Sublethal toxicity of organophosphate, diazinon on some organs of great sturgeon (*Huso huso*)

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Received: March 2014 Accepted: August 2014

Abstract

Sublethal toxicity effects of diazinon on the structure of some organs of great sturgeon (*Huso huso*) were investigated. Diazinon was applied at a sublethal concentration of 1.5 mg L^{-1} at 22±1 ºC for nine weeks as a constant bath. Tissues of liver, kidney and gills were sampled and examined histopathologically after 1, 14, 28, 42 and 63 days post exposure to the toxicant. The results of light microscopic examinations of tissues revealed congestion of blood vessels, pyknosis and cloudy swelling in hepatocytes. Congestion of blood vessels and thickening of the basal membrane of bowman capsule in glumerol of the kidney were also observed. The gill of fish exposed to toxicant also showed congestion and dilation of blood vessels, swelling of the basement membrane, hyperplasia, fusion and necrosis of lamellae. This data showed that a long term exposure of fish to diazinon can cause several pathological changes in respiratory organ, haematopoietic tissues and liver.

Keywords: diazinon, great sturgeon, sublethal toxicity, histopathology.

Introduction

The progressive growth of anthropogenic pressures on the coastal and marine ecosystems has caused a remarkable increase in the level of contamination in the Caspian Sea. The main sources of such pollution are land-based/offshore sources and diazinon in large quantities is one of the major herbicidal, insecticide and fish antiparasite chemicals currently used in these areas. Diazinon [O-O-diethyl O-(2-isopropyl-1-6-methyl-4-pryimidinyl) hosphorothioate] is an organophosphorus pesticide that is extensively used, both in agriculture and households to control insects in plants, fruit and vegetable crops (Dutta & Meijer 2003), as well as nematodes and soil insects in lawns and croplands (Eisler 1986). After its application on crops and plants, diazinon is easily washed into surface waters and enters the ground water. Finally, it enters the aquatic environments in large quantities (Coppage & Mathews 1974). Diazinon can cause severe pathological changes if aquatic animals are continuously in contact with chronic and long sublethal concentrations of diazinon. Such pathological damages can be dominant, particularly if the exposure period occurs under water quality parameters of low temperature, low moisture, high alkalinity and lack of suitable microbial degraders (Hamm, Wilson & Hinton 1998). Diazinon LC_{50} values vary widely and depend on the organism's age, weight and water quality conditions. Sublethal doses may lead to reduced growth and reproduction in aquatic invertebrates (Eisler 1986). In fishes, exposure to diazinon in sublethal doses is known to affect the nervous system by inhibition of acetylcholinesterase activity (Pan & Dutta 1998; Dutta & Arends 2003), to cause abnormalities in the gills (Dutta, Munshi, Roy, Singh, Adhikari & Killius 1996; Sharifpour, Pourgholam, Soltani, Hassan, Akbari & Nouri 2006), changes in the immune system (Dutta *et al.* 1996; Khoshbavar Rostami, Soltani & Hassan 2006), to reduce the olfactory functioning (Moor & Waring 1996), to disrupt ovarian structure (Dutta & Maxwell 2003) and reduction in size and

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diameters of the lumen of seminiferous tubules and germ cells (spermatogonia) (Dutta & Meijer 2003). Sturgeons are among the aquatic organisms that are severely influenced by the pollution impacts. The great sturgeon (also named beluga, giant sturgeon) (*Huso huso*) is the major economical species of northern part of Iran. This fish plays a significant role in the source income of the Iranian south Caspian Sea fisheries. These commercially valuable species of fish generally spend their adult lives in both freshwater and salt water environments with spawning in upriver. The main aim of this study was to determine whether sublethal toxicity of diazinon has significant histopathologic effects on the organs of gill, liver and kidney of great sturgeon (*Huso huso*).

