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Ameliorative effects of Turmeric (*Curcuma longa*) on Sawdust Effluent-induced physiological stress in *Heteroclarias*

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ABSTRACT

The release of sawdust effluent into the aquatic environment increases turbidity and induces oxidative stress in fish. This study investigated the ameliorative effects of stresses induced by sawdust effluent in *Heteroclarias*. Juveniles *of Heteroclarias* were exposed to lethal concentrations (1.0, 2.0, 3.0, 4.0, and 5.0 g/l) of sawdust effluent for 96 h and fed on turmeric (0, 0.5, 1, 2, and 2.5%) for 30 days. After exposure periods, the 96 h LC_{50} was 3.1g/l, blood and organs were collected for hematological and biochemical assays. Activities of Alanine aminotransferase (ALT); Aspartate aminotransferase (AST); Lactate dehydrogenase (LDH); Acetylcholinesterase (AChE); Superoxide dismutase (SOD); Catalase (CAT); Glutathione peroxidase (GPX); melanodehydehyde (MDA), total protein and glucose increased ($P < 0.05$) in Group F compared to control Group A. The red blood cell (RBC), white blood cell (WBC), hemoglobin, hematocrit, lymphocytes, and platelets decreased ($P < 0.05$) as the concentration of toxicant increased. However, enzyme activities and haematological levels revealed a gradual reduction and increase in Groups C, D & E fed 1%, 2%, and 2.5% turmeric respectively. While a great reduction and elevation ($P < 0.05$) was, observed in Group B fed 0.5% turmeric respectively. This implies that 0.5% turmeric supplementation in diet is capable of minimizing oxidative stress in sawdust-exposed-*Heteroclarias*.

Keywords: Amelioration, Biochemical, Haematology, *Heteroclarias*, Sawdust Effluent. Turmeric.

INTRODUCTION

Fish serves as a source of protein nutrients, providing about 3.0 million people with almost 20% of their animal protein and improving national food security (FAO, 2012). However, the population of fish species has been greatly reduced as a result of the adverse effects of different contaminants released from domestic, industrial, and agricultural sectors, which bio-accumulate in the tissues of the fish. Amongst these contaminants, is sawdust that is released from the sawmill industry into the nearest water body (Okoh et al., 2015).

The occurrence of this sawdust causes changes in the physical properties of water by changing the colour, with an unpleasant smell and suspended particles, which increase the turbidity that eventually makes the water lose its purity, hence reducing some physicochemical properties like dissolved oxygen (Idise et al., 2012). A reduction in the dissolved oxygen in the water body will affect the respiration rate and induce oxidative stress in fish.

Suspended particles of sawdust tend to spread on the surface of water bodies and reduce the penetration of sunlight into the water, thereby reducing the photosynthetic activities of phytoplankton. Sawdust could contain trace metals like Lead, Chromium, Arsenic, Mercury, and Barium depending on the preservatives or chemicals added to the wood during growth, processing, and preservation (Ali and Khan, 2018).

Bioaccumulation of these heavy metals in the tissues of aquatic organisms, especially fish,

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when in excess in the water bodies could cause damage to the tissues of the fish and have adverse effects on the functions of these tissues, which cause oxidative stress that could eventually lead to fish death (Ju-Wook and Jun-Hwan, 2019). Fish exposure to sawdust have been reported to either caused hyperactivity and induce the production of reactive oxygen species (ROS), or altered the erythrocytes levels in different fish species.

Turmeric (*Curcuma longa*) is a widely used antioxidant that react with free radicals, and thus, protects animals against stress. Studies have shown that dietary supplementation with turmeric is very effective in ameliorating the effects of pollutants on fish. Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant of the ginger family, *Zingiberaceae* with an active ingredient called Curcumin that makes it a potent antioxidant with hepatoprotective properties (Pal et al., 2001; El-Bahr, et al., 2007; Salama and El-Bahr, 2007; Chan , et al., 2009). The main component of the root is a volatile oil, containing turmerone and there are coloring agents called curcuminoids in turmeric, which can improve digestion and nutrient metabolism (Al-Sultan and Gameel, 2004).

