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Effects of sumithion on histopathology of liver and kidney in silver barb, *Barbonymus gonionotus*

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Abstract. Sumithion is an organophosphorous pesticide that has a negative impact on fish organ such as liver and kidney. This study was conducted to assess the effects of sumithion on histological changes in liver and kidney of silver barb (*Barbonymus gonionitus*). Four treatments such as T_o (control), T_1, T_2 and T_3, designated for 0.00, 0.025, 0.05 and 0.1 ppm of sumithion respectively with two replications for each were designed. Histopathological alterations in liver and kidney were found after 7, 15 and 30 days of exposure to sumithion. Hypertrophy of hepatocytes, mild to severe necrosis, ruptured central vein, necrosis, pyknosis, hemorrhage, irregular shaped nucleus, clear cell foci, nuclear degeneration and vacuolation were found in liver of fish exposed to 0.05 and 0.1 ppm sumithion for 15 and 30 days. Degeneration of kidney tubules, vacuolization, mild to severe necrosis, pyknosis, ruptured renal corpuscle, ruptured collecting duct and hemorrhage were also recorded in kidney. At the earlier stage of the experiment the cells of kidney and liver were compact but with the progress of the experimental period and increasing doses the cells became degenerated and intracellular space was increased. Severe changes were found at 0.1 ppm after 30 days of exposure in case of liver and kidney. The histopathological changes observed in the tissues of liver and kidney indicates that sub lethal concentrations of sumithion caused moderate to severe alterations in liver and kidney.
Keywords: Silver barb, Organophosphorus pesticide, Histopathology

Introduction

In Bangladesh approximately 35% of the crop producing area is sprayed with organophosphorus pesticides for many different purposes associated with crop protection (Chowdhury *et al.* 2012). Sumithion is an organophosphate insecticide that is extensively used in aquaculture ponds to eradicate undesirable insects mainly tiger bug before releasing the fish larvae. It is also used effectively against pests including penetrating, chewing and sucking insects, on cereals, cotton, orchard fruits, rice, vegetables, and forests. Sumithion is considered somewhat toxic to fish (Thomson 1989), moderately toxic to warm and cold water fish. In stinging catfish larvae mortality rate of 57.8%±4.1% and 62.67%±7.6% in 150 μg/L and 250 μg/L of sumithion was reported respectively (Shahjahan *et al.* 2017). Pesticides may also get their way into water bodies through many other ways including spray drifts, surface runoff, leaching of ground water land careless disposal of empty containers and equipment washing water (Sankararamakrishnan *et al*. 2005; Hossain *et al.* 2015; Sumon *et al*. 2016).

Silver barb (*Barbonymus gonionotus*) is native to Southeast Asia and is cultivated in freshwater ponds in Thailand, Indonesia and Vietnam (Tangtrongpiros *et al.* 1990) and in Bangladesh. Silver barb is a short-cycle species that can be farmed with low cost technology and relatively less effort than other species which has made the specie a popular aquaculture candidate to the fish farmers of Bangladesh. It grows to a marketable size within 8–12 months but matures at 4–6 months of age. In Bangladesh most of the fish farms are adjacent to the agriculture fields and the water they use are continuously contaminated with the pesticides used in the agricultural lands most specifically in paddy fields. Oruc (2010) observed mortality of juvenile and adult tilapia (*Oreochromis niloticus*) by insecticide chloropyrifos. Histopathological changes in various tissues (e.g. liver, kidney, gill and gonad)of fish may be caused by insecticides. Moreover several histopathological changes such as cytoplasmic and karyoplasmic clumping, cytoplasmic reactions, atretic oocytes, adhesion, necrosis and thinning of follicular lining in bluegill sunfish ovary were reported by Dutta and Maxwell (2003). However to date no study has been done to elucidate the toxic effect of sumithion on liver and kidney of *B. gonionotus*. The objective of the present study was to investigate the histopathological changes in liver and kidney of *B. gonionotus* exposed to 3 different concentrations of sumithion in culture condition. Theresult of the study could be used to elucidate the toxic effect of sumithion at different concentration in aquaculture condition and improving guideline of sumithion use and management in agricultural lands for the farmers.

