The Journal of Basic and Applied Zoology

RESEARCH Open Access

# *Carica papaya* and *Mangifera indica* modulate haematological, biochemical and histological alterations in atrazine-intoxicated fish, *Clarias gariepinus* (Burchell 1822)

Olufemi D. Owolabi* and Saratu I. Abdulkareem

## Abstract

**Background:** Atrazine has impacted negatively on fish by inducing significant alterations in their haematological, biochemical and histological parameters. Mitigating such alterations to enhance fish survival becomes pertinent. Unfortunately, readily available and eco-friendly remedies are scarce. The study, therefore, investigated the potential ameliorative effects of dietary supplementation of aqueous *Carica papaya* and *Mangifera indica* leaf extracts on atrazine-induced toxicity and oxidative damage in the tissues of *Clarias gariepinus*. Fish (average weight: 10.57 ± 1.69 g, and average length: 10. 36 ± 1.26 cm) were randomly divided into six groups of ten samples each. Group I served as control and administered borehole water only, group II was exposed to 8.50 µg/l atrazine corresponding to ¼ of 96 h $LC_{50}$, group III was treated with low (0.25 mg/g) concentration of each extract alone, group IV was exposed to 8.50 µg/l atrazine and treated with the low concentration of each extract, group V was administered with high (0.75 mg/g) concentration of each extract alone, and group VI was exposed to 8.50 µg/l atrazine and treated with the high concentration of each extract. The experiment spanned 28 days after which the haematological, biochemical and histopathological alterations were assayed.

**Results:** Atrazine intoxication significantly induced oxidative damage in the gill and liver, culminating into different histopathological disorders, decreased haematological parameters, increased serum, gill and liver levels of malondialdehyde and enzyme biomarkers (alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase and acetylcholinesterase). On the other hand, atrazine exposure caused decreased levels of glucose, protein and superoxide dismutase in the tissues. Treatment with diets fortified with both extracts significantly increased all the decreased haematological parameters, reduced the serum levels of the elevated malondialdehyde and tissues enzyme biomarkers in a concentration-dependent manner. Biochemical parameters in the tissues were also improved with dietary supplementation of the extracts. Histopathological examination of both tissues showed ameliorating effects of both extracts in restoring the structural and functional integrity of each tissue.

**Conclusions:** These results suggest that the extracts have ameliorative potentials against atrazine-induced peroxidative injury in *C. gariepinus*. The utilisation of these extracts could enhance better health management practices, particularly in a rice-cum fish culture, where atrazine application is common.

*Correspondence: olulabi47@yahoo.com; olulabi@unilorin.edu.ng
Department of Zoology, University of Ilorin, P.M.B. 1515, Ilorin 240003, Nigeria

**Keywords:** Atrazine toxicity, *Clarias gariepinus*, Oxidative stress, Amelioration, Plant extracts

## Background

Natural water bodies around the world are fast degrading as a result of run-offs and underground water leachates resulting from the use of synthetic fertilizers, pesticides and herbicides to boost agricultural productions (Fiorino et al., 2018; Stara et al., 2019). This negative water quality trend and its resultant toxic effects on biological systems and human health were predicted over a decade ago by Aktar et al. (2009), and it has become a serious public and environmental health concern. Unfortunately, there seems to be no readily available and eco-friendly remedies. Antofie et al. (2010) also predicted that the use of herbicides would be on a large-scale as long as herbicide-tolerant, genetically modified crop is on the increase. Therefore, proffering solutions to these effects to enhance the survival and conservation of fish species become pertinent. Various species of plants have been reported to contain naturally occurring phytochemicals with antibiotic, antioxidant, immunosuppressing, immunostimulating, anti-inflammatory, antiulcerogenic activities (Rafieepour et al., 2019). The use of plants in aquaculture has become widely accepted because they serve as alternatives to synthetic chemicals, drugs and antibiotics for disease prevention and curative purposes (Hamed et al., 2021; Reveter et al., 2017; Sinha et al., 2021a). *Mangifera indica* (Mango) is one of the commercially important species in the family Anacardiaceae. It is an evergreen plant native to the tropics and subtropics. In Nigeria, the plant is very common and popular as its different parts are used as decoctions in the treatment of many ailments such as gastrointestinal disorders, respiratory and urinary infections, dysentery, toothaches and diarrhoea (Madunagu et al., 1990). *M. indica* has been documented to possess antibiotic (Madunagu et al., 1990), immunostimulating (Makare et al., 2001), antioxidative (Sanchez et al., 2001) and antidiabetic (Kemasari et al., 2011) properties among others.

*Carica papaya* (Pawpaw) is an herbaceous plant belonging to the family Caricaceae. It originated from Mexico, Central America, but has been spread to other tropical areas of the world, where they are mostly cultivated either for its succulent fruits or different parts used medicinally. The seeds are used as worm expeller, and the flowers used as an infusion for menstrual induction in females (Duke, 1984). The latex from the plant is used for wound healing, ulcers and boils. Nigeria has been rated as the world sixth-largest producer of *C. papaya* in the world and the largest in Africa (FAO, 2017). Apart from eating the fleshy and succulent fruits, *C. papaya* is majorly used in Nigeria as an antisickling agent (Oduola et al., 2006). According to Indran et al. (2008), *C. papaya* offered protection by reducing plasma lipid peroxidation and increased erythrocyte glutathione peroxidase activity in rats. It, therefore, has the potential to serve as a therapeutic/ameliorative agent of protection against oxidative damage. Thus, both *M. indica* and *C. papaya* hold a promise regarding the amelioration of induced toxic effects of synthetic herbicides such as atrazine, which hitherto have not been used to modulate atrazine-induced toxicity and oxidative stress in fish.

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), a synthetic herbicide, is a photosynthetic inhibitor that is selective, moderately toxic and is currently one of the most widely used herbicides around the world (Santos & Martinez, 2012). Rohr and McCoy (2010) rated it as one of the heavily used herbicides in the USA and around the world. It has been used intensively and satisfactorily to control broadleaf weed of field crops over the last 40 years (Paulino et al., 2012). It is, therefore, one of the most widely occurring and most detectable herbicides around the world (Graymore et al., 2001). Despite the prohibition of atrazine in most developed countries of the world (European Commission, 2004), its importation into Nigerian markets and other developing countries continues unabated. Due to its persistence in the environment, atrazine may find its way into the aquatic system via run-offs and can persist therein for a long time (ATSDR, 2003). A concentration range of between 0.00 and 0.94 μg/g has been reported in some fish and sediment samples in Nigerian waters (Ezemonye et al., 2015). The possible debilitating effects of atrazine on the haematological, biochemical, histological and embryological profiles of fish have been described by various authors (Blahova et al., 2013, 2014, 2020; Nwani et al, 2011; Owolabi & Omotosho, 2017; Paulino et al., 2012; Santos & Martinez, 2012). Free radicals' generation has been reported as one of the major factors contributing to the atrazine-induced toxicity in fish (Owolabi & Omotosho, 2017). They are known to cause disequilibrium in prooxidant-antioxidant levels (Afzal & Armstrong, 2007), and this disequilibrium could predispose any aerobic organism to the risk of adverse physiological and biochemical disorders. An efficient modulatory strategy to regulate and enhance the redox stability, therefore, becomes imperative to reduce oxidative stress and its attendant consequences.

Although fishes are known to have high xenobiotic bioaccumulation potentials (Oritz et al., 2012), they

suffer serious oxidative stress resulting from the impact of atrazine (Blahova et al., 2013; Santos & Martinez, 2012). The African catfish, *Clarias gariepinus*, is a common freshwater fish in Nigerian water bodies with enhanced capability to tolerate poorly aerated water and oxidative stress; hence it is widely cultivated. It is a high protein-rich food fish widely relished and valued by people due to its tasty flesh. It has been increasingly studied not only to increase its production output but also to examine its response to a wide spectrum of xenobiotics. Earlier studies on the importance of *C. papaya* and *M. indica* to animals focused more on their nutritive and medicinal values; their roles in mitigating atrazine toxicity in fish and other aquatic organisms are under-explored. This study was, therefore, designed to investigate the ameliorative potentials of *C. papaya* and *M. indica* plant extracts on *C. gariepinus* exposed to sub-lethal concentrations of atrazine using biochemical and physiological endpoints. It is hoped that these plants would offer maximum protection to the tissues of *C. gariepinus* and essentially no devastating effects would be deployed to their metabolic functions to maintain homeostasis.

## Methods

### Collection of chemicals

The chemical, atrazine, was procured from Sigma-Aldrich (St Louis, USA) and used for the experiment without further purification.

### Fish collection and handling

Juvenile African catfish, *Clarias gariepinus* ($n=600$, average weight: $10.57 \pm 1.69$ g, and average length: $10.36 \pm 1.26$ cm) were procured from the hatchery of Ministry of Agriculture, Ilorin (8° 29′ 47″ N, 4° 32′ 31″ E), Kwara State, Nigeria. They were transported to the rearing compartments in the Fisheries and Hydrobiology Laboratory, Department of Zoology, University of Ilorin, Ilorin, Nigeria; after a 6-h feeding holiday during transportation and introduction to the laboratory. The fishes were acclimatized in aerated borehole water stored in 140-L plastic tanks under laboratory condition for 2 weeks, during which they were fed twice daily with Coppens commercial feed pellets at 3% body weight. Physicochemical parameters of the test media were monitored every day using standard methods (APHA, 2005) and were as follows: temperature $22.86 \pm 1.09$ °C, pH $6.79 \pm 0.91$, dissolved oxygen $7.19 \pm 1.89$ mg/l, conductivity $0.91 \pm 0.44$ µ/s and biological oxygen demand $22.08 \pm 2.35$ mg/l. The test media was renewed every 48 h and a 12 h light: 12 h dark photoperiod was maintained.

