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Full Length Research Paper

Protective efficiency of *Thunbergia laurifolia* leaf extract against lead (II) nitrate-induced toxicity in *Oreochromis niloticus*

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***Thunbergia laurifolia* leaf was extracted by using 50% ethanol and supplemented with normal fish food. *Oreochromis niloticus* (n = 120) were divided into 6 groups by treating with or without 45 ppm of waterborne lead (II) nitrate and feeding normal fish food, fish food supplemented with *T. laurifolia* leaf extract in low or high dose. After 28 days of treatment, peripheral blood and organs were collected. Growth rate, blood chemistry, hematology and histology were investigated. Fish which were exposed to $Pb(NO_3)_2$ and were fed with fish food supplemented with *T. laurifolia* leaf extract especially in high dose, exhibited higher specific growth rate than fish which were exposed to $Pb(NO_3)_2$ and were fed with normal fish food. Moreover, *T. laurifolia* leaf extract can normalized blood chemistry, hematological and histological parameters in $Pb(NO_3)_2$-treated *O. niloticus*. We conclude that *T. laurifolia* leaf clearly reduced toxicity and is able to promote growth performance in *O. niloticus* after $Pb(NO_3)_2$ exposure.**

Key words: *Thunbergia laurifolia*, *Oreochromis niloticus*, lead, blood chemistry, hematology, histopathology.

INTRODUCTION

Heavy metal contamination in environment is one of the most important problems worldwide. It cannot only destroy through biological degradation, but heavy metals also have ability to accumulate for a long time leading to deleterious effect on the aquatic ecosystem and consequently possess serious health complications in human through food chain. In Thailand, heavy metal contamination in aquatic systems has extensively become the concern of this study. Heavy metals are reported as pollutants which caused the metabolic, physiological and structural alterations in fish (Jiraungkoorskul et al., 2006; 2007a; 2008). Lead (Pb) is an important pollutant in aquatic environment with increasing used in industry. Pb accumulation was found to increase in various organs including gills, liver, kidney, spleen and muscle of fish compared with control group leading to toxic effects in fish and reduced human food safety (Cicik et al., 2004; Jiraungkoorskul et al., 2008; Dai et al., 2009). Fish can uptake this metal through both gill and gastrointestinal tract. Gill is the primary site for Pb accumulation while digestive tract exhibited the greatest burden. Liver tissue always accumulated relatively high concentrations of Pb either through gill or gut (Rogers et al., 2003; Alves and Wood, 2006).

Thai traditional herb, *Thunbergia laurifolia*, which is a shrub with small oblong or ovate leaves dividing into 3 types including white, yellow and purple, of which the purple types are believed to possess health benefits. In Thai traditional medicine, *T. laurifolia* leaves are widely

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used as antipyretic and antidote against poisonous agents (Tejasen and Thongthapp, 1980). Previous studies had showed its therapeutic effect in drug addiction and hepatoprotective activity in ethanol-induced liver (Thongsaard and Marsden, 2002; Pramyothin et al., 2005). Aritajat et al. (2004) found that *T. laurifolia* extract decreased levels of blood glucose and also aids in the recovery of some β-cells in diabetic rats. It not only showed various pharmacological properties, but it also exhibited strong anti-mutagenic activity (Oonsivilai et al., 2007).

Fish are largely being used for assessment of aquatic environmental quality and can serve as bio-indicators of environmental pollution (Farombi et al., 2007). In the present experiment using freshwater fish, Nile tilapia (*Oreochromis niloticus*) as toxicological model due to its high efficiency to adapt in diverse diets, great resistance to diseases and handling practices as well as good tolerance to a wide variety of husbandry conditions. *O. niloticus* is a teleost fish, whose wide distribution around the world, is of economic importance for fishery and aquaculture. In this study, we aimed to evaluate the efficiency of *T. laurifolia* leaf extract to reduce pathotoxicological alterations in Nile tilapia after $Pb(NO_3)_2$ exposure.

MATERIALS AND METHODS

Plant extraction

Thunbergia laurifolia leaf was collected in Ratchaburi province, Thailand and was identified by Dr. Thaya Jenjittikul (Department of Plant Science, Faculty of Science, Mahidol University, Bangkok, Thailand). Voucher specimen (Palipoch 001) was deposited at Suan Luang Rama IX herbarium, Bangkok. Fresh leaves of *T. laurifolia* were washed several times in running water, dried at 60°C for 48 h in the hot air oven (Thelco®, GCA/Precision scientific, USA) and it was made powder using blender (Otto, Thailand). Ten grams of leaf powder were extracted with 100 ml of 50% ethanol and incubated in room temperature on shaker (Germmy Orbit Shaker model VRN-480, Taiwan) at 250 rpm for 48 h, after that centrifuged at 4,000 rpm for 10 min. Supernatant was collected and dried under reduced pressure in rotary evaporator (Rotavapor® R-200, BUCHI, USA). Samples were stored at -20°C until use.

Fish food supplementation

The dietary supplementation was made by using the technique of Lamchumchang et al. (2007) with minor modification. The fish foods were grounded in blender, hydrated with distilled water 0.7 ml/g of fish food, mixed with *T. laurifolia* leaf extract and extruded through minced-meat processing machine. The mixture was break into small pellet and air-dried at 70°C for 48 h in hot air oven. Fish food was stored at room temperature.