Materials and Methods

Fish

One hundred fish weighing 450±50 g from Rajai Fish Farm in Mazandran province were used. Fish were held in 2000 L tanks containing 25 fish per tank at 22±1 ºC, dissolved oxygen of 7.5-9 mg L^{-1} and pH 7.5. Other water quality parameters including ammonia, nitrite and total hardness were <0.02 mg L^{-1}, <0.1 mg L^{-1} and 145 mg L^{-1}, respectively. Fish were acclimatized to the new conditions a few weeks prior to the experiments. Fish were fed with commercial trout pellet containing 50% fish meal.

Application of diazinon

Fish were divided into two groups with each group containing 50 fish. Diazinon was applied to the water at a sublethal concentration of 1.5 mg L^{-1} at 22±1 ^{o}C for 9 weeks as a constant bath (Khoshbavar Rostami *et al.* 2006). To maintain the toxicant concentration during the experiment, whole water in each tank was replaced with fresh diazinon treated water (1.5 mg L^{-1}) every 12 h. Control fish were kept in the same system separately with water replacement every 12 h.

Collection and processing of samples

Samples were collected from both groups after 24 h and thereafter every week until 9 weeks post-exposure to the toxicant. Five fish per treatment were sampled at each sampling time.

Histopathological study

Tissues of gills, liver and kidney were sampled for each treatment at each sampling time. Tissues were immediately fixed in cold 10 % buffered formalin for at least 24 h before processing. The fixed tissues were processed in an automatic tissue processor using standard procedure (Roberts 2001). The paraffin embedded tissues were sectioned (5 µm) using a Rotary Microtome. The sections were then stained using Hematoxylin and Eosin staining, mounted and examined under compound microscope. The indices below were used to show the severity of the lesions in the tissues:

- = no significant microscopic changes
+ = mild changes (10 percent change in 40x objective microscope view)
++ = moderate changes (20 percent change in 40 x objective microscope view)
+++ = severe changes (more than 20 percent change in 40 x objective microscope view)

Results

Liver

Congestion of blood vessels and sinus dilation were seen almost in all samples (Table 1). Different stages of degeneration leading to complete necrosis of hepatocytes, cytoplasmic degeneration, pyknotic nuclei, vacuolation in hepatic cells and rupture of

Table 1 Histopathological scores of great sturgeon liver exposed to continuous exposure of diazinon at 1.5 mg L^{-1} at 20-22 ºC for 9 weeks

Histopathological changes	Sampling days (post-exposure)				
	1	14	28	42	63
Congestion of blood vessels	+++	+++	++	+	+
Hemorrhage	+	-	-	-	-
Sinus dilation	-	-	+	++	++
Vacuolar degeneration of hepatocytes	+	-	-	-	-
Pyknosis of hepatic cells	+	+++	+	+	+
Cloudy swelling	-	+++	-	-	-
Focal necrosis	-	+	-	-	+
General necrosis of hepatocytes	-	-	+	+	-

Table 2 Histopathological scores of great sturgeon kidney exposed to continuous exposure of diazinon at 1.5 mg L^{-1} bath at 20-22ºC for 9 weeks

Histopathological changes	Sampling days (post-exposure)				
	1	14	28	42	63
Congestion of blood vessels	+	-	-	-	-
Hemorrhage	-	-	-	-	-
Cellular infiltration	+	-	-	-	-
Congestion of glummerul	+++	-	-	-	-
Vacuolar degeneration of kidney cells nuclei	-	+	-	-	-
General necrosis of kidney tissue	-	+++	-	+	+
Degeneration of tubules	-	-	+	-	-
Necrosis of kidney glummerul	-	-	+	-	-
Thicken of basal membrane of bowman capsule	-	-	++	-	-
Pyknosis of different types of kidney cells nuclei	-	+	-	+	+

Table 3 Histopathological scores of great sturgeon gill exposed to continuous exposure of diazinon at 1.5 mg L^{-1} at 20-22ºC for 9 weeks

