Spirulina maxima and its effect on antioxidant activity in fructose induced oxidative stress with histopathological observations

Original research article/Review

Urmila Jarouliya^{1✉}, Anish Zacharia¹, Raj K. Keservani², Godavarthi B.K.S Prasad¹

¹Jiwaji University, School of Studies
in Biotechnology, Gwalior (M.P.), India.

²Rajiv Gandhi Proudyogiki Vishwavidyalaya,
School of Pharmaceutical Sciences, Bhopal (M.P)

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Abstract Diabetes mellitus is a metabolic disorder characterised by hyperglycemia and oxidative stress. The aim of the present study is to explore the antioxidant effect of *Spirulina maxima* in rat model along with the histopathological observations. Diabetes was induced by feeding 10% fructose solution orally to Wistar rats (n = 6) for 30 days, analysed for plasma blood glucose and the markers of the oxidative stress [catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) and thiobarbituric acid reactive substances (TBARS)]. These biochemical studies were associated with histopathological examination of liver and kidney sections. The microalga *Spirulina maxima* being rich in proteins and other essential nutrients is widely used as a food supplement. *S. maxima* at a dose of 5 and 10% per kg and the metformin (500 mg/kg) as reference drug were given orally for 30 days to the diabetic rats. Diabetic rats showed significant (p < 0.001) elevations in plasma blood glucose, thiobarbituric acid-reactive substances and significant reduction in catalase, superoxide dismutase and reduced glutathione activity. Oral administration of 5 and 10% aqueous extract of *S. maxima* for 30 days restored not only of blood glucose levels but also markers of oxidative stress. Histopathological observations of tissues manifested that the *S. maxima* administration had the protective and therapeutic effects against fructose-induced abnormalities in diabetic rats. It is concluded that *S. maxima* is effective in reinstating the antioxidant activity in addition to its antidiabetic effect in type 2 diabetic rats.

Keywords Antioxidant – Diabetes – Oxidative stress – *Spirulina maxima*

INTRODUCTION

Diabetes mellitus is a group of metabolic disorder in which a person has high blood sugar that is hyperglycaemia or insulin resistance characterised by reducing insulin secretion (American Diabetes Association 2013). The long-term chronic hyperglycaemia is cognated with damage, dysfunction and failure of different organs with generation of oxidative stress. Oxidative stress results due to the increased production of free radicals and the reduction in antioxidant defence mechanism. In the internal environment of cells infectious diseases, toxins and nutritional imbalance form the reactive oxygen and nitrogen species. These reactive molecules in excess damage the protein, lipids and DNA (Naviaux 2012) that is associated with complications in type 2 diabetic patients.

Recent studies by researchers reported that long-term fructose feeding promotes oxidative damage and exerts detrimental effects by reducing antioxidant defences, and increasing generation of free radicals. Abnormally high

levels of free radicals that cause membrane damage due to peroxidation of membrane lipids and protein glycation and the simultaneous decline of antioxidant defence mechanisms lead to cell and tissue damage (Maritim *et al.* 2003; Tang *et al.* 2006).

The oral hypoglycaemic agents currently used in clinical practice have characteristic profiles of serious side effects (Prout *et al.* 1974; Holman and Turner 1991; Williams and Pickup 1991). Hence, there is a need to search for newer antidiabetic agents that retain therapeutic efficacy and are devoid of side effects. *Spirulina maxima* is a helical microscopic cyanobacteria and blue-green aquatic plant. It appears to be a good source of organic nutrition, especially plant protein. These blue-green algae occurs naturally in alkaline water and is a complete food because of the presence of nutrients that include β-complex, vitamins, minerals, proteins, γ-linoleic acid, nutraceutical pigments (Keservani *et al.* 2015) and the

* E-mail: urmi_gudia@rediffmail.com

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β -carotene as well as the trace elements that make it helpful for the treatment of various diseases. Some therapeutic properties, such as hypocholesterolemic effect (Nakaya *et al.* 1998), antioxidant and immunomodulatory activity (Kulshreshtha *et al.* 2008), anti-herpes simplex virus activity (Hayashi 1993), body weight reduction in obese patients and antimutagenic potential, anticoagulative and antithrombotic activity (Kamble *et al.* 2013) and the arsenic-induced toxicity (Kulshreshtha *et al.* 2014) and other pharmacological properties have indicated an added advantage of an algal-protein diet for human and animal consumption (Belay 1997). This study was designed to investigate the protective and therapeutic effect of *S. maxima* extract on antioxidant enzyme status in fructose-induced diabetic rats.

MATERIALS AND METHODS

Animals

Male *Rattus norvegicus* (Wistar strain) aged 8–10 weeks (weighing about 180–220 g) were used and the animals were procured from Defence Research & Development Establishment, Gwalior (M.P). All the rats were individually housed in plastic cages with stainless steel covers and maintained on a 12 h light/dark cycle at a room temperature of $25 \pm 2^\circ\text{C}$. They were acclimatised to the laboratory condition for 1 week with access to standard diet and tap water *ad libitum*. All animals were treated according to the ethical guidelines approved by Institutional Animal Ethics Committee [(IHEC-09/2008), Jiwaji University, Gwalior].

Chemicals

All the chemicals used in this experiment were of analytical grade and purchased from E. Merck India Limited (Mumbai, India).

Culture maintenance

S. maxima axenic culture was acquired from algal laboratory of Indian Agriculture Research Institute (IARI), New Delhi and cultured in Zarrouk's medium at alkaline pH (8.5) under the light intensity of 2500 lux at 30°C temperature for 16 h light and 8 h dark period. On the 20th day, exponential growth phase of the algae was observed, and then the cultures were harvested, filtered, collected, dried below 40°C for overnight and powdered by electric grinder for further use.

Induction of diabetes

The experimental animals were fed orally with 10% fructose solution for 30 days and the blood glucose were monitored at an interval of 15 days until the 60th day while various antioxidant parameters were analysed at an interval of 30 days, that is, at 0, 30 and 60th day.

Experimental design

The care and maintenance of animals were as per the approved guidelines of the 'Committee for the Purpose of Control and Supervision of Experiment on Animals' (CPCSEA, India). Forty-two male Wistar rats were divided into the following groups having six rats in each group and treated as below:

Group I (normal control): Given only tap water and standard feed.

Group II (diabetic control): Diabetes was induced by giving 10% fructose for 30 days and no treatment was given.

Group III (diabetic treated with 5% *S. maxima* extract): Rats of this group were fructose-diabetic and treated with 5% *S. maxima* extract once daily for 30 days.

Group IV: These diabetic rats were treated with 5% *S. maxima* extract once daily for 30 days along with co-administration of 10% fructose solution continuously.

Group V (diabetic treated with 10% *S. maxima* extract): Rats of this group were fructose-diabetic and treated with 10% *S. maxima* extract once daily for 30 days.

Group VI: Diabetic rats treated with 10% *S. maxima* aqueous extract once daily for consecutive 30 days along with the co-administration of fructose solution.

Group VII (diabetic treated with metformin): This group rats were treated with metformin (500 mg/kg) once daily for 30 consecutive days.

Preparation and administration of Spirulina maxima extract

The *S. maxima* aqueous extract was prepared by suspending dry powder in water at a concentration of 5 and 10 g/100 ml of water. The prepared infusion was given orally once to diabetic animals throughout the experimental period by intragastric tube (cannula).

BIOCHEMICAL ANALYSIS

Blood glucose estimation

Blood glucose was estimated spectrophotometrically by using the commercial diagnostic kits (E. Merck India Ltd, Mumbai, India) based on glucose oxidase peroxidase method (Trinder 1969).

Estimation of oxidative stress markers in blood

Before collecting the blood sample from retro orbital plexus of rat eyes, the animals were fasted for 6 hrs, anaesthetised by mild ethyl ether and the blood was collected through heparinised capillary into heparinised vials once before and at 30 day intervals for biochemical analyses. Reduced glutathione (GSH) was estimated in a whole blood (0.2 ml) and remaining sample was used for preparation of haemolysate

for estimation of catalase (CAT), superoxide dismutase (SOD) and thiobarbituric acid reactive substances (TBARS). All estimations were carried out immediately after collecting the samples.

Estimation of GSH level in blood was carried out by the method originally described by Ellman (Ellman 1959). TBARS were determined as an index of lipid peroxidation (LPO) (Ohkawa *et al.* 1979). The SOD activity was assayed by the method of Winterbourn *et al.* (Winterbourn *et al.* 1975). CAT was determined by monitoring the decomposition of hydrogen peroxide as described by Sinha (Sinha 1972). Protein concentration in the haemolysate was determined according to Lowry *et al.* method (1951), using bovine serum albumin (BSA) as standard (Lowry *et al.* 1951).

Histopathological study

At the end of experiment on the 61st day, rats were fasted for overnight before sacrifice. They were anaesthetised with ethyl ether and the blood was collected by cardiac puncture. The liver and kidneys were removed, pruned, blotted and weighed and these tissues were fixed in aqueous Bouin's fluid solution. After proper fixation, small pieces were dehydrated by passing successfully through a different mixture of ethyl alcohol–water, cleaned in xylene and embedded in paraffin wax. Sections of liver and kidney (5–6 µm thick) were prepared and then stained with haematoxylin and eosin dye, which were mounted in neutral deparaffinated xylene (DPX) for microscopic observations (McManus and Mowry 1965).

Statistical analysis

The data were analysed using one-way Analysis of variance (ANOVA) (Bonferroni t-test) employing Sigma Stat, statistical software, version 1.0 (Jandal corporation, USA). $P < 0.001$ and $P < 0.05$ were regarded as statistically significant.

RESULTS

Figure 1 shows that by giving 10% fructose solution to Wistar rats daily for 30 days significantly ($p < 0.001$) increases the blood glucose level by 85–98% and the lipid peroxidation (TBARS) products by 70% (Figure 2) in diabetic rats when compared with normal control group rats. The activities of SOD and CAT were significantly ($p < 0.001$) reduced by 60 and 40%, respectively (Figures 3 and 4). GSH level was marginally affected by 15% (Figure 5).

Effect of *S. maxima* aqueous extract on blood glucose level in fructose-induced diabetic rats after 30-day treatment

While given treatment to diabetic rats with 5 and 10% *S. maxima* extract, blood glucose levels were significantly reduced by 60 ($P < 0.001$) and 54% ($P < 0.001$) compared with normal control rats.

Figure 1. Effect of *S. maxima* administration on blood glucose level. Abbreviations: SM - *Spirulina maxima*, CF - Continuous fructose. Units: mg/dl. The values expressed as mean $\pm$ SE, $n = 6$.

^a $p < 0.05$ (*), ^a $p < 0.001$ (**) significant compared with normal control group.

^b $p < 0.05$ (*), ^b $p < 0.001$ (**) significant compared with diabetic control group.

The diabetic group treated with continuous fructose feeding and *S. maxima* had reduced the blood glucose levels significantly by 45–46%. While in the allopathic drug (metformin) treated group, reduction in the blood sugar level was observed by 45% within 30 days of the *S. maxima* therapy (Figure 1).

Effect of *S. maxima* aqueous extract on oxidative stress markers in fructose-induced diabetic rats after 30-day treatment

Thiobarbituric acid reactive substances (TBARS) level

The level of elevated lipid peroxidation was considerably reduced to 60.33% ($P < 0.001$) with 5% *S. maxima* and 39.25% with 10% *S. maxima* compared with normal control group. Whereas the diabetic group treated with *S. maxima* and on continual fructose feeding generated reduction in TBARS levels by 32–45%. In the metformin (500 mg/kg) treated diabetic group, there is reduction in TBARS levels by 29.64% within 30 days of therapy (Figure 2).

SOD activity

The administration of 5 and 10% *S. maxima* to diabetic rats increased the SOD activity by 190 ($P < 0.001$) and 230% ($P < 0.001$) after 30 days of the therapy. The *S. maxima*-treated and constant fructose-fed diabetic rats had elevation of 185–250% ($P < 0.001$) in the SOD activity. Whereas the metformin (500 mg/kg) treated diabetic rats had marginal change in SOD activity (7%) (Figure 3).

Catalase activity

The administration of 5 and 10% *S. maxima* to diabetic rats increased the CAT activity by 70.64 ($P < 0.05$) and 96.29% ($P < 0.001$) after 30 days of the therapy. The diabetic group

Figure 2. Effect of *S. maxima* administration on TBARS level
Abbreviations: SM - *Spirulina maxima*, CF- Continuous fructose.
Units: nmole/mg protein. The values expressed as mean $\pm$ SE, $n = 6$.
 $^a p < 0.05$ (*), $^a p < 0.001$ (**) significant compared with normal control group.
 $^b p < 0.05$ (*), $^b p < 0.001$ (**) significant compared with diabetic control group.

Figure 3: Effect of *S. maxima* administration on SOD activity
Abbreviations: SM - *Spirulina maxima*, CF- Continuous fructose.
Units: Unit/min/mg protein. The values expressed as mean $\pm$ SE, $n = 6$.
 $^a p < 0.05$ (*), $^a p < 0.001$ (**) significant compared with normal control group.
 $^b p < 0.05$ (*), $^b p < 0.001$ (**) significant compared with diabetic control group.

explored with *S. maxima* and ongoing fructose feeding increased the CAT activity by 41–49% ($P < 0.05$) and the CAT activity in metformin (500 mg/kg) treated diabetic rats were increased by 22% (Figure 4).

Reduced GSH level

The results in Figure 5 showed that the GSH level was found to be elevated by 21% ($P < 0.05$) and 9% with 5 and 10% *S. maxima*, respectively, in the diabetic rats after 30 days of the

Figure 4. Effect of *S. maxima* administration on CAT activity
Abbreviations: SM - *Spirulina maxima*, CF- Continuous fructose.
Units: μ mole/min/mg protein. The values expressed as mean $\pm$ SE, $n = 6$.
 $^a p < 0.05$ (*), $^a p < 0.001$ (**) significant compared with normal control group.
 $^b p < 0.05$ (*), $^b p < 0.001$ (**) significant compared with diabetic control group.

Figure 5. Effect of *S. maxima* administration on reduced GSH level
Abbreviations: SM - *Spirulina maxima*, CF- Continuous fructose.
Units: mg/ml. The values expressed as mean $\pm$ SE, $n = 6$.
 $^a p < 0.05$ (*), $^a p < 0.001$ (**) significant compared with normal control group.
 $^b p < 0.05$ (*), $^b p < 0.001$ (**) significant compared with diabetic control group.

therapy. There was a marginal change in GSH level by 4% in the *S. maxima* and 10% continuous fructose-fed diabetic rats. While the metformin (500 mg/kg) treatment had marginal changes in blood GSH level by 8% after 30 days of therapy (Figure 5).

Histopathological observations

Liver histopathology

Liver histology of control rats showed normal hepatic cord pattern, hepatic lobules and hepatocytes with normal

Figure 6. (a–f): Effect of *S. maxima* administration on liver histology of rats (haematoxylin and eosin $\times 100$)

- Normal control liver
- Diabetic control liver
- Diabetic liver treated with 5% *S. maxima*
- Diabetic liver treated with 5% *S. maxima* along with 10% continuous fructose
- Diabetic liver treated with 10% *S. maxima*
- Diabetic liver treated with 10% *S. maxima* along with 10% continuous fructose

Figure 7(a–f). Effect of *S. maxima* administration on kidney histology of rats (haematoxylin and eosin $\times 100$).

- Normal control kidney
- Diabetic control kidney
- Diabetic kidney treated with 5% *S. maxima*
- Diabetic kidney treated with 5% *S. maxima* along with 10% continuous fructose
- Diabetic kidney treated with 10% *S. maxima*
- Diabetic kidney treated with 10% *S. maxima* along with 10% continuous fructose

architecture (Figure 6a). Liver histology of diabetic rats showed congestion of blood sinusoids around the central vein with sporadic haemorrhage and the large number of hepatocytes were pyknotic in appearance, deposition of exudates in the portal tract with signs of necrotic hepatocytes (Figure 6b). The hepatic lesions were minimal in rat liver treated with *S. maxima* alone. However, few inflammatory cells were observed in the vicinity of central canal. The general hepatic architecture was normal (Figure 6c and e). It is observed that the severity of degenerative changes in

the liver of rats co-administered with fructose and *S. maxima* were reduced significantly as compared with the diabetic rats (Figure 6d and f).

Renal histopathology

Renal histology of control rats showed normal packed malpighian corpuscles, renal tubules and blood vessel which were supported by scanty reticular fibre (Figure 7a). Histological examination of kidneys of diabetic rats showed multiple areas of tubular vacuolations with parenchymal hypertrophy. Renal tubules also revealed degenerative changes in the epithelium characterised by eosinophilic appearance with pyknotic nuclei (Figure 7b). Renal histology of diabetic rats treated with *S. maxima* alone showed minimal lesions and renal histology appeared normal (Figure 7c and e). These vacuolative degenerative changes were also significantly reduced in the renal sections of rats co-administered with fructose and *S. maxima* (Figure 7d and f).

DISCUSSION

Recently, much attention has been focussed on the role of oxidative stress and it has been suggested that oxidative stress may be the key and common factor in the pathogenesis of different diabetic complications (Sepici-Dincel *et al.* 2007). The mechanisms that contribute to increased oxidative stress in diabetes include non-enzymatic glycosylation, auto-oxidative glycosylation and metabolic stress. Diabetes and experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, which depletes the activity of antioxidative defence system and thus, prevents the de novo free radicals elimination/neutralisation (Kamalakanan and Prince 2006). Oxygen-free radicals react with all biological substances; however, the most susceptible ones are polyunsaturated fatty acids. Reactions with these cell membranes constituents lead to lipid peroxidation (LPO). These elevated levels of LPO disrupt the membrane transport and signalling function (Arulselvan and Subramanian 2007). Thus, this study exhibits a potent anti-oxidant function of *S. maxima* against fructose-induced oxidative stress. In a preliminary study done by me to see the hyperglycemic and hyperlipidemic effect in fructose-fed Wistar rats, the findings suggested that the *S. maxima* administration results the anti-hyperglycemic and anti-lipidemic along with the hepatoprotective activity (Jarouliya *et al.* 2012). This finding confers that by inducing diabetes through fructose feeding to Wistar rats for the period of 30 days resulted in a significant elevation in TBARS levels with simultaneous reductions in GSH, SOD and CAT activity. As shown in Figure 2, the fructose-fed diabetic rats with elevated level of TBARS in the blood may be due to high level of circulating glucose, which, in turn, increase free radicals production, glucose auto-oxidation and glycation (oxidative modification) of protein (Wolf *et al.* 1990). The present study shows that the administration of *S. maxima* significantly decreased the blood TBARS level (Figure 2).

In this study, the administration of *S. maxima* extracts to fructose-fed rats significantly increased the SOD and CAT activities in blood (Figures 3 and 4). The SOD and CAT are the two major scavenging enzymes that remove toxic-free radicals *in vivo*. A decrease in the activity of these antioxidants can lead to an excess availability of superoxide anion $O_2^{\cdot -}$ and hydrogen peroxide in biological systems, which, in turn, generate hydroxyl radicals, resulting in initiation and propagation of lipid peroxidation. The elevated activities of SOD and CAT suggest that *S. maxima* have antioxidant property, which shows the beneficial effect against pathological complications caused by the presence of free radicals (Aebi 1984). Also, the *S. maxima* therapy elevated the GSH level in fructose-induced diabetic rats. The increased GSH level protects from the toxic effects of reactive oxygen species or peroxidative damages and plays an important role in the removal of peroxides and foreign compounds (Shan and Jones 1990). *S. maxima* is found to significantly increase the GSH level in blood (Figure 5), it may be because of the activation of GSH synthetase that increases the biosynthesis of GSH by *S. maxima* extract administration. Histopathological observations of tissues (liver and kidney) revealed that the *S. maxima* administration had the protective and therapeutic effects against fructose-induced abnormalities in male Wistar rats (Figures 6 and 7). This study shows that the administration of aqueous extract of *S. maxima* decreased TBARS level with concurrent increase in key antioxidants SOD, CAT and

GSH levels in blood. The antioxidant effects of *S. maxima* may be attributed to β -carotene, tocopherol, and phenolic compounds working individually or in synergy (Miranda *et al.* 1998; Hsiao *et al.* 2005; Nagakao *et al.* 2005).

CONCLUSIONS

The present investigation shows that the oral administration of 5 or 10% aqueous extract of *S. maxima* for 30 days restored significantly not only the blood glucose levels but also markers of oxidative stress. The activities of antioxidant enzymes (TBARS, SOD, CAT and GSH) in diabetic rats were reverted back to near-normal level after treatment with 5 or 10% *S. maxima* extract. The histopathological studies also revealed the therapeutic effects of *S. maxima* extract on liver and kidney tissues. The effect of 5% *S. maxima* extract was more pronounced than 10% extract.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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