TOXICOLOGY | RESEARCH ARTICLE

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Evaluation of abamectin induced hepatotoxicity in Oreochromis mossambicus

Shweta Kushwaha1,2, Isha Anerao1,3, Shweta Rajput1, Poonam Bhagriya1 and Hetal Roy1*

Abstract: Abamectin (ABM) is a naturally fermented product of Streptomyces avermitilis. It is used to control pests in livestock and agriculture. In the present study, it has been hypothesized that intoxication of ABM to Oreochromis mossambicus impairs the function of the hepatocyte. Fishes were exposed to ABM with 40, 45, and 55 ppb for 48 h. Test animals were observed at regular intervals of time and sacrificed at the end of the regimen. Liver function tests, oxidative stress parameter, and histopathological alterations were taken into account to analyze hepatotoxicity induced by the test compound. Plasma transaminase activities were increased significantly in all the treated groups. The activity of lipid peroxidation was measured higher due to ABM intoxication, whereas catalase activity was depleted. The marked focal necrotic alteration was examined in liver tissue. The low-dose group showed a less adverse effect on liver, whereas the medium and high dose induced moderate-to-severe hepatotoxicity. Data from this study demonstrate that ABM exposure generates reactive oxygen species (ROS) and alter liver function of fishes, which may lead to liver necrosis. The authors’ emphasis on the regulatory use of ABM to protect fish health against ABM-induced toxicological effects.

Subjects: Environmental and Ecological Toxicology; Pesticides toxicity; Hepatotoxicity

Keywords: Abamectin; hepatotoxicity; reactive oxygen species; transaminase; histopathology

1. Introduction

Abamectin (avermectin B1, ABM) is a family of macrocyclic lactones produced by the soil microorganism Streptomyces avermitilis and used as a pesticide for crop protection (Abd-Elhady & Abou-Elghar, 2013; Boonstra et al., 2011; Chung et al., 1999). ABM comprises of two active components,
Avermectin B1a (≥80%) and avermectin B1b (≤20%), which affect insect nervous system by acting on GABAergic neurons (Maioli et al., 2013; Raftery & Volz, 2015). The toxicological properties of ABM are due to these two active components that affect inhibitory synapses via a mode of action involving glutamate-sensitive chloride channels (Yoon et al., 2004). On the scale of toxicity, ABM is classified under class II category of pesticide and used extensively all over the world although ABM is more toxic than ivermectin (Kolar et al., 2008; Mossa et al., 2018).

The intoxication of ABM not only impairs coordination of neurons but also affects the function of hepatocytes and induces liver toxicity (Hsu et al., 2001; Terali et al., 2018; Zanoli et al., 2012). Higher activity of serum aminotransferase was measured in the rat after oral intoxication of ABM (Abd-EIhady & Abou-Elghar, 2013; Hsu et al., 2001; Mossa et al., 2018). The study reports of Kennedy et al. (2014) suggested that ABM exposure alters the fish physiology. Neuronal degeneration and liver dysfunction were the results of ABM exposure to cyprinids fish (Thiripurasundari et al., 2014). Moreover, the accumulation of ABM in liver tissue was confirmed by Howells and Sauer (2001).

Aquatic ecotoxicological research for ABM has become very important since its residue regularly enters the aquatic environment after application on a crop by drift or runoff. The presence of ABM in the aquatic body as well as in fish body (ABM accumulates as it is lipophilic) was quantified for ecotoxicity measure (Novelli et al., 2012). Fish play an important role to maintain the aquatic ecosystem. It is a key organism for the food chain and biomagnifications that is consumed by human also. Fengmei et al. (2011) quantified the presence of avermectin in fish sample that clarify the accumulation of ABM pesticide in fish which is one of the food sources of human. Furthermore, expansion of fish industries suggests increased demands for fish as a source of animal protein. Hence, there is a need for analyzing ABM-induced toxicity in the fish, although it is a non-target organism. Xenobiotic acquisition from any route of exposure comes to the liver for biotransformation as it is primary site of detoxification and facilitated clearance. During high level of metabolism, oxidative stress is generated as well as secondary metabolites make liver possible target of damage (Hong et al., 2016; Lushchak et al., 2018). It is also known that acute liver intoxication is also associated with chronic pesticide exposure which results into irreversible alteration in liver tissue and modulate whole body homeostasis (Wahlang et al., 2013). This study, therefore, aims to evaluate the hepatotoxicity of ABM to provide the first detailed description of liver pathology in O. mossambicus during ABM exposure.