A dearth of information, however, exists on sawdust toxicity in catfish and the sub-lethal effects of sawdust exposure, and potential ameliorative effects of turmeric are yet to be understood. This study, therefore, attempts to fill the information gap by examining the biochemical, haematological and growth responses of sawdust in *Heteroclarias* and ameliorative potentials of turmeric through evaluation of erythrocytes and leucocytes levels and enzymes activities in the blood, gills and liver and determination of growth rate of *Heteroclarias* exposed to sawdust and ameliorated with turmeric. *Heteroclarias* is a hybrid catfish with high efficient air breathing organ, which allows them to survive in oxygen depleted water (Anyanwu, 2009).

MATERIALS AND METHODS

Experimental Setup

Juveniles *Heteroclarias* (average weight 18.40 ± 2.75 g and average length 11.37± 1.14 cm) were purchased from a fish farm in Ilorin, Kwara state, and transported in the early hour of the day to prevent stress that could lead to mortality. They were acclimatized in the Department of Zoology Laboratory for 14 days according to USEPA (1996). These fish were not fed before and after transportation to avoid indigestion. The pond water was partly replaced by an aerated chlorine-free borehole water. The water was completely renewed after 24 hours, and feeding commenced with commercial feeds (diameter 2 mm) at 4% of initial body weight twice daily (Bekeh, *et al*., 2017). The renewal continued after every 24 hours with aerated borehole water, where faeces and unconsumed feed were removed to prevent disease outbreaks and mortality, and the behavioural activities of the fish were monitored throughout the experimental period. Water quality parameters, such as temperature, hydrogen ion concentration (pH) and dissolved oxygen (DO), Biochemical oxygen Demand (BOD); Chemical oxygen Demand (COD), Turbidity, Conductivity, and total suspended solids were measured following the standard method of water assessment by APHA (2005).

Turmeric Collection and Processing

The turmeric was purchased from a local market in Ilorin, Kwara state, Nigeria. The turmeric rhizomes were thoroughly washed, pounded with a pestle in a mortal and air-dried for seven days in the Laboratory. The dried coarse turmeric was grinded in an electric grinder to make powder and kept in a tightly tied polythene bag prior to formulation of fish diet.

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Feed Formulation

Five different experimental diets with graded levels of turmeric powder (0.0, 0.5, 1.0, 2.0 & 2.5%) were prepared and fed on six different groups of fish for 30 days. The diet was formulated with various measured ingredients such as fish meal (30%), maize corn (30%), groundnut cake (18%), toasted soya bean (20%), vitamin C (0.2%), methionine (0.4%), lysine (0.4%), salt (0.2%), premix (0.2%), turmeric (0%). These ingredients were weighed, mixed, grinded, milled and pelleted with mincers of 2 mm die. The pellets were air-dried and packaged in separate polythene bags and kept in a dry place for later use. (8). Each of the six groups (A -F) was fed on different diets formulated with varying percentage inclusions of turmeric. Group A (control) was fed on 0% turmeric, and groups B, C, D & E were exposed to sawdust effluent (3.1 g/l-1/10 LC_{50}) and fed on 0.5, 1.0, 2.0 & 2.5% turmeric respectively, and group F was exposed to 1/10 of Sawdust effluent and fed on 0% turmeric.

Chronic Toxicity Test (Ameliorative Effect

Based on the estimated value of LC_{50}, the fish exposed to $1/10^{th}$ LC_{50} value and fed on diets with different percentage inclusions (0.0, 0.5, 1.0, 2.0 and 2.5%) of turmeric for 30 d. The toxicant was renewed every 24 hours to maintain the toxicity level of the toxicant, while the faecal and unfed diet were removed to avoid pollution and fed at 4% of their body weight (Olusola and Nwokike, 2018). Fish from each aquarium were weighed on the 7th, 14th, 21st and 28th day to determine the growth rate. After each experiment, blood was collected from five fish in each group and dissected to remove gills and liver for biochemical and haematological assays.