Fish maintenance

Freshwater fish, Nile tilapia (*Oreochromis niloticus*) from Chacheongsao province, Thailand with similar size were used. The average body weight was 54.18 g, of which the length of the longest fish was not more than 1.5 times of the shortest fish. The fish were kept in the glass flow through aquaria (50 × 50 × 120 cm) with continuous air and filled with 200 L of dechlorinated tap water (APHA, 2005). The temperature and pH of water was monitored and light-dark cycles (16:8 h) were controlled in the fish facilities. The fish were fed twice a day (2% of body weight per day) with commercial fish food containing 28% proteins, 4% fibers and 3% fats (Charoen Pokphand Group, Bangkok, Thailand).The fish, *O. niloticus* were acclimatized and closely cared under laboratory condition for 4 weeks before performing the experiment.

Fish treatment and specimen collection

The 96 h LC_{50} value of Nile tilapia exposed to lead (II) nitrate ($Pb(NO_3)_2$) was determined in our laboratory as 182.12 ppm (Jiraungkoorskul et al., 2007b). In this study, fish were exposed to 45 ppm of waterborne $Pb(NO_3)_2$ which corresponded to 25% of the 96 h LC_{50}. Fish (n = 120) were weighted on day 0, randomly divided into 6 groups and treated as follows:

Group 1: Fed with normal fish food (control group).
Group 2: Treated with 45 ppm of $Pb(NO_3)_2$ and fed with normal fish food.
Group 3: Fed with Fish food mixed with 0.2 mg of leaf extract/g of fish food (low dose)
Group 4: Treated with 45 ppm of $Pb(NO_3)_2$ and fed with fish food mixed with 0.2 mg of leaf extract/g of fish food (low dose)
Group 5: Fed with fish food mixed with 2 mg of leaf extract/g of fish food (high dose)
Group 6: Treated with 45 ppm of $Pb(NO_3)_2$ and feeding fish food mixed with 2 mg of leaf extract/g of fish food (high dose).

On day 28 of treatment, fish were weighted, anesthetized with tricaine methan sulphonate (0.2 g/L of distilled water) and the peripheral blood on mid-ventral line was collected. After that fish were euthanized by anesthetizing overdose. Operculum and peritoneal cavity were opened and the organs including gills, kidney and liver were collected.

Growth measurement and biochemical studies

Specific growth rate was calculated using the following formula:
$(\ln W_f - \ln W_i)/ T \times 100$,

Where
ln = natural logarithm;
W_f = final weight (g);
W_i = initial weight (g);
T = time in days (Metwally, 2009).

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein and albumin in blood were measured using Random access chemistry analyzer (BT-2000 plus, Biotechnica instrument, Italy).

Hematological studies

Hematocrit (Hct) was determined by filling blood into the microhematocrit tube (Vitrex 0705427, Herlev, Denmark) by using capillary action. The tube was filled at least 5 cm, sealed with modeling clay and centrifuged in the microhematocrit centrifuge (BOECO, Germany) at 10,000 to 12,000 rpm for 5 min. Hct level was measured with hematocrit reader. Hemoglobin (Hb) was measured by using cyanmethemoglobin method. The 20 µl of blood was added into 5 ml of Drabkin's solution, measured with

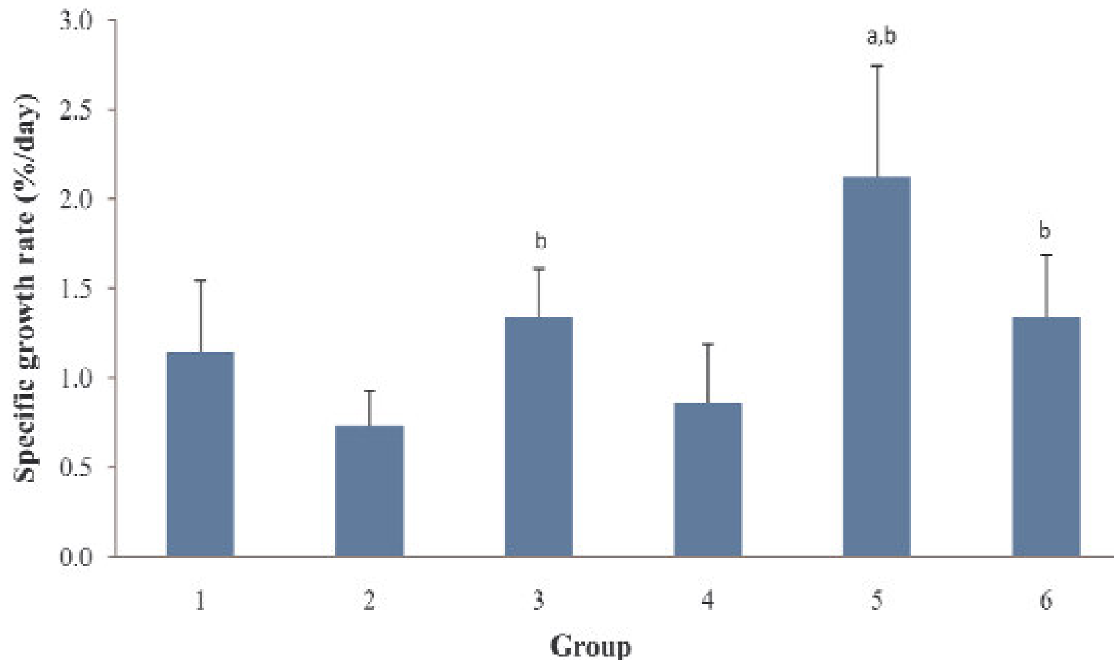

Figure 1. Specific growth rate of Nile tilapia (*Oreochromis niloticus*) in each group (n=20). Note: [a] and [b] were the mean difference significant at P<0.05 compared with group 1 and 2, respectively.

spectrophotometer at 530 nm and calculated from hemoglobin standard curve. For determining the number of red and white blood cells, 10 µl of blood was added into 2 ml of Natt and Herrick diluting solution (Natt and Herrick, 1952), mixed together, dropped into the hemocytometer and determined under light microscope.

