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Biochemical stress response in freshwater fish *Channa punctatus* induced by aqueous extracts of *Euphorbia tirucalli* plant

Sudhanshu Tiwari *, Ajay Singh

Natural Products Laboratory, Research Block, Department of Zoology, Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur 273009, Uttar Pradesh, India

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Abstract

Piscicidal activities of aqueous extracts of *Euphorbia tirucalli* were very well established, but their ultimate mode of action on fish metabolism was not yet known. Exposure of fishes over 24 h or 96 h to sub-lethal doses (40% and 80% of LC_{50}) of aqueous extract of *E. tirucalli* stem-bark and latex, significantly ($P < 0.05$) altered the level of total protein, total free amino acids, nucleic acids, glycogen, pyruvate, lactate and activity of protease, alanine aminotransferase, aspartate aminotransferase, acetylcholinesterase and cytochrome oxidase enzyme in liver and muscle tissues of freshwater fish *Channa punctatus*. The alterations in all these biochemical parameters were significantly ($P < 0.05$) time- and dose-dependent. After 7 d of withdrawal of treatment of 80% of LC_{50} of *E. tirucalli* extracts shows that there was a partial recovery in the levels of glycogen but nearly complete recovery in total protein, total free amino acids, pyruvate, lactate, nucleic acids level and activity of protease, aspartate aminotransferase, alanine aminotransferase, acetylcholinesterase and cytochrome oxidase enzyme in both the tissues of fish. Thus aqueous extracts of *E. tirucalli* adversely affect respiratory pathway of fish and cause energy crisis during stress by suppressing ATP production. The reversibility of the action of the aqueous extracts would be an additional advantage in their use.

Keywords: *Euphorbia tirucalli*; Euphorbiaceae; *Channa punctatus*; Fish metabolism

1. Introduction

Human beings have been using plants for catching fishes from time immemorial. Several plant parts such as seed, flower, leaf, stem-bark or latex are thrown by tribal people into water to stupefy the fish (Wallis, 1985). After a time that varies according to conditions the fish begins to rise to the water-surface and it can readily be taken out by hand and can be eaten without any problem (Neuwinger, 2004). *Euphorbia tirucalli* (family—Euphorbiaceae) is a common medicinal plant of India and plant juice is purgative, carminative and useful in gonorrhoea, whooping cough, asthma, dropsy, leprosy, spleen enlargement, jaundice, tumors and stone in gall bladder (Satyavati and Gupta, 1987). *E. tirucalli* is also used to stupefy fish on the west coast of Maharashtra (Kamat and Muthe, 1995) and in Africa (Neuwinger, 2004). The piscicidal activities of aqueous latex and stem-bark extracts of *E. tirucalli* against freshwater fish *C. punctatus* have been established (Tiwari et al., 2001). But there is little literature available for ultimate mode of action of *E. tirucalii* and their effect on fish metabolism.

In course of present study we are interested to ascertain the biochemical effects of *E. tirucalli* stem-bark and latex extracts and their ultimate mode of action, short- as well as their long-term effect on metabolism of freshwater air-breathing fish *Channa punctatus* under exposure to sub-lethal doses, as these extracts cannot be put to commercial use without a study of these aspects as well.

* Corresponding author. Tel.: +91 551 2201171.
E-mail address: sudhansh4@rediffmail.com (S. Tiwari).

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2. Materials and methods

2.1. Collection and maintenance of experimental animal

C. punctatus (Bloch.) (17.5 ± 1.50 cm total length; 12.0 ± 2.5 g weight) were collected from Ramgarh Lake of Gorakhpur district of Uttar Pradesh. They were stored in glass aquaria containing 100 l of de-chlorinated tap water. Prior to experiment fish were allowed to acclimatise under laboratory conditions for one week. The aquaria water was aerated continuously and food was provided in the form of dried, powdered small prawn, goat liver etc. Water was changed at every 24 h. Average sized adult animals were used for the experiment. Experimental water conditions were atmospheric temperature, 31.5–32.5 °C; water temperature, 28.5–30.0 °C; pH, 7.2–7.5; dissolved oxygen, 7.7–8.2 mg l^{-1}; free carbon dioxide, 4.5–5.5 mg l^{-1}; bicarbonate alkalinity, 104.5–106.5 mg l^{-1} (APHA/AWWA/WEF, 1998).

2.2. Collection and preparation of aqueous extracts of plant material

The plant *E. tirucalli* (family—Euphorbiaceae) commonly known as Barki-thohar was collected locally from Botanical Garden of Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur and identified by Prof. S.K. Singh, Plant taxonomist, Department of Botany, Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur, Uttar Pradesh, India where a voucher specimen was deposited.

Preparation of aqueous stem-bark extract: *E. tirucalli* stem-bark was dried in incubator at 37 °C. Dried stem-bark powdered with the help of mechanical device and stored in airtight desiccator for further use. At the time of treatment the dried, fine, powdered (particle size, 10^{-4}–10^{-5} cm) stem-bark of *E. tirucalli* was dissolved in distilled water and centrifuged at $1000 \times g$ for 10 min. The supernatant was used as a water extract for biochemical experiments.

Preparation of aqueous latex extract: The white, milky latex produced by this plant was drained into glass tubes by cutting the stem apices, this latex was lyophilized at −40 °C and lyophilized powder was stored for further use. The freeze-dried powdered latex was mixed with appropriate volume of distilled water to obtain the desired concentration, which was used for biochemical experiments. The wet weight of 1 ml latex of *E. tirucalli* was 1.370 g and dry weight (lyophilized at −40 °C) was 0.315 g.

2.3. Biochemical experiments

The acclimatised fishes were treated with 40% and 80% of 24 h or 96 h LC_{50} of aqueous extracts of *E. tirucalli* stem-bark and latex for 24 h or 96 h. Six aquaria were set up for each dose and each aquarium contained ten fishes in 6 l de-chlorinated tap water. The LC_{50} values of aqueous extract of *E. tirucalli* stem-bark were 140.37 mg l^{-1} for 24 h, 127.19 mg l^{-1} for 96 h and for latex extract was 9.01 mg l^{-1} for 24 h against *C. punctatus,* respectively (Tiwari et al., 2001). Forty percent and 80% of 24 h or 96 h LC_{50} of both the aqueous extracts of *E. tirucalli* were selected as sub-lethal doses in the present study to analyses its time and dose-dependent effects and at that dose there was no mortality observed in the treated animals and measurable changes were found to have occurred in biochemical parameters of the fish. After the stipulated time (24 h and 96 h), the test fishes were removed from aquaria and washed with water and killed and liver, muscle tissues were quickly dissected, freed from adipose and connective tissues, frozen in liquid nitrogen and stored at −70 °C which was used for biochemical analysis. Control animals were held in the same condition without any treatment. Each experiment was replicated six times and the values were expressed as mean ± SE of six replicates. Students '*t*' test was applied to locate significant changes with controls (Sokal and Rohlf, 1973; Prasad, 2003), ANOVA and LSD test was also applied for appropriate statistical analysis (Paterson, 1939) for interpretation.

Total protein: Total protein level was estimated by the method of Lowry et al. (1951). Homogenates (5 mg ml^{-1}) were prepared in 10% trichloroacetic acid (TCA). Bovine serum albumin was used as standard.

Total free amino acids: Total free amino acids level was estimated by the method of Spices (1957). Homogenates (10 mg ml^{-1}) were prepared in 95% ethanol. Glycine was used as standard.

Nucleic acids: Nucleic acids (DNA and RNA) were estimated by the methods of Schneider (1957). Homogenates (10 mg ml^{-1}) were prepared in 5% TCA. Calf thymus DNA and yeast RNA was used as standard for DNA and RNA, respectively.

Glycogen: Glycogen level was estimated by the method of Van der Vies (1954). Homogenate (10 mg ml^{-1}) was prepared in 5% TCA. Glucose was used as standard.

Pyruvate: Pyruvate level was estimated by the method of Friedemann and Haugen (1943). Homogenate (50 mg ml^{-1}) was prepared in 10% TCA. Sodium pyruvate was used as standard.

Lactate: Lactate level was estimated by the method of Huckabee (1961). Homogenate (50 mg ml^{-1}) was prepared in 10% TCA. Sodium lactate was used as standard.

Protease: Protease enzyme activity was estimated by the method of Moore and Stein (1954). Homogenate (50 mg ml^{-1}) was prepared in cold distilled water. Tyrosine was used as standard.

Alanine aminotransferase (ALAT) and aspartate aminotransferase (AAT): ALAT and AAT enzyme activities were estimated by the method of Reitman and Frankel (1957). Homogenates (50 mg ml^{-1}) were prepared in 0.25 M cold sucrose solution. Oxaloacetic acid was used as standard.

Acetylcholinesterase (AChE): AChE enzyme activity was estimated by the method of Ellman et al. (1961). Homogenate (50 mg ml^{-1}) was prepared in 0.1-M-phosphate