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RESEARCH ARTICLE

Haematological, biochemical, enzymological and histological responses of *Labeo rohita* exposed to methyl orange dye solution treated with *Oedogonium subplagiostomum* AP1

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Abstract

The present investigation is an attempt to assess the impact of untreated methyl orange and *Oedogonium subplagiostomum* AP1 treated methyl orange dye solutions on *Labeo rohita.* The behavioural response, mortality, haematological (red blood corpuscles (RBC), packed cell volume (PCV), haemoglobin (Hb), white blood corpuscles (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)), biochemical (plasma glucose and protein), enzymological (aspartate amino transaminases (AST) and alanine amino transaminases (ALT)) and histological examination (gills, liver and kidney) of *Labeo rohita* are exposed to untreated and treated methyl orange dye solutions were assessed on 7th day. The fish exposed to tap water and treated dye solution showed normal behavioural response whereas abnormal behaviour was noted in fish exposed to untreated dye solution. Similar trend was recorded in the mortality rate of the fishes. Fish exposed to untreated dye solution showed reduction in RBC, PCV, Hb, MCHC, plasma glucose and plasma protein, increased level of WBC, MCV and MCH and also alteration in AST and ALT thereby indicating the toxicity of the dye. No such reduction and alteration were observed in haematological, biochemical and enzymological levels of fishes exposed to tap water and treated dye solution indicating the non-toxic nature of the degraded metabolites of dye. Histological examination of fishes exposed to methyl orange dye revealed necrosis and haemorrhage in the gills and hepatocytes, congested and shrunken glomeruli in kidney thereby indicating the toxicity of the dye. The histoarchitecture of control and algae-treated fishes showed no structural changes indicating the non-toxic nature of the degraded metabolites of the dye. The results concluded that methyl orange dye solution treated with *O. subplagiostomum* AP1 can be explored for aquacultural purposes owing to its non-toxic nature.

Keywords Fish bioassay · Dyes · *Labeo rohita* · *Oedogonium subplagiostomum* AP1 · Toxicity

Introduction

Rapid industrialization and market demands have substituted natural dyes with synthetic dyes due to their better fastness, brightness, wide ranges of colours and easy application as well as its cost-efficiency. Dyes are not only used by textile industries but also utilized in many other industries such as leather, tanning, paper and pulp, food, colour and paint, photography, pharmaceuticals and medicine, cosmetic, hair colourings, wood staining, agricultural and also in biological and chemical research (Jagruti 2018; Shabban et al. 2020). Enhanced industrial and anthropogenic activities with technological advancement along with increased population have led to the extreme release of textile dye contaminated wastewater into the ecosystems. Particularly, azo dyes used in textile dyeing industries are highly toxic, and its discharge into aquatic ecosystem has modified them as a sink for water pollution with serious impacts on aquatic faunal resources (Athira and Jaya 2018).

Methyl orange, a water-soluble anionic azo dye, was chosen in the study due to its wide application in textile, paper manufacturing, pharmaceutical, printing, food industries and research laboratories. When methyl orange unintentionally enters the body, it causes cancer, allergy, hypersensitivity and dermatitis and eventually gets metabolized into aromatic amines by intestinal microbes (Mittal et al. 2007; Chen et al.

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2010, 2011; Pillai 2017). Exposure of untreated dyes into water bodies may cause behavioural changes, biological and physiological alterations, allergic, mutagenic, carcinogenic and detrimental effects to the living creatures especially the fishes (Amte and Mhaskar 2013; Amaya et al. 2018; Parmar and Shah 2020; Vigneshpriya et al. 2017). Aspiring the results of the various researchers on the impact of toxicants on fishes, meagre studies have been carried out to assess the impact of dye solution treated with algae on Indian carps. Thus, this study gains significance through zootoxicity assay which would provide complete and effective solution to be implemented in the polluted riverine systems without any hazard to aquatic fauna. Environmental toxicologists extensively employ zootoxicity tests using fish as a bioindicator for assessing the effect of pollutants on the molecular, biochemical, enzymological and histological responses which have been employed as the biomarkers of various environmental stresses (Tufekci et al. 2007). The treated wastewater of dyeing industries has been used in aquacultural practices around the world for the production of fish biomass which is the primary goal with marginal concern in wastewater renovation (Das et al. 2012).

Oedogonium subplagiostomum AP1, a freshwater dominant, unbranched, filamentous non-toxic green alga economically obtained on a large scale from the nearby water resources was experimented as a biosorbent in the removal of methyl orange from aqueous solutions and used for fish bioassay studies (Maruthanayakam et al. 2020). The Indian major carp *Labeo rohita*, a bottom feeder which mainly consumes algae and aquatic plants was selected as the experimental animal in the present study due to its rapid growth, high sustainability, tolerance at high stocking density and survival ability in oxygen-depleted water. Studies associated to the exposure of Indian major carps to methyl orange treated with *O. subplagiostomum* AP1 are meagre. Keeping this in view, the present study aims in assessing the haematological, biochemical, enzymological activities and histopathology of *L. rohita*.

