

## Hepatorenal protective action of *Spirulina platensis* against beryllium induced hepatorenal dysfunction and histopathological alterations in rats

Suchita Raghuvanshi, Narottam Das Agrawal<sup>#\*</sup>, Purneema Rawat, Sadhana Srivastava & Sangeeta Shukla

<sup>1</sup>UNESCO Trace Element and Satellite Centre, Reproductive Biology and Toxicology Laboratory, School of Studies in Zoology, Jiwaji University, Gwalior, Madhya Pradesh-474 011, India

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Beryllium (Be) is highly toxic to human, induces oxidative stress and leads to Chronic Beryllium Disease (CBD) or berylliosis, apoptosis and cancer. In this study, we have investigated the antioxidative and hepatorenal protective potential of *Spirulina platensis* (SP) against beryllium (Be) induced alterations in hepatorenal biochemical parameters, oxidative stress and histopathological alterations in rats. Different doses of SP (50, 100, 200 and 400 mg/kg, p.o.) were administered (once a day daily for 7 days) against Be(NO<sub>3</sub>)<sub>2</sub> (1 mg/kg, i.p. once a day daily, for 28 days) induced toxic manifestations in female Wistar rats. Be decreased GSH, SOD, CAT, G-6-Pase, ALP and ATPase activities and increased TBARS and ACP activity in liver and kidney. Be enhanced ALT, AST, bilirubin, uric acid, creatinine, total cholesterol and decreased haemoglobin, total protein, sugar in blood. Be deposited in vital organs of rats and altered the histoarchitecture of liver and kidney. Different doses of SP showed dose dependent recovery. SP @400 mg/kg showed significant recovery against Be induced oxidative stress, maintained hepatorenal function towards normal and reduced beryllium body burden. Results of the study, well supported by histopathological observations, conclude that SP possesses hepatorenal protective potential against Be induced toxicity in rats.

**Keywords:** Berylliosis, Beryllium body burden, Chronic Beryllium Disease (CBD), Kidney, Liver, Oxidative stress

Beryllium (Be) is a light weight metal, possess unique chemical and physical properties (high melting and boiling points, corrosion resistance, metal hardening capacity, high elasticity, etc.). Due to many desirable properties, beryllium is continuously used in various industries and have wide range of applications with increasing demands, such as in medical diagnostics, nuclear/fusion reactors, golf clubs, aerospace applications, ceramics, electronics and nuclear defense and dental prosthesis industries<sup>1,2</sup>. However, Be exhibits wide range of toxic effects on all life forms including humans, and is classified as group I carcinogen<sup>3-5</sup>. Toxic effects of Be envisage immunotoxic<sup>3</sup>, allergic<sup>6</sup>, mutagenic<sup>7</sup> and carcinogenic<sup>8</sup> action. While the primary

health concern related to Be is the lung disease (chronic beryllium disease or berylliosis), other health issues are hepato-, nephro-, neuro- and testicular toxicities<sup>5,9-11</sup>.

Majority of studies on Be toxicity focus on occupational pathologies<sup>5,12</sup> caused by exposure to Be dust. However, Be pollution may affect wide population groups through other exposure routes<sup>5</sup>. The use of Be-containing rocket fuels results in rapid and dense emissions of high Be amounts to the environment. Thus, people residing in proximity to or working at the launching (and rocket trace) sites may be exposed to high amounts of Be through transdermal, respiratory, and gastrointestinal routes. Thus, the pollution affects a higher number of people than those, who are exposed to occupational factors<sup>5</sup>. Be is found accumulated in soils, close to electronic waste disposal grounds<sup>13</sup>, and therefore, also to increased environmental exposure, through drinking water and diet<sup>5</sup>. General human population may also expose to Be via air, water, diet as well as smoking on daily basis<sup>14</sup>. Workers are exposed to beryllium containing dusts during crushing and grinding of ores, and to soluble beryllium salts during extraction and processing of beryllium and through welding fumes<sup>2</sup>.

\*Correspondence:

Phone: +91 9719464802 (Mob.)

E-mail: [agrawalnarottam@gmail.com](mailto:agrawalnarottam@gmail.com)

<sup>#</sup>Present add.: Department of Biochemistry, Rajkiya Medical College, Jalaun (Orai), Uttar Pradesh-285 001, India

**Abbreviations:** ACP, Acid phosphatase; ALP, Alkaline phosphatase; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ATPase, Adenosine triphosphatase; Be(NO<sub>3</sub>)<sub>2</sub>, Beryllium nitrate; CAT, Catalase; G-6-Pase, Glucose-6-phosphatase; SOD, Superoxide- dismutase; SP, *Spirulina platensis*; TBARS; Thiobarbituric Acid Reactive Substance.

Be exposure induced oxidative stress and lung inflammation, play an important role in development of beryllium induced chronic inflammatory disorders (CBD) and apoptosis<sup>15</sup>. Accumulated data reported that antioxidants inhibited beryllium antigen stimulated CD4+T-cell proliferation in peripheral blood mononuclear cells from CBD patients, suggesting a potential therapeutic utility of antioxidant molecules in this and similar granulomatous lung diseases<sup>8</sup>. Exact mechanism of Be toxicity still remains to be elucidated. Be altered major metabolic pathways of iron, protein, lipid and carbohydrate, leads to membrane damage of vital organs and altered the histoarchitecture of liver and kidney<sup>10,11</sup>. Be deposited in the vital organs and altered the physiological efficiency<sup>11,16</sup>. Be exposed animals showed behavioural changes, including increased motor activity, aggressive reactions and complete spasticity<sup>5</sup>. Thus, inhibition of beryllium induced oxidative stress may be an approach in prevention of Be induced hepatorenal diseases. A number of various synthetic compounds and some natural products have been tried earlier against Be toxicity but they achieve limited success<sup>9-11,17,18</sup>. Thus, there is urgent need for alternative treatment to ameliorate Be toxicity that may also be applicable to many other toxicants and many species. Use of natural products of plant origin, having antioxidant potential may be an alternative in treatment of beryllium toxicity. In this regard, an interest in using *Spirulina platensis* (SP), a group of blue-green algae, in diet has gained attention in recent years.