Materials and Methods

Experimental Animal and Culture facilities: Healthy and disease free *B. gonionotus* juveniles (average length and weight 5.00$\pm$0.34cm and 11.0$\pm$0.5g, respectively) were collected from a fish farm named "Sagor Motsho Khamar", Digharkanda, Mymensingh for the experiment. Fish were released in 4ponds with 2 replicates at 40 fish/decimal, each pond having about 40m^3 of water and reared for 30 days. Three different concentration of sumithion such as 0.025 mg/L, 0.05mg/L and 0.1 mg/L was used in three treatment T_1, T_2 and T_3 respectively and no sumithion was applied in treatment T_o (control). No supplemental feed were provided and the fish were solely depended on natural foods.

Pesticide: Sumithion (50E/C) was collected from a local retail pesticide shop, Mymensingh. The experimental procedures followed the guidance approved by the Animal Care and Use Committee of Bangladesh Agricultural University, Mymensingh. All the reagents including Haematoxylene-eosin were collected from local supplier.

Experimental design: The experiment was conducted using three treatments and a control each with two replications. We used concentrations of sumithion such as

0.025mg/L as T_1, 0.05mg/L as T_2, and 0.1mg/L as T_3. Two ponds were under T_0 (control) having no pesticides. The ponds received water from a water-supply system of a deep-tube well and also from rain. Water volume (in m^3) of the ponds was calculated and sumithion was applied in the ponds according to the water volume. Each pond having water volume of around 40.5 $m^3$and total amount of sumithion applied was 1.0, 2.40 and 4.40 ml in each pond of T_1, $T_2$and T_3 respectively.

Histopathology of liver and kidney: The fish from both control and treated ponds were collected on prefixed sampling days and the liver and kidney were carefully collected by dissection and immediately kept in vials containing 10% formalin**.** Theformalin-fixed tissues were washed, processed, dehydrated in graded alcohol, cleared in benzene, embedded in paraffin, sectioned and stained with hematoxylin and counterstained with eosin. Changes in histopathology of kidney and liver were observed under a microscope photographed using digital photomicroscope (Olympus CX 41).

Results

Histopathological alteration in the liver of B. gonionotus: Hepatocytes and other cells of the liver in control group (T_0) were found in normal and systematically arranged (Fig. 1A). Several changes, such as necrosis (N), degenerated hepatocytes (DH), vacuolation (V), clear cell foci (CCF), pyknotic nuclei (PN), nuclear degeneration (ND), irregular shaped nucleus (ISN), and hemorrhage (H)were found in the liver of the test fish in T_1, T_2 and T_3. Pyknosis and vacuole were observed at the dosage of 0.05 ppm at 7 days of exposure (Fig. 1C).ISN and cytoplasmic vacuolation (CV) were observed at the dosage of 0.05 ppm and 0.10 ppm at 15 days exposure (Fig. 2C and 2D). CCF, ND and PN were observed at the dosage of 0.025, 0.05 and 0.10 ppm at 30 days of exposure, respectively (Fig. 3). The intensity of the histoarchitectural changes was more severe in higher doses and exposure time. The intensity of the histoarchitectural changes was more severe in higher dose and exposure time indicating that this pesticide has long term effects on fish body.