Materials and methods

Zootoxicity assay

Procurement and acclimatization of the experimental fish

Freshwater fingerlings of *L. rohita* (length 7.2 ± 0.4 cm and weight 8.3 ± 1.0 g) were procured and transported to laboratory from National fish seed farm, Department of Fisheries, Bhavanisagar, Erode, Tamil Nadu, India, in clean aerated polythene bags. The collected fishes were safely brought to the laboratory and acclimatized under laboratory conditions for 20 days in fish tank disinfected with potassium permanganate solution to prevent from fungal infection. Dechlorinated tap water with pH 7, temperature 25 °C, 5.3 mg/L of dissolved oxygen and 20 mg/L of total hardness (APHA 1998) was filled in the fish tank. During the acclimatization period, the fishes were fed daily with rice bran and groundnut oil cake (2:1) which had no detectable amount of dye. The water in the acclimatization tank was renewed daily to remove the excess amount of feed and excretory materials. Fishes showing abnormal characteristic behaviour was removed from the tank. At the end of the acclimatization period, healthy fingerlings were separated and subjected to experimental study.

Preparation of treated dye solution

Oedogonium subplagiostomum AP1 at a concentration of 400 mg/L was introduced into the aqueous solution amended with methyl orange (500 mg/L), and the decolourization efficiency was recorded at room temperature (Maruthanayakam et al. 2020). The physico-chemical characterization (colour, odour, pH, electrical conductivity, total dissolved solids (TDS), total suspended solids (TSS), total solids (TS), biological oxygen demand (BOD), chemical oxygen demand (COD), total hardness, total alkalinity, chloride, sulphate and nitrate) of untreated and treated methyl orange dye solution was assessed as per the standard method (APHA 1998), and the decolourized/treated dye solution is used for the zootoxicity study (Alaguprathana and Poonkothai 2018).

Experimental design

Fifteen fishes were introduced into the tubs filled with 20 L of dechlorinated tap water which served as control (T_1), untreated dye (T_2) and treated dye (T_3) solutions separately. For each treatment, triplicates were maintained, and the experimental water samples were renewed daily. The feeding level of the fishes was 5% of their body weight. Dead fishes were removed immediately to prevent the contamination and to maintain desired concentration of oxygen. The fishes were maintained at 12:12 light: dark cycle.

Mortality rate

The mortality of *L. rohita* was recorded for every 24 h during the observation period of 168 h. If there is no visible movement (e.g. gill movements) or no reaction while touching the caudal peduncle of the fish, it is considered to be dead and removed. The mortality of the fishes were recorded as per the cumulative percentage of fishes dead (Amte and Mhaskar 2013). At the end of the experimental period, the fishes from each treatment were collected and subjected to toxicological studies.

Collection of samples for toxicological studies

The blood was collected from the experimental fishes by cardiac puncture using heparinized syringes and transferred into heparin-coated vials. The whole blood was used for haematological studies, and the remaining was centrifuged (Remi, Mumbai) at 10,000 rpm for 20 min to separate the plasma and used for biochemical assays. After drawing blood from fishes, they were washed with distilled water and blotted dry with absorbent paper. The liver was isolated from the control and experimental fishes. One hundred milligrams of each tissue were weighed and homogenized with 2.5 ml of 0.25 M sucrose solution in ice-cold condition. The homogenates were centrifuged at 6000 rpm for 20 min, and the clear supernatant fluid was taken for enzyme assay. The collected whole blood, plasma and the supernatant obtained from the liver samples were subjected haematological, biochemical and enzymological analyses.

Haematological, biochemical and enzymological analyses

RBC and WBC were counted by haemocytometer (Rusia and Sood 1992), packed cell volume was determined by microhematocrit reader (Nelson and Morris 1989) and the haemoglobin concentration was estimated by cyanmethemoglobin method (Drabkin 1946). Erythrocyte indices like MCV, MCH and MCHC were determined by calculation method (Blaxhall and Daisley 1973). Plasma glucose and plasma protein (Trinder 1969) and Doumas et al. 1971) and liver AST and ALT activities (Reitman and Franckel 1957) were estimated using spectrophotometer (Labman, Chennai).

Statistical analyses

The data obtained were statistically analysed by mean ± SD. The data obtained was statistically analysed by one-way analysis of variance ($P < 0.05$) using statistical software Sigma stat 3.1.

