigs with dimethoate induced degenerative effects in the testis and (Sakr and Salib, 1987) stated that, the process of spermatogenesis was inhibited after feeding hamsters with dimethoate.

Saleh (1996) found cytoplasmic vacuolation in testes of mice exposed to lannate. Amarose and Czajka (1962) considered that the intercellular vacuolation found in the testis of mouse exposed to deutrium oxide is due to degeneration and detachment of spermatogenic cells. A single intraperitoneal injection of ethyl parathion, methyl parathion and DDT induced degenerative cellular changes in the seminiferous tubules of rats included necrosis, karyopyknosis, vacuolation of the cytoplasm and formation of multinucleated giant cells (Dikshith and Datta, 1972).

The physiology of the reproductive system in animals exposed to insecticides was reported. Fikes (1990) demonstrated a marked increase in corticosterone and decrease in the ACTH and testosterone concentration in the serum of mammalian animals after exposure to organophosphate and carbamate insecticides. The role played by androgenic steroids, such as testosterone, in spermatogenesis is well proved (Steinberger *et al.*, 1973). It is speculated here that, in hedgehogs, insecticides reduced the level of testosterone in the blood resulting in the inhibition of spermatogenesis.

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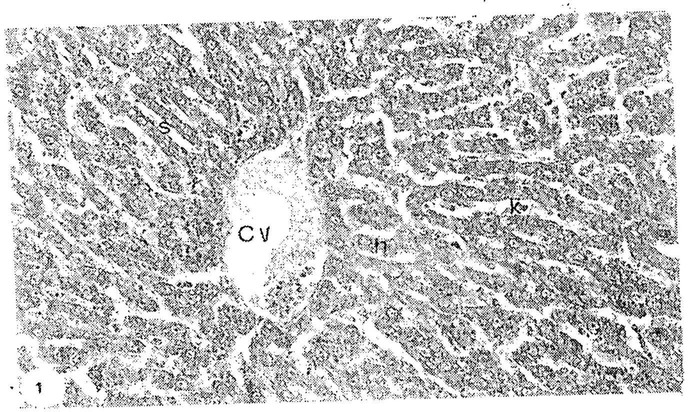

Fig.1. Section in the liver of control hedgehog showing hepatic cells (h). Sinusoidal spaces (s) with Kupffer cells (k) and central vein (CV) (X125).

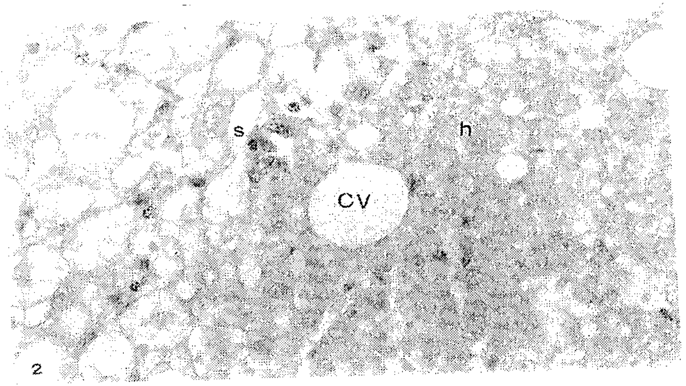

Fig.2. Section in the liver of an exposed hedgehog. Disorganization of the hepatic strands is clearly visible. A severe fatty infiltration is noticed. Necrosis appears in most cells, while others appeared with irregular nuclear membrane. Central vein (CV) (X250)

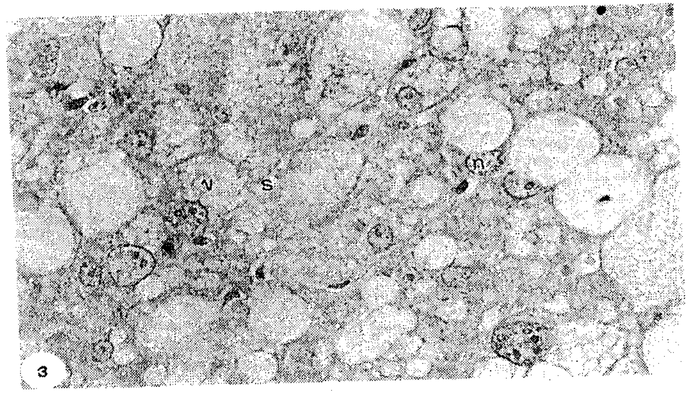

Fig.3. Section in the liver of an exposed hedgehog showing vacuolation (v) of the cytoplasm of hepatocytes. Accumulation of chromatin in some nuclei. Sinusoid spaces(s) were dilated and filled with RBCs. Kupffer cells appeared swollen. (X500)

