

# MERCURY CHLORIDE (HgCl<sub>2</sub>) EXPOSURE CHANGES THE HISTOPATHOLOGICAL FIGURE OF EYE AND BRAIN OF TILAPIA FISH (*Oreochromis mossambicus*)

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## ABSTRACT

Mercury pollution brings harmful effects to aquatic animals, the environment and eventually to human health. Mercury accumulates in the liver, kidney, eye lens and brain of fish, resulting in organ damage. This study aimed to determine the effect of HgCl<sub>2</sub> exposure on anatomical pathology and histopathology of tilapia fish eye and brain. A total of 36 male tilapia fish were allotted into 4 treatment groups with 3 replications. Fish were exposed to 0.00, 0.25, 0.50, and 0.75 ppm of HgCl<sub>2</sub> for 10, 20, and 30 days. Subsequently, the anatomical pathology was observed followed by histopathological examination. Anatomical pathology examination of fish eye on day 30 showed white membrane on the eye lens surface, pupil diminution, and sunken eyes. The brain demonstrated hemorrhage, necrosis, discolorations, and granulated area. The retina showed necrosis, retina pigmentation flexiform layer widened, and cone cell atrophy. Brain depicted structural and cellular damage such as degeneration necrosis and vacuolation. HgCl<sub>2</sub> exposure changes the anatomical pathology and histopathology of tilapia fish eye and brain.

**Keywords:** cellular damage, fish brain, fish eye lens, histopathology, mercury chloride

## INTRODUCTION

Heavy metals polluted the waters of the sea and rivers. Among heavy metals pollutants is mercury (Hg). Mercury has volatile properties, takes the form of liquid at a certain temperature, capable of dissolving various metals and very toxic element to all living things (Gworek *et al.* 2020; Wang *et al.* 2017). Mercury in nature comes in two forms, namely inorganic mercury such as mercury chloride (HgCl<sub>2</sub>) and mercury oxide (HgO), and organic/organomercury consisting of methylmercury (CH<sub>3</sub>Hg<sup>+</sup>) and ethylmercury (C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup>). Both types are toxic, however the natural and anthropogenic emissions occur as inorganic form (Zulaikhah *et al.* 2020; Macirella *et al.* 2016).

Mercury chloride in sediments on the seabed and rivers can be converted by microorganisms into organic metal mercury (R-O-Hg) compounds, which remain soluble in water and

accumulate in sediments. High Hg concentrations can cause death in fish, whereas sublethal concentrations lead to disruption of organ function (Saturday 2018; Rauf *et al.* 2018). Mercury accumulated in the body of aquatic animals will damage or stimulate the enzymatic system, which resulting in the decreased adaptability of the animal concerned to the polluted environment (Mehouel *et al.* 2019; Lee & Wendy 2017).

Mercury enters the tissues of living organisms through several ways, namely the respiratory, digestive, and penetrating through the skin (Dogan & Canli 2018; Ismail & Mahboub 2016). Mercury that enters the body of aquatic organisms cannot be digested, however, it can be dissolved in fat. The fat-soluble metal is able to penetrate the cell membrane, so that eventually the mercury metal ions will accumulate in the cells and other organs. The highest accumulation is usually in the detoxification organs (liver) and excretory

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organs (kidneys) (Macirella *et al.* 2016; Jyotsna *et al.* 2014; Moustafa *et al.* 2016). In fish, the organs that accumulate the most heavy metal of mercury are the kidneys, liver, brain, and lens of the eye (Sobhanardakani *et al.* 2018; Gupta & Kumar 2006; Pereira *et al.* 2014; Kimakova *et al.* 2018).

Severe sublethal toxicity affects the morphology of tissue histology, physiological conditions such as metabolism, growth and development of fish morphology, biochemistry (blood chemistry and enzyme activity), behavior (neurophysiology) and fish breeding (Korbas *et al.* 2017; Nazaruddin *et al.* 2020). According to Morcillo *et al.* (2017), exposure to Hg can produce highly variable effects which depend on the magnitude and duration of exposure. This phenomenon was proven by Nazaruddin *et al.* (2020) which found the most severe histopathological condition on male gonad organs of tilapia fish exposed to the longest time period of HgCl<sub>2</sub>.