Histological examination

For histological investigation, with the fishes exposed to tap water, untreated and treated dye solutions were dissected to collect the tissues of the gills, liver and kidney. The dissected tissues were immediately fixed in 10% formalin and washed in distilled water, dehydrated in graded ethanol series (30%, 50%, 70%, 85% and 100%), infiltrated with xylene and embedded in paraffin wax at 56–60 °C. The tissues embedded in paraffin wax were sectioned using a rotator microtome (5 μm) and treated with xylene to remove paraffin and subsequently washed in 90%, 70%, 50% and 30% alcohol (Bancroft and Cook 1994). Finally, paraffin-free sections were washed with distilled water, stained with haematoxylin for 3 min and washed in running tap water for 1 min. Finally, the tissues were stained in eosin for 45 s, examined under microscope (Labomed, California) and photographed. The changes in the tissues of the experimental fishes were compared with the control fishes.

Results and discussion

Behavioural responses and mortality of *Labeo rohita*

The levels of the physico-chemical parameters analysed in the untreated and treated methyl orange dye solution were within the BIS limit prescribed for the discharge of industrial effluent (Alaguprathana and Poonkothai 2018). The *L. rohita* exposed to different treatments selected for the present study showed a variety of behavioural changes. The irregularities observed prior to mortality are a signal of depleted oxygen content due to high concentration of toxicant (Dahunsi and Oranusi 2012). Based on visual observation and the physical behaviour, the fishes exposed to tap water (T_1) exhibited normal swimming, vision and proper body motion whereas those exposed to untreated dye solution (T_2) initially showed abnormal movements such as erratic swimming, hyperexcitation and variation in opercular beat rates. After 24 h, the fishes became weaker, showed sluggish movement and started to settle at the bottom of the trough. At the end of 168 h, morphological changes such as reddening in the gills, faded body colour, gulping of air and shedding of scales from the skin were observed. Thus, methyl orange directly affects the fishes by reducing the reflex actions and equilibrium thereby causes abnormalities in growth and its behaviour. Similar such observation was noticed in *Oreochromis niloticus* exposed to acid dye 53 (Amwele et al. 2015) and *L. rohita* to direct green 6 (Barot and Bahadur 2013). Kane et al. (2005) also noticed the absorption of environmental pollutants onto the skin of fish causing damage in the dermal layer and thereby lead to shedding of the scales. The fishes exposed to treated dye solution (T_3) showed normal swimming and stable movement indicating its tolerance level and depicting the reduction in the toxicity of dye.

Labeo rohita being a fresh water fish can tolerate disturbances and contaminants in the aquatic environment, and the mortality depends on the sensitivity and duration of exposure to the toxicant (Cavas and Gozukara 2005). However, introduction of fishes into the toxicant medium leads to severe damage of organ systems and causes mortality (Suvetha et al. 2015). The percentage mortality of fishes was high in untreated dye solution (88%) when compared with fishes exposed to treated dye solution (33%) and tap water (1%), respectively (Fig. 1). Roopadevi and Somashekar (2012)

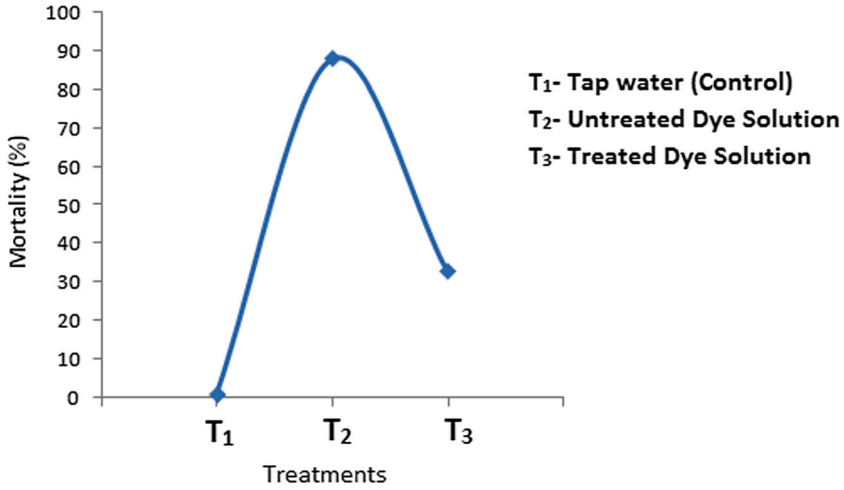

Fig. 1 Percentage mortality of *Labeo rohita* exposed to different treatments

observed the death of fishes stocked in higher effluent concentration for longer period, and also, the homeostatic behaviour of the test organism was eventually disturbed. This confirms with the results of the present study stating the toxicity of methyl orange to the experimental fish, *L. rohita*.

