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Antioxidant Property of Hypersaline Cyanobacteria, *Phormidium tenue* (KMD 33)

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Abstract: The antioxidant property of cyanobacterial isolates was analyzed by physical (bodyweight change and swimming time) and biochemical parameters (superoxidedismutase activity and total reduced glutathione activity) by using swiss mice at Animal House, J.J. College of Arts and Science, Pudukkottai, Tamil Nadu, India. The efficiency of cyanobacterial isolates was determined by comparing with the antioxidant property of Spirulina (Commercial grade). The results showed that *Phormidium tenue* (KMD 33) possess significant antioxidant property when compared to other cyanobacterial isolates and Spirulina (commercial grade).

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

INTRODUCTION

Oxidative damage to cells is thought to be causative factor of disease and aging. One etiologic agent implicated in diseased state is oxidative stress which involves excess generation of prooxidants. The prooxidants in biological systems include excited states, free radicals and other related species mainly derived from oxygen and nitrogen. As such prooxidants are generated in our body during exposure to adverse pathophysiological conditions (Sies, 1986).

Reactive species are also generated during Phagocytosis a manifestation of innate immunity. (Kanofsky, 1989; Moan and Berg, 1992; Devasagayam and Sainis, 2002). Oxidative damage in biomolecules mediated by $^1O_2$ is rather frequent in lipids, proteins and DNA. As a whole oxidative stress is defined as an imbalance between oxidants and antioxidants in favour of the former, resulting in oxidative damage to molecules. Reactive Oxygen Metabolites (ROM) which include super oxide amion (O_2^-), H_2O_2, OH^+ and exogenous causes of oxidative stress are tobacco smoke, smog, UV light, fatty acids in food, iron and copper ions, ethanol, pesticides, drugs and xenobiotics which implicated several cancers (Ronald, 1998; Athar, 2002), Cataractogenesis, insulin dependent diabetes mellitus, ischemo-reperfusion cardiac injury, (Daniel *et al*., 1998; Dhuley, 1999) neuro degenerative diseases, Alzheimer's disease, Parkinsonism and neurological conditions like epileptic seizures, stroke, brain damage, neuro trauma etc. (Srinivasan, 2002). This clinical disorder due to free radical oxidative stress occurs usually from deficient natural antioxidant defenses. So potential antioxidant therapy should, therefore, include either natural free radical scavenging antioxidant enzymes or agents, which are capable of augmenting the activity of these enzymes, which include-Super Oxide Dismutase (SOD), CATalase (CAT) and Glutathione PeroXidase (GPX).

An antioxidant is any substrate that when present at low concentrations compared to these of an oxidizable substrate significantly delays or prevents certain types of cell damage and oxidation of substrates. The normal antioxidant defense mechanisms in biological systems consist of both enzymatic and non-enzymatic reactions. Although both are important in biological systems, the non enzymatic antioxidant include nutrient antioxidants like water and fat soluble vitamins, carotenoids, α tocopherol, ascorbic acid, glutathione, flavonoids, uric acid and plasma proteins such as albumin, transferrin, ceruloplasmin, metalothionein etc. (Ronald, 1998; Dureja *et al*., 2003). The use of synthetic antioxidants has decrease due to their suspected activity as promoters of carcinogenesis (Namiki, 1990) as well as general consumer rejection of synthetic food additions (Mirada *et al*., 1998). There is a current worldwide interest in finding new and safe antioxidants from natural sources for example plant material to prevent oxidative deterioration of food and to minimize oxidative damage to living cells (Pratt, 1992). Prokaryotic organisms without a nuclear membrane especially cyanobacteria display a more diverse array of antioxidant pigments and a broader selection of carotenoids than terrestrial plants and most green algae (Robbins, 1987). Carotenoids represent one of the most widely distributed and structurally diverse classes of natural pigments with important functions in photosynthesis, nutrition and protection against photo-

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oxidative damage. The present study is an attempt to screen the antioxidant property of hypersaline cyanobacteria isolated from the saltpans of Kattumavadi, Palk Strait region of Tamil Nadu, India.

