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Effect of carbofuran on mortality and histo-pathological lesions in different vital organs of *Channa punctatus* (Bloch)

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Abstract: The freshwater fish, *Channa punctatus* was exposed to 1, 10, 25, 50 and 100ppm of insecticide named carbofuran. Variation in mortality rate was observed in different doses and exposure time. Histopathologoical changes were observed in kidney, stomach, intestine, heart and liver of the treated fish. Carbofuran treatment resulted in rupturing of blood capillary endothelium and severe damage to bile duct and connective tissue in the liver of treated fish. Hepatic necrosis was also observed. Extensive histological lesions occur in renal tubules, glomerulus and Bowman's capsule of the fish. The stomach of *C. punctatus* showed disintegration of epithelium duct and desquamation of gastric mucosa. Degenerative lesions at the tip of villi, swelling and necrosis were observed in the intestine of treated fish. The treated heart of *C. punctatus* showed fragmentation in muscle fibers and degeneration of longitudinal cardiac muscle showed pyknosis in muscle bundle.

Key words: Carbofuran, *Channa,* lesion, histopathology, mortality

Introduction

Pesticides are useful tools in agriculture and forestry but their contribution to the gradual degradation of the aquatic ecosystem cannot be ignored. Application of pesticides in aquatic ecosystems and their indirect uses caused erosion from agricultural lands and agricultural waste water infiltration which eventually washed into deep water environment and ecosystem (Dutta & Arends, 2003). Indiscriminate use of insecticides is one of the notorious problems of decreased fish production. A survey conducted by Bangladesh Fisheries Research Institute (FRI, 1991), Reverine Station, Chandpur reveals that about 34% of the farmers applied insecticide as over doses. Seventy six percent of the farmers have little or no idea about the methods of insecticide application and 70% of them observed fish mortality after the use of insecticides in their crop fields. Insecticides may enter into the natural water bodies destroying spawning grounds and feeding areas, restrict fish migration, reduce resistance to disease and deteriorate fish production (Metelev *et al.,* 1983). Lead can alter the physiological activities and can cause histopathological changes of various organs in fish (Jiraungkoorsku *et al.,* 2003). Histopatological changes in the carp (*Labeo rohita)* exposed to hexachlorocyclohexane was observed (Das & Mukherjee, 2000). Fish may die when large quantities of toxic substances from agricultural fields run off. Histopathological changes in the rainbow trout when exposed to sublethal concentrations of methiocarp or endosulfan have been studied (Altidok & Capkin, 2007). Among the considered endangered fish species, snake heads or murrels are the most familiar in Bangladesh and *Channa punctatus* is one of the most rare fish species of Bangladesh flood plains (Hossain *et al.,* 2000).

Histological and biochemical changes occur due to the toxic effects of malathion in *C. punctatus* (Pugazhvendan *et al.,* 2009). A

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comparative study on the piscicidal activity of synthetic pesticides and plant origin pesticides to *C. punctatus* was conducted (Shahi & Singh, 2010). Due to the residual effects of pesticides important organs like the kidney, liver and heart are damaged (Akter & Saha, 2013). Carbofuran has been extremely used to control crop field pests in Bangladesh. It is a granular insecticide of the carbamate group (durability of its action in water is 15-180 days), which may produce serious damage to the fish resulting in death. Nowadays the insecticides are the main cause of toxicity in fish. *C. punctatus* is one of the most popular fish in Bangladesh and is now one of the most endangered fish species in Bangladesh.

Therefore, the purpose of the present work is to determine the effects of carbofuran on the mortality and histological changes of specialized organs like kidney, stomach, intestine, heart and liver of *C. punctatus*.

Materials and Methods

Experimental fish

Sixty live and healthy specimens of *C. punctatus* (Mean length = 15.01 ± 0.27 cm, Mean weight = 37.58 ± 0.79g) were purchased from a fish landing and marketing center of Rajshahi city corporation. The specimens were transported carefully to the laboratory and were acclimatized for two weeks under laboratory conditions. The fishes were fed with twice a day (at 9:00 a.m. and 6:00 p.m.) with pellets of basal control diets (Osaka, Perfect Companion Group Co., Ltd. Samutprakarn 10540 Thailand).

