Effect of *Thunbergia laurifolia* (Linn) Leaf Extract Dietary Supplement Against Lead Toxicity in Nile Tilapia (*Oreochromis niloticus*)

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**Abstract:** This experiment was carried out to evaluate protective effects of *Thunbergia laurifolia* (Linn.) leaf extract against lead toxicity in *Oreochromis niloticus* (L.). Fish (n=120) were divided into 6 groups: groups 2, 4 and 6 treated with 45 mg L\(^{-1}\) of lead nitrate, whereas groups 1, 3 and 5 no exposed. Fish were fed basal fish food (groups 1 and 2), fish food supplemented with 0.2 mg of *T. laurifolia* leaf extract g\(^{-1}\) of fish food (groups 3 and 4) and 2 mg g\(^{-1}\) of fish food (groups 5 and 6). After 28 days of treatment, lead concentration, oxidative stress biomarkers and gill ultrastructure were investigated. Fish food supplemented with *T. laurifolia* leaf extract was able to reduce levels of lead in liver and muscle. Lead caused oxidative stress by reducing the content of reduced glutathione (GSH) and the activities of catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx) and increasing lipid peroxidation (LPO). Fish food supplemented *T. laurifolia* leaf extracts were able to increase the activities of intrinsic antioxidant and able to deplete LPO in gill, kidney and liver upon lead exposure. Surprisingly, it was able to reduce gill pathology in lead-exposed *O. niloticus*.

**Key words:** *Thunbergia laurifolia* %Antioxidant %Lipid peroxidation %Fish %*Oreochromis niloticus*

**INTRODUCTION**

Heavy metals are metallic elements which present in both natural and contaminated environments. They show the ability to accumulate in long times resulting in the deleterious effects in various organs of fish and consequently, important risk for human safety [1]. Lead is one of the toxic heavy metals which distributed into aquatic environment by various sources such as mining and refining of ores. It has pointed to either elevated lipid peroxidation (LPO) or decreased intrinsic antioxidant defense in various tissues of animals [2-4]. It inhibits the intrinsic antioxidants such as reduced glutathione (GSH), glutathione reductase (GR), glutathione peroxidase (GPx) and catalase (CAT) [5,6].

Fish are a good model for assessing aquatic ecosystem health and in toxicology [7]. *Oreochromis niloticus* (L.) is an important economic cultured fish in worldwide [8]. It grows fast and is characterized by easily breeding. It also provides a high quality food source to humans. From a previous study performed in *O. niloticus* exposed for one month to sediments from Mae Klong River, Samutsonkram province, Thailand which contained elevated levels of heavy metals, lead and chromium, Peebua *et al.* [9] demonstrated abnormalities of gill, liver and kidney.

*Thunbergia laurifolia* (Linn.), a vine distributed in Southeast Asia, is a shrub with small oblong or ovate leaves. In Thai traditional medicine, *T. laurifolia* used as anti-inflammatory, anti-pyretic and anti-bacterial agents.
It is also used as anti-dote against insecticides and toxic chemicals. Crude water extract of *T. laurifolia* leaf significantly increased K*-stimulated dopamine release from rat striatal slices and synergized with amphetamine on K*-stimulated dopamine release comparing with amphetamine alone [10]. *Thunbergia laurifolia* leaf extract increased up to 2-3 folds viability of primary cultures of rat hepatocyte upon ethanol treatment. It is promoted rat liver recovery decreasing the severity of rat liver injury and normalizing the levels of hepatic triglyceride, alanine aminotransferase and aspartate aminotransferase [11]. It is also exhibited strong anti-mutagenic activity [12]. In mice, co-treatment with aqueous *T. laurifolia* leaf extract at 100 mg kg⁻¹ or 200 mg kg⁻¹ body weight was found to restore the levels of caspase-3 activity and maintain total antioxidant capacity and antioxidant enzymes in the brain [13]. In the present study, we aimed to evaluate protect effect of *T. laurifolia* leaf extract-supplemented fish food against lead toxicity in *O. niloticus* for applied the extract as a fish food supplementation in the future.

