

Full Length Research Paper

Efficiency of *Moringa oleifera* dietary supplement reducing lead toxicity in *Puntius altus*

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***Moringa oleifera* was investigated to reduce the lead toxicity in *Puntius altus* via histopathological analysis. *P. altus* (n = 30) was divided into 3 groups, for feeding program with the different *M. oleifera* concentration; 0, 20 and 60 mg g⁻¹ fish food. After 4-weeks feeding time, all fish were exposed to 93.8 mg L⁻¹ (50% of 24 h LC₅₀) of lead for 24 h. The histopathologic alterations were observed in the gill, kidney and liver. Alterations like hyperplasia, epithelial lifting and telangiectasis were found in gill. The kidney lesions were shown cloudy swelling, tubular narrowing and hyaline droplet. Anomalies such as nuclear pyknosis, cytoplasmic vacuolation and melano-macrophages aggregation were found in liver. The *M. oleifera* feeding groups especially in high dose group were found lesser scores in the histological alteration when compared with the control group. Therefore, these results suggested that pre-feeding this plant will be protective in reducing lead burdens in fish exposed to environments contaminated with waterborne lead.**

Key words: *Moringa oleifera*, lead, *Puntius altus*, histopathology, fish, barb.

INTRODUCTION

Environmental pollutions caused by industrial and domestic wastes are nowadays the greatest concern in public health (Chiesa et al., 2006; Pandey et al., 2008). Thus contaminants change water quality and can cause serious damages to the organisms, including fish (Jemal et al., 2002; van der Oost et al., 2003). Lead (Pb) is a particular concern in this aspect because fish are able to bio-accumulate it in the body tissues due to reduce human food safety, especially protein source. Pb is a non-essential metal and contemporary contaminant throughout the world. Moreover, Pb is often used in varieties of industrial applications and products such as battery productions, chemicals, pigments and paints (WHO, 1977; Cavas, 2008). According to the previous studies, Pb can alter the physiological activities and cause histopathological changes of various organs in fish

(Jiraungkoorskul et al., 2007; Lamchumchang et al., 2007; Singhadach et al., 2009; Vinodhini and Narayanan, 2008). Considering that lead toxicity is currently one of the serious problems worldwide, there is still no specific, reliable and safe treatment. Our laboratory reported the efficiency of ascorbic acid and calcium to reduce lead toxicity in Nile tilapia, *Oreochromis niloticus* (Jiraungkoorskul et al., 2008; Singhadach et al., 2009).

Among the most abundant of natural plant, *Moringa oleifera* Lam. (drumstick tree, horse-radish tree, and synonym: *Moringa pterygosperma* Gaerth) is one of the best known and most distributed species of Moringaceae family. *Moringa* is an important tropical crop that is used as human food, medicine and in oil production (Becker and Makkar, 1999; Anwar et al., 2005; Soliva et al., 2005; Hamza, 2010). All parts of this plant are applied in traditional medicine for the treatment of human diseases, whereby the leaves are rich in protein, carotenoids, ascorbic acid and iron (Sanchez et al., 2006). The biological activities that is hepatoprotective (Pari and Kumar, 2002), hypocholesterolemia (Mehta et al., 2003),

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antifungal (Chuang et al., 2007), antioxidant (Sanchez et al., 2006), and anti-tumor (Bharali et al., 2003) are documented. Its leaves are also used as nutritional supplement and growth promoters due to the significant presence of protein, Se, P, Ca, β -carotene and α -tocopherol (Foidl et al., 2001; Sanchez et al., 2006). Although many researchers have reported these beneficial effects in mammal, there is no information regarding the utilization of *Moringa* leaves in fish feed. Therefore, this study was conducted to examine the potential protection by evaluated dietary *M. oleifera* against the acute lead toxicity in *P. altus* via histopathological analysis.