2. Material and methods

2.1. Fish maintenance

Oreochromis mossambicus, commonly known as Tilapia (weighing 30 ± 3 gm and 15 ± 2 cm length), used in this study was obtained from the pure brooders. Glass tanks containing 40 l of de-chlorinated tap water (temperature 27 ± 4 °C, dissolved oxygen 7.3 ± 0.5 mg/L, pH 7.4) was used. Ten fishes were placed in each tank and then acclimatized to environment for 15 days. The fishes were fed daily with commercial fish pellets equal to 2.5% of their body weight. A commercial formulation of ABM benzoate (Proclaim—5.27% SG, manufactured by Syngenta Agri. Crop Ltd., Batch No. PGSC000002) was purchased from a local distributor. Tilapia was separated into four groups: (1) control (C); (2) low dose (40 ppb, LD); (3) medium dose (45 ppb, MD) and (4) high dose (55 ppb, HD). The fishes were exposed to calculated (1/10th (LD), 1/20th (MD) and 1/30th (HD) concentration of LC50 value of ABM) concentrations of ABM for 48 h under controlled laboratory conditions. At the end of the treatment period, fishes were sacrificed and tissues were collected from each fish for biochemical estimation and analysis of histopathology.

2.2. Behavioral study

At regular interval of time, the behavior of fishes was recorded after ABM exposure to analyze the toxicant induced behavioral changes. Operculum movement, surfacing on water, swimming efficiency, and swimming patterns of tilapia were recorded to analyze the altered behavioral pattern due to ABM exposure.
2.3. Liver function test
Fish blood was collected in K2-EDTA coated tubes. Blood was centrifuged at 3000 rpm for 15 min to separate plasma. Enzyme activity of aspartate transaminase (AST) and alanine transaminase (ALT) were determined at 340 nm using commercially available Reckon diagnostic kit (Bergmeyer et al., 1978). The activity of alkaline phosphatase (ALP) was measured by adapting the protocol of Tietz et al. (1983). 0.5 ml of buffered substrate (0.01 M PNPP in 0.1 M phosphate buffer) and 100 µl of liver supernatant was mixed and incubated for 15 min at room temperature. 0.5 ml of 0.5 N NaOH was added as stopping reagent and intensity of color was measured at 405 nm.

2.4. Biochemical analysis
The liver was homogenized, centrifuged, and the supernatant was collected for biochemical analysis. At 750 nm, total protein was measured using Folin–Ciocalteu’s phenol reagent (Lowry et al., 1951). Two hundred microliters of liver homogenate and 500 µl of Lowry reagent (alkaline CuSO₄ solution) were incubated for 15 min. Folin phenol reagent (0.5 ml) was added in it and incubated for 5 min at room temperature. Optical density was measured at 750 nm. Direct bilirubin was estimated using Jendrassik and Grof (1938) method using Reckon diagnostic kit. Estimation of glycogen was performed using Seifter et al. (1950) method. Hundred milligrams of tissue digested in boiling KOH and glycogen was precipitated in Ethyl alcohol. Five hundred microliters of aliquot and 2 ml of anthrone reagent were carefully added and heated in boiling water bath for 5 min. The intensity of the green color was read at 620 nm.

2.5. Oxidative stress parameter
Using the method of Buege and Aust (1978), lipid peroxidation product, malondialdehyde (MDA) level was measured by thiobarbituric acid reactive substance (TBARS). In 100 µl of liver homogenate, 200 µl of 8% SDS, 500 µl of 20% acetic acid, 500 µl of 2% thiobarbituric acid (TBA) and 1 ml phosphate buffer were incubated in a water bath at 95℃ for 60 min. After cooling, 2 ml of 10% TCA was added and centrifugation at 3000rpm for 10 min, the supernatant was removed and its absorbance was read at 532 nm.