Dose preparation

Doses of carbofuran *viz*. 1, 10, 25, 50 and 100ppm were prepared by requisite amount of carbofuran in water maintaining standard method.

Test aquaria

Six glass aquaria (50 cm × 30 cm × 20 cm) were washed with detergent and 0.1% of potassium permanganate to make the aquaria germ free and used for the experiment.

Exposure to carbofuran

The fishes were exposed to 1, 10, 25, 50 and 100ppm of carbofuran solution in 5 glass aquaria separately for a period of four days. Mortality among the insecticide treated fishes was observed and dead fishes were immediately preserved in deep freeze for histopathological studies. A control group of fishes was also maintained under the similar laboratory conditions.

Preservation of target organs

The fishes were sacrificed by decapitation. The target organs of both live and dead fishes were surgically removed and thoroughly washed in cold normal saline solution (0.85% NaCl). The tissues of target organs viz. kidney, stomach, intestine, heart and liver were fixed in Bouin's fluid. After washing in running tap water, the tissues were subjected to the different concentrations of alcohol and were preserved for routine microtome technique.

Microtomy

In the process of microtome, preserved tissues were subjected to the dehydration and infiltration, where alcohol and paraffin were used respectively. The tissues were kept in hot molten wax, which facilitate the penetration of wax to the available spaces inside the tissue. As soon as the tissue was thoroughly infiltrated with paraffin, it is ready for embedding. The block to be used for mounting was trimmed to the correct shape. For the trimming, one side of the block was shaved to get a flat surface. The other side of the block is shaved parallel to the first. Then the block was set to its holder and the

microtome machine was adjusted for sectioning in the thickness of 4-6µ. The microtome machine was allowed to rotate moving forward and backward in horizontal plane until the block started cutting. A considerable number of sections were cut. Thus the desired sections were produced with easy rhythm. For stretching, small pieces of ribbon were kept in a petridish which contained 2 ml of distilled water and 1-2 drops of Mayer`s albumen and the tissues were fixed to the slides. The petridish was gently heated over the sprit lamp. After affixing the tissues, the slides were subjected to deparafining and staining. Then the tissues were stained with Haematoxylene and Eosin. Finally each individual slide was mounted by Canada Balasum. Then the slides were ready for microscopic examination.

Microscopic observation

Tissues stained with Haematoxylene and Eosin was observed under the microscope. Histopathological lesions were observed in different vital organs of the fish.

Results

Mortality

The dose mortality response of *C. punctatus* due to carbofuran treatment has been shown in Fig. 1 and Table 1. The toxicity of the insecticide depended on both concentration and exposure time.

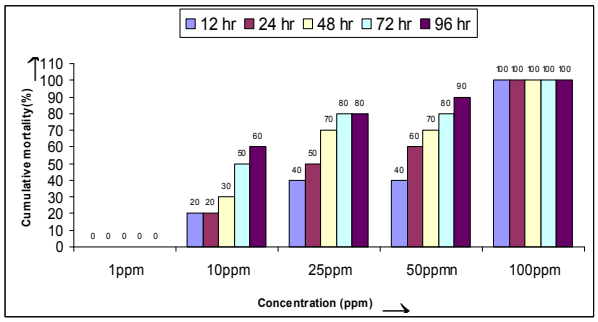

Fig. 1. Mortality of *C. punctatus* at defferent doses and exposure periods

Table 1. Lethal concentration, 95% confidence limit and regression equation of carbofuran in treated *C. punctatus*

Exposure period (hours)	LD_{50} (ppm)	95% confidence limits		Regression equation	χ^2-value
		Lower	Upper		
12 h	75.11	6.40	882.02	Y = 3.35 + 0.88x	0.226
24 h	30.69	14.80	63.64	Y = 2.67 + 1.57x	0.249
48 h	17.72	8.60	36.50	Y = 3.02 + 1.58x	0.898
72 h	8.86	2.24	35.03	Y = 3.72 + 1.35x	0.390
96 h	6.25	1.06	36.97	Y = 3.92 + 1.35x	0.001

Histo-pathological studies

The histopathologoical lesions in various vital organs of *C. punctatus* due to Carbofuran toxicity were presented in Tables 2 & 3.