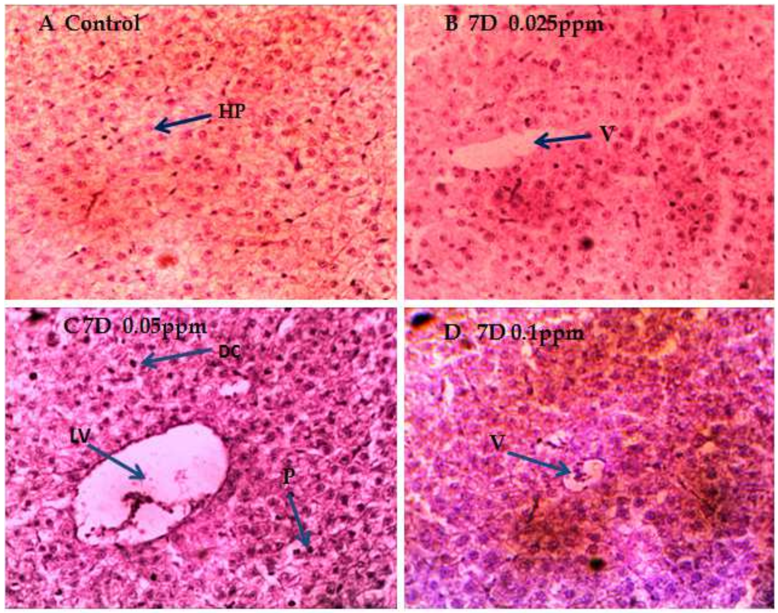

Fig. 1. Histological changes in liver (H and E stained, X100) exposed to sumithion (A) Control (T_0); (B) 0.025 ppm (T_1); (C) 0.05 ppm (T_2); (D) 0.1 ppm (T_3) at 7 days of exposure. Arrows indicate large vacuolation (LV), small vacuolation (SV), pyknosis (P), degenerated cell (DC) and vacuolation (V).

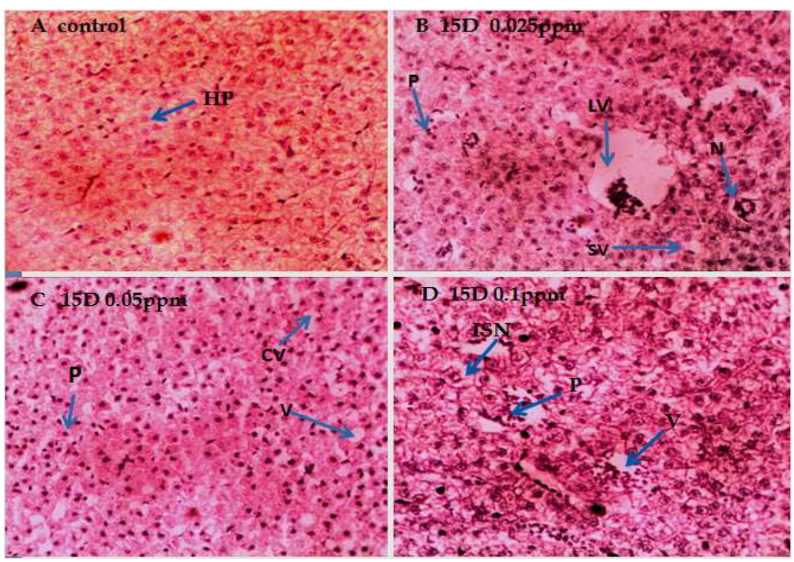

Fig. 2. Histological changes in liver (H and E stained, X100) exposed to sumithion (A) Control (T_0); (B) 0.025 ppm (T_1); (C) 0.05 ppm (T_2); (D) 0.1 ppm (T_3) at 15 days of exposure. Arrows are indicating hepatic cell (HC), pyknosis(P), hepatocytes with irregular shaped nucleus (ISN), large vacuolation (LV), small vacuolation (SV), cytoplasmicvacuolation (CV), necrosis (N).