### Plant-source, handling and extraction process

Fresh *C. papaya* and *M. indica* leaves collected from farmland on the main campus of the University of Ilorin, Ilorin, Nigeria, were authenticated in the herbarium of the Department of Plant Biology, University of Ilorin, Nigeria, where the voucher number UILH009/2018 was deposited. The leaves were rinsed with distilled water, air-dried under shade atmosphere and good ventilation at room temperature for 7 days. Crude water extracts were individually obtained from crushed 200 g air-dried leaves of each plant in 15 L of distilling water for 24 h. The extracted solution was filtered through a muslin cloth and subsequently through filter paper. The filtrate was then evaporated to dryness by a rotary evaporator steam bath at 40 °C. The solid extract was weighed, wrapped in aluminium foil and preserved in the refrigerator prior use.

### Fish food/diet formulation

Fortified fish feed diets were prepared from Coppens commercial fish feed (Helmond Netherland) using the solid-aqueous extracts obtained from *C. papaya* and *M. indica* leaves. Two concentrations of each extract were prepared by dissolving 250 g and 750 g of the solid extracts in 1000 ml of distilled water to form a concentration of 0.25 mg/g and 0.75 mg/g of feed, respectively. Thus, forming low and high concentrated diets for each extract, respectively. Fish feed supplementation with the extract was achieved using the technique of Lamchumchang et al. (2007). Briefly, the commercial feed was ground using a blender, to which 0.7 ml distilled water was added for hydration and thoroughly mixed with the powdered extract in the appropriate proportion aforementioned. The mixture was passed into a meat grinder and then broken into smaller pellets, air-dried for 2 days and stored for use at room temperature.

### Experimental design

The concentration of atrazine chosen for the experiment was based on the earlier results of the previous assay conducted (Owolabi & Omotosho, 2017). A preliminary acute toxicity assay in which fishes were exposed to different definitive concentrations, based on the results of a presumptive test, was carried out using OECD (1992) procedures; to determine the 96 h $LC_{50}$ value. Samples of acclimatized juvenile *C. gariepinus* were then exposed to 8.50 µg/l atrazine (corresponding to ¼ of 96 h $LC_{50}$ value estimated to be 34.50 µg/l) and borehole water media for 28 days in ten experimental groups, namely:

*Group I* Borehole water and normal diet without plant extract (normal control).
*Group II* Atrazine and normal diet without plant extract.
*Group IIIa* Borehole water and low concentrated (0.25 mg/g) *C. papaya* extract fortified diet.
*Group IIIb* Borehole water and low concentrated (0.25 mg/g) *M. indica* extract fortified diet.
*Group IVa* Atrazine and low concentrated (0.25 mg/g) *C. papaya* extract fortified diet.
*Group IVb* Atrazine and low concentrated (0.25 mg/g) *M. indica* extract fortified diet.
*Group Va* Borehole water and high concentrated (0.75 mg/g) *C. papaya* extract fortified diet
*Group Vb* Borehole water and high concentrated (0.75 mg/g) *M. indica* extract fortified diet.
*Group VIa* Atrazine and high concentrated (0.75 mg/g) *C. papaya* extract fortified diet.
*Group VIb* Atrazine and high concentrated (0.75 mg/g) *M. indica* extract fortified diet.

Groups I, III and V served as control groups while II, IV and VI served as the experimental groups. Each group had three replicates and each replicate consisted of ten randomly selected and weighed acclimatized juvenile *C. gariepinus* in a basin containing 10 L of the respective medium, making a total of 300 fish used for the experiment. The respective media were renewed every 48 h. All fishes were fed with respective diets at a rate of 3% of their body weight twice daily, and dead fishes were instantly retrieved. After 28 days, the survived fishes were killed for haematological and biochemical assays.

#### Haematological and biochemical assays

At the end of the experiment, fish were randomly collected from each treatment for blood sampling. Bloodletting was achieved through a right-angle laceration of the ventral part near the caudal artery using a dissecting set. Blood (approximately 2 ml) was then collected using a capillary tube and stored in a vial coated with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Haematological assays were achieved in the laboratory the same day the blood samples were collected. Blood was split into two portions: the first portion (~ 1 ml) was used immediately to estimate the haematological parameters, while the second portion (~ 1 ml) was allowed to clot and centrifuged at 3000 rpm at 4 °C for 15 min to obtain the serum subsequently used to determine the biochemical parameters. RBCs and WBCs were estimated using Neubauer haemocytometer following Dacie and Lewis' (2001) procedure. Haemoglobin (HB) was determined using cyanmethemoglobin method (Blaxhall & Daisley, 1973), while packed cell volume (PCV) was estimated using the microhaematocrit protocols (Morris & Davey, 2001). Erythrocyte indices: mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were assessed using the formulae described by Dacie and Lewis (2001). Approximately, one gramme each of the gill and liver samples was collected, weighed and homogenised with 5 ml 0.1 M phosphate buffer saline in ice-cold condition using Glass/PTFE potter Elvehjem tissue homogeniser (Omni International, Kennesaw, GA). The homogenates were filtered and centrifuged at 1600 rpm at 4 °C for 15 min. The supernatants thus obtained were stored at − 20 °C prior examination. Serum and tissue ALT and AST activities were assessed following the procedure of Reitman and Frankel (1957). LDH was estimated according to the procedure of Vassault (1983). Serum protein and glucose levels were measured using the methods of Lowry et al. (1951) and Trinder (1969), respectively. The procedure of Knedel and Bottger (1967) was used for the determination of acetylcholinesterase (AChE), while superoxide dismutase (SOD) was assayed by the method of Misra and Fridovish (1972). Thiobarbituric (MDA) levels were estimated following the method of Mihara and Uchiyama (1978).

#### Histopathological assay

Fish was killed by decapitation and the gill and liver samples from each treatment group and control were carefully dissected out and fixed in Bouin's fluid after which they were dehydrated through ascending grades (50–100%) of alcohol. The samples were subsequently embedded in paraffin wax, sectioned at 5 μm thick using a rotary microtome (SLEE, CUT 5062, Nikon Instrument, Europe) and stained with haematoxylin and eosin (HE) solution. The stained sections were evaluated for histopathological disorders and microphotographed using an optical microscope (Nikon E200 POL) fixed with a digital camera (Bancroft & Stevens, 1990). Histopathological alterations were semi-quantitatively assessed using a four-graded assessment scheme of Peebua et al. (2008): − (none), + (mild), ++ (moderate) and +++ (severe) and five slides per treatment were observed.

#### Statistical analysis

The data obtained from haematological and biochemical assays were pooled per replicate and expressed as mean ± SD. The General Linear Model (GLM) programme of SPSS 19 software for Windows (IBM SPSS Inc., USA) was used for data analysis. The normality of data distribution was examined using the Shapiro–Wilk test. This was followed by parametric one-way analysis of variance (ANOVA) with Tukey multiple-range test to compare any statistically significant differences among the treatment groups. Multivariate analysis of variance

(MANOVA) was also used to assess the relationship, if any, among the tissue type, atrazine treatment and extract type. Atrazine concentration, tissue type and extract type were factored as a within-subject covariate. Tukey's Least Square Difference (LSD) tests were used, where necessary, for post hoc comparisons of means ($p < 0.05$).

## Results

### Haematological parameters

Figure 1a, b shows the effects of dietary *C. papaya* (CPE) and *Mangifera indica* extracts (MIE) on the haematological parameters of *C. gariepinus* exposed to atrazine-polluted water. All the haematological parameters in the control group (group III) treated with low (0.25 mg/g) concentration of dietary CPE except mean cell haemoglobin concentration (MCHC) did not show significant ($p < 0.05$) alterations compared to the normal control (Fig. 1a). In the control group (group V) which received high (0.75 mg/g) concentration of CPE), red blood cell (RBC), haemoglobin (HB), packed cell volume (PCV) and mean cell haemoglobin (MCH) did not show significant ($p < 0.05$) alteration, whereas mean cell volume (MCV), MCHC, white blood cell (WBC) and lymphocytes (LYM) were significantly ($p < 0.05$) increased compared to the normal control. Upon exposure to atrazine alone, all the haematological parameters were significantly ($p < 0.05$) reduced when compared to the normal control. However, simultaneous administration of diets fortified with both the low and high concentrations of CPE showed similar ameliorative effect against the atrazine-induced decrease of all haematological parameters except for RBC and PCV in which low dietary CPE did not significantly prevent the atrazine caused decrease and had no significant ($p > 0.05$) effect, respectively (Fig. 1a).

In the control group recipient of low (0.25 mg/g) concentration of dietary MIE alone, HB, MCH and MCHC did not show alterations as evidenced by their non-significant ($p > 0.05$) values from the normal control, while other parameters were either significantly ($p < 0.05$) increased (RBC, LYM) or decreased (PCV, MCV, WBC) (Fig. 1b). In the control group treated with high (0.75 mg/g) concentration of dietary MIE, HB, PCV and MCHC were not significantly ($p > 0.05$) different from the normal control, while RBC, MCV, WBC and LYM significantly ($p < 0.05$) increased and MCH significantly decreased in comparison with the normal control (Fig. 1b). Exposure to atrazine alone caused a significant reduction of all the haematological parameters investigated. Treatment with diets supplemented with low (0.25 mg/g) concentration of MIE did not reverse any of the atrazine-caused decreases in the levels of haematological parameters. Treatment with diets fortified with high (0.75 mg/g) concentration of MIE, however, significantly ($p < 0.05$) prevented the atrazine-caused reduction in the levels of HB, MCV, MCH, MCHC, WBC and LYM but did not prevent the reduction in RBC and PCV (Fig. 1b).

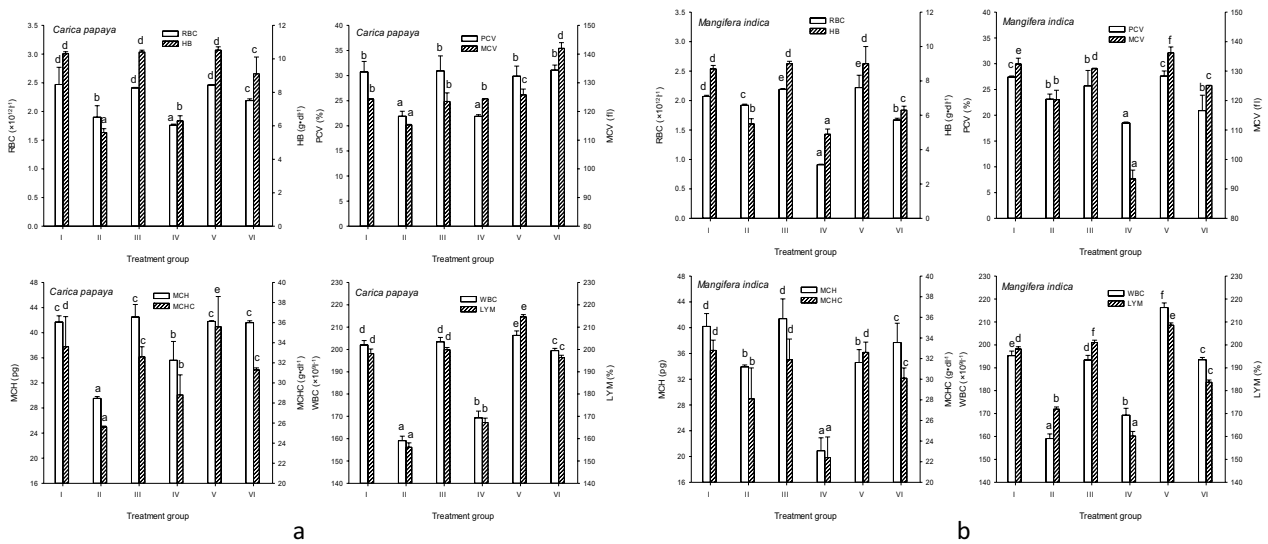


**Fig. 1** Effects of **a** *Carica papaya*- **b** *Mangifera indica* fortified diets on the haematological parameters of *C. gariepinus* exposed to atrazine for 28 days. Bars are means (± SD) of 3 replicates. Bars with different superscripts were significantly different ($p < 0.05$). I control II atrazine-exposed group III low concentration of *C. papaya* alone IV atrazine + low concentration of *C. papaya* V high concentration of *C. papaya* alone VI atrazine + high concentration of *C. papaya*

### Biochemical parameters

Figures 2a, 3a and 4a show that significant alterations were observed for all the biochemical parameters in all the tissues investigated in control groups which received the low (0.25 mg/g) and high concentration (0.75 mg/g) of dietary CPE alone (group III and group V) compared to the normal control group (group I), except serum glucose level from group III and serum AChE and MDA levels from group V that did not show significant ($p > 0.05$) difference from the normal control. Treatment with atrazine alone resulted in the significant ($p < 0.05$) increase in serum MDA level and the activities of ALT, AST, LDH and AChE, whereas the levels of glucose, protein and SOD activity were significantly ($p < 0.05$) reduced (Fig. 2a) compared to the normal control. Exposure to atrazine significantly ($p < 0.05$) elevated MDA level and decreased the levels of other biochemical parameters in the gill (Fig. 3a) and liver (Fig. 4a) as compared to the normal control. Following supplementation with a low (0.25 mg/g) concentration of dietary CPE, significant ($p < 0.05$) atrazine-induced elevations of serum MDA level and activities of ALT, AST and LDH; and significant ($p < 0.05$) atrazine-induced depletions of glucose and protein levels and the activities of SOD and AChE were

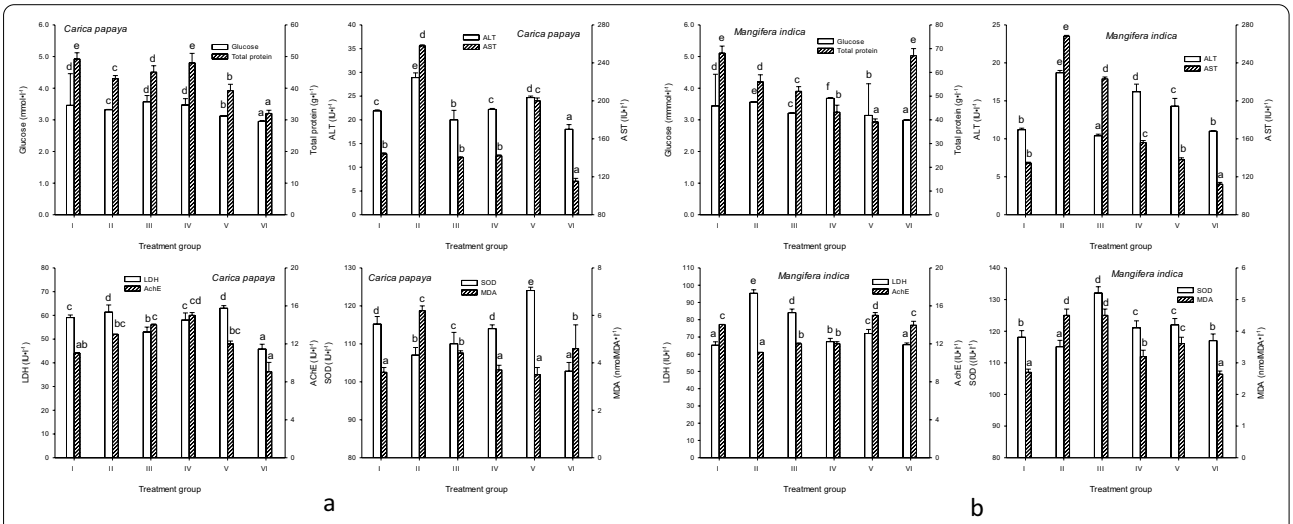


**Fig. 2** Effects of **a** *Carica papaya*- **b** *Mangifera indica* fortified diets on the biochemical parameters in the blood of *C. gariepinus* exposed to atrazine for 28 days. Bars are means (± SD) of 3 replicates. Bars with different superscripts were significantly different ($p < 0.05$). I control II atrazine-exposed group III low concentration of *C. papaya* alone IV atrazine + low concentration of *C. papaya* V high concentration of *C. papaya* alone VI atrazine + high concentration of *C. papaya*

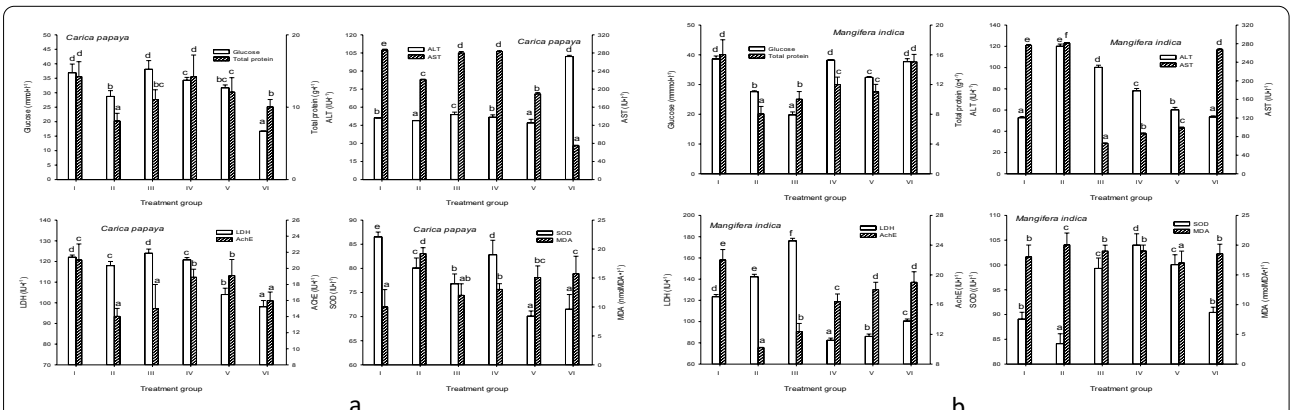


**Fig. 3** Effects of **a** *Carica papaya*—**b** *Mangifera indica* fortified diets on the biochemical parameters in the gill of *C. gariepinus* exposed to atrazine for 28 days. Bars are means (± SD) of 3 replicates. Bars with different superscripts were significantly different ($p < 0.05$). I control II atrazine-exposed group III low concentration of *M. indica* alone IV atrazine + low concentration of *M. indica* V high concentration of *M. indica* alone VI atrazine + high concentration of *M. indica*

