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ORIGINAL ARTICLE

BIOASSAY OF *TRICHODESMIUM ERYTHRAEUM* (Ehr.) (MICRO ALGA)ON HISTOPATHOLOGY AND BIOCHEMICAL STUDY IN THE MICE

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ABSTRACT

The present study is aimed to investigate the *Trichodesmium erythraeum* on the histological and biochemical study in the bain tissue of mice, Mus musculus The *Trichodesmium erythraeum* (25, 50, 100 and 150 mg/kg body wt. i.p) was administrated in mice. In the present study, the histophathological and biochemical changes such as Serum Cholinesterase (ChE) and Acetyl cholinesterase (AchE) were observed in brain tissue of mice. During the *Trichodesmium erythraeum* treatment, the brain tissue shows the necrosis and degeneration of neuroglial cell were observed and simultaneously cholinesterase and acetylcholinesterase were slightly decreased when compared to control.

Keywords: *Trichodesmium erythraeum*, Brain, Histology, Biochemical Study

1.INTRODUCTION

The term “bioassay” is a common short form for “biological assay”. Bioassay can be defined as those tests which are used to detect the biological or functional activity of an extract or pure substance from herbs and/or natural products using the living organism, tissue or cell. Bioassay may be used for qualitative and/or quantitative characterizations. Qualitative bioassays are used for assessing the biological effects of a substance that may not be quantified, such as abnormal development or deformity. Quantitative bioassays involve the estimation of effective concentration or potency of a substance by measurement of the biological response that it produces. Bioassays are essential tools in the development of new drugs. The most important societal contributions of marine natural products had been isolation and identification of marine toxins responsible for seafood poisoning. Outbreaks of seafood poisoning are usually sporadic and unpredictable because toxic fishes and shellfish do not produce the toxins themselves, but concentrate them in the organisms that they eat. Most marine toxins are produced by microorganisms such as dinoflagellates, diatoms and cyanobacteria and which may pass through several levels of the food chains till they reach the humans. The toxins which are produced by cyanobacteria are grouped into two main categories based on the type of bioassay used for their screening. Compounds toxic to cultured cell lines are termed as cytotoxins while those to whole animals are called biotoxins (Carmicheal, 1990).

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Plants have been an indispensable source of natural products for relief from illness for many years (Graham *et al.,* 2000). Secondary metabolites of plants possess many biological activities and hence they serve as protective agents against various pathogens (eg. Insects, fungi and bacteria). Many plant extracts and isolated compounds have been tested invitro for cytotoxicity by using different human cell lines (prostrate, stomach, liver, colon etc.) as well as animal cells such as monkey kidney cells (Don *et al.,* 2006; Jo *et al.,* 2005). Cell culture toxicity testing is a valuable and inexpensive approach for short term testing. A test should be able to provide information on the dose effect relationship including the dose range for potential exposure and risk to humans.

Some neurotoxic- like substances have been recently characterized from *Trichodesmium erythraeum* and *T.thiebauti* (Jackson *et al.,* 2001).But literature pertaining to the Indian seas still group them as non-toxic, which needs to be relooked (Bhat *et al.,* 2006). *Trichodesmium spp*. has also been described as non-toxic, toxic or sometimes toxic to range of organisms. Mouse bioassay is the typical first test for toxicity and widely used, either for a simple determination of outright toxicity or a more sophisticated determination of toxin concentration (Jones *et al.,* 1993). Hence the present investigation was undertaken to examine the toxicity of *Trichodesmium* blooms of Parangipettai coastal waters.