SP is a filamentous cyanobacterium microalgae with potent dietary phyto-antioxidant, widely used as health food in many countries including India. *Spirulina*'s nutritional qualities are truly "one-of-a-kind" and promoted as 'the food of the future' with exceptional constituents that contribute to high energy levels. SP is rich source of vitamins, minerals, protein, carotenoids, phycocyanins, essential fatty acids, and other bioactive molecules such as phenolic acids, tocopherols, and  $\gamma$ -linolenic acid<sup>19</sup>. SP has been broadly studied in the field of medicine and food industry due to its high nutritive value, lack of toxicity, and therapeutic effects. Accumulating evidences have shown that SP exerts health promoting functions, including antioxidant, anti-inflammatory, hepatoprotective, anticancer, antimicrobial, strengthening immune system, metalloprotective activities, as well as positive effects against hyperlipidemia, obesity and diabetes<sup>19-23</sup>. But it's antitoxic and hepatorenal protective potential against beryllium induced hepatorenal toxicity,

is still unclear. Thus, *Spirulina platensis* (SP) was investigated against beryllium induced oxidative stress, hepatorenal dysfunction, beryllium body burden and histopathological alterations in rats.

## Materials and Methods

### Maintenance of animals and their feeding

Female Albino rats (*Wistar* strain) (8-10 weeks old having  $150 \pm 10$  g body weight) were randomly selected from departmental animal facility. Animals were housed under standard conditions ( $25 \pm 2^\circ\text{C}$  temp, 60-70% relative humidity, 12 h photo period). Animals were fed on commercially available standard animal diet (Pranav Agro Industries Ltd., New Delhi, India) and drinking water *ad libitum*. Animals used in this study were treated and cared for in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Chennai, India and experimental protocols were approved by Institutional Ethics Committee (CPCSEA/501/01/A) of Jiwaji University, Gwalior (MP), India.

### Chemicals and plant material

Pure form of SP powder was procured from NB Laboratories Pvt. Ltd., Nagpur (Maharashtra), India and stored in a desiccator to avoid oxidation and thermal decomposition. Beryllium nitrate was purchased from Sigma Aldrich Co. USA. All other chemicals used in this study, were of pure and analytical grade. Kits used for serum biochemical estimation were purchased from E-Merck (Germany).

### Experiment design and dose preparation

Beryllium nitrate (35% w/v) was diluted in triple distilled water making up doses of 1 mg/2 mL/kg and administered intraperitoneally<sup>10</sup>. SP powder was dissolved in triple distil water and administered orally with the help of an intragastric rubber catheter. Forty two adult female rats were divided into seven groups of six animals in each group as follows: Group I, negative control (Normal); Group II, *Spirulina platensis* (SP) *per se* (400 mg/kg, p.o.) once a day, daily for last 7 days; Group III, positive control; received  $\text{Be}(\text{NO}_3)_2$  (1 mg/kg, i.p.) once a day, daily for 28 days; Groups IV-VII,  $\text{Be}(\text{NO}_3)_2$  (as in group III) + SP 50, 100, 200 and 400 mg/kg p.o., once a day, daily for 7 days, respectively.

### Processing samples for biochemical analysis

Twenty-four hr after the last treatment, blood was collected by puncturing the retro-orbital venous sinus (retro-orbital plexus) under mild anesthesia<sup>24</sup>. Blood

was allowed to clot for 30 minutes at room temperature (25°C), and then the clot was gently detached from the wall of the test tubes with the help of a thin sterilized needle. The test tubes were centrifuged for 20 minutes at 3000 rpm to harvest serum, which was stored at -20°C until it was analyzed. For other assays, the tissues were homogenized with a Remi motor homogenizer (RQ-122), using a glass tube and Teflon pestle, in different media according to the estimation. Tissue homogenates (10% w/v; 0.15 M KCl) were prepared for lipid peroxidation. Homogenates (5% w/v) were prepared in 0.25 M sucrose solution for the estimation of reduced GSH and in hypotonic solution (0.008% NaHCO<sub>3</sub>) for enzymatic assay.

#### Blood biochemical analysis

Serum was used to determine aspartate aminotransferase (AST) and alanine aminotransferase (ALT)<sup>25</sup>, serum protein<sup>26</sup> and blood sugar, bilirubin, uric acid, creatinine and total cholesterol were assessed by kit methods as per instructions provided by the company (E-Merck, Germany). Hemoglobin was estimated in the blood using Sahli's apparatus<sup>27</sup>.

#### Assessment of tissue biochemical assay

Fresh tissues of liver and kidney were immediately processed to determine lipid peroxidation (LPO)<sup>28</sup>, reduced glutathione (GSH)<sup>29</sup>, superoxide dismutase (SOD)<sup>30</sup>, catalase (CAT)<sup>31</sup>, alkaline phosphatase (ALP) and acid phosphatase (ACPase)<sup>32</sup>, adenosine triphosphatase (ATPase)<sup>33</sup>, and tissue protein<sup>26</sup>. Activity of glucose-6-phosphatase (G-6-Pase) was determined in liver<sup>34</sup>.

#### Determination of beryllium body burden

Beryllium contents in blood, liver, kidney and spleen were determined with atomic absorption spectrophotometer (Thermo-Japan) at wavelength 235 nm according to the Bokowski method<sup>35</sup>.

