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Effects of tartrazine on growth and brain biochemistry of Indian major carps on long-term exposure

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Abstract

Background: Synthetic azo dyes are very extensively using various industries such as food items and non-food items. Tartrazine is well known azo dye due to its wide range of applications, also known as acid yellow 23. Most industries release these dyes containing effluents into water bodies without proper treatment. There is no well-documented study about the toxicological effect of tartrazine in fishes. Most studies with tartrazine are concentrated in humans and rats. However, there is a high chance of reaching the dye in natural water bodies either through direct or indirect deposition and there is a necessity of understanding its effects on fish.

Objective: The study was conducted to assess the effect of synthetic azo dye tartrazine on fishes due to long-term exposure.

Materials and Methods: *Labeo rohita* commonly known as Indian major carp was selected as experimental fish and the major parameters discussed are the growth of the fish in terms of condition factors and the biochemical changes in the brain. Experimental fishes were grouped after the acclimatization of seven days as control (C), test 1 (T1), and test (T3). The major biochemical parameters analyzed include total proteins, carbohydrates, lipids, free amino acids; antiperoxidative enzymes- GST, GR, GPx, SOD, CAT; lipid peroxidation product MDA and activity of AChE. After the chronic exposure experiment of 75 days, the control and test fishes were subjected to morphometric measurements and biochemical analysis.

Results: The results show that the weight of experimental fishes reduced significantly ($P < .001$) with respect to control groups. Antiperoxidative enzymes were also altered in the experimental fishes with respect to control. AChE activity also decreased significantly. Lipid peroxidation product MDA increased in the brain tissues of the experimental fishes.

Conclusion: The study concluded that tartrazine has detrimental effects on the fish by altering the biochemical as well as physiological attributes.

Keywords: Tartrazine, chronic exposure, condition factor, MDA, antiperoxidative enzymes

Introduction

Synthetic dyes are the major class of chemicals proficient to destroy aquatic ecosystems through various sources. Partially treated and untreated industrial effluents take part in the release of synthetic dyes into the environment. There are different types of synthetic dyes such as Vat dyes, Acid dyes, Direct dyes, Azo dyes, Sulphur dyes, Reactive dyes, Basic dyes, Oxidation dyes, Mordant dyes, Solvent dyes, Optical brighteners and Disperse dyes. Among the synthetic dyes, azo dyes are one of the largest groups of synthetic colourants used in industries^[1]. Azo dyes are characterized by the presence of the azo or nitrogen- nitrogen ($-N=N-$) bond in the centre and hence they are highly electron-deficient^[2]. These azo dyes are found to be complex in nature and show carcinogenic evidence on reductive cleavage. There are different types of azo dyes used in various foods and non-food items such as Tartrazine, Alizarine yellow R, Allura red AC, Azo violet, Azorubine, Congo red, Eriochrome black T, Metanil yellow. Most of them degrade into aromatic compounds such as amine, benzidine and amino-azo-toluene. These dyes and their degradation products are capable of altering the physical and chemical properties of soil, and water and causing harm to the biota of the environment^[3]. Tartrazine is a bright orange-yellow coloured synthetic azo dye derived from coal tar. Generally, the dye is well-known as a food dye, however, its application ranges are very much diverse in recent years.

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Tartrazine replaced the use of non-synthetic colouring materials (annatto, malt colour) and is considered a low-cost alternative to saffron and beta carotene in foods and drugs [4]. The foodstuffs containing tartrazine include sweetmeat, soft drinks, cotton candy, cereals (corn flakes and muesli), flavoured chips, cake combinations, soups, jam, sauces, ice cream, some rice, candy, chomping gum, marzipan, jelly, mustard, marmalade, yoghurt, noodles, fruit pleasant and product, chips and several expediency foods together with glycerine, lemon and honey products, soft drinks (Mountain Dew and Mirinda), energy drinks, prompt desserts etc. [5]. In the case of drugs and medicines, tartrazine is extensively used in syrups, antacids, vitamins and capsules. Non-food items like soaps, cosmetics, shampoos and other hair products, conditioners, nail polishes, nail polish removers, temporary tattoos, tanning lotions, cleaning products, pastels, crayons, face paints, pet foods and some stamp dyes also contain tartrazine in varying quantities. Tartrazine is also used in textiles and garments to impart yellow shades and as a dyeing mixture with other dyes like brilliant blue and malachite green to impart different shades of green.

