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Research Article

Furadan Induced Histopathological Changes in *Channa punctatus*, *Heteropneustes fossilis* and *Anabas testudineus*

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ABSTRACT

Insecticide furadan is one of the common pollutants in aquatic environment and has become destructive for aquatic organisms. The objective of the present investigation was to observe the effects of furadan in different organs including gills, liver, heart, intestine and kidney of three indigenous fish species *Channa punctatus*, *Heteropneustes fossilis* and *Anabas testudineus.* Adult fishes were exposed to six different concentrations (2.5, 5.0, 10.0, 15.0, 20.0 and 25.0 mg/l) of the insecticide in the test aquaria (Clay pot `Chari') to explore the major alterations of the vital organs for 24, 48, 72 and 96 hours of exposure period. Lower concentrations of furadan (2.5 to 10.0 mg/l) did not modify significantly on the organs of the fishes but in the concentration from 15.0 to 25.0 mg/l showed curling, necrosis, fusion of lamellae and destruction of gill arches. Rupture of blood vessels and damage of sinusoids in liver, muscle fiber fragmentation and damage of blood vessels in heart, villi were fused and renal tubules damaged in *C. punctatus* from different exposure time period. Fusion of secondary lamellae of gills, damage of sinusoid, vacuolated in liver and heart, fused villi and hemorrhagic renal tubules were observed in kidney of *H. fossilis.* Damage of lamellae in gills, blood vessels irregular in liver, muscle fibers fragmented in heart, ruptured/ fused villi and blood vessels, hemorrhage and necrotic change were noticed in kidney of *A. testudineus* in the same doses. The study indicates that different concentrations of insecticide damaged the important organs of the fish, even a small amount of pesticide (Furadan) in fresh water ecosystem causes harmful effects on fish physiology and consequently make death of the fishes.

INTRODUCTION

Currently pesticides are considered as one of important kits for pest control in agricultural sectors to enhance crop production by eliminating unwanted diseases and insects (Begum and Mytra, 2015). In agricultural sector in Bangladesh has aggregated with agro-chemicals and insecticides for more production from a limited land to meet the demand of ever increasing population (Akhter and Shaha, 2013). Though in agricultural sector of Bangladesh was negligible to use of pesticides until 1960s but from the year 1992 to 2010 has recorded increase of pesticides from 7350 metric tons to 45,172 metric tons (Ali *et al*, 2018). Presently in Bangladesh, 141 active ingredients are in use and among them 48 are registered as public health pesticides and 93 are registered as agricultural pesticides represented by 303 trade names (MoEF, 2005). Like other country of the world, pesticides (Malathion, Furadan, Diazinon etc.) are being used legally or illegally in substantial quantities in Bangladesh which can easily contaminate the water resources (Hasanuzzaman *et al*, 2016). Among the pesticides, carbamate insecticide 'Furadan' is commonly used in crops and vegetable cultivation (Begum *et al*, 2015). Konar (1975) noticed pesticides have become the contributor of steady aquatic ecosystem degradation that are recommended as a greater part of the natural environment. About 15 million hectors of crop cultivated area that used pesticides in cereal crops like rice, wheat, maize and other important crops like vegetables, pulses, jute, fruits, tea, sugarcane and tobacco (BBS, 2016). In addition, Bangladesh encompass various wetland ecosystems viz., inland water resources, brackish water and saline water bodies, Rivers, streams, lakes, marshes, haors and beels are include in the inland water bodies comprise an area of about 3.9 million hectors that comprises in haors, ponds, lakes and beels (Kizar et al, 2018).