#### Histological observations

For histopathological study, liver and kidney tissues were fixed immediately in Bouin's fixative and paraffin sections of 5 µm thickness were cut. Hematoxylin-eosin (HE) stained slides were observed under light microscope.

#### Statistical analysis

Results are presented as mean ± S.E. of six animals used in each group. Data were subjected to statistical analysis through one-way analysis of variance (ANOVA) taking significance at 5% level of probability followed by Student's t-test taking significance at  $P \leq 0.05$ ,  $P \leq 0.01$  and  $P \leq 0.001$ <sup>36</sup>. Percent protection was calculated by the following formula.

$$\% \text{ Protection} = 1 - (D - N / T - N) \times 100 \quad [D = \text{Drug}, N = \text{Normal}, T = \text{Toxicant}]$$

## Results

#### Blood3 biochemistry

Table-1 represents the toxic manifestations of Be and its response to SP administration in various blood biochemical indices that include liver and kidney function tests. Activities of AST and ALT significantly elevated in Be treated rats as compared to normal control rats ( $P \leq 0.001$ ). The level of bilirubin, uric acid, creatinine and total cholesterol were significantly ( $P \leq 0.001$ ) increased in Be treated

Table 1— Efficacy of *Spirulina platensis* against beryllium induced alteration(s) in blood biochemistry

Treatment	AST (IU/L)	ALT (IU/L)	T.Cholesterol (mg/dL)	T.Bilirubin (mg/dL)	Serum protein (mg/100 mL)	Haemoglobin (g/dL)	Glucose (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)
Control	65.8±3.6	47.2±2.6	39.1±2.16	0.33±0.01	32.8±1.18	14.5±0.80	107.2±5.9	1.18±0.065	0.32±0.01
SP <i>per se</i>	66.0±3.6	47.5±2.6	39.4±2.17	0.34±0.01	32.0±1.76	14.3±0.79	106.9±5.9	1.19±0.065	0.33±0.01
Be <i>per se</i>	152.1±8.4 <sup>C</sup>	126.0±6.9 <sup>C</sup>	80.4±4.44 <sup>C</sup>	0.76±0.04 <sup>C</sup>	24.0±1.32 <sup>B</sup>	11.3±0.62 <sup>B</sup>	69.3±3.83 <sup>C</sup>	2.86±0.15 <sup>C</sup>	0.78±0.04 <sup>C</sup>
Be+SP(50 mg/kg)	143.3±7.9	115.0±6.3	72.2±3.99	0.68±0.03	25.6±1.41	11.7±0.64	77.2±4.26	2.63±0.14	0.69±0.03
% Protection	10.1%	13.9%	19.8%	18.6%	18.8%	12.5%	20.8%	13.6%	19.5%
Be+SP(100 mg/kg)	128.3±7.0 <sup>a</sup>	97.2±5.3 <sup>c</sup>	65.3±3.60 <sup>b</sup>	0.61±0.03 <sup>b</sup>	26.8±1.48	12.2±0.67	82.8±4.57	2.28±0.12 <sup>b</sup>	0.58±0.03 <sup>c</sup>
% Protection	27.5%	36.5%	36.5%	34.8%	31.8%	28.1%	35.6%	34.5%	43.4%
Be+SP(200 mg/kg)	103.2±5.7 <sup>c</sup>	80.6±4.4 <sup>c</sup>	55.6±3.07 <sup>c</sup>	0.52±0.02 <sup>c</sup>	28.8±1.59 <sup>a</sup>	13.4±0.74	86.1±4.75 <sup>a</sup>	1.90±0.10 <sup>c</sup>	0.52±0.02 <sup>c</sup>
% Protection	56.6%	57.6%	60.0%	55.8%	54.5%	65.6%	44.3%	57.1%	56.5%
Be+SP(400 mg/kg)	95.80±5.2 <sup>c</sup>	68.3±3.7 <sup>c</sup>	48.3±2.67 <sup>c</sup>	0.43±0.02 <sup>c</sup>	30.5±1.68 <sup>a</sup>	13.9±0.76 <sup>a</sup>	90.2±4.98 <sup>b</sup>	1.63±0.09 <sup>c</sup>	0.42±0.02 <sup>c</sup>
% Protection	65.2%	73.2%	77.7%	76.7%	73.8%	81.2%	55.1%	73.2%	78.2%
ANOVA (F-value)	37.8 <sup>@</sup>	49.3 <sup>@</sup>	29.1 <sup>@</sup>	36.7 <sup>@</sup>	5.22 <sup>@</sup>	3.85 <sup>@</sup>	10.0 <sup>@</sup>	41.7 <sup>@</sup>	41.0 <sup>@</sup>

[Values are mean ± SE from six rats in each group]. P value Be vs. control at <sup>A</sup> ≤0.05; <sup>B</sup> ≤0.01; <sup>C</sup> ≤0.001; P value treatment vs. Be at <sup>a</sup> ≤0.05; <sup>b</sup> ≤0.01; <sup>c</sup> ≤0.001 for student's t test; <sup>@</sup>significant (analysis of variance) F= P ≤0.05. AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; Be, Beryllium; SP, *Spirulina platensis* crude; T.Bilirubin, Total Bilirubin; and T.Cholesterol, Total Cholesterol]

rats, whereas serum protein, haemoglobin ( $P \leq 0.01$ ) and blood sugar were significantly decreased ( $P \leq 0.001$ ) as compared to normal control rats. Post treatment of SP showed dose dependent recovery in aforesaid blood biochemical variables, and more significant recoupment was observed by SP 400 mg/kg as compared with Be treated rats, which was confirmed by one way statistical analysis using ANOVA. The extent of recovery was calculated as percent protection. SP 400 mg/kg showed protection in AST (65.2%), ALT (73.2%), bilirubin (76.7%), uric acid (73.2%), creatinine (78.2%), total cholesterol (77.7%), haemoglobin (81.2%), serum protein (73.8%) and blood sugar (55.1%) respectively. SP *per se* treatment at doses of 400 mg/kg did not induce any significant alterations in serum and blood biochemical variables as compared with normal control rats.