Histopathological changes	Sampling days (post-exposure)				
	1	14	28	42	63
Congestion	+	-	++	-	-
Dilation of blood vessels	-	-	-	-	-
Mild cellular infiltration	-	-	-	-	-
Swelling of the basement membrane	-	-	++	-	-
Increasing in lymphocytes number	-	+	-	-	-
Separating and sloughing of basement membrane	-	+	-	-	-
Necrosis of lamellae	-	+++	+++	+++	+++
Hyperplasia and fusion of lamellae	-	-	+	-	-
Fusion of lamellae	-	-	-	++	++
Sloughing of basement membranes of lamella	-	-	-	++	++
Necrosis of lamellae	-	+++	+++	+++	+++

-vessels were also seen in the fish exposed to the toxicant. No pathological abnormalities were observed in the control group (Fig. 1).

Kidney

As shown in Table 2, congestion and hemorrhage were observed in the early times of exposure of fish to the toxicant. Tubular and haematopoietic cells degeneration, vacuolation, pyknosis and karyolysis of nuclei, necroses of the kidney haematopoietic tissue and to some extent, cellular infiltration in intercellular spaces of the tissue were also observed in kidney sections of fish exposed to diazinon, while no pathological changes were found in control fish (Fig. 2).

Gills

Histopathological changes in gills of fish exposed to the toxicant are shown in Table 3 and Figure 3. Congestion and dilation of blood vessels and swelling, separating and sloughing of basement membrane of the secondary lamella were the most common degenerative changes observable in the fish exposed to diazinon. After 28 days of exposure to the toxicant signs of hyperplasia, fusion and necrosis of lamellae were the more severe pathological findings (Fig. 3). The degree of histopathological changes was varied and time-dependent. No abnormality was found in the gill sections of control group.

Discussion

Exposure to diazinon produced remarkable alterations in the microscopic structure of the liver, kidney and gill of the great sturgeon. The pathological changes observed are not pathogonomic for organophosphorus poisoning but are similar to those produced by other pesticides. Cytoplasmic vacuolation, pyknosis, degeneration and necrosis of hypatocytes, hemorrhage, congestion of blood vessels and sinus dilation were observed in the liver tissue. The hepatocytes have undergone different stages of degeneration leading to complete necrosis after a longer exposure period to the toxicant. Therefore, the severity of some microscopic changes was time-dependent. Also, such changes in hepatocytes showed that these cells were quite sensitive to diazinon. Similar histopathological findings were also reported by Rahman, Hossain, Mollah & Ahmad (2002) and Sharifpour *et al.* (2006) when *Anabas testudineus*, *Channa punctatus* and *Barbodes gonionotus* were exposed to diazinon for a short and/or