MATERIALS AND METHODS

Animal model

Red-tail tinfoil barb, *P. altus* (37.48 ± 9.95 g in body weight and 13.5 ± 0.67 in total length) from Bangkok, Thailand, with similar size were used. Under laboratory condition, fish were acclimated for 30 days and supplied with dechlorinated tap water at $29.0 \pm 1.0^\circ\text{C}$, pH = 6.6 to 7.0, total hardness = 68 to 80 mg L⁻¹ (as CaCO₃), alkalinity = 75 to 80 mg L⁻¹ and conductivity = 190 to 220 $\mu\text{mhos cm}^{-1}$. A 16:8 h light-dark cycle was maintained throughout. Chlorine residual and ammonia were below detection limits. The manufacturer's specifications for the fish food were 37% protein, 14% fat, 3% fiber, 12% ash, and 1% sodium (Charoen Pokphand Group, Bangkok, Thailand). The quantity of food was 2% of the initial body weight per day. The animal care and handling in this research was approved by the Mahidol University-Institutional Animal Care and Use Committee (MU-IACUC). Therefore, this research followed the mammal animal care and use, that is, (1) Use, care and transportation of fish for toxic pathological testing was complied with all applicable animal welfare laws. (2) Number of fish was kept to the minimum requirement for achieve scientifically valid results. (3) All protocols were taken to avoid the discomfort, distress or pain in the fish. (4) The appropriate dosage of the anesthesia was 200 mg L⁻¹ ethyl-3-aminobenzoate methanesulfonate salt (MS222, Sigma) and the euthanasia was overdose of this chemical.

Preparation of dry leaf with fish food

Fresh *M. oleifera* leaves were washed several times in water, dried at 45°C for 72 h and made semi-powder by crushing using a mortar and pestle. The extraction was done by following the method of Lamchumchang et al. (2007) with modifications. Briefly, fish food was grounded in a blender and hydrated with distilled water 0.7 ml g⁻¹ of fish food, mixed with the leaf semi-powder extract in 20 and 60 mg g⁻¹ fish food, and extruded through a minced-meat processing machine. They were then broken into small pellets by hands and dried at 60°C for 48 h.

Experimental design

Fish ($n = 30$) were placed in aquaria-containing de-chlorinated tap water under laboratory condition. 10 fish per group were maintained on their respective diets for 4 weeks as follows: group 1, control group feeding with normal fish food; Groups 2 and 3, feeding with *M. oleifera* in 20 and 60 mg g⁻¹, respectively. The doses were selected on the basis of previously reported suggesting that *M. oleifera* is not toxic to animals (Grabow et al., 1985). Fish was

observed daily to monitoring its behavior. After the 4-weeks feeding program, five fish from each group was anesthetized with MS-222, weighed, and measured. Gill, liver and kidney were removed and prepared for histopathological analysis. The five fish remaining in each group were further investigated for lead toxicity. The 24 h LC₅₀ value of barb exposed to lead was determined in our laboratory as 187.60 mg L⁻¹ (Jiraungkoorskul et al., 2007). After the 4-weeks feeding program, fish were exposed to 93.8 mg L⁻¹ Pb(NO₃)₂, corresponding to 50% of the 24 h LC₅₀. After 24 h of lead exposure, fish from each group was anesthetized with MS-222, weighed, and measured. Gill, liver and kidney were removed and prepared for histopathological analysis.

Histopathological analysis

Histopathological procedure was followed by Humason (1972) with modification. Gill, liver, and kidney were excised and fixed in 10% neutral buffered formalin, dehydrated in graded series of ethanol, cleared in xylene and embedded in paraffin. Five micron sections were cut and stained with hematoxylin and eosin for the histopathological examination.

Semi-quantitative scoring

The lesion was evaluated semi-quantitatively by ranking tissue lesion severity. Ranking from - to + + + depending on the degree and extent of the alteration as follows: (-) no histopathology, (+) histopathology in < 20% of fields, (++) histopathology in 20 to 60% of fields, (+++) histopathology in > 60% of fields. This ranking was used by Benli et al. (2008) to establish an overall assessment value of the histopathological lesion for each individual fish tissues. Five slides were observed from each organ and treatment.