Though various types of insecticides increase the crop yields but contaminate the aquatic environment through several ways such as direct deposition, spray, surface runoff and water leaching from the soil surface that induce the ecological balance and hazardous health effects on non-target creatures including fish (Hossain *et al*, 2015; Haider *et al*, 2014). At high concentrations, pesticides are known to reduce the survival, growth and reproduction of the aquatic organisms and create severe alterations in their organs like gonads, gills, liver, stomach, kidney, kidney, brain, muscle etc. (Mckim *et al*, 1975). Nowadays, various types of pesticides are contaminated with the aquatic environment that not only a great threat to the overall ecosystem but also destructive for human health (Faruk, 2014). Pesticides were contaminating with surface water that reported since about half century ago in USA, Canada and Europe. After that a huge number of documents had been revealing the toxic effects of these pollutants to the aquatic ecosystem (Miller *et al*, 2002; Galloway, 2003). Some of the fish species including *C. punctatus*, *H. fossilis*, *A. testudineus* and some other indigenous small fishes use paddy fields and adjacent aquatic environment (lakes, canals, ponds, swamps, beels and other small water bodies) as breeding and nursing of their fries. Hence, they played as biological indicators of eco-toxicological studies. Carbofuran is one of the most toxic carbamates that marketed under the trade name Furadan which is extensively used to control insects in a wide variety of field crops. Furadan (2,2-Dimethyl -2,3-dihydro-1-benzofuran-7-yl methylcarbamate) is a broad spectrum N-methyl carbamate insecticide widely used in agriculture for the control of insects, mites and nematodes in soil or for protection of fruit, vegetables and crops (Jongeneelen *et al*, 2013). However, Furadan is banned in Canada, European Union and by Environmental Protection Agency announced in United States for protection of birds and risk to human health (EPA, 2008). It is mainly granular form used as toxic to birds, insects and domestic pest control And its usage has increased in recent years to restrain a variety of insects including aphids, beetles, bugs as well as rats. After application in the agricultural lands, Furadan easily washes away into the surface water and ultimately brings into adjacent water bodies, beels, ponds, rivers and lakes where they contaminate the aquatic ecosystems and effects the aquatic organisms. A number of researches have been performed to evaluate the effects of Furadan on different species of fishes and others (Banaee, 2011). Though carbofuran pesticides are widely used in agricultural field that contaminated aquatic ecosystem and effects in fish's important organs are damaged by it's residual effects.

The fish species used in the current investigations viz., *C. punctatus*, *H. fossilis*, *A. testudineus* are common fresh water fishes present in local water bodies of Bangladesh and play vital role in terms of animal protein in human food and among other aquatic organisms. Therefore, it is necessary to investigate the adverse effects of pesticide on fish health. As it has a direct link with food chain as well as contamination of water bodies occurring by pesticides imbalanced the aquatic ecosystem (Al-Otaibi *et al*, 2019). Fishes are severely affected by pesticides in different ways and mostly affected essential organs. The important respiratory organ, gills are attracted firstly because it is the first organs to be exposed by contaminated water / pollutants (Gallagher, 1992). Kidney infected by water-born toxic particles and it regulates the extracellular and composition of fluid volume as well as acid-base balance of fish. Insecticides interrupt the kidney's functions and most cases it refers to imbalance of homeostasis (Miller, 2002). Through these backgrounds, the present study has been conducted to investigate the toxicological effects of Furadan in different organs of the three indigenous fresh water fish species *i.e. C. punctatus*, *H. fossilis* and *A. testudineus* that would facilitate knowledge for the management of fresh water reservoirs regarding Furadan application in agriculture fields.

MATERIALS AND METHODS

Experimental species and their holding

Three fish species *C. punctatus* with 12 to 17 cm length and 23 to 29 gm weight, *H. fossilis* with 13.5 to 19 cm length and 23.5 to 31 gm weight and *A. testudineus* with 4.5 to 7.5 cm length and 10.0 to 26.5 gm weight were collected from the local water bodies (beels/lakes) in Dhamrai area located at Savar in Dhaka district, Bangladesh. Selected fishes were transported in plastic container and reared in 5 clay pots locally called Chari of 18×5×10 inches in size with 20 liters of water volume. Experimental species were disinfected with 0.5% $KMNO_4$ solution for five minutes to set free external infections and acclimatized them under tap water in a large glass tank with 100 liters capacity for a week. Water temperature 25±0.47º C with pH 6.7±0.03 and 7.4±0.12 mg/l dissolve oxygen were maintained in the test aquaria. The experiments were conducted in the photoperiod of 12D:12L. Experimental fishes were fed using earth worm twice daily and dead fishes were immediately removed to avoid possibility of decline of water quality. Water of the experimental aquaria (Chari) was changed frequently that maintained the better possible effects of Furadan.

Formulation of Insecticide

Insecticide 'Furadan 3GR' was purchased from the local authorized dealerAnd 200 ml stock solution was prepared following the EC% active ingredient (mg/l) with the formula of (200×60)/1000= X (X= amount of Furadan 3GR).The desired dose concentrations for 20L tap water were formulated with the aphorism of $S_1V_1= S_2V_2$. After dilution with distilled water of granular insecticide 'furadan', selected concentrations were poured into the 20L tap water in the test aquaria with micropipette and stirred the solution gently with a glass rod for mixing absolutely.