Cyanobacterial toxins are alkaloids, peptides and lipopolysaccharides. The most rapidly acting of these are the

alkaloids which are neurotoxins producing death by paralysis of peripheral skeletal muscles, then respiratory muscles leading to respiratory arrest in a few minutes to a few hours following exposure. *Trichodesmium* bloom material Mouse bioassay revealed the presence of a highly potent neurotoxic compound similar in action to that of anatoxin-a (Carmichael, 1998; Harda *et al.,* 1989). In some expedition members experienced coughing and respiratory difficulties while collecting the material used in the study as reported by others previously following contact with *Trichodesmium erythraeum* blooms (Sato *et al.,* 1966). The objective of this study was to evaluate acute toxicity of the orally administered *T. erythraeum* blooms in male Wister mice based on the results of hematological, biochemical and histopathological investigation. In our study ChE enzyme was chosen as the biochemical indicator of neurotoxicity in addition to brain histopathology.

2.MATERIALS AND METHODS

Trichodesmium collection and extraction

Trichodesmium was collected from the surface water, by towing the phytoplankton nets (mouth diameter 0.35m) made up of bolting silk cloth (No. 30, Mesh size - 48 µm), the collected samples were frozen and lyophilized. After that, one gram of lyophilized form of *Trichodesmium erythraeum* was prepared by extraction, 100 ml methanol. Then the dried extract was diluted with various amounts of saline solution to get four different concentrations such as 25mg, 50mg, 100mg, and 150mg (Tzong-Huei Lee *et al.*, 1999.).

Animal and Experimental Design

This study was approved by the Animal Ethical Committee of Annamalai University (IAEC/160/1999/CPCSEA/583, dated; 03.10.2008). Animals were maintained by Animal House Rajamuthaiah Medical College, Annamalai University. This experiment was conducted on 40 male Wister mice *(Mus musculus*), (20 g in weight) acclimatized for 10 days in laboratory condition (by feeding them with food and water *ad labitum*), After the 10 days of acclimation, the animals were randomly assigned to the experimental labeled cages with solid plastic sides and stainless-steel grid tops and floors. Animals of the control group were orally fed daily with a normal diet in standard laboratory chaw. They were maintained in controlled laboratory conditions of 12hr dark /light cycle, 23±1^{o}C, relative humidity (55±10%). The extracted samples were administrated through intraperitoneal (i.p) injection.

Group I: Mice received standard chow diet (Control)

Group II : Mice received crude extract (25 mg/kg of Bw, i.p)

Group III : Mice received crude extract (50 mg/kg of Bw, i.p)

Group IV : Mice received crude extract (100 mg/kg of Bw i.p)

Group V : Mice received crude extract (150 mg/kg of Bw, i.p)

The entire animals were sacrificed at the end of the day and then blood samples were collected for biochemical estimation. Section of brain tissue was set aside for histological studies.

Histopathological study

Brains were excised from the envenomated mice, fixed in 10% formalin. They were then rinsed in three changes of 70% (V/V) alcohol to remove the excess fixative. The tissues were dehydrated in ascending grades (50%, 70%, 90%, and 100%) of alcohol for 1 hour each. The samples were then cleared in xylene for two hours and embedded in paraffin wax thrice, each time for 45 minutes. Once the samples were blocked, they were allowed to solidify and the surplus amount was trimmed off. Sections of 5-6µm thickness were made using a hand rotary microtome. The "ribbon" thus obtained was kept with its shiny side downwards onto the surface of warm water in the water bath where the temperature was maintained at 2-3°C lesser than the melting point of paraffin wax.

The best sections among them were picked up on microscopic slide. The excess water was removed using a blotting paper. Dewaxing was done by drying the slides in hot plate for 2-3 hours and by clearing them in xylene. Samples were then hydrated in decending grades of alcohol. Staining was done by using delafield's haematoxylene for 7 min, dipped once in acid alcohol and then for 3 min in scotts tap water. These were then passed through descending grades of alcohol (3 min. each), followed by eosin stain (3 min) and absolute alcohol (1 dip) and finally cleared in xylene. These were mounted on DPX (Gurr, 1959). Prepared sections were examined and photographed later.

Biochemical Assay

The blood (serum cholineesterase and brain acetylcholineesterase) activities were spectrophotometric method by Ellman *et al.* (1961).

Preparation of Enzyme Source

The method as previously described by Ellman *et al.* (1961) was followed to assay the AChE activity. Brain and blood isolated from a male albino mice weighing 1g of tissue was homogenized with 0.25 M ice cold sucrose solution and 2% (w/v) tissue homogenate was prepared in the same sucrose solution and stored in the freezer as enzyme source.

Three milliliters of phosphate buffer (pH 8.0) was taken in each test tube to with 0.1ml of enzyme source (2% w/v homogenate) was added and stirred. Then 100µl of 0.01 M DTENB (5-5-dithiobis-2 nitro benzoic acid) was added and initial colour was measured spectrophotometrically at 412 nm. The test solution of 100µl sample obtained from the bloom sample *T. erythraeum* in different concentrations such as 25, 50, 100 and 150mg was added, control experiment was also run simultaneously with 100µl of triple distilled water.

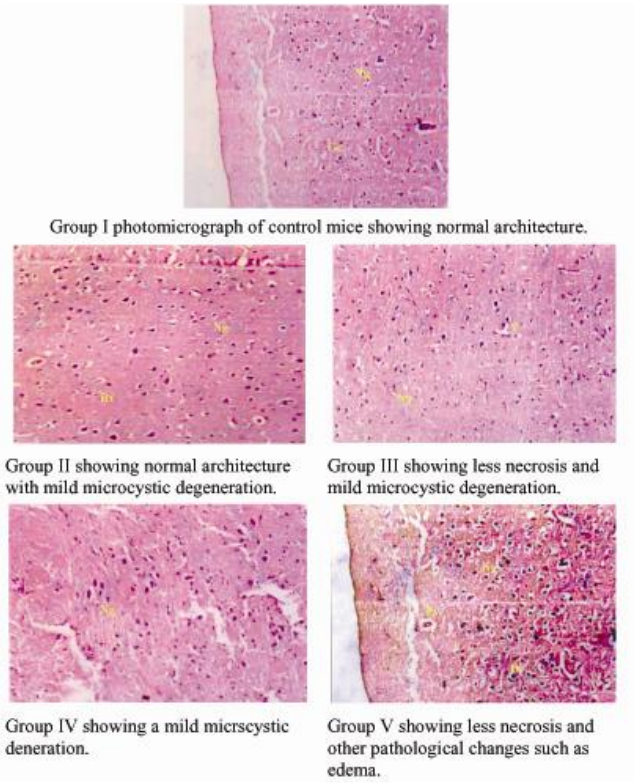
To start the reaction, 20µl of actylthiocholine iodide (ATCI) was added to each test tube as substrate and the reaction was allowed to continue for 15 min at room temperature. The colour developed was measured as final reading spectrophotometrically at 412 nm. Duplicates were maintained for all experiments.

3.RESULTS

Histopathological observation

The normal untreated mice brain tissue showed the complete histoarchitecture. In the cerebral area, grey matter was located on the surface area; it was surrounded by white matter. The cerebral cortex contained nerve cells, fibers, neuroglial and blood vessels. These cells were pyramided granular layer and spindle types and completely arranged in layers. The molecular layer contained fibre cells. The pyramidal and granular cells, neuroglial cells were present in all layers. During the period, the size and shape of the neuroglial cells were not uniform in some area. In grey region, granulated stellate cells and pyramidal cells were seen; neuroglidal cells degenerated. During the treatment, the blood vessels were damaged and vacuoles were formed (Fig.1).

Fig. 1. Effect of *T .erythraeum* crude extract on brain histology of control and experimental animals



(Ng- Neuroglial cells; Bv- Blood vessels; V- Vocuole; Gc- Granular cells; Py- Pyramid cells).