Effect of carbofuran on the kidney

The kidney tubules and haematopoetic cells of control group were normal (Fig. 2A). Slight histopathologoical changes occurred at 10ppm of carbofuran treatment but no changes were observed at 1ppm of carbofuran treatment for 4 days exposure. Histological changes occurred at 25ppm of carbofuran treatment on *C. punctatus* (Bloch) but more conspicuous and prominent changes were visible at 50 and 100ppm showing large vacculation, necrosis, degeneration of kidney tubules, pyknosis, swelling of epithelial tubules and fragmentation of glomerulii (Fig. 2B).

Effect of carbofuran on the stomach

In control fish, stomach showed normal epithelial duct, mucosa and sub mucosa with blood capillary (Fig. 3A) whereas the stomach of treated *C. punctatus* showed hyperchromasia of epithelium cells, disintegration of epithelium duct and desquamation of gastric mucosa (Fig. 3B).

Table 2. Degree and intensity of carbofuran on different vital organs of *C. punctatus*

Doses (ppm)	Examined fish	Mortality		Effects				
		number	(%)	Stomach	Intestine	Kidney	Liver	Heart
0 (Control)	10	0	0	0	0	0	0	0
1	10	0	0	0	0	0	0	0
10	10	6	60	+	++	+	+	0
25	10	8	80	++	++	+	++	+
50	10	9	90	+++	+++	+++	+++	++
100	10	10	100	++++	++++	+++	+++	++

Note: Number of signs shows the degree and intensity of effects: 0 = No Effect, + = Low Effect, ++ = Higher Effect, +++ = Severe Effect, ++++ = Extremely Severe Effect.

Table 3. Effects of carbofuran on different vital organs of *C. punctatus*

Doses (ppm)	Examined fish	Affected fish (%)	Effects				
			Kidney	Stomach	Intestine	Heart	Liver
0 (Control)	10	No	No	No	No	No	No
1	10	No	No	No	No	No	No
10	10	60	MD	MD	AV	MD	HS
25	10	80	S	S	AV	CMA	SR
50	10	90	KTV	V	VD,CMD	PD,MMD	NH
100	10	100	KTD	CED	VD,SD,MD	MMD,PD	CR,BDD

Note: MD, minor damage; AV, abnormal villi; HS, hepatocytic swelling; S, swelling; CMA, cardiac muscle alteration; SR, sinusoid rupturing; KTV, kidney tubule vacculation; V, vacculation; VD, villi degeneration; CMD, circular muscles degeneration; PD, pericardial damage; MMD, myocardial muscle damage; NH, nuclear hypertrophy; KTD, kidney tubule degeneration; CED, columnar epithelial degeneration; SD, serosal damage; MD, muscularis degeneration; CR, cell rupturing; BDD, bile duct degeneration

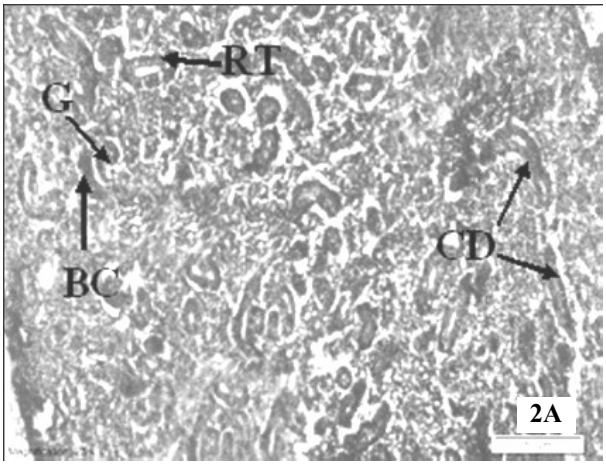

Fig. 2A. T.S. of kidney (300x) of control *C. punctatus* showing the normal structure (G, glomerulus; RT, renal tubules; CD, collecting duct; BC, Bowmen`s capsule)