The major environmental concern of synthetic dye is water pollution due to the bright coloured industrial effluents. Colour is the first wastewater contaminant to be recognized since a very small amount of azo dye in water (<1 mg/L) was highly visible [6]. The dyes can reduce the transparency, gas solubility and aesthetical pleasantness of the water. Some dyes are used as weedicides in aquatic ecosystems, lead to the destruction of beneficial aquatic micro-fauna as well as flora and ultimately reduces the fish population that depends on this vegetative environment for spawning and metamorphosis. In addition to these, dye-containing effluents also cause high COD, BOD and other chemical loads to water bodies.

Tartrazine is identified to cause allergies such as urticaria and asthma, besides the emphasis of studies on its carcinogenesis and mutagenesis because of its 13 metabolic conversions into aromatic amine (sulphanilic acid) via the gut microflora [7] and possibly by mammalian azo reductase in the hepatic or intestinal wall after consumption [8]. There is a lack of documentation about the effects of tartrazine in water and fish. The present study attempts to find out various effects of the dye in lower animals such as fish. Fish have often been used as sentinel organisms for ecotoxicological studies because they play a number of roles in the trophic web, accumulate toxic substances and respond to low concentrations of toxicants [9]. Most of the biomarker studies using fishes were point out that successful assessment of the surrounding environment with its physiological and biochemical changes.

A biomarker is a measurable change at molecular, biochemical, cellular, or physiological levels of an individual representing a larger group population. Therefore, they can reveal the present and past exposure of the individual to at least one contaminant and the quality of the living environment [10]. The changes in molecular level, biochemical level, genetic level, mortality, behaviour and associated growth and development can be employed as successful biomarkers of toxicity. Some of the biomarkers are specific to a class of toxicants or general stress proteins [11]. Xenobiotics are specific to certain effects such as some effects on the blood, (haemotoxicity), neurons (neurotoxicity), cells (cytotoxicity), and proteins (proteotoxicity). Organisms cope with oxidative stress in

two vital ways such as antioxidant production to react with oxyradicals and enzyme production to reduce the quantity of oxyradicals. The antioxidant enzymes include superoxide dismutase (reduce O_2^-), catalase and glutathione peroxidase (reduce H_2O_2). These metabolizing enzymes are produced by peroxisomes and the endoplasmic reticulum of cells. Elevated levels of these antioxidant enzymes in xenobiotics exposed organisms represent the rise in oxidative stress. Modification in the levels of these enzymes might be noticeable in individuals from contaminated sites and those exposed to contaminants in the laboratory [12] [13]. The growth of an organism is a sum total of overall physiological health and it can be affected by any stress such as toxicity. The length-weight relationship or condition factor is an important parameter that indicates the health and growth of fishes in cultural conditions [14].

So, through the wastewater from domestic activities and small-scale industries, there may be the release of tartrazine in nearby effluent receiving water bodies and it can cause toxicological effects in aquatic organisms including fishes. In this context, the present experimental study has been carried out to evaluate the toxicological and biochemical effects of tartrazine on fishes.

Materials and Methods

Experimental Fish and housing condition

For the present study Indian major carp- *Labeo rohita* (Hamilton, 1822) [15] commonly known as 'Rohu' was procured from the National Fish Seed Farm, Department of Fisheries, Kerala State Govt. at Neyyar Dam, Thiruvananthapuram. After disinfection, the fishes were acclimatized to the laboratory conditions for 21 days with an *ad libitum* diet. For both acclimatization and experiments, chlorine-free water was used and major water quality parameters were analysed according to the standard procedures in APHA (2012) [16].