Biochemical Assay

The tissues were homogenized and centrifuged at 4,700g (4°C) for 20 minutes to obtain supernatant. Activities of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) enzymes in the tissues were analyzed using the Reitman and Frankel method or Colorimetric method (Wright et al., 1970). Deutshe Gessellschaft fur kilns method was adopted for the analysis of lactate dehydrogenase (LDH), Superoxide dismutase (SOD), acetylcholinesterase (AchE) and malondialdehyde (MDA). Glucose and Total protein were analysed according to the methods of Mistra and Fridovich (1972).

Haematological Assay

Blood samples were collected from both the control and the exposed groups following the procedure of Owolabi and Abdulkareem (2021). The haematological parameters such as RBCs and WBCs were estimated with the use of Neubauer haemocytometer as described by Dacie and Lewis (2001). Haemoglobin (HB) was evaluated by the cyanmethemoglobin procedure (Blaxhall, and Daisley, 1973), while packed cell volume (PCV) was determined following the microhaematocrit method (Morris, & Davey, 2001). Erythrocyte indices: mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were evaluated by using Dacie and Lewis, 2001) formulae.

Growth

The specific growth rate was estimated using the formula of (Pack, (1991):

$$r^3 = \frac{\log Wt_2 - \log Wt_1}{t_2 - t_1} \times 100$$

Statistical Analysis

Analysis of variance (ANOVA) and Duncan's (20) multiple range test were used to test for differences in the treatment levels and to separate means respectively. The test of significance was at 95% ($P < 0.05$).

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RESULTS

Activities of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and antioxidant enzymes superoxide dismutase (SOD); and glutathione peroxidase (GPX) were significantly ($P < 0.05$) elevated in the group exposed to sawdust without turmeric (Group F), compared to the control group (A). In contrast, gradual reductions in enzyme activities were observed in groups supplemented with increasing concentrations of turmeric (C, D, E), with significant ($P < 0.05$) reductions in Group B that received 0.5% turmeric (Figures1-6). Activities of antioxidant enzymes significantly ($P < 0.05$) increased in sawdust-exposed-fish after 30 days and the increase is time dependent compared to control. There was insignificant ($P > 0.05$) reduction in the activities of the enzymes in the ameliorative groups, except in Group B that was fed on 0.5% turmeric with a great ($P < 0.05$) reduction in the enzymes activities compared to Group F that was fed on 0% turmeric (Figures 1 - 6).

The levels of malondialdehyde (MDA), total protein, and glucose increased ($P < 0.05$) in-Group F exposed to sawdust only compared to control Group A (Figures 7 -10). While a reduction in the activities of total protein and glucose in the group fed 0.5%, turmeric compared to the control Groups A and F was recorded.

The red blood cell (RBC), haemoglobin, and haematocrit decreased ($P < 0.05$), while the white blood cell (WBC), lymphocytes and platelets increased ($P < 0.05$) as concentration of sawdust increased (Figures 11 - 12). However, the erythrocytes and leucocytes levels revealed gradual increase and decrease in Groups C, D & E fed 1%, 2% and 2.5% turmeric respectively, while a significant ($P < 0.05$) increase and decrease were observed in Group B fed 0.5% turmeric respectively (Figures 11 -12). The erythrocytes levels increased ($P < 0.05$) in Group B compared to those of Group F with reduced levels of erythrocytes (Figures 11-12).

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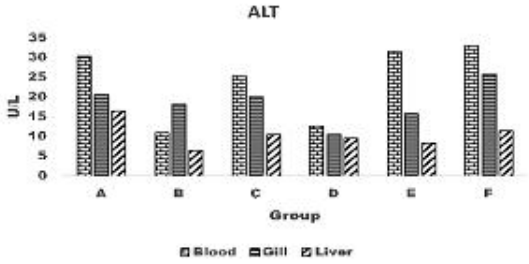

Figure 1: Alanine aminotransferase (ALT) level in the serum, gills and liver in *Heteroclarias* exposed to sub-lethal concentration of sawdust and ameliorated with turmeric for the period of 30 days.