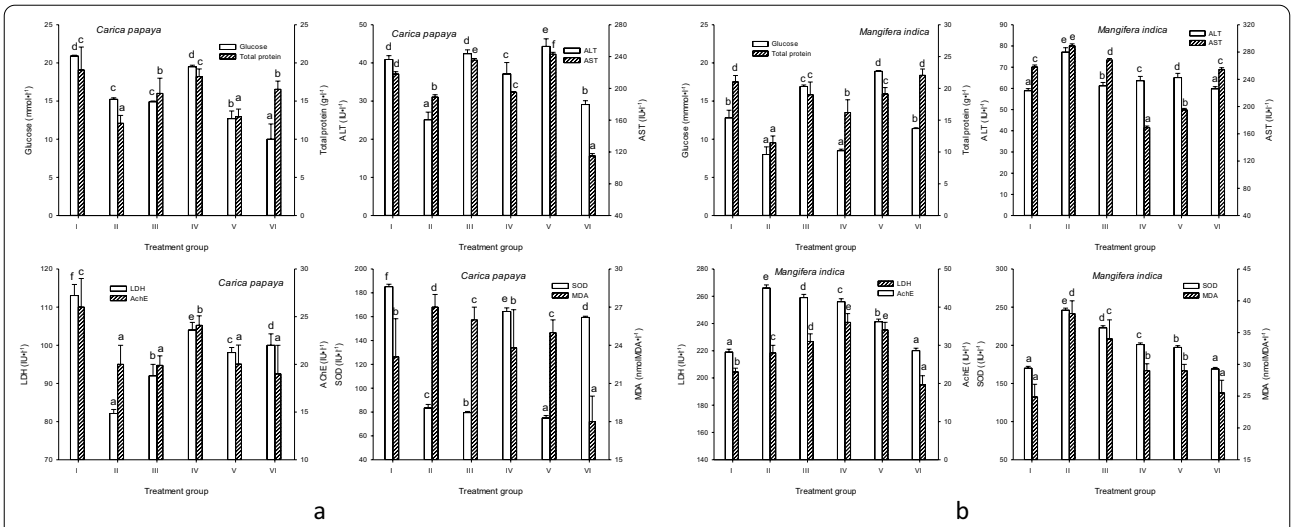


**Fig. 4** Effects of **a** *Carica papaya*—**b** *Mangifera indica* fortified diets on the biochemical parameters in the liver of *C. gariepinus* exposed to atrazine for 28 days. Bars are means (± SD) of 3 replicates. Bars with different superscripts were significantly different ($p < 0.05$). I control II atrazine-exposed group III low concentration of *M. indica* alone IV atrazine + low concentration of *M. indica* V high concentration of *M. indica* alone VI atrazine + high concentration of *M. indica*

prevented as compared to the group treated with atrazine alone. In both the gill (Fig. 3a) and liver (Fig. 4a), fortification with the low concentration of dietary CPE significantly ($p < 0.05$) attenuated the depletion of glucose and protein levels and ALT, AST, LDH, SOD and AChE activities compared with the atrazine-exposed group. Similarly, supplementation with the low dietary CPE significantly ($p < 0.05$) inhibited the elevation of MDA level in both the branchial (Fig. 3a) and hepatic tissues (Fig. 4a) compared to the atrazine-exposed group. As shown in Fig. 2a, treatment with the high concentration of dietary CPE prevented a significant elevation of MDA level but did not prevent the depletion of other serum biochemical parameters compared to the atrazine-treated group. In the gill, treatment with the high dietary CPE showed ameliorative effect against an increase in MDA and decrease in protein levels and activity of ALT but did not show such effect on other parameters (Fig. 3a). In the liver, however, while the high dietary CPE thwarted the enhancement of MDA level and the depletion of ALT, LDH, SOD activities and protein level, ALT activity and glucose level remained reduced with AChE activity insignificantly ($p > 0.05$) different compared to the liver homogenate of atrazine-exposed fish (Fig. 4a).

Figures 2b, 3b and 4b show that significant ($p < 0.05$) alterations (either increase or decrease) were recorded for all the tissues' biochemical parameters examined in control groups treated with the low (group III) and high dietary MIE (group V) alone compared to the normal control, except for insignificant ($p < 0.05$) value recorded for the gill MDA level in the low dietary MIE control group. Exposure to atrazine alone caused a significant ($p < 0.05$) increase in the levels of MDA and the activities of ALT, AST and LDH in the serum (Fig. 2b), gill (Fig. 3b) and liver (Fig. 4b) compared to the control devoid of atrazine. Whereas serum glucose level, and liver SOD and AChE activities were significantly ($p < 0.05$) enhanced upon exposure to atrazine, the levels of protein in all the tissues, glucose in both the gill and liver and activities of SOD and AChE in serum and gill, respectively, were inhibited. Treatment with dietary MIE at low (0.25 mg/g) concentration prevented significant atrazine-induced increases in the levels of serum MDA, ALT, AST, and LDH and atrazine-induced decreases in the levels of glucose, SOD and AChE compared to the atrazine group (Fig. 2b). Serum protein remained significantly ($p < 0.05$) reduced even after treatment with the low dietary MIE. In the gill, MIE treatment at low (0.25 mg/g) concentration ameliorated glucose, protein and MDA levels and the activities of SOD and AChE, while ALT, AST and LDH activities remained depleted compared to the atrazine-exposed group (Fig. 3b). A decrease in protein and AChE levels and an increase in MDA level were attenuated in the hepatic tissue upon administration of low dietary MIE, while the rest biochemical parameters remained inhibited, except AChE that showed no significant ($p < 0.05$) difference compared to the atrazine-treated group (Fig. 4b). Dietary supplementation with a high (0.75 mg/g) concentration of MIE significantly ($p < 0.05$) decreased MDA level and increased the level of glucose and activities of SOD and AChE in the serum (Fig. 2b).

High concentration (0.75 mg/g) of dietary MIE also had preventive effects against the induction of serum ALT, AST and LDH activities; however, the level of protein remained reduced in comparison with the group exposed to atrazine (Fig. 2b). In the gill, high dietary MIE inhibited the increase in MDA level but enhanced the levels of glucose, protein, SOD and AChE, whereas the levels of ALT, AST and LDH declined compared to the atrazine group (Fig. 3b). Similar effects were observed in the liver except that glucose and protein were the elevated parameters, while others were significantly ($p < 0.05$) reduced upon treatment with high dietary MIE (Fig. 4b).

### Histopathology

Histopathological changes in the atrazine-exposed fish gill and liver and the ameliorative effects of CPE and MIE are shown in Figs. 5 and 6 and Tables 1 and 2. The normal architecture of the gill was observed in control fish (Figs. 5a, 6a). In contrast, the atrazine-exposed fish showed alterations in the gill, including degeneration of cartilaginous core, lamella fusion, curling of