Catalase activity was estimated at 590 nm (Sinha, 1972). Two hundred microliters of liver homogenate and 500 µl of phosphate buffer (0.1 M, pH 7.0) were mixed. At the interval of 15 and 30 s, 0.3 ml H₂O₂ (0.2 M) was added. Dichromate acetic acid reagent (1 ml) was mixed and incubated in boiling water bath for 10 min. Absorbance was recorded at 590 nm.

Reduce Glutathione (GSH) level was measured using Beutler et al. (1963) protocol. This method was based on the development of yellow color when thiol reagent (Elman reagent) 5,5’-dithio-bis-2-nitrobenzoic (DTNB) reacts with GSH present in tissue sample forming 5-thio nitrobenzoic acid (TNB). Intensity of color was measured at 412 nm.

2.6. Histopathology
Liver tissue was collected and fixed in 10% neutral buffered formalin for 24 h. Tissue was dehydrated in an ascending series of ethanol and embedded in paraffin. Sections were cut and stained by hematoxylin-eosin. Slides were examined under light microscope. The degree of tissue changes (DTC) was calculated according to Poleksic & Mitrovic-Tutundžić method (Poleksic & Mitrović-Tutundžić, 1994). The histological alteration was classified based on semi-quantitative estimation of degree of damage with reference to observed histomorphological changes in tissue section (stages I, II, and III). Fewer damage of tissue is classified as stage I in which tissue damage is reversible itself; stage II alterations are more severe and it disturb the homeostasis of tissue. This is reversible up to certain level with treatment. Stage III changes are extremely severe and irreversible. The DTC value in tissue section was calculated by summation of pathological lesions found in each stage and multiplying with the stage using given equation below:

\[ \text{DTC} = 10^0 \times \sum \text{Stage I} + 10^1 \times \sum \text{Stage II} + 10^2 \times \sum \text{Stage III} \]

For each group, five tissue sections were observed under the microscope and average DTC value was calculated. These average DTC values were used to evaluate liver damage due to ABM based
on standard classifications given: (1) 0–10 DTC—physiological normal, (2) 11–20 DTC—slightly damaged, (3) 21–50 DTC—moderately damaged, (4) 50–100 DTC—severe damaged, and (5) more than 100 DTC—highly severe and irreversibly damaged.

2.7. Statistical analysis
The statistical analysis of all parameters was performed by one-way analysis of variance (ANOVA) followed by post hoc Dunn–Bonferroni test to determine the significance level with control. Statistical analysis was performed using GraphPad Prism 6 software. The statistical significance was taken to be p < 0.05. All the results were expressed as mean ± standard error of the mean (SEM) for each group.

3. Results

3.1. Behavior analysis
Unusual altered behavior of living organisms is one of the clues of impaired physiology and disturbed homeostasis. Stress behavior of fish, due to exposure of xenobiotic can be determined using swimming, feeding, and respiratory (Operculum movement) behaviors. There were no observed change in body movement (swimming) and pigmentation of the control group of fish. One-way ANOVA followed by post hoc Bonferroni comparison of the frequency of operculum movements of treated fishes showed significantly increased movements up to 36 h, after this period operculum movement was decreased at 45 ppb and 55 ppb doses of ABM per minute (Graph 1(A)), whereas nonsignificant increased movement was observed in LD group of fishes. Increased movement of operculum and consistent gulping of air were observed behavioral changes in treated fish that supports induction of hypoxia due ABM exposure. Surfacing behavior of fish is commonly observed when xenobiotics induce the stress by reducing the carrying capacity of O₂. Abnormal behavior like excessive mucus secretion, shedding of scale, loss of pigmentation, loss of balance, restlessness, and swimming on the back were recorded during ABM intoxication. The intensity of altered behavioral activities of the fish was increased with increasing concentration and duration of ABM exposure. However, fishes of the control group maintained normal behavior during 48 h of experiment period.