RESULTS

Gill histopathological analysis

The gill had four gill arches on each side of the body and each one supported numerous filaments name primary lamellae. The regular secondary lamellae were seen in all groups before lead exposure (Figure 1a, b and c). After 24 h lead exposure, the degenerative changes were observed in all groups (Figure 1d, e and f). The histopathological alteration in the gill was the least severity in Group 3, which feeding with 60 mg g⁻¹ of *M. oleifera* leaves (Figure 1f). On the other hand, Group 1, which not feed with this plant, presented the most severity alterations that is, primary lamellae hyperplasia; lifting of the lamellar epithelium; and telangiectasis. The semi-quantitative scoring of gill lesion is shown in Table 1.

Kidney histopathological analysis

The kidney of freshwater fish was composed of a variety of cells including parenchyma cells, lymphoid and hematopoietic tissue, which in the head kidney, and also groups of malano-macrophages. The normal appearance of glomerulus and renal tubule were seen in all groups

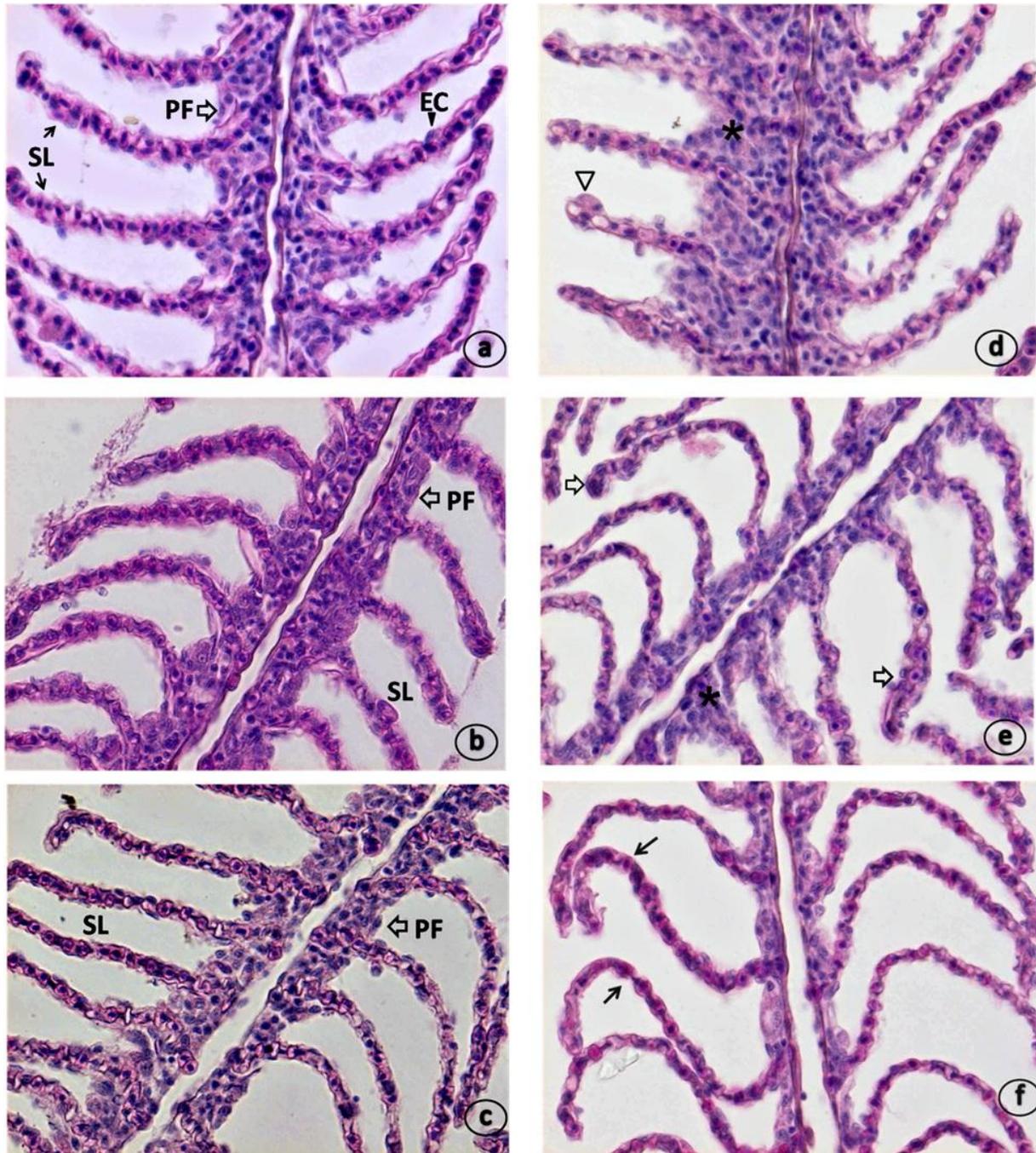


Figure 1. Photomicrographs of the gill of *P. altus* after the 4-weeks feeding program (a, b and c) showing normal appearance in control, low and high doses of *M. oleifera* groups, respectively. After 24 h lead exposure; (d) Control group showing hyperplasia of the primary filament (*) and hypertrophy of the lamellae epithelium (arrowhead); (e) In low dose treated group showing partial hyperplasia of filament (*) and telangiectasis (arrows); (f) In high dose treated group showing bend lamellae (arrows) (H and E stain, 40x). Note: Primary filament (PF), secondary lamellae (SL) and epithelial cell (EC).

before lead exposure (Figure 2a, b and c). After 24 h lead exposure, renal tissues in the control group were shown the most conspicuous alterations. Glomerulus atrophy and increase in the number of lymphocytes in parenchyma were observed. Cloudy swelling, tubular

narrowing and hyaline droplet were also found in renal tubule of group 1 (Figure 2d). Less frequently, regenerating tubules were seen in Groups 2 and 3 (Figure 2e and f). The semi-quantitative scoring of kidney lesion is shown in Table 1.

Table 1. Histopathologic analysis of *P. altus* after 24 h lead exposure.

Tissue and histopathology	<i>M. oleifera</i> concentration (mg g ⁻¹)		
	Group 1 (0)	Group 2 (20)	Group 3 (60)
Gill			
Primary filament hyperplasia	+++	+	-
Lamella hypertrophy	++	+	-
Epithelial lifting of lamellae	+++	+	-
Kidney			
Cloudy swelling	+++	+	+
Hyaline droplet degeneration	++	-	-
Liver			
Pyknotic nucleus	+	-	-
Cytoplasmic vacuolation	+++	+	-
Focal necrosis	+	-	+

Note: (-) no histopathology, (+) histopathology in < 20% of fields, (++) histopathology in 20 to 60% of fields, (+++) histopathology in > 60% of fields.

Liver histopathological analysis

Liver from fish fed *M. oleifera* crude leaves had slightly different histological characteristics from those of the control group. Lipid bodies were visible in the cytoplasm of hepatocytes (Figure 3b and c). A homogeneous hepatic parenchyma was observed in the control group. Hepatocytes were arranged in the cords (Figure 3a). The most alterations of liver were founded in the control group after lead exposure.

The main lesions were hepatocyte hypertrophy, nuclear pyknosis, cytoplasmic vacuolation and moderate melanomacrophages aggregation (Figure 3d). Like those of the control group, the histopathology in both treated groups (Groups 2 and 3) showed the similar changes but less severe (Figure 3e and f). The semi-quantitative scoring of liver lesion is shown in Table 1.

DISCUSSION

Histopathological alterations can be used as indicators of the effects of various pollutants on the organism including fish, and reflection of the overall health of the entire pollution. According to studies by Mohamed (2009) shown that the exposure of fish to pollutants, that is agricultural and industrial chemicals, were resulted in several pathological changes in different tissues of fish. Similar alterations in histopathology were also reported in the *Oreochromis spp.* exposed to hexavalent chromium (Abbas and Ali, 2007).

The present study examined the histopathological changes of lead exposure through non pre-feeding (control) and pre-feeding *P. altus*, with 20 and 60 mg g⁻¹

of *M. oleifera* leaves. According to Jiraungkoorskul et al. (2007), 50% of 24 h LC₅₀ of lead on freshwater fish, barb was 93.8 mg L⁻¹. Results of this study revealed that fish fed with 60 mg g⁻¹ of *M. oleifera* leaves had slightly histological changes, when compare with the control group. The observed changes in fish gills such as hyperplasia, telangiectasis and lifting of lamellar epithelium were generally attributed to toxic effects of lead. Similar alterations in the gills have also been reported in the fish exposed to metals (Cerqueira and Fernandes, 2002; Camago and Martinez, 2007; Altinok and Capkin, 2007).

In fish, as in higher vertebrates, the kidney performs an important function to maintain the homeostasis. The kidney is one of the first organs to be effected by contaminants in water (Thophon et al., 2003; Mela et al., 2007). In the present study, kidney often showed cloudy swelling in tubule cells after lead exposure. More hyaline droplet degeneration was observed in control group. Cengiz (2006) observed degeneration in the renal tubule, pyknotic nuclei in the hematopoietic tissue and degeneration of glomerulus. Similar alterations in the kidney have also been reported in Nile tilapia exposed to ammonia (Benli et al., 2008).

Pathological findings in liver included cytoplasmic vacuolation with partial nuclei hypertrophy in hepatocytes. The control group showed severe alterations when compared to the treated group. It has previously been shown that anomalies such as irregular shaped hepatocytes and cytoplasmic vacuolation were also founded in *Corydoras paleatus* contaminated by organophosphate pesticides (Fanta et al., 2003). Mobarak and Sharaf (2011) also reported the similar histopathological changes in gill and digestive system in

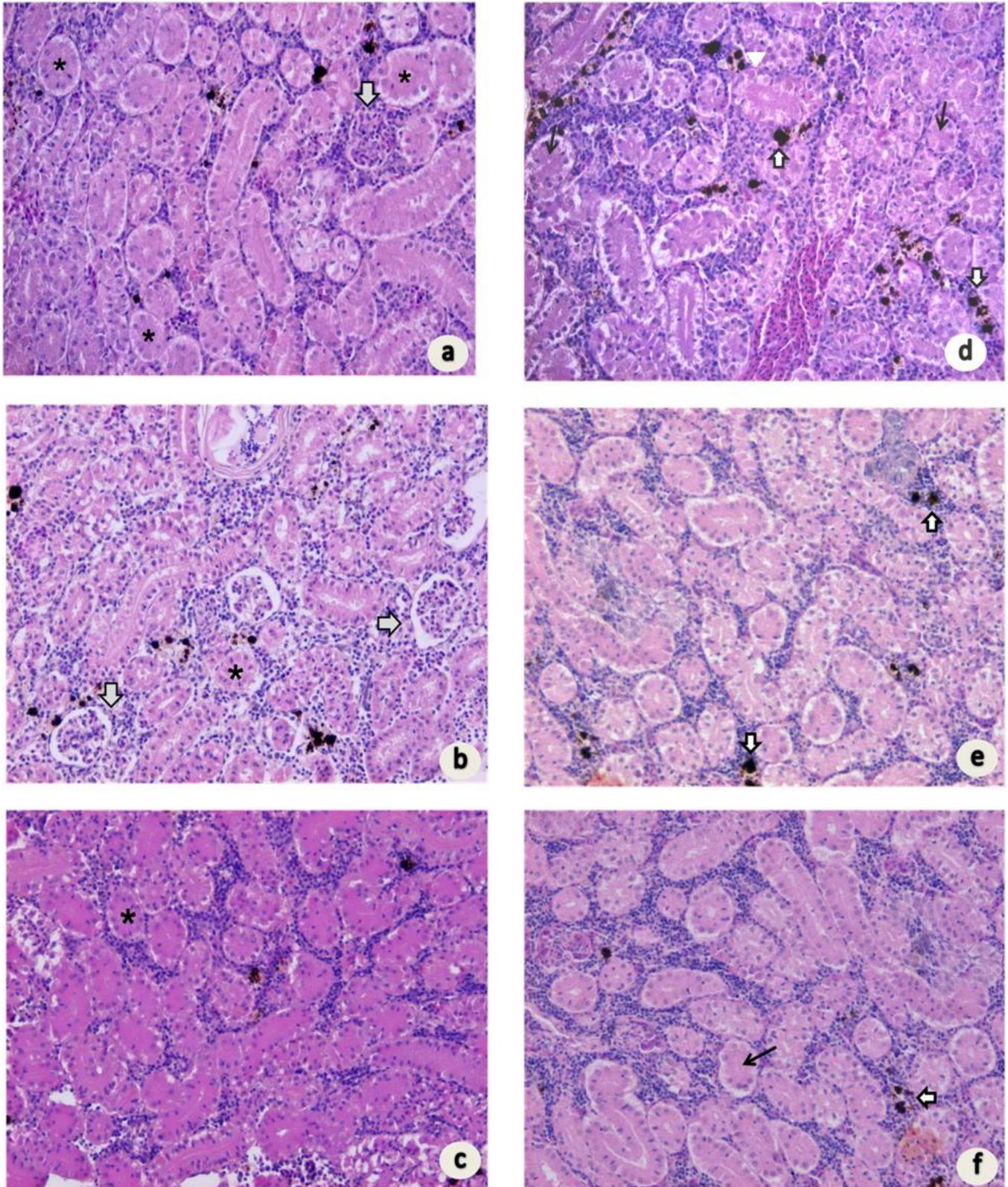


Figure 2. Photomicrographs of the kidney of *P. altus* after the 4-weeks feeding program (a, b and c) showing normal renal corpuscle, glomerulus, Bowman's space (arrow) and renal tubule (*) in control, low and high doses of *M. oleifera* groups, respectively. After 24 h lead exposure, (d) control group showing renal tubule cells with hypertrophy nucleus, cloudy swelling degeneration (arrow) and tubule with hyaline droplet (arrowhead), (e and f) low and high dose treated groups showing cloudy swelling with cellular occlusion of the tubular lumen and slightly melano-macrophages aggregation (white arrow) (H and E stain, 20 \times).

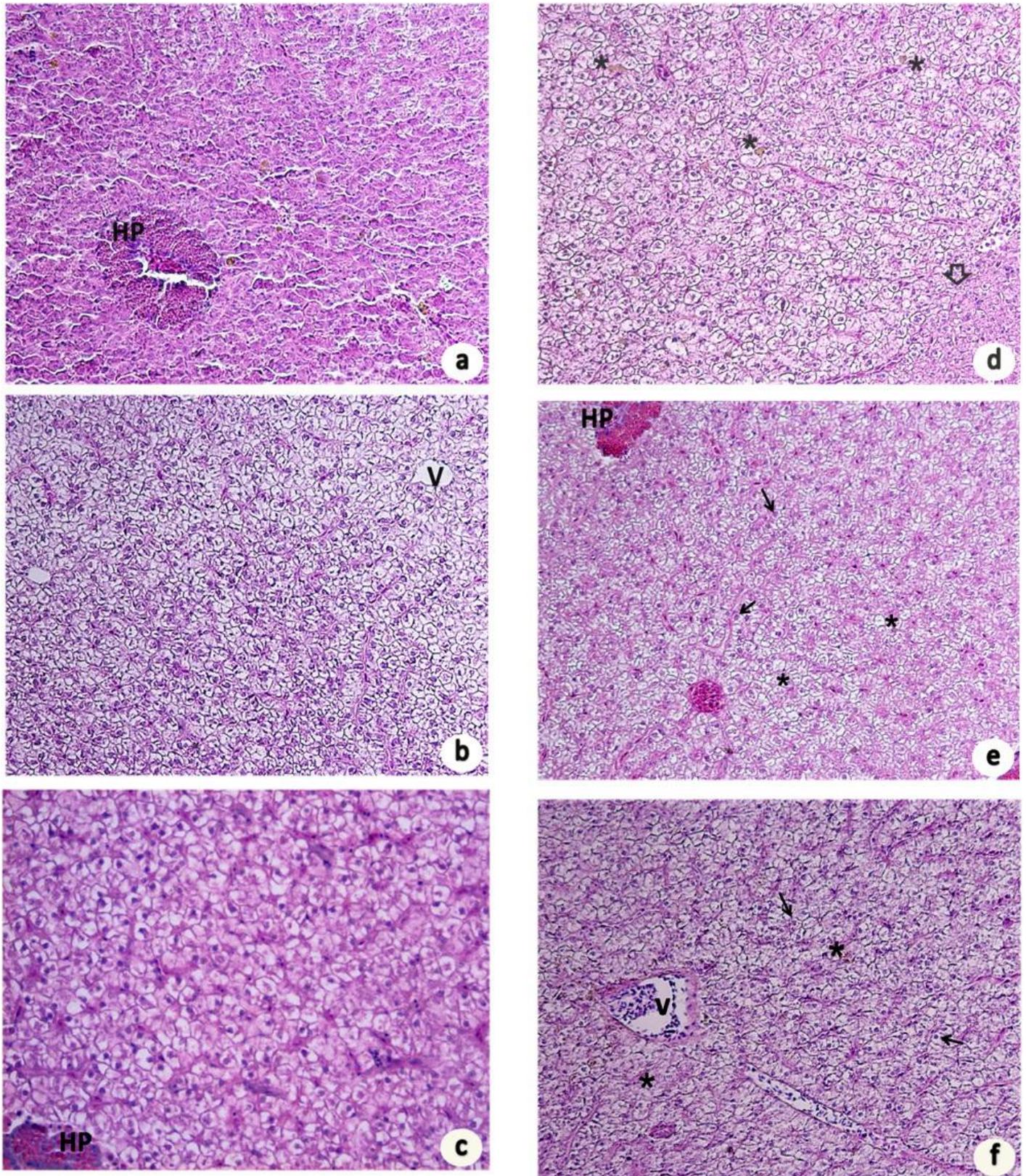


Figure 3. Photomicrographs of the liver of *P. altus* after the 4-weeks feeding program (a, b and c) showing normal hepatocytes with sinusoid, vein (V) and hepatopancreas (HP) in control, low and high doses *M. oleifera* groups, respectively. After 24 h lead exposure, (d) control group showing hepatocytes with cytoplasmic vacuolation (*) and focal necrosis of hepatic tissue (arrow), (e and f) low and high dose treated groups showing partial hypertrophy hepatocytes (arrows) and cytoplasmic vacuolation (*) (H and E stain, 20×).

system in *Poecilia latipinna*, freshwater fish, after lead acetate exposure. Additional, Fakurazi and co-workers (2008) reported the hepatoprotective effect of *M. oleifera* leaves via the restoration of the liver enzymes in rat induce with acetaminophen. They also reported that the pre-treated with these leaves significant preservation of rat liver histology. They suggested that the plant extract have some roles in preserving structural integrity of hepatocellular membrane, thus prevented enzymes leakage into the blood circulation. However, they also suggested that protective effects afforded by *M. oleifera* leaves against chemical induced hepatotoxicity is due to its ability to induce phase II detoxification pathway via promoting reduced glutathione (GSH) conjugation with toxic metabolites generated from CYP450 pathway (Fakurazi et al., 2008). The further research should investigate the mechanism of protective activities of *M. oleifera* leaves and the role of bioactive components of this plant responsible for this action.

Conclusion

There is a growing concern that some fish lovers are consuming high dose of heavy metals along with their fish dishes and could be suffering from health problems. This study emphasizes that *M. oleifera* leaves can reduce metal toxicity. Results showed that less histological changes were found in the pre-feeding with 60 mg g⁻¹ of *M. oleifera* group.

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