#### Oxidative stress markers

Table-2 represents Be induced oxidative stress and its response to SP administration. Administration of Be induced oxidative stress, resulted in elevation of LPO ( $P \leq 0.001$ ), reduction in GSH ( $P \leq 0.001$ ,  $P \leq 0.01$ ) and higher activities of SOD ( $P \leq 0.001$ ) and CAT significantly ( $P \leq 0.001$ ,  $P \leq 0.01$ ) in liver and kidney as compared to normal control rats. Post treatment of SP showed dose dependent recovery in altered oxidative stress markers in liver and kidney. SP at doses of 400 mg/kg showed better results in alteration of oxidative stress markers more towards normal. The extent of recovery by SP 400 mg/kg was calculated by percent protection as seen in LPO liver

(73.1%) and kidney (80.6%), GSH in liver (77.7%) and kidney (65.0%), SOD liver (70.6%) and kidney (78.6%), CAT liver (77.6%) and kidney (66.8%) respectively. SP *per se* treatment at doses of 400 mg/kg did not induce any significant alteration on oxidative stress markers as compared with normal control rats.

#### Tissue biochemistry

Tables 2-3 represents Be induced alterations in tissue biochemical variables and its response to SP administration. Administration of Be significantly decreased the total protein content ( $P \leq 0.01$ ), the activities of ALP ( $P \leq 0.001$ ), ATPase ( $P \leq 0.001$ ) in liver and kidney, G-6-Pase ( $P \leq 0.001$ ) in liver and significantly increased the activity of acid phosphatase ( $P \leq 0.01$ ,  $P \leq 0.05$ ) in liver and kidney. Post treatment of SP against Be intoxication showed recovery in dose dependent manner in aforesaid altered tissue biochemical variables, thus more significant recoupment was observed by SP 400 mg/kg. The extent of recovery by SP 400 mg/kg was calculated by percent protection as in ALP liver (77.5%) and kidney (61.5%), ATPase liver (62.9%) and kidney (65.5%), G-6-Pase liver (76.0%), ACP liver (69.2%) and kidney (77.2%), and protein in liver (60.6%) and kidney (68.4%), respectively. SP *per se* treatment at doses of 400 mg/kg did not induce any adverse effect on tissue biochemical variables as compared with normal control rats.

#### Determination of beryllium body burden

Fig.1, A-D represents the effect of SP on Be mobilization from different tissues. Be concentration

Table 2 — Efficacy of *Spirulina platensis* on oxidative stress markers and G-6-Pase activity

Treatment	LPO (n moles of TBARS/mg protein)		GSH ( $\mu$ mole/g)		CAT ( $\mu$ mole H <sub>2</sub> O <sub>2</sub> /min/mg protein)		SOD (U/mg protein)		G-6-Pase ( $\mu$ moles pi/min/g tissue)
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver
Control	0.24 $\pm$ 0.01	0.47 $\pm$ 0.02	7.8 $\pm$ 0.43	6.9 $\pm$ 0.38	68.2 $\pm$ 3.7	65.6 $\pm$ 3.6	64.3 $\pm$ 3.5	60.3 $\pm$ 3.3	7.2 $\pm$ 0.39
SP <i>per se</i>	0.25 $\pm$ 0.01	0.49 $\pm$ 0.02	7.7 $\pm$ 0.42	6.8 $\pm$ 0.37	68.0 $\pm$ 3.7	65.4 $\pm$ 3.6	63.9 $\pm$ 3.5	60.0 $\pm$ 3.3	7.0 $\pm$ 0.38
Be <i>per se</i>	0.91 $\pm$ 0.05 <sup>C</sup>	1.35 $\pm$ 0.07 <sup>C</sup>	5.1 $\pm$ 0.28 <sup>C</sup>	4.9 $\pm$ 0.27 <sup>B</sup>	44.5 $\pm$ 2.4 <sup>C</sup>	46.3 $\pm$ 2.5 <sup>B</sup>	39.1 $\pm$ 2.1 <sup>C</sup>	38.8 $\pm$ 2.1 <sup>C</sup>	4.28 $\pm$ 0.23 <sup>C</sup>
Be+SP (50 mg/kg)	0.73 $\pm$ 0.04 <sup>b</sup>	1.12 $\pm$ 0.06 <sup>b</sup>	5.8 $\pm$ 0.32	5.3 $\pm$ 0.29	49.9 $\pm$ 2.7	52.6 $\pm$ 2.9	43.9 $\pm$ 2.4	44.6 $\pm$ 2.4	4.76 $\pm$ 0.26
% Protection	26.8%	26.1%	25.9%	20.0%	22.7%	32.6%	19.0%	26.9%	16.4%
Be+SP (100 mg/kg)	0.62 $\pm$ 0.03 <sup>c</sup>	0.93 $\pm$ 0.05 <sup>c</sup>	6.3 $\pm$ 0.34	5.9 $\pm$ 0.32	53.8 $\pm$ 2.9	55.8 $\pm$ 3.0	47.6 $\pm$ 2.6	47.5 $\pm$ 2.6	5.1 $\pm$ 0.28
% Protection	43.2%	47.7%	44.4%	50.0%	39.2%	49.2%	33.7%	40.4%	28%
Be+SP(200mg/kg)	0.51 $\pm$ 0.02 <sup>c</sup>	0.78 $\pm$ 0.0 <sup>c</sup>	6.8 $\pm$ 0.37 <sup>b</sup>	6.0 $\pm$ 0.33 <sup>a</sup>	58.7 $\pm$ 3.2 <sup>b</sup>	57.6 $\pm$ 3.1 <sup>b</sup>	52.7 $\pm$ 2.9 <sup>b</sup>	52.0 $\pm$ 2.8 <sup>b</sup>	5.9 $\pm$ 0.32 <sup>b</sup>
% Protection	59.7%	64.7%	62.9%	55.0%	59.9%	58.5%	53.9%	61.3%	55.4%
Be+SP (400 mg/kg)	0.42 $\pm$ 0.02 <sup>c</sup>	0.64 $\pm$ 0.0 <sup>c</sup>	7.2 $\pm$ 0.39 <sup>b</sup>	6.2 $\pm$ 0.34 <sup>a</sup>	62.9 $\pm$ 3.4 <sup>b</sup>	59.2 $\pm$ 3.2 <sup>b</sup>	56.9 $\pm$ 3.1 <sup>b</sup>	55.7 $\pm$ 3.0 <sup>b</sup>	6.5 $\pm$ 0.35 <sup>c</sup>
% Protection	73.1%	80.6%	77.7%	65.0%	77.6%	66.8%	70.6%	78.6%	76.0%
ANOVA (F-value)	73.1 <sup>@</sup>	54.7 <sup>@</sup>	8.64 <sup>@</sup>	5.74 <sup>@</sup>	9.41 <sup>@</sup>	5.53 <sup>@</sup>	12.9 <sup>@</sup>	9.55 <sup>@</sup>	14.5 <sup>@</sup>

