

Biochemical studies on the toxic effects of Metanil Yellow on Teleostean catfish *Heteropneustes fossilis* (Bloch)

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Abstract

The experiment was conducted on fresh water teleost fish *Heteropneustes fossilis* (Bloch). The chronic toxicity for an exposure of 30 and 45 days with a sub-lethal dose of 2.0 g/l was studied on *H. fossilis*. Different biochemical investigations like protein content, amylase, protease and lipase activity were carried out on different tissues like oesophagus, stomach, intestine (anterior, middle and posterior), liver, pancreas and muscle of control and Metanil Yellow treated *H. fossilis*. The present study showed the significant changes in protein content and also in amylase, protease and lipase activity of *H. fossilis* due to the toxicosis of Metanil Yellow.

Keywords: Biochemical Study, Metanil Yellow, *Heteropneustes fossilis*

Introduction

There are different types of colour additives used in foods for enhancing the flavor, colour and texture of foods. But nowadays different synthetic colour additives are used in food items because they are not so expensive. Metanil Yellow, a non-permitted synthetic azo dye related monosodium salt is used in food items, particularly different kinds of sweets, soft drinks, biriyani and snacks *etc* (FD & C ACT, passed in 1958). The effect of oral and parental administration of Metanil Yellow on hepatic and intestinal biochemical parameters were investigated (Ramchandani *et al.*, 1997). Protein and lipids are pivotal in diet composition for the most carnivorous fish (Chou *et al.*, 2001). High protease activities

in carnivorous fish and high carbohydrase activities in omnivorous and herbivorous fish has been reported (Ugolev and Kuz'mina, 1994). Proteins are important biomolecules involved in a wide spectrum of cellular functions. Palanichamy *et al.*, (1989) reported that protein content of tissue such as muscle, liver, gill and intestine of fertilizer treated fresh teleostean fish *Oreochromis mossambicus* was decreased. Dabrowski *et al.*, (1992) reported ten-times higher amylase activity in the charr *Salvelinus alpinus* as the feeding was high protein content and controversially, Das and Tripathi (1991) observed higher amylase activity in *Ctenopharyngodon idella* fed on low protein levels. Sabapathy and Teo (1993) reported that lower amylase activity

in *Lates calcarifer* due to carnivorous in nature. Alkaline protease activity was previously reported in the gastric juice of other species and highest protease activities in omnivorous and herbivorous one had been reported (Kuz'mina, 1991; Ugolev and Kuz'mina, 1994). Malachite Green found to cause significant alterations in biochemical parameters in the blood of *Heteropneustes fossilis* and it caused depletion of serum calcium and protein levels and also increased the total cholesterol level of blood in catfish (Srivastava *et al.*, 1995b). On the otherhand, Malachite Green impaired protein synthesis in certain fish tissues (Svobodova *et al.*, 1997). Orange II caused no change in the serum and tissue cholesterol content in rats (Singh *et al.*, 1987). The background on the enzyme specific activities is fundamental in animal comparative biochemistry. The total hydrolytic capacity of the digestive tract is a clue to evaluate the animal potential for the best uses of the feeding. However, this demands enzyme analysis of all gut sections as the best way to assess the whole digestive capacity (Buddington *et al.*, 1997). The level of carbohydrate and protein in the muscle, liver and kidney of *Labeo rohita* considerably decreased when the fish exposed individually to sublethal concentration of DDT and BHC, the organochloride (OCL), dichlorovos, monocrotophos and organophosphorous compounds (Rajamannar and Manohar, 1998). The impact of pesticides on protein content of fish had been discussed by many workers (Jawale, 1985; Malla Reddy and Bashamohideen, 1988; Susan *et al.*, 1999; Jha and Verma, 2002; Ramani *et al.*, 2002; Prashanth, 2007). In stomach, the gastric mucosa and gastric glands are the main sites of enzyme secretion. In the intestine, the mucous epithelial cells, blood vessels and lymph spaces show strong activity. The enzyme is distributed in higher concentration in the proximal two third portions. The liver and pancreas are lipase positive. The