To observe the swimming efficiency of fishes after ABM exposure, they were released into a channel of 1 m. Swimming patterns and distance travelled by fishes were recorded to observe ABM induced physiological modification. Low and slow stroke of swimming was resulted into significant drop in length travelled by fish per minute due to ABM treatment of MD and HD groups. The difference in distance covered by fishes was increased with an increased exposure period and concentration of ABM (Graph 1(B)). After 24 h of the exposure, frequency to swim on the back was increased in MD and HD group of tilapias. It was also observed that fishes could not maintain body balance during swimming after 36 h of ABM exposure at 55-ppb level. Nonsignificant change was noticed in 40 ppb ABM exposed fishes after 36 h. There was no record of altered behavior of swimming in reference group of tilapias.
3.2. Liver function test
To validate ABM-induced hepatic injuries, serum enzymatic activities of ALP, AST, and ALT were estimated. There were no observed changes in mean plasma ALT activity of control and low-dose group of fishes. ALT activity was estimated nonsignificantly higher in the MD group of *O. mossambicus*, whereas the significantly increased activity of ALT was reported in 55 ppb ABM-treated tilapia (*p* < 0.05). Aspartate aminotransferase activity was measured significantly higher when compared to the HD groups (*p* < 0.01) of fishes with experimental control group. There was nonsignificant higher activity of AST measured at 45-ppb treatment, whereas no change of activity was recorded after 40-ppb exposure of ABM to tilapias (Table 1). The AST/ALT ratio was <1.0 in the high-dose group of *O. mossambicus* that indicates the fibrotic insult of liver tissue. ALP activity was nonsignificantly elevated in fishes that were exposed to 40 ppb and 45 ppb of ABM for 48 h as compared to that of the reference group of tilapias. However, test organisms of high dose group showed a statistically significant increase in the activity of ALP due to biliary obstruction generated by ABM exposure (Table 1, Figure 2(b)).

| Enzymes | Control | LD     | MD     | HD      |
|---------|---------|--------|--------|---------|
| ALT (IU/L) | 10.66 ± 0.03 | 10.75 ± 0.06 | 10.9 ± 0.1 | 11.08 ± 0.14* |
| AST (IU/L) | 10.4 ± 0.34 | 10.79 ± 0.49 | 11.09 ± 0.3 | 13.19 ± 0.39** |
| ALP (μmol bound PNP/100 mg tissue/hr) | 42.43 ± 3.21 | 52.63 ± 4.05 | 58.63 ± 5.56 | 74.57 ± 5.76** |

(Data values are shown as mean ± SEM. One-way analysis of variance was followed by post hoc Dunn-Bonferroni test with control at: * = *p* < 0.05; ** = *p* < 0.01).

3.3. Biochemical test
There were no significant changes found in total protein levels in low toxicant exposed fish. The quantified level of protein in mid- and high-dose groups was measured significantly low after 48 h of ABM treatment (Table 2). Majority of serum proteins are synthesized in the liver; therefore, the altered level of total protein is an indicator of liver function impairment. On the contrary, the test organism from mid- and high-dose groups showed a significant decrease in glycogen level (*p* < 0.05 and *p* < 0.01, respectively), whereas low-dose group showed unremarkable depletion in the level of glycogen after ABM intoxication as compared to control fishes (Table 2). There were no significant changes in the total bilirubin levels in LD and MD groups of ABM-exposed tilapia as compared to the value obtained from the control fishes. The significantly higher level of bilirubin was estimated on 55 ppb intoxication of ABM to fishes.

| Bio-molecules | Control | LD     | MD     | HD      |
|---------------|---------|--------|--------|---------|
| Protein (mg/gm tissue) | 18.28 ± 0.51 | 16.85 ± 0.75 | 14.58 ± 0.90* | 12.67 ± 0.75** |
| Glycogen (mg/gm tissue) | 37.97 ± 0.58 | 32.7 ± 2.0 | 29.9 ± 1.1* | 26.27 ± 2.06** |
| Bilirubin (mg/dL) | 0.72 ± 0.01 | 0.77 ± 0.03 | 0.79 ± 0.03 | 0.89 ± 0.06* |

