Antioxidant property of fresh and marine water cyanobacterial extracts in Swiss mice

R. Navanietha Krishnaraj[1], S. Venkatesh Babu[2], B. Ashokkumar[3], P. Malliga[4] and P. Varalakshmi[2]*

ABSTRACT

Continuous usage of pesticide is the main cause of cellular damage by generation of free radicals. Antioxidants are intimately involved in the prevention of cellular damage. Hence the present investigation is mainly focused to study the antioxidant property of cyanobacterial extracts from diverse environments in order to prevent the free radicals toxicity. The alcoholic extracts of different cyanobacterial isolates including *Oscillatoria salina Synechcococcus, Oscillatoria annae, Oscillatoria chlorina, Spirulina sabsalsa and Spirullina platensis* were analyzed for their antioxidant property by physical body weight change, swimming time and biochemical parameters (superoxide dismutase activity and total reduced glutathione activity) by using Swiss mice. Stress was induced by forced swimming test and the antioxidant efficiency of cyanobacterial extracts was determined. The results showed that *Spirulina platensis* possess significant antioxidant property and *Synechcococcus sp* possess least activity when compared to other cyanobacterial isolates and control.

Key words: Body weight, cyanobacteria, reactive oxygen radicals, super-oxide dismutase

INTRODUCTION

Pesticides are toxic; they are also potentially hazardous to humans, animals and other living beings in the environment. In this present scenario the continuous exposure to pesticides causes severe cellular and molecular damage to humans and other animals by generating free radicals. Antioxidants are substances or nutrients in our food which can prevent or slow the oxidative damage to our body. Phytonutrients and pigments present in the cyanobacteria act as antioxidants which facilitate the formation of the body's defense against free radical damage to cells. Antioxidants act as free radical scavengers and prevent and repair damage done by the free radicals. Reactive oxygen species (ROS) are often generated either as byproducts of biological reactions or from exogenous factors (Cerutti, 1991). It includes superoxide radicals, hydroxyl radicals, singlet oxygen, and hydrogen peroxide. ROS generally play a positive role such as energy production, phagocytosis, regulation of cell growth and intercellular signaling, or synthesis of biologically important compounds (Halliwell, 1997). But, ROS may also play a negative role; they can attack lipids in cell membranes and also attack DNA, inducing oxidations that cause membrane damage such as membrane lipid peroxidation and a decrease in membrane fluidity, and also cause DNA mutation leading to cancer (Pietta, 2000). An antioxidant is a substance that present at low concentrations compared to an oxidizable substrate has the ability to prevent or delay different types of cell damage. The antioxidant defense mechanisms in biological systems are of two types namely enzymatic and non-enzymatic reactions. The enzymatic antioxidants include catalase and hydroperoxidase. The non enzymatic antioxidants include nutrient antioxidants like carotenoids, *a* tocopherol, ascorbic acid, glutathione, flavonoids, uric acid and plasma proteins such as transferrin, albumin, metalothionein etc. (Luximon Ramma *et al.,* 2002; Serena *et al*., 2010).

There is a great demand throughout the world in finding new natural sources for antioxidants to prevent oxidative damage to living cells and to reduce the deterioration of food by oxidation (Pratt, 1992). Traditionally, some antioxidants such as tea, wine, fruits, vegetables and spices are used from the ancient days. Cyanobacteria are prokaryotic organism contains a wide variety of antioxidant pigments than the plants and most algal source (Robbins, 1987). Screening of cyanobacteria for antibiotics and other pharmacologically active compounds, has received ever-increasing interest as a potential source for new drugs. Cyanobacteria are known to produce metabolites with diverse biological activities such as antibacterial (Jaki *et al.,* 2000), antifungal (Kajiyama *et al.,* 1998), antiviral (Patterson *et al.,* 1994), anticancer and antiplasmodial activity (Papendorf *et al.,* 1998). Recently antioxidant property of cyanobacteria especially from *O.annae* has been reported by Rajavel *et al.* (2011). Carotenoids are the most widely distributed and structurally diverse classes of natural pigments predominantly

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produced by cyanobacteria and that are doing important functions in photosynthesis and nutrition. Also they have potent anti oxidant activity. With this background of this present study mainly focused to screen are the antioxidant property of five different cyanobacterial isolates like *Oscillatoria annae*, *O. chlorina, Spirullina sabsalsa, Synechococcus and S. platensis* in order to prevent the oxidative damage caused by the pesticides because that are absorbed on the surface of vegetables and fruits would cause severe damage to the health of the animals while they consume the fruits and vegetables.