MATERIALS AND METHODS

The analysis was carried out by using Swiss mice at Animal House, J.J. College of Arts and Science, Pudukkottai, Tamil Nadu, India at June 2005. Mid log phase culture of different hypersaline cyanobacterial isolates including *Phormidium tenue* KMD 33, *Phormidium tenue*, *Phormidium fragile* and *Phormidium angustissimum* (1 g fresh weight) was crushed with glass powder and 75% alcohol using mortar and pestle and centrifuged (5000 rpm/10 min). The clear extract was separated and dried using speed vac concentrator. Carotenoids and Phycobilins pigments of *Phormidium tenue* (KMD 33), *Phormidium tenue*, *Phormidium fragile* and *Phormidium angustissimum* were studied by using the procedure of Bennet and Bogorad (1973) and Boussiba and Richmond (1979). Antioxidant effect of different hypersaline cyanobacterial extracts were compared with commercially available Spirulina by measuring the level of antioxidant activity before and after giving the animal stress on 1st, 14th and on 28th day. Stress was induced by forced swimming test. Induction of Stress (Nagaraja and Jeganathan, 1999) was carried out in polypropylene tub 90 cm's height, 90 cm's diameter and 60 cm's depth of water. The water was maintained at 18°C by adding ice cubes to the container in between. Male Albino Rats of Swiss Strain weighing 130 to 200 g were purchased from Sri Ramachandra Medical College and Research Institute, Porur, Chennai after getting the ethical clearance and isolated into 4 groups of 6 animals each. They were examined carefully, weighed and placed at room temperature (30°C) in normal environmental conditions in Animal house J.J. College of Arts and Science, Pudukkottai, Tamil Nadu, India. They were fed with normal diet (pellets obtained from Hindustan Liver Ltd).

The male albino rats segregated into 4 groups, 6 animals each were fed with 1 mL of distilled water, 0.5 μg g^{-1} body weight of hypersaline cyanobacterial extracts (*Phormidium tenue* (KMD 33), *Phormidium tenue*, *Phormidium fragile* and *Phormidium angustissimum*), 1 μg g^{-1} body weight Spirulina extract, 1.5 μg g^{-1} body weight of hypersaline cyanobacterial extracts (*Phormidium tenue* (KMD 33), *Phormidium tenue*, *Phormidium fragile* and *Phormidium angustissimum*) directly into the oesophagus using curved feeding tube daily at 11:00 am and named as Group 1, 2, 3 and 4, respectively. 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 (Cat. No. 31-00-011-Sewa Medical Lab Ltd., Chennai) into a test tube containing 0.1 mL of heparin. The physiological parameters like bodyweight changes and swimming time, the biochemical parameters like 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 analysed and compared with the anti oxidant activity between commercial grade Spirulina and hypersaline cyanobacterial extracts. The statistical analysis was carried out by using ANOVA followed by Dunett's multiple comparison tests (SPSS version 10). The intergroup difference was considered significant when $p<0.05$.

RESULTS AND DISCUSSION

The formation of free radicals include reactive species of oxygen, nitrogen or chlorine, super oxide, hydroxyl ions, hydrogen peroxide and nitric acids. Free radicals can have negative effects when they damage protein, lipids and nucleic acids. The biological antioxidant system is an integrated assay of enzyme, antioxidant and free radical scavengers. These include reduced glutathione (GSH), glutathione reductase, glutathione-S-transferase, glutathione peroxidase, Super Oxide Dismutase (SOD), catalase, ascorbic acid (Vitamin C), tocopherol (Vitamin E) and carotenoids (Frimer 1985; Devasagayam *et al*., 1993; Santhosh Kumar *et al*., 1999).

Antioxidants are biomolecules that protect organisms from the damaging effects of reactive oxygen species that are constantly formed in biological systems. Systematic screening for therapeutic substances from algae, particularly cyanobacteria, has received greater attention recently (Patterson *et al*., 1993; Skulberg, 2000). Cyanobacteria contain a wide range of antioxidants in the form of specific trace minerals, amino acids, vitamins and especially pigments.