Haematological parameters

Blood is a pathophysiological reflector of the whole body, and therefore, its parameters are important in diagnosing the altered physiological status of fish exposed to toxicants. The haematological elements of fish are widely used as an indicator in toxicological research and environmental monitoring studies (Carvalho and Fernandes 2006). The results revealed that *L. rohita* exposed to untreated dye solution (T_2) showed a notable decrease in the levels of RBC, PCV, Hb and MCHC when compared with T_1 and T_3 fishes. The WBC, MCV and MCH levels increased in the T_2 fishes when compared with T_1 and T_3 fishes (Table 1). The haematological profile, plasma glucose and plasma protein levels and enzyme activities were significant ($P < 0.05$) in fishes exposed to treated dye solution when compared with untreated dye solution. Decrease in the haematological parameters in T_2 fishes depicts its anaemic condition which might have resulted from the haemolysis or damage caused in the gills leading to impaired respiratory capacity by the pollutant, methyl orange. The significant reduction of RBC content in T_2 fishes might be due to the failure of erythrocyte production, impaired osmoregulation, internal haemorrhage and inhibition of oxygen production during stress conditions (Kaoud et al. 2011; Kavitha et al. 2010). Tariq et al. (1996) observed an increase in the treatment and carbon dioxide content in the blood of fishes exposed to pollutants. He also noticed the swelling of RBCs, cytotoxic effect on erythropoietic tissues leading to disturbances in bone marrow and alteration in the cell cycle and erythropoiesis.

PCV is a measure of percentage of RBCs present in a volume of whole blood. Decrease in the PCV of fishes exposed to untreated dye solution may be due to anaemia or destruction of red blood cells or nutritional deficiency, disturbances occurred in metabolic and haemopoietic activities leading to impairment in haemopoietic organs (Sharma and Langer 2014). Thus, PCV appears to be positively correlated with erythrocyte count where decrease in the number of RBCs followed by PCV confirms anaemia in *Labeo rohita* (Palanisamy et al. 2011). The results are in agreement with the findings of Srivastav and Roy (2015) who observed a significant decrease in PCV of fishes exposed to malachite green when compared with the control and treated fishes.

Hb seems to be reliable and best blood indicator of environmental stress. Increase in its concentration could be a persistent sign of an adaptational improvement in oxygen-transporting capacity of blood (Khalesi et al. 2014). Lower level of haemoglobin in T_2 fishes might decrease the ability to enhance the activities required to meet demands such as seeking of food and escaping from predators (Barot and Bahadur 2013). The depletion or reduction in Hb content in T_2 fishes could also be attributed to the production of reactive oxygen species under the influence of toxicant resulting in the destruction of red blood cell membrane and its function, inhibition of the enzymes involved in Hb synthesis (Pamila et al. 1991), impaired intestinal absorption of iron or transferrin dysfunction (Joshi et al. 2002) and impairment in the immunological reactions to produce antibodies to cope up with stress induced by the toxicant (Ramdas 2013). Similar decrease in the amount of RBC, Hb and PCV could be corroborated with the findings of earlier investigations in *Tilapia mossambica* exposed to textile dyeing effluent (Deepika and Noorjahan 2018), *Catla catla* exposed to acid red 97 (Avni and Jagruti 2017) and *Carassius auratus gibelio* exposed to azo red 120 (Al-Sabti 2000), respectively.

Leucocytes or white blood corpuscles are cells of immune system which play a vital role in both specific and non-specific immune responses in protecting the body against toxicants. One of the most elementary ways to assess the immune

Table 1 Haematological, biochemical and enzymological analyses in *Labeo rohita* exposed to different treatments

Parameters analysed	Treatments		
	T_1	T_2	T_3
Haematological parameters			
RBC (million/cu mm)	1.42 ± 0.02	0.33 ± 0.07	0.96 ± 0.05
PCV (%)	16.31 ± 0.57	7.02 ± 0.21	15.69 ± 0.66
Hb (g/dl)	4.95 ± 0.32	1.67 ± 0.08	4.16 ± 0.24
WBC (1000/cu mm)	1.37 ± 0.13	2.23 ± 0.24	1.50 ± 0.02
Erythrocyte indices			
MCV (fl)	81.25 ± 4.80	167.11 ± 2.44	125.08 ± 5.35
MCH (pg)	26.04 ± 2.16	42.40 ± 1.63	33.67 ± 1.69
MCHC (g/dl)	23.86 ± 0.47	17.65 ± 1.63	20.45 ± 0.81
Biochemical parameters			
Plasma glucose (mg/100 ml)	56.37 ± 2.49	25.15 ± 1.63	55.76 ± 2.05
Plasma protein (μg/ml)	2.85 ± 0.12	1.59 ± 0.08	2.60 ± 0.16
Enzymological parameters			
AST (IU/L)	78.21 ± 2.86	143.69 ± 5.79	119.25 ± 11.2
ALT (IU/L)	15.22 ± 0.81	58.91 ± 0.70	36.73 ± 2.59