Histological studies

Histopathology of the tissue organs was followed by the guidelines of Department of Pathobiology, Faculty of Science, Mahidol University, Thailand. Tissue organs including kidney, gills and liver were preserved in 10% formaldehyde for 24 h and washed with 70% ethanol.

Then the tissue organs were placed in small metal caskets, stirred by a magnetic stirrer (Lab-Line Pyro Magnestir, Melrose Park), they were dehydrated using alcohol series from 70 to 100% alcohol and embedded in paraffin (Paraplast X-TRA, Oxford Labware, 8889-503002, USA). The tissue organs were embedded in the paraffin using embedding machine (Axel Johnson Lab system, USA). Paraffin blocks were sectioned using a rotary ultra microtome (Histo STAT, Reichert, USA) and distributed onto glass slides with drying overnight. After staining with hematoxylin and eosin dyes and mounting, slides were observed under the light microscope (Humason, 1972).

Histological alterations were semi-quantitatively evaluated in terms of degree of tissue change (DTC) based on the severity of lesions. Organ changes were classified into 3 progressive stages:

Stage I = changes that do not damage the tissue to such an extent that the organ can repair itself.
Stage II = changes that are more severe and affect the associated tissue function.
Stage III = changes that preclude the restoration of the structure of the organ (Camargo and Martinez, 2007; Silva and Martinez, 2007).

The DTC was calculated by using the following mathematical equation (Poleksić and Mitrović-Tutundžić, 1994):

$$DTC = \sum I + 10\sum II + 100\sum III$$

where; I, II and III are the number of lesions of Stages I, II and III, respectively.

The average DTC was divided into five categories:

(i) 0 to 10: functionally normal.
(ii) 11 to 20: slightly to moderately damage.
(iii) 21 to 50: moderately to heavily damage.
(iv) 51 to 100: severely damage.
(v) >100: irreparable damage and the severity of tissue damage was compared among group.

Statistical analysis

All data were expressed as mean ± SD. Analysis of variance (ANOVA) with LSD test was performed for differences between each group. Significance of differences was considered at 5% level ($P<0.05$).

RESULTS

Specific growth rate

Growth rate of teleost fish, *O. niloticus* in each group was summarized in Figure 1. After 28 days of experimental period, fish in Group 6 which was exposed to $Pb(NO_3)_2$ and fed with fish food supplemented with high dose of *T.*

Table 1. Hematological parameters of Nile tilapia (*Oreochromis niloticus*) in each group expressed as mean ± SD. * The mean difference was significant at P<0.05 compared with Group 2.

Parameter	Group					
	1	2	3	4	5	6
Hct (%)	35.80 ± 0.84	34.00 ± 2.16	35.20 ± 2.28	33.00 ± 4.00	36.33 ± 7.64	33.80 ± 4.60
Hb (g/dl)	6.42 ± 0.74*	4.95 ± 0.51	6.35 ± 0.33*	5.68 ± 1.02	6.43 ± 1.69*	6.20 ± 0.29*
MCV (fl)	161.24 ± 11.54	177.88 ± 8.45	162.95 ± 12.03	162.48 ± 13.98	155.00 ± 1.42	160.38 ± 18.24
MCH (pg)	33.06 ± 4.20*	25.70 ± 2.07	32.43 ± 2.24*	32.63 ± 2.24*	31.13 ± 1.27*	31.28 ± 0.96*
MCHC (g/dl)	20.70 ± 1.39*	15.93 ± 0.61	20.18 ± 1.56*	20.88 ± 1.33*	20.93 ± 0.25*	20.80 ± 1.76*
RBC ($x10^6$ /cu.mm)	1.95 ± 0.24	1.74 ± 0.24	1.98 ± 0.27	1.87 ± 0.14	2.06 ± 0.54	1.85 ± 0.15
WBC ($x10^3$ /cu.mm)	7.00 ± 50.33	9.00 ± 34.64	7.50 ± 25.16	8.50 ± 50.00	7.33 ± 41.63	7.50 ± 44.33
Lymphocyte (%)	91.25 ± 1.26	92.75 ± 0.96	91.75 ± 1.26	91.75 ± 2.22	91.00 ± 1.29	91.75 ± 0.96
Neutrophil (%)	4.50 ± 0.58	3.75 ± 0.96	4.75 ± 0.50	4.75 ± 0.96	4.50 ± 1.29	4.50 ± 0.58
Monocyte (%)	4.25 ± 0.96	3.50 ± 1.29	3.50 ± 0.96	3.50 ± 1.29	4.50 ± 1.29	3.75 ± 0.50

Table 2. Plasma chemistry of Nile tilapia (*Oreochromis niloticus*) in each group expressed as mean ± SD. * The mean difference was significant at P<0.05 compared with Group 2.