Among the different hypersaline cyanobacterial isolates (*Phormidium tenue* (KMD 33), *Phormidium tenue*, *Phormidium fragile* and *Phormidium angustissimum*) tested only *P. tenue* KMD 33 showed potent antioxidant property. The efficiency of the organisms was analysed, by measuring body weight changes and swimming time on 1st, 14th and 28th days. There was significant increase in body weight from day 1st to 28th in all four groups (Table 1). The body

Table 1: Physiological and biochemical parameters at 1st, 14th and 28th among different groups

Groups	Day 1	Day 14	Day 28
Body weight changes (g)			
Group 1	161.830±8.416[a]	212.670±8.123[a]	218.670±6.280[a]
Group 2	161.600±9. 807[a]	198.400±9.132[a]	201.170± 9.297[a]
Group 3	158.970±8.050[a]	204.000±8.748[a]	208.830±7.337[a]
Group 4	162.630±4.913[a]	191.000±6.424[a]	193.500±5.841[a]
Changes in swimming time (sec)			
Group 1	178.000±3.786[b]	179.000±2.892[b]	181.000±2.955[b]
Group 2	176.670±6.097[b]	184.670±3.964[b]	193.670±4.104[b]
Group 3	163.800±7.375[a]	196.330±7.588[a]	214.000±7.971[a]
Group 4	147.000±5.737[a]	206.000±5.328[a]	226.170±4.976[a]
Superoxide dismutase activity (µg)			
Group 1	21.200±0.9059[b]	21.517±0.9357[b]	21.633±0.7649[b]
Group 2	20.417±0.5839[b]	22.050±0.9251[b]	24.517±1.0650[b]
Group 3	21.733±0.8947[a]	23.533±0.8999[a]	30.317±0.9138[a]
Group 4	21.600±0.8095[a]	28.683±0.8994[a]	33.017±0.7233[a]
Total reduced glutathione activity (µg)			
Group 1	35.000±2.000[b]	35.167±1.740[b]	35.833±1.8510[b]
Group 2	35.667±1.358[a]	38.167±1.138[a]	41.333±0.7601[a]
Group 3	36.333±1.606[a]	43.833±1.249[a]	50.500±1.2040[a]
Group 4	35.333±1.563[a]	46.500±1.057[a]	53.500±0.8466[a]

Values are mean±SD from ten replicates. [a]Values followed by identical letter(s) in the same row are statistically significant, [b]Values followed by identical letter(s) in the same row are not statistically significant

weight was less in animals treated with *P. tenue* (KMD 33) and this decrease in body weight was in a dose dependent manner. The results showed that *P. tenue* (KMD 33) has an anti obesity effect. Dhuley (1999) observed similar anti obesity effects by giving cinnamon and cardamom. Becker *et al.* (1986) reported the anti obesity effects of Spirulina extract of 5 mg day^{-1} in rats.

In group 1 and 2 the change of swimming time was minimal from the base line reading on day 1st, 14th and 28th days. The p-value was not significant. But in-group III and IV there was a significant increase ($p<0.05$) in swimming time from 14th to 28th days (Table 1). The swimming time changes were significant when fed with

P. tenue (KMD 33), indicating a dose dependent anti stress activity. At a standard daily dose of 5 mg of Spirulina, there was a rise in swimming time, which indicates that, the *P. tenue* extract was functioning as a scavenger of free radicals, therefore the test animal swam by overcoming the stress. The present result are in support of the results obtained by Zhi-Gang *et al.* (1997).

Antioxidant activity was found by evaluating the biochemical parameters of Super oxide dismutase activity and total reduced glutathione activity. Super Oxide Dismutase (SOD) activity was not increased significantly ($p>0.05$) in group 1 and group 2. But in-group 3 and 4 the activity of Super Oxide Dismutase showed significant ($p<0.05$) increase. Super Oxide Dismutase (SOD) an enzymatic antioxidant, catalytically scavenging the super oxide free radical providing the first line of defense against free radical damages. The rise of SOD level was extremely significant when *P. tenue* (KMD 33) extract was use in 7.5 mg day^{-1} concentration, which indicates dose dependent SOD activity (Table 1). It was observed that increasing level of SOD corresponds to the increase in TRG activity. The increase in SOD level with 5 mg Spirulina extract was 40% from the base line that has already been verified from different studies (Belay, 2002; Hayashi *et al.*, 1993, 1994; Yang *et al.*, 1997; Manoj *et al.*, 1992; Zhi-Gang *et al.*, 1997; Mirada *et al.*, 1998). Forman and Broveris (1982) reported that marked increase in SOD activity followed by the administration of cinnamon and cardamom showed the adaptive nature of the system against damaging effects of superoxide radical.