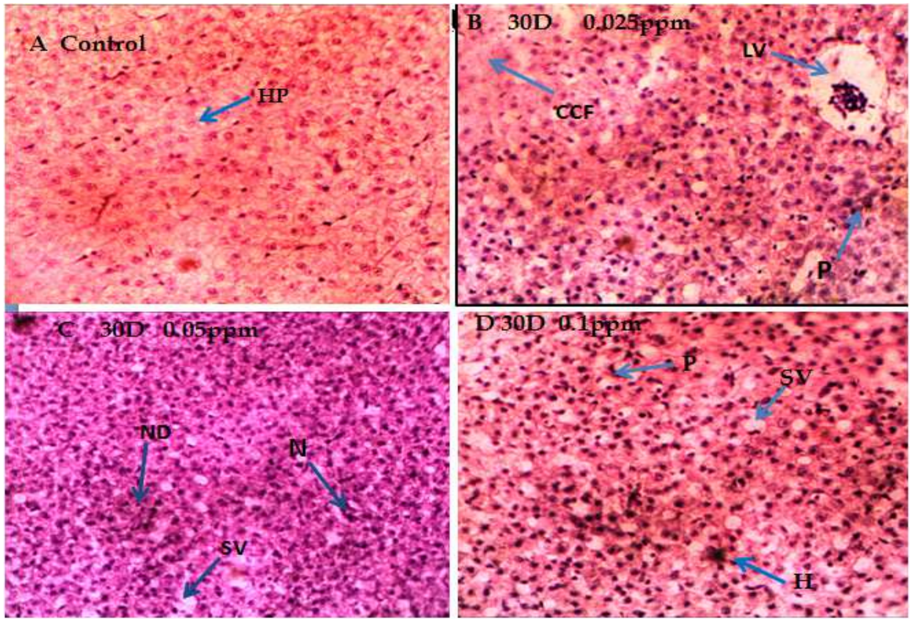

Fig. 3. Histological changes in Liver (H and E stained, X100) exposed to sumithion: (A) Control (T_0); (B) 0.025 ppm (T_1); (C) 0.05 ppm (T_2); (D) 0.1 ppm (T_3) at 30 days of exposure. Arrows are indicating, large vacuolation (LV), small vacuolation (SV), necrosis (N), pyknosis(P), clear cell foci (CCF), pyknotic nuclei (PN), nuclear degeneration (ND).

Histopathological alteration in the kidney of B. gonionotus: Kidney tubules and hematopoietic cells were found to be normal and systematically arranged in the control group (Fig. 4A). Kidney sections affected by Sumithion showed disintegration of convoluted tubules with large intra-cytoplasmic vacuoles in the epithelial cells and lumen. Kidney tissue from *B. gonionotus* after 7 days of exposure to 0.025, 0.05 and 0.10 ppm showed necrosis, degenerated kidney tubules and vacuolation (Fig. 4B, 4C and 4D). Degeneration of kidney tubules, necrosis, ruptured renal corpuscle and pyknosis were also recorded after 15 days of exposure at the dosage of 0.025,0.05 and 0.1 ppm (Fig. 5A, 5B and 5C). Intra-cellular space and ruptured collecting duct and large vacuolation were found at the dosage of 0.025, 0.05 and 0.10 ppm at 30 days of exposure (Fig. 6B, 6C and 6D). Shrinkage and degeneration of the glomeruli, dilation within the Bowman's space were also recorded. We also found irregular shaped blood vessels in kidney tissue. At the first stage of the experiment the cells of kidney were compact but with the progress of the study period cells became degenerated and some intracellular space was created within the cells. Higher concentration of sumithion exposure also caused cell rupture.

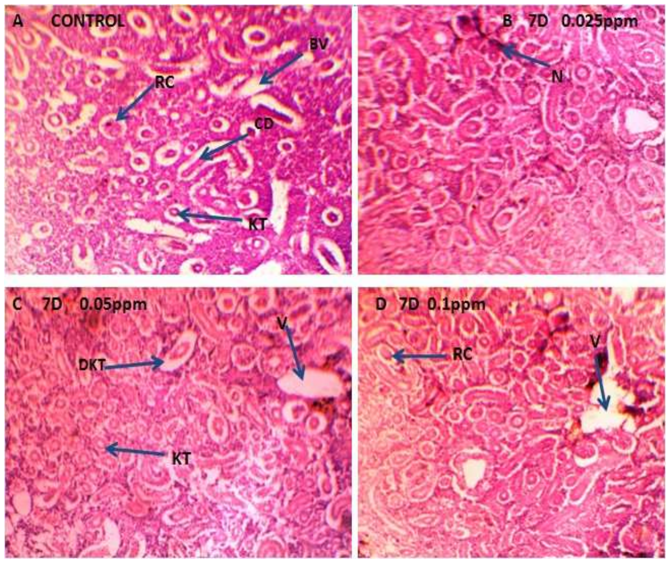

Fig. 4. Histological changes in kidney (H and E stained, X100) exposed to sumithion (A) Control (T_0); (B) 0.025 ppm (T_1); (C) 0.05 ppm (T_2); (D) 0.1 ppm (T_3) at 7 days of exposure. Arrows are indicating-kidney tubules (KT), renal corpuscle (RC), collecting duct (CD), necrosis (N), blood vessel (BV), degenerated kidney tubules (DKT) and vacuolation (V).