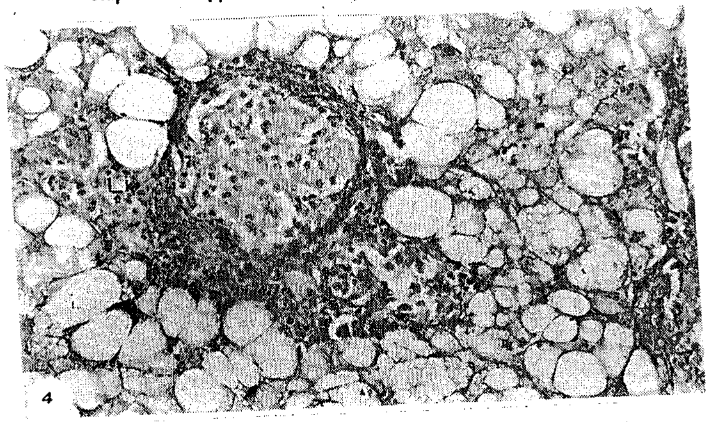

Fig.4. Section in the liver of an exposed hedgehog showing marked leucocytic infiltration (Li) and vacuolation of most cells (X500)

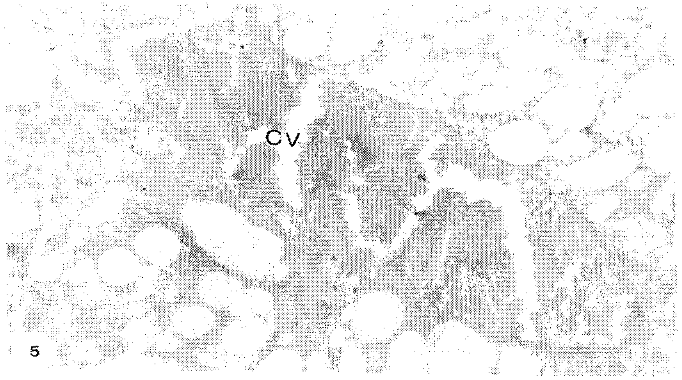

Fig.5. Section in the liver of an exposed hedgehog showing distended central vein (CV) congested with blood and massive fatty infiltration in a macro vesicular pattern (X500).

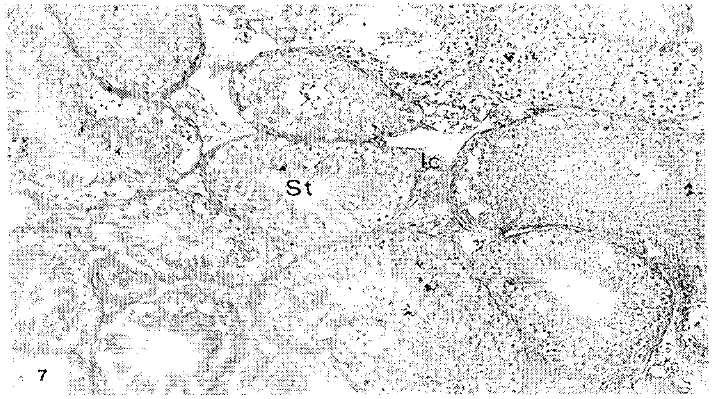

Fig.7. Portion of transverse section of testis of a control hedgehog showing seminiferous tubule (St) and interstitial cells (Ic). (X125).

Fig.6. Section in the liver of an exposed hedgehog showing congested portal vein (PV) and an increase in collagen fibrous tissue (X500).

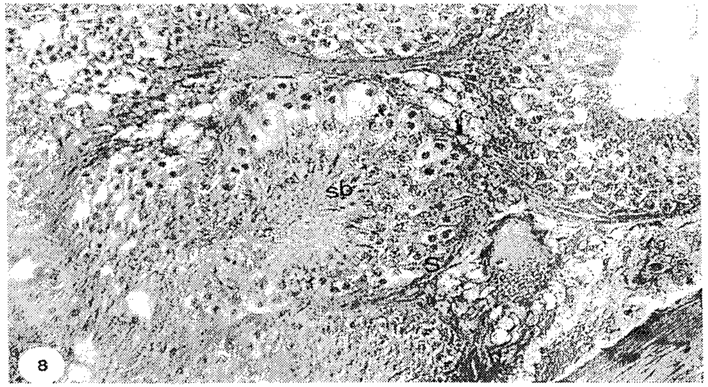

Fig.8. Enlarged seminiferous tubule of a control hedgehog showing all types of normal spermatogenic cells, sperm bundles (sb) and Sertoli cell (S). (X250).