Total Reduced Glutathione (TRG) activity was increased in group 2, 3 and 4 showing significant p-value. But in group 1 the p-value was not significant (Table 1). There was a gradual increase in TRG level based on increasing concentration of the *P. tenue* extract which confirms the role of glutathione in protecting endothelial cells against H_2O_2 or free radicals (Andreoli *et al.*, 1986). In context of using *P. tenue* as an antioxidant it was worth noting that it contains about 24 µg phycocyanin, relatively high content of SOD (33.017 µg) and 53.5 µg of TRG, when compared to Spirulina in addition to a high content of carotenoids (20 µg). The above findings show that there is dose dependent rise in free radical scavenging activity of *P. tenue* (KMD 33) that can be almost equal to that of Spirulina.

The carotenoid content of *P. tenue* (KMD 33) maintained at 100 ppt salinity was 20 µg g^{-1} fresh weights. Carotenoids represent one of the most widely distributed and structurally diverse classes of natural pigments, with important functions in photosynthesis, nutrition and protection against photo-oxidative damage (Johmson and Schroedar, 1996.). β-Carotene is just one of the over 500 carotenoids β carotene (536.85 M.W., $C_{40}H_{56}$) is a water-soluble precursor or to Vitamin A, but is an antioxidant in itself. β-carotenes showed promising result on oral leukoplakias, skin cancer and sarcomas.

Many researchers namely, Johmson and Schroedar (1996), Ben-Amotz *et al.* (1989), Levin and Mokady (1994), Kelly (1998) and Frei (1994) confirmed that β-carotene has greater therapeutic value. Overall evidence suggests that micro algae demonstrates at least four antioxidant properties, viz., scavenging of reactive oxygen species (free radicals), regeneration of endogenous antioxidants, such as SOD and glutathione reductase, chelation of heavy metals and repair of oxidation-damaged proteins.

Among the biological compounds, carotenoids are the most efficient quenchers. There are two types of quenchers based on the mechanisms of action. Compounds such as carotenoids and nickel complexes quench $^1O_2$ by energy transfer with high rate constants, compounds like diazabicyclo (2.2.2) octane, phenols, sulphides and azides are known to quench $^1O_2$ by electron transfer mechanisms with lower rate constants. The biologically occurring open chain isomer of β-carotene shows the greatest quenching ability (Devasagayam *et al.*, 1992).

The phycocyanin content of *P. tenue* (KMD 33) maintained at 100 ppt salinity was 24 μg g^{-1} fresh weight. The phycocyanin also one of the pigments of cyanobacteria appears to have both antioxidants and anti-inflammatory properties. The phycocyanin is water-soluble blue pigment, synthesized from a common precursor and their production is controlled by the presence of light and available nitrogen. Very few researchers reported that phycocyanin having antioxidant, anti-inflammatory, hepato protective effects, prevention and mitigation of health problems like cancer and heart disease (Schwartz *et al*., 1987; Romay *et al*., 1998a, b; Vadiraja *et al*., 1998; Torres- Duran *et al*., 1999; Bhat and Madayastha, 2000; Rimbau *et al*., 1999; Romay and Gonzalez, 2000; Reddy *et al*., 2000; Liu *et al*., 2000).

Spirulina has been produced commercially for food and feed. It has 60-70% protein and a rich source of vitamins, especially vitamin B_{12}, Pro vitamin A (β-carotene) and minerals. It provides an adequate amount of a spectrum of carotenoid pigment, zeaxanthin, phycocyanins and polysaccharides. In this respect Spirulina is considered as micro vegetable that can provide potential therapeutic applications in the areas of immuno modulation antioxidant anti cancer, antiviral and cholesterol reduction effects (Belay, 2002; Hayashi *et al*., 1993, 1994; Yang *et al*., 1997; Manoj *et al*., 1992; Zhi-Gang *et al*., 1997; Mirada *et al*., 1998).

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