[Values are mean  $\pm$  SE from six rats in each group]. P value Be vs. control at <sup>A</sup>  $\leq 0.05$ ; <sup>B</sup>  $\leq 0.01$ ; <sup>C</sup>  $\leq 0.001$ ; P value treatment vs. Be at <sup>a</sup>  $\leq 0.05$ ; <sup>b</sup>  $\leq 0.01$ ; <sup>c</sup>  $\leq 0.001$  for student's t test; <sup>@</sup> significant (analysis of variance)  $F = P \leq 0.05$ . Be, Beryllium; CAT, Catalase; G-6-Pase, Glucose-6-phosphatase; GSH, Reduced Glutathione; LPO, Lipid peroxidation; SOD, Superoxide dismutase; SP, *Spirulina platensis* crude]

Table 3 — Efficacy of *Spirulina platensis* against beryllium induced alteration(s) in enzymatic activities and total protein

Treatment	ALP (mg Pi/100 g/h)		ACP (mg Pi/100 g/h)		ATPase (mg Pi/100 g/min)		Total Protein (mg/100 mg tissue)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
Control	70.2±3.8	2451±135	185±10.2	196±10.8	2340±129	2325±128	15.2±0.84	15.4±0.85
SP <i>per se</i>	69.9±3.8	2446±135	189±10.4	200±11.0	2330±128	2312±127	15.0±0.82	15.2±0.84
Be <i>per se</i>	30.2±1.6 <sup>C</sup>	1235±68.2 <sup>C</sup>	250±13.8 <sup>B</sup>	246±13.5 <sup>A</sup>	1260±69.6 <sup>C</sup>	1350±74.6 <sup>C</sup>	11.9±0.65 <sup>B</sup>	11.6±0.64 <sup>B</sup>
Be + SP(50 mg/kg)	38.4±2.1	1578±87.2	238±13.1	230±12.7	1478±81.7	1540±85.1	12.4±0.68	12.0±0.66
% Protection	20.5%	28.2%	18.4%	32.0%	20.1%	19.4%	15.1%	10.5%
Be+SP(100 mg/kg)	44.2±2.4 <sup>a</sup>	1680±92.8 <sup>a</sup>	225±12.4	222±12.2	1678±92.7 <sup>a</sup>	1710±94.5 <sup>a</sup>	12.8±0.70	12.4±0.68
% Protection	35.0%	36.5%	38.4%	48.0%	38.7%	36.9%	27.2%	21.0%
Be+SP(200 mg/kg)	55.6±3.0 <sup>c</sup>	1879±103 <sup>c</sup>	216±11.9	215±11.8	1856±102 <sup>c</sup>	1870±103 <sup>b</sup>	13.5±0.74	13.4±0.74
% Protection	63.5%	52.9%	52.3%	62.0%	55.1%	53.3%	48.4%	47.3%
Be+SP(400 mg/kg)	61.2±3.3 <sup>c</sup>	1984±109 <sup>c</sup>	205±11.3 <sup>a</sup>	207.4±11.4 <sup>a</sup>	1940±107 <sup>c</sup>	1989±109 <sup>c</sup>	13.9±0.76	14.2±0.78 <sup>a</sup>
% Protection	77.5%	61.5%	69.2%	77.2%	62.9%	65.5%	60.6%	68.4%
ANOVA (F-value)	31.9 <sup>@</sup>	20.9 <sup>@</sup>	4.92 <sup>@</sup>	2.58 <sup>NS</sup>	18.4 <sup>@</sup>	14.8 <sup>@</sup>	3.39 <sup>NS</sup>	5.02 <sup>@</sup>

[Values are mean ± SE from six rats in each group]. P value Be vs. control at <sup>A</sup> ≤0.05; <sup>B</sup> ≤0.01; <sup>C</sup> ≤0.001; P value treatment vs. Be at <sup>a</sup> ≤0.05; <sup>b</sup> ≤0.01; <sup>c</sup> ≤0.001 for student's t test; <sup>@</sup>significant (analysis of variance) F= P ≤0.05. ACP, Acid phosphatase; ALP, Alkaline phosphatase; ATPase, Adenosine triphosphatase; Be, Beryllium; and SP, *Spirulina platensis* Crude]