Histology

For histological investigation, fishes (20×3) were exposed to six various concentrations of Furadan (2.5. 5.0, 10.0, 15.0, 20.0 and 25.0 mg/l) with three replications for each group. The control group (20 fishes) had not treated with insecticide. Throughout 96 hours of experiment, both control and insecticide treated fishes were dissected and examined for histological study according to Keneko

(1989) and Schalm *et al,* (1995). Gill, Liver, Heart, Intestine and Kidney were dissected sophisticatedly and kept in 10% formaldehyde. After 24 hours, the sections of tissue were dehydrated by ethyl alcohol and then embedded using paraffin. The paraffin embedded tissue blocks were sectioned using a microtome. The thin sections were mounted on individual microscope slides and stained with hematoxylin and eosin. of the stained sections werewere viewed under OLYMPUS CH40 microscope (X10) and photographed.

RESULTS AND DISCUSSION

Histological observation of different concentrations of Furadan were performed on gills, liver, heart, intestine and kidney of three treated fish species are presented in Figure 1, 2 and 3. The specific changes of different organ of the insecticide 'furadan' exposed fishes are described.

Channa punctatus

Gills

Gills are primary respiratory organs and all metabolic pathways depend upon the effectiveness of gills. In lower concentrations (0.5-10.0 mg/l) of insecticide no notable changes observed in treated samples. Lamellar fusion, deterioration of secondary lamellae and gill arch were observed in 15.0 to 25.0 mg/l concentrations of Furadan. Damage of lamellae was noticed in 15.0 mg/l, gill filament and test buds apparently changed at 20.0 mg/l concentrations. In 25.0 mg/l concentration level of insecticides showed hemorrhage and destruction of gill arches (Fig. 1, A* - A++).

Liver

Histological observation of liver in control fish in the present study showed sinusoids and central vein were systematically arranged and with no abnormalities. Normal structures of liver tissues were also found upto 10.0 mg/l concentrations of insecticide treated fishes. In 15.0 to 20.0 mg/l concentration, destruction of sinusoids and central vein ruptured found (Fig. 1, B*-B++).

Heart

At lower concentrations (0.5-10.0 mg/l) of insecticide treated for 96 hours exposure period in the fishes, no remarkable changes were found. In concentration of 15.0, 20.0 and 25.0 mg/l the sections of tissue showed destruction of blood vessels and fragmentations of muscle fibers (Fig. 1, C*-C++).

Intestine

In the intestine of control fish muscularis, mucosa; sub mucosa, and serosa's membrane were systematically arranged. Also in lower concentrations level (2.5- 10.0 mg/l) had showed no changes in different part of intestine but in higher concentrations (15.0 to 20.0 mg/l) muscularies and submucosa were disintegrated. In 25.0 mg/l concentration blended of mucosa and villi were ruptured (Fig. 1, D*-D++).

Kidney

Renal tubules, bowman's capsules and blood vessels were normal in 15.0 mg/l. Concentration from 20.0 to 25.0 mg/l level, degeneration of tubules, blood vessels and Bowman's capsule with necrotic changes in glomerulus were observed (Fig. 1, E*-E++) in the furadan treated species.

Heteropneustes fossilis

Gills

In the gills of control fish, primary and secondary gill filaments arranged and gill arch well established. There is no significant changes were found in gills of the treated fishes in lower concentrations upto 15.0 mg/l doses of insecticide for 96 hours**.** In 20.0 mg/l concentration, showed lamellar fusion and gill filaments and gill arches were found destructed in 25.0 mg/l level of concentration (Fig. 2, F*-F++).

Liver

In the histological observations of liver of both treated and control fishes exposed upto 10.0 mg/l concentrations of insecticide showed normal structure. From 15 mg/l dose, destruction of blood vessels had started. Central vein and sinusoids were disordered appeared in 15.0 mg/l concentration level and in 20-25 mg/l concentration revealed that central vein necrosis and blood vessels were disrupted and vacuoles appeared in sinusoids (Fig. 2, G*- G++).

Heart

The heart of control fish showed normal arrangement of muscle and blood vessels. In lower concentrations of insecticides upto 10 mg/l level tissues showed minor changes. In higher concentrations from 15.0 to 25.0 mg/l, the organs demonstrated remarkable changes including necrosis, destruction of blood vessels and fragmentation of muscle fibers of heart (Fig. 2, H*- H++).

Intestine

The intestine of control fish *H. fossilis* usually comprised of four layers, i.e. muscularis, mucosa, sub-mucosa and serosa. Sub-mucosa is a connective tissue layer containing blood vessels, lymphatic and nerves. Treated fish intestine was not significantly infected in lower concentrations of insecticide (2.5-10.0 mg/l). In 15 to 25.0 mg/l concentrations muscularis was swollen, serosa slightly damaged and destruction of villi were found in 96 hours exposure (Fig. 2, I* - I++).