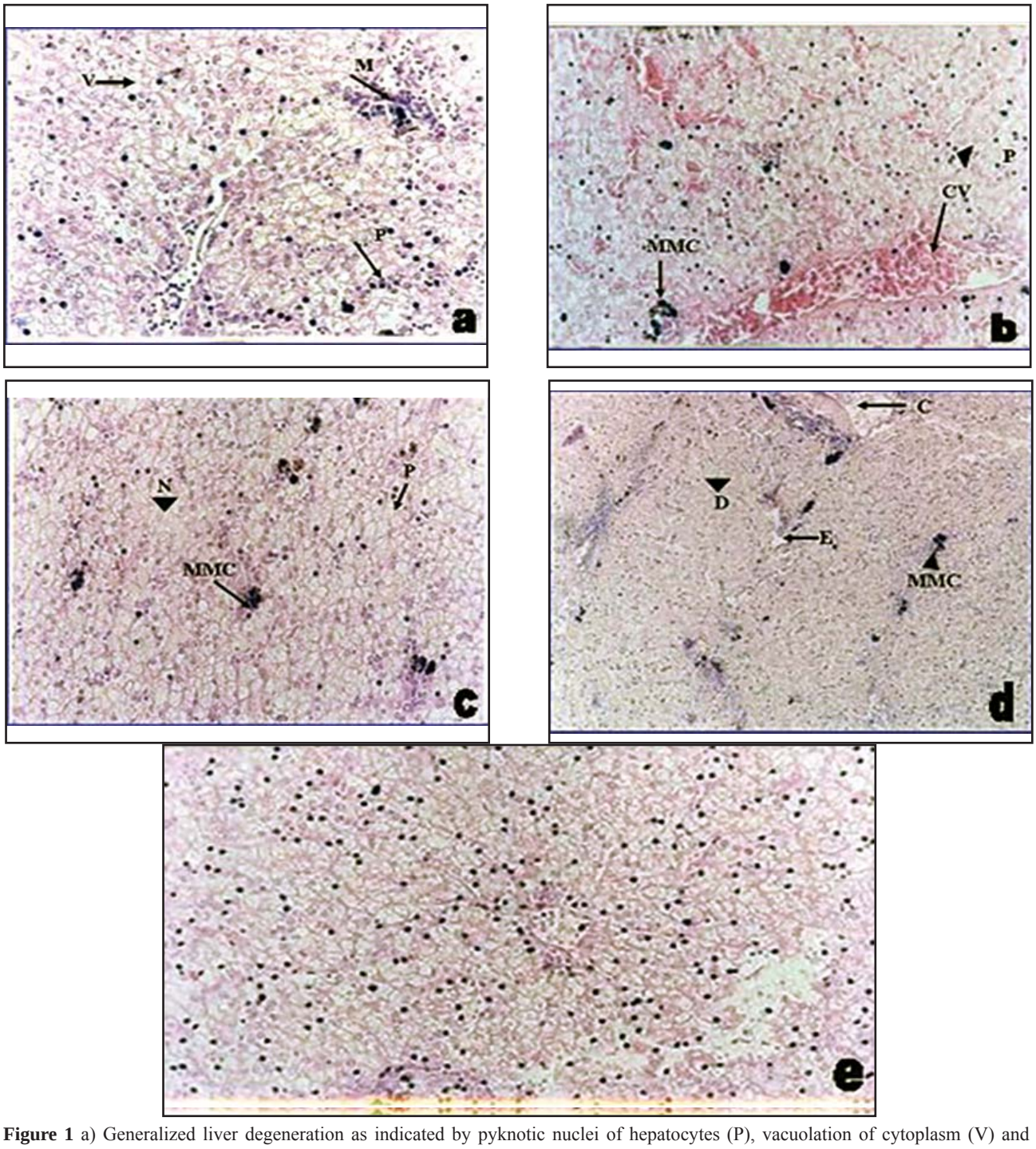

Figure 1 a) Generalized liver degeneration as indicated by pyknotic nuclei of hepatocytes (P), vacuolation of cytoplasm (V) and melanomacrophage centre (MMC) in fish exposed to 1.5 mg L^{-1} diazinon at 1 day post-exposure (H & E, x 1075). b) Liver 15 days post exposure to 1.5 mg L^{-1} diazinon in great sturgeon. Pyknotic nuclei of hepatocytes (P), congestion of blood vessel (CV) and melanomacrophage centre (MMC), (H & E, x 860). c) 28 days post exposure to 1.5 mg L^{-1} diazinon in liver of great sturgeon as indicated by pyknotic nuclei of hepatocytes (P), necrosis of liver tissue (N) and melanomacrophage centre (MMC) (H & E, x 860). d) 42 days post exposure to 1.5 mg L^{-1} diazinon in liver of great sturgeon as indicated by expansion of sinusoid (E), congestion of vessel (C), degeneration of hepatocytes (D) and melanomacrophage centre (MMC) (H & E, x 344). e) Liver 63 days post exposure to 1.5 mg L^{-1} diazinon in great sturgeon indicated by increased number of melanomacrophage centre, degeneration of hepatocytes, pyknosis and necrosis of hepatocytes and rupture of liver tissue (H & E, x 860).

a long time period.