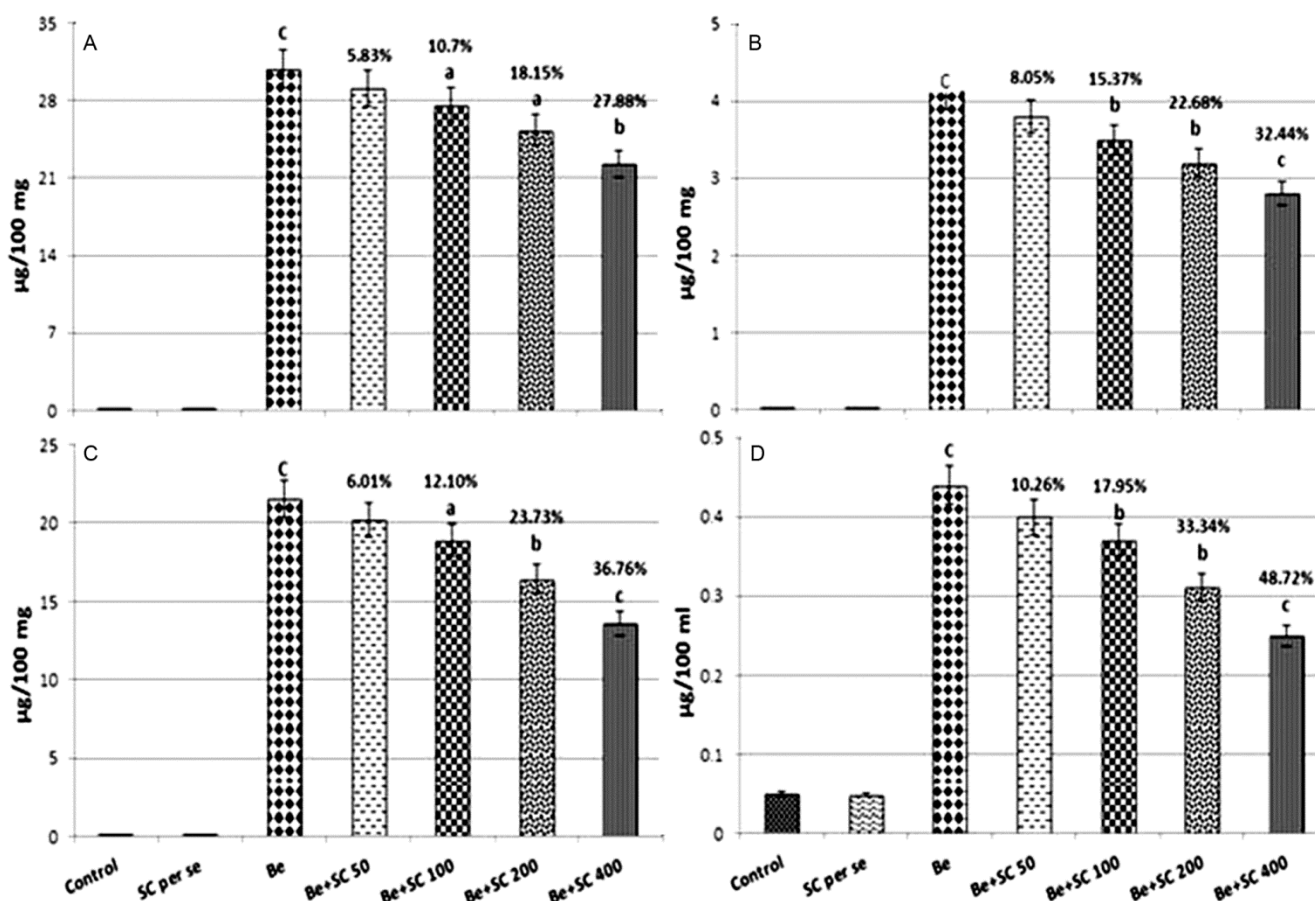


Fig. 1 — (A-D) Protective effect of *S. plantensis* on Be mobilization in (A) liver; (B) kidney; (C) spleen; and (D) blood. [Values are mean ± SE from six rats in each group]. P value Be vs. control at <sup>A</sup> ≤0.05; <sup>B</sup> ≤0.01; <sup>C</sup> ≤0.001; P value treatment vs. Be at <sup>a</sup> ≤0.05; <sup>b</sup> ≤0.01; <sup>c</sup> ≤0.001 for student's t test; <sup>@</sup>significant (analysis of variance) F= P ≤0.05. Abbreviations: Be, Beryllium; SC, *Spirulina platensis* crude. F variance for ANOVA in Liver=188<sup>@</sup>; Kidney=188<sup>@</sup>; Spleen=152<sup>@</sup>; Blood=194<sup>@</sup>]

in liver, kidney, spleen and blood was significantly increased after its administration for 28 days ( $P \leq 0.001$ ). Post-treatment with SP significantly reduced the accumulation of Be in these tissues and blood in a dose dependent manner, thus more significant protection was observed by SP 400 mg/kg ( $P \leq 0.001$ ). ANOVA was found to be significant ( $P \leq 0.05$ ) for all the tissues.