*Values are shown as mean ± SEM, *n* = 10; * = *p* < 0.05; ** = *p* < 0.01.
3.4. Oxidative stress parameters
The effects of ABM on certain oxidative stress parameters are summarized in Graph 2. Treated groups of *O. mossambicus*, LD, MD, and HD, exhibited an increase in the level of MDA that indirectly shows higher the activity of LPO (Graph 2(A)). The MDA levels in the liver tissues of fish exposed to higher concentration of ABM were significantly higher than those in the control (p < 0.01). However, the MD and HD treatment of ABM to tilapias depleted the activity of catalase significantly when compared to the control group (Graph 2(B)). Data also show a significant decrease in catalase activity of hepatocytes observed in the fishes of MD and HD groups, whereas 40-ppb treatment of ABM did not result in a significant change in catalase activity compared to the control fishes. Graph 2(C) shows the effects of ABM on reduced glutathione content in liver tissues from the fishes of ABM treated groups. Reduced glutathione content in the liver was significantly decreased (p < 0.005) in the HD group of fishes as compared to the value obtained from the control tilapias. Nonsignificant depletion of GSH level was recorded in 40 ppb and 45 ppb ABM-intoxicated groups of tilapias.

Graph 2. Effect of abamectin on oxidative stress parameters of liver after 48 h intoxication to tilapias. (A) Alteration in MDA level in hepatic tissue of fishes; (B) modulation in catalase activity after ABM treatment; (C) GSH level in liver tissue of tilapias (each value is the mean ±SEM, n = 10, * = p < 0.05; ** = p < 0.01; *** = p < 0.005).

3.5. Histopathology
Common pathological alteration found in the liver tissue was centrilobular destruction, vacuolar degeneration, dilation of sinusoid, fibrosis, and bile stagnation. Histology of control fish liver (Figure 1(a,b)) shown a regular arrangement of hepatocytes in glandular pattern with an intact central vein. The hepatic histopathology of a low dose group of fishes showed sinusoidal dilation due to ABM exposure (Figure 1(c)). On 45 ppb exposure of ABM to tilapia induced vacuolar degeneration of hepatocytes. Sinusoid dilation was also a commonly observed feature at a medium dose of ABM exposure (Figure 1(d)). Liver fibrosis was commonly observed histoarchitectural damage of high dose group of test organism. Centrilobular destruction and bile stagnation were marked identified histopathological alteration on 55 ppb intoxication of ABM (Figure 2(b,d)). Bile stagnation was observed as yellow-brown patches in hepatic tissue. Observed hepatic alteration of the HD group was severe and irreversible. Calculated DTC value was 53.18 for 45 ppb ABM exposure, whereas at 55 ppb ABM exposure, DTC value was raised on 139.6 in comparison to control hepatic tissue. DTC value above 100 indicates irreversible damage to the liver (Graph 3).
Figure 1. Photomicrograph of Liver histology of O. mossambicus (a) control fish liver histoarchitecture 4X; (b) control hepatic section 45X; (c) 40 ppb ABM-treated liver histopathology of tilapia with mild sinusoid dilation, 10X; (d) sinusoid dilation after 45 ppb ABM intoxication to fishes 10X, * shows centrilobular destruction and arrow shows vacuolar degeneration.