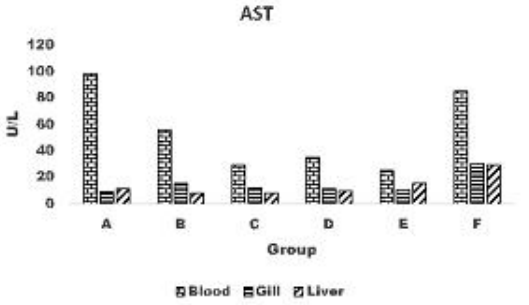

Figure 2: Aspartate aminotransferase (AST) level in the serum, gills and liver in *Heteroclarias* exposed to sub-lethal concentration of sawdust and ameliorated with turmeric for the period of 30 days.

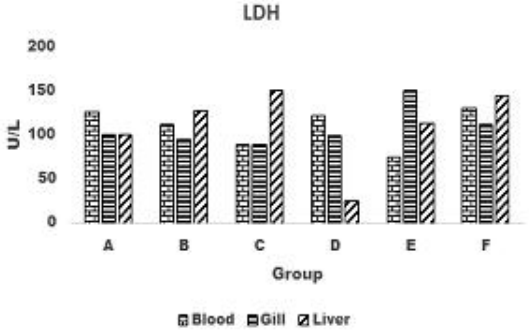

Figure 3: Lactate dehydrogenase (LDH) level in the serum, gills and liver in *Heteroclarias* exposed to sub-lethal concentration of sawdust and ameliorated with turmeric for the period of 30 days.

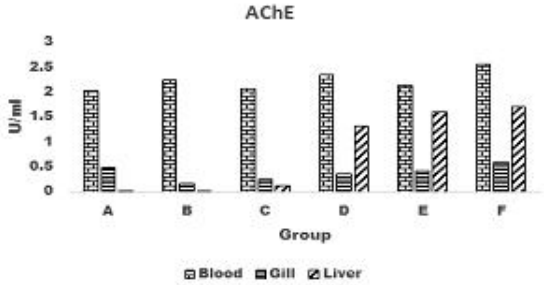

Figure 4: Acetylcholinesterase (AChE) level in the serum, gills and liver in *Heteroclarias* exposed to sub-lethal concentration of sawdust and ameliorated with turmeric for the period of 30 days.

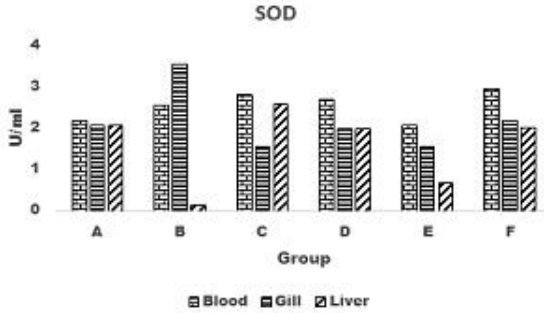

Figure 5: Superoxide dismutase (SOD) level in the serum, gills and liver in *Heteroclarias* exposed to sub-lethal concentration of sawdust and ameliorated with turmeric for the period of 30 days.

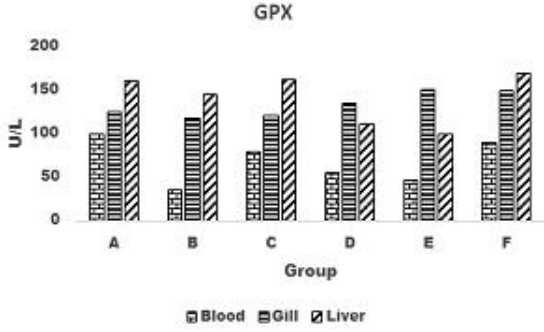

Figure 6: Glutathione peroxidase (GPX) level in the serum, gills and liver in *Heteroclarias* exposed to sub-lethal concentration of sawdust and ameliorated with turmeric for the period of 30 days.

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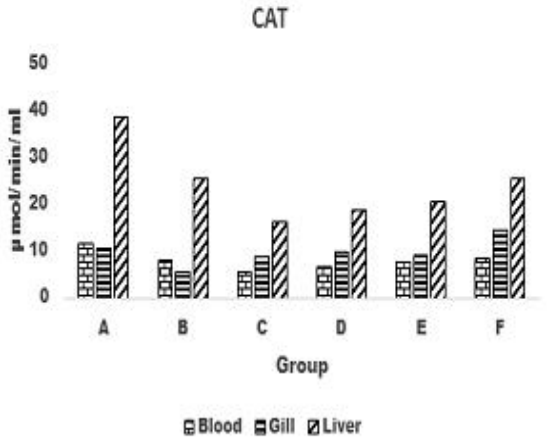

Figure 7: Catalase (CAT) level in the serum, gills and liver in *Heteroclarias* exposed to sub-lethal concentration of sawdust and ameliorated with turmeric for the period of 30 days.

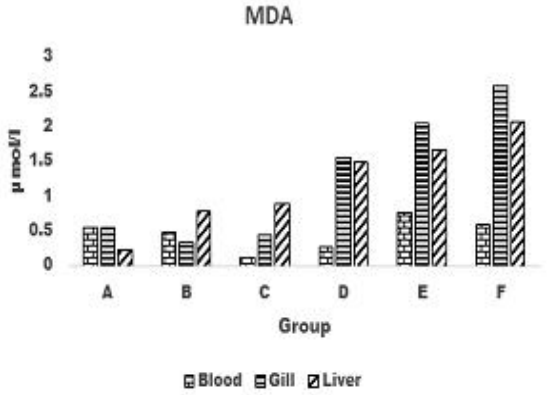

Figure 8: Malondialdehyde (MDA) level in the serum, gills and liver in *Heteroclarias* exposed to sub-lethal concentration of sawdust and ameliorated with turmeric for a period of 30 days.

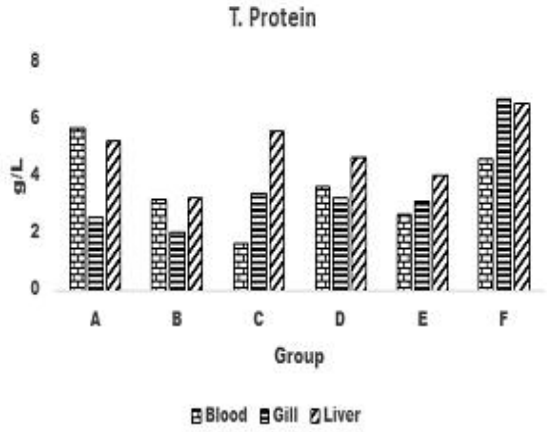

Figure 9: Total Protein (T. Protein) level in the serum, gills and liver in *Heteroclarias* exposed to sub-lethal concentration of sawdust and ameliorated with turmeric for a period of 30 days.

Figure 10: Glucose level in the serum in *Heteroclarias* exposed to sub-lethal concentration of sawdust and ameliorated with turmeric for the period of 30 days.

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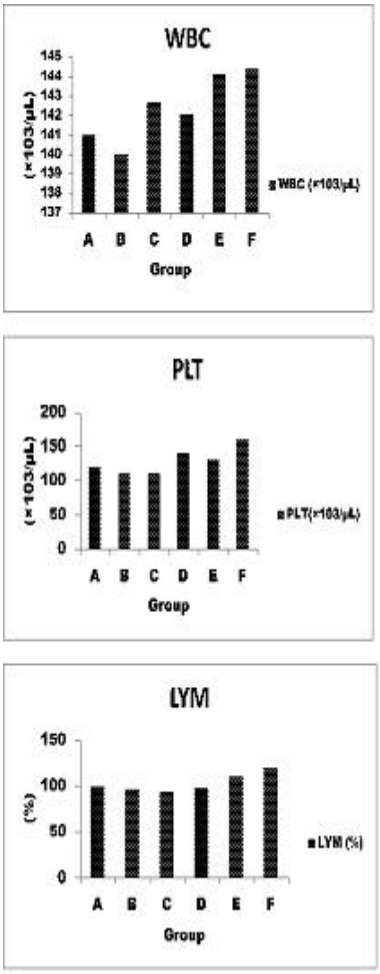

Figure 11: Erythrocytes of *Heteroclarias* juvenile exposed to sub-lethal concentration of sawdust and ameliorated turmeric powder for the period of 30 days.

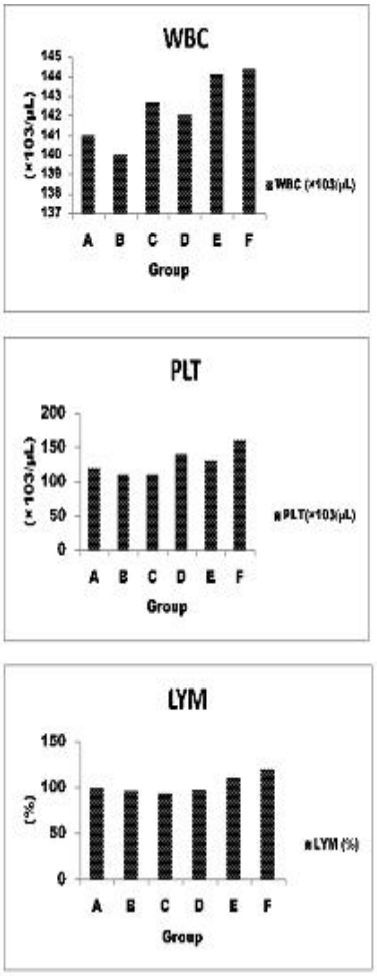

Figure 12: Leucocytes of *Heteroclarias* juvenile exposed to sub-lethal concentration of sawdust and ameliorated turmeric powder for the period of 30 days.

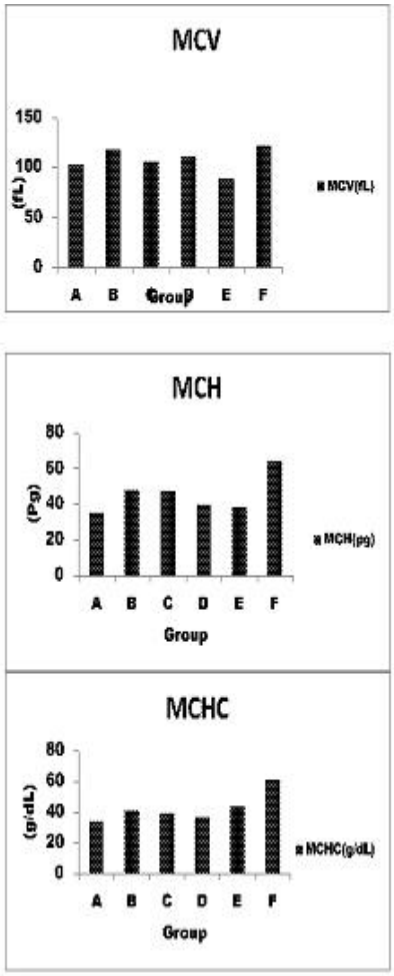

Figure 13: Haematological indices of *Heteroclarias* juvenile exposed to sub-lethal concentration of sawdust and ameliorated turmeric powder for the period of 30 days.

N.B: Normal feed (A), Sawdust (3.1 g/l) + 0.5% turmeric diet (B), Sawdust (3.1 g/l) + 1% turmeric diet (C), Sawdust (3.1 g/l) + 2% turmeric diet (D), Sawdust (3.1 g/l) + 2.5% turmeric diet (E), Sawdust (3.1 g/l) + normal feed (F).

There was significant ($P < 0.05$) reduction in the growth rate of Group F exposed to sawdust with 0% turmeric compared to control Group A that was also fed on 0% turmeric without exposure to sawdust. However, a gradual elevation in the growth rate of the sawdust-exposed fish fed on 1.0, 2.0, 2.5% were recorded, while a significant ($P < 0.05$) increased occurred in the Group B fed on 0.5% turmeric (Figure 14).