#### Histological observation in Liver and Kidney

Liver of control rat showed well-formed cord arrangement of hepatocytes having prominent nucleus, normal appearance of central vein and were separated by sinusoids containing Kupffer cells (Fig. 2A, 400X). Liver of Be treated rat showed structural loss and

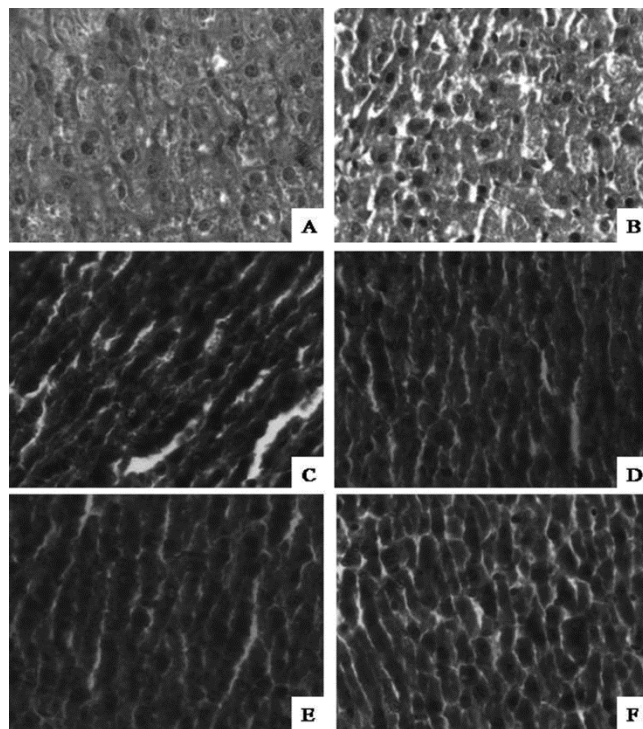


Fig. 2 — (A) Liver of control rat showed well-formed cord arrangement of hepatocytes having prominent nucleus and normal appearance of central vein (400X). (B) Liver of beryllium rats showed structure loss, disintegration of hepatocytes, massive hepatic necrosis, hyperchromatism and pyknosis of nuclei with vacuolation in hepatocytes (400X). (C) Liver of SP (50 mg/kg) treated rats showed slight improvement than Be *per se* with well-formed hepatocytes with conspicuous nucleus. Kupffer cells were also observed (400X). (D) Liver of SP (100 mg/kg) treated rats showed better cord arrangement of hepatocytes, no lymphocytic infiltration however, vacuolation and hyperchromatism of nuclei were still present (400X). (E) Liver of SP (200 mg/kg) treated rats showed well-formed cord arrangement of polygonal hepatocytes with marked nuclei and less vacuolation of hepatocytes (400X). (F) Liver of SP (400 mg/kg) treated rats showed better formed cord arrangement of well-formed hepatocytes, no vacuolation, clear central vein and few numbers of kupffer cells are seen (400X).

disintegration of hepatocytes, massive hepatic necrosis, hyperchromatism of nuclei, vacuolation in hepatocytes, degenerated nucleus and heavy lymphocytic infiltration. A considerable number of Kupffer cells are observed in the sinusoid walls. (Fig. 2B, 400X). Treatment with SP (50 mg/kg), liver showed slight improvement in hepatocytes structure and central canal with degenerated endothelium (Fig. 2C, 400X). In SP (100 mg/kg) treated rats liver showed better cord arrangement of well formed hepatocytes, no lymphocytic infiltration however, less vacuolation and hyperchromatism of nuclei were still present with empty sinusoids (Fig. 2D, 400X). In SP (200 mg/kg) treated rats, liver showed well-formed polygonal hepatocytes with normal nucleus, less vacuolation and well-formed hepatic cord arrangement (Fig. 2E, 400X). In SP (400 mg/kg) treated rats, liver showed almost normal hepatocytes with intact cord arrangement of hepatocytes, no vacuolation with normal nuclei (Fig. 2F, 400X).

Kidney of control rats showed well-formed renal tubules and Bowman's capsule with normal glomeruli (Fig. 3A, 400X). Kidney of Be treated rats showed degenerated glomeruli and capsule wall, constriction of glomeruli, hypertrophy of epithelial cells and tubular obstruction in most of the tubular lumens, losing brush-like edges of tubular cells and heavy vacuolation (Fig. 3B, 400X). In SP (50 mg/kg) treated rats, kidney showed slight improvement with apparently normal renal cortex and better formed glomeruli than be *per se* treatment (Fig. 3C, 400X). In SP (100 mg/kg) treated rats, kidney also showed slight improvement with better formed uriniferous tubule with decrease in hyperchromatism of nuclei (Fig. 3D, 400X). In SP (200 mg/kg) treated rats, kidney showed well formed Bowman's capsule and uriniferous tubule with decrease in hyperchromatism of nuclei (Fig. 3E, 400X). In SP (400 mg/kg) treated rats, kidney showed maintained Bowman's capsule with compact glomeruli, well-formed endothelial lining of uriniferous tubule with wider lumen and intact basement membrane (Fig. 3F, 400X).

#### Discussion

Be is highly toxic to living organism. Nearly all insoluble and soluble forms of Be are toxic as function of the concentration and the exposure degree. Toxicological action of Be is due to production of ROS, depleted major internal thiol antioxidants and results in oxidative stress<sup>8,17</sup>. In present study, Be