Parameter	Group					
	1	2	3	4	5	6
Total protein (g/dl)	2.65 ± 0.30	2.45 ± 0.46	2.35 ±0.24	2.45 ± 0.13	2.67 ± 0.40	2.52 ± 0.22
Albumin (g/dl)	1.28 ±0.05	1.21 ± 0.10	1.23 ± 0.05	1.20 ± 0.08	1.30 ±0.10	1.26 ± 0.05
AST (U/L)	112.50 ± 46.87	139.00 ± 16.39	82.50 ± 20.79	135.50 ± 46.69	61.00 ±34.83*	62.20 ± 14.20*
ALT (U/L)	31.00 ± 9.83	39.00 ± 16.39	18.50 ± 3.87*	39.25 ± 14.43	19.67 ± 5.86*	25.60 ± 9.56

laurifolia leaf extract exhibited significantly (P<0.05) higher specific growth rate than fish in Group 2 which was exposed to $Pb(NO_3)_2$ and fed with normal fish food. Moreover, fish in Group 5 which were not exposed to $Pb(NO_3)_2$ and were fed with fish food supplemented with high dose of *T. laurifolia* leaf extract showed the highest growth rate and significant (P<0.05) difference compared to fish in Group1 (control group) and Group 2.

Blood chemistry and hematology

Results of blood chemistry were given in Table 1. Total protein and albumin revealed no significant difference when compared with each group. Fish in Groups 5 and 6 could significantly (P<0.05) reduce AST, while fish in Groups 3 and 5 significantly (P<0.05) reduce ALT compared with fish in Group 2.

Hematological parameters of *O. niloticus* in each group were illustrated in Table 2. Hct level, mean corpuscular volume (MCV), red blood cell (RBC) count, white blood cell (WBC) count and blood cell differential showed no significant difference when compared with fish in each group. Fish in Groups 3, 5 and 6 showed significantly (P<0.05) higher Hb level than fish in Group 2. Fish in Groups 3, 4, 5 and 6 revealed significantly (P<0.05) higher mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) level than fish in Group 2.

Histology

Efficiency of *T. laurifolia* leaf extract to reduce histological alterations was observed in kidney, gills and liver of *O. niloticus* (Table 3; Figures 2, 3 and 4). Fish in Group 2 were exposed to $Pb(NO_3)_2$ and were fed with normal fish food showed various histological alterations including: occlusion of proximal tubular lumen, cloudy swelling with cellular and nuclear hypertrophy, tubular necrosis and enlargement of glomerulus with reduction of Bowman's space in kidney (Figure 2B); hyperplasia of gill epithelium, epithelial lifting and lamellar fusion in gills (Figure 3B); cytoplasmic degeneration in hepatocyte, vacuolization of nucleus and irregular shape of hepatocyte (Figure 4B). Moreover, it showed narrowing of tubular lumens and dilatation of glomerulus capillaries in kidneys; hypertrophy of epithelium, blood congestion, lamellar disorganization, lamellar aneurysm and rupture of epithelial cells in gills; and nuclear and cellular hypertrophy, irregular shaped of nucleus and cellular rupture in liver (Table 3). Fish that were exposed to $Pb(NO_3)_2$ and were fed with fish food supplemented with *T. laurifolia* leaf extract (Groups 4 and 6) illustrated few pathological changes in kidney (Figure 2C and E), gills (Figure 3C and E) and liver (Figure 4C and E). Fish in

Table 3. Histopathological alterations of kidney, liver and gills in Nile tilapia (*Oreochromis niloticus*) in each group (n = 5) indicating the respective stage of tissue damage and frequency of occurrence. Stage I: do not alter normal physiology of tissue; stage II: more severe and causing abnormal physiology of tissue; stage III: marked severe and causing irreparable damage.

Histopathological alteration	Stage	Group					
		1	2	3	4	5	6
Kidney							
Nuclear hypertrophy of renal tubule	I	+	+++	+	++	0	+
Cellular hypertrophy of renal tubule	I	+	+++	+	++	+	+
Narrowing of tubular lumen	I	+	++	+	+	+	+
Enlargement of glomerulus	I	0	+++	0	0	0	0
Dilatation of glomerulus capillaries	I	+	++	0	+	0	0
Reduction of Bowman's space	II	0	+++	0	0	0	0
Occlusion of tubular lumen	II	0	++	0	+	0	0
Tubular necrosis	III	0	+++	0	0	0	0
Gills							
Hyperplasia of gill epithelium	I	+	+++	+	++	+	+
Hypertrophy of gill epithelium	I	0	++	0	+	0	+
Blood congestion	I	+	+	0	+	0	0
Lifting of lamellar epithelium	I	0	++	0	+	0	0
Lamellar fusion	I	0	+	0	+	0	0
Lamellar disorganization	I	0	++	0	+	0	0
Lamellar aneurysm	II	0	++	0	+	0	0
Rupture of epithelial cells	II	0	+	0	0	0	0
Liver							
Nuclear hypertrophy	I	0	++	0	+	0	0
Cellular hypertrophy	I	0	++	0	+	0	0
Irregular shaped nucleus	I	0	+++	0	0	0	0
Irregular shaped cell	I	+	+++	+	++	+	+
Cytoplasmic degeneration	II	0	+	0	+	0	0
Nuclear vacuolization	II	0	+	0	0	0	0
Cellular rupture	II	0	+	0	0	0	0

Note: absent (0); rare (+); frequency (++); very frequency (+++).

