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**ORIGINAL ARTICLE**

# BIOASSAY OF *TRICHODESMIUM ERYTHRAEUM* (Ehr) (MICRO ALGA) ON HAEMATOLOGICAL INVESTIGATION IN THE MICE, FROM SOUTH EAST COAST OF INDIA

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**ABSTRACT**

The present study is aimed to investigate *Trichodesmium erythraeum* (Ehr) activity on the haematological parameters in the blood of *Mus musculus*. In the present study, the Red blood cells(RBC), White blood cells(WBC), Haemoglobin (Hb), Hamatocrit (Ht), Thrombocytes, Lymphocytes, Monocytes and Granulocyte were observed in the blood of *Mus musculus* treated with *Trichodesmium erythraeum* extract. The present study showed the RBC counts, Ht and Hb and WBC and lymbocytes were significantly decreased and thrombocytes, monocytes and granulocytes were significantly increased when compared with control.

**Keywords:** *Trichodesmium erythraeum*, *Mus musculus*, Haematology

## 1.INTRODUCTION

Phytoplanktonic organisms, as primary producers, constitute an important component of marine and fresh water food sources. However, excessive growth of these organisms, called blooms, has an adverse impact on the sustainable functioning of the ecosystems as well as on environmental health (Hanna Mazur-Marzec, 2006). It has been estimated that approximately 300 marine phytoplankton species can, at times, proliferate to such an extent that they form blooms which results in change of the colour of the surface water to red (red tides), or green (Hallegraeff, 1993). The toxic blooms occur in areas where the fish and shellfish are formed and hence they can cause serious economic losses as well as threats to human health

The microscopic planktonic algae in the marine environments are crucial food for filter-feeding bivalve shellfish (Oysters, mussels, scallops, clams, etc.) as well as the larvae of commercially important crustaceans. In most cases, the proliferations of planktonic algae is beneficial to aquaculture but in some situations it can have a negative effect, causing economic losses due to major environmental and human health impacts.

Among over 5000 known species of marine phytoplankton, about 300 species can sometimes proliferate in such numbers that they discolour the surface of the sea, which is called red tides and brown tide phenomena. Only about 40 of these species have the capacity to produce potent toxins that can find their way through fish and shellfish to humans (Sournia *et al*., 1991).

Microalgae occur worldwide in fresh and coastal waters; recently, microalgae have become targets for screening programmers in search of novel compounds of potential value. The toxins of cyanobacteria constitute a major source of natural product toxin, 'biotoxin', that are found in freshwater species and strains in all of the common planktonic cyanobacterial genera including *Anabaena*, *Aphanizomennon*, *Microcystis*, *Nodularia*, *Nostac* and *Oscillatoria*. Other genera including coelosphaerium, cylindrospermposis, *Fischerella*, *Gloeotrichia*, *Scytonema*, *Spirulina*, *Symploca*, *Tolypothrix*, and *Trichodesmium* have been found to be toxic but as yet no toxin has been isolated and characterized from these genera (Scott, 1991; Skulberg *et al*., 1992). *Trichodesmium erythreaum*, a marine cyanobacteriaum occurs in tropical and subtropical seas. Toxic cyanobacteria occur worldwide in freshwater and coastalwaters. The cyanobacterial toxins can be classified in to five functional groups: Heptotoxins Neurotoxins, Cyanotoxins, Dermatotoxins, and Irritanttoxins (Lipopolysaccharides). Some neurotoxic- like substances have been recently characterized from *Trichodesmium erythraeum* and *T.thiebauti* (Jackson *et al*., 2001).But

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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. 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 investigation.

## 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.

### Mice bioassay for lethality

Male Wister albino mice (*Mus musculus*) 20±2 g weight were used following standard AOAC methods (AOAC 1984), the Crude extracts when injected intraperitoneally (i.p) to mice at doses 25mg 50mg 100mg and 150mg and the time at completion of injection was noted, control were run simultaneously.

### Hematological Analysis

### Blood sampling

The blood drawn at sinuaricular puncture was collected from the treated animals with the help of 24 gauze sized needles. The collected samples were stored in vials containing di-sodium salt of EDTA. Total red blood cells count (RBC), total white blood cell counts (WBC), total thermbocytes count, hemoglobin (Hb), haematocrit, values were analyzed by the method of Brown (1993).

### White blood cells (WBC)

The coulter counter instrument was used for the **w**hite blood cells counting. The sample was diluted in an electrically charged solution which passes through an aperture across which a specific voltage passed. As each passed through, the voltage changes created a pulse. The voltage magnitude varied also with cell size. This way the cells were counted.

### Red blood cells (RBC)

Red blood cells were counted with an instrument called coulter counter. The sample is diluted in an electrically charged solution and more slowly through an aperture across which a specific voltage passes. As each cell passes through, the voltage charges, creating a pulse. The voltage magnitude varied also with cell size. This way the cells were counted. Particles greater than 36 FL were counted as RBCs.

### Blood Haemoglobin (Hb)

An instrumental method, using a spectrophotometer, measured the blood sample less light transmittance equated to more haemoglobin (HB).

### Hematocrit (HCT)

This was calculated as volume from the values of the red cell counts and mean corpuscular volume (MCV).

HCT = RBC X MCV

### Lymocyte (LYM)

LYMP were nucleated cells of 35-90 fL measured by using coulter counter method.

## 3.RESULTS

### Haematological parameters

The results of haematological analysis of mice in control and treatment groups are present in Table 1. In mice treated with *T. erythraeum* extracts, RBC counts, Ht and Hb and WBC

and lymbocytes values showed a dose dependent decrease, but a significant levels increase in thrombocytes, monocytes and granulocyte when compared to control.