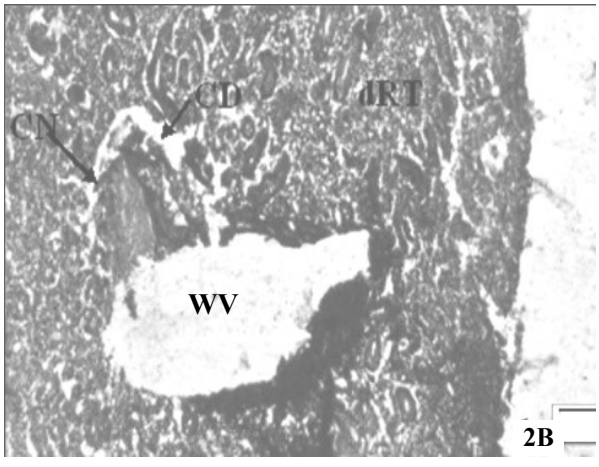

Fig. 2B. T.S. of kidney (300x) of treated (100ppm) *C. punctatus* showing degeneration of renal tubule, cell necrosis and wide vacculation (dRT, degenerative renal tubules; WV, wide vacculation; CD, cell disruption; CN, cell necrosis)

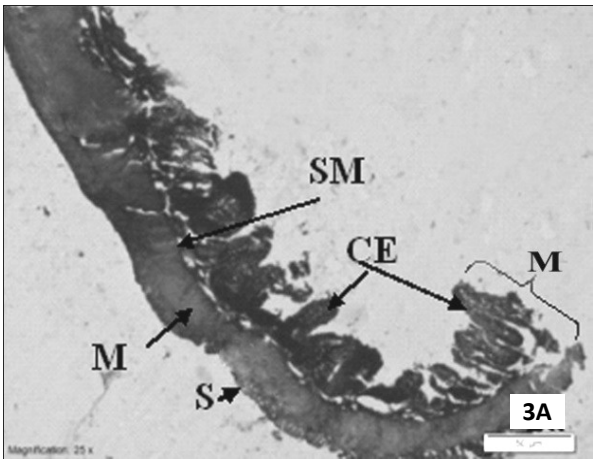

Fig. 3A. T.S. of stomach (300x) of control *C. punctatus* showing the normal structure (S, serosa; M, muscularis; SM, submucosa; M, mucosa; CE, columnar epithelium)

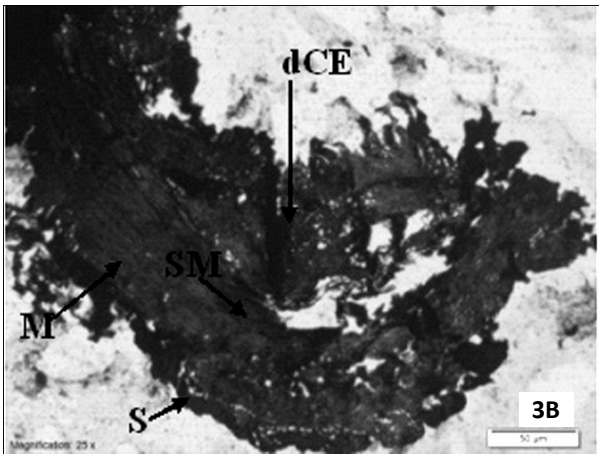

Fig. 3B. T.S. of stomach (300x) of treated (25ppm) *C. punctatus* showing the abnormal structure (cdCE, complete degeneration of columnar epithelium)

Effect of carbofuran on the intestine

In control fish, the intestine showed the normal structures containing mucosa, sub-mucosa, serosa and villi during microscopic observation (Fig. 4A). The present investigation indicated that the destruction in tissues of intestine are progressively increased depending on doses of 1, 10, 25, 50, and 100ppm. Degenerative changes at the tip of villi, circular muscle and necrosis were observed in 50ppm (Fig. 4B).