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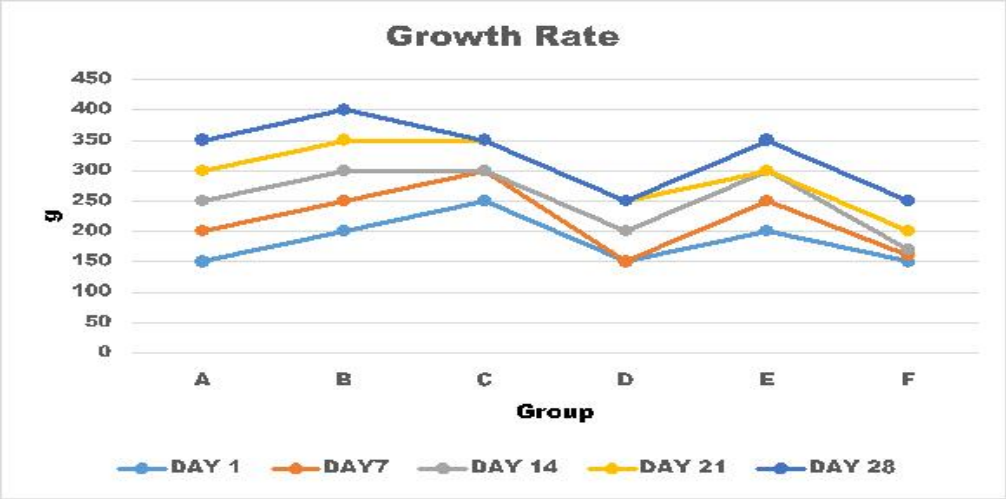

Figure 14: Growth rate of *Heteroclarias* juvenile exposed to sub-lethal concentration of sawdust and ameliorated with turmeric for 30 d.

N.B: Normal feed (A), Sawdust (3.1 g/l) + 0.5% turmeric diet (B), Sawdust (3.1 g/l) + 1% turmeric diet (C), Sawdust (3.1 g/l) + 2% turmeric diet (D), Sawdust (3.1 g/l) + 2.5% turmeric diet (E), Sawdust (3.1 g/l) + normal feed (F).

DISCUSSION

Elevated ALT and AST enzymes activities in *Heteroclarias* exposed to sawdust likely indicate hepatic damage resulting from metal bioaccumulation (Kaoud, *et a*l., 2011). The Increased LDH suggest stress response due to oxygen depletion. LDH is an anaerobic glycolytic enzyme that converts pyruvate and lactate using NAD as co-enzyme (Abdulkareem and Utuedor, 2016).

Increased antioxidant enzymes SOD and GPx in the blood stream as a result of oxidative stress induced by the production of reactive oxygen demand (ROS) triggered by the accumulation of metals. Increased levels of the SOD activities could be due to defense mechanism of SOD to detoxify ROS and radicals to H_2O_2, and conversion of H_2O_2 to $2H_2O$ and release oxygen by GPx.

Increased MDA indicates the extent of lipid peroxidation (Destruction of membrane lipids peroxide production). Increased antioxidant enzymes could be an antioxidant defense mechanism needed to protect biomolecules from the harmful effect of ROS (Madhuri et al., 2014) and to quench oxyradicals (Orbea Amaia et al., 2000).

The significant decrease in biochemical and antioxidant enzyme activities in fish-fed turmeric implies its protective role against oxidative stress. The antioxidant activities of turmeric could be due to the presence of curcumin which is the active ingredients that play the role of antioxidants (El-Bahr, et al., 2007; Salama and El-Bahr, 2007).

Decreased glucose level could be due to the breakdown of glucose to build up energy to enable the fish to withstand the stress situation. Increased glucose levels may indicate stress coping mechanisms, whereby energy is diverted to manage the toxic effects of sawdust

The decreased level of the total serum protein could be due to breakdown of protein as an alternative source of energy or liver damage,

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which inhibits protein synthesis as a response to the toxicity of sawdust.