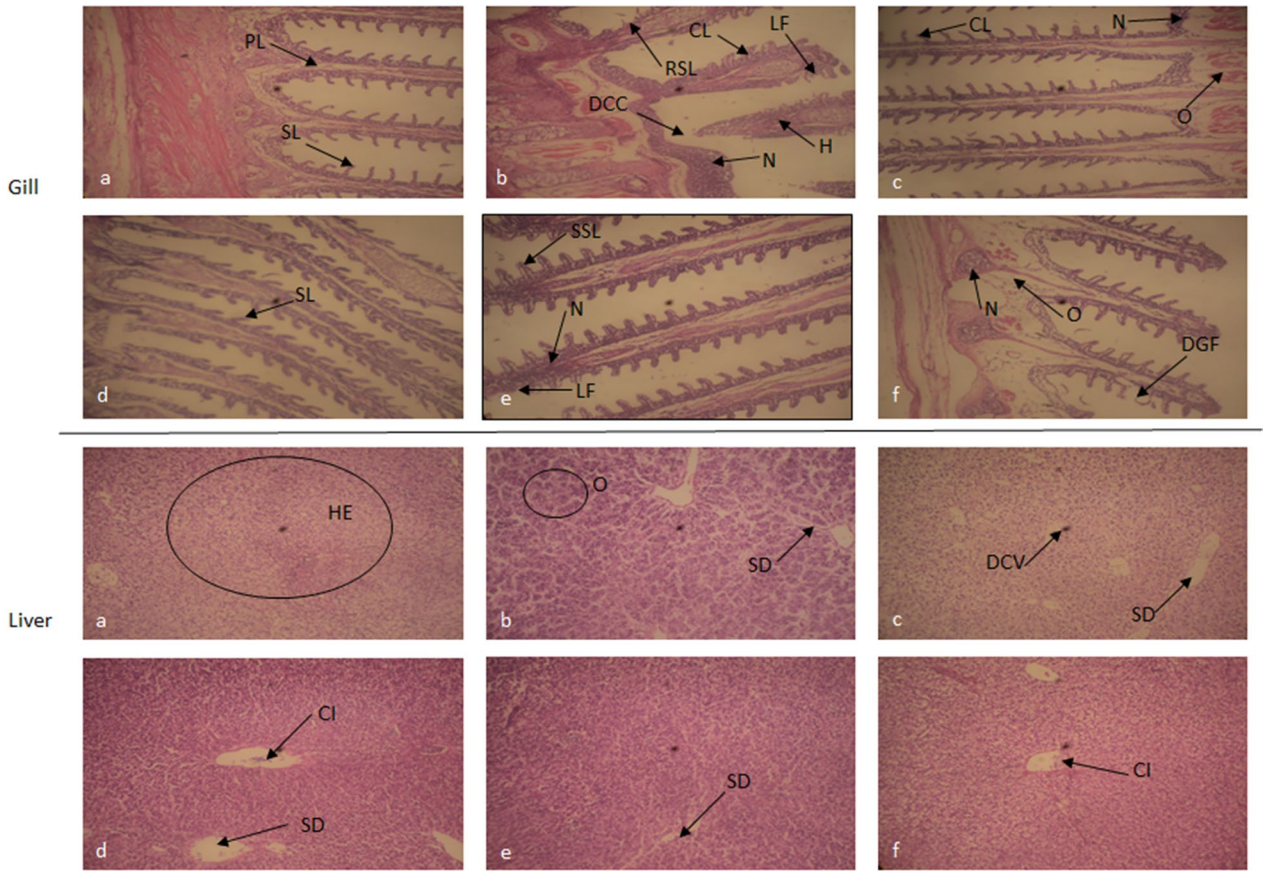


**Fig. 5** Histopathological changes in the gill and liver of *Clarias gariepinus* exposed to atrazine for 28 days and ameliorated with *Carica papaya* (H & E × 100). **a** *Gill* of *C. gariepinus* in the control showing normal architecture of primary lamellae (PL) and secondary lamellae (SL); **b** degeneration of cartilaginous core (DCC), curling of lamella (CL), hyperplasia (H), lamellar fusion (LF), necrosis (N) and rupture of secondary lamella RSL) in the gill of fish exposed to atrazine alone; **c** curling of lamella (CL), oedema (O) and necrosis (N) in the gill of fish exposed to low (0.25 mg/g) concentration of *C. papaya* alone; **d** gradual reversal of lamella fusion (LF) and improvement in curling of lamella (CL) and lamella swelling (LS) in the gill of atrazine-exposed fish ameliorated with low (0.25 mg/g) concentration of *C. papaya*; **e** Lamella fusion (LF), necrosis (N) and swelling of secondary lamella (SSL) in the gill of fish exposed to high (0.75 mg/g) concentration of *C. papaya* alone; **f** displacement of gill filament (DGF), necrosis (N) and oedema (O) in the gill of atrazine-exposed fish ameliorated with high (0.75 mg/g) concentration of *C. papaya*; **a** *Liver* of *C. gariepinus* in the control showing normal arrangement of hepatocytes (H); **b** sinusoidal distortion (SD) and oedema (O) in the liver of fish exposed to atrazine alone; **c** displacement of central vein (DCV) and sinusoidal distortion (SD) in the liver of fish exposed to low (0.25 mg/g) concentration of *C. papaya* alone; **d** gradual reversal of cellular infiltration (CI) and sinusoidal distortion (SD) in the liver of atrazine-exposed fish ameliorated with low (0.25 mg/g) concentration of *C. papaya*; **e** sinusoidal distortion (SD) in the liver of fish exposed to high (0.75 mg/g) concentration of *C. papaya* alone; **f** cellular infiltration (CI) in the liver of atrazine-exposed fish ameliorated with high (0.75 mg/g) concentration of *C. papaya*

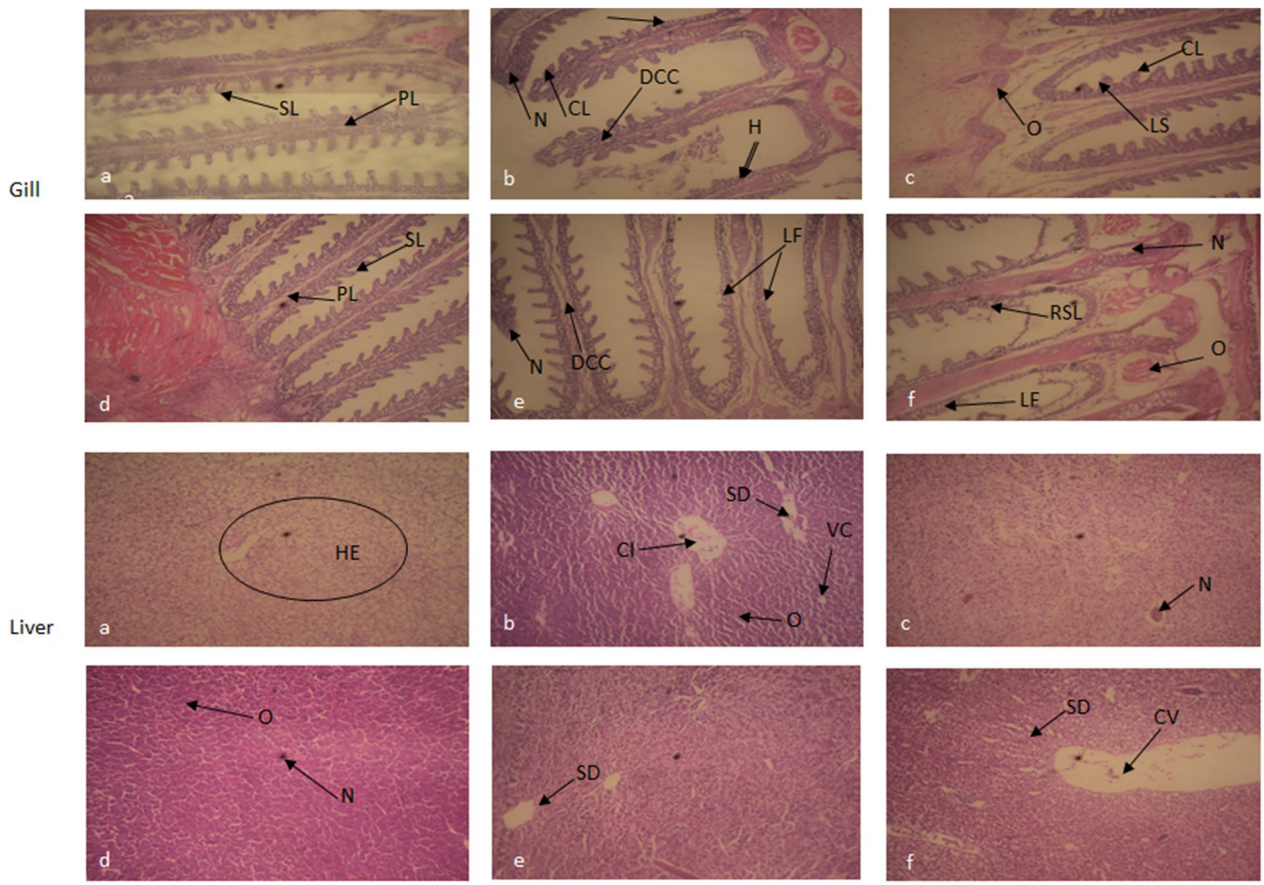


**Fig. 6** Histopathological changes in the gill and liver of *Clarias gariepinus* exposed to atrazine for 28 days and ameliorated with *Mangifera indica* (H & E × 100). **a** *Gill* of *C. gariepinus* in the control showing normal architecture of primary lamellae (PL) and secondary lamellae (SL); **b** degeneration of cartilaginous core (DCC), curling of lamella (CL), hyperplasia (H), lamellar fusion (LF), necrosis (N) and rupture of secondary lamella RSL) in the gill of fish exposed to atrazine alone; **c** curling of lamella (CL), oedema (O) and lamella swelling in the gill of fish exposed to low (0.25 mg/g) concentration of *M. indica* alone; **d** gradual reversal of lamella fusion (LF) and improvement in curling of lamella (CL) and lamella swelling (LS) in the gill of atrazine-exposed fish ameliorated with low (0.25 mg/g) concentration of *M. indica*; **e** Lamella fusion (LF); necrosis (N) and degeneration of cartilaginous core (DCC) in the gill of fish exposed to high (0.75 mg/g) concentration of *M. indica* alone; **f** necrosis (N), oedema (O), lamella fusion (LF) and rupture of secondary lamella (RSL) in the gill of atrazine-exposed fish ameliorated with high (0.75 mg/g) concentration of *M. indica*. **a** *Liver* of *C. gariepinus* in the control showing normal arrangement of hepatocytes (H); **b** Sinusoidal distortion (SD), oedema (O) and cellular infiltration (CI) in the liver of fish exposed to atrazine alone; **c** necrosis (N) in the liver of fish exposed to low *M. indica* alone; **d** Gradual reversal of oedema (O) and necrosis (N) in atrazine-exposed fish ameliorated with low *M. indica*; **e** Sinusoidal distortion (SD) in the liver of fish exposed to high (0.75 mg/g) concentration of *M. indica* alone; **f** Sinusoidal distortion (SD), central vein (CV) in the liver of atrazine-exposed fish ameliorated with high (0.75 mg/g) concentration of high *M. indica*