Korbas *et al.* (2017) stated that fish eye has the potential for Hg absorption since it has a wide surface of exposure and continuously contact with external media and thus, becomes an effective absorption route of Hg. High accumulation of mercury in the eye lens causes ocular anomalies such as cataracts or opacity, as previously reported in humans affected by Hg (Lemire *et al.* 2010). Pereira *et al.* (2014) also stated that eye is the often-neglected organ in accumulation of heavy metals mercury studies. A thoroughly histopathological study on changes of fish eyes exposed to Hg has never been reported.

In the brain, mercury will accumulate in the cerebrum cortex and cerebellum where it will be oxidized to a form of mercuric (Hg<sup>++</sup>). Mercury ions will bind to sulfhydryl from enzyme and cellular proteins that interfere with enzyme function and cell transport (Morcillo *et al.* 2017), leading to degeneration of nerve cells in cerebellum of the brain (Berntssen *et al.* 2003).

Although there is a considerable amount of information available on toxicity of mercury to fish tissues and cells, which is among the most sensitive biological responses due to mercury exposure. Furthermore, this study focusing on eye and brain which proved to be the most impacted organs physiologically affected by mercury pollution.

## MATERIALS AND METHODS

### Fish and Experimental Design

This study used 36 male tilapia (*Oreochromis mossambicus*) fish within similar sexual maturity stage and body weight of 250-300 g. Fish were obtained from farmer's ponds at Cadek Village, Banda Aceh, Indonesia. Fish were apparently healthy and free from skin lesions or external parasites. The fish were maintained in glass aquaria (80 x 60 x 40 cm capacity) having 100 L of dechlorinated tap water. Each aquarium was equipped with aerator. Fish were acclimatized for 7 days to the laboratory environment and fed three times daily.

This experimental study implemented a completely randomized design. Fish were allotted into 4 groups (n = 9) with 3 replications. Group 1 (K1) was negative control, while group 2, 3, and 4 (K2, K3 and K4) were treated with HgCl<sub>2</sub> 0.25, 0.5 and 0.75 ppm. The treatments were exposed to HgCl<sub>2</sub> for 10, 20, and 30 days.

### Histopathological Examination

The fish were dissected to collect the eye and brain then subsequently fixed with Davidson solution for 24 h. The fish tissues were then dehydrated in an ascending series of alcohol, cleared in xylene and embedded in paraffin wax. Section of 5 µm thick were cut, processed and stained with haematoxylin and eosin (H&E). The observations of the slides were done using Olympus light microscope and the histopathological changes of the tissues were documented using the photo microscope.

## RESULTS AND DISCUSSION

### The Anatomical Pathology of Tilapia Fish Eye

The observation of the anatomical pathology of tilapia fish showed that fish eye in the control group (K1) was normal indicated by clear, fresh, and intact eye ball and lens (Fig. 1). The observation did not show any changes of eye performance of the fish exposed to HgCl<sub>2</sub> on day 10 and 20, however on day 30 fish in Group K2 and K3 demonstrated soft and transparence white layer on the surface of eye ball. This layer became thicker and wider, which almost cover

all surface of the eye ball, thus, looks like cataracts. Furthermore, the diminution of the eye pupil and sunken eyes was also clearly observed (Fig. 1).

Normal fish eye is bright, intact, transparent, and no mucos layer on the eye ball surface. Fish eye, although modified a lot, is basically similar to other vertebrates, consisting of the cornea, corneal epithelium, lens, retina, choroid, optic nerve and iris (Takashima & Hibiya 1995). Fish eye anatomy is rather horizontal in the anterior part, with the intention that the convex lens almost touches the cornea which is an important transparent part of the eye ball scleroid coat. The lens has a layer that resembles a layer of onions, the layer on the lens is transparent, non-nucleated and inelastic. Not like the lenses in mammals which are elastic and can be modified by the contraction of the eye muscles, the lenses in fish are not elastic, thus they must be pulled inward toward the retina with the retractor lentic muscles to accommodate changes in vision (Azab *et al.* 2017).