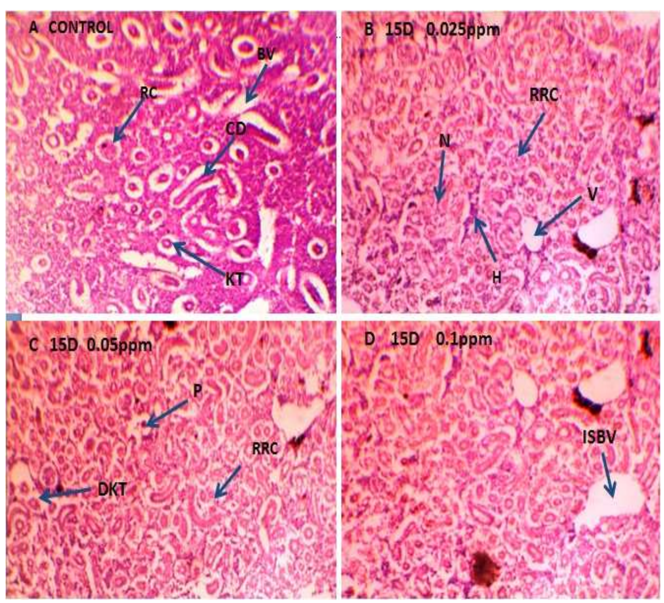

Fig. 5. Histological changes in kidney (H and E stained, X100) exposed to sumithion.(A) Control (T_0); (B) 0.025 ppm (T_1); (C) 0.05 ppm (T_2); (D) 0.1 ppm (T_3) at 15 days of exposure. Arrows are indicating-kidney tubules (KT), renal corpuscle (RC), collecting duct (CD), necrosis (N), degenerated kidney tubules (DKT), vacuolation (V), pyknosis (P), ruptured renal corpuscle (RRC) and haemorrhage (H).

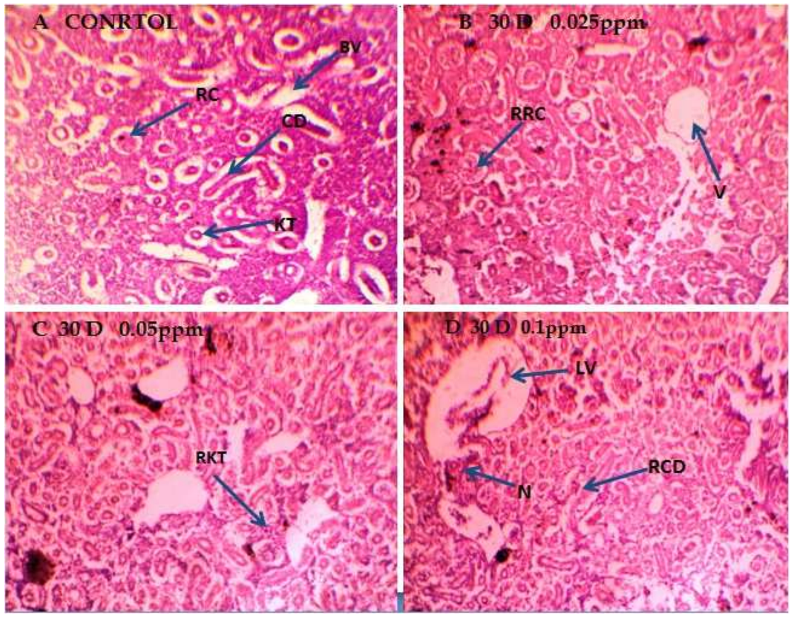

Fig. 6. Histological changes in kidney (H and E stained, X100) exposed to sumithion. (A) Control (T_0); (B) 0.025 ppm (T_1); (C) 0.05 ppm (T_2); (D) 0.1 ppm (T_3) at 30 days of exposure. Arrows are indicating-kidney tubules (KT), renal corpuscle (RC), collecting duct (CD), ruptured kidney tubules (RKT), vacuolation (V), pyknosis (P), ruptured renal corpuscle (RRC), large vacuole (LV), ruptured collecting duct (RCD).