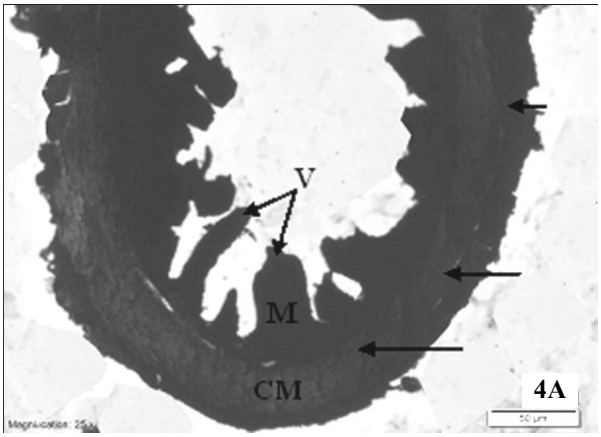

Fig. 4A. T.S. of intestine (300x) of control *C. punctatus* showing the regular arrangement (S, serosa; LM, longitudinal muscle; M, muscularis; CM, circular muscle; SM, submucosa; M, mucosa; V, villi)

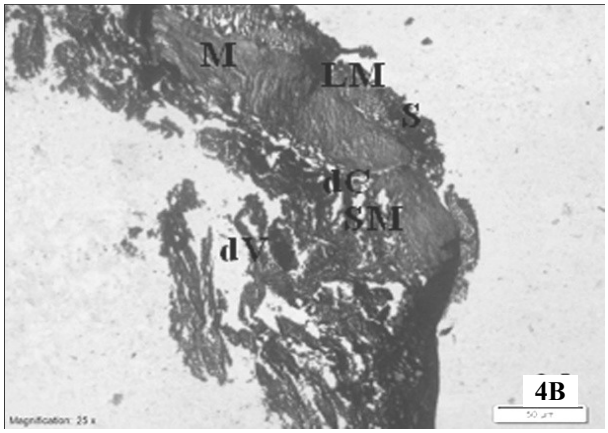

Fig. 4B. T.S. of intestine (300x) of treated (50ppm) *C. punctatus* showing the degeneration of villi and circular muscle (S, serosa; LM, longitudinal muscle; M, muscularis; SM, submucosa; dCM, degenerative circular muscle; dV, degenerated villi).

Effect of carbofuran on the heart

In treated fish, heart tissue exhibited remarkable changes in the pericardium as well as myocardium due to insecticide treatment (Fig. 5A). Severe changes, including fragmentation of muscle fibers, pyknosis and necrosis occurred at 100ppm of carbofuran (Fig. 5B).

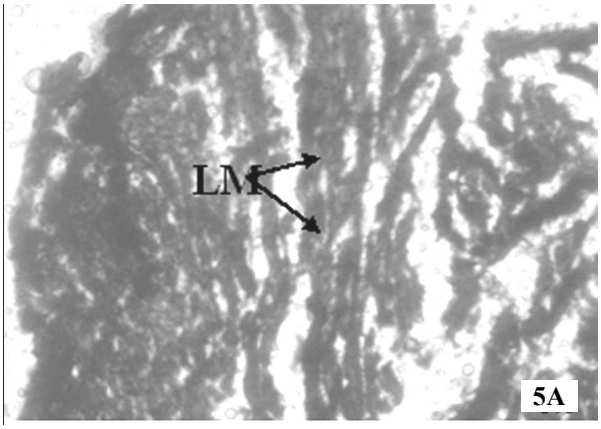

Fig. 5A. T.S. of heart (300x) of control *C. punctatus* showing the regular arrangement (LM, longitudinal muscle; CM, cardiac muscle)

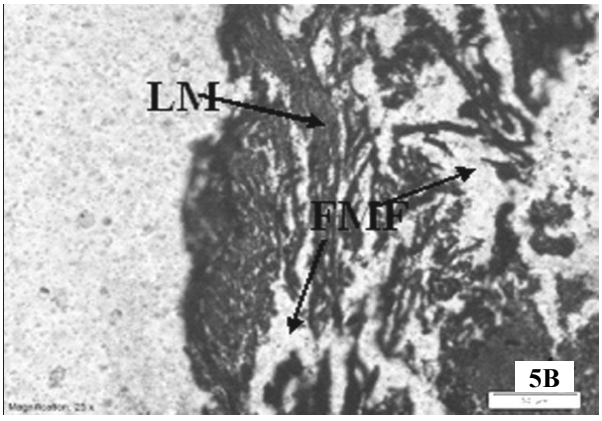

Fig. 5B. T.S. of heart (300x) of treated (50ppm) *C. punctatus* showing the fragmentation of muscle fibres (LM, longitudinal muscle; CM, cardiac muscle; FMF, fragmentarion of muscle fibers)