Fig.9. Portion of transverse section of testis of exposed hedgehog Note seminiferous tubules appeared loosely packed with irregularly shaped (X125)

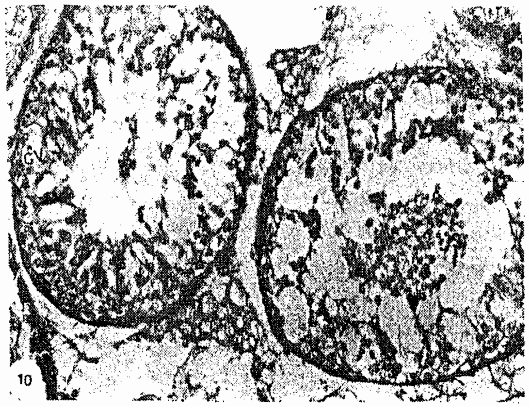

Fig.10 Portion of transverse section of testis of exposed hedgehog, showing reduced spermatogenic cells and marked cytoplasmic vacuolation (cv) (X250)

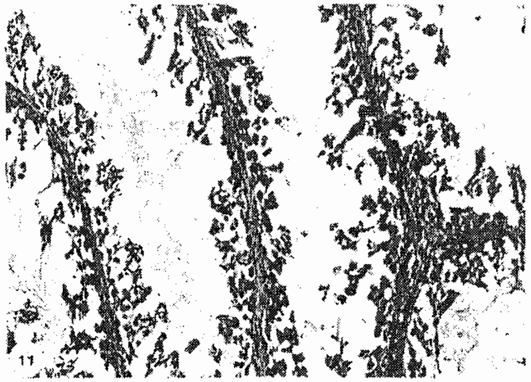

Fig 11. Transverse section of testis of exposed hedgehog showing two elongated seminiferous tubules Note decrease in germinal epithelium height, and complete absence of sperm bundles were completely absent (X250)

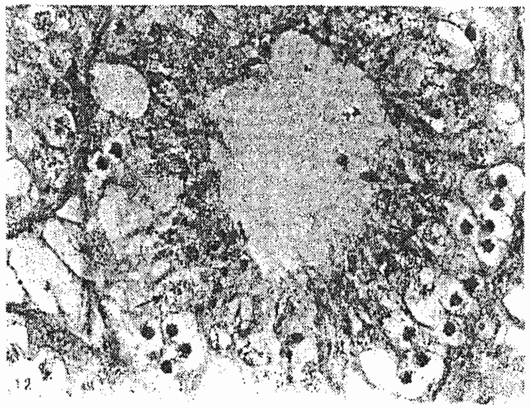

Fig.12. Portion of transverse section of testis of exposed hedgehog Note vacuolization of cytoplasm and necrotic spermatocytes (arrow)

التغيرات النسيجية المرضية المحدثة في كبد وخصية القنفذ تحت تأثير المبيدات الحشرية

ميســاء محمد الراوي

قسم الأحياء ــ كلية التربية للبنات ــ مكة المكرمة

اشتمل هذا البحث على دراسة التأثيرات الضارة التي يسببها اثنان من المبيدات الحشرية وهمــا الديازيــنون والسيبرميثرين المستخدمان من قبل وزارة الزراعة في منطقة الخنساء بمكة المكــرمة فــي المملكة العربية السعودية لمكافحة الآفات الزراعية. أوضحت النتائج أن تعرض القنافذ لهذين المبيدين أدت إلى حدوث تغيرات نسيجية في الكبد حيث ظهر تلف نسيجي وخلوي ، لوحظــت الفجــوات الســيتوبلازمية في خلايا الكبد وزيادة رشح الخلايا الالتهابية وتلف في الأوعــية الدموية حيث امتلأت الأوردة المركزية والبابية بالدم كدليل على حدوث نزف داخلي، واتســعت الجــيوب الكبدية وامتلأت بالكرات الدموية الحمراء ونشطت خلايا كوفر الابتلاعية. أيضاً من التغيرات النسيجية المرضية التي تدل على مظاهر تحطم وتلف النسيج الكبدي، ظهور تــنكرز بــؤري واحــتقان وتفتت لأنوية الخلايا الكبدية وفقد الترتيب المورفولوجي لخلايا الكبد وزيادة شديدة في الدهون وظهور التليف.أما خصي القنافذ المعرضة لهذين المبيدين فقد أظهرت نقصــاً معــنوياً في قطر الأنيبيبات المنوية وكذلك نقصت الطلائية الجرثومية. وأظهر الفحص النســيجي للخصية وجود نقص في الخلايا الجرثومية بأنواعها المختلفة كما شوهدت الأنيبيبات المــنوية خالية تماماً من الحيوانات المنوية. ظهرت فجوات سيتوبلازمية في الخلايا الجرثومية كما لوحظ ظهور تنكرز بؤري واحتقان وتفتت لأنوية خلاياها.

يتضح من هذه النتائج أن استخدام كلا من المبيدين الديازينون والسيبرمثرين يحدث تغيرات مرضية شديدة تعتبر دليلاً على التلف الكبدي وكذلك الإخلال بعملية تكوين الحيوانات المنوية في القنافذ.