Figure 2. Photomicrograph of histopathological of hepatic tissue after 55 ppb exposure of ABM. (a) Histological damage of liver, 4X; (b) bile stagnation (arrow) and necrosis with gross hepatocellular damage, 10X; (c) Kupffer cell proliferation (arrow), 45X; (d) centrilobular destruction (*) and gross necrosis, 45X.)
4. Discussion
Alteration in behavior patterns is one of the most sensitive indicators of environmental stress (Byrne & O’Halloran, 2001). The behavior parameter provides the information of external (morphological) and internal (physiological) adaptive changes of an organism due to the insult of any chemical (Legradi et al., 2018). The increased opercular movement of gills, surfacing, and gulping was observed after ABM exposure. Increased surfacing and gulping during exposure periods of ABM suggests an elevated rate of metabolism and altered physiology of fish due to hypoxia. Hypoxia triggers numerous potential detrimental metabolic disturbances in fish because aerobic metabolism gradually becomes more compromised as oxygen levels decreases, which results into loss of appetite, growth, and locomotory activity (Oldham et al., 2019). In the state of hypoxia, fishes struggled to meet the oxygen demands required to maintain basal homeostasis, making them acidic from anaerobic metabolism that eventually damage the tissue or become fatal to fishes (Hvas & Oppedal, 2019). Exposures of fishes to different concentrations of ABM-altered swimming pattern and impaired movement (Ballesteros et al., 2009; Gormley & Teather, 2003; Pereira et al., 2012). In current study, increased mucus secretion in treated fishes was due to nonspecific adaptive response against ABM which provides additional protection against irritation or form barrier between body and pesticide so absorption of pesticide decreases (Saleh et al., 2019).

The nervous system controls muscle movement by the neurotransmitter. The inhibition of the neurotransmitter by ABM may affect swimming pattern and equilibrium of body of tilapia (Bretaud et al., 2000; Golombieski et al., 2008; Varo et al., 2003). Our results show that fish exposed to ABM during 48 h exhibit decreased swimming speed and movement percentage with respect to the reference group. The drop of locomotor activity as adaptive phenomenon is called Hypoactivity Syndrome. Lowered swimming activity in fishes could be adaptive response to restrain energy against higher metabolism of biotransformation (Ballesteros et al., 2009; Cazenave et al., 2008). ABM induces neurotoxicity by activation of GABA-gated chloride channels and develops hypoactivity of muscle; moreover, consistent exposure of ABM persuades paralyzing effect to organism (Dawson et al., 2000; Novelli et al., 2012; Raftery & Volz, 2015; Xu et al., 2017). Decline travel distance in treated group was attribution of GABAergic inhibition. ABM is designed to control the pest through GABA-gated chloride flux disrupt to seize the movement (Casida, 1993). Due to this mechanical action of ABM, swimming activity and balancing of body was modulated after treatment.
Liver function tests are important to evaluate liver physiology on the exposure of pesticides. Hence, activities of serum ALT and AST have been routinely used to assess altered fish physiology as well as for the detection of tissue damage by pesticide exposure (McGill, 2016; Rao, 2006). The present results showed that the treatment of ABM to fish caused a significant increase in the plasma activities of AST and ALT when compared to the control fish. An increased activity of these enzymes in serum is a sensitive indicator of cellular damage (Firat et al., 2011; Jee et al., 2005; Palanivelu et al., 2005). Elevated liver enzyme activity in serum indicates stress-based tissue impairment, degenerative changes and hepatic hypofunction as the effects of the toxicant on hepatocytes induce to release cellular enzymes into blood. A high level of liver specific enzyme activity in serum is one of the indicators of necrosis which could be result of hypoxia-induced ABM exposure and elevation of ROS due to biotransformation.

Elevated activity of LPO is in support of the production of hydroxyl radicals that causes oxidative damage to hepatocyte. The present study recorded significant increased MDA level in HD group of fishes. Lipid peroxidase peroxides the lipid of the plasma membrane and makes the membrane leaky which escorts the necrotic event (Zambo et al., 2013). Thus, elevated level of hepatocyte specific enzymes ALT and AST activities were recorded higher in serum. Ogueji et al. (2020) reported depletion in antioxidant level and increased hepatic LPO and ROS levels in Clarias gariepinus exposed to acute concentrations of ivermectin.

There was a negative correlation with catalase activity and GSH level due to ABM exposure. Catalase removes H$_2$O$_2$ by converting it into O$_2$ and H$_2$O and protects the cell. Present study showed lower the activity of catalase on ABM exposure. A low level of GSH was observed in the treated group during experiment. GSH involved in elimination of H$_2$O$_2$. Therefore, reduction in GSH level and catalase activity directly escort overproduction of H$_2$O$_2$ that enhance the activity of LPO that supports our results. Notably reduction in GSH level was observed in the liver tissue of O. mossambicus after 96 h of ABM exposure (Al Ghais et al., 2019). Juliana et al. (2018) have shown that ABM exposure perturbs mitochondrial bioenergetics and participate in the mitochondrial dependent pathological event via a reduction in GSH level and elevation of ROS.