The values are mean ± SD. T_1—tap water (Control), T_2—untreated dye solution, T_3—treated dye solution. All the values are significant at 5% level ($p<0.05$)

system is to explore the changes in WBC count (Bujjamma and Padmavathi 2018). The WBC count was observed to be high in T_2 fishes when compared with T_1 and T_3 fishes where the count was decreased or lowered. Increase in the WBC count in T_2 fishes can be attributed to the stimulation of the immune system in response to tissue damage caused by the dye. Tiago et al. (2008) reported that the increase in antibody production helps in the survival and recovers the fishes exposed to toxicants. Significant increase in the total WBC count was observed in *Heteropneustes fossilis* exposed to malachite green and pyceze (Srivastav and Roy 2015) which supports the findings of the present investigation.

For examining the health status, blood index analyses have proven to be a valuable method, which provides reliable information on metabolic ailments, deficiency and chronic stress status. The RBC indices, namely, MCV, MCH and MCHC are the part of whole blood count, which express the size and Hb content of erythrocyte. MCV is one of the important blood parameters, which gives an indication of the status of the size of RBC and reflects the normal or abnormal cell division during erythropoiesis (Zhou et al. 2009).

In the present study, fishes exposed to untreated dye solution showed a higher level of MCV and MCH when compared with the control and treated fishes. This increase might be due to the enlargement or swelling of RBCs because of osmotic disturbances or hypotonic condition or uptake of electrolytes and water into the cells accompanied by acidification of the cytoplasm of RBCs leading to macrocytic anaemia in fishes (Suvetha et al. 2015). Ferrando and Moliner (1991) also reported that higher concentration of smaller immature erythrocytes in the circulation due to hyperplasia in erythrocyte forming sites leads to increased MCV. The decreased MCHC value in the present study may be due to the binding of the pollutant to Hb which prevents the oxygen-carrying capacity and leads to metabolic stress and death of fishes (Lemly 1993). These results coincide with the findings of Amte and Mhaskar (2013), Barot and Bahadur (2014) and Afaq and Rana (2009) who observed an increase in the values of MCV and MCH in *Oreochromis mossambicus*, *Labeo rohita* and *Cirrhinus mrigala* exposed to textile dyeing effluent, direct green 6, Bismarck brown and acid leather brown, respectively. In contrast, brilliant green treated with *Sargassum wightii* did not induce any observable hazardous health effects on the fish (Vigneshpriya et al. 2020). Thus, haematological parameters also determine the changes in the levels of biomarkers namely the enzymes, normal functioning and histomorphology of the organs.

Biochemical parameters

To monitor the presence of toxicants in aquatic media, biochemical analysis offers as an important bioindicator. Carbohydrates and proteins serve as nutrient and energy stores in all-biological processes, but under severe stress condition, they provide energy in metabolic pathways and biochemical reactions according to the need of an organism (Olaganathan and Patterson 2013). Plasma glucose has been extensively used as a sensitive biochemical indicator to study the stress of fish under unfavourable environment (Sancho et al. 2000). Glucose is one of the most important compounds of carbohydrates, which serve as an

immediate and major metabolic fuel for all the biological activities (Pal and Reddy 2018). The decreased level of plasma glucose in untreated fishes (T_2) might be due to the hypoxic condition caused by the toxicant that reflects an excess utilization of stored carbohydrate during treatment period (Agrahari et al. 2007). In addition, the accumulation of the untreated dye in the kidney may cause renal injury which in turn reflects the concentration of glucose during stress conditions. Protein is an important biochemical parameter, which has been used to understand the general state of health and biological mechanism and metabolism under the stress of a toxicant (Saravanan and Ramesh 2013). Decrease in the plasma protein in T_2 fishes might be due to necrosis or liver cirrhosis or alteration in the enzyme involved in the biosynthesis of proteins (Palaniappan and Vijayasundaram 2009). The decrease in the plasma glucose and plasma protein in the present investigation coincides with the studies carried out in *Clarias gariepinus* and *Oreochromis niloticus* exposed to textile dye industry wastewater (Agbon et al. 2014), *Oncorhynchus mykiss* treated with malachite green (Atamanalp 2007) and *Cyprinus carpio* with textile industrial effluent (Dhanalakshmi et al. 2018) respectively.

Enzymological parameters

Various physiological activities are regulated by the vital organ, the liver, which performs metabolism, storage, secretion and detoxification in fishes. Enzyme activities in the blood serum are considered an important biochemical indicator in hepatic dysfunction and damage (Jung et al. 2003). Hence, enzyme assays are widely used to assess the health of an organism in aquatic toxicology (Gul et al. 2004). Amongst the battery of enzymes, aspartate transaminase and alanine transaminase are widely used as pathological and potential markers to detect the function of liver or toxicant-induced hepatotoxicity (Huang et al. 2006). In the present study, the activities of serum AST and ALT were high in T_2 fishes when compared with T_3 and control fishes. When the fishes were exposed to untreated dye solution, the hepatic parenchyma cells were damaged, liberated AST and ALT into the blood stream, and their activities were elevated. Increased or enhanced AST and ALT indicated increased or active transamination of amino acids, possibly providing ketoacids which are utilized for energy synthesis through TCA cycle to favour gluconeogenesis (Beyer et al. 1996; Javed et al. 2016; Naveed et al. 2010; Neelimal et al. 2013).

Histopathological examination of *Labeo rohita*

Histological changes in the gill

Gills are a good indicator of water quality and possess respiratory, osmoregulatory and excretory functions. Absorption of pollutants from an aquatic medium through gills makes fish a vulnerable target to its toxicity (Fant et al. 2003). Histological observation on the gills of *L. rohita* exposed to tap water (T_1) showed normal architecture of gill filaments such as primary and secondary gill lamella (Fig. 2a). The gills of *Labeo rohita* fingerlings exposed to untreated dye solution (T_2) showed necrosis of secondary lamella and severe haemorrhage and erosion in the lamella of gills (Fig. 2b). These histopathological changes may be a reaction to pollutant intake or an adaptive response to prevent their entry through the gill surface. The necrosis of respiratory epithelial cells can adversely affect the gaseous exchange and ionic regulation in fishes. Such similar necrosis and haemorrhage in the gills of fishes exposed to untreated dye solution were also evidenced in *Catla catla* to reactive red 120 (Avni and Jagruti 2016) and *Labeo rohita* to textile mill effluent (Nikalje et al. 2012), respectively. However, the fingerlings exposed to treated dye solution did not show any deformities in the gills, and the regeneration of respiratory epithelium of the gills was observed (Fig. 2c). Such similar findings were reported in *Catla catla* exposed to treated sago effluent (Ramesh and Nagarajan 2014) and *Etheostoma olmstedi* exposed to *Bacillus pumilus*–treated textile wastewater (Watharkar et al. 2014), respectively.

Histological changes in the liver

The liver plays an important role in the detoxification of contaminants because it acts as a main storage organ for many substances. Hence, the accumulation of toxicants may affect its functions and thereby decreases the blood supply to all parts of the body (Nagai et al. 2002). The histology of liver in control fishes showed normal hepatocytes arrangement. The hepatocytes were located amongst the sinusoids that form a cord-like structure and possess large nuclei cords (Fig. 3a). No remarkable changes were observed in the hepatocytes of fishes exposed to treated dye solution, and the histoarchitecture was similar to that of control fishes (Fig. 3c). Fishes exposed to untreated dye solution showed vacuolar degeneration, sinusoidal congestion, necrosis and haemorrhage of hepatocytes (Fig. 3b). Intoxication of the pollutant in the fish changes the general architecture of liver indicating the degree of structural heterogeneity that enhances with the inhalation of the toxicant. The histoarchitecture of the fishes exposed to untreated dye is in confirmation with the findings of *Poecilia reticulata* exposed to textile dyeing industry effluent (Selvaraj et al. 2015), and *Channa punctatus* exposed to vat blue 4 and vat green 1 (Olaganathan and Patterson 2012), respectively.

Histological changes in the kidney

The kidney is a primary organ important for the elimination of waste and osmoregulation in fish. Histology of kidney in control fishes showed well-characterized glomeruli and renal tubules (Fig. 4a). Fishes exposed to untreated dye solution