Anatomical pathology changes occurred in the form of white membranes in the eye ball surface might be caused by physical and systemic

factors. Physical factors occur due to direct contact between HgCl<sub>2</sub> and the surface of the eye mucosa, while systemic factors occur through inhalation or ingestion of toxic compounds which are then carried by blood circulation and reaches the eye organ resulted in nerves and balance disorders such as blindness and swim abnormally. This happens since the eyes are connected to the optic lobe of the brain through the optic nerve (Rajeshkumar *et al.* 2017). Furthermore, Korbas *et al.* (2013) stated that fish eyes have wide surface which continuously contact with external media leading to an effective absorption route of Hg.

In addition, fish eyes appeared sunken, indicated high accumulation of HgCl<sub>2</sub> in the eye tissue which lead to contraction and sunken of the eye ball. According to Pereira *et al.* (2013), sunken eyes are caused by physiological reaction and fish resistance to exposure of pollutants, bacterial infections or viruses which changes the morphology of fish eyes such as sunken and cloudy. In this study cataracts were also found due to HgCl<sub>2</sub> exposure.

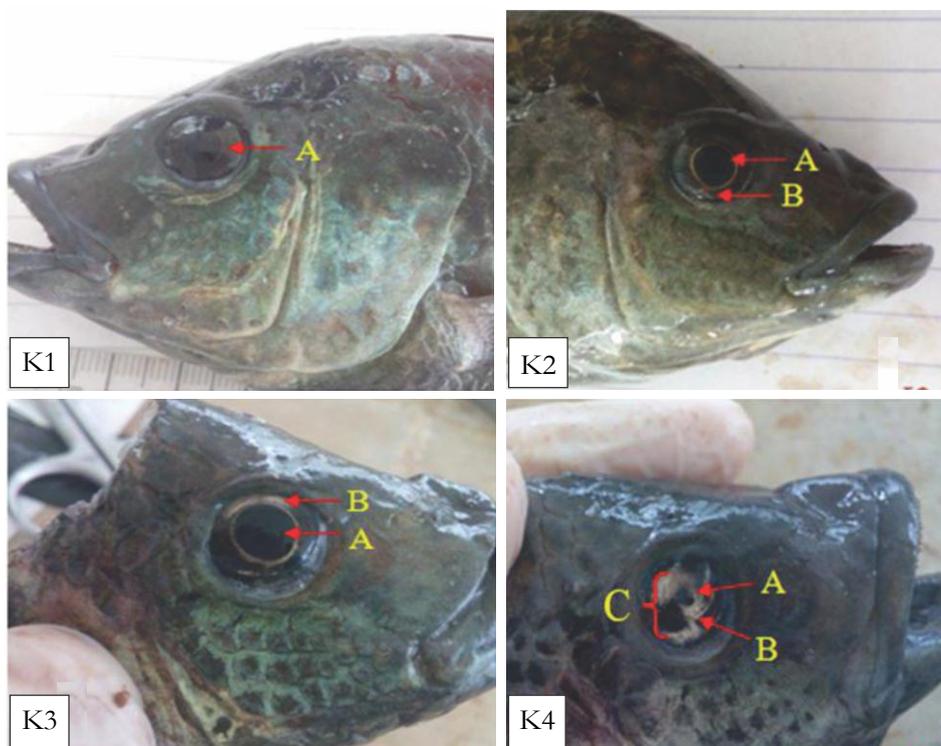


Figure 1 Anatomical pathology of tilapia fish exposed to HgCl<sub>2</sub> on day 30  
 Notes: K1 = control group; K2 = 0.25 ppm HgCl<sub>2</sub>; K3 = 0.50 ppm HgCl<sub>2</sub>; K4 = 0.75 ppm HgCl<sub>2</sub>; A = Eye pupil; B = White layer on eye ball surface; C = Sunken eye.

