ORIGINAL ARTICLE

Gastro-protective potentials of *Spirulina*: role of vitamin B$_{12}$

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Abstract Gastric ulcer is a chronic health problem world over. *Spirulina* is known to contain significant amounts of vitamin B$_{12}$ hence *Spirulina* was evaluated for gastroprotective properties against gastric ulcerations. *Spirulina* biomass (SB) and *Spirulina* extract (SE) were evaluated in swim stress induced gastric ulcers in adult male albino rats. Both SB and SE inhibited 45% and 60% of ulcers formation induced by swim stress respectively. Gastroprotection has been elucidated to be due to inhibition of (a) upregulated H$^+$, K$^+$-ATPase activity that induced ulcer condition; (b) lipid peroxidation and (c) altered antioxidant enzyme levels. The data highlighted the importance of vitamin B$_{12}$ in protecting the gut against gastric ulcerations and suggested that both *Spirulina* biomass and *Spirulina* extract contain bioavailable B$_{12}$. *Spirulina* based product/food can be used as alternatives to gastroprotective agents that are known to cause acidity themselves upon long term use.

Keywords *Spirulina* · Vitamin B$_{12}$ · Gastric ulcer · Deficiency of vitamin B$_{12}$

Introduction

The usual dietary sources of vitamin B$_{12}$ are animal-derived foods, although a few plant-based foods contain substantial amounts of vitamin B$_{12}$. Vitamin B$_{12}$ deficiency

thus results in high-risk of vitamin B$_{12}$ associated disorders in vegetarian population.

Deficiency of vitamin B$_{12}$ is one of the emerging global problems (Allen 2010), since this deficiency is affecting infants to elders, and both the genders throughout the lifespan. Vitamin B$_{12}$ deficiency is more common in pregnant and lactating women and young children in developing countries. Elderly persons above the age of 60 are at equally greater risk as performance of gut decreases and vitamin B$_{12}$ deficiency is known to affect the gut epithelium, there by affecting the regular absorption of food (Schjonsby 1989).

Vitamin B$_{12}$ deficiency manifested symptoms in humans includes neurologic injury, disability, demyelinating nervous system disease, dementia, psychiatric illness, anemia, vascular occlusions and suppressed immune system (Durand et al. 2003). The biochemical abnormalities include low serum B$_{12}$, holo-transcobalamin (holo TC), elevated plasma total homocysteine (tHcy), urinary methylmalonic acid.

Microalgae are rich source of several nutrients like vitamins, minerals, essential fatty acids, protein, chlorophyll, carotenoids and a vast spectrum of phytochemicals. Vitamins are found in their natural forms in microalgae (Andrade et al. 2018). Recent reports have emphasized the necessity to identify plant-derived foods that contain significant amount of vitamin B$_{12}$ to prevent its deficiency in vegetarians (Watanabe et al. 2014). Cyanobacterial species are reported to contain a good amount of vitamin B$_{12}$, but the forms of vitamin or their bioavailability is not elucidated. Among green algae, *Chlorella vulgaris* is reported to contain the true/active form of vitamin B$_{12}$ (Watanabe et al. 2014). Dried purple laver is one of the good sources of vitamin B$_{12}$. However, many of the studies have focused on the identification of the vitamin B$_{12}$ in these algae, and

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none of these studies have analyzed the bioactivity aspects, except in the macro alga Nori, the purple laver (Helliwell et al. 2016).

Radiological studies of vitamin B_{12} deficient experimental animals indicated the damages in the gastrointestinal lining (Briani et al. 2013). Indeed, intact gastrointestinal lining by gastric epithelium is one of the robust factors in gut defense and hence gut health (Pastorelli et al. 2013). A cohort study conducted in Japan indicated that most patients with complicated peptic ulcers may have deficiency of one or more water-soluble vitamins including vitamin B_{12} in the early phase of the disease before the onset of ulcer complications (Miyake et al. 2013). It becomes therefore imperative to address the effect of vitamin B_{12} deficiency during gastric ulcer condition and the status of ulcer during the treatment with vitamin B_{12} enriched source, in this case, *Spirulina platensis*.

Current study thus focusses on (1) effect of vitamin B_{12} on ulcer condition in albino Wistar male rats; (2) ulcer status in normal and vitamin B_{12} deficient Wistar rats; (3) modulatory effect if any upon feeding vitamin B_{12} containing *Spirulina* biomass and extract in comparison with standard vitamin B_{12} at same concentrations. The present study provided the impact of vitamin B_{12} effect on gastric ulcerations and the potential anti ulcerative effect of *Spirulina* biomass and *Spirulina* extract.

Materials and methods

Spirulina platensis biomass and extract

Spirulina platensis CFTRI strain was used for the experiment. *Spirulina* was grown in outdoor raceway pond in Zarrouk's medium. The biomass was harvested by gravity filtration after the culture reached stationary phase. The biomass was acid washed to reduce the ash content and later washed twice with distilled water. The harvested biomass was dried by lyophilization and stored at − 80 °C in air tight container until further use. *Spirulina* extract was prepared in the aqueous medium from 100 g biomass in batches for the oral intubation to rats.

Reagents

Corn starch, cane sugar and refined groundnut oil were procured from local market. Vitamins, minerals, cellulose, choline chloride and L-cystine were obtained from Himedia Laboratories (Mumbai, India). Casein was purchased from Nimesh Corporation (Mumbai, India). Clinical enzyme kits namely SGOT, SGPT, were obtained from Agape Diagnostics, Kerala (India).

Extraction and analysis of vitamin B_{12}

HPLC analysis was carried out to estimate the vitamin B_{12} levels in the *Spirulina* biomass (SB) and the extract (SE). Since vitamin B_{12} is water soluble, water extraction method (Kumudha et al. 2010) was adopted wherein *Spirulina* biomass was continuously stirred in water at high temperature followed by centrifugation and collected the supernatant. Further, it was purified with XAD and sep-pak columns. After purification, vitamin B_{12} content of the *Spirulina* was determined by both HPLC and microbiological assay.

Microbiological assay

For microbiological assay *Lactobacillus delbrueckii* MTCC 911 strain procured from Microbial Type Culture Collection, CSIR-IMTECH, Chandigarh, India) was used (Kumudha and Sarada 2015).

HPLC analysis

Purified vitamin B_{12} extract from *Spirulina* sample and serum samples were analysed by C18 column. Serum samples were incubated in water bath for 20 min at 45 °C, centrifuged and supernatant were analyzed by HPLC that was equipped with C-18 Column (4.6 × 300 mm, particle size 10 μm) procured from Waters Corp., USA. The absorbance was measured at 546 nm. The solvent systems used were (A) 50% methanol containing 0.1% (v/v) acetic acid (B) water, for 40 min with a flow rate of 1 ml/min. Vitamin B_{12} was eluted with a linear gradient of methanol (from 0 to 90% of a 50% (v/v) methanol solution).

Assessment of gastric ulcers; protection by *Spirulina* biomass and extract

Healthy albino wistar male rats (120 ± 10 g) were used for the experiments, and the animals were fed with SB and SE for 30 days through guavage. All the experimental animals were maintained under standard conditions of temperature, humidity and light. The study was permitted by the institutional ethical committee, that follows the guidelines of "CPCSEA (Committee for the purpose of control and supervision of experiments on animals, reg. No. 49, 1999), Government of India, New Delhi, India". All the experimental animals were classified into 9 groups of 6 animals each. Group 1: Rats were given AIN-93 diet (Reeves 1997), which is designated as normal diet. Group 2 and 3 serve as *Spirulina* Biomass (SB) and standard vitamin B_{12} controls along with normal diet. Group 4 and 6 are ulcer control groups where group 4 with normal diet; while group 6 with vitamin B_{12} deficient diet. Group 5 received *Spirulina* Extract (SE) along with normal diet, followed by

ulcer induction (UI). Group 7–9 are vitamin B_{12} deficient groups fed with standard vitamin B_{12}, SB and SE respectively. The amount of *Spirulina* biomass and *Spirulina* extract fed to rats was based on vitamin B_{12} equivalent to the amount as defined by "The Nutritional requirements of rats" set by the National Research Council, USA. At the end of the experiment, rats were sacrificed.

Induction of ulcer and determination of ulcer index

Ulcer was induced by forced swim stress as per Srikanta et al. 2007 where the rats were subjected to forced swim stress by taking them in a jar of 30-cm high and 10 cm diameter. The jar contained water up to 15 cm height and animals were allowed to swim for 5 h. Rats were sacrificed under deep ether anesthesia. The inner layer of the stomach was examined for the incidence of ulcers. The stomach was removed and opened along the greater curvature. It was washed with normal saline, stretched and flattened on a piece of cardboard. The inner surface was examined for mucosal integrity and occurrence of the ulcer. The total number of mucosal lesions/ulcers per stomach was counted and expressed as ulcer index (UI) (Srikanta et al. 2007). "Lower to higher grade was assigned to milder to severe symptoms. The following are the ulcer scores: 0.5 = red coloration, 1.0 = spot ulcers, 2.0 = hemorrhagic streaks more than 3 mm and less than 5 mm, 3.0 = ulcers/hemorrhagic streaks more than 5 mm. The total ulcer score calculated for each experimental group and mean ulcer score of each experimental group were expressed as the ulcer index (UI)" (Kamath et al. 2008; Kulkarni and Goel 1996). Stomach and liver tissue homogenate and serum were collected from all experimental animals and analyzed for various biochemical parameters.

Estimation of gastric mucin

Gastric wall content of mucin was estimated by Alcian blue-binding method (Corne 1974). A sample of 100 mg of stomach tissues from animals of each group was taken and incubated for 2 h in acetate buffer (pH 5.8, 0.05 M) containing 0.16 M sucrose and 1.0% Alcian blue dye. The absorbance of the supernatant was read at 498 nm.

Histopathological studies

Gastric tissue samples were fixed in 10% buffered formalin for 24 h. The processed tissues were fixed in paraffin blocks and sections made were stained with hematoxylin and eosin dye (Sibilia et al. 2003). The histochemical sections were analyzed by observing under light microscope at 10× magnification.

Preparation of tissue homogenate for biochemical analysis

The stomach and the liver tissues collected were weighed and homogenized in chilled phosphate buffer 20 mM, pH 7.4. The homogenates were centrifuged at $1000g$ at 4 °C for 20 min using a high-speed cooling centrifuge (Remi C 24, Mumbai, India). The clear supernatants were used for various biochemical parameters.

Estimation of H^+, K^+-ATPase

Known weight of gastric tissue from animals of each group was homogenized in Tris-HCl buffer 16 mM, pH 6.5. The homogenates were centrifuged at $6000g$ at 4 °C for 20 min, and the parietal cell extract thus prepared was used to determine the H^+, K^+-ATPase. The reaction mixture contained Tris-HCl, 2 mM $MgCl_2$, 2 mM KCl. The reaction was initiated with the addition of 2 mM ATP, and the incubation was continued for 30 min at 37 °C. The reaction was terminated by adding ammonium molybdate and trichloroacetic acid mixture followed by centrifugation at $2000g$. The amount of inorganic phosphate released from ATP was estimated spectrophotometrically as described by Yoda and Hokin (1970).

TBARS assay

Lipid peroxidation products of serum, stomach liver homogenates were determined as thiobarbituric acid reactive substance (TBARS) as described by Buege and Aust (1978). Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) to form a diadduct that is measured spectrophotometrically at 532 nm. To 1 ml of the above homogenates, 1 ml of 0.15 M KCl and 0.1 ml of 0.2 mM ferric chloride were added to initiate peroxidation and the solution was incubated for 37 °C for 30 min. 2 ml of ice-cold mixture of TCA–TBA–BHT (15% trichloroacetic acid, 0.3% thiobarbituric acid, 0.05% butylated hyroxytoluene in 0.25 N HCl) was added to terminate the reaction and was heated at 80 °C for 60 min. The reaction mixture was cooled, centrifuged and the absorbance of the supernatant was measured at 532 nm.

Antioxidant enzyme assays

Catalase activity

Catalase activity was determined as described by Aebi (1984). H_2O_2 decomposition on the addition of sample was followed at 240 nm. A unit of catalase was defined as the amount of enzyme required to decompose 1 μmol H_2O_2 per minute at 25 °C at 7.0 pH.

Glutathione peroxidase activity

Glutathione peroxidase activity was estimated as described by Flohe and Gunzler (1984). The mixture containing the sample, 0.1 ml of 10 mM glutathione reductase and 0.1 ml of 10 mM glutathione were preincubated for 10 min at 37 °C and 0.1 ml of NADPH solution was added. The hydroperoxide independent consumption of NADPH was monitored for 3 min. The reaction was started by adding 0.1 ml of the pre warmed hydroperoxide solution and the decrease in absorption at 340 nm was monitored for 3 min.

Superoxide dismutase activity

Superoxide dismutase (SOD) activity was assayed by measuring the reduction in the NBT in the presence of SOD. Activity was expressed as unit per milligram protein per min. (Flohe and Otting 1984).

Results

Analysis of vitamin B_{12} content in *Spirulina* biomass and *Spirulina* extract

Total vitamin B_{12} content in *Spirulina* biomass and *Spirulina* extract was found to be 220 ± 1.8 μg/100 g (Kumudha and Sarada 2015). The true form of vitamin B_{12} in *Spirulina* is found to be methyl cobalamin.

Assessment of gastric ulcers, protection by *Spirulina* biomass and extract

Ulcer score was calculated based on number and intensity of ulcers in each rat in the group as described under materials and methods. Group-7 animals that were induced with ulcers under vitamin B_{12} deficient diet were considered to have 100% ulceration and relative percent ulceration was calculated in other groups. Relative percent protection by SB and SE was calculated taking the group of animals that have received normal diet as well as vitamin B_{12} deficient diet as base. In sample and standard control groups, no ulcers were observed suggesting that samples selected for treatment were not causing ulcers per se (Fig. 1).

Animals treated with *Spirulina* biomass and *Spirulina* extract showed significant reduction (2.5 folds) in ulcer index as opposed to untreated animals. Rats treated with *Spirulina* biomass and *Spirulina* extract under normal diet condition showed ulcer score of 12% and 23% respectively suggesting 88% and 77% gastro protection respectively.

Under B_{12} deficient condition *Spirulina* biomass showed 75% protection as opposed to 68% by standard vitamin B_{12} suggesting increased gastro protective potential by SB in addition to vitamin B_{12}. It is possible that SB may contain some additional compounds that can contribute to enhanced gastroprotection in addition to vitamin B_{12} in SB. It is interesting to observe that animals that were receiving vitamin B_{12} deficient diet showed $\sim$ 10–13% less than those which were receiving normal diet, indicating that vitamin B_{12} may play a crucial role in terms of ulcer/tissue healing (Fig. 1).

Swim stress induces severe lesions including inflammatory patches, bleeding in mucosa and ulcers with different size and degree in ulcerated rats. In the present study also the rats treated with forced swim stress showed damage in the gastric wall with a hemorrhagic form of lesions and intraluminal bleeding (Fig. 1) while no such gastric lesions and bleeding were noted in healthy controls. Results were substantiated by histopathological studies. In addition, in ulcerous rats the gastric mucin levels reduced compared to control. Rats that were treated with SB and SE showed increase in mucin level (Table 1).

Histopathological studies

Deep erosions with discontinuous mucosal layer were observed in ulcer-induced rats. Rats pre-treated with *Spirulina* extract and biomass showed normal histology or only very superficial lesions. Healthy controls showed intact mucosal epithelium. The recovery of the mucosal layer was observed in *Spirulina* extract and biomass treated groups when compared to ulcer-induced stomach (Fig. 1). Results also showed that tissue regeneration and formation of mucosal epithelium was complete with complete layer formation and intact mucosal glandular tissues in sections from animals treated with SB and Standard vitamin B_{12} along with normal diet. However the treatment of the same in mucosal gland and epithelium recovery was not efficient as evidenced in Fig. 2.

H^+, K^+-ATPase, activity

H^+, K^+-ATPase activity was increased in ulcerous animals when compared to healthy controls. *Spirulina* biomass and *Spirulina* extract could normalize the levels in the stress induced models (Table 2). Around threefold increase in H^+K^+-ATPase activity during ulcerations were normalized ($\sim$ 50%) in both normal diet as well as vitamin B_{12} deficient groups of animals supplemented with *Spirulina* biomass and *Spirulina* extract.
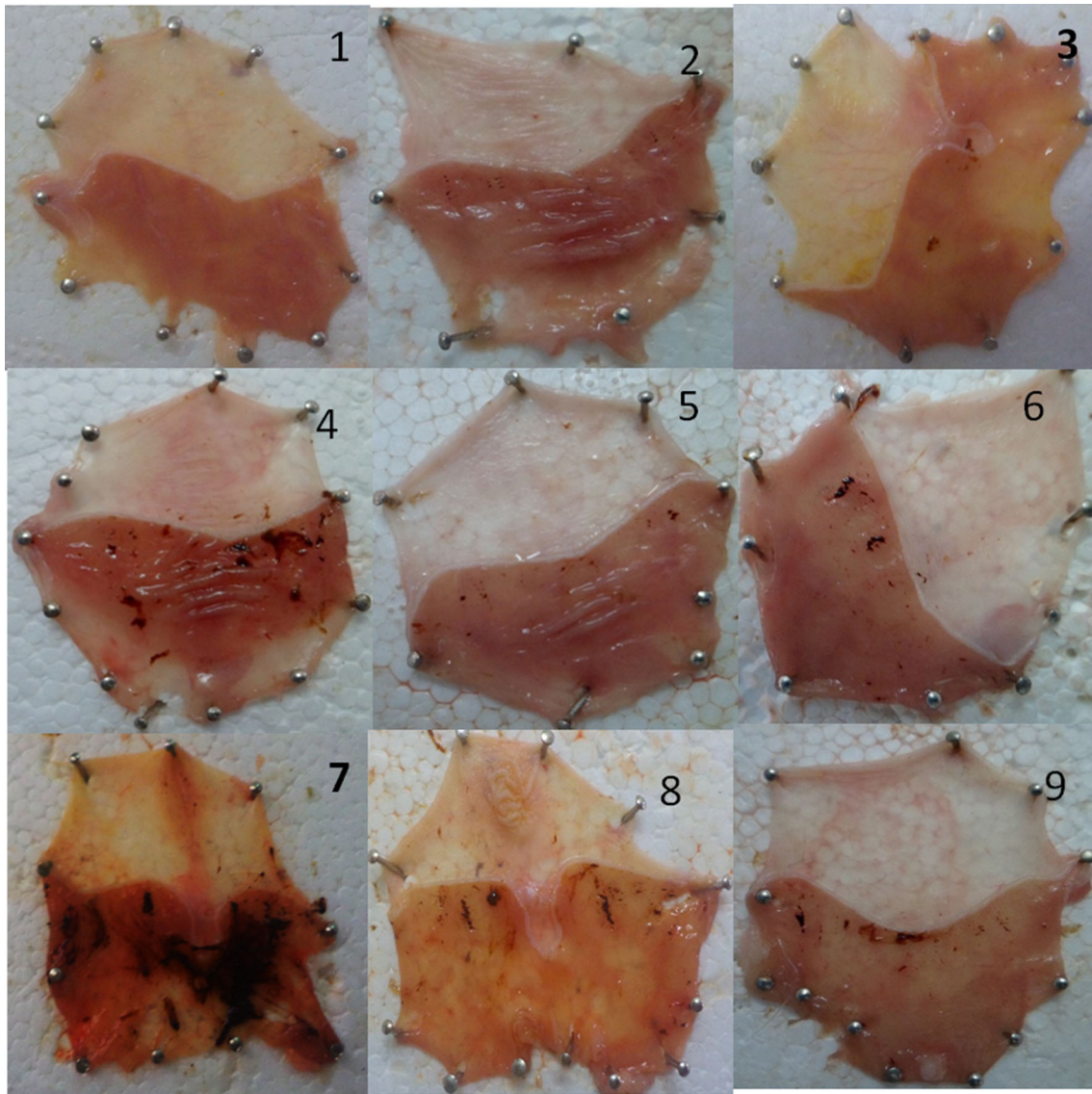
Fig. 1 Macroscopic observation of ulcers in the stomach from ulcer induced and *Spirulina* biomass/*Spirulina* extract treated animals. Group 1: Healthy with normal diet. Group 2 and 3 serves as *Spirulina* biomass (SBM) and standard vitamin B_{12} controls. Group 4 and 6 are ulcer control groups. Group 5 with (SE), followed by ulcer induction. Group 7–9 are vitamin B_{12} deficient groups with standard vitamin B_{12}, SBM and SE

Changes in antioxidant enzymes

The increase in TBARS levels shown in the stomach homogenate in ulcer condition was normalized by *Spirulina* biomass as well as *Spirulina* extract. Decrease in the antioxidant enzymes such as SOD, CAT and glutathione peroxide during ulcer condition were normalized with the treatment of rats with *Spirulina* biomass and *Spirulina* extract as evident from Tables 3 and 4.

Discussion

Deficiency of vitamin B_{12} is observed among people of all ages who consume vegetarian diet. Reason for malabsorption appears to be many. Lack of intrinsic factors in the parietal cells and some genetic factors are known to affect vitamin B_{12} absorption the most and appear to be the important cause for vitamin B_{12} malabsorption (Pocock et al. 2013). In this context it is important to understand the absorption levels of vitamin B_{12} during gastric ulceration condition, where parietal cellular damage and hence loss of regulation of H^+K^+-ATPase that controls acidity in the lumen of the stomach has been observed.

Table 1 Gastric mucin levels in healthy, ulcerated and *Spirulina* biomass and extract treated rats

S. no.	Group name	mg/g tissue
1	Normal diet	58.09 ± 7.2
2	Normal diet + *Spirulina* biomass	57.75 ± 4.2
3	Normal diet + Std Vit B_{12}	59.68 ± 3.8
4	Ul + normal diet	18.95 ± 2.5
5	Ul + *Spirulina* extract	43.33 ± 5.2
6	Ul + B_{12} deficient	18.73 ± 3.8
7	UI + B_{12} deficient +Std B_{12}	39.44 ± 2.9
8	UI + B_{12} deficient + *Spirulina* extract	38.48 ± 4.3
9	UI + B_{12} deficient + *Spirulina* biomass	41.68 ± 3.7

UI ulcer induced, *Std B_{12}* standard B_{12}

Vitamin B_{12} is required as a coenzyme for the metabolism of the amino acids methionine, threonine and valine and for the transformation of methyl-tetrahydrofolate to tetrahydrofolate, which is necessary for DNA synthesis (Al Aisari et al. 2010). During ulcer conditions, there is a lot of erosion of gastric mucosal cells and this has to be regenerated in order to complete the repair process during ulcer healing process. DNA synthesis obviously is essential for ulcer healing process. In the current study therefore, we evaluated the susceptibility to gastric ulcerations under normal and vitamin B_{12} deficient and the extent of ulcer prevention.

Our results showed that under B_{12} deficient condition, the gastroprotective potential of *Spirulina* biomass as well as standard vitamin B_{12} is reduced and this could be attributed to the delayed ulcer healing processes. Results were also evident in histopathological analysis, where *Spirulina* biomass (SB) and standard vitamin B_{12}, could regenerate gastric mucosal cells better with the normal diet than with vitamin B_{12} deficient diet. This can be attributed to the role of vitamin B_{12} in cellular proliferation. Proliferation is an important phenomenon for regeneration. Supplementation of vitamin B_{12} thus can augment the process of mucosal proliferation as evidenced by histopathological analysis and production of gastric mucin that can envelope the gastric epithelium for effective defense.

Antiulcer agent together with vitamin B_{12} may offer speedy recovery of gastric ulcerations, which is evident, when we observed better ulcer protective effect by *Spirulina* biomass (SB) than vitamin B_{12} per se. Better effect
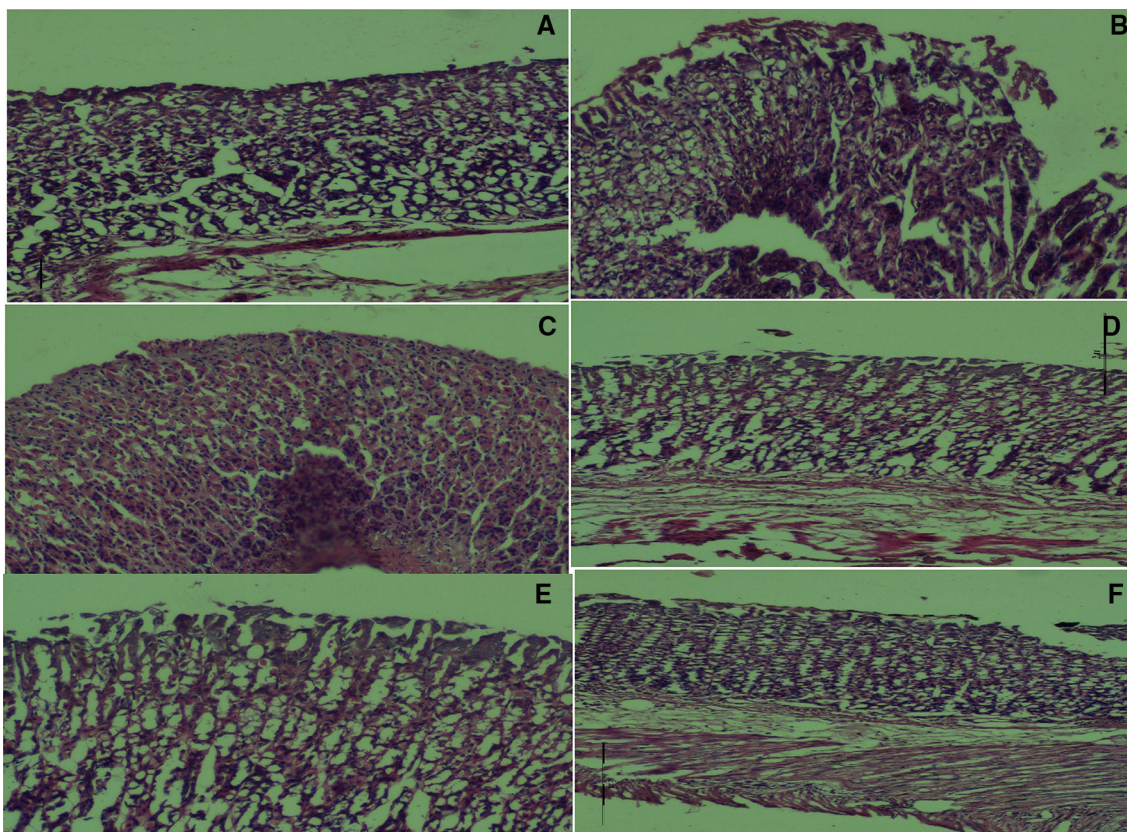

Fig. 2 Histopathological observation of stomach from ulcer induced and *Spirulina* biomass *Spirulina* extract treated animals. **a** Control, **b** ulcerated showing damaged mucosal epithelium, **c**, **d** and **e**, **f** showed recovery in mucosal epithelium treated with *Spirulina* biomass and *Spirulina* extract respectively

Table 2 H^+, K^+-ATPase levels in healthy, ulcerated and treated rats

S. no.	Group name	μ moles pi release/mg protein/h
1	Normal diet	1.39 ± 0.12
2	Normal diet + *Spirulina* biomass	1.22 ± 0.07
3	Normal diet + Std Vit B_{12}	1.27 ± 0.12
4	Ul + normal diet	3.65 ± 0.28
5	Ul + *Spirulina* extract	1.83 ± 0.52
6	Ul + B_{12} deficient	3.87 ± 0.38
7	UI + B_{12} deficient +Std B_{12}	1.92 ± 0.17
8	UI + B_{12} deficient + *Spirulina* extract	2.18 ± 0.11
9	UI + B_{12} deficient + *Spirulina* biomass	1.93 ± 0.18

UI ulcer induced, *Std B_{12}* standard B_{12}

Table 3 SOD activity and TBARS level in serum of healthy, ulcerous and treated rats

S. no.	Group name	SOD U/mg (protein)	TBARS μmol/mg protein
1	Normal diet	16.6 ± 1.2	3.77 ± 0.3
2	Normal diet + *Spirulina* biomass	16.65 ± 0.8	4.16 ± 0.3
3	Normal diet + Std Vit B_{12}	17.32 ± 2.5	3.59 ± 0.6
4	Ul + normal diet	8.2 ± 0.5	$8.62 \pm 0.0.6$
5	Ul + *Spirulina* extract	13.46 ± 1.7	5.17 ± 0.5
6	Ul + B_{12} deficient	7.66 ± 1.4	9.39 ± 0.8
7	UI + B_{12} deficient + Std B_{12}	14.75 ± 0.8	5.55 ± 0.7
8	UI + B_{12} deficient + *Spirulina* extract	13.85 ± 1.6	4.81 ± 0.3
9	UI + B_{12} deficient + *Spirulina* biomass	13.65 ± 0.9	5.14 ± 0.8