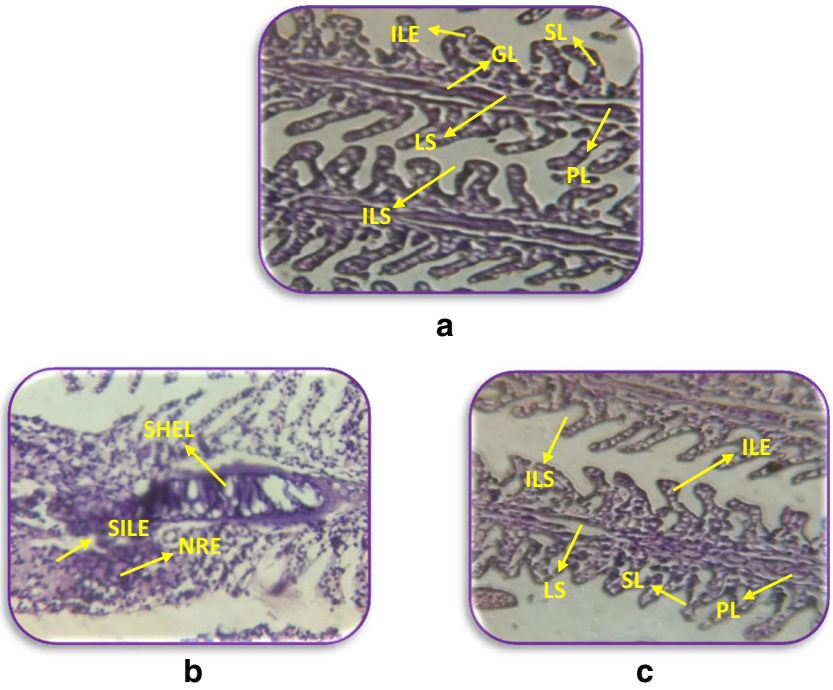

Fig. 2 Histology of gills exposed to control (T_1), untreated (T_2) and treated (T_3) methyl orange dye solution at × 45 magnification (PL—primary lamellae, SL—secondary lamellae, ILE—inter-lamellar epithelium, ILS—inter-lamellar space, LS—lamellar space, GL—gill filament, SILE—swelling of inter-lamellar epithelium, NRE—necrosis of respiratory epithelial cells, SHEL—severe haemorrhage and erosion in the lamella of gills and RRE—regeneration of respiratory epithelium). **a** Gill section of *L. rohita*—control (T_1). **b** Section of gills in *L. rohita* exposed to untreated dye solution (T_2). **c** Section of gills in *L. rohita* exposed to treated dye solution (T_3)

showed highly degenerative and necrotic changes in the renal tubules and congested and shrunken glomeruli (Fig. 4b). Degeneration of renal tubules and necrotic changes in the kidney has also been observed in *Labeo rohita* exposed to direct green 6 (Barot and Bahadur 2013), *Clarias lazera* exposed to dyestuff wastewater (Abdel-Moneium et al. 2008), *Cyprinus carpio* exposed to textile industrial effluent (Dhanalakshmi et al. 2018) and *Labeo rohita* exposed to textile dyeing effluent (Rana and Raizada 2000), respectively. El-Neweshy and Srag (2011) observed necrotic changes in the tubular epithelium of the kidney exposed to acid orange 7. The intoxication of the dye may result in necrotic cell death, which

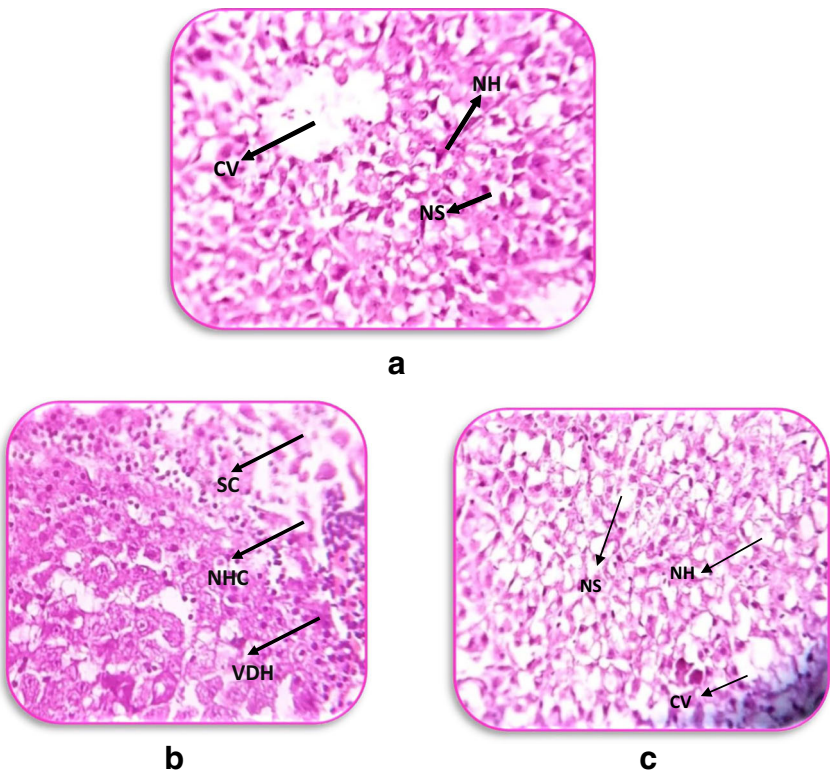

Fig. 3 Histology of liver exposed to control (T_1), untreated (T_2) and treated (T_3) methyl orange dye solution at × 45 magnification (CV—central vein, NH—normal hepatocytes, NS—normal sinusoids, SC—sinusoidal congestion, NHC—necrosis of hepatic cells, VDH—vacuolar degeneration of the hepatocytes). **a** Liver section of *L. rohita*—control (T_1). **b** Section of liver in *L. rohita* exposed to untreated (T_2) dye solution. **c** Section of liver in *L. rohita* exposed to treated (T_3) dye solution