Chemicals

Tartrazine (C.I. 19140, CAS No 1934-21-0, Mw 534,37, synonyms: E 102, Food Yellow 4, Acid Yellow 23, FD & C Yellow No.5) is an azo dye with the chemical formula 4,5-Dihydro-5-oxo-1-(4-sulfophenyl)-4-((4-sulfophenyl) azo)-1H-pyrazole-3-carboxylic acid and the trisodium salt (95%) was purchased from TCI chemicals (Tokyo Chemical Industries), India. All chemicals and biochemicals were of AR grade and purchased from TCI Chemicals, E Merck and Nice (India).

Experimental design

After acclimatization, 54 healthy fishes of 15 ± 2 cm length and 28 ± 2 g body weight were selected for the experimental study, and are divided into test and control groups. After the range-finding test, 96 hours LC_{50} was calculated (probit analysis - Finney *et al.*, 1971) [17] and subsequent sublethal concentrations were selected for the chronic studies, 5 mg/L (CT1) and 10 mg/L (CT2) concentrations of the dye. In the chronic experiment, fishes were exposed for 75 days under controlled conditions in the aquarium (25L). The control group fishes were also maintained in each set of tests. Static renewal type of assays was conducted for chronic experiments. The important water quality parameters like temperature, pH, DO, and conductivity was determined [16] and maintained at an optimum level throughout the experiment. During the experiments fish behaviour,

swimming patterns etc. were also noticed. Gross observation and morphological measurements of fish bodies were done before, after and at regular intervals during the experiment.

Determination of condition factor

The condition factor was calculated from the morphometric measurement such as body weight and length. Condition factor (CF) is considered an important morphological index of the fish and is a measure of fitness. The length was measured from the tip of the snout to the rear edge of the fork at the tail fin and weighed in a normal weighing balance. Fulton's condition factor (K) [18] was calculated using the formula.

$$K = W/L^3 \times 100$$

Where 'K' is Fulton's condition factor, W = Body wet weight in gram and L^3 = length in cm; factor 100 is used to bring close to unity.

Biochemical analysis

After the chronic experiments, fishes were sacrificed and organs were separated and washed with physiological saline for biochemical studies. Biochemical analyses were carried out by using standard procedures. For the analysis, tissues were homogenized using respective substrates and reagents, and spectrophotometrically determined absorbance with UV-Visible Spectrophotometer (Systronics 118, India) at corresponding wavelengths. The components and corresponding references are included as follows.

Total proteins [19], total carbohydrates [20], total lipids [21], total free amino acids [22], glutathione peroxidase (GPx, EC.1.11.1.9) [23], glutathione reductase (GR, EC.1.6.4.2) [24], Glutathione-S-transferase (GST, EC.2.5.1.18) [25], superoxide dismutase (SOD, EC.1.15.1.1) [26], catalase (CAT, EC.1.11.1.6.) [27], acetylcholinesterase (AChE, EC 3.1.1.7) [28], and Malondialdehyde (MDA) [29] were estimated. All the biochemical analyses were carried out in triplicates, and statistical analyses were also carried out to check the significance of values obtained for test groups and control groups.

Statistical Analysis

The statistical analysis was conducted to assess the change in condition factor of fishes in control (CC), chronic test 1 (CT1) and chronic test 2 (CT2) groups by analysis of variance (ANOVA) in SPSS 16 for windows. The level of significance was considered at $P < .001$.

Results

In the present study, 96 hours LC_{50} value for tartrazine in the fish *L. rohita* (weight 28 ± 2 g; length 15 ± 2 cm) was found to be 71.54 mg/L. From the LC_{50} values, sublethal concentration was calculated as 1/15th of LC_{50} as 4.769 and rounded as 5 mg/L, which was the lowest sublethal concentration. Therefore 5 mg/L and 10 mg/L of tartrazine as CT1 and CT2 were selected for the chronic experiment.