lamella, hyperplasia, necrosis and rupture of epithelial cells (Figs. 5b, 6b, Tables 1, 2). In each of the control group treated with the low dietary CPE (Fig. 5c, Table 1) and MIE (Fig. 6c, Table 2) alone, curling of lamella and oedema were mildly observed compared to the atrazine-exposed group. In addition to this, necrosis was seen only in the low CPE group (Fig. 5c, Table 1) but not in low MIE group (Fig. 6c, Table 2), while swelling of lamella was seen only in low MIE (Fig. 6c, Table 2) but not in low CPE (Fig. 5c, Table 1) group. Compared to the atrazine group (Figs. 5b, 6b, Tables 1, 2) the administration of both the high CPE (Fig. 5e, Table 1) and MIE (Fig. 6e, Table 2) alone triggered similar gill disorders except degeneration of cartilaginous core that was seen only in high MIE group (Table 2). Treatment of atrazine-exposed fish with both low dietary CPE (Fig. 5d, Table 1) and MIE (Fig. 6d, Table 2) showed nearly normal general architecture of the gill as a partial or total reversal of lamellae rupture, curling and necrosis and other lesions were observed. However, treatment with both the high CPE (Fig. 5f) and MIE (Fig. 6f) exhibited similar lesions such as necrosis and oedema. Also, a degenerative disorder

**Table 1** Ameliorative effects of *Carica papaya* on the histopathological changes in the gill and liver of *Clarias gariepinus* exposed to atrazine for 28 days

| Tissue | Lesion | Treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F |
| Gill | Curling of lamella | − | +++ | + | + | − | − |
| | Degeneration of cartilaginous core | − | + | − | − | − | − |
| | Displacement of gill filament | − | − | − | − | − | + |
| | Hyperplasia | − | ++ | − | − | − | − |
| | Lamella fusion | − | ++ | − | + | + | − |
| | Lamella swelling | − | − | − | − | ++ | − |
| | Necrosis | − | + | ++ | − | + | ++ |
| | Oedema | − | − | + | − | − | + |
| | Rupture of secondary lamella | − | ++ | − | − | − | − |
| Liver | Cellular infiltration | − | − | − | + | − | + |
| | Displacement of central vein | − | − | + | − | − | − |
| | Necrosis | − | − | − | − | − | − |
| | Oedema | − | ++ | − | − | − | − |
| | Sinusoidal distortion | − | ++ | ++ | + | + | − |

− (none), + (mild), ++ (moderate) and +++ (severe); A control; B atrazine-exposed group; C low (0.25 mg/g) concentration of *C. papaya* alone; D atrazine + low (0.25 mg/g) concentration of *C. papaya*; E high (0.75 mg/g) concentration of *C. papaya* alone; F atrazine + high (0.75 mg/g) concentration of *C. papaya*

**Table 2** Ameliorative effects of *Mangifera indica* on the histopathological changes in the gill and liver of *Clarias gariepinus* exposed to atrazine for 28 days

| Tissue | Lesion | Treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F |
| Gill | Curling of lamella | − | +++ | + | − | − | − |
| | Degeneration of cartilaginous core | − | + | − | − | + | − |
| | Displacement of gill filament | − | − | − | − | − | − |
| | Hyperplasia | − | ++ | − | − | − | − |
| | Lamella fusion | − | ++ | − | − | ++ | + |
| | Lamella swelling | − | − | ++ | + | − | − |
| | Necrosis | − | + | − | − | + | + |
| | Oedema | − | − | + | − | − | + |
| | Rupture of secondary lamella | − | ++ | − | − | − | ++ |
| Liver | Cellular infiltration | − | + | − | − | − | − |
| | Displacement of central vein | − | − | − | − | − | − |
| | Necrosis | − | − | + | + | − | − |
| | Oedema | − | ++ | − | + | − | − |
| | Sinusoidal distortion | − | ++ | + | − | ++ | + |

− (none), + (mild), ++ (moderate) and +++ (severe); A control; B atrazine-exposed group; C low (0.25 mg/g) concentration of *M. indica* alone; D atrazine + low (0.25 mg/g) concentration of *M. indica*; E high (0.75 mg/g) concentration of *M. indica* alone; F atrazine + high (0.75 mg/g) concentration of *M. indica*

such as displacement of gill filament was found in the former (Fig. 5f), while lamella fusion and rupture of secondary lamella were observed in the latter (Fig. 6f).

Degenerative changes observed in the liver of atrazine-exposed fish were vacuolation, cellular infiltration, sinusoidal distortion and oedema (Figs. 5b, 6b, Tables 1, 2) compared to the control (Figs. 5a, 6a) with a normal arrangement of the hepatocytes. In the group treated with the low CPE (Fig. 5c) and MIE (Fig. 6c) alone, moderate sinusoidal distortion and mild displacement of central vein became prominent in the former (Table 1), whereas necrosis was mildly exhibited in the latter (Table 2). In the groups treated with high concentrations of both extracts (Figs. 5e, 6e, Tables 1, 2) sinusoidal

**Table 3** Multivariate analysis for the effects of tissue type (blood, gill, liver), atrazine treatment (I, II, III, IV, V, VI)[a] and extract type (*C. papaya*, *M. indica*) on biochemical parameters in *C. gariepinus*

| Variables | *Df* | Glucose (mmol/l) | | Total protein (g/l) | | ALT (IU/l) | | AST (IU/l) | | LDH (IU/l) | | SOD (IU/l) | | AChE (IU/l) | | MDA (nmol MDA/l) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| Tissue | 2 | 101.30 | **< 0.001** | 127.32 | **< 0.001** | 25.44 | **< 0.001** | 1.76 | 0.192 | 20.30 | **< 0.001** | 19.05 | **< 0.001** | 31.40 | **< 0.001** | 204.57 | **< 0.001** |
| Atrazine | 5 | 1.145 | 0.361 | 1.67 | 0.175 | 0.43 | 0.823 | 1.69 | 0.171 | 0.37 | 0.865 | 0.24 | 0.939 | 1.47 | 0.233 | 2.99 | **0.029** |
| Extract | 1 | 0.067 | 0.771 | 5.45 | **0.027** | 4.53 | **0.042** | 0.00061 | 0.980 | 17.19 | **< 0.001** | 11.08 | **0.003** | 3.03 | 0.093 | 12.48 | **0.002** |
| Residuals | 27 | | | | | | | | | | | | | | | | |

The bold values were significant at $p < 0.05$

[a] I control II atrazine-exposed group III low concentration of plant extract alone IV atrazine + low concentration of plant extract V high concentration of plant extract alone VI atrazine + high concentration of plant extract

distortion was markedly exhibited but was mildly shown in the group treated with the low CPE (Table 1). Simultaneous administration of both the low (Figs. 5d, 6d) and high (Figs. 5f, 6f) concentrations of the two extracts to the atrazine-exposed fish reversed the hepatic lesions to some extent, more so, with the low concentrations but not with the high concentrations (Tables 1, 2). Generally, there were more consistencies between improvement in tissue biochemical parameters and histopathological disorders in atrazine group treated with the low and high CPE than in atrazine group treated with the low and high MIE.

Multivariate analysis (Tables 3, 4) summarised the effects of tissue type, atrazine treatment and extract type on the biochemical parameters of *C. gariepinus*. The analysis showed that the overall trend in the ameliorative effects of the plant extracts on the biochemical parameters of the atrazine-exposed fish was markedly affected by tissue type ($p < 0.001$) and extract type, but not by the concentration of atrazine except for MDA parameter (Table 3). Four of the parameters (LDH, SOD, AChE and MDA) showed a higher significant ($p < 0.05$) response to the ameliorative activities of the extracts in the liver, two (glucose and ALT) in the gill and one (protein) in the blood; but the response of AST was not significantly ($p > 0.05$) different among the tissues (Table 4). The ameliorative activities of MIE were significantly ($p < 0.05$) higher in five of the parameters than CPE (Table 4).

## Discussion

Most resident fish species in aquatic ecosystems recipient of run-offs and drainages from farmlands have become susceptible to tissue damage from xenobiotic accumulation (Faria et al., 2021; Sula et al., 2020). The evaluation of blood profile in animals has been known to be advantageous in disease diagnosis and early detection of warning signs of environmental contamination (Abdel-Tawwab et al., 2019; Qyli et al., 2020). In this study, the significant decrease ($p < 0.05$) in the haematological parameters of *C. gariepinus* upon exposure to atrazine alone when compared to the control is consistent with earlier reports on the effects of atrazine on fish (Blahova et al., 2014; Ramesh et al., 2009).