Groups 3 and 5 that were not exposed to Pb $(NO_3)_2$ but were fed with fish food supplemented with *T. laurifolia* leaf extract alone exhibited normal histology of kidney (Figure 2D and F), gills (Figure 3D and F) and liver (Figure 4D and F) as well as those of kidney (Figure 2A), gills (Figure 3A) and liver (Figure 4A) in control group.

In kidney, DTC of fish in Groups 3, 5 and 6 were 2.20 ± 0.84, 1.80 ± 0.45 and 2.80 ± 0.45, respectively, indicating normal function of kidney as well as fish in control group as shown in Table 4.

DTC of fish in Group 4 was 19.80 ± 5.76, indicating slightly to moderately damage of kidney but less pathological alteration than fish in Group 2 which shown irreparable damage. In gills, DTC of fish in Groups 3, 5 and 6 were 0.60 ± 0.55, 0.80 ± 0.45 and 1.40 ± 0.89, respectively, indicating normal function of gills as well as those in control group. DTC of Group 4 were 15.40 ± 0.89, indicating slightly to moderately damage of gills but less pathological alteration than fish in Group 2 which shown moderately to heavily damage. In liver, DTC of fish in Group 3, 5 and 6 were 0.60 ± 0.55, 0.80 ± 0.45 and 0.80 ± 0.45, respectively, and indicated normal function of liver as well as those in control group. DTC of fish in Group 4 were 12.40 ± 0.89, indicating slightly to moderately damage of liver but less pathological alteration than Group 2 which shown moderately to heavily damage.

DISCUSSION

From previous studies the leaf of *T. laurifolia* exhibited

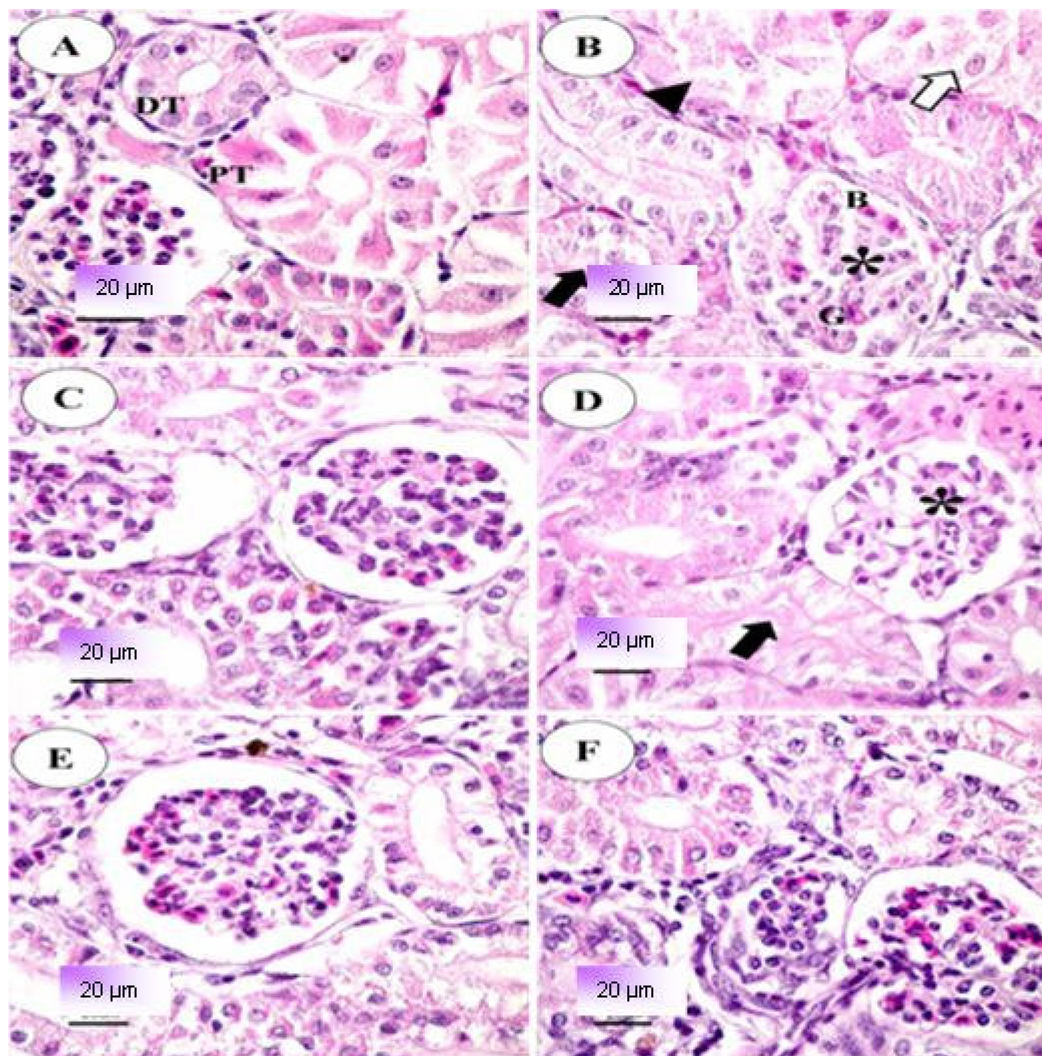

Figure 2. Histopathological alterations of kidney in Nile tilapia (*Oreochromis niloticus*) in each group. (A) Normal renal tubule both proximal (PT) and distal tubule (DT) and normal corpuscle of group 1 (control); (B) occlusion (black arrow) of proximal tubular lumen, cloudy swelling with cellular and nuclear hypertrophy (white arrow), tubular necrosis (arrowhead) and enlargement of glomerulus (G) with reduction of Bowman's space (B) (asterisk) of group 2; (D) narrowing of tubular lumen (arrow) and dilatation of glomerulus capillaries (asterisk) of group 4; (C, E and F) normal renal tubule and corpuscle of group 3, 5 and 6, respectively. Scale bar = 20 µm.