### Histopatology of Tilapia Fish Eye Lens and Retina

Histopathology of tilapia eye lens in control group (K1) showed lens layer was transparent and pink in color. Similar results were observed on tilapia fish lens in group K2 on observation days 10 and 20. In contrast, on day 30, K2 showed a change in the form of hemorrhage in the iris but the lens was still intact. K3 treatment showed a severe necrosis area in the lens of the eye as well as in the K4 treatment. Moreover, in K4 groups a change in the color of the lens that was pink turned into purple in the middle of the lens and became granulated were observed (Fig. 2).

Normal tilapia retina were shown in the control group (K1). The structure of the tissue showed very clear layers and there were no changes of the structure, color and size. The fundamental layers of the retina were still complete and remain unchanged. The structure of the retina layers from inner to outer layer consists of the optic nerve layer, the ganglion layer, the inner flexiform layer, the inner nucleus layer, the outer flexiform layer, the outer nucleus layer, cone cells and stem cells, the retinal pigment cell layer, and choroid (Genten *et al.* 2009) (Fig. 3).

The histopathological examination of tilapia fish eye retina exposed by HgCl<sub>2</sub> revealed necrosis in inner and outer layer of nucleus in K2 and K3 on day 10 observation, while K4

showed cell necrosis in outer layer of nucleus, cone cell and rod cell atrophy which resulted in unarranged of both cell structure and widened inner flexiform layer. Moreover, on day 20, fish eye retina in K2 showed cell necrosis in the inner and outer layers of nucleus, while in K3 cell necrosis of inner nucleus layer and cone cell necrosis leading to widened rod cell was revealed. Fish in K4 group showed cell necrosis in inner and outer nucleus layer and cone cell atrophy, furthermore, retina pigment cell started to widened (Fig. 3).

Severe damages of the eye retina tissue were observed on day 30. K2 demonstrated inner nucleus cell necrosis, cone cell and rod cell atrophy, and widened retina pigment. Fish in K3 groups showed cell necrosis in almost all layers, thus the borders between layers disappeared. Severe condition was shown in K4 group, eye retina was damaged which resulted in total changes of all layers. All cells were necrosis causing the borderless condition between the layers and became one-piece tissue. In addition, hyperplasia of the cells also found in K2, K3, and K4.

The highest HgCl<sub>2</sub> concentration and the longest exposure time caused cells degeneration and lost of their function which end with cell death. This observed in day 20 and 30 observation, which cell damages were clearly seen in each component of retina layers.

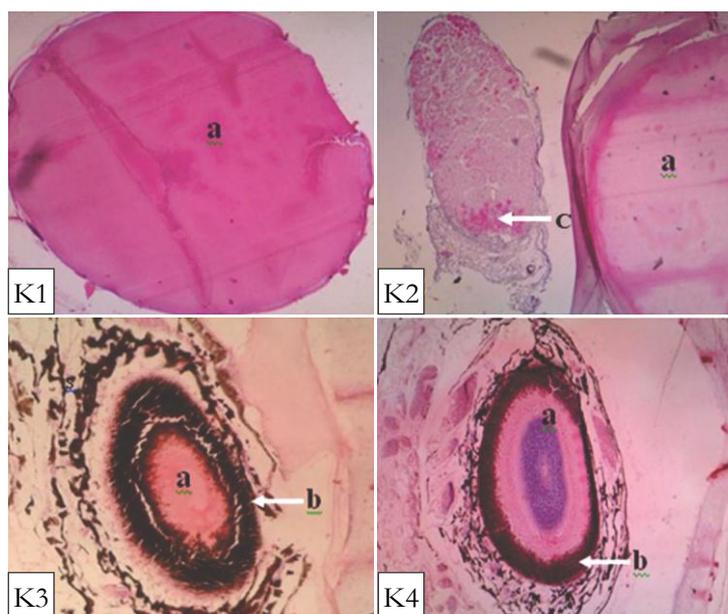


Figure 2 Histopathology of tilapia fish eye lens exposed to HgCl<sub>2</sub> on day 30

Notes: K1 = control group; K2 = 0.25 ppm HgCl<sub>2</sub>; K3 = 0.50 ppm HgCl<sub>2</sub>; K4 = 0.75 ppm HgCl<sub>2</sub>; a = eye lens; b = necrotic area; c = haemorrhage. (HE 40X).

Histologically, eye lens is composed of collagen produced by the epithelium cells of the eyepiece, forming a layer that resembles a layer of onions, transparent, thin, has no core, and not elastic (Mumford *et al.* 2007). Results of this study indicated that exposure to HgCl<sub>2</sub> for 30 days has the potential to cause damage to eye tissue and high concentration of HgCl<sub>2</sub> also show obvious histopathological damage. This is in accordance with the statement of Mela *et al.* (2013) that the longer exposure and the higher concentration of Hg can produce severe and varied forms of effects. For example, purple discoloration of fish eye lens was caused by a chronic HgCl<sub>2</sub> exposure since the discoloration was only observed on the 30<sup>th</sup> day of observation.

Other lesion found was hemorrhage that occurred in the iris of fish eye due to the accumulation of HgCl<sub>2</sub> in the blood leading to blood vessels to rupture. When hemorrhaging occurs, the intake of nutrients and other substances to the eye will stop, thus the cells will experience the lack of nutrients which eventually cause cell necrosis followed by lost in its function (Pereira *et al.* 2013). In this study most necrosis was found in the retina layer which was marked by the loss of cell parts and appeared empty (vacuole). This is in accordance with Takashima & Hibiya (1995) that necrosis describes a condition where a decrease in tissue activity is characterized by the loss of several parts of the cell, one by one from one tissue, so that in a short time the cell will be dead. Jeevanaraj *et al.* (2016) also reported that there was necrosis in retinal tissue due to chronic mercury exposure in *H. malabaricus traira* fish.

The observations also showed hyperplasia of retina cells as a response of cell adaptation to the stimulation of toxic compounds. It was found that cellular proliferation caused the retina layer to undergo hyperplasia and caused the boundary layer components to be irregular. This can affect the function of the retina which is very sensitive to light (Azab *et al.* 2017). Similar mechanism of proliferation occurred to the brain cerebellum tissue causing the lost of layers boundaries of the tissue.

Atrophy occurred in the cone and rod cells of fish eye in group K2, K3, and K4 on day 10, 20 and 30. Atrophy is suspected to be caused by inhibited vascularization activity caused

blood intake to the eye is inadequate. Cone cells and rod cells are receptor cells in the retina that are very sensitive to light. Fish tend to use their vision to adapt to light when looking for food, because according to Hunt *et al.* (2015) cone cells have the ability in terms of sensitivity to light and sharpness of vision. The damage of the cone cells or rod cells will affect the behavior of fish related to the sharpness of vision, axis of vision, and maximum visibility in fish (Pereira *et al.* 2014).

### Histopathology of the Brain

Microscopic examination of the brain cerebellum of tilapia fish in control group demonstrated that the fish brain cerebellum consists of two parts namely cortex (gray matter) dan medulla (white matter). Cortex consists of three layers, the outer layer called molecular layer, middle layer (ganglioner), and inner layer called granular layer (*periventriculare*). Every layer has specific cells. Satellite cell, neuroglia cell and basket cell were found in molecular layer, while Purkinje cell was found in ganglioner layer, and granuler cells and golgy cells were found in granuler layer.