Degeneration and thinning of epithelial lining of the lamellae, hyperplasia, fusion and necrosis of lamellae, separation of vascular layer from the epi-

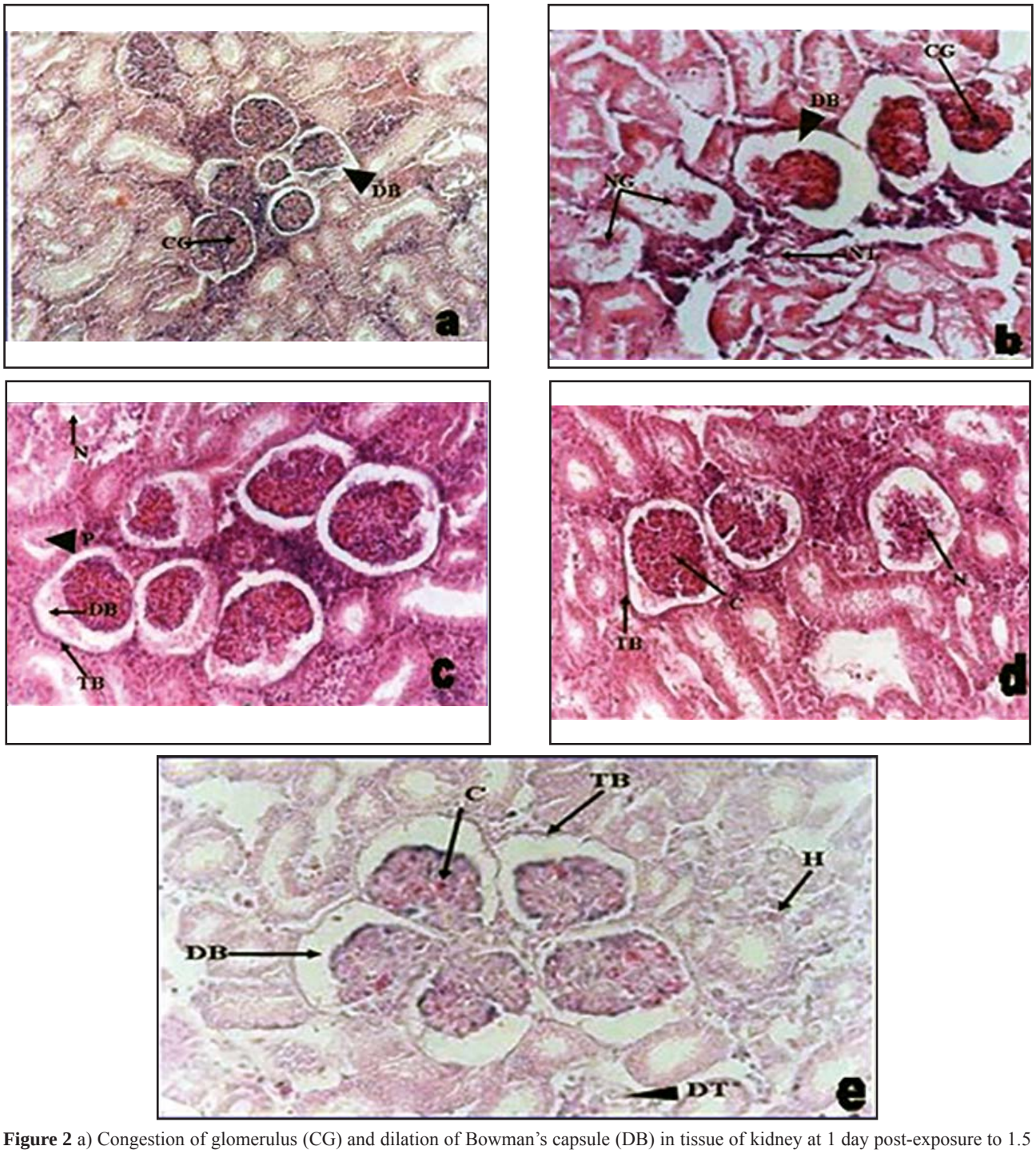

Figure 2 a) Congestion of glomerulus (CG) and dilation of Bowman's capsule (DB) in tissue of kidney at 1 day post-exposure to 1.5 mg L^{-1} diazinon (H & E, x 860). b) Congestion of glomerulus (CG), dilation of Bowman capsule (DB), necrosis of glomerulus (NG) and necrosis of tubules (NT) in tissue of kidney at 15 day post-exposure to 1.5 mg L^{-1} diazinon (H & E, x 860). c) Thickening of basal membrane of Bowman's capsule (TB), dilation of Bowman's capsule (DB), necrosis of glomerulus (NG) and protein deposition in tubule (P) in tissue of kidney at 28 days post-exposure to 1.5 mg L^{-1} diazinon (H & E, x 752). d) Thickening of basal membrane of Bowman's capsule (TB), necrosis of glomerulus (NG) and congestion of glomerulus (CG) in tissue of kidney at 42 days post-exposure to 1.5 mg L^{-1} diazinon (H & E, x 860). e) Thickening of basal membrane of Bowman's capsule (TB), dilation of Bowman's capsule (DB), degeneration of tubules (DT), hemorrhage of kidney (H) and congestion of glomerulus (C) in tissue of kidney at 63 days post-exposure to 1.5 mg L^{-1} diazinon (H & E, x 860).

thelium, and in some cases fusion of the epithelium of the adjacent villi were the conspicuous signs observable in the gill sections. Furthermore, disruption and sloughing of respiratory epithelium from