various pharmacological properties including antipyretic, antidote (Tejasen and Thongthapp, 1980), hepatoprotective activity (Pramyothin et al., 2005) and antioxidant (Oonsivilai et al., 2007). To our knowledge, the present study is the first report which clearly demonstrates the efficiency of *T. laurifolia* leaf extracted with 50% ethanol to reduce pathotoxicology of $Pb(NO_3)_2$ in blood chemistry, hematology and histology of freshwater fish, Nile tilapia (*O. niloticus*).

The evaluation of blood chemistry parameters is a routine and important tool which provides the vital information on the physiological status of animals (Chen et al., 2003). The present study showed no significant difference of total protein and albumin in each group indicating that $Pb(NO_3)_2$ at concentration of 45 ppm may not affect hepatic function of *O. niloticus*. AST and ALT are frequently used as biomarker of various tissue damages, such as liver, muscle and gills, but more specific in liver damage (De la Tore et al., 2000). Increased blood AST and ALT level of fish was caused mainly by the leakage of these enzymes from the hepatocyte into blood circulation as a result of liver damage. Fish that were exposed to $Pb(NO_3)_2$ and was fed with normal fish food significantly ($P<0.05$) demonstrated higher AST than fish in Groups 5 and 6 and higher ALT than fish in Groups 3 and 5. We thought

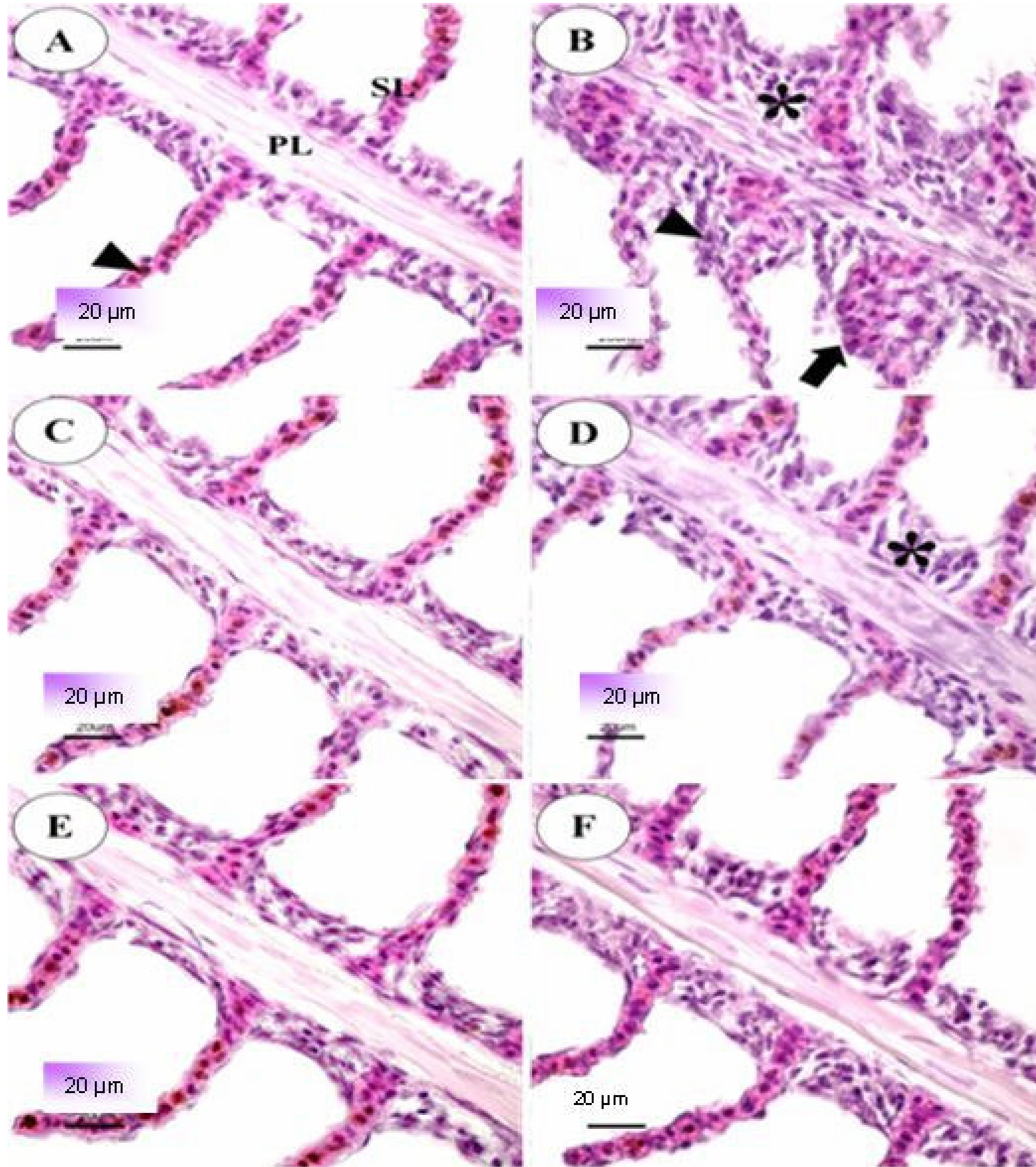

Figure 3. Histopathological alterations of gills in Nile tilapia (*Oreochromis niloticus*) in each group. (A) Normal primary lamellae (PL), lamellar epithelium (arrowhead) and secondary lamellae (SL) of Group 1 (control); (B) hyperplasia of gill epithelium (asterisk), epithelial lifting (arrowhead) and lamellar fusion (arrow) of Group 2; (D) hyperplasia of gill epithelium (asterisk) of group 4; (C, E and F) normal primary lamellae and secondary lamellae of Groups 3, 5 and 6, respectively. Scale bar = 20 µm.

that *T. laurifolia* leaf extract may act as hepatoprotective activity. Similar observations were reported in Wistar rats which demonstrated the ability of *T. laurifolia* leaf extract to reduce severity of rat liver injury and to normalize the level of ALT and AST after 14 days of ethanol exposure (Pramyothin et al., 2005).

Hematological indices are the vital parameters for evaluation of fish physiological status and they are more related to fish survival, reproduction and growth (Moiseenko, 1998). In the study, fish which exposed to $Pb(NO_3)_2$ and feeding normal fish food significantly ($P<0.05$) reduce Hb level, MCH and MCHC compared with fish in control group which were not exposed to $Pb(NO_3)_2$ but were feed with normal fish food alone (Table 1). Like other vertebrates, Pb-exposed fish provides abnormal heme synthesis. Normally, the erythrocyte enzyme delta-aminolevulinic acid dehydratase (ALA-D, E.C.4.2.1.24) catalyzes formation of porphobilinogen from aminolevulinic acid (ALA). Fish ALA-D is sensitive to the action of Pb, essential sulfhydryl groups in ALA-D are inactivated result in reduced hemoglobin formation (Sorensen, 1991). Fish that were

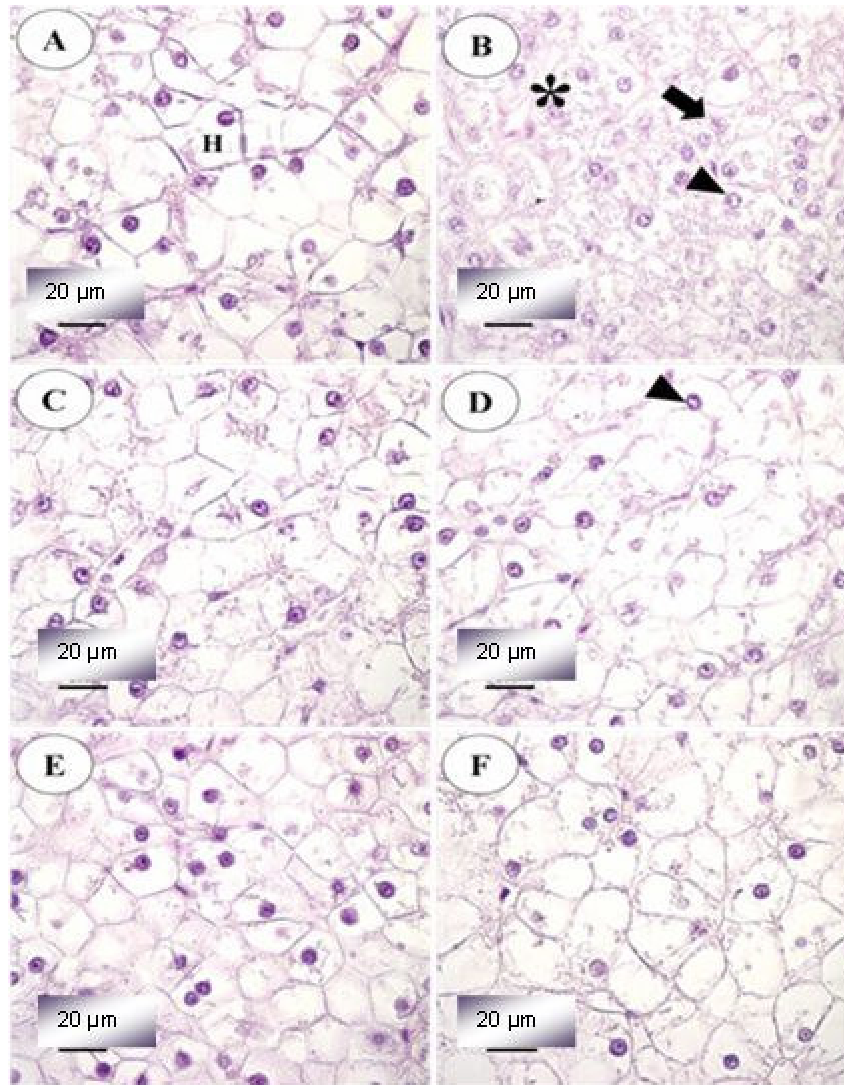

Figure 4. Histopathological alterations of liver in Nile tilapia (*Oreochromis niloticus*) in each group. (A) Normal hepatocyte (H) and showing sinusoid (S) of Group 1 (control); (B) cytoplasmic degeneration in hepatocyte (asterisk), vacuolization of nucleus (black arrowhead) and irregular shape of hepatocyte (arrow) of Group 2; (D) vacuolization of nucleus (arrowhead) of group 4; (C, E and F) normal hepatocyte of group 3, 5 and 6, respectively. Scale bar = 20 µm.

exposed to $Pb(NO_3)_2$ and fed with fish food supplemented with high dose of *T. laurifolia* leaf extract (Group 6) revealed significantly ($P<0.05$) higher Hb level, MCH and MCHC than fish in Group 2 (Table 1). The exact mechanism of action is still unknown. We hypothesize that *T. laurifolia* leaf extract may play a role in Pb chelation.

