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Studies on the toxicity of 2-Methyltetrahydrofuran on the histopathology of gills of African catfish *Clarias gariepinus*

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Abstract
In the present study, investigation were carried out on gills of African cat fish *Clarias gariepinus* exposed to sub lethal concentrations (80mg/ml,400mg/ml and 800mg/ml) of 2 -Methyltetrahydrofuran for 10 days. Lesions were observed in gills tissue of treated fish for long term exposure to Methyltetrahydrofuran (2MTHF). The occurrence and degree of alteration were positively related with the concentration of 2MTHF. Histological examination of the gills of fish treated with 80 mg/ml of 2MTHF for 10 days showed architectural loss, necrosis and desquamation of epithelial layer. Histological examination of the gills of fish treated with 400 mg/ml of 2MTHF for 10 days showed architectural loss, necrosis and mild vaccuolation. The gill filament exhibited telangiectesis, disorganisation of secondary gill lamellae and complete vaccuolation of gills treated with 800 mg/ml of 2MTHF for 10 days. The study indicated that 2MTHF had marked effects on the cyto-architecture of the gills of *C. gariepinus.* The degree of vaccuolation and necrosis were positively related with the concentration of 2MTHF.

Keywords: Gill lamellae, 2-Methyltetrahydrofuran (2MTHF), Nacrosis, Telangiectesis, Vaccuolation

INTRODUCTION

At present a large number of pollutants and waste are eliminated to the environment because of human activities. A wide range of man-made chemicals used for several industrial and household activities have been shown to disturb normal physiology and endocrinology of aquatic organisms (Balabanic *et al*., 2011, Rhind., 2009). In the present study, investigation were carried out on gills of African cat fish (*Clarias gariepinus*) exposed to 2-MTHF. 2-MTHF is mainly used as a higher boiling substitute for tetrahydrofuran and used in secondary lithium electrodes, as a component in alternative fuels. This compound is widely used as a reaction medium for Grignard and metal hydride reactions, in the fabrication of articles for packaging, transporting, and storing of foods, as a solvent for dyes and lacquers and as a chemical intermediate in polymerization solvent for fat oils, unvulcanized rubber, resins, and plastics. It is also an indirect food additive when it is in contact with the surface of articles intended for use in food processing. It is discharge form power plants, plastic industries and electrolyte industries (Aycock *et al.,2007, (Man et al., 2003)*.

Its high toxicity, lower sensitivity to photo-oxidation, high persistence in water and low molecular weight, 2-MTHF accumulates rapidly in aquatic animals and affects normal vital functions. It is generally found in greater concentrations in fish tissues which are direct and continuous contact such as gills. 2-MTHF in fish organs are not directly responsible for the death of the organism, but sublethal concentrations may affect its functionality and normal physiology by damaging biological structures (Liqun *et al*.,2014). Tetrahydrofuran has been reported as carcinogenic effects in rat and mice (Chhabra *et al.,* 1998). Its mutagenic effects have been reported in Chinese hamster ovary cells and in mouse bone marrow cells

(NTP., 1998). Histological changes associated with different derivatives of tetrahydrofuran and tetrahydrofuran like chemicals in fishes have been studied by many authors (Leino *et al.,*1987. Roy *et al*.,1991. Tilak *et al.,* 2001). There are no articles available on gill histology of fish exposed to 2 -MTHF. Moreover, studies on the histopathology of different fish organs exposed to other contaminants are often carried out with freshwater or brackish-water species (El-Sayed *et al*. 1995) (Dwivedi *et al.* 1997).

The objective of the present study was to describe the effects of 2-Methyltetrahydrofuran on the gills of African catfish *Clarias gariepinus,* assessing alterations in the gills function in relation to concentration. To our knowledge, this is the first investigation on alterations of gill morphology in African catfish *Clarias gariepinus* exposed to sublethal concentrations of 2-MTHF**.**

MATERIALS AND METHODS

The fresh water African catfish, *C. gariepinus*, was selected for the present study because of its availability from local tanks or market and or its convenient size. All the fishes used in the present study were brought from the local market. The body weight of fish ranged between 250-350 gm and their length varied between 30-37cm. The fish were mainained in glass aquarium containing 50 liter of tap water, under normal conditions of light and temperature.The fishes were acclimatised for one weak by keeping 6 fishes in each aquarium prior to their use in the experiment.