Discussion

Histoarchitectural alterations in liver of test fish: The results show that sumithion is excessively toxic to *B. gonionotus*. The toxicity of sumithion was increased with increasing concentration and exposure time. Histopathological studies are effective tools to assess the effects of toxicants on fish (Pimpao *et al.* 2007). In the present study, several histopathological changes of liver were observed upon exposure to sumithion. Under control (T_0), liver was composed of a mass of large hexagonal hepatic cells. Large number of blood vessels and intercellular bile canaliculi were scattered among the hepatic cords. Hypertrophy of hepatocytes, mild to severe necrosis, ruptured central vein, necrosis, pyknosis, hemorrhage, irregular shaped nucleus, clear cell foci, nuclear degeneration and vacuolation were found in liver of fish exposed to higher concentrations of sumithion (T_2 and T_3).The intensity of histoarchitectural changes was more severe in higher doses and exposure time. These changes may be due to direct toxic effects of sumithion on hepatocytes since liver is the site of detoxification of all types of toxins and chemicals (Parikh *et al.* 2010). Almost same deformities including necrosis of the hepatic cells, rupture of sinusoids with hemorrhages at several points in *Heterobranchus bidorsalis* exposed to different concentrations of cypermethrin (0.038

and 0.040 ml/L)was observed (Olufayo and Alade 2012). Kabir and Begum (1978) reported cytoplasmic degeneration, pyknotic nuclei in liver tissues, vacuolation in hepatic cells and rupture of blood vessels, degenerative hepatic cells and necrotic nuclei. Shastry and Sharma (1979) exposed *C. punctatus*to a sub-lethal concentration (0.01 mg/L) of endrin and observed hypertrophy of hepatic cells and liver cord disarray, vacuolation of cytoplasm and necrosis, rupture of hepatic cell membrane and necrotic centrolobular area. The qualitative changes of liver tissues of *Channa punctatus* exposed to malathion were reported by Dubala and Shah (1979). Sharmin *et al.*(2015)found abnormal conditions of liver in fish exposed to malathion which are similar to those of the present findings. The intensity of the histoarchitectural changes was more severe in higher dose and exposure time. These changes may be due to direct toxic effects of sumithion on hepatocytes, as the liver is the site of detoxification of all types of toxins and chemicals (Parikh *et al.* 2010). The intensity of the histoarchitectural changes was more severe in higher doses and exposure time indicating that this pesticide has long term effects on fish body.

Histoarchitectural alterations in kidney of test fish: In sub-lethal dosages of sumithion several alterations such as degeneration of kidney tubules and hematopoietic tissue, vacuolization, mild to severe necrosis, pyknosis, ruptured renal corpuscle, ruptured collecting duct and hemorrhage were recorded. Similar findings also have been reported in *Heterobranchus bidorsalis* exposed to different doses of cypermethrin (Olufayo and Alade 2012), in *Labeo rohita* exposed to organochlorine pesticide endosulfan (Indirabai *et al.* 2010), in *B. gonionotus* exposed to an organophosphorous pesticide Quinalphos 25 EC (Mostakim *et al.* 2015).

The present study revealed that sumithion is toxic to fish and little amount of this pesticide can cause histological changes in liver and kidney of sarpunti (*Barbonymus gonionotus*). So indiscriminate use of pesticides should be stopped and the use of pesticides in agricultural lands and aquaculture ponds must be carefully evaluated and controlled for the benefit of fisheries and eventually for the welfare of human being.

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