Kidney

Kidney's functional unit is nephron and it consists of renal corpuscle and renal tubule. The renal corpuscle of nephron consists of glomerulus and Bowman's capsule. Upto 15.0 mg/l concentration of furadan the normal histological structure of kidney showed the control and treated group. In 20.0 to 25.0 mg/l of insecticide in the transverse section of kidney appeared necrosis and hemorrhage of renal tubules and glomerulus (Fig. 3, J* - J++).

Anabus testudineus

Gills

Anabus testudineus bears four pairs of gills which help respiration. In the lower concentration of Furadan had not remarkably affected to gills of the fishes upto 10.0 mg/l. Bent and curly form of lamellae were noticed from 15.0 to 25.0 mg/l concentration level. In 25.0 mg/l level of concentration the gill lamellae were destructed ((Fig. 3, K*- K++).

Liver

The photomicrograph of liver showed tissues, blood vessels, sinusoids etc. It was normal upto 10.0 mg/l concentrations of both control and insecticide treated fishes. Abnormal structure of blood vessels and sinusoids started from 15.0 mg/l concentrations level. In 15.0 mg/l concentration level, irregular blood vessels and hemorrhages in 20.0 mg/l level were noticed. Severe hemorrhage in liver tissues was observed in this concentration. In 25.0 mg/l some vacuoles were also seen in sinusoids in the highest exposed concentration of insecticide at 96 hours exposer time period (Fig. 3, L* - L++).

Heart

Histological changes after exposure to insecticide Furadan for the concentration upto 10.0 mg/l showed no distinguished changes apparently in both control and treated fishes. In higher concentrations (15 to 20 mg/l) of Furadan treated fishes compared with the control fishes while two major changes were noticed including fragmented muscle fibers and destructed blood vessels. In 25.0 mg/l level treated fish tissue showed necrosis and fragmented muscle fibers (Fig. 3, M* - M++).

Intestine

Furadan concentrations upto 10.0 mg/l and control had no effect on the intestine of *A. testudineus*. In 15.0 and 20.0 mg/l concentration, submucosa tissue layer was found disintegrated blood vessels and vacuolated and in the highest treated dose of 25.0 mg/l level intestinal villi were attained in fused and ruptured. (Fig. 3, N*- N++).

Kidney

Kidneys of the treated fishes became infected by Furadan at 15.0 mg/l concentration and renal tubules and blood vessels were hemorrhagic and necrotic. Glomeruli were changed in 20.0 mg/l concentration level. Severe necrosis was observed in 25.0 mg/l dose at 96 hours of exposer period (Fig. 3, O*- O++).

DISCUSSION

Histological studies on different tissues of organs of exposed fish are useful tools for toxicological investigation and monitoring of water quality of aquatic ecosystem. Structural changes of tissues of the organs in the tested fishes induced to different concentrations of insecticides play significance role facilitate knowledge of the nature of toxicity (Banaee, 2013). These changes of tissues depend on the levels of insecticides and exposure duration to the toxicants (Fanta *et al*, 2003). Tissues injuries and damages in organs can result in the reduced survival, growth, fitness and reproduction of fish species (Banaee, 2012). Furadan is known to be very toxic to aquatic biota including fish species and some histopathological studies evidence such as gill, liver and kidney alterations was found in freshwater fish *Cyprinus carpio* at the end of 96 h in treatment (Cengiz, 2006). Histopathological alterations of different tissues in *Cirrhinus mrigala* exposed to various concentrations of dichlovos and organophosphate pesticide on the gill and liver tissues were hyperplasia, congestion, vacuolar degeneration, karyolysis, karyohexis, dilation of sinusoids, necrosis of epithelial, oedema, lameller fusion, collapsed secondary lamellae and curling of secondary lamellae (velmurugan, 2009, Cengiz and Unlu, 2006). The lesions of different sophisticated organs *i.e.* gills, liver, heart, kidney and digestive truck of various insecticides treated fishes disturbed homeostasis that lead to physiological disorders and consequently dead of these fishes.

The results of the present study revealed that Furadan strictly affected in the gills of the three treated (*C. punctatus*, *H. fossilis* and *A. testudineus*) fish species and showed curly and bent of lamellae, fusion and destruction of gill filaments and gill arches in different concentration levels. Similar results described from other studies of Chatterjee *et al*, (1997); McKim, 1975 and Yeldrimet *et al*, (2005) showed that gills were the main target tissue induced by Carbofuran that used as the main route of arrival pesticides. Gills are the vital organ of respiration, osmoregulation and secretory functions. Irregular and decrease of respiration are the early symptoms of insecticide toxicity (Cengiz, 2006) that dominate the physiological functions and may cause death. A number of scientists have studied on different fish tissues with their histological and histochemical effects by various pollutants. Some related worth mentioning contributions to date are those of Poleksik and Karan (1999), Begum *et al*, (2001) and Akter *et al*, (2008) and fully supported the result of the present study. Other studies also noticed similar effects of insecticides on gills of different species of fish. For example, histopathological effects of Deltamethrin on gills of Nile tilapia *Oreochromis niloticaus* were studied by Yeldrim *et al*, (2006), Atrazine induced of deteriorating effects in the gill epithelium of *Gnathonemus petersii* by Alazemi *et al*, (1996). Epithelial layer lifting, hyperplasia and necrosis, shortening of the lamellae, frequent epithelial rupture, lamellar fusion, mucous cells hypertrophy, extensive fusion and clavate lamellae were found in the experimented fishes. In *H. fossilis* gill lamellae were distorted, gill filaments disintegrated, gill filament were swollen and destroyed and gill arches were separated after being treated with Cassia siamea seed extract that supporting the findings of the present study.

In the present study liver samples of all the three species revealed destruction of blood vessels, tissues fragmentation and hemorrhage in blood vessel as well as vacuoles were found in sinusoids. These results are assimilated to Cattaneo *et al*, (2008) who reported that fragmentation and rupture in cell membrane, vacuoles in sinusoids of the liver tissues of silver catfish, *Rhamdia* *quelen* for the effect of 2,4-dichlorophenoxiacetic acid. It also followed the results of Cengiz and Unlu (2006), Mishra and Mohanty, (2008), Vinodhini and Narayanan, (2009). They reported that hemorrhagic alterations with necrosis, narrowing of sinusoids were seen in *Gambusia affinis* and *C. punctatus* and *C. carpio* exposed to deltamethrin and heavy metal. The present study also followed the finding of Matos *et al*, (2007) and Sepici-Dincel *et al*, (2009). They notice the similar histopathological changes in liver tissues of *O. niloticus* and *C. carpio* exposed to sub-lethal concentrations of carbaryl and cyfluthrin to the experimented fish species.

Different concentrations of Furadan rigorously affected the kidney tissues of the three species of treated fish. Renal tubules, glomerulus were changes to necrosis in the treated fishes. Similar results were found from the studies of Glover *et al*, (2007). They exposed endosulfan on Atlantic salmon (*Salmo salar*) at concentration range from 4 to 710 µg kg^{-1} for 35 days duration and found irregular structure and malfunction of kidney from low concentration to high degree of insecticide formulation. Similar result were also observed by Boran *et al*, (2010) of the acute toxicity of maneb and carbarys on juvenile rainbow trout, *Oncorhynchus mykiss*. The work revealed lamellar fusion, abnormality of lamellae, fusion of lamellae, necrosis in epithelial cell of the treated fishes infected by insecticide and found both insecticides were almost similar in histological tissue injuries in the treated fishes and the major affected organs were gills, liver and kidney.

Matton and LaHam, (1969) experimented the effect of Furadan on *A. testudineus, H. fossilis* where they found destruction of kidney tubules, necrosis, haemorrhage and Vacuolation in kidney tissues. Ram & Singh, (1988), Stalin, (2019) reported necrosis in hepatocytes, bleeding in hepatic blood vessels alterations in liver, the histological lesions such as lamellar fusion, necrosis, fused lamellae and lifting of lamellae epithelium in gills, blood conjunctions and necrotic hepatocytes in liver tissue and fusion, flattened of villi in the intestine were obtained from the Furadan treated fishes. It supported the present results. Velmurugan *et al*, (2009) also reported that pathological changes on gills, liver and kidneys of *Clarias gariepinus*, founding with tubular fusion, hyperplasia, epithelial lifting, oedema, fusion of secondary lamellae, necrosis in the gills, swelling of hepatocytes, necrosis, pycnosis and vacuoles in liver and heart. Epithelial hypertrophy, glomerular condensation, hemorrhage and necrosis of Bowman's capsule were found in kidney tissues of the experimented fishes in various concentrations of cypermethrin supporting the present results of the study.

CONCLUSION

The results of the study concludes that even a small amount of pesticide (Furadan) in fresh water ecosystem causes harmful effects on fish physiology and consequently make death of the fishes. Therefore, necessary precautions are imperative to applied pesticides in natural environment or use of pesticide in minimum quantity to protect the aquatic creatures. In conclusion, further investigations are required to select the optimum concentrations of pesticides in agriculture fields to protect the aquatic environments for the conservation purposes.

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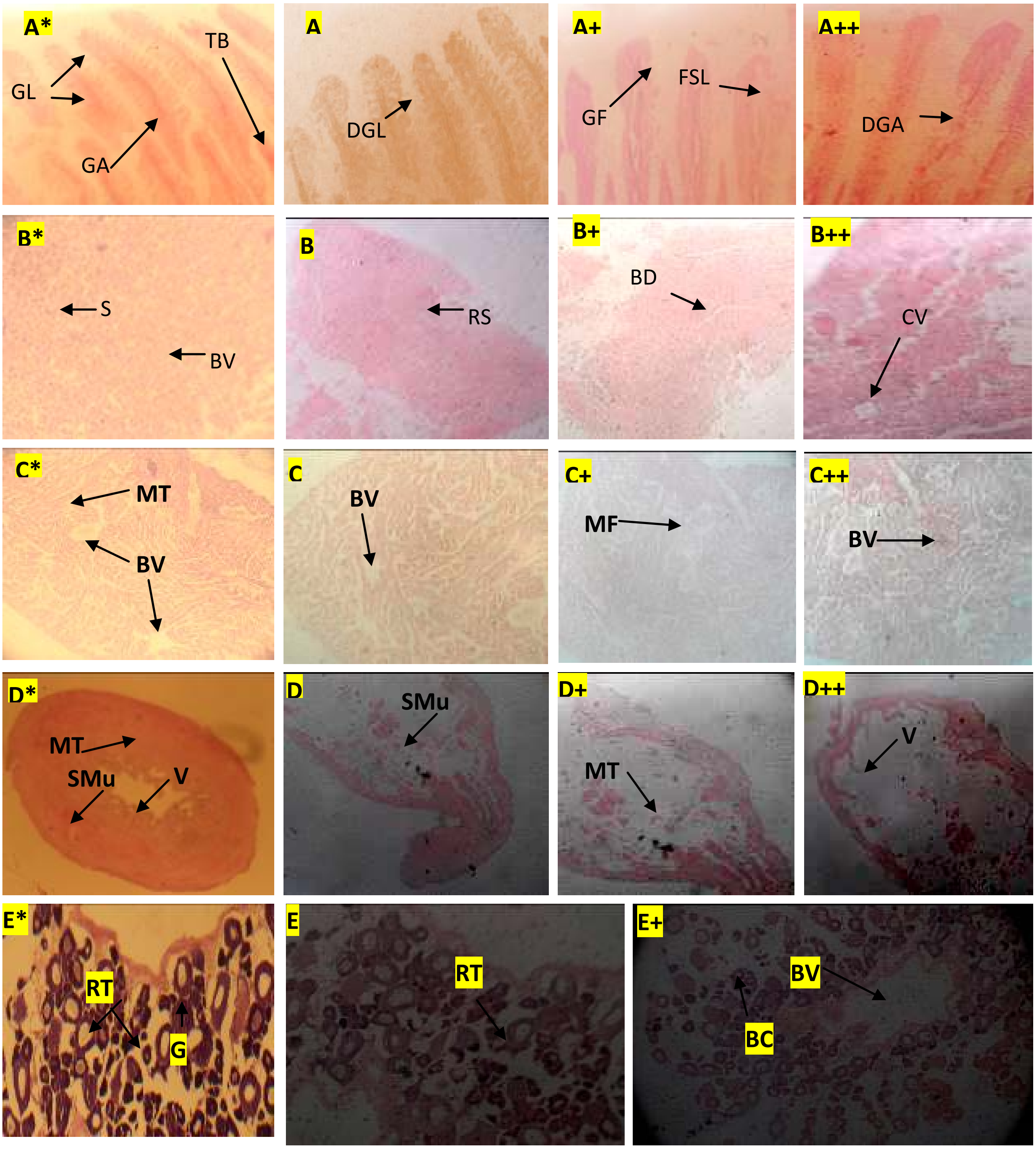

Fig 1: Histological photomicrograph of control and furadan affected gill, liver, heart, intestine and kidney tissues of *C. punctatus* (Top to bottom sequential way). * = control, A-E = 15 mg/l, + = 20 mg/l and ++ = 25 mg/l concentration. GL= Gill Lamellae, GA= Gill Arch, TB= Test buds, FSL= Fusion of Secondary Lamellae, DGL= Damage of Gill Lamellae, GF= Gill Filament, DGA= Damage of Gill Arch, S= sinusoids, BV= Blood vessels, RS= Rupture of sinusoids, BD= Bile Duct, CV= Central vessel, MT= Muscular tissue, MF= Muscle Fiber, SMu= Sub-mucosa, V= Villi, RT= Renal Tubule, G= Glomerulus, BC= Bowman's capsule.

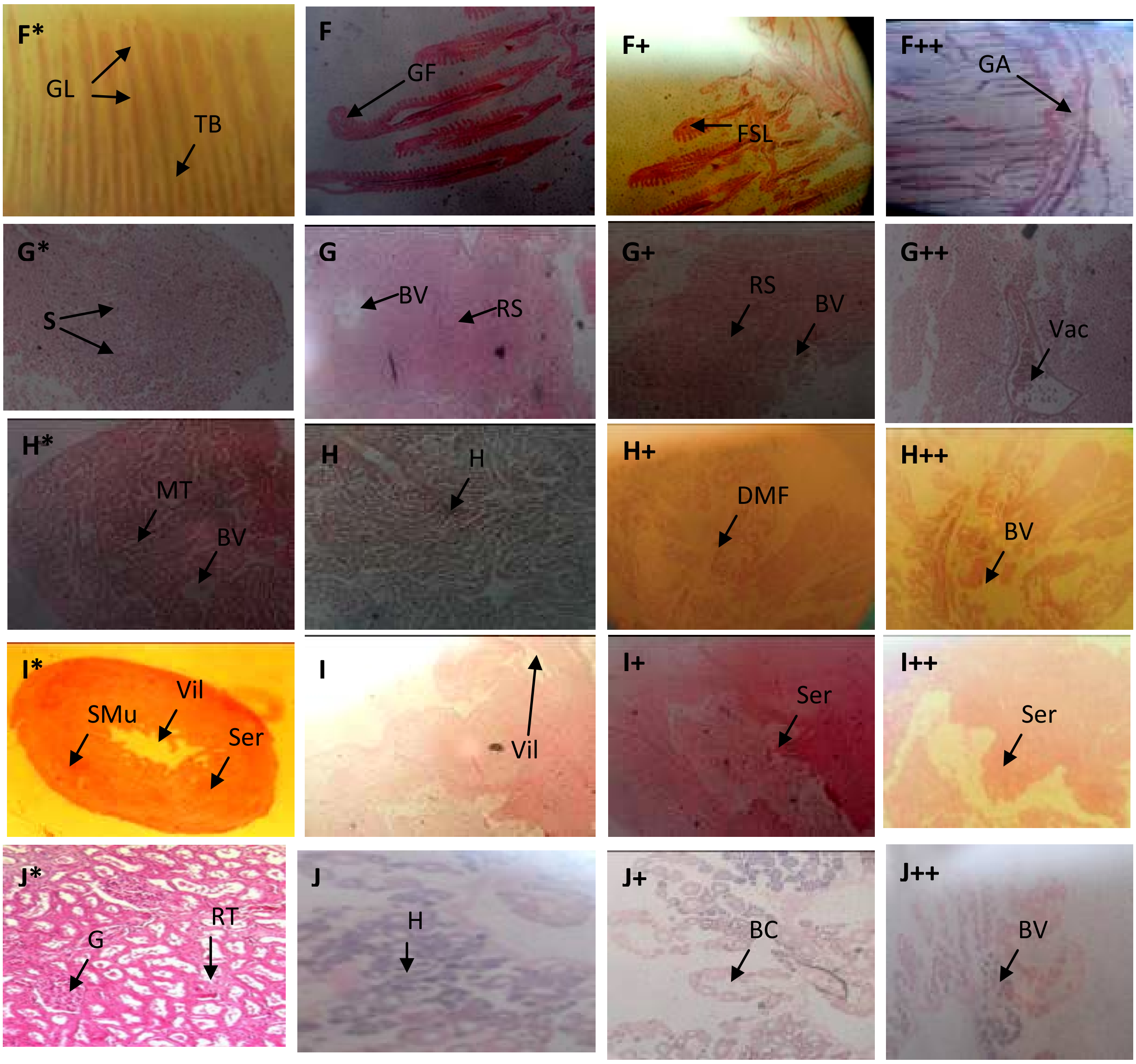

Fig 2: Histological photomicrograph of control and furadan affected gill, liver, heart, intestine and kidney tissues of *H. fossilis* (in order to top to bottom). * = control, F-J = 15 mg/l, + = 20 mg/l and ++ = 25 mg/l concentration. GL= Gill Lamellae, GA= Gill Arch, GF= Gill filament, FSL= Fusion of Secondary Lamellae, DGA= Damage of Gill Arch, S= sinusoids, RS= Rapture of Sinusoids, BV= Blood vessels, RS= Rupture of sinusoids, Vac= Vacuole, DMF= Destruction of Muscle Fiber, H= Hemorrhage, MT= Muscular tissue, SMu= Sub-mucosa, Ser= Serosa, Vil= Villi, RT= Renal Tubule, G= Glomerulus, BC= Bowman's capsule, BV= Blood vessels

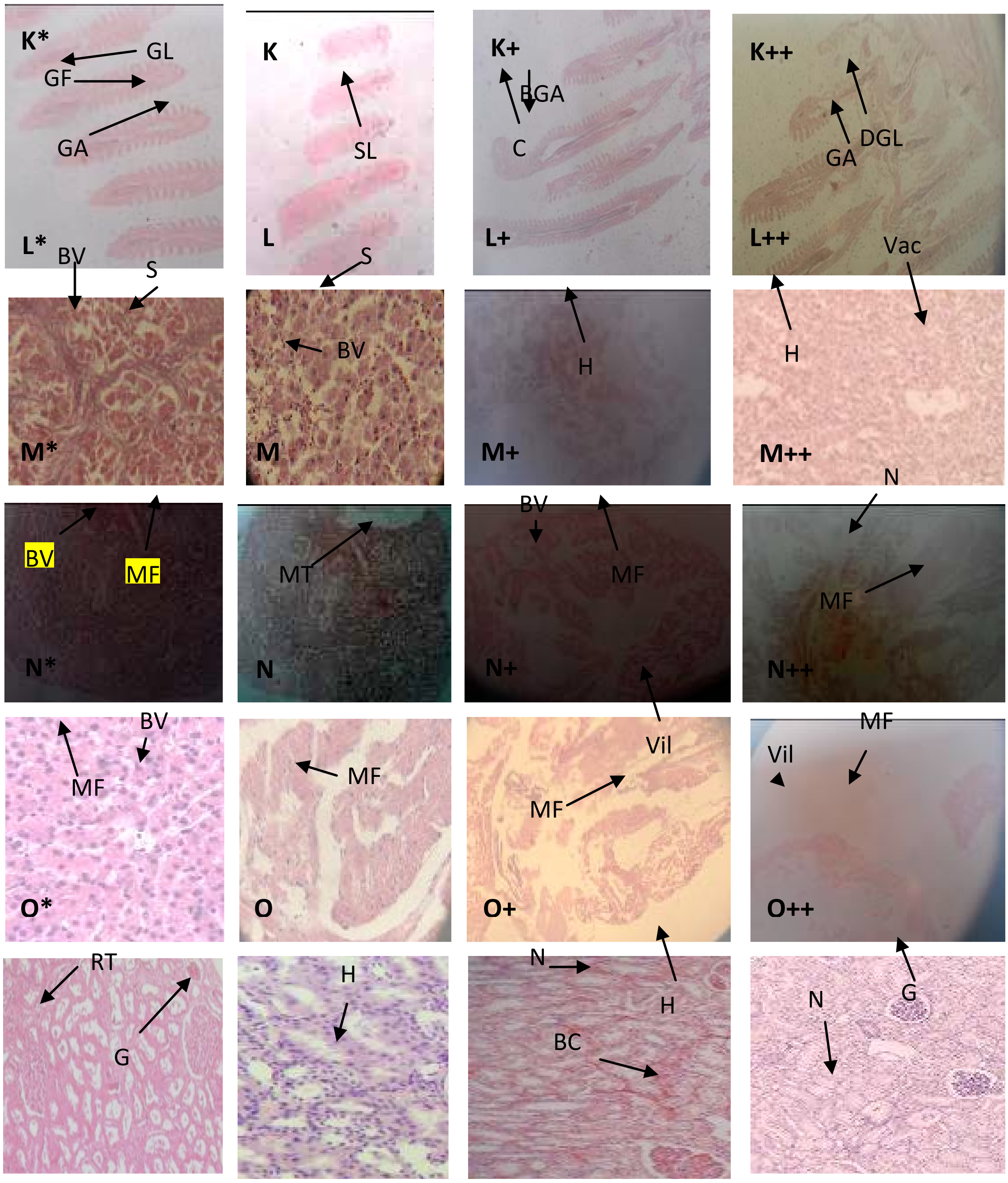

Fig 3: Histological photomicrograph of control and furadan affected gill, liver, heart, intestine and kidney tissues of *A. testudineus* (Top to bottom sequential way). * = control, K-O = 15 mg/l, + = 20 mg/l and ++ = 25 mg/l concentration. GL= Gill Lamellae, GF= Gill Filament, GA= Gill Arch, SL= Secondary Lamellae, BGA= Bend of Gill Arch, C= Curly, DGL= Destructed of Gill Lamellae, S= sinusoids, BV= Blood vessels, H= Hemorrhage, Vac= Vacuole, MT= Muscular tissue, MF= Muscle Fiber, N= Necrosis, Vil= Villi, RT= Renal Tubule, G= Glomerulus, BC= Bowman's capsule