Biochemical Assay

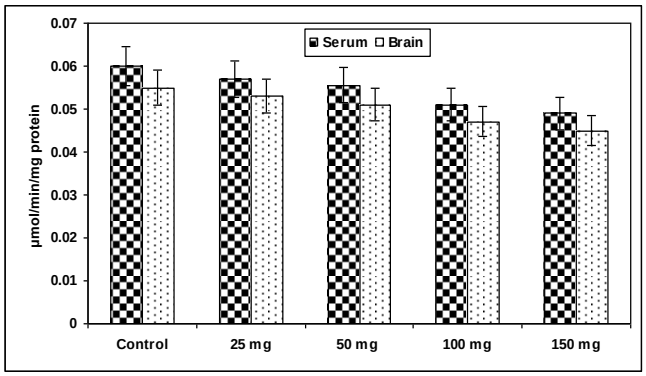
The crude extracts of *T. erythraeum* at the concentrations of 25, 50, 100, and 150 mg. The brain AchE and blood ChE enzyme activities of exposed groups are shown in Fig. 2. However, brain AchE and blood chE activities were slightly decreased when compared to control.

4.DISCUSSION

Histopathology of Brain

The brain is an extremely heterogenous organ with a large number of different neuronal and non neuronal cell types, with extensive morphological differentiation within the cell (Raner *et al*., 2002). It is particularly vulnerable to oxidative damage due to the high utilization of inspired oxygen, the large amount of easily oxidizable poly unsaturated fatty acids.

Fig.2 . Serum ChE and brain AChE enzyme activities of mice in both different doses of treated groups



In the present study, histomorphological changes have been observed in brain tissue of mice *Mus musculus* when injected with crude extract intraperitonialy for 7 days. Histopathology is used to study the impact of toxic materials. Some toxic cyanobacteria cause damages to central nervous system; it consists of a variety of highly specialized cells including many different types of neurons and ganglia. In addition, neurous influence their structure and function in the central nervous system (Deleve and Koplowitz, 1990). The nervous system is comprised primarily of nerve cells and neuroglial cells. The neuroglial cells not only provide physical support but also respond to injury, regulate the ionic and chemical composition of the extra cellular fluid, precipited in the blood-brain barrier, form neuronal migration during development and exchange of metabolites with neurons (Niemi *et al.,* 1991). In the present study, *Trichodesmium* caused mild microcystic changes and edema in the brain tissue. It showed a reduced number of neuroglidal cells and pyramidal cells. The irregular arrangements of neuroglidal cells were seen in some regions. The size and shape of these cells had changed. Similar results were observed by Basu *et al.* (2000) in rat treated with calcium and nifedipine. In the present study, the numerous neuroglidal cells had been regenerated because it was non toxic compound. Similar types of results were also observed by Margorat (2001) in brain tissue of mice treated with toxic compound. Sureshkumar (1999) has also observed the regaining of cytoaerchitecture in the heavy metal intoxicated mice when treated with penicillamin and *Tribulus terrestris* extract. This result indicated the recovery from toxins. The regenerated capacity in the brain tissue had been studied by Margarat and Jagadeesan (1999).

Biochemical Assay

Acetylcholineestrase (AchE) is the enzyme involved in the hydrolysis of the neurotransmitter actylholine during neurotransmission and conduction. This enzyme contributes to the integrits and permeability of the synaptic membrane (Grafius *et al*., 1971). The crude extracts of *T. erythraeum* were administered at the concentrations of 25, 50, 100 and 150 mg. The brain AchE and blood ChE enzyme decreased in all concentrations (when compared to the control). The most toxic of all the cyanobacterial toxins are those which block sodium channels. The LD 50 i.p. for saxitoxin and its analogue neosaxitoxin is 5µg/kg. These toxins have been isolated from *Aphanizomenon flos-aquae* and are fast-acting (Mohmood and Carmicheal, 1986). The inhibition of nerve condition by the sodium channel-blocking effect of saxitoxin

produces violent spasm and convulsion in mice with death occurring in one or two minutes (Codd, 1992).Treated animals of total protein serum and brain in all concentration slightly increased (compared to the control). However, the crude extract of *Trichodesmium erythraeum* on the treated animals had no significant effect in total protein levels of serum and brain.

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