Histological analysis is a very sensitive parameter which is crucial for determining the cellular change generated by pollutant exposure in target organs (Hinton et al., 2001). In our study, it provides information of $Pb(NO_3)_2$-induced toxicity in target organs of fish including kidney, gills and liver. $Pb(NO_3)_2$ toxicity in kidney of *O. niloticus* demonstrated several pathological alterations such reduction of Bowman's capsule and occlusion of tubular lumen. Since kidney is the organ that filters the large volume of blood, $Pb(NO_3)_2$ present in blood can caused some pathological changes in Bowman's capsule, such as epithelial cell proliferation and thickening of basal lamina leading to the reduction of Bowman's capsule (Table 3; Figure 2D) similar to result which was reported by Silva and Martinez (2007). Occlusion of proximal and distal tubules can occur by the accumulation of some materials in lumen and also consequence of epithelial cells swelling (Takashima and Hibiya, 1995). Gills are participated in various vital

functions including respiration, osmoregulation and excretion and considered as the main target of Pb uptake causing various pathologies (Table 3). Lifting of lamellar epithelium, hypertrophy and hyperplasia of gill epithelium indicated defense mechanism of gills. Hypertrophy of gill epithelium may be caused by an increase in the cellular metabolism resulting in an imbalance of osmotic regulation by impairing ionic active transport (Mazon et al., 2002). Liver is the essential organ for the metabolism and detoxification which is also one of the organs most affected by contaminants in water (Rodrigues and Fanta, 1998; Crestani et al., 2007). After $Pb(NO_3)_2$ exposure, liver exhibited several lesions as shown in Table 3 which has also been described in freshwater fish, *Prochilodus lineatus*, subjected to *in situ* tests along the upper reaches of Cambé stream (Camargo and Martinez, 2007). Increased nuclear vacuolization of hepatocytes indicated a signal of degenerative process that suggested metabolic damage possibly related to exposure of contaminated water (Pacheco and Santos, 2002). In the study, fish that were exposed to $Pb(NO_3)_2$ and were feed with fish food supplemented with *T. laurifolia* leaf extract could reduce histopathology in kidney, gills and liver. Normally, Pb has the ability to produce toxic by both direct and indirect pathways. In the indirect pathway, it can induce oxidative stress through either overproduction of free radical especially reactive oxygen species (ROS) or accompanying change of enzymatic antioxidants (Shi et al., 2005). Oxidative stress caused tissue damage leading to various diseases in humans (Young and Woodside, 2001; Valko et al., 2007). An accumulation of ROS caused deleterious effect on cell structure including DNA, lipids and proteins (Evans et al., 2004). Furthermore, ALA-D inactivation may also result in the accumulation of δ-aminolevulinic acid which can cause overproduction of ROS (Bhadauria and Flora, 2003). The present study revealed the health benefit of *T. laurifolia* leaf extracted to reduce histopathological alterations in fish, but actual mechanism of action is still unknown. HPLC analysis of *T. laurifolia* leaf extract identified caffeic acid and apigenin as primary constituents of water extracts, while acetone and ethanol extracts contained primarily chlorophyll a and b, pheophorbide a, pheophytin a, and lutein (Oonsivilai et al., 2007). These substances can act as antioxidant which indicated that substances in *T. laurifolia* leaf extract may play a key role to scavenge Pb-generated free radical resulting in reduce histopathological changes of fish after Pb exposure. This study selects 0.2 mg of leaf extract/g of fish food as low dose due to this concentration which is enough to reduce free radical including ROS *in vitro* (Data not shown). Further studies are necessary to investigate HPLC analysis for identified substance in 50% ethanol extract of *T. laurifolia* leaf. Moreover, Tejasen and Thongthapp (1980) reported that *T. laurifolia* leaf had the ability to antidote the poisonous agents which may involve in the reduction of Pb toxicity in fish.

Comparing specific growth rate in each group, fish which were not exposed to $Pb(NO_3)_2$ while been fed with fish food supplemented with high dose of *T. laurifolia* leaf extract showed the highest growth rate. Moreover, fish which were exposed to $Pb(NO_3)_2$ and were been fed with fish food supplemented with high dose of *T. laurifolia* leaf extract exhibited significantly ($P<0.05$) higher specific growth rate than fish which were exposed to $Pb(NO_3)_2$ and fed with normal fish food. We suggest that *T. laurifolia* leaf extract supplemented in fish food had the ability to promote growth performance and protect fish against $Pb(NO_3)_2$ toxicity. Thus, it may be use as food supplementation of economic fish, *O. niloticus* which is important for fishery and aquaculture in worldwide. We conclude that *T. laurifolia* leaf extract clearly improved growth performance, blood chemistry, hematology and histology against $Pb(NO_3)_2$ toxicity in Nile tilapia (*O. niloticus*).

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