The experimental fishes in both CT1 and CT2 groups show several behavioural changes such as unstable movements, increased mouth and opercular movements, increased

surface activity, frequent changes in activity levels such as hyperactivity and general weakness, food avoidance behaviour with severe diarrhoea and lack of schooling behaviour. The morphological changes in test fishes include, over mucous coating, depigmentation, yellow colouration in some parts of the body, shredded scales, swellings in snout portions, weight loss, and bending or curving of the body. The overall body of the fish became thin and curved after the experiment, while the control (CC) group fishes gained some weight and length. The changes in the condition factor are given in figure.1 and the photographs (Plate 1, 2 and 3) show visible changes.



Plate 1: Control fish after Experiment



Plate 2: Test 1 fish after Experiment



Plate 3: Test 2 fish after Experiment

Biochemical Analysis

The results of the biochemical analyses are given in Figures 2, 3 and 4. The highest total protein (TP) content was observed in chronic control (CC) group fishes as 89.09 ± 0.41 mg/g wet wt. and the lowest was in chronic test 2 (CT2) as 75.62 ± 0.21 mg/g wet wt. It is a highly significant ($P < .001$) decrease in TP content of the brain tissues of the test fishes with respect to control. The total free amino acids (TFA) in brain tissues CT2 fishes were high (13.01 ± 0.19 mg/g wet wt. with respect to CC (12.92 ± 0.06 mg/g wet wt.)). However, there was no significant change. The highest brain TC content was observed in CC group fishes as 10.59 ± 0.22 mg/g wet wt. and the lowest was observed in CT2 group fishes as 7.42 ± 0.16 mg/g wet wt. There was a highly significant ($P < .001$) reduction of TC content in the brain tissues of CT1 and CT2 group fishes. The highest TL content in gill tissues was observed in CT1 group fishes as 49.77 ± 1.01 mg/g wet wt. and the lowest was in CT2 group fishes as 44.94 ± 1.01 mg/g wet wt. CT2 group fish brain tissue shows a highly significant ($P < .001$) increase of TL content with respect to control (CC) fishes.

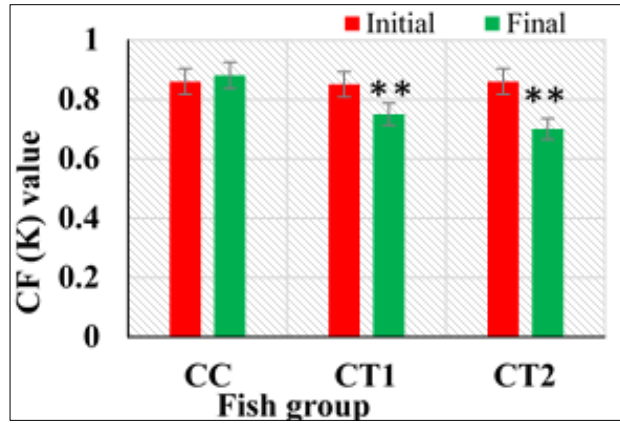


Fig 1: Initial and final Condition Factor (K) of fishes in chronic experiment (** $P < .001$)

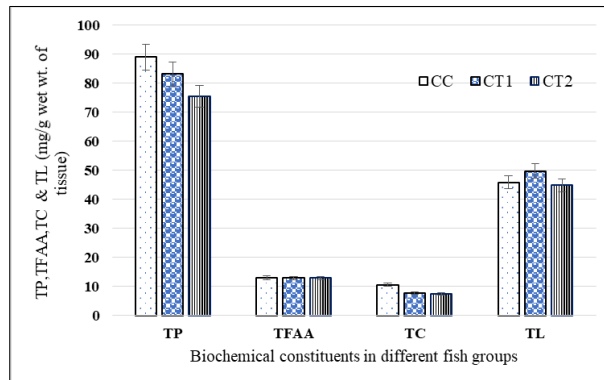


Fig 2: Variation in biochemical constituents in control & test fishes (All values are Mean \pm SD)

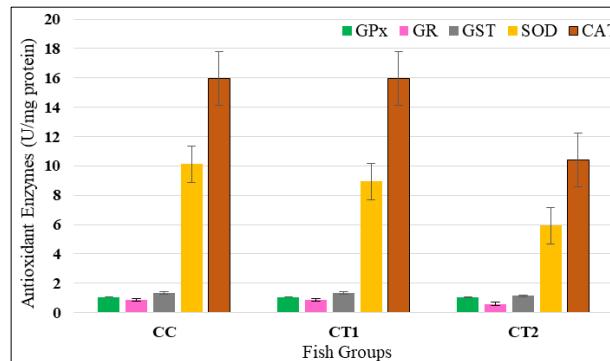


Fig 3: Variation in antioxidant enzymes in control & test fishes (All values are Mean \pm SD)

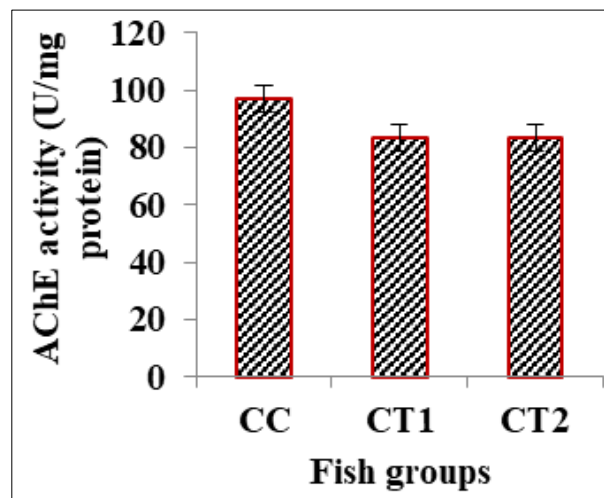


Fig 4: Variation in activity of AChE in control & test fishes (All values are Mean \pm SD)

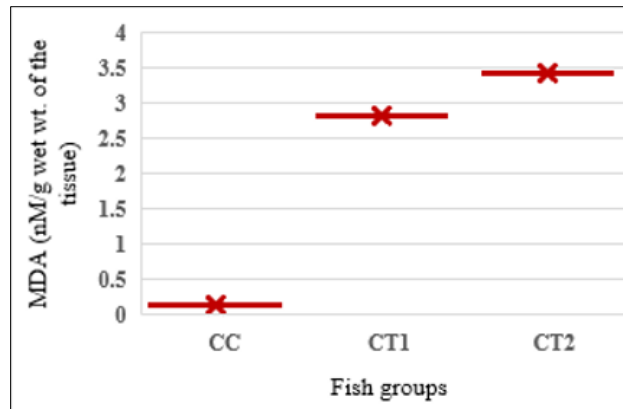


Fig 5: Variation in MDA in control & test fishes (All values are Mean \pm SD)

The activity of antiperoxidative/antioxidant enzymes

The activity of GPx IN brain tissues was high in CT1 group fishes as 1.08 ± 0.09 U/mg protein with respect to control (CC) and CT2 but it is not significant. The lowest activity of GPx (1.03 ± 0.06 U/mg protein) was observed in CT2 group fishes. However, the activity of GR was altered in the brain tissues of experimental fish. The highest activity of GR has observed in the control (CC) group fishes as 0.88 ± 0.08 U/mg protein and the lowest was observed in the CT2 group fishes as 0.59 ± 1.14 U/mg protein. CT1 and CT2 group fish brain tissues show a highly significant ($P < .001$) decrease in the activity of GR with respect to control (CC). There is no significant change in the activity of GR among CT1 and CT2 group fishes.

The highest activity of GST in brain tissues was observed in the control (CC) group fishes as 1.33 ± 0.56 U/mg protein and the lowest was observed in CT2 group fishes as 1.13 ± 0.13 U/mg protein. There is no significant change in brain activity of GST in test groups (CT1 and CT2) with respect to control (CC) group fishes. The highest activity of SOD in brain tissues of control (CC) group fishes as 10.11 ± 1.11 U/mg protein and lowest in CT2 group fishes as 5.94 ± 1.12 U/mg protein. A significant ($P < .001$) decrease in the activity of SOD of brain tissues in CT2 with respect to control (CC) group fishes was observed. The highest activity of CAT in brain tissue was found in the control (CC) group fishes as 15.93 ± 1.27 U/mg protein and the lowest was found in CT2 group fishes as 10.43 ± 0.44 U/mg protein. Test groups (CT1 and CT2) show a highly significant decrease ($P < .001$) in brain activity of CAT with respect to control (CC). There is no significant change in brain activity of CAT among CT1 and CT2 group fishes.

AChE enzyme activity in brain tissues of CT1 and CT2 group fishes was not much varied but significantly ($P < .001$) reduced with respect to control. The highest activity was recorded in CC group fishes (95.03 U/mg protein) and the lowest was in CT2 group fishes (85.78 U/mg protein).

In the chronic experiment, the highest MDA content in brain tissues was found to be in brain tissues as 3.41 ± 0.27 μ moles/100g wet wt. in CT2 group fishes and the lowest was in control (CC) group fishes as 0.12 ± 0.01 μ moles/100g wet wt. CT1 and CT2 group fish brain tissues show a highly significant ($P < .001$) increase in MDA content with respect to control (CC) group fishes. There is no significant change in the MDA content of brain tissues among the CT1 and CT2 groups.

Discussion

Determination of LC₅₀ gives a measure of acute toxicity of xenobiotics. In the case of tartrazine, there is no previous documentation of tartrazine toxicity in fish. Ai-Mashhedy

and Fijer (2016) [30] reported the LD₅₀ of tartrazine in mature Sprague-Dawley white mice (25-30 g weight) as 6.250g/kg body weight. In the studies attempted by Sharma *et al.* (2009) [31] using another dye methyl red, the LC₅₀ was found to be 54.80 mg/L in *Gambusia affinis*. In the present study, acute toxicity studies lead to chronic experiments. The behavioural, morphological and biochemical alterations as a result of tartrazine exposure are well determined.

In the presence of high concentrations of toxicants fish become lethargic [32]. The general tiredness and weakness are also found in exposed fishes due to the state called lethargy. The total body weight of fish was reduced after the chronic exposure of 75 days. The stress-induced food avoidance behaviour in fishes may be due to loss of appetite and may be of gradual accumulation of dyes in the gastrointestinal tract of exposed fishes. The avoidance of feeding might be due to the impairment of olfactory organs due to the effect of contaminants as reported by Oliveira Ribeiro *et al.* (1995) [33]. The feeding and social behaviour have been considered substantial in the exposure experiments. The fishes were inactive and swim slowly and never came close to each other. Sometimes, CT2 fishes were swims faster and more hyperactive and then become inactive and tired. Toxicity studies by Scherer (1992) [34] reported that loss of appetite, weight, equilibrium; erratic swimming, nervousness and gradual onset of inactivity as a result of inorganic mercury intoxication. The disrupted schooling behaviour was also reported in toxicity studies by Kumar *et al.* (2015) [35] in *Clarias batrachus* exposed to copper sulphate. The behavioural changes like hyperactivity, antisocial behaviour and anxiety in male Wistar rats at 0, 1 and 2.5% doses of tartrazine in drinking water were recorded in the study by Kamel and El-Iethy (2011) [36].

In the chronic experiment, the experimental fishes' depigmentation and shedding of scales happened may be due to the dermal toxicity of azo dye tartrazine. According to Kane *et al.* (2005) [37], the depigmentation and shredding of scales were due to the damage to the skin especially the dermis part because of the direct contact with contaminants. In addition to the depigmentation and colour change, a very thick mucus covering all over the body was observed. The alteration in the body colour was the most detectable feature in the exposed fishes. The mucous secretion in fishes due to the contaminant exposure has an ameliorative effect against the toxicants [38]. Several toxicity studies reported heavy mucus on the surface of all exposed fishes as in *L. rohita* and *C. mrigala* exposed to fenvalerate [39].

The body of the test fishes became brittle and curved may be due to the muscle spasm and body convulsions due to the

toxicity of exposed chemicals [40]. The food avoidance and tartrazine-induced stress led to the growth reduction of fishes subjected to chronic assays (CT1 and CT2). In chronic experiments, there is no growth in test fishes (CT1 and CT2) in terms of weight and length with respect to control fishes. The length-weight relationship was measured as Fulton's condition factor (F). Condition Factor of Fishes The condition factor (K) was determined in the chronic test because of the long-term experiment and the changes in weight and growth were visible. Condition Factor (Ponderal Index) is a physiological indicator of the well-being of any fish living in a given environment. Low values of condition factor, as it is normally referred to as a definite sign of non-allometric fish growth probably owing to the competition for food and space or other physiological stress within the different fish communities in a water body [41]. There was a significant ($P < .001$) reduction in weight of the test fishes with respect to control and there was a difference in length also. Therefore, the condition Factor also changed accordingly.

The decrease in total proteins in brain tissues may be due to the utilisation of protein reservoirs and imbalanced enzyme production. The lack of nutrition due to stress may cause the destruction of cells and which leads to the inhibition of protein synthesis. Catabolism of proteins makes a major contribution to the total energy production in fish [42]. Whole-body protein concentrations are influenced by a variety of environmental factors [43]. The reduction in the tissue proteins may be due to increased catabolism of proteins as a result of a defence mechanism against the stress. The altered behavioural patterns and frequent hyperactivity may require a large amount of energy. In the fish body, the protein catabolism may act as an immediate source of energy. Brain tissues of experimental fishes clearly indicated the impairment in amino acid and protein synthesis and also the destruction of large protein molecules. Which leads to the rise of total free amino acids.

The reduction in glycogen content in tartrazine-exposed fishes has been attributed to the utilization of glycogen reserve to meet the high energy requirement during stress. Therefore, the unfavourable environmental situation due to tartrazine exposure may be the probable cause of change in carbohydrate metabolism especially the exploitation of glycogen reserves. The results were in agreement with that of *Labeo rohita* exposed to copper as reported by Radhakrishnaiah *et al.* (1992) [43] also reported a decrease in glycogen content. The glycogen is considered the major reservoir of carbohydrates in an animal body. The depletion of total lipids in tartrazine-treated fishes recorded in this study may be due to their active mobilization towards the blood and/or tissue metabolism. The reduction might be due to the utilization of lipid to meet the additional energy requirement under stress [45]. The degradation products of tartrazine, such as amines are well known for their cytotoxicity and neurotoxicity in animals.

Abnormally high production of reactive oxygen species (ROS) or free radicals is considered one of the obvious symptoms of oxidative damage in animals [46]. For protection from xenobiotics and for combating oxidative stress, aerobic organisms have evolved complex antioxidant defence systems [47]. Antioxidant enzyme systems comprise superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), which are protective agents against free radical-induced oxidative damage [48].

Superoxide (O₂⁻) is one of the powerful intracellular reactive oxygen species (ROS) and it is highly reactive. It could be converted to H₂O₂ by SOD and then to oxygen and water by CAT and glutathione reductase (GR). CAT protects fishes from oxidative stress by converting hydrogen peroxide to oxygen and water and thereby protecting cells from the toxicity of hydrogen peroxide [49, 50]. To counteract the impact of ROS generated by xenobiotics like organic pollutants, endogenous defence mechanisms including antioxidant enzymes such as superoxide dismutase, catalase and glutathione-S-transferase are produced. Besides enzymes, vitamins and minerals from nutrition also play important role in combating oxidative stress and promoting growth as suggested by Craig and Helfrich (2009) [50]. Free radicals damage cells and contribute to the development of cardiovascular diseases, cancer or tissue injury of the liver, brain, kidney, lung and nervous system of animals [52].

The antioxidant enzymes in most cases work in a cooperative or synergistic manner for protection against oxidative stress and tissue-specific damage in organisms exposed to contaminants. The ROS are detoxified by antioxidant enzymes that protect macromolecules such as proteins, lipids and nucleic acids against oxidative damage and the maintenance of high constitutive levels of antioxidant enzymes is essential to prevent oxyradical mediated lipid peroxidation [53, 54]. The SOD-CAT system provides the first line of defence against oxygen toxicity. Usually, a simultaneous induction response in the activity of SOD and CAT was observed in *Channa punctatus* exposed to paper mill effluents [55]. Ferreira *et al.* (2005) [55] in their studies on *Mugil cephalus* and *Platichthys flesus* have shown that exposure to contaminants, especially trace metals in aquatic ecosystems is capable of enhancing the intracellular formation of ROS causing severe oxidative damage to biological systems. In the test fishes of the chronic experiment, activities of almost all antioxidant enzymes were in decreasing trends. The elevated levels of antioxidant enzymes indicate lipid peroxidation. Similar observations were also made by Yonar (2018) in fish *Cyprinus carpio* exposed to chlorpyrifos in which SOD, CAT and GST were decreased with a high level of lipid peroxidation.

The inhibition of the acetylcholinesterase by toxicants can affect the locomotion and equilibrium of exposed organisms [58, 59]. The aromatic amines, formed by the degradation of azo dye tartrazine has considered carcinogenic and neurotoxic. The reduction in the enzyme activity may be due to the action of aromatic amine.

Several toxicity studies using xenobiotic compounds have reported the presence of thiobarbituric acid reactive substances (TBARS) in several tissues like the liver, brain, kidney and gills of exposed or treated fishes. Zhang *et al.* (2012) [58] reported elevated levels of MDA in zebrafish exposed to textile effluent. Chang *et al.* (2020) [59] reported oxidative stress by revealing the increased level of MDA in fish *Carassius auratus* exposed to municipal sewage treatment plant effluent. Similar observations were also made by Ahmad and Ali (2013) [60] in *C. punctata* as a result of exposure to battery manufacturing effluent.

In the study, all aspects such as behaviour and morphometry and biochemical attributes were shown substantial changes in the tartrazine-treated fishes in the long term. In the field conditions, dye-containing effluents can affect the indigenous fish populations even in low concentrations also

apart from the dilution factor and external factors. And there may be a chance of synergetic effects of azo dyes with other toxic combinations. For the sake healthy environment, it is very important to have proper wastewater treatment technologies.

Conclusion

In conclusion, the study shows that biochemical alterations can be used as bioindicators of toxicity. The biochemical profile of an organism can indicate its overall health and surrounding environment. In the present study, the visible changes in the growth of experimental fishes with respect to control were proved by the morphometric measurements and length-weight ratio. The major biochemical constituents like total proteins, total free amino acids, total carbohydrates and total lipids were reduced, which indicates impairment in biomolecule production and cellular destruction. The variation in antioxidant enzymes indicates the stress associated with the toxicity of tartrazine. The brain is the centre of all coordination and control of any animal and any type of destruction in the organ affects the entire well-being. The biochemical alterations in the brain resulted in the behavioural pattern and growth of the experimental fishes. The study also confirmed the cytotoxicity and neurotoxicity of the azo dye in fishes.

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