In the present study, the chemical 2-MTHF (Product code: 99983) was purchased from SRL laboratories Pvt. Ltd (CAS No. : 96-47-9) (where 0.855gm/ml. The four aquaria were taken filled with 50 liter tap water, and 6 fishes were kept in each aquarium.The fishes in the one aquarium were treated as control and other three aquarium exposed with three different concentrations group (80mg/ml,400mg/ml and 800mg/ml respectively).The control and treated fishes were dissected out for histopathological examination. For microscopic examination, surviving fishes of each group were removed and dissected. Small pieces of the gills tissue were taken and immediately fixed in Bouins fixative (Saturated aqueous picric acid : 75 ml, 40% aqueous formaldehyde: 25 ml, glacial acetic acid: 5 ml) . Fixed tissues were processed routinely for paraffin embedding technique. Embedded tissues were sectioned at 5µ in thickness and then stained with Eharlich hematoxylin (Haematoxylin crystals: 2gm, Absoute alcohol:100 ml, glycerine:100 ml, Glacial acetic acid:10ml, Distilled water:100 ml, Alum in excess) and Eosin stain (Eosin powder: 1gm, 70% alcohol: 100 ml.) and mounted in DPX. The slides were then observed Light microscope (Trinocular Compound Microscope SF40T).

RESULTS AND DISCUSSION

The gill is made up of filaments of primary lamellae arranged in double rows. Secondary lamellae arise from these filaments. The secondary lamellae are lined by a squamous epithelium. Squamous epithelium is composed of pavement cells and non-differentiated cells. Below that epithelium are lamellar blood sinuses separated by pillar cells. Between the lamellae, the filament is lined by a thick stratified epithelium constituted by several cellular types, such as chloride, mucus and pavement cells (Jayachandran and Pugazhendy., 2009).

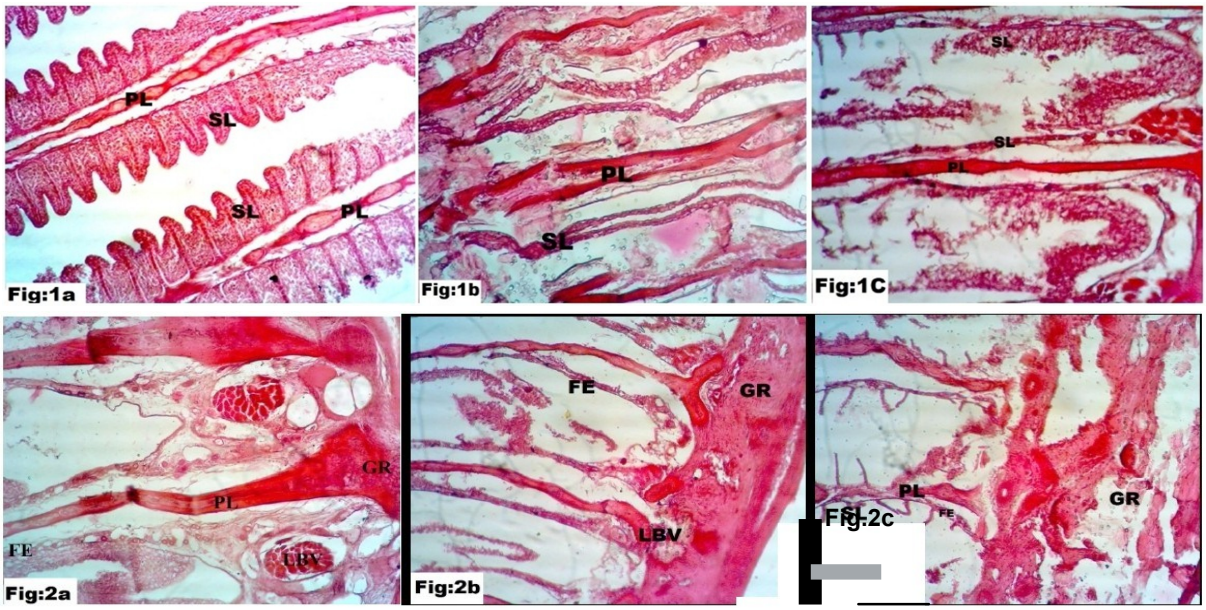
In the present investigation, no histopathological abnormalities were observed in the gills of the control fish (Fig. 1a, 2a and 3a). Lesions were observed in the gills tissue of *Clarias gariepinus* exposed to 2-MTHF. The occurrence and degree of alterations were positively related with the concentration of 2-MTHF. The histological changes observed in the 2-MTHF exposed and control fishes are shown in Table 1.

Histological examination of the gills of fish exposed to 80mg/ml 2-MTHF for 10 days showed mild architectural loss, necrosis and desquamation of epithelial (Fig:1b). Histological examination of the gills of fish exposed to 400mg/ml 2-methyltetrahydrofuran for 10 days showed moderate architectural loss, necrosis, desquamation of epithelial, collapsed and curling of secondary lamellae, vaccuolation and mild telangiectesis (Fig. 1c, 2b, 3b and 4b, 4c). Histological examination of the gills of fish exposed to 800mg/ml 2-methyltetrahydrofuran for 10 days showed severe architectural loss, necrosis, desquamation of epithelial, collapsed and curling of secondary lamellae, vaccuolation and telangiectesis (Fig. 1c, 2c and 3c).

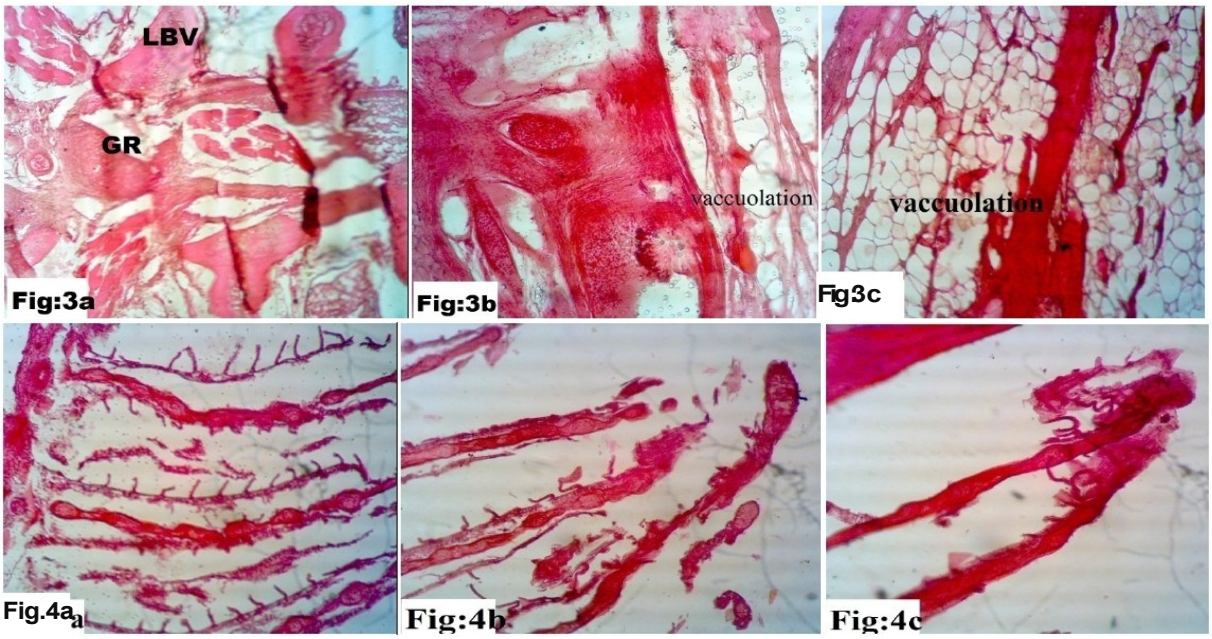
Histopathological results indicated that gill was the primary target tissue affected by 2MTHF. Gills are generally considered good indicator of water quality being models for studies of environmental impact. Several other studies have shown similar

Table 1. Histological changes observed in the 2-MTHF exposed and control fishes.

Histological changes	Control	80 mg/ml	400 mg/ml	800mg/ml
Architectural loss and necrosis		+	++	+++
Desquamation of epithelial		+	++	++++
Collapsed and curling of secondary lamellae		-	+++	++
Vaccuolation			++	++++
Telangiectesis			+++	+



Primary lamellae= PL; Secondary lamellae=SL;Lamellar blood vessels=LBV; Filament Epithelial=FE: Gill Raker=GR; **Fig. 1a.** *L.S. of gill filaments of Clarias gariepinus from the control group showing intact Primary lamellae and Secondary lamellae;* **Fig.2a.** *L.S. of Gill arch of Clarias gariepinus from the control group showing intact Gill raker;* **Fig.1b.** *L.S. of gill filaments of Clarias gariepinus from the exposed group (80mg/ml) showing mild architectural loss, necrosis and desquamation of eqithelial layer*; **Fig1c. and 2b.** *L.S. of gill filaments of Clarias gariepinus from the exposed group (400mg/ml) showing moderate architechtural loss, necrosis and desquamation of eqithelial layer and collapsed Secondary lamellae;* **Fig. 2c.** *L.S. of gill filaments of Clarias gariepinus from the exposed group (800mg/ml) showing severe architechtural loss, necrosis, desquamation of eqithelial layer, collapsed and curling of Secondary lamellae.*



Primary lamellae= PL; Secondary lamellae=SL;Lamellar blood vessels=LBV; Filament Epithelial=FE: Gill Raker=GR; **Fig. 3a.** *T.S. of gill of Clarias gariepinus from the control group showing Primary lamellae, Secondary lamellae, Chondrocytes, Lamellaer epith elialeell, Lamellar blood vessels, Gill raker;* **Fig.3b.** *T.S. of gill filaments of Clarias gariepinus from the exposed group (400mg/ml) showing moderate Vaccuolation*; **Fig.3c.** *T.S. of gill filaments of Clarias gariepinus from the exposed group (800mg/ml) showing severe Taccuolation*; **Fig.4a and 4b.** *T.S. of gill filaments of Clarias gariepinus from the exposed group (400mg/ml) showing collapsed and curling of secondary lamellae*; **Fig. 4c.** *T.S. of gill filaments of Clarias gariepinus from the exposed group (400mg/ml) showing telangiectesis.*

effects in the gill architecture of fish have been exposure to Mercury (Gupta and Rajbanshi., 1995, Singh and Datta., 1996), zinc (Sharma and Sharma.,1991), pesticides (Tilk *et al.,*2001) Vijayalakshmi, and Tilak.,1996) as well as effluents from tannery (Sakthive.,1994) and textile mills (Dhanapakiam *et al.,* 2004). Mucus extrusion, lamellar swelling, fused and reduced microridges, were observed in bluegill sunfish, *Lepomis macrochirus* to different sublethal concentrations of diazinon (Dutta *et al.,* 1997). Histopathological changes observed were hemorrhage in the primary and secondary gill lamellae, degeneration and necrosis of epithelial cells, distortion of the secondary lamellae, disruption of epithelial cells from pillar cells. Shorter gill lamellae, fusion, complete destruction of lamella, increased vacuolation, irregular appearance of gill lamellae were observed in guppy *Poecilia reticulate* exposed to chlorpyrifos (De Silva and Samayawardhena, 2002). All the histopathological observation indicated that exposure to sublethal concentrations of 2MTHF caused destructive effect in the gill tissues of *C. gariepinus*. Gill histopathological alterations, observed in this study and findings from previous studies, could result in severe physiological problems, ultimately leading to the death of fish.

Conclusion

As a conclusion, the findings of the present histological investigations demonstrated a direct correlation between 2MTHF exposure and histopathological disorders observed in gills. After chronic exposure of 2MTHF to *C.gariepinus* at a concentration of 400mg/ml and 800mg/ml the fish exhibited the greatest amount of tissue damage architectural loss, necrosis, desquamation of epithelial layer, dilation of marginal vascular channels, and disorganization of secondary gill lamellae, shortening of secondary lamellae and telangiectesis. To our knowledge, this is the first investigation on alterations of gills morphology in African catfish *Clarias gariepinus* exposed to sublethal concentrations of 2MTH.

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