MATERIALS AND METHODS

Swiss mice were the animal model used for this experiment. Mid log phase culture of different cyanobacterial isolates including *O. salina, Synechcococcus, O .annae, O. chlorina , S. sabsalsa and S. platensis* were collected from National Facility for Marine Cyanobacteria, Bharathidasan University, Tiruchirappalli. The cultures were grown BG11 and ASN medium in a culture flask separately. The cultures were allowed to grow till they reached the mid log phase. Five different strains of cyanobacteria (1 g fresh weight) were homogenized separately with glass powder and 75% alcohol using Mortar and Pestle. The homogenized extracts were centrifuged at 5000 rpm for 10 minutes. The clear extract was separated and dried using speed vac concentrator. Antioxidant effect of different cyanobacterial extracts were analyzed by measuring the level of antioxidant activity before and after the stress induction to the experimental animal on 1st, 14th and on 28th day.

Swimming test

Stress was induced by forced swimming test. Induction of Stress (Nagaraja and Jeganathan, 1999) was carried out in polypropylene tub 90 cm height, 90 cm diameter and 60 cm depth of water. The water was maintained at 18°C by adding ice cubes to the container. Male albino rats of Swiss strain (130 to 200g) were isolated into 19 groups and each group contains 6 animals.

Analysis of superoxide dismutase and total reduced glutathione activity

Animals were examined carefully, weighed and placed at room temperature (30°C) in normal environmental conditions. They were fed with normal diet (pellet) directly into the oesophagus using curved feeding tube daily at 11:00 am. On 1st, 14th and 28th days the animals were weighed and were given stress. The blood samples (2 mL) were taken for the analysis of antioxidant effect by puncturing the retro orbital plexus directly into heparinised micro capillary tube into a test tube containing 0.1 ml of heparin. The physiological parameters (bodyweight changes), the biochemical parameters super oxide dismutase activity in haemolysate (Marklund and Marklund, 1974) and total reduced Glutathione activity in haemolysate (Patterson and Lazarow, 1975; Gul and Kutay, 2000) were analyzed.

Experimental animal groups

Five groups of animals were used for this study. They were: Group A - A1 = 0.5 µg/L of *Spirullina sabsalsa;* A2 = 1.0 µg/L of *Spirullina sabsalsa;* A3 = 1.5 µg/L of *Spirullina sabsalsa*; Group B - B1 = 0.5 µg/L of *Synechococcus;* B2 = 1.0 µg/L of *Synechococcus;* B3 = 1.5 µg/L of *Synechococcus*; Group C-C1 = 0.5 µg/L of *Spirullina platensis;* C2 = 1.0 µg/L of *Spirullina platensis;* C3 = 1.5 µg/L of *Spirullina platensis*; Group D - D1 = 1.0 µg/L of *Oscillatoria annae;* D2= 1.0 µg/L of *Oscillatoria annae;* D3 = 1.5 µg/L of *Oscillatoria annae*; Group E - E1 = 0.5 µg/L of *Oscillatoria chlorina;* E2 = 1.0 µg/L of *Oscillatoria chlorina;* E3 = 1.5 µg/L of *Oscillatoria chlorina*; Group F - F1 = 0.5 µg/l of *Oscillatoria salina;* F2 = 1.0 µg/L of *Oscillatoria salina* F3 = 1.5 µg/L of *Oscillatoria salina.*