pancreas exhibits more intense activity than the liver. A significant increase in serum total lipid may be correlated with reduced lipoprotein lipase activity as reported by Agarwal and Sharma (1999) due to SO₂. Protein contents of muscle, liver, gill and intestine decreased with increasing concentrations of dye effluent (Amutha *et al.*, 2002). Shukla *et al.*, (2007) also observed decrease in total protein, lipid level and inorganic constituents of muscle of Cd treated *Channa punctatus*. The protein content in the gill of *Clarius batrachus* showed significant change when exposed to sublethal concentration of malathion (Khare and Singh, 2002). It was observed that cadmium stress induced protein synthesis and total protein level in liver and gill of fish increased after exposure with cadmium (Basha and Rani, 2003; Hilmy *et al.*, 1985). Mathur *et al.*, (2005) reported that the toxicity of sunset yellow at both the dose also effect serum and testis lipid content. Determination of protein concentration of various tissue on the freshwater fish *Labeo rohita* exposed to different concentrations of pesticides was observed by Radha and Rajendran (2009). There was a significant decline in protein fractions of skeletal muscle and total protein content of brain of *Channa punctatus* exposed to Cadmium chloride (Patnaik *et al.*, 2010). The source of lipase in the digestive system of teleost fishes is not clearly understood. While most of the workers had detected the enzyme in tissue extracts (Babkin and Bowie, 1928; Sarbahi, 1940), very few demonstrated its presence histochemically (Al-Hussaini, 1949). Opinions differ regarding the distribution of lipase in the intestine of fishes, as its presence is regarded due to the adsorption of the enzyme secreted by the pancreas. The pyloric caeca considered to be mainly absorptive, are reported to secrete lipase (Kitamkaddo and Tachino, 1960). Fluoride at high concentration had been found to inhibit protein synthesis (Holland,

1979; Aardema *et al.*, 1989). Effect of chocolate colour agents on body weight, serum cholesterol as well as on liver enzymes, total protein and globulin fraction on male albino rat after an exposure of 60 days was recorded by AbuEl-Zahab *et al.*, (1997). In the present study toxic responses of Metanil Yellow of chronic exposure, for a period of 30 and 45 days was investigated on biochemical changes in different tissues of *H. fossilis*. It mainly focuses on changes in the protein content and also in the amylase, protease and lipase activity in different tissues of *H. fossilis*.

Materials and methods

The test fishes, *Heteropneustes fossilis* used in this experiment were collected from local ponds and then they were acclimatized to ambient laboratory glass aquaria of 250 litre capacity for two weeks supplied with *Tubifex* sp. twice daily as food. The fishes were 25 ± 12 cm long with an average weight of 65 ± 20 g. After two weeks these glass aquaria were used one for control and other two for treatment. Each aquarium containing ten specimens and a dose of 2.0 g/l of Metanil Yellow for 30 and 45 days was given to two sets of the test fishes. After thirty first and on forty sixth day the desired tissues like oesophagus, stomach, intestine (anterior, middle and posterior), liver, pancreas and muscle were collected from control as well as treated one. Protein content was determined by using the Lowry technique (Lowry *et al.*, 1951). To determine the amylase activity Bernfeld method was followed (after Bernfeld, 1955). Protease activity was done by using Snell and Snell method (after Snell and Snell, 1971) and lipase activity was done by using Cherry and Crandall method (after Cherry and Crandall, 1932).

Results

Protein assay: Protein constitutes one of the important components of fish food.

Carnivorous fishes fed on aquatic animals have a higher percentage of protein in their diet. Protein content in different parts of the alimentary canal of *H. fossilis* showed in different quantities. Posterior intestine had less protein content in *H. fossilis* than other parts of alimentary canal. In the present experiment maximum protein content under control condition were found in stomach, liver and muscle of the *H. fossilis* (Fig. 1). After 30 and 45 days exposure in Metanil Yellow significant changes were found in protein content in stomach, liver and muscle. The maximum reduction in protein content was noticed in stomach and liver. In stomach, liver and muscle the protein levels were 73.68, 97.90 and 73.27 mg/g protein respectively as recorded after 30 days treatment while 62.74, 83.24 and 69.01 mg/g protein respectively estimated after 45 days treatment (Fig. 1). Changes were also found in the anterior and middle intestine. In the anterior and middle intestine the amount of protein were reduced to 60.86 and 34.25 mg/g protein respectively after 30 days treatment but after 45 days of exposure it became more less *i.e.*, 53.12 mg/g protein and 27.18 mg/g protein respectively (Fig. 1).