UI ulcer induced, *Std B_{12}* standard B_{12}

Table 4 Catalase and Glutathione peroxidase levels in healthy, ulcerous and treated rats

S. no.	Group name	Catalase μmol/min/mg/protein	Glutathione peroxidase μmol/min/mg protein
1	Normal diet	35.9 ± 6	1.17 ± 0.08
2	Normal diet + *Spirulina* biomass	32.3 ± 4.5	1.07 ± 0.6
3	Normal diet + Std Vit B_{12}	36.7 ± 5	1.02 ± 0.06
4	Ul + Normal diet	15.9 ± 2.1	3.18 ± 0.1
5	Ul + *Spirulina* extract	28.8 ± 5.1	1.37 ± 0.06
6	Ul + B_{12} deficient	14.1 ± 3.5	3.28 ± 0.09
7	UI + B_{12} deficient + Std B_{12}	29.1 ± 9.6	1.38 ± 0.06
8	UI + B_{12} deficient + *Spirulina* extract	26.4 ± 4.1	1.48 ± 0.08
9	UI + B_{12} deficient + *Spirulina* biomass	24 ± 3.5	1.57 ± 0.1

UI ulcer induced, *Std B_{12}* standard B_{12}

with SB could be due to the presence of additional probable gastroprotective compounds in them.

Upregulation of H^+K^+-ATPase, mucosal damage, decreased antioxidant status and increased oxidative stress conditions are common events observed during gastric ulcerations (Suzuki et al. 2012). Generally all ulcer inducing conditions such as stress, ethanol intake or drug like indomethacin have been known to encompass these disease pathogenic steps. Vitamin B_{12} has direct influence on all these since its absorption is dependent on the intrinsic factor which is present in the parietal cell, perturbation of this cellular integrity has been known to affect H^+K^+-ATpase, which releases acid, that acts directly on the gastric mucosal epithelium in addition to imbalance in oxidant and antioxidant condition. Various studies have also suggested that vitamin B_{12} deficiency correlates with lowering of antioxidant status (Herrmann et al. 2001). Our studies thus were directed to evaluate oxidant and

antioxidant status in gastric ulceration condition in group of animals that had normal diet as well as the group that had vitamin B_{12} deficient diet. There is increase in oxidative stress condition in vitamin B_{12} deficient group than those getting normal diet. Since vitamin B_{12} fed group also recovered all antioxidants to similar extent, data may suggest that vitamin B_{12} alone possesses the capability to regulate antioxidant status of cells or tissue. Results were supported by the effective balancing of antioxidant enzymes by vitamin B_{12} supplementation.

Overall data suggests that although, there is effective normalization of antioxidant enzymes, after vitamin B_{12} treatment, gastro protection under deficient condition is slightly lesser than the group of animals, which were getting normal diet. Marginal reduction in gastro protective efficiency could be attributed to reduced mucosal recovery as evidenced also by histopathological analysis (Fig. 2). Reduced mucosal recovery could be assigned to reduced proliferation of damaged mucosal cells, which is due to vitamin B_{12} deficiency. Even upon feeding of vitamin B_{12}, comparatively slow recovery in the vitamin B_{12} deficient group suggests that the proliferative programme is affected with vitamin B_{12} deficiency. Current data has direct relevance on its role in increasing susceptibility to gastric ulcerations in experimental animals besides its delayed recovery effect.

Conclusion

The stressful lifestyles, inadequate intake of nutritious food/nutraceuticals, continuous use of non-steroidal anti-inflammatory drugs by the global population are some of the reasons for the increased incidence of ulcers worldwide (Langman et al. 1991; Miller 1987). More than 30 million people in the world are known to take non-steroidal anti-inflammatory drugs. Drugs that are commercially available for treatment, when used for long term are known to cause unpredictable side effects and hence this warranted a safer alternative source for ulcer management. The study demonstrated for the first time that orally administered *Spirulina* biomass and *Spirulina* extract containing vitamin B_{12} exerts gastro protective effect on swim-stress induced gastric lesions in rats.

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