The present results demonstrate a significant decrease in the mean level of serum proteins with increase in the dose of ABM and developed hypoproteinemia. The results indicate that ABM caused alterations in the protein metabolism of fish. Low content of protein level may be due to increased proteolytic activity to compensate pesticide metabolic stress or ABM treatment may be attributed to impairment in protein synthesis (Bradbury et al., 1987; Mastan & Rammaya, 2010). Depletion of protein content may be attributed to cell damage by ABM treatment and consequent inability of cell to synthesize protein or protein may be utilize for alternative source of energy for repair of the damaged cells caused by ABM intoxication (Al-Kahtani, 2011; Thanosmit, 2016). Hypoproteinemia is a result of the high requirement of glucose to compensate stress and homeostasis which may be balanced by gluconeogenesis from protein. Observed low level of glycogen is in support with resultant hypoproteinemia due to ABM exposure. Known metabolic response of fish to toxic effects is elevation in blood glucose level which is produced by lysis of glycogen. Hence, the level of glycogen was reported low (Luskova et al., 2002; Ogueji et al., 2020) To After 48 h of treatment of ABM, direct bilirubin level was measured high due to the disruption of hepatic architecture or altered hemoglobin breakdown. Measured higher activity of ALP in serum of ABM treated fishes is in patronage with elevated level of bilirubin.

The elevation of ALT, AST, and MDA level and induction of hypoxia in the present study suggest probable liver damage due to ABM exposure. The damage alters the histoarchitecture of tissue that can be seen in the histopathological lesions. Marked degenerative changes like vacuolar degeneration, centrilobular degeneration, and fibrosis were noticed in the liver of treated fish. After ABM exposure to fish, a reduction in antioxidant enzyme might occur due to the overproduction of free radicals that hamper the defense mechanism of hepatocyte and leads necrosis. Bile stagnation was reported during histopathological analysis that is in support of increased...
activity of alkaline phosphatase. Repeated administration of avermectin causes significant histopathological alteration in liver tissues. Necrotic changes were observed in hepatocyte of rat on avermectin exposure (Ahmed et al., 2020). Cellular degeneration and atrophy of liver tissue indicated the direct toxicosis of ABM due to oxidative stress generated on ABM exposure.

5. Conclusion
In summary, the present results demonstrated that intoxication of ABM negatively affects fish physiology. Exposure to ABM, change the swimming behavior of tilapias and induce hypoxia which can be fatal for fish survival. Dose dependent altered liver physiology was observed on 48 h of ABM exposure. ABM intoxication impaired hepatic function of fishes by inducing oxidative stress and modulate antioxidant enzymes. Forty-eight hours of ABM treatment alters the activity of liver marker enzymes (ALT, AST, and ALP). Severe histoarchitectural damage was also observed in hepatic tissue. Hepatic necrosis suggests that ABM is potential toxic to tilapias which develops toxicological manifestation and impaired fish health. Hence, ABM must be used with utmost caution.

Abbreviation

| Abbreviation | Description                           |
|--------------|---------------------------------------|
| ABM          | Abamectin                             |
| ppb          | Parts per billion                     |
| ALT          | Alanine transaminase                  |
| AST          | Aspartate transaminase                |
| ALP          | Alkaline Phosphatase                  |
| ROS          | Reactive oxygen species               |
| PBS          | Phosphate buffer saline               |
| LPO          | Lipid peroxidation                    |
| MDA          | Malondialdehyde                       |
| TBA          | Thiobarbituric acid                   |
| TBARS        | Thiobarbituric acid reactive substance|
| PNPP         | 4-Nitro phenyl phosphate              |
| SEM          | Standard error of the mean            |

Compliance with ethical standards
The research carried out was in accordance with the guidelines of APHA-AWWA-WEF (1998).

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Graphical abstract
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