Improvement in the levels of glucose and total protein in the group fed on 0.5% turmeric diet could attribute to the capability of 0.5% turmeric diet to reduce stress induced by sawdust, thereby reducing the use of glucose and breakdown of protein for energy purpose. The capability of medicinal plants to neutralize the toxicity of contaminant is similar to reports of some of our works where *Clarias gariepinus* was exposed to Dichlorvos and ameliorated with *Moringa oliefera* (Madhuri *et al*., 2014). This therefore helps to improve the immune system of the fish under the stress of sawdust and its metal components.

The ability of turmeric to reduce the stress induced by sawdust depends on the percentage supplementation of turmeric in the diet. The lower the percentage inclusion of turmeric in the fish diet, the more the immune system and the less the stress, thereby minimizing the breakdown of glucose. Decreased erythrocytes could be due to structural damage of the liver that affect the production of red blood cells and bioaccumulation of the metals in the blood stream could destroy the red blood cells, leading to reduction in the blood counts. Increase in the white blood cells could be attributed to the production of ROS that helped to recruit more WBCs to attack the pollutant and strengthened the affected cells. Increase in the levels of platelet and lymphocytes could be due to the production of ROS in response to toxicity of the contaminant (Abdulkareem *et al*., 2021).

The improvement and reduction in the levels of erythrocytes and leucocytes in the group fed on 0.5% turmeric diet could be attributed that 0.5% turmeric supplementation in the fish diet is capable of neutralizing the toxic effects of sawdust, reducing stress and improving the immune system of the fish respectively. Similar ameliorative effects was reported in vitamin E that was fed on *Heteroclarias* exposed to chlorpyrifos (Abdulkareem *et al*., (2021). The reduction in the growth rate and the specific growth rate could be due to hyperactivity that lead to excessive use of glucose and protein for energy purposes to escape the sawdust-polluted water. Avoidance of the sawdust particle in the water body by the fish could contributed to their inability to feed well, thereby affecting the growth of the fish. While the great improvement in the growth rate and the specific growth rate in the group fed on turmeric diet could indicate that 0.5% turmeric diet is capable of neutralizing the toxic effect of sawdust that prevented normal growth.

The enhanced growth in the group fed on turmeric could be attributed to the higher specific enzymes of digestive enzymes in turmeric (Rojtinnakorn, *et al*., 2012). This result corroborate with the increased growth in *Cirrhinus mrigala* fed on turmeric that enhanced immune system (Sivagurunathan e*t al*., 2011). Mahmoud *et al*., (2014) also reported enhanced growth in *Oreochromis niloticus* fed on turmeric because of improved feed consumption and feed utilization that indicated increased nutrient digestibility because of antioxidant activity of turmeric. This is also following the reports of Abdulkareem and Utedor, (2016) and Abdulkareem *et al*., (2021) who fed C*larias gariepius* and *Heteroclarias* with *Moringa oliefera* and vitamin E when exposed to Diclorvos and Chlorpyrifos respectively. The results of this study therefore indicate that 0.5% turmeric inclusion in the diet of *Heteroclarias* is capable of neutralizing the toxic effects of sawdust and minimizing oxidative stress in sawdust-exposed-*Heteroclarias* juveniles. \

CONCLUSION

Sawdust effluent induced oxidative stress and altered the physiology of *Heteroclarias,* but

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0.5% supplementation of the fish diet with turmeric ameliorated the toxic effects in the fish. This therefore, indicates that 0.5% turmeric supplementation in the diet of *Heteroclarias* is capable of neutralizing the toxic effects of sawdust and minimizing oxidative stress in sawdust-exposed-*Heteroclarias* juveniles.

Induction of oxidative stress stimulated the production of reactive oxygen species, which may in turn cause DNA damage. Further investigation on the effect of sawdust on the quality of DNA in *Heteroclarias* is therefore, suggested.

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