RESULTS AND DISCUSSION

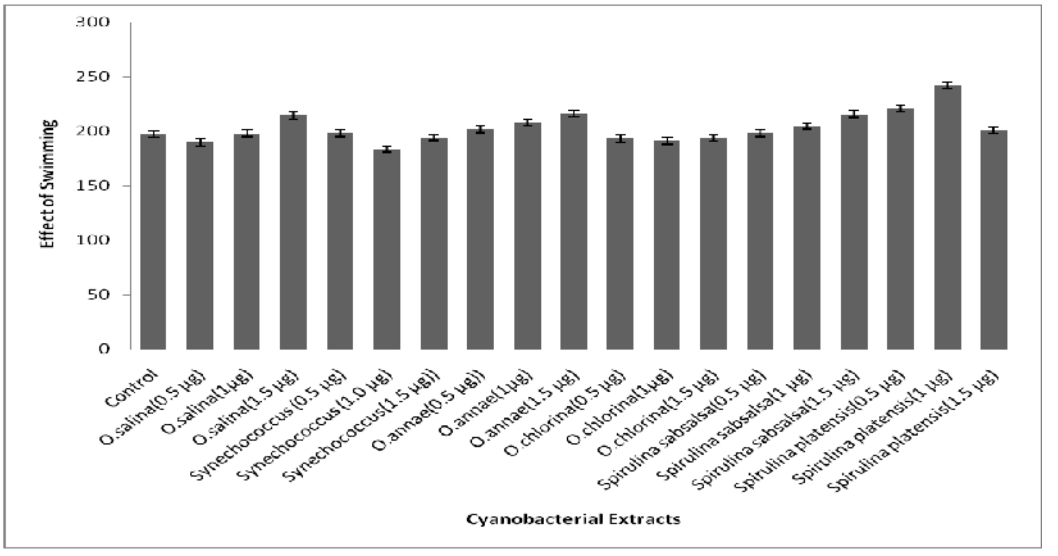
Total reduced glutathione activity

The biological antioxidant system has several enzymes to protect the body from free radicals. Reduced glutathione is one of the enzymes which can be considered as a marker for antioxidant activity. Reduced glutathione is directly proportional to the amount of biological antioxidant activity. GSH based antioxidant study shows that *S. platensis* has higher reduced glutathione activity when compared with *O. salina*, *O. annae*, *O. chlorina Synechococcus* sp and *S. sabsalsa*. *O. chlorina* and *Synechococcus* sp has the least GSH activity. *O. salina, O. annae* and *S. sabsalsa* have intermidiate activity. So the experiment reveals that *S. platensis* extract may prevent the oxidative damage caused by pesticides.

Body weight changes

A body weight change is one of the physical parameters to study the oxidative stress. Induction of stress increases the body weight of mice. On supplementing the antioxidants, the body weight decreases. The body weight has significantly reduced in *S. platensis* when compared to *O. salina*, *O. annae*, *O.chlorina* and *S. sabsalsa*. Obviously, *S. platensis* has higher antioxidant activity. *O. chlorina* and *Synechococcus* sp. has least GSH activity. *O. salina*, *O. annae* and *S. sabsalsa* have intermidiete activity.

Figure 3. Influence of cyanobacterial extracts in swimming effect of Swiss Albino mice



shows least SOD activity. *O. salina*, *O. annae*, *O. chlorina* and *S. sabsalsa* exhibit moderate SOD activity.

The extract of *S. platensis* has the potent anti oxidant activity in swiss albino mice. Hence *S. platensis* can be a new pharmaceutically valuable source for the animals ingested the with toxic pesticides in order to reduce the free radicals formation.

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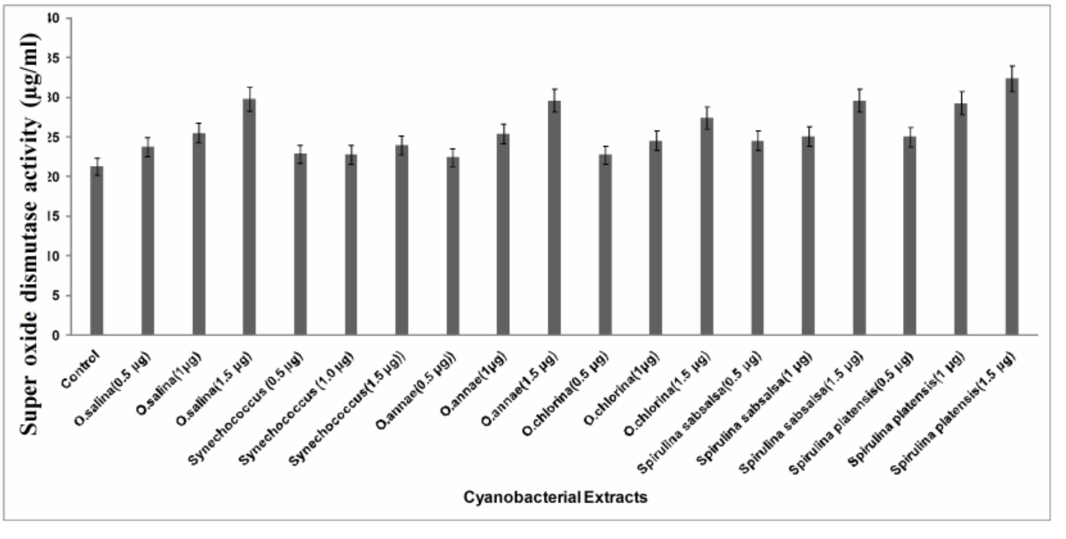
Fig 4. Effect of cyanobacterial extracts in scavenging of SOD in Swiss Albino mice



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R. Navanietha Krishnaraj[1], S. Venkatesh Babu[2], B. Ashokkumar[3], P. Malliga[4] and P. Varalakshmi[2]*

[1]Department of Biotechnology, Kalasalingam University, Krishnan Koil, Tamil Nadu, India.

[2]Department of Molecular Microbiology, School of Biotechnology, Madurai Kamaraj University, Madurai, Tamil Nadu, India.

[3]Department of Genetic Engineering, School of Biotechnology, Madurai Kamaraj University, Madurai, Tamil Nadu, India.

[4]Department of National Facility Marine Cyanobacteria, Bharathidasan University, Tiruchirappalli, Tamil Nadu India.

*E.Mail: vara5277@gmail.com

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