The decrease in RBC, HB and PCV may be attributed to the malfunctioning of the erythropoietic tissue due to the haemolysing and shrinkage effects of atrazine, or haemodilution which might have resulted from the osmoregulatory dysfunction of the gill. These findings concur with the previous observations of Velisek et al. (2010) and Marzouk et al. (2012), who reported a marked decrease in RBC, HB and PCV in *Cyprinus carpio* and *Clarias gariepinus* exposed to terbutryn and atrazine, respectively. Hamed and El-Sayed (2018) also recorded similar observations in *Oreochromis niloticus* treated with pendimethalin herbicide. The atrazine-induced decrease in MCV, MCH and MCHC may be due to direct responses of erythrocyte membranes to structural damage resulting in haemolysis and impairment in synthesis of haemoglobin or stress related release of erythrocytes from the spleen and hypoxia (Mekkawy et al., 2013). WBC is important in regulating the immune system and they usually increase in number as a protective response to oxidative stress. The decrease in WBC and LYM is suggestive of a compromised immune system in the face of stress due to atrazine. Such a decline in WBC and LYM has been reported in *Cyprinus carpio* acutely exposed to atrazine (Blahova et al., 2014) and in *Oncorhynchus mykiss* and *Cyprinus carpio* exposed to metribuzin (Velisek et al., 2008, 2009). In this study, the simultaneous treatment of atrazine-exposed fish with both the low and high concentrations of CPE that prevented the decrease in the levels of the haematological parameters, and the significant increase in the blood parameters close to their respective values as a result of the treatment with the high concentration of MIE were to maintain the levels of these indices in the blood. Hence, both extracts reversed the atrazine injurious effects and improved the haematological parameters of atrazine-exposed fish.

Biochemical parameters are routinely used as biomarkers of tissue damage in fish (Qyli et al., 2020; Rashidian et al., 2018) as their assessment could portray the structural and/or functional status or condition of the tissues (Banaee et al., 2011). Enhanced activities of ALT, AST and LDH in the serum of atrazine-exposed fish are consequences of tissue dysfunction due to alterations in membrane permeability resulting in leakage from the tissues into blood circulation. The concentration dependent elevations of ALT and AST in the tissues may be ascribed to the increased utilisation of amino acids for protein synthesis triggered by the toxicant. Elevated LDH as observed in the tissues of atrazine-exposed fish is suggestive of a shift towards anaerobic condition as a result of hypoxia caused by atrazine in attempt to ensure the availability of sufficient energy to sustain the detoxification of the toxicant (Silvaperumal & Sankar, 2013b). These enhanced activities of ALT, AST and LDH are consistent with the findings of some authors who examined the effects of atrazine and other pesticides on the biochemical profile of fish (Blahova et al., 2014; Silvaperumal & Sankar, 2013a). Remarkably, atrazine toxicity treatment using CPE and MIE at either 0.25 or 0.75 mg/g prevented the elevations of ALT, AST and LDH and the depletion of glucose, protein, SOD and AChE levels in either the serum, gill or liver of exposed fish, an indication that the two extracts possess ameliorative properties. These findings are in consonance with Adeneye et al. (2015) and

**Table 4** Least square means of various biochemical parameters for tissue type, atrazine exposures and extract type

| Parameter | Tissue | | | | Atrazine* | | | | | | | Extract | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Blood | Gill | Liver | SE | I | II | III | IV | V | VI | SE | *C. papaya* | *M. indica* | SE |
| Glucose | 3.33$^{a}$ | 31.73$^{c}$ | 14.15$^{b}$ | 1.42 | 19.35$^{a}$ | 14.41$^{a}$ | 16.08$^{a}$ | 17.94$^{a}$ | 16.99$^{a}$ | 13.62$^{a}$ | 2.01 | 16.64$^{a}$ | 16.16$^{a}$ | 1.16 |
| Total protein | 48.50$^{c}$ | 11.83$^{a}$ | 16.98$^{b}$ | 1.76 | 31.28$^{a}$ | 23.12$^{a}$ | 25.53$^{a}$ | 25.31$^{a}$ | 22.25$^{a}$ | 27.13$^{a}$ | 2.49 | 23.40$^{a}$ | 28.14$^{b}$ | 1.44 |
| ALT (IU/l) | 18.13$^{a}$ | 68.31$^{c}$ | 50.39$^{b}$ | 5.04 | 39.48$^{a}$ | 53.13$^{a}$ | 48.03$^{a}$ | 44.83$^{a}$ | 42.61$^{a}$ | 45.57$^{a}$ | 7.13 | 39.41$^{a}$ | 51.81$^{b}$ | 4.12 |
| AST (IU/l) | 169.29$^{a}$ | 200.82$^{a}$ | 219.10$^{a}$ | 19.01 | 219.56$^{a}$ | 251.04$^{a}$ | 202.11$^{a}$ | 171.91$^{a}$ | 177.32$^{a}$ | 156.48$^{a}$ | 26.88 | 196.13$^{a}$ | 196.67$^{a}$ | 15.52 |
| LDH (IU/) | 65.82$^{a}$ | 116.48$^{b}$ | 170.90$^{c}$ | 11.66 | 116.98$^{a}$ | 127.53$^{a}$ | 131.37$^{a}$ | 114.77$^{a}$ | 110.76$^{a}$ | 105.01$^{a}$ | 16.50 | 89.82$^{a}$ | 145.65$^{b}$ | 9.52 |
| SOD (IU/l) | 116.54$^{b}$ | 86.24$^{a}$ | 162.77$^{c}$ | 8.83 | 127.36$^{a}$ | 119.29$^{a}$ | 120.12$^{a}$ | 131.23$^{a}$ | 114.74$^{a}$ | 118.37$^{a}$ | 12.49 | 104.88$^{a}$ | 138.82$^{b}$ | 7.21 |
| AChE (IU/l) | 12.68$^{a}$ | 16.84$^{b}$ | 25.06$^{c}$ | 1.12 | 19.52$^{a}$ | 16.06$^{a}$ | 17.38$^{a}$ | 20.40$^{a}$ | 19.70$^{a}$ | 16.10$^{a}$ | 1.59 | 17.06$^{a}$ | 19.32$^{a}$ | 0.92 |
| MDA | 3.93$^{a}$ | 16.40$^{b}$ | 26.94$^{c}$ | 0.81 | 13.71$^{a}$ | 19.16$^{b}$ | 16.65$^{ab}$ | 15.29$^{ab}$ | 15.53$^{ab}$ | 14.18$^{ab}$ | 1.14 | 14.11$^{a}$ | 17.40$^{b}$ | 0.66 |

Glucose (mmol/l), Total protein (g/l), ALT (Alanine transaminase), AST (Aspartate transaminase), LDH (Lactate dehydrogenase), SOD (Superoxide dismutase), AChE (Acetylcholinesterase), MDA (Malondialdehyde, nmolMDA/l)

*I control II atrazine-exposed group III low concentration of plant extract alone IV atrazine + low concentration of plant extract V high concentration of plant extract alone VI atrazine + high concentration of plant extract

Means along the same row for tissue type, atrazine exposures and extract type with different superscripts were significantly different at $p < 0.05$

Awodele et al. (2016) who reported that CPE and MIE, respectively, can mitigate acetaminophen- and carbon tetrachloride-induced toxicity, respectively.

Protein and glucose could serve as an indicator of stress and general well-being of fish. The reduction in protein (hypoproteinemia) with increasing atrazine concentration is due to possible utilisation of protein for energy source for the repair of damaged cells caused by atrazine. Similar reduction in protein level was reported by Mekkawy et al. (2013) in atrazine-exposed *C. gariepinus*. Hamed and El-Sayed (2018) and Ogueji et al. (2019) also made a similar observation in *Oreochromis niloticus* treated with pendimethalin and *C. gariepinus* exposed to acute concentrations of ivermectin, respectively. Changes in carbohydrate metabolism in fish may be due to stressful conditions. Ogueji et al. (2017) reported catecholamines and adrenocorticoid secretions in fish during stressful conditions which could lead to significant changes in carbohydrate reserves. The concentration-dependent decrease in glucose in the exposed fish may be due to kidney dysfunction as suggested by Ogueji et al. (2019), where damaged kidneys release glucose into the urine or inhibition of glucose synthesis due to hepatocytes damage. Simultaneous treatment of the atrazine-exposed fish with the two extracts improved the levels of protein and glucose.

The present study, in tandem with the previous ones (Blahova et al., 2014), has clearly shown that chronic atrazine exposure induces oxidative stress in the tissues of *C. gariepinus* as evidenced by the elevated levels of MDA, tissue enzymes biomarkers (ALT, AST and LDH) with concomitant depletion of glucose, protein and SOD levels in all the tissues examined. SOD, considered as the first line of the defence system, is a widely known and accepted method of ascertaining free radicals-induced oxidative stress in fish exposed to xenobiotics (Burgos-Aceves et al., 2018; Sehonova et al., 2019). The decreased SOD activity in fish tissues after atrazine exposure validates the previous observations of different pesticides-induced diminution in the activity of SOD in fish species (Abdelkhalek et al., 2015; Hamed & El-Sayed, 2018). The increased level of MDA as observed in the tissues of atrazine-exposed fish can be explained by the excessive production of ROS, which disrupted the cell membranes due to the inhibition of SOD, since this antioxidant protects the cell by eliminating free radicals from ROS. Thus, indicating the reduction in potency of the natural antioxidant defence mechanism of the fish, which supposed to have scavenged the ROS during atrazine insult. The disruption of cell membranes due to lipid peroxidation could have resulted in the loss of membrane functional integrity causing damage in the gill and liver of atrazine-exposed *C. gariepinus*. Several studies have also confirmed atrazine related induction of oxidative stress vis-à-vis high lipid peroxidation in many fish species (Blahova et al., 2013; Kadry et al., 2012; Mekkawy et al., 2013; Mela et al., 2013; Owolabi & Omotosho, 2017). In the present study, dietary supplementation with both plant extracts prompted a significant decrease in the MDA levels and enhancement of SOD in all the tissues of atrazine-exposed fish towards normal, indicating free-radicals prevention potentials of these plants against oxidation of tissues' cellular membranes. In rats, CPE and MIE were reported to abrogate stress-induced lipid peroxidation (Adeneye et al., 2015; Awodele et al., 2016). Furthermore, corroborating the present results, increased SOD was observed in *C. gariepinus* and *O. niloticus* exposed to deltamethrin and pendimethalin, respectively, following

treatment with dietary *Spirulina platensis* and *Moringa oleifera* extracts, respectively (Hamed, 2016; Hamed & El-Sayed, 2018).

AChE is widely used as a sensitive biomarker of neurotoxic substances. The inhibition of AChE activity in *C. gariepinus* following atrazine exposure could be attributed to the oxidative stress of atrazine exposure (Salbego et al., 2010). In several studies, many authors have demonstrated the inhibitory effects of pesticides on AChE activity in different fish species (Abdel-Tawwab & Hamed, 2018; Hamed & El-Sayed, 2018)). Interestingly, the neuro-ameliorative potential of the extracts was demonstrated by the reversal of the atrazine-induced inhibition of AChE activity upon fortification of the fish diets with CPE and MIE. Neuroprotective potentials of plants' extracts against xenobiotic-induced suppression of AChE activity have also been observed in fish (Gombeau et al., 2019; Hamed & El-Sayed, 2018).

Histology of tissues is a well-accepted indicator of xenobiotics exposure and it is considered a useful tool in the assessment of the degree of pollution, especially for sublethal and chronic effects (Plhalova et al., 2020; Vajargah et al., 2019). The observed gill and liver abnormalities is indicative of the negative effects of atrazine. The increased activities of the tissue enzymes biomarkers (ALT, AST and LDH) in the atrazine-exposed group validated the histopathological observations herein. Similar or more pronounced abnormalities in fish gill and liver resulting from atrazine exposure have been reported (dos Santos et al., 2015; Popoola, 2018). These abnormalities occurred due to diminution of the endogenous antioxidant status in response to oxidative damage resulting from the excessive generation of ROS. The gradual return of the tissue enzyme biomarkers close to their normal values observed to be more consistent with the data on histopathology after treatment with the low dietary CPE, and to a certain extent with the high CPE; is suggestive of the efficient suppression ability of the CPE against tissue damage. The widespread damage of the tissues may have prevented a clear correlation between improvement in tissue enzyme biomarkers and histological observations in the case of MIE, where partial ameliorative effects on the histology were observed at both concentrations of the extract. Similarly, it has been reported that strict correlation between histopathological observations and tissue enzyme biomarkers may not exist and the histological spectrum of disease condition can be noticed even in individuals with normal ALT values (Molfrad et al., 2003; Yilmaz-Ozden et al., 2015).

Although the ameliorative effects of both the CPE and MIE on atrazine-induced toxicity in fish have not been reported so far, previous studies have suggested the in vivo ameliorative potentials of the two extracts against toxins in mammalian models (Adeneye et al., 2015; Awodele et al., 2016; Ojo et al., 2018). The results of these studies are in tandem with those observed in the present study regarding the prevention of leakage of tissue enzyme biomarkers from the cytoplasm, where they are originally domiciled, into adjacent cells and general circulation, and the antioxidant property of the extracts. The efficacy of any plant may be hinged on its capability to suppress, improve and/or reverse to normal the anomalies caused by chemical insults. In this context, the non-improvement of some parameters in atrazine-exposed fish even after treatment with the plants' extracts at low concentration is probably suggestive of insufficient 'dosage' to prevent increase or decrease of the parameters, while at high concentration; it may be due to the suppressing effects of the extracts.

Plant secondary metabolites have been used, and still in use, as therapeutics, either as protective or ameliorative agents against toxins and this has been encouraged by the World Health Organisation (WHO) (Noureen et al., 2018). Previous reports on the analyses of the two plants' extracts phytochemical constituents have identified alkaloids, tannins, flavonoids, glycosides, saponins, terpenoids, phenols, amino acids and reducing sugars as the main bioactive compounds of these plants (Naggayi et al., 2015; Awodele et al., 2016; Hamed et al., 2021; Kurian et al., 2021). These bioactive compounds are well known for their protective or ameliorative properties of mediating their actions as primary antioxidants combating oxidative stress (Aruoma et al., 2006; Kang & Kim, 2018) or as scavengers abrogating the effects of free radicals (Rashidian et al., 2019; Shahidi & Ambigaipalan, 2015). Therefore, these bioactive constituents of the plants' extracts might have accounted for the observed ameliorative effects of atrazine-induced toxicity in this study.

Accumulation of xenobiotics might cause biochemical and physiological changes that could culminate in tissue malfunction which is inimical to the survival of an organism. Ecologically, fish are at the top of the aquatic food chain and are capable of accumulating contaminants and transfer them to man through the food chain (Abdel-Warith et al., 2020). Several studies have confirmed the possibility of atrazine accumulation and propensity to cross the brain-barrier, hence transmittable from mother to offspring (Lin et al., 2014; Rastegar-Moghaddam et al., 2018). The use of CPE and MIE, therefore, may not only serve as ameliorative or protective agents for fish in atrazine contaminated water but also for the riparian rice farmers cum fish-eating communities that depend on fish food protein.

The increased number of hepatic ameliorated parameters than those in gill and blood may depict a homeostatic

or adaptive mechanism, which necessitated the supply of more exogenous antioxidants from the extracts to enhance its defence status against atrazine toxicity. This might have accounted for the skewed activities of the extracts towards the liver. Tissue antioxidant capacity is crucial in combating free radicals and ensuring normal physiological functions in fish (Banaee et al., 2015). Atrazine treatment which did not show marked variations among the biochemical parameters (except MDA) in the atrazine-exposed fish could be due to the differential counter effects of the extracts at both the low (0.25 g) and high (0.75 g) concentrations, though both concentrations are capable of ameliorating some, if not all, of the haematological and biochemical/physiological disorders in atrazine-exposed *C. gariepinus*. However, the effect shown on the MDA undermines the appropriateness of the concentration of the extracts in a total reversal of lipid peroxidation damage in the atrazine-exposed fish. Therefore, the choice of concentration of extract appears to be critical in reducing the devastating effects of xenobiotics and maintaining balanced biochemical homeostasis in the tissue. Further investigations are, however, required to unravel the appropriate concentration and mechanisms of the extracts mediated amelioration against atrazine toxicity in fish. The ameliorative activities of MIE that were significantly higher than the CPE may be due to the abundant antioxidant capacity of the former than the latter.

## Conclusions

In conclusion, the results of this study demonstrate that the aqueous leaf extracts of *Carica papaya* and *Mangifera indica* can significantly ameliorate the oxidative injury caused by atrazine exposure in a concentration-dependent manner by increasing the haematological parameters, reducing the tissue enzyme biomarkers and increasing some metabolite levels such as glucose and protein. The improvement of these parameters was further accompanied by the improvement in gill and liver histopathology in the extracts-treated fish. The ameliorative effects of these extracts also validate their traditional use in several ailments earlier mentioned. Thus, the plant extracts could be exploited for fish health management practices, particularly in a rice-cum fish culture, where atrazine application is more prevalent.

#### Abbreviations

CPE: *Carica papaya* Extract; MIE: *Mangifera indica* Extract; RBC: Red blood cells; HB: Haemoglobin; PCV: Packed cell volume; MCH: Mean cell haemoglobin; MCHC: Mean cell haemoglobin concentration; MCV: Mean cell volume; WBC: White blood cells; LYM: Lymphocytes; ALT: Alanine aminotransaminase; AST: Aspartate aminotransaminase; LDH: Lactate dehydrogenase; SOD: Superoxide dismutase; AChE: Acetylcholinesterase; MDA: Malondialdehyde.

#### Acknowledgements

We sincerely appreciate the painstaking assistance of our undergraduate students who did the preliminary bench works and the useful suggestions of Prof. A.T. Ande.

#### Authors' contributions

ODO conceptualised, designed and participated in the supervision of the study. He wrote the first draft of the manuscript. SIA assisted in supervising the study, read and corrected the manuscript. Both ODO and SIA read, corrected and approved the final draft of the manuscript. All authors have read and approved the final manuscript.

#### Funding

The study was self-funded as the authors did not receive any financial support from any donor organisation or agency.

#### Availability of data and materials

Not applicable.

## Declarations

#### Ethical approval and consent to participate

All experiments were carried out following the principles guiding the use and handling of experimental animals as approved by the University of Ilorin Ethical Review Committee (UERC); reference number not applicable and were in accordance with both the national and international safety regulations and ethical principles for animal welfare.

#### Consent for publication

Not applicable.

#### Competing interests

The authors have no competing interests to declare as no funding was received for the study.

Received: 2 November 2020 Accepted: 15 September 2021
Published online: 26 September 2021

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