Effects of Crude Methanol Extract of *Euphorbia hirta* on Hematological and Biochemical Indices and Histological Changes of Liver in African Catfish *Clarias gariepinus* (Burchell, 1822)

A. Sheikhlar, A.R. Alimon, H. Daud, C.R. Saad and E. Ramezani-Fard

1Department of Animal Science, Faculty of Agriculture, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
2Department of Veterinary Clinical Studies, Aquatic Animal Health Unit, Faculty of Veterinary Medicine, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
3Department of Aquaculture, Faculty of Agriculture, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Corresponding Author: A.R. Alimon, Department of Animal Science, Faculty of Agriculture, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Methanol extract of *Euphorbia hirta* was tested in *in vivo* experiment to investigate the effect on hematological and biochemical indices and histological changes of liver in the African catfish (*Clarias gariepinus*). The plant methanol extract was included in the feed and administered to the fish. The experiment was conducted for eight weeks and with five diets including control (*E. hirta*-0), 2 (*E. hirta*-2), 5(*E. hirta*-5), 7(*E. hirta*-7) and 9(*E. hirta*-9) g *E. hirta* kg⁻¹ of dry matter diet. Fish fed the *E. hirta*-9 diet had lowest (p<0.05) RBC and TP compared with the other dietary groups. Histological analyses revealed this group (*E. hirta*-9) had histopathologic alterations in the liver. Results of this study indicated that methanol extract of *E.hirta* by 7 g kg⁻¹ can be safely used to improve hematological indices of African catfish without any adverse effect on the liver.

Key words: African catfish, *Euphorbia hirta* extract, hematological and biochemical indices, liver histology

INTRODUCTION

*Euphorbia hirta* belongs to the genus *Euphorbiacea*, is a small, annual herb which grows 30-40 cm in height, the stem is slim and almost reddish in colour. The plant is also commonly known as *Euphorbia pilulifera*. *Euphorbia hirta* extensively found in all around the world particularly in tropic and sub-tropics (Khan et al., 1980). It prefers sandy and loamy soils and the soil must be properly drained, so it can simply be grown in waste places, roadsides, gardens and rice fields (Blomquist and Oosting, 1940).

*Euphorbia hirta* has been known as traditional drug to cure cough, coryza, hay asthma, bronchial affections, bowel complaints, worm infestations, kidney stones and low milk yield (Lanthers et al., 1991; Anjaria et al., 1997). Moreover, the whole plant plays significant roles as antibacterial (Vijayna et al., 1995) antiamoebic (Guissou et al., 1992; Tona et al., 2000), antifungal (Masood and Ranjan, 1991; Raja and Kurucheve, 1999), antiviral (Verma and Awasthi, 1973), spasmylytic (Tona et al., 2000), antidiarrhoeic (Galvez et al., 1993), sedative, anxiolytic
(Lanbers et al., 1990), analgesic, antipyretic, anti-inflammatory (Lanbers et al., 1991), antimalarial (Tona et al., 1999) and anti-hypertensive (Johnson et al., 1999; Williams et al., 1997) agents.

Analyzing *Euphorbia hirta* showed that the constituents quantitatively were amino acids, a-amylase, b-amylase, carbohydrate, glutamine, protein, proline and phenolics (Krishnaraju et al., 2005). Furthermore, the components qualitatively analyzed were alkaloids, anthroquinone, catechol, flavonoids, phenols, saponins, steroids, triterpenoids and tannins (Perez et al., 1990; Palombo and Semple, 2001; Nweze et al., 2004; Draughon, 2004; Owais et al., 2005; Krishnaraju et al., 2005). Cytotoxicity test of methanol extract of *Euphorbia hirta* was conducted by the cell line and the non-cytotoxic doses of the extract were assessed for antibacterial property against the cytopathic concentration of the bacteria. The methanol extract was shown to be non-cytotoxic and efficient antibacterial medicine (Vijayua et al., 1995).

However, there are no reported investigations in the literature concerning the possible therapeutic capacity and cellular toxicity of the crude methanol extract of *E. hirta* in Fish. Therefore, the objective of the current study was to assess the bioactivity of the crude methanol extract of *E. hirta* on the hematological and biochemical parameters and histological changes of liver in African catfish (*Clarias gariepinus*).

**MATERIALS AND METHODS**

**Herbal collection and preparation of the methanol extract:** The aerial parts of *E. hirta* were collected at the local University (University Putra Malaysia) campus. The collected *E. hirta*, was washed in sterile distilled water. Afterward, they were air dried for 10 days until constant weight. The herb was powdered using electric blender. To prepare methanol extracts, sample (100 g) of the plant powder was added into the conical flask containing 1 L of 95% methanol; conical flask was covered and left for 7 days at room temperature with daily agitation. Then, the supernatant was removed and the methanol was evaporated using a rotary evaporator; the residue was freeze-dried (Labconco, USA) and stored in a sterile bottle (Harikrishnan et al., 2009).

**Fish, facility and water quality:** Healthy fingerling African catfish (*Clarias gariepinus*) were obtained from a local farm and fed a commercial diet for 2 weeks to acclimatize. Thereafter the fish were weighed and stored in 100 L aquaria connected to a recirculating system with 30 fish per aquarium. The initial body weight (Mean±SE; n = 30) per fish was 9.5±0.4 g. In the experiment, a basal diet was formulated (Table 1) according to the NRC (1993) recommendation. This diet was considered as control diet (diet 1) and additional four diets were the control diet supplemented with 2 (EHE-2), 5 (EHE-5), 7 (EHE-7), or 9 (EHE-9) g EHE kg⁻¹ of Dry Matter (DM). All dry ingredients were ground and mixed by a food mixer. Distilled water was added into each diet (1.2 mL mg⁻¹ DM) and mixed. The mixed diets were pelleted (2 mm diameter), air-dried and kept frozen (-20°C) in airtight plastic bags. Each experimental diet was fed to three replicates of fish over a period of 60 days. All fish were hand-fed two times daily (8:00 h and 18:00) at 4% of body weight. All aquaria were cleaned weekly and the water quality parameters were checked daily; Dissolved oxygen and temperature were determined by YSI oxygen meter and temperature meter. Ammonia was measured by AG-2 kit. pH was measured by pH meter. Dissolved oxygen was not less than 6.0 mg L⁻¹. The temperature was between 28 and 30°C. Ammonia nitrogen was lower than 0.05 mg L⁻¹ and pH were 6.00±0.03.

**Blood sampling:** At the end of the experiment, blood samples were taken 24 h after the last feeding. Fish were anesthetized by 120 mg L⁻¹ tricaine methanesulfonate (MS-222) prior to
Table 1: Composition of basal diet (dry matter basis)

<table>
<thead>
<tr>
<th>Item</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn flour</td>
<td>320</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>380</td>
</tr>
<tr>
<td>Fish meal</td>
<td>170</td>
</tr>
<tr>
<td>Blood meal</td>
<td>50</td>
</tr>
<tr>
<td>Palm oil</td>
<td>50</td>
</tr>
<tr>
<td>Mineral premix*</td>
<td>10</td>
</tr>
<tr>
<td>Vitamin premixA</td>
<td>10</td>
</tr>
<tr>
<td>Binder</td>
<td>10</td>
</tr>
</tbody>
</table>

**Chemical analysis (%)**

<table>
<thead>
<tr>
<th>Item</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>87.8</td>
</tr>
<tr>
<td>Crude protein</td>
<td>35.1</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>7.76</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>6.62</td>
</tr>
<tr>
<td>Ash content</td>
<td>7.26</td>
</tr>
<tr>
<td>Digestible energy (kcal kg⁻¹)</td>
<td>3493</td>
</tr>
</tbody>
</table>

1Contains per kg of premix: dibasic calcium phosphate, 500 g; calcium carbonate, 215 g; sodium chloride, 40 g; potassium chloride, 90 g; magnesium hydroxide, 124 g; iron sulfate, 20 g; zinc sulfate, 4 g; manganese sulfate, 3 g; cobalt sulfate, 0.02 g; potassium iodide, 0.04 g; sodium selenite, 0.03 g and sodium fluoride, 1 g. 2Contains per kg premix: retinol palmitate, 2,500,000 IU; cholecalciferol, 500,000 IU; tocopherol acetate, 30 g; menadione, 2 g; thiamine, 2 g; riboflavin, 5 g; pantothenic acid, 10 g; niacin, 5 g; pyridoxine, 4 g; folic acid, 2 g; cyanocobalamin, 4 mg; ascorbic acid, 20 g; biotin, 200 mg and inositol, 80 g.

Handling. Blood samples (heparinized and non-heparinized) were collected from the caudal vein of fish randomly selected from each replicate for determination of blood parameters (Larsen and Snieszko, 1961; Larsen, 1964; Houston, 1990). Briefly, the Red Blood Cell (RBC) were counted in a 1:20 dilution of the blood sample in Hayem’s solution and the White Blood Cell counts (WBC) from a 1:200 dilution of the blood sample in Turke’s solution with a Neubauer hemocytometer. Hematocrit concentration (Ht) was immediately measured after sampling by placing fresh blood in glass capillary tubes and centrifuged for 10 min in a microhematocrit centrifuge. Hemoglobin (Hb) was determined colorimetrically by measuring the formation of cyanmethemoglobin following Van Kampen and Zijlstra (1961) method.

The serum from non-heparinized samples was applied for measurement of Total Protein (TP), Albumin, Globulin and activities of alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) using commercial kits and an auto-analyzer (Technicon RA-1000). Total lipid was determined colorimetrically following Joseph et al. (1972) method. The collected fish were euthanized in a solution of 200 mg L⁻¹ MS-222 and livers were removed for histological examination. Tissues were fixed in 10% formalin over night. Afterward, for dehydration of the tested tissues, serial ethanol cycles (70 to 100%) were applied. The tissues were clarified and then embedded in paraffin. Slices of 5 μm were prepared with a microtome (JB, 2035; Leica). After that, deparaffinization was conducted and triplicate slides of each block were prepared. The slides were stained with hematoxylin and eosin. Specimens were then screened using the light microscope (Nikon, Eclipse, E 800).

**Statistical analysis:** The data analyzed by the GLM procedure of SAS (SAS Institute, 1990). Duncan’s multiple range test was used to determine significance of differences among means at p<0.05. Aquarium means were considered as the experimental unit.
RESULTS

Hematological and biochemical parameters: The effect of dietary treatments on hematological and biological parameters is presented in Table 2. The concentrations of RBC, albumin, globulin and TP in groups fed the EHE-5 and EHE-7 diets increased (p<0.05) significantly compared to the other dietary groups while the concentrations of RBC and TP decreased significantly (p<0.05) for fish fed EHE-9 diet. There was no significant effect of the dietary EHE on the WBC, Ht and total lipid concentrations. The concentrations of AST, ALT and ALP increased when fish fed EHE-9 diet but numerically.

Light microscopy: The livers of control, EHE-2, EHE-5 and EHE-7 groups did not reveal any histopathological changes in the tissues tested by the light microscope (Fig. 1a). Livers of the diets (control, EHE-2, EHE-5 and EHE-7) exhibited homogenous size hepatocytes with vacuolised cytoplasm. While, the liver of fish fed EHE-9 diet showed numerous inflammatory cells (Fig. 1b).

Table 2: Changes in red blood cells, white blood cells, hemoglobin, hematocrit, albumin, globulin, total lipids, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) (Means±SE) of African catfish fed different amounts (2, 5, 7 and 9 g kg⁻¹ dry diet) of *Euphorbia hirta* extract (EHE) at the end of a 60 day experimental period

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>EHE-2</th>
<th>EHE-5</th>
<th>EHE-7</th>
<th>EHE-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cell (10¹² L⁻¹)</td>
<td>33.3±2.8³</td>
<td>29.9±2.4³</td>
<td>38.5±2.2³</td>
<td>40.5±2.5³</td>
<td>22.1±1.7³</td>
</tr>
<tr>
<td>White blood cell (10³ L⁻¹)</td>
<td>19.4±3.3</td>
<td>17.2±3.1</td>
<td>15.7±4.5</td>
<td>21.1±2.7</td>
<td>20.1±3.5</td>
</tr>
<tr>
<td>Hemoglobin (g L⁻¹)</td>
<td>14.5±6</td>
<td>13.3±0</td>
<td>14.2±8</td>
<td>13.8±9</td>
<td>12.0±8</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>36.1±2.5</td>
<td>39.9±4.1</td>
<td>37.3±3.9</td>
<td>35.9±3.7</td>
<td>40.5±3.6</td>
</tr>
<tr>
<td>Albumin (g L⁻¹)</td>
<td>17.5±2.7³</td>
<td>18.7±2.9³</td>
<td>27.6±2.0³</td>
<td>23.9±2.9³</td>
<td>15.1±1.9³</td>
</tr>
<tr>
<td>Globulin (g L⁻¹)</td>
<td>12.5±1.3³</td>
<td>11.1±1.1³</td>
<td>15.9±1.5³</td>
<td>17.5±1.0³</td>
<td>10.3±1.4³</td>
</tr>
<tr>
<td>Total lipids (g L⁻¹)</td>
<td>0.79±0.00</td>
<td>0.85±0.11</td>
<td>0.77±0.10</td>
<td>0.70±0.00</td>
<td>0.81±0.08</td>
</tr>
<tr>
<td>Total protein (g L⁻¹)</td>
<td>30.0±2.0³</td>
<td>29.8±1.8³</td>
<td>43.5±2.2³</td>
<td>41.4±2.0³</td>
<td>25.4±1.6³</td>
</tr>
<tr>
<td>AST (IU L⁻¹)</td>
<td>19.7±7.9</td>
<td>17.5±7.8</td>
<td>26.1±8.2</td>
<td>23.0±8.4</td>
<td>30.4±7.7</td>
</tr>
<tr>
<td>ALT (IU L⁻¹)</td>
<td>17.4±9.8</td>
<td>15.6±7.9</td>
<td>22.7±7.5</td>
<td>28.3±9.7</td>
<td>31.1±9.0</td>
</tr>
<tr>
<td>ALP (IU L⁻¹)</td>
<td>28.5±5.7</td>
<td>25.3±4.4</td>
<td>29.2±4.2</td>
<td>31.7±5.9</td>
<td>34.5±6.6</td>
</tr>
</tbody>
</table>

³ Means within the same row with different superscript letters are significantly different (p<0.05). ³EHE-2, EHE-6, EHE-7 and EHE-9 were basal diet plus 2, 5, 7 and 9 g kg⁻¹ of dry diet of *Euphorbia hirta* extract, respectively.

Fig. 1(a-b): Histological appearance of the liver tissue (a) control fish (H and E stain, 200X) (b) fish fed 9 mg kg⁻¹ of extract of *E. hirta* after 60 days of feeding dietary extract (H and E stain, 200X)
DISCUSSION

Hematological assessment showed that RBCs counts were higher compared to control group when fish fed diet included 5 and 7 g kg$^{-1}$ of EHE. Also, albumin, globulin and total protein levels in fish fed EHE (5 and 7 g kg$^{-1}$ diet) supplemented diets increased profoundly in comparison to control group. These improvements may reflect the good nutrients intake and immunity enhancement in fish. Babatunde and Pond (1987) pointed out that RBC, hemoglobin and hematocrit concentration are correlated with the nutritional status of the animal and are directly exhibited the dietary nutritional balance. Similarly, there are several reports that show several herbs have been indicated some changes in blood parameters by inhibition of the uptake and utilization of some minerals (Sokunbi and Egbunike, 2000; Ihekwumere et al., 2002). Equal concentration values for WBC for all treatments in the current experiment show the fish ability to battle disease invasion was not impaired by the dietary supplementations of EHE.

In this experiment, no significant changes were observed in total lipid, aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) among dietary treatments by 7 g kg$^{-1}$ (EHE). By increasing the level of EHE and MLE to 9 g kg$^{-1}$, the concentration of ALT, AST and ALP increased numerically. Increased activity of AST, ALT and ALP could be due to damage of liver, kidney, muscle and other tissues (Viveros et al., 2002; Abdel-Tawwab et al., 2007).

Histological assessments revealed that inclusion of EHE by 7 g kg$^{-1}$ diet did not show any changes in the liver’s tissues, while EHE-8 indicated some abnormalities in the liver compared to the other treatments. Our finding is in line with the Fiuza et al. (2009) who revealed that there were significant changes in the hepatopancreas of Nile tilapia induced by inclusion of crude ethanol extract and fractions of Eugenia uniflora (Myrtaceae) in the diet.

CONCLUSION

Based on the results obtained from this study, methanol extract of E. hirta by 7 g kg$^{-1}$ of diet can be safely and effectively used to improve the general health of African catfish.

REFERENCES