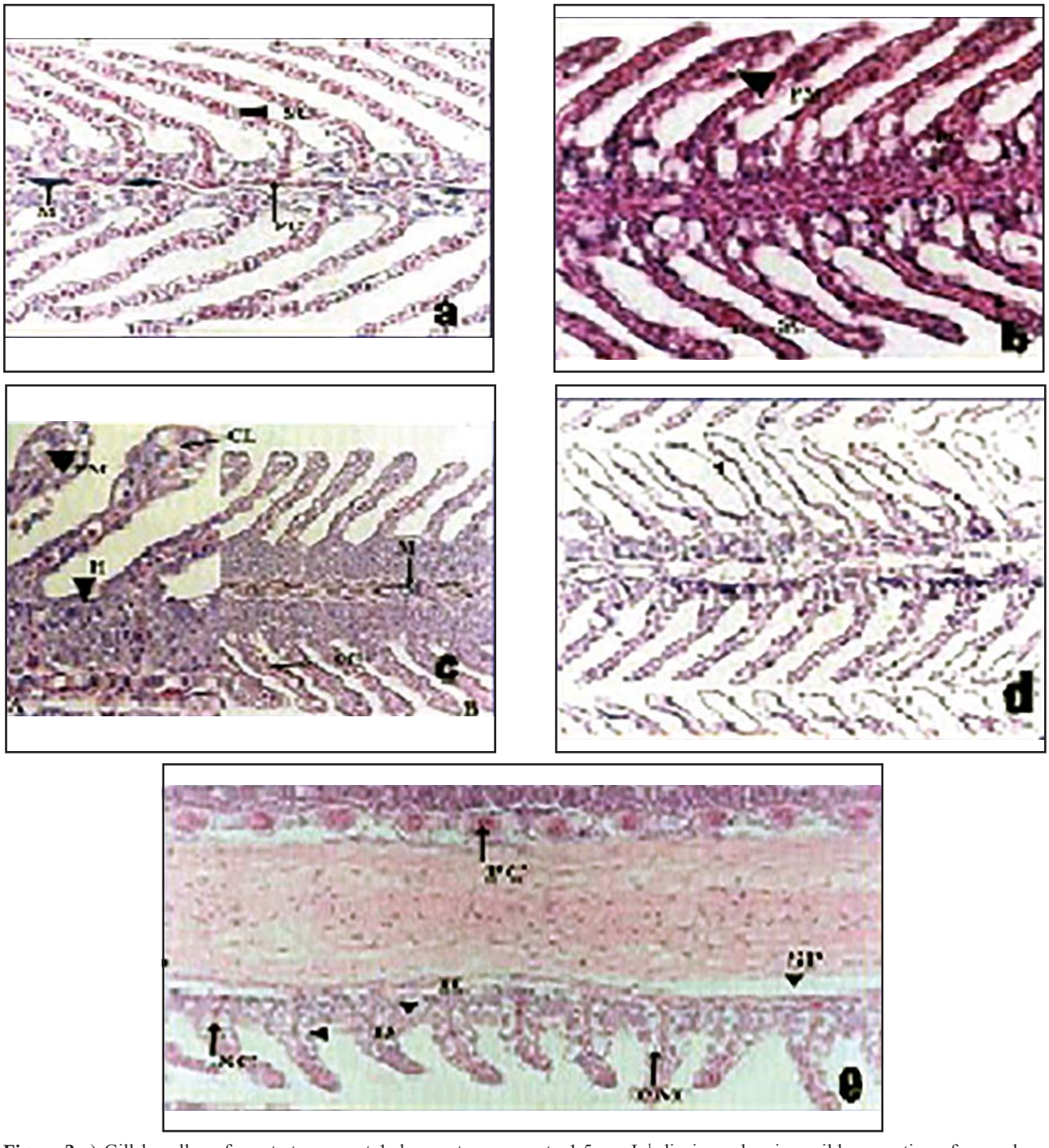

Figure 3 a) Gill lamellae of great sturgeon at 1 day post-exposure to 1.5 mg L^{-1} diazinon showing mild congestion of secondary lamellae (SC), congestion of primary lamellae (PC) and presence of melanin in blood vessel (M), (H & E, x 1075). b) Gills lamellae of great sturgeon at 14 days post-exposure to 1.5 mg L^{-1} diazinon showing congestion of secondary lamellae (SC), congestion of primary lamellae (PC) and proliferation of mucus cells in secondary lamellae (PM) (H & E, x 860). c) Gills lamellae of great sturgeon at 28 days post-exposure to 1.5 mg L^{-1} diazinon showing clubbing of secondary lamellae (CL), Hyperplasia (H), proliferation of mucus cells in secondary lamellae (PM) (picture A in C) and presence of melanin in blood vessel (M), congestion of secondary lamellae (SC) (picture B in C) (H & E, A x 537 and B x 430). d) Gills lamellae of great sturgeon at 42 days post-exposure to 1.5 mg L^{-1} diazinon showing separation of epidermis from the base of secondary lamellae (arrow head). (H & E, x 677). e) Gills lamellae of great sturgeon at 42 days post-exposure to 1.5 mg L^{-1} diazinon showing congestion of secondary lamellae (SC), congestion of primary lamellae (PC), dilation of secondary lamellae (D), proliferation of mucus cells in secondary lamellae (PM), hyperplasia (H) and separation of epidermis from the base of primary lamellae (SP). (H & E, x 677).

the basement membrane of gills in response to diazinon have been reported in other species fish (Dut-

ta, Richmonds & Zeno 1993; Rahman *et al.* 2002; Sharifpour *et al.* 2006). Skidmor & Tovel (1972) believed that lifting and swelling of the epithelium serve to increase the distance through which chemical irritants must diffuse to reach the bloodstream. However, Jagoe & Haines (1983) reported that this could adversely affect oxygen uptake by increasing the distances over which respiratory diffusion occurs. Hyperplasia, fusion and necrosis of lamellae which were seen in this study have also been reported by previous researchers. Similar histopathological changes in gills of Bluegill sunfish (*Lepomis macrohirus*) exposed to diazinon have been reported. They reported that diazinon concentrations used in their experiments caused various types of changes, such as lifting of the epithelial layer, hyperplasia and necrosis, lamellar fusion, epithelial rupture, as observed in the present study. Dutta *et al.* (1993) believed that the microstructural changes in the affected cells might serve as a defense mechanism in protecting the fish from diazinon-contaminated water by increasing the diffusion distance. If the diffusion distance and secretion of mucus increase, it could affect the respiration of the fish, leading to its death. Also, Mallatt (1985) and Pourgholam (2005) demonstrated that gill alterations such as necrosis and rupture of lamellae are poisoning dose-dependent. Observation of congestion and hemorrhage in kidney tissue in the early times of exposure could be due to the diazinon effect on the blood vessels resulting in diapedes of blood vessels because no abnormalities were seen in the control fish. Also, pathological changes including tubular and haematopoietic cells degeneration, vacuolation, pyknosis and karyolysis of nuclei, necrosis of immunocompetent cells and cellular infiltration in tissue intercellular spaces were observed in the kidney sections of only the experimental fish. Similar histopathological changes were also reported by Rahman *et al.* (2002) and Sharifpour *et al.* (2006) when other fish were exposed to different concentrations of diazinon. Similar to liver and gill tissues the severity of some pathological changes in kidney sections was also time-dependent.

In conclusion, long term exposure of great sturgeon to diazinon can cause several pathological changes in respiratory organ, haematopoietic tissues and liver causing morbidity and mortality in fish. Therefore entrance of such chemical toxicant to Caspian Sea, the main natural source of this species, should be avoided.

Acknowledgments

We are grateful to professor M. Soltani, department of aquatic animal health, university of Tehran for guidance to this manuscript.

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مطالعه اثرات سمیت غلظت تحت حاد دیازینون بر روی ساختار برخی اندام‌های فیل ماهی (*Huso huso*)

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چکیده

در این مطالعه اثرات سمیت تحت حاد دیازینون برروی ساختار برخی از اندام‌های داخلی فیل ماهی مورد بررسی قرار گرفت. بدین منظور فیل ماهیان با متوسط وزن ۵۰±۴۵۰ گرم در تانکهای حاوی دیازینون با غلظت ۱/۵ میلی گرم در لیتر به مدت ۹ هفته در دمای ۱±۲۲ درجه سانتی گراد نگهداری شدند. سپس هیستوپاتولوژی بافت‌های کبد، کلیه و آبشش در ۱، ۱۴، ۲۸،۴۲ و ۶۳ روز پس از در معرض قرارگیری با استفاده ازمیکروسکوپ نوری مطالعه گردید. نتایج حاصل پرخونی، پیکنوزیس و تورم ابری مانند در سلول‌های هپاتوسیت را نشان می‌داد. همچنین پرخونی و افزایش ضخامت غشاء پایه کپسول بومن در گلومرول‌های کلیه مشاهده شد. بررسی آبشش ماهیان در معرض آلودگی قرار گرفته حاکی از پرخونی و آماس رگ‌های خونی ، تورم در غشاء پایه، هایپرپلازی، چسبندگی و نکروز در تیغه‌های آبشش بود. بر اساس این مشاهدات می‌توان نتیجه گرفت که قرار گرفتن طولانی مدت فیل ماهی در معرض سم دیازینون منجر به بروز و ظهور صدمات پاتولوژیک متعدد در اندام‌های حیاتی از قبیل آبشش، بافت خونساز و کبد در این ماهی می‌شود.

واژه‌های کلیدی: دیازینون، فیل ماهی، سمیت تحت حاد، هیستوپاتولوژی.

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