The Thrombocytes count of blood in the normal mice was 148.12±11.28. During the different concentration (25, 50,100 and 150 mg/kg of Bw, i.p) of crude extract of *T. erythraeum*, the thrombocytes were increased upto 152.02±11.57, 154.37±11.81, 159.15±12.14 and 160.32±12.20 respectively.

**Table 1. Shows of Haematological Analysis of Mice in control and treated groups**

| Parameters | Group I | Group II | Group III | Group IV | Group V |
|---|---|---|---|---|---|
| RBC ($x10^{12}$/l) | 6.44 ± 0.49 | 6.32±0.48 | 6.18±0.47 | 5.62±0.43 | 5.57±0.42 |
| Hb (g/dl) | 12.34±0.94 | 12.14±0.92 | 11.98±0.91 | 10.12±0.77 | 9.94±0.75 |
| Ht (%) | 40.51±3.0 | 38.14±2.90 | 37.24±2.85 | 35.92±2.74 | 34.84±2.65 |
| WBC ($x10^{9}$/l) | 12.90±0.98 | 11.80±0.89 | 11.40±0.87 | 10.56±0.80 | 10.21±0.77 |
| Thrombocytes ($x10^{9}$/l) | 148.12±11.28 | 152.02±11.57 | 154.37±11.81 | 159.15±12.14 | 160.32±12.20 |
| Lymphocytes ($x10^{9}$/l) | 8.44±0.64 | 8.12±0.61 | 7.34±0.56 | 6.72±0.51 | 6.15±0.46 |
| Monocytes ($x10^{9}$cells/l) | 0.75±0.28 | 0.81±0.33 | 0.88±0.25 | 0.92±0.31 | 0.96±0.19 |
| Granulocyte ($x10^{9}$cells/l) | 2.96±0.22 | 3.04±0.22 | 3.25±0.24 | 3.56±0.26 | 3.79±0.28 |

Mean ± S.D of six individual observations; Group I Compared with in all groups; Values are insignificant ($p$<0.05) ANOVA-DMRT.

**Heamatological Study**

**Red blood corpuscles (RBC) in the blood**

The Red blood cells (RBC) count of blood in the normal mice was 6.44 ± 0.49. During the different concentration (25, 50,100 and 150 mg/kg of Bw, i.p) of crude extract of *T. erythraeum*, the Red blood cells were decreased upto 6.32±0.48, 6.18±0.47,5.62±0.43 and 5.57±0.42 respectively.

**Haemoglobin content in the blood**

Haemoglobin content in the blood of normal mice was 12.34 ± 0.94. During the different concentration (25, 50,100 and 150 mg/kg of Bw, i.p) of crude extract of *T. erythraeum*, the haemoglobin contents were decreased upto 12.14±0.92,11.98±0.91,10.10.12±0.77 and 9.94±0.75 respectively.

**Haematocrit (Ht) in the blood**

Haematocrit value in the blood of normal mice was 40.51± 3.0. During the different concentration (25, 50,100 and 150 mg/kg of Bw, i.p) of crude extract of *T. erythraeum*, the haematocrit were decreased upto 38.14±2.90, 37.24±2.85,35.92±2.74 and 34.84±2.65 respectively.

**White blood corpuscles (RBC) in the blood**

The White blood cells (WBC) count of blood in the normal mice was 12.90± 0.98. During the different concentration(25, 50,100 and 150 mg/kg of Bw, i.p) of crude extract of *T. erythraeum*, the White blood cells were decreased upto 11.80±0.89, 11.40±0.87,10.56±0.80 and 10.21±0.77 respectively.

**Thrombocytes in the blood**

The Lymphocytes count of blood in the normal mice was 8.44±0.64. During the different concentration (25, 50,100 and 150 mg/kg of Bw, i.p) of crude extract of *T. erythraeum*, the Lymphocytes were decreased upto 8.12±0.61, 7.34±0.56,6.72±0.51 and 6.15±0.46 respectively.

**Monocytes in the blood**

The Monocytes count of blood in the normal mice was 0.75±0.28. During the different concentration (25, 50,100 and 150 mg/kg of Bw, i.p) of crude extract of *T. erythraeum*, the Monocytes were increased upto 0.81±0.33, 0.88±0.25, 0.92±0.31 and 0.96±0.19 respectively.

**Granulocyte in the blood**

The Granulocyte count of blood in the normal mice was 2.96±0.22. During the different concentration (25, 50,100 and 150 mg/kg of Bw, i.p) of crude extract of *T. erythraeum*, the Granulocyte were increased upto 3.04±0.22, 3.25±0.24, 3.56±0.26 and 3.79±0.28 respectively.

## 4.DISCUSSION

The results of our study showed that the crude extract of *T. erythraeum* caused a dose dependent decrease in some haematological parameters of the mice such as RBC, WBC, Lymphocytes and Ht values. The high dose crude extract treatment in this experiment caused a significant decrease in Hb concentration and the decrease in Hb value could be due to an increase in the rate at which Hb is destroyed and thus the results indicating a disruption of erythropoiesis or an increase in destruction of blood cells. This finding was observed in cypermethrin (fungicide) treated rats by Vural *et al*. (1986). The thermocyte values were slightly increased when compared to the control group. The increase in

granulocyte, monocyte in treated mice compare to the control group, this result is consistent with the extract having no immune suppressive effect on mice; the results indicate that cypermethrin (insecticide), not an immune suppressive effect on rats (Institoris *et al*., 1999 ;2001). The haematological parameters were extensively investigated, but very little attention has been paid to morphological changes induced by crude extract of *T. erythraeum.*

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