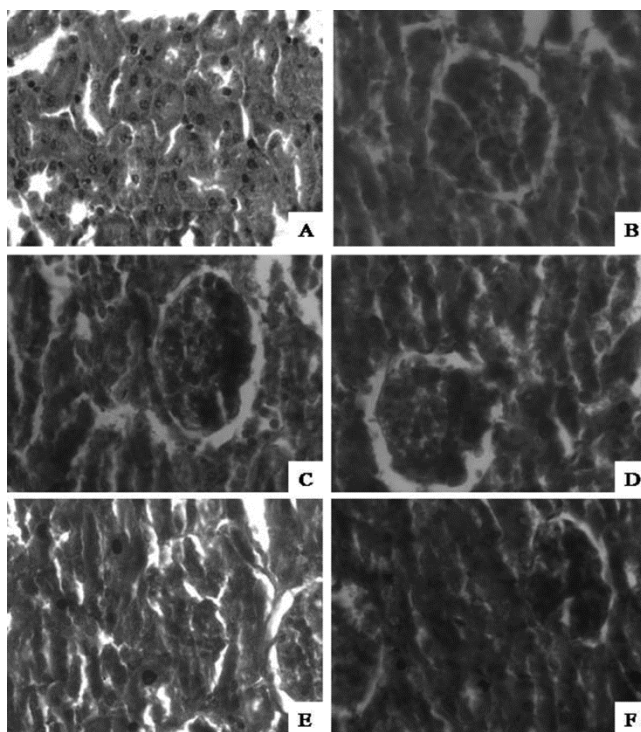


Fig. -3 — (A) Kidney of control rats showed well-formed renal tubules and Bowman's capsule with normal glomeruli (400X). (B) Kidney of beryllium treated rats showed degenerated glomerulus and capsule wall, constriction of glomeruli, tubular obstruction in the tubular lumen with tubular hypertrophy and heavy vacuolation (400X). (C) Kidney of SP (50 mg/kg) treated rats showed better formed glomeruli than *per se* (400X). (D) Kidney of SP (100 mg/kg) treated rats showed distinct renal tubules with well-formed Bowman's capsule and compact glomeruli (400X). (E) Kidney of SP (200 mg/kg) treated rats showed well-formed tubules with wider lumen, maintained Bowman's capsule with glomeruli (400X). (F) Kidney of SP (400 mg/kg) treated rats showed maintained Bowman's capsule with compact glomeruli, well-formed endothelial lining was noted. The proximal and distal tubules were well organized with intact basement membrane (400X).

induced oxidative stress by depletion of cellular reduced glutathione (GSH), increase in lipid peroxidation of membrane lipids together with inhibition of antioxidant enzymes SOD and CAT activities. Metal toxicity due to Be similar to lead, aluminium, cadmium, arsenic and mercury generate reactive oxygen species (ROS), causes peroxidation of membrane lipids and alters the structure and functional behaviour of cellular membranes and membrane linked enzymes. Be enhanced the production of hydroxyl and super-oxide free radicals (ROS). These ROS readily induce oxidative damage to various biomolecules including DNA, lipids, proteins, and lipoproteins and results in damage to

vital organs (Liver, kidney, heart, brain and lungs). Be is considered as an important etiological factor implicated in development of several chronic diseases such as CBD, neurodegenerative diseases, diabetes mellitus, atherosclerosis, arthritis, and cancer<sup>8,9,16</sup>.

GSH act as internal antioxidant, protect the biological membrane from oxidative damage and help in protection of vital organs of the body. Depleted GSH, SOD and catalase activities and increased TBARS in lipid peroxidation after Be intoxication indicated the failure of the antioxidant defence mechanism. Antioxidants are the compounds which bind with free radicals and neutralise their effects. Some of the antioxidants have been proven to be effective in preventing beryllium induced toxicity in many previous interventions<sup>8-11,14-18</sup>. SP is a potent antioxidant, has been reported earlier in amelioration of toxic manifestations induced by chemotherapeutic agents, some metals as well as pesticides<sup>19,21,23</sup>. SOD and catalase are the cellular major antioxidant enzymes, play an important role in elimination of ROS and act as the key component of cellular defence mechanism. These antioxidant enzymes depend on various essential trace elements (bivalent metal ion) and prosthetic groups for proper molecular organization and enzymatic action. Be is divalent metal ion, may bind to the enzymatic active site and inhibited enzymatic action. Decreased SOD and catalase activity in Be toxicity reflects decrease in mRNA expression of these two antioxidant enzymes<sup>10</sup>. Treatment with SP induced SOD and CAT activity may be due to increase in mRNA expression of these two antioxidant enzymes and inhibition of ROS decrease in lipid peroxidation of liver and kidney. Previous study reports that aqueous extract of SP and C-phycoyanin are known for its anti-oxidant and anti-inflammatory activities<sup>22</sup>. Due to its better antioxidant potential, SP quenches free radicals and maintains the antioxidant pool inside the cellular environment as also evidenced by previous studies against various toxic compounds<sup>20-22</sup>.

Liver is a target organ of various xenobiotics and major site of metabolism whereas kidney acts as drug and xenobiotic excretion. A toxic effect of chemicals, drugs and xenobiotic usually appears primarily in liver and kidney. After intraperitoneal administration of Be and its salts, Be enters in to blood circulation and transported as Be-protein complex primarily to liver and then rest of vital organs of rats. Thus, significant amount of beryllium deposited in vital

organs. Liver act as a primary elimination route of Be and occupy maximum amount of beryllium in rats<sup>5</sup>. Be produces ROS, leads to hepatocytes membrane damage and interrupted liver function thus transaminases leak out in to blood circulation and increase it's level higher than normal. In histological sections, Be degenerated hepatocytes membrane, disturbed cord arrangement of hepatocytes and heavy vacuolation, indicates the pro-oxidant effect of Be. Treatment with SP showed dose dependent recovery in histoarchitecture of liver and prevented the membrane damage thus the level of serum transaminases returns back towards normal. Hyperbilirubinaemia indicated impaired liver function, rise significantly with Be intoxication, this may be due to more destruction of RBCs, thus hemoglobin level decreases and catabolism of free hemoglobin results in more production of bilirubin. SP is a rich source of minerals, vitamins and antioxidants, neutralizes Be induced ROS and showed membