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Effect of Euphorbia Tirucalli L. Extracts on Brain and Muscle Proteins of Fish

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ABSTRACT

Euphorbia tirucalli has a number of medicinal uses along with toxic effects attributed to the plant. This plant is mainly used for its fish stupification and piscicidal activities by tribals of Gond tribe of Kawal Wild Life Sanctury, Andra Pradesh, southern Rajasthan, southern Maharashtra in India and Africa [2][3][4][5]. The present study aims to identify the effect of E. tirucalli extracts on skeletal muscles and brain protein composition, as the contraction and relaxation of skeletal muscles is responsible for normal swimming movements of the fish. The altered swimming movements are indicative of effect of plant extracts on these proteins. For the current study three different extracts of E. tirucalli were prepared and their effects were observed on the fish, Tilapia. Protein samples were prepared from skeletal muscles and brain tissues. These samples were separated with the help of SDS-PAGE and Agarose Gel Electrophoresis. The results indicated denaturation of certain protein bands in the plant extract treated muscle and brain proteins of the fishes.

Keywords: Euphorbia tirucalli, Piscicidal activity, Stupification of Fish, Protein, SDS-PAGE, AGE

I. INTRODUCTION

Fish catching with the aid of plants is an ancient practice. Plants have been used in various parts of the world by people from times immemorial for poisoning or stupifying fish. This practice is one of the great biological and ethnological interest. All parts of the plants are used; but in most cases a certain part such as the bark or root in which the toxic principles are located are utilized. Such plants contain different phytochemicals like alkaloids, coumerins, resins, saponins, diosgenin etc. [1] [2][4][5]

Family Euphorbiacae has a reputation of having incredibly toxic plants. Some of the Euphorbia plants are used in folk medicine to cure skin diseases, gonorrhoea, migraines, intestinal parasites, and warts. The genus Euphorbia has been the source of large number of biological active compounds. Tannins, flavonoids, unsaturated sterols/triterpenes, carbohydrates, lactones and proteins/amino acids were reported as major active constituents of some Euphorbia species [6]. Euphorbia tirucalli L. (Family: Euphorbiacae) is a succulent cactus-like plant growing to a height of about 10 M. It was introduced from Africa as a garden plant. E. tirucalli grows in arid zones as well as zones that are more mesophytic, the species makes a good living fence post. E. tirucalli is also called petroleum plant because it produces a hydrocarbon substance similar to gasoline [6]. Whole plant harvesting is worthwhile from energy point-of-view with rubber, petroleum, and alcohol as energy products and resins, which may find use in the linoleum, oilskin and leather industries. The charcoal derived from plant can be used in gunpowder[7][8]. The dried latex contains resin which is a principle constituent (75.8-82.1%). The stem contains

hentriacontene, hentriacontanol, the antitumor steroid 4-deoxy-phorbol ester, beta-sitosterol, caoutchouc, casuariin, corilagin, cycloeuphordenol, cyclotirucanenol, ellagic acids, euphorbins, euphol, euphorone, euphorcinol, gallic acids and glucosides[9][10]. A variety of diterpenoids with antibacterial, anticancer, prostaglandin E2-inhibitory, antifeedant, anti-HIV, and analgesic activity have also been isolated from different Euphorbia species [22]. They include jatrophane, ingenol and myrsinane diterpenoids. These diterpenoids are reported to act in diverse ways; they are found to be skin- irritants, tumour-promoters [11]. In addition to anti-tumour activity, several species of this genus have been investigated for their immunomodulatory activity and some immunotoxic, immunosuppressive and immune-stimulatory effects. These broad range and diversity of biological activities in the Euphorbia genus, is perhaps due to the presence of various components with different modes of action in the plants [13][14][15][16]. Adverse effect of aqueous extracts of Euphorbia tirucalli on respiratory pathway of fish is studied. The extract also cause energy crisis during stress by suppressing ATP level [17][18].

Many workers in India have documented their work on piscicidal effect of various plants and plant parts including Euphorbia tirucalli. Piscicidal effect of some common plants were studied against target animals of fresh waterbodies in India by Digvijay Singh and Ajay Singh in 2002 [19]. In 1995, Poisonous effects of Euphorbia tirucalli on fish was studied by Kamat and Muthe [20]. Effects of Euphorbia tirucalli latex on blood calcium and phosphate of the freshwater air-breathing catfish Heteropneustes fossilis was studied by Abhishek Kumar et al(2010) [18]. In 2003, Toxicity of Euphorbia tirucalli plant against fresh water target and non-target organisms were studied by Tiwari et al.[21] From literature survey it is evident that though piscicidal effect of Euphorbia tirucalli is studied, toxic effects of Euphorbia tirucalli on skeletal muscles and brain protein of fish are not investigated. The current study gives an insight on the effects of various extracts of Euphorbia tirucalli on fish muscles and brain, which are required for swimming action and its co-ordination. During the current investigation proteins from muscles and brain are analyzed using electrophoretic technique so as to observe any degenerative changes.

II. METHODS AND MATERIAL

A. Extraction of Plant material and Treatment Given to Tilapia Fish:

A plant material of Euphorbia tirucalli was collected from college campus. The twigs and stem of plant Euphorbia tirucalli were dried in shade and were powdered. Extractions were prepared using dried plant powder as well as fresh plant materials. Three types of extracts were prepared as follows-

1. **Extract 1: Crude aqueous extract:** Fresh stem and twigs were macerated in 3 ml of distilled water using mortar and pestle and filtered using muslin cloth.
2. **Extract 2: 100% Ethanolic Soxhlet extract:** Fresh stem and twigs of the plant were macerated and then extracted using ethanol in Soxhlet apparatus for 8hrs. The ethanolic extract thus obtained was dried using flash evaporator. A dried extract was dissolved in 10 ml of ethanol. This extract was used as ethanolic plant extract for estimation of change in protein profile (Kamath &Muthe, 1995) [7].
3. **Extract 3: Alkaloid extract:** Plant alkaloids are major fraction playing important role in piscicidal activity; hence alkaloids were extracted from the stem and twigs of E. tirucalli.

These extracts were used for the study of stupification and piscicidal activity on commonly available fresh water fish Tilapia. The fishes were divided in four groups and each group containing six fishes. They were acclimatized for a period of 24 hrs to the laboratory conditions and the doses of above

mentioned extracts were prepared as mentioned in table 1

Table 1

EXTRACT	CONCENTRATION	QUANTITY	TIME PERIOD
1 2 3	2ppm 2ppm 2ppm	100 µl 100 µl 100 µl	6 hrs 6 hrs 6 hrs
1 2 3	10 ppm 10 ppm 10 ppm	100 µl 100 µl 100 µl	6 hrs 6 hrs 6 hrs
1 2 3	20 ppm 20 ppm 20 ppm	100 µl 100 µl 100 µl	6 hrs 6 hrs 6 hrs
Control-1 Control-2	Ethanol Distilled Water	100 µl 100 µl	6 hrs 6hrs

During the treatment period of 6hrs, the fishes were observed for behavioural and physiological changes. After the treatment period, fishes from all four groups were sacrificed and skeletal muscles and brain tissue was procured. The tissues thus procured were then used for analysis of proteins.

B. Extraction Of Protein From Fish and Quantitative Protein Estimation by Barfords' Method:

Fishes were dissected to obtain muscle and brain tissue at 4-6 ^{0}C.

↓

Muscle and brain tissue were taken in separate petri-plates containing 1X Phosphate buffer (pH 7.4)

↓

Homogenize the tissues with chilled 1X phosphate buffer (pH 7.4)

↓

The homogenized tissue sample was cold centrifuged at

↓

4 ^{0}C at 3000 rpm for 10 minutes (Gupta & Mullins, 2010) [23]

Supernatant was collected in pre-chilled centrifuge tube and was used as protein sample.

Fishes from control group were used for quantitative protein estimation by Barfords' method.

The protein samples extracted from all four groups were separated by SDS-PAGE and Agarose gel electrophoresis.

C. Electrophoresis of Fish Brain and Muscle protein:

1. SDS-Polyacrylamide Gel Electrophoresis was performed by using standard HIMEDIA SDS-PAGE Kit (HTP001) for all four groups with brain and muscle proteins extracts. The gels thus obtained were stained with Coomassie Brilliant Blue R (CBB-R) stain and Silver Stain. Silver staining is the most sensitive protein staining method available for gel electrophoresis. It can efficiently detect minute quantity such as 5 ng protein in a 2.5 mm wide band in 0.75 mm thick gel. Different proteins give different intensity of the staining. [22]
2. Agarose Gel Electrophoresis was performed for separation of muscle and brain protein samples from fish.

It was carried out using HIMEDIA Genomic DNA isolation AGE kit. A standard procedure of gel preparation was followed.

III. RESULTS AND DISCUSSION

The fishes were exposed to 2ppm, 10ppm and 20ppm concentrations of crude aqueous extract, 100%

ethanolic Soxhlet extract and alkaloid extract for a period of 6hrs as mentioned in the Table II.1.

The results indicated that on exposure to low concentration of 2ppm of all three extracts, fishes survived the treatment period of 6 hrs without any signs of discomfort or irritation. When the fishes were treated with 10ppm concentration of all three extracts, they exhibited primary irritation for 15min. to 20 min. Redness near gills region was also observed. After exposure to 20ppm concentrated extracts, fishes showed increased levels of discomfort and irritation. There was a prominent redness near gills. Fishes were also observed to gasp for air. For crude extract and ethanolic extract, these symptoms lasted for 30mins. But the fishes eventually recovered. In case of 20 ppm alkaloid extract, these symptoms persisted for almost 40 mins and fishes also exhibited stupification. Surfacing of the fishes was seen with no swimming activity.

Muscle and brain proteins were extracted from treated and control fishes. Proteins from muscles and brain were quantified calorimetrically by Barford's Method. The amount of protein in muscle was found to be 0.3194 % and in brain it was 0.8429 %. The protein quantity was observed to be sufficient to carry out protein separation by SDS-PAGE and Agarose gel electrophoresis (AGE).

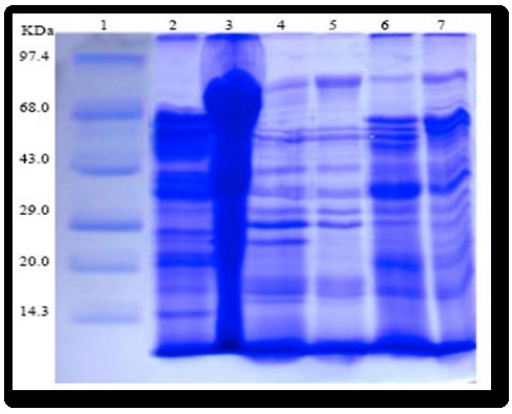

Figure 1. Brain Protein Separation By SDS- PAGE

Lane 1- Protein Marker.
Lane 2- Control Muscle Protein.
Lane 4- Muscle Protein Treated with Extract 1.
Lane 5- Muscle Protein Treated with Extract 2.
Lane 6- Muscle Protein Treated with Extract 3.
Lane 7- Muscle Protein Treated with Extract 3

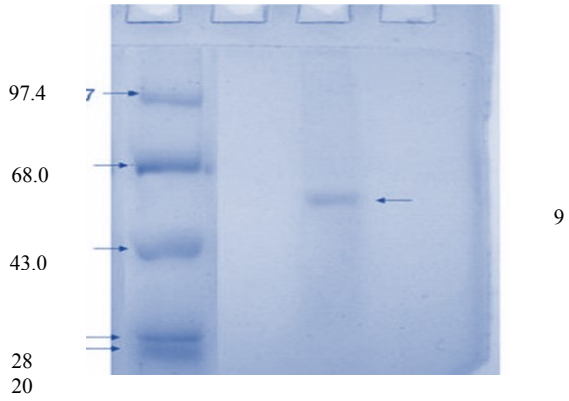

Figure 2. Brain Protein Separation By SDS- PAGE

Lane 1- Protein Marker
Lane 3- Control Fish Brain Protein
Lane 4- Treated Fish Brain Protein

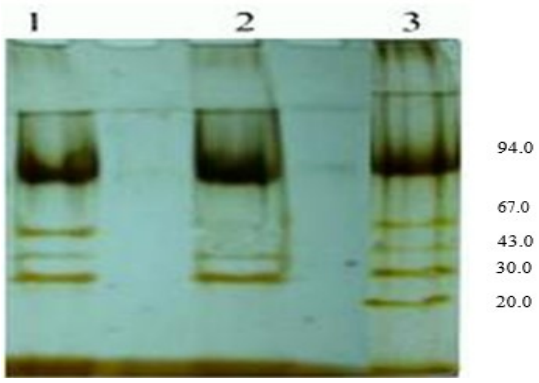

Figure 3. Silver Staining of SDS-PAGE of Muscle Protein

Lane 1- Control Fish Muscle Protein.
Lane 2- Extract 3 Treated Fish Muscle Protein.
Lane 3- Protein Marker.

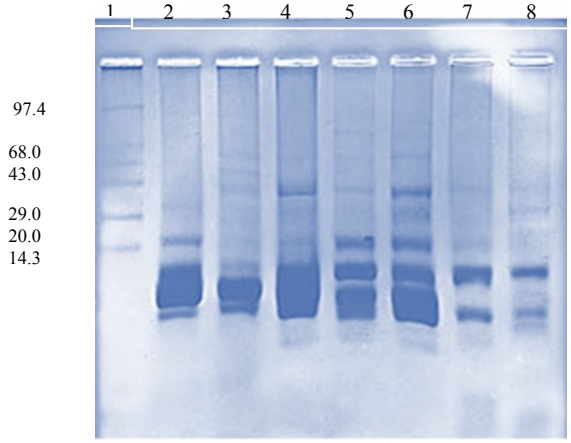

Figure 4. AGE Separation of Fish Muscle and Brain Protein By CBB Staining

Lane 1- Protein Marker.
Lane 2- Control Brain Protein.
Lane 3- Extract 2 Treated Brain Protein.
Lane 4- Extract 3 Treated Brain Protein.
Lane 5- Extract 1 Treated Muscle Protein.
Lane 6- Control Muscle Protein.
Lane 7- Extract 2 Treated Muscle Protein.
Lane 8– Extract 3 Treated Muscle Protein.

Muscle and brain protein samples extracted from treated and control fish were separated by SDS- PAGE and Agarose Gel Electrophoresis

Separation of Muscle Protein:

Muscle protein separation carried out using SDS-PAGE, resulted in an altered protein behavior between 97.4 KDa and 68.0 KDa molecular weight region. Hence, it can be concluded that protein in this region is sensitive to the phytochemical constituents of Euphorbia tirucalli extract.

Silver Staining Evaluation of Muscle Protein:

Silver staining of muscle protein showed alteration pattern in protein, where in it was observed that a single band of molecular weight 68.0 KDa was absent. This also indicates alteration of protein structure of fish under the influence of phytochemical fractions of the Euphorbia tirucalli extract.

Separation of Brain Protein:

When brain protein samples were separated on SDS-PAGE, it showed a single band from control protein sample. Treated protein samples did not show any band separation on electrophoresis. These type of results may be observed due to denaturation of brain proteins under the influence of phytochemicals of the extracts or due to unfavourable laboratory conditions.

Separation of Proteins By Agarose Gel Electrophoresis (AGE):

When muscle and brain protein samples were separated by agarose gel electrophoresis, it showed following results-

When alcoholic and alkaloid extracts treated fish muscles and brain proteins were separated on AGE; it was observed that there was absence of bands situated at position 97.4 KDa, 68.0 KDa and one more band at 43.0 KDa molecular weight region. This indicated that fish muscle and brain contains proteins which are sensitive to phytochemicals of Euphorbia tirucalli. This result was more prominent in alkaloid extract of E. tirucalli.

IV. CONCLUSION

The present study, thus reveals the protein denaturation from muscles and brain of fish Tilapia under the influence of extracts of Euphorbia tirucalli. The alkaloid fraction of plant extract was observed to show degenerative effect on muscle protein of molecular weight 97.4 KDa to 68.0 KDa as these bands were absent in the separated brain and muscle proteins by SDS-PAGE and AGE of treated fishes. It was also observed that one more band of protein situated at 43.0 KDa region was absent, when brain and muscle proteins were separated by Agarose gel electrophoresis. All these results thus indicate that the piscicidal and stupification activities exhibited by Euphorbia tirucalli are due to denaturation of certain proteins which are required for normal swimming behavior of the fish. The contraction and relaxation of skeletal muscles is responsible for normal swimming movement. The degeneration of brain proteins lead to loss of control and co-ordination. The altered swimming movement was indicative of effect of Euphorbia tirucalli extracts on these proteins. It was also observed that alkaloid extract (Extract 3) was most effective in denaturation of these proteins as compared to other two extracts. The alkaloid component was also observed to cause air grasping problem which leads to redness near gill region of Tilapia.

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