Cerebellum tissue of tilapia fish in group K1, K2, and K3 showed relatively similar figure between 10, 20, and 30 days of treatment that the molecular layer was widened, while the ganglioner layer was thinner compared to control group (Fig. 4). The highest the mercury concentration was exposed the widest the molecular layer observed. The widening of molecular and granular layers of brain cerebellum was caused by cell proliferation of one layer to the others. In contrast, ganglioner layer was thinner along with the higher dosage of HgCl<sub>2</sub> exposure. Granular layer showed different patterns compared to the two previous layers. In the low dose of HgCl<sub>2</sub> exposure the granular layer was thinner than normal tissue, however, in higher dose of exposure the granular layer became thicker. The ganglioner and granulare layer disappeared after being exposed to HgCl<sub>2</sub> for 30 days. Other than structural damage of cerebellum tissue caused by cell proliferation, cellular changes were also observed such as cloudy swelling degeneration, cell swelling, necrosis and vacuolation.

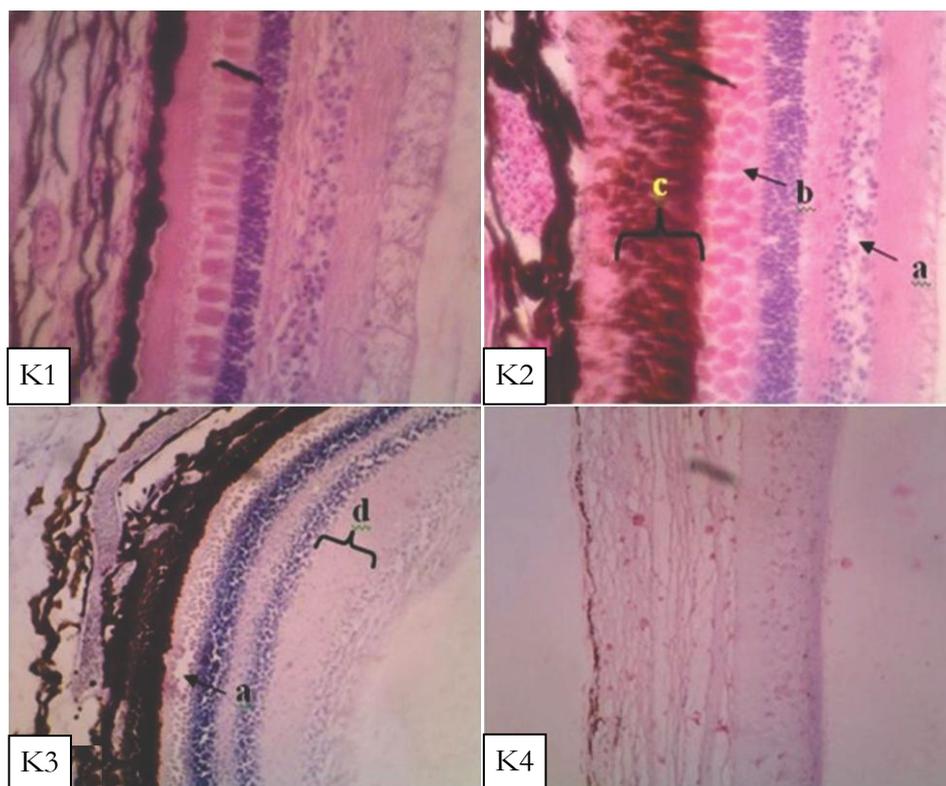


Figure 3 Histopathology of tilapia eye retina exposed to  $\text{HgCl}_2$  on day 30.  
Notes: a = Cell necrosis; b = Cone cell atrophy; c = Widened of eye retina pigment; d = Widened of inner flexiform layer. (HE 400X).

The tilapia fish brain cerebellum exposed to  $\text{HgCl}_2$  also showed the presence of vacuolation in all treatment groups. Similar results were reported by Berntssen *et al.* (2003) in Atlantic salmon (*Salmo salar*) fish exposed to chronic dietary mercury. Vacuolysis has characteristics such as a round, hollow hole that occurs because of the accumulation of fat. The contributing factors of vacuolations are the accumulation of toxic substances, oxygen damage and excess fat consumption (Chamarthi *et al.* 2013). Vacuolysis will lead to disruption of cell metabolism process which eventually resulted in cell lysis (Lakshmaiah 2017). These vacuoles are seen more in brain samples with the highest dose of mercury exposure. This is consistent with the statement of Rajeshkumar *et al.* (2017), that the degree of pathological damage is highly

dependent on the dose and duration of exposure.