Effect of carbofuran on the liver

Liver tissues of the control group exhibited normal condition (Fig. 6A). No prominent damages were observed at 1ppm of carbofuran treatment on the liver of treated fish. But fish exposed to 10, 25, 50 and 100ppm of carbofuran for 7 days showed degenerative changes in the liver. The most degenerative changes were observed at 50ppm of carbofuran treatment that included nuclear hypertrophy, bile duct degeneration, swelling of hepatocytes, pyknotic nuclei, large vacculation and haemorrhages (Fig. 6B).

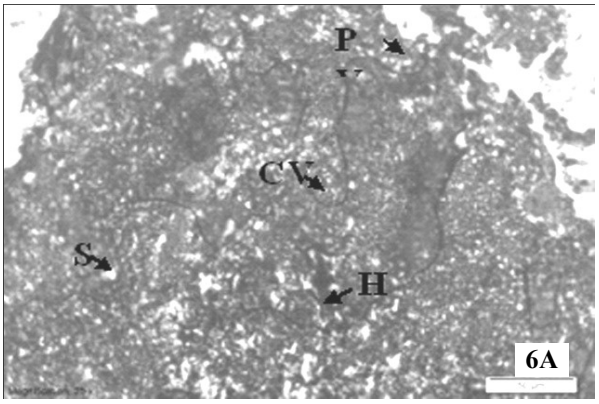

Fig. 6A. T.S. of liver (300x) of control *C. punctatus* showing the normal structure (PV, portal vein; CV, central vein; S, sinusoid; H, hepatocyte)

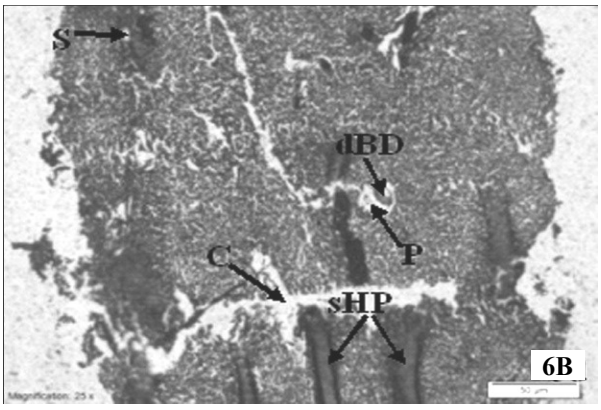

Fig. 6B. T.S. of liver (300x) of treated (50ppm) *C. punctatus* showing cell rupturing, bile duct degeneration and swelling of hepatocyte (PV, portal vein; CV, central vein; S, sinusoid; H, hepatocytes; CR, cell rupture; SHPN, swelling of hepatocyte with pycnotic nuclei)

Discussion

The effects of carbofuran treatment on various organs of *C. punctatus* have been discussed below. In present study, the exposure of carbofuran leads to the alterations in the histological architecture of targeted organs.

Kidney: The kidney of treated *C. punctatus* showed damage and degeneration of glomerulus, renal tubules and glomerular capillary, shrunken Bowman's capsule and

haemorrhages in epithelial tissues. Histopathological lesions observed in the present study are severe enough to cause impairment in the functioning of the kidney. Sublethal ammonia exposure of Nile tilapia showed detrimental effects on Kidney (Benli *et al.,* 2008). Kidneys showed histopathological lesions in fresh water fish, *Cyprinus carpio* after an acute exposure to deltamethrin (Cengiz, 2006). The kidney showed histological changes due to toxic effect of mercuric chloride in *Heteropneustes fossilis* (Soni & Gupta, 2006). The present investigation showed the degeneration of kidney tubule and haematopoetic cells, necrosis, pyknosis and hemorrhage in treated kidney of *C. punctatus*.

Stomach: Carbofuran produced hyperchromatic epithelial cells, disintegration of glandular epithelium duct and desquamation of gastric mucosa in the stomach of *C. punctatus*. Hyperchromasia, disintegration of epithelium duct and desquamation of gastric mucosa was also observed due to the effect of cadmium chloride in *C. punctatus* (Bais & Lokhande, 2012). Tunica muscularis with nuclear depolarization and cytoplasmic heperchromasia, general edema, loss of pepsinogen granules, disintegration of epithelium duct with desquamation of gastric mucosa in *H. fossilis* when exposed to copper (Bhatnager & Shrivastava, 1975). Hypersecretion of pepsin led to the degradation of tissue of protein in *C. punctatus* and an increase in ammonia and urea have also been observed in this fish after exposure to lead nitrate (Sastry and Gupta, 1978). In present study exposure of carbofuran leads to alteration in histology of experimental organ (stomach). Carbofuran might have an adverse effect on the stomach of *C. punctatus*.

Intestine: Toxic lesions are the most common in the intestine which include hyperemia, degenerative changes in the tips of villi, loss of structural integrity of mucosal folds, degenerative mucosal epithelium, necrosis and desquamation of mucosal epithelium in intestine of different fishes exposed to cadmium chloride (Gutierrez *et al.,* 1978; Newman and MacLean, 1974; Establier *et al.,* 1978, and Walsh and Rubalin, 1975). Histochemical changes due to induction of endosulfan in the stomach and intestine of *H. fossilis* (Shrivastava, 2013). The present investigation also shows similar effects due to carbofuran treatment in *C. punctatus*.

Heart: The fish (*C. punctatus)* exposed to carbofuran showed significant alteration in histology of cardiac tissue. Acute toxicity of heavy metal vandate oligomers on cardiac muscle of toadfish, *Halobatrachus didactylus* (Borges *et al*., 2003). The myocardial fibers degeneration could attribute to the excessive calcium accumulation (Lennard & Haddart, 1992). Protein profile and histopathology in heart tissue of rat was studied (Nagarjuna & Das, 2009). *C. punctatus* exposed to sub lethal concentration of malathion showed decreased the level of protein content in cardiac muscle (Magar & Duve, 2013). Heart tissue exhibited remarkable changes in the pericardium as well as myocardium due to carbofuran toxicity in *C. punctatus.*

Liver: The liver of C*. punctatus* showed various histological changes when exposed to carbofuran. Dilation of blood sinusoid, partial vacculation in hepatocyte and pyknosis occurred in the liver of *C. punctatus* due to sublethal exposure of carbaryl and cartab (Mirsha *et al.,* 2006). Ruptured blood sinuses and disintegration and vacculation of the hepatic cells were recorded in the liver of *Catla catla* when exposed to fenitrothion (Tomar *et al*., 2006). Carbofuran and cypermethrin induced histopathological alterations in the

liver of *L. rohita* (Sarkar *et al.,* 2005). The hepatocytes exhibited reduction in the size and there was a peripheral accumulation of cytoplasm due to exposure to the increased concentrations of the organophosphate pesticide, Nuvan (Shukla *et al.,* 2005). *C. punctatus* exposed to a sublethal concentration (0.01 mg/l) of endrin showed hypertrophy of hepatic cells and liver cord disarray and vacculation of cytoplasm (Sastry & Sharma, 1979). Inflammation, central necrosis and cell degeneration in the liver tissue of *Oreochromis aureus* juveniles were noted due to phenol (Fatma, 2008 and Nasar- Allah & Hameid, 2007). Liver showed the swelling of hepatocytes, destruction of sinusoid, pyknotic nuclei and vacculated hepatocytes when *C. punctatus* exposed to dimethoate (Paithane, 2012). In present investigation, *C. punctatus* exposed to carbofuran showed marked histopathological changes in liver like cell necrosis, swelling of hepatocytes with nuclear hypertrophy and rupture of sinusoid with hemorrhages at several places.

It is evident from the above discussion that *C. punctatus* is vulnerable to the toxicity of various chemical agents, including carbofuran. Carbofuran might have the adverse effects on the different organs of *C. punctatus*. The fish has a great economic value and as such must be protected against toxicity of the insecticide among others.

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