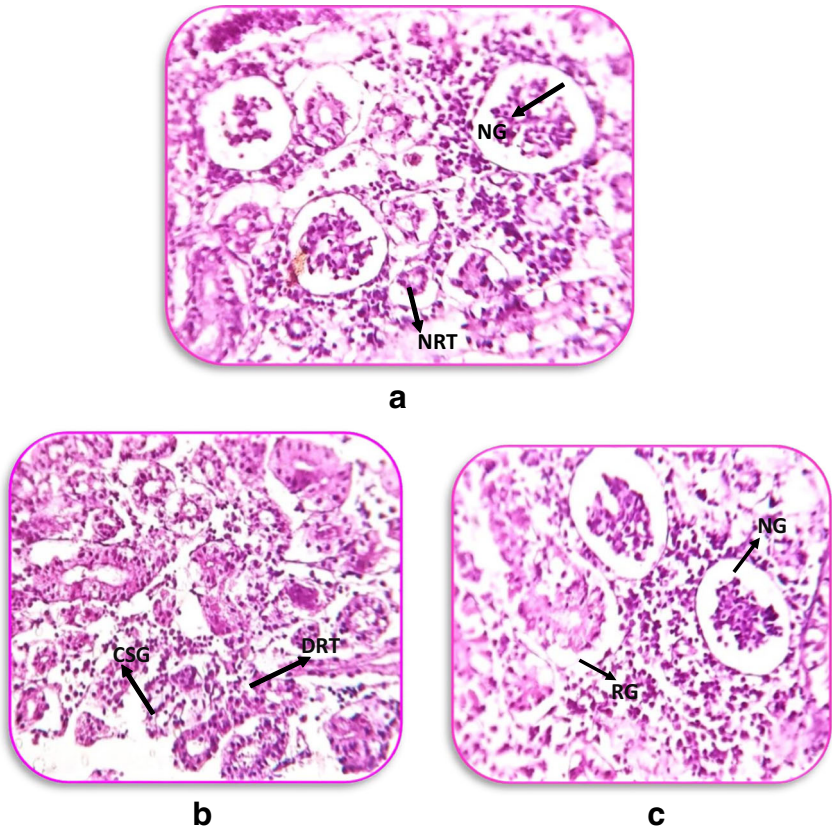

Fig. 4 Histology of kidney exposed to control (T_1), untreated (T_2) and treated (T_3) methyl orange dye solution at × 45 magnification (NG—normal glomeruli, NRT—normal renal tubules, DRT—degeneration of renal tubules, CSG—congested and shrunken glomeruli, RG—regeneration of glomeruli). **a** Kidney section of *L. rohita*—control (T_1). **b** Section of kidney in *L. rohita* exposed to untreated (T_2) dye solution. **c** Section of kidney in *L. rohita* exposed to treated (T_3) dye solution

might be caused by the swelling and eventual failure of cell membrane, leading to rupture of the renal cells and release of its content into the extracellular space. The renal tubules and glomeruli were regenerated in the fishes exposed to treated dye solution (Fig. 4c). The structural features were found to be alike of those observed in the control fishes. Literature on haematological, biochemical, enzymological and histological parameters analysed in fishes exposed to treated dye solution are meagre, and the present study forms a platform to carry out research in this area.

Conclusions

The present study infers that methyl orange treated with *O. subplagiostomum* AP1 does not have profound influence on haematological, biochemical and enzymological profiles of the Indian major carp *L. rohita*, hence portraying the less toxic nature of the degraded products. On the contrary, unusual conditions were noticed in fish exposed to untreated methyl orange indicating the toxic nature of the dye. Histopathological lesions in the gills, liver and kidney tissues of fish exposed to untreated dye solution might be due to the stress induced by the dye. The parameters studied could be utilized as potential biomarkers in assessing the toxicity of the freshwater fish in the field of environmental biomonitoring and can also be used as a warning indicator for dye exposure to aquatic fauna. Thus, methyl orange–treated *O. subplagiostomum* AP1 could be explored for aquacultural purpose and paves a platform for an ecofriendly approach to mankind.

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Author contributions MP contributed to the study conception and design. MA performed experiment and data collection. The data was analysed and interpreted by MP and MA. The draft of the manuscript was written by MP. All authors read and approved the final manuscript.

Data availability All data generated or analysed during this study are included in this published article.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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