Under conditions of higher mercury exposure the vacuole turned into expanding empty spaces. Similar results are reported by Lakshmaiah (2017), in catfish exposed by various concentrations of glyphosate herbicides which is neurotoxic, characterized by severe degeneration, edema and vacuole changes into large empty spaces. The vacuolation that occurred in this study might share similar effect as the herbicide since mercury is also neurotoxic. Cell swelling or vacuole degeneration is reversible. Thus, cell returns to normal when the exposure to toxic substances is terminated. Bose *et al.* (2013) reported that increasing the dose of heavy metal exposure causes vacuolation in the molecular layer of cerebellum.

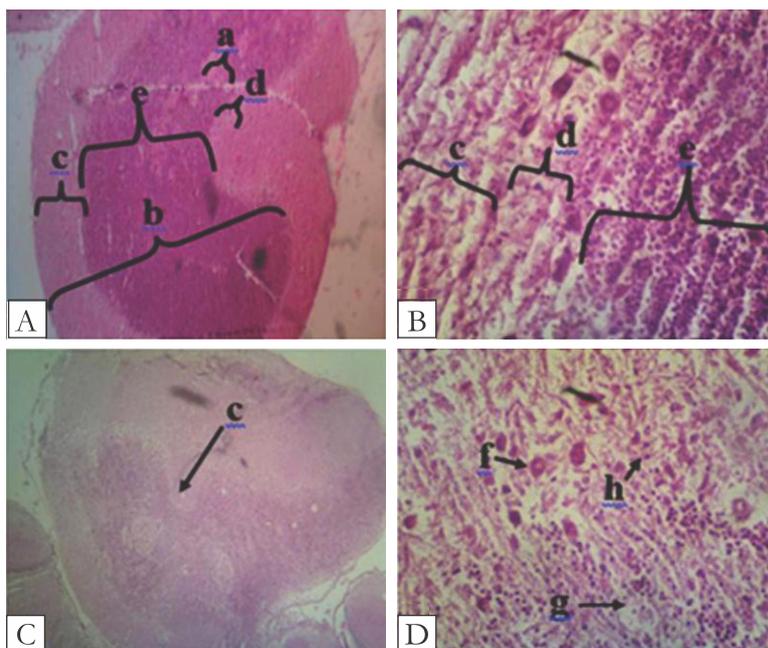


Figure 4 Histopathological feature of tilapia fish brain cerebellum  
 Notes: A-B = Control group; C-D = K3 group; a = medulla; b = cortex;  
 c = molecular layer; d = ganglioner layer; e = granulare layer;  
 f = proliferative Purkinje cell; g = proliferative neuroglia cell;  
 h = proliferative granulare cell. (HE 40x, 400x).

Cell changes mainly occurred in neuroglia cells in the molecular layer, Purkinje cells in the ganglioner layer and granulare cells in the granular layer. It is clearly seen that necrosis were observed on Purkinje cells and granulare cells. The cell swelling was shown by Purkinje cells as well as neuroglia cells. Swelling of Purkinje cells was reported by Chamarthi *et al.* (2014) in the cerebellum of the teleost catfish induced by benzene petrochemical. The neuroglial cell swelling was reported by Erhunmwunse *et al.* (2014) in tilapia exposed to glyphosate herbicide.

### CONCLUSION

HgCl<sub>2</sub> exposure changes anatomical pathology and histopathological of tilapia fish eye and brain. The higher the HgCl<sub>2</sub> concentration and the longest time of exposure, the most severe the changes of tilapia fish eye and brain tissues.

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