**MATERIALS AND METHODS**

**Plant Extraction:** *Thunbergia laurifolia* leaves were collected in Ratchaburi province, Thailand and identified by at the 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 (Theclo®, GCA/Precision scientific, USA) and made powder using a blender (Otto, Thailand). Ten grams of leaf powder extracted with 100 mL of 50% ethanol were incubated on shaker (Germy Orbit Shaker model VRN-480, Taiwan) at 250 rpm and room temperature for 48 h and then centrifuged at 4,000 rpm for 10 min. Leaf extract was concentrated and dried under reduced pressure in a rotary evaporator (Rotavap® R-200, BUCHI, USA). Samples were stored at -20°C until used.

**Supplementation of Fish Food:** The fish foods were grounded in blender, hydrated with distilled water 0.7 mL g⁻¹ of fish food, mixed with leaf extract (0.2 and 2 mg g⁻¹ of fish food) and extruded through a 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 Treatments and Specimen Collections:** *Oreochromis niloticus* (L.) from Chacheongsao province, Thailand with similar size was used. The average body weight was 54.18 g. The fish were kept in glass aquaria (50 x 50 x 120 cm) with continuous air and filled with 200 L of dechlorinated tap water. The temperature and pH of water were monitored. Light-dark cycles (16:8 h) were applied to the fish facilities. 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). Fish were acclimatized and closely cared under laboratory condition for 28 days before performing the experiment.

The 96 h LC₅₀ value of Nile tilapia (182.12 mg L⁻¹) exposed to lead nitrate [Pb(NO₃)₂] was determined in our laboratory [14]. In this study, fish were exposed to 45 mg L⁻¹ of waterborne Pb(NO₃)₂ which corresponded to 25% of the 96 h LC₅₀. Fish (n = 120) were divided into 6 groups and treated as following:

**Group 1:** Fed basal fish food
**Group 2:** Treated with 45 mg L⁻¹ of Pb(NO₃)₂ and fed basal fish food
**Group 3:** Fed fish food supplemented with 0.2 mg of leaf extract g⁻¹ of fish food
**Group 4:** Treated with 45 mg L⁻¹ of Pb(NO₃)₂ and fed fish food supplemented with 0.2 mg of leaf extract g⁻¹ of fish food
**Group 5:** Fed fish food supplemented with 2 mg of leaf extract g⁻¹ of fish food Group 6: treated with 45 mg L⁻¹ of Pb(NO₃)₂ and fed fish food supplemented with 2 mg of leaf extract g⁻¹ of fish food

After 28 days of treatment, fish were euthanized with tricaine methane sulphonate (0.2 g L⁻¹ of distilled water) by anesthetizing overdose. Operculum and peritoneal cavity were opened and collected the organs including gill, kidney, liver and muscle.

**Determination of Lead Concentrations:** For determining the concentration of lead, gill, kidney, liver and muscle were placed in a test tube and transferred to hot air oven at 65°C for 48 h. After adding 6 mL of solution containing nitric acid and perchloric acid (2:1) at 120°C for 3 h, small pieces of tissue samples were filtrated through a 0.45 im Millipore filter (Whatman, NJ, USA). The solution was then analyzed using the GBC 932 plus flame atomic absorption spectrophotometer [15].

Preparation of Post-mitochondrial Supernatant (PMS): Gill, kidney and liver were homogenized in chilled 0.1 M Phosphate buffer saline (PBS, pH 7.4) containing 1.17% potassium chloride using a Potter Elvehjem homogenizer. The homogenate was centrifuged at 800 g, 4°C for 5 min to separate the nuclear debris. The supernatant was centrifuged at 10,500 g, 4°C for 30 min to obtain PMS.

GSH Level: GSH content was assayed by the method of Jollow et al. [16]. An aliquot of 1.0 mL of 10% PMS in distilled water was precipitated with 1.0 mL of 4% sulphosalicylic acid. The samples were kept at 4°C for 1 h and centrifuged at 1,200 g, 4°C for 15 min. The assay mixture contained 0.1 mL filtered aliquot, 2.7 mL of 0.1 M PBS (pH 7.4) and 0.2 mL of 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB, 40 mg in 10 mL of 0.1 M PBS, pH 7.4) in a total volume of 3 mL. The yellow color was measured at 412 nm.

CAT Activity: CAT activity was assayed by the method of Claiborne [17]. The mixture consisted of 1.95 mL of 0.05 M PBS (pH 7.0), 1.0 mL of 0.019 M H$_2$O$_2$ and 0.05 mL of 10% PMS. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated in terms of nmol H$_2$O$_2$ consumed min$^{-1}$Gmg$^{-1}$ protein.

GR Activity: GR activity was assayed according to Mohandas et al. [18]. The assay system consisted of 1.65 mL of 0.1 M PBS (pH 7.6), 0.1 mL of 0.5 mM EDTA, 0.05 mL of 1 mM oxidized glutathione, 0.1 mL of 0.1 mM NADPH and 0.1 mL of PMS in a total volume of 2.0 mL. The enzyme activity was measured by measuring disappearance of NADPH at 340 nm and was calculated as nmol NADPH oxidized min$^{-1}$Gmg$^{-1}$ protein using a molar extinction coefficient of 6.22 x 10$^4$ M cm$^{-1}$G.

GPx Activity: GPx activity was assayed by the method of Mohandas et al. [18]. Reaction mixture consisted of 1.44 mL of 0.05 M PBS (pH 7.0), 0.1 mL of 1 mM EDTA, 0.1 mM sodium azide, 0.05 mL of GR (1 U ml$^{-1}$G), 0.1 mL of 1 mM GSH, 0.1 mL of 2 mM NADPH, 0.01 mL of 0.25 mM H$_2$O$_2$ and 0.1 mL of 10% PMS in a total volume of 2 mL. Disappearance of NADPH was recorded at 340 nm. Enzyme activity was calculated as nmol NADP reduced min$^{-1}$Gmg$^{-1}$ protein using a molar extinction coefficient of 6.22 x 10$^4$ M cm$^{-1}$G.

Protein Concentration: Protein content was estimated by Bradford assay (Sigma, USA) by using bovine serum albumin as protein standard.

LPO: LPO was measured by the procedure of Wright et al. [19]. The reaction mixture, in a total volume of 1.0 mL, contained 0.58 mL of 0.1 M PBS pH 7.4, 0.2 mL of 10% PMS, 0.2 mL of 100 mM ascorbic acid and 0.02 mL of 100 mM ferric chloride was incubated at 37°C in a shaking water bath for 1 h. The reaction was stopped by the addition of 1.0 mL of 10% trichloroacetic acid. Then, 1.0 mL of 0.67% TBA was added and all the tubes were placed in a boiling water bath for 20 min. Malondialdehyde (MDA) formed was measured at 535 nm. The rate of LPO was calculated as nmol of thiobarbituric reactive substances (TBARS) formed hG g$^{-1}$ of tissue using a molar extinction coefficient of 1.56 x 10$^4$ M cm$^{-1}$G.

Determination of Gill Ultrastructure: Small pieces of gill were fixed with 4% glutaraldehyde in 0.1M PBS pH 7.4 for 24 h at 4°C, post-fixed in 1% osmium tetroxide for 1 h, dehydrated through series of alcohol and dried in a drye r. Then, gill was mounted on aluminium stubs and coated with platinum and palladium in an ion-sputtering apparatus (Hitachi E-102, Japan) for 6 min at 10-15 mA. Then, gill was examined under the scanning electron microscope with a digital camera (Hitachi S-2500, Japan).

Protein Concentration: Protein content was estimated by Bradford assay (Sigma, USA) by using bovine serum albumin as protein standard.

Statistical Analysis: All data were expressed as mean ± SD. Analysis of Variance (ANOVA) with Least Significant Difference (LSD) post-hoc test was performed for differences between each group. Significance of differences was considered at 5% level ($P < 0.05$).

RESULTS

Lead Concentrations: As shown in fig. 1, fish belonging to the group treated with Pb(NO$_3$)$_2$ and receiving not leaf extract (group 2) elicited the highest lead accumulation in kidney following gill, liver and muscle, respectively. Fish which were exposed to Pb(NO$_3$)$_2$ and fed fish food supplemented with 0.2 mg of T. laurifolia leaf extract gG of fish food (group 4) and 2 mg gG of fish food (group 6) were able to significantly reduce ($P<0.05$) lead concentration in liver and muscle, whereas in gill and kidney showed no significant difference of lead.
Fig. 1: Lead concentration in gill, kidney, liver and muscle of *O. niloticus*. The mean difference significant at \((P>0.05)\) compared with fish belonging to group 1 (a) and group 2 (b).

Note: Group 1: Basal fish food  
Group 2: Pb(NO\textsubscript{3})\textsubscript{2} + basal fish food  
Group 3: 0.2 mg of leaf extract g\textsubscript{G} of fish food  
Group 4: Pb(NO\textsubscript{3})\textsubscript{2} + 0.2 mg of leaf extract g\textsubscript{G} of fish food  
Group 5: 2 mg of leaf extract g\textsubscript{G} of fish food  
Group 6: Pb(NO\textsubscript{3})\textsubscript{2} + 2 mg of leaf extract g\textsubscript{G} of fish food

Table 1: LPO and the activities of antioxidants in gill, kidney and liver of *O. niloticus*. The mean difference significant at \((P > 0.05)\) compared with fish belonging to group 1 (a) and group 2 (b).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Organ</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO (nmol TBARS formed gG tissue)</td>
<td>Gill</td>
<td>2.48 ± 0.10</td>
<td>5.35 ± 0.69\textsuperscript{a}</td>
<td>2.12 ± 0.34</td>
<td>3.12 ± 0.19\textsuperscript{b}</td>
<td>1.40 ± 0.20\textsuperscript{a}</td>
<td>1.41 ± 0.05\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>2.13 ± 0.25</td>
<td>6.86 ± 0.21\textsuperscript{a}</td>
<td>1.40 ± 0.13\textsuperscript{b}</td>
<td>2.59 ± 0.26\textsuperscript{a}</td>
<td>2.26 ± 0.24</td>
<td>1.44 ± 0.34\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>7.85 ± 0.12</td>
<td>12.64 ± 1.46\textsuperscript{b}</td>
<td>11.34 ± 1.65</td>
<td>8.41 ± 0.51\textsuperscript{b}</td>
<td>5.71 ± 0.39</td>
<td>7.21 ± 0.35\textsuperscript{a}</td>
</tr>
<tr>
<td>GSH (mM)</td>
<td>Gill</td>
<td>46.67 ± 3.21</td>
<td>28.33 ± 4.16\textsuperscript{b}</td>
<td>49.33 ± 0.58</td>
<td>32.00 ± 1.73</td>
<td>50.67 ± 1.53</td>
<td>43.00 ± 2.00\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>49.33 ± 5.86</td>
<td>32.00 ± 3.00\textsuperscript{a}</td>
<td>47.67 ± 2.89</td>
<td>33.00 ± 3.61</td>
<td>51.00 ± 5.29</td>
<td>40.33 ± 4.16\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>68.33 ± 3.60</td>
<td>43.67 ± 3.06\textsuperscript{a}</td>
<td>70.67 ± 7.51</td>
<td>46.33 ± 8.08</td>
<td>73.67 ± 6.66</td>
<td>55.00 ± 3.46\textsuperscript{b}</td>
</tr>
<tr>
<td>CAT (nmol H\textsubscript{2}O\textsubscript{2} consumed mgG protein)</td>
<td>Gill</td>
<td>189.23 ± 4.51</td>
<td>101.67 ± 5.86\textsuperscript{a}</td>
<td>192.33 ± 9.29</td>
<td>149.00 ± 10.58\textsuperscript{b}</td>
<td>197.67 ± 21.57</td>
<td>176.33 ± 13.20\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>101.33 ± 2.89</td>
<td>57.33 ± 3.06\textsuperscript{a}</td>
<td>113.00 ± 14.11</td>
<td>68.67 ± 8.96</td>
<td>150.67 ± 22.90\textsuperscript{a}</td>
<td>112.00 ± 6.00\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>248.33 ± 7.51</td>
<td>112.67 ± 4.04\textsuperscript{a}</td>
<td>257.67 ± 6.03</td>
<td>143.00 ± 9.85\textsuperscript{b}</td>
<td>264.33 ± 10.12\textsuperscript{a}</td>
<td>221.33 ± 7.09\textsuperscript{b}</td>
</tr>
<tr>
<td>GR (nmol NADPH oxidized mgG protein)</td>
<td>Gill</td>
<td>111.33 ± 10.02</td>
<td>81.33 ± 2.08\textsuperscript{a}</td>
<td>81.67 ± 2.08</td>
<td>88.67 ± 9.07</td>
<td>120.67 ± 1.53</td>
<td>96.00 ± 2.65\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>81.33 ± 8.02</td>
<td>56.67 ± 4.73\textsuperscript{a}</td>
<td>81.67 ± 11.37</td>
<td>57.00 ± 7.94</td>
<td>86.33 ± 3.06</td>
<td>76.33 ± 7.51\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>164.33 ± 6.11</td>
<td>131.00 ± 4.00\textsuperscript{a}</td>
<td>165.33 ± 6.51</td>
<td>133.33 ± 8.96</td>
<td>172.33 ± 3.06</td>
<td>145.33 ± 5.86\textsuperscript{b}</td>
</tr>
<tr>
<td>Gpx (nmol NADP reduced mgG protein)</td>
<td>Gill</td>
<td>122.67 ± 10.02</td>
<td>130.00 ± 6.24</td>
<td>126.00 ± 4.58</td>
<td>132.67 ± 3.21</td>
<td>133.67 ± 9.45</td>
<td>142.00 ± 4.58\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>99.67 ± 4.32</td>
<td>73.00 ± 6.00\textsuperscript{a}</td>
<td>100.33 ± 8.33</td>
<td>74.67 ± 11.24</td>
<td>107.67 ± 3.21</td>
<td>86.33 ± 10.50\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>171.33 ± 2.52</td>
<td>189.00 ± 4.00\textsuperscript{a}</td>
<td>173.67 ± 13.32</td>
<td>195.00 ± 8.72</td>
<td>177.33 ± 7.02</td>
<td>191.00 ± 3.91\textsuperscript{b}</td>
</tr>
</tbody>
</table>

Note: Group 1: Basal fish food  
Group 2: Pb(NO\textsubscript{3})\textsubscript{2} + basal fish food  
Group 3: 0.2 mg of leaf extract g\textsubscript{G} of fish food  
Group 4: Pb(NO\textsubscript{3})\textsubscript{2} + 0.2 mg of leaf extract g\textsubscript{G} of fish food  
Group 5: 2 mg of leaf extract g\textsubscript{G} of fish food  
Group 6: Pb(NO\textsubscript{3})\textsubscript{2} + 2 mg of leaf extract g\textsubscript{G} of fish food
Fig. 2: Gill ultrastructure of *O. niloticus*. (A, C, D, E and F) indicated normal gill structure of primary lamellae (PL), secondary lamellae (SL) and microridges (M) of *O. niloticus* belonging to groups 1, 3, 4, 5 and 6, respectively; (B) demonstrated aneurisms in SL (red asterisk) of group 2.

Note: Group 1: Basal fish food
Group 2: Pb(NO$_3$)$_2$ + basal fish food
Group 3: 0.2 mg of leaf extract gG of fish food
Group 4: Pb(NO$_3$)$_2$ + 0.2 mg of leaf extract gG of fish food
Group 5: 2 mg of leaf extract gG of fish food
Group 6: Pb(NO$_3$)$_2$ + 2 mg of leaf extract gG of fish food
Table 2: Semi-quantitative scoring of gill lesions of *O. niloticus* where ranking from (-) no, (+) mild, (+++) moderate and (++++) severe pathology.

<table>
<thead>
<tr>
<th>Lesion</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal structure of secondary lamellae</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lamellar aneurism in secondary lamellae</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Thickness of the gill filament</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hypertrophy of secondary lamellae</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: Group 1: Basal fish food  
Group 2: Pb(NO$_3$)$_2$ + basal fish food  
Group 3: 0.2 mg of leaf extract g of fish food  
Group 4: Pb(NO$_3$)$_2$ + 0.2 mg of leaf extract g of fish food  
Group 5: 2 mg of leaf extract g of fish food  
Group 6: Pb(NO$_3$)$_2$ + 2 mg of leaf extract g of fish food

concentration compared with group 2. Fish belonging to group 6 significantly decreased (P<0.05) lead concentration in liver compared with group 2.

Effect of *T. Laurifolia* Leaf Extracts on Oxidative Stress Biomarkers: Oxidative stress biomarkers including GSH, CAT, GR, GPx and LPO in each group of *O. niloticus* were demonstrated in Table 1. Fish belonging to group 2 significantly depleted (P<0.05) of the intrinsic antioxidant status and significantly elevated (P<0.05) LPO in gill, kidney and liver compared with group 1 which were not treated with Pb(NO$_3$)$_2$ and fed basal fish food.

In gill, comparing with group 2, fish belonging to groups 4 and 6 were able to significantly increase (P<0.05) the activity of CAT and to significantly reduce (P<0.05) LPO. They were able to increase the activity of GR, but the differences did not reach the significance level. Fish belonging to group 6 were able to significantly increase (P<0.05) the level of GSH and the activity of GPx.

In kidney, comparing with group 2, fish belonging to groups 4 and 6 significantly depletes (P<0.05) the level of GSH and the activities of CAT and GR and fish belonging to groups 4 and 6 significantly depletes (P<0.05) LPO. They increased the activity of GPx, but no differences were observed.

In liver, comparing with group 2, fish belonging to groups 4 and 6 were able to significantly increase (P<0.05) the activity of CAT and to significantly reduce (P<0.05) LPO. They were able to increase the activity of GPx, but no differences were detected. Fish belonging to group 6 were able to significantly increase (P<0.05) the level of GSH and the activity of GR.

Effect of *T. Laurifolia* Leaf Extracts on Gill Ultrastructure: Fish belonging to group 2 showed markedly pathological changes including abnormal structure of secondary lamellae, lamellar aneurism in secondary lamellae, thickness of the gill filament and hypertrophy of secondary lamellae. Surprisingly, fish belonging to groups 4 and 6 showed no or few pathological alterations observed on the gill epithelium of primary lamellae and secondary lamellae as well as groups 1, 3 and 5 (Fig. 2 and Table 2). Microridges of fish in all groups exhibited well-defined contours, concentric and long appearances.

DISCUSSION

Lead is a ubiquitous environmental metal which can induce a broad range of the physiological, biochemical and behavioral dysfunctions in fish [21,22]. Oxidative stress has been proposed as a possible pathogenesis of lead toxicity [23]. Previous studies reported that lead either decreased in antioxidant status such as CAT, GPx and GSH [24,25] or elevated LPO in lead-exposed animals [2,3,4,26]. Lead showed the inhibition of several enzymes having functional SH groups [27]. Reduced glutathione is a tripeptide containing cysteine that has a reactive SH group. Normally, GSH plays a key role in the cellular protection against oxidative stress by direct interaction of the SH group with reactive oxygen species (ROS) or involvement in the enzymatic detoxification reactions of ROS as a cofactor or a coenzyme [28]. Glutathione reductase possesses a disulfide at its active site [29] which was suggested as a lead target [2]. The inhibition of GR results in decreased GSH:GSSG ratio causing cells more susceptible to oxidative stress. On the other hand, GPx and CAT are metalloproteins which accomplish their antioxidant functions. Due to these enzymatic antioxidants depend on various essential trace elements for proper molecular structure and enzymatic activity, they are potential targets for lead toxicity [30].
Medicinal plant extracts are of gradually high interest for an alternative choice to treatment lead toxicity. In this experiment, *T. laurifolia* leaf extracts possess the chelating ability which demonstrated the depletion of lead levels in liver and muscle. Similarly to another plant, Zhang et al. [31] indicated that pre-germinated brown rice was able to decrease the lead accumulation in rats. Xia et al. [32] demonstrated *Smilax glabra* extract as chelating agent to reduce blood and tissue lead burden in rats. On the other hand, this experiment showed that *T. laurifolia* leaf extract-supplemented fish foods were not affected the levels of lead in gill and kidney. Agreement with previously reported of Tangpong and Satarug [13], they found that co-treatment with aqueous *T. laurifolia* leaf extract did not affect levels of lead in blood and brain of mice given lead in drinking water at 1 g L$^{-1}$ for 8 weeks. This study demonstrated the protective regimen of *T. laurifolia* leaf extract-supplemented fish food by reducing LPO and increasing the intrinsic antioxidant status including GSH, CAT, GR and GPx in gill, kidney and liver of *O. niloticus* upon Pb(NO$_3$)$_2$ exposure. Similar results were illustrated in another extracts. *Smilax glabra* extract individually enhanced GSH content and co-treatment of *S. glabra* extract and meso-2,3-dimercaptosuccinic acid (DMSA) increased CAT activity and GSH level in brain, liver and kidney of lead-exposed rats [31]. The ethanol extract of *Aquilegia vulgaris* (L.) increased the level of GSH and improved the histological picture of liver and kidney of lead-treated rats [33]. Treatment with *Etlingera elatior* extract reduced lipid peroxides and protein carbonyl contents and increased antioxidant enzyme activities. Eventually, *E. elatior* extract was able to protect the lead acetate-induced bone marrow oxidative damage in rats [34].

From previous studies, 95% ethanol leaf extract of *T. laurifolia* was found to be good sources of chlorophyll derivatives, whereas water extract composed of apigenin and caffeic acid [14]. Endo et al. [35] suggested that chlorophyll derivatives may be acting as electron donors as evidenced by their ability to reduce free radicals such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Both porphyrin structure and nature of the central metal were considered important to antioxidant activity of chlorophyll derivatives. Apigenin was capable to inhibit LPO and to increase the antioxidant status [36]. Caffeic acid acts as natural antioxidant by both direct contribution and sparing á-tocopherol [37]. Moreover, it inhibited DNA fragmentation and caspase-3 activity during exposure to ROS [38]. Potential antioxidants of these substances in *T. laurifolia* leaf extract may play the key role to reduce LPO and increase the intrinsic antioxidant status against lead-induced oxidative stress and eventually, it was able to reduce gill pathology in lead-exposed *O. niloticus*.

**In conclusion,** *Thunbergia laurifolia* leaf extract-supplemented fish food was able to reduce lead concentration specifically in liver and muscle of *O. niloticus* upon Pb(NO$_3$)$_2$ exposure. It protects the lead-induced oxidative stress by reducing LPO and increasing the GSH level and the activities of CAT, GR and GPx in gill, kidney and liver of *O. niloticus*. Moreover, it was able to reduce gill pathology in lead-exposed *O. niloticus*. This data indicated that *T. laurifolia* leaf extract-supplemented fish food was able to protect *O. niloticus* against lead toxicity and able to apply as a fish food supplementation in *O. niloticus*.

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