Amylase activity: Enzyme amylase is an important carbohydrate digesting enzyme. The maximum amylase activity was found to be associated with the pancreas in *H. fossilis* (17.25 unit/mg protein/minute) and in middle intestine (14.23 unit/mg protein/minute) and anterior intestine (12.06 unit/mg protein/minute) (Fig. 2). But comparatively low amylase activity was obtained in the oesophagus (3.79 unit/mg protein/minute) and in muscle (2.12 unit/mg protein/minute) (Fig. 2). Moderate amount of amylase activity was found in the liver (7.32 unit/mg protein/minute) and in stomach (5.57 unit/mg protein/minute) (Fig.2). After the chronic toxicity of Metanil Yellow with its sublethal concentration for a period of 30 and 45 days the amylase

activity in different regions of the alimentary canal of *H. fossilis* became changed. Maximum changes were found in the anterior and middle intestine and in pancreas. The activity became 9.31, 9.66 and 8.21 unit/mg protein/minute respectively after 30 days exposure but these were reduced to 7.46, 4.47 and 2.29 unit/mg protein/minute respectively after 45 days treatment (Fig. 2). In *H. fossilis* pancreas became highly affected with Metanil Yellow. But in oesophagus and stomach the alterations were not so marked.

Protease activity: The intense activity of this enzyme was observed in stomach, intestinal parts and pancreas of *H. fossilis*, whereas moderate quantity of this enzyme was measured in posterior intestine and lowest in muscle. In *H. fossilis* the protease activity in stomach was 15.38 unit/mg protein/minute (Fig. 3). In the anterior and middle intestine of *H. fossilis* the protease activity were 9.23 and 11.26 unit/mg protein/minute respectively (Fig. 3). Higher amount of protease were present in the pancreas *i.e.*, 17.74 unit/mg protein/minute) (Fig. 3). But in liver the amount of the protein splitting enzyme was moderate (11.91 unit/mg protein/minute) and lowest amount was found in muscle *i.e.*, 1.26 unit/mg protein/minute (Fig. 3). Due to the toxicosis of Metanil Yellow the quantity of protease enzyme became severely affected in the teleostean catfish *H. fossilis*. Maximum changes were shown in the stomach and pancreas after 30 and 45 days exposure. But considerable changes were shown in the intestinal parts. In stomach the activity became 11.27 and 7.21 unit/mg protein/minute after an exposure of 30 and 45 days respectively but in pancreas it became 8.89 and 2.81 unit/mg protein/minute respectively (Fig. 3). But in muscle the changes were not so marked.

Lipase activity: Lipase is water-soluble enzyme that catalyzes the process of hydrolysis of ester bonds in water-insoluble, lipid substrate. Lipase was an important lipolytic enzyme in *Heteropneustes fossilis* and other carnivorous fishes. Maximum amount of lipase activity was found in the pancreas and stomach *i.e.*, 8.08 and 6.77 unit/mg protein/minute (Fig. 4) respectively. But moderate amount was found in the intestinal parts *i.e.*, in anterior (4.22 unit/mg protein/minute) and in middle intestine (4.34 unit/mg protein/minute). Owing to Metanil Yellow exposure, the activity of lipase became severely affected. The activity became highly depressed in intestinal parts and in pancreas. In the anterior and in middle intestine the enzyme activity was 3.57 and 3.64 unit/mg protein/minute respectively (Fig. 4) after 30 days treatment and it became reduced to 2.81 and 2.93 unit/mg protein/minute respectively after 45 days of exposure. But in pancreas the amount reduced to 7.11 and 6.22 unit/mg protein/minute for 30 and 45 days respectively (Fig. 4). In oesophagus and muscle the activity of this enzyme was not so marked.

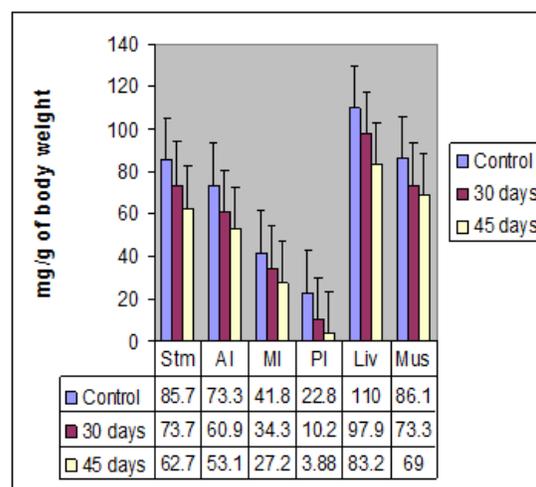


Fig. 1: Protein content of control and Metanil treated fish (*H. fossilis*) tissues.

Shetty, 1980; Raj and Kutty, 1984; Patra and Ray, 1988; Singh, 1990). Mukherjee and Sinha, 1993 observed the influence of different dietary protein and estimation of total protein content in muscle, liver and blood and to correlate these parameters with the growth of an Indian freshwater carp, *Labeo rohita* (Hamilton), Malachite Green has been found to cause depletion of serum calcium and protein levels and has also been increased the total cholesterol level of blood in catfish (Srivastava *et al.*, 1995a).

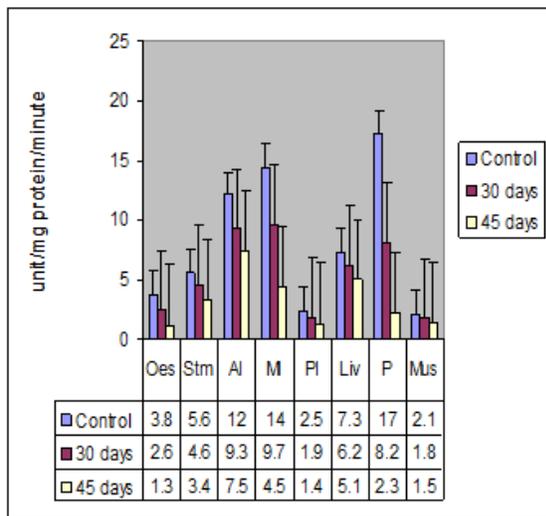


Fig. 2: Amylase activity of control Yellow and Metanil Yellow treated fish (*H. fossilis*) tissues.

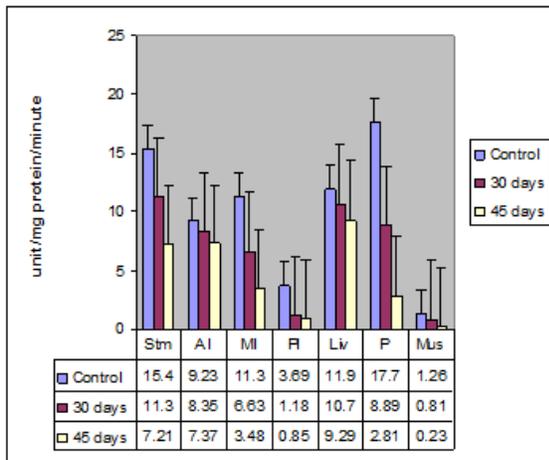


Fig. 3: Protease activity of control and Metanil Yellow treated fish (*H. fossilis*) tissues.

Discussion

Dubale and Awasthi (1984) recorded time dependent depletion of protein content in liver and kidney of *Heteropneustes fossilis* treated with organophosphate (OPP) pesticide dimethoate. It is known that proteins, the main component of biological constituency, are essential for any diet for normal growth of animals. The importance of administering protein-rich diets to different fish species for gaining accrued yields has been recorded (Jayaram and

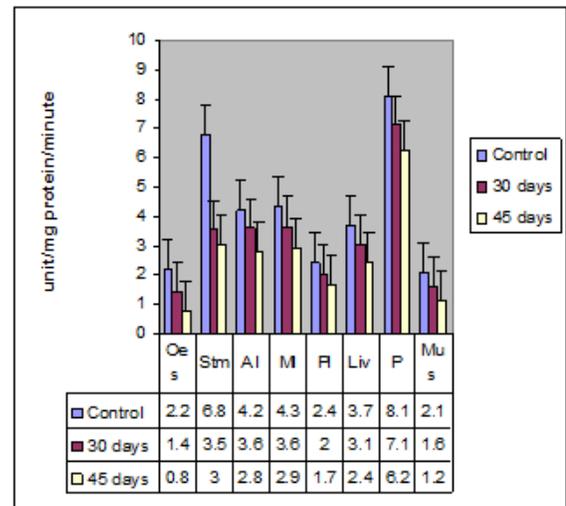


Fig. 4: Lipase activity of control and Metanil Yellow treated fish (*H. fossilis*) tissues.

Where, Oes = Oesophagus, Stm = Stomach, AI = Anterior intestine, MI = Middle intestine, PI = Posterior intestine, Liv = Liver, P = Pancreas, and Mus = Muscle

The present experiments have shown that maximum protein activities found in the stomach, anterior intestine, liver and muscle *H. fossilis* which has been found to become reduced after treatment. Teleostean fishes ingest the food materials which are comprised of complex molecular components. Specific enzyme *viz.*, amylase, protease and lipase are required for breakdown the complex components into simpler form. After ingestion food materials

are passed through buccal cavity and pharynx and the subsequent physiological processes of digestion take place in the post-pharyngeal part of the alimentary canal in teleostean fishes. Various digestive enzymes viz., amylase, protease and lipase activity have been recorded in the extracts of oesophagus, stomach, intestine (anterior, middle, and posterior), liver, pancreas and muscle tissues. In the present study, significant changes in the enzyme activity in both *H. fossilis* and *R. norvegicus* have been observed after an exposure in Metanil Yellow for 30 as well as 45 days. After ingestion, the contaminated food is metabolized in the alimentary canal of fish and the production of residual metabolites affect the activity of the aforesaid digestive enzymes. Present experiment has shown that the maximum amylase activity in the pancreas, anterior as well as in the middle intestine of *H. fossilis*. Amylase activity in the alimentary canal of some carnivorous fishes has been studied (Falge and Shapannkhof, 1976; Olatunde and Ogunbiyi, 1977; Dhaliwal, 1977; Kuzmina and Golovanova, 1980; Shapannkhof and Plantikow, 1983). The amylase activity in the liver is also found in moderate amount. After treatment with sublethal dose of Metanil Yellow alteration of amylase activity is found in the liver, pancreas, middle intestine while least affect found in the oesophagus and stomach. Therefore, a considerable amount of amylase seems to be formed in the small-intestinal mucosa itself. Activity of protease enzyme has been recorded by various authors (Kitamikado and Tachino, 1960; Croston, 1966; Kapoor *et al.*, 1975; Falge and Shapannkhof, 1976; Yoshinaka *et al.*, 1981; Tue, 1983). In the present study, maximum protease activity has been found in stomach, pancreas as well as in liver of *H. fossilis*. But lowest amount has been found in the posterior intestine and in muscle. Reduction of the activity of the aforesaid enzyme has been observed after

treatment. Alteration in gastric pH value, excessive secretion of neutral mucin and disruption in the gastric gland which have been affected the production of proteolytic enzyme. In the pancreas the destruction of zymogen granule has found to cause the reduction of protease activity after treatment. But in case of intestine the destruction of mucosal epithelial cells and brush border has found to show effect on enzyme activity. Lipase activity has been found to be highest in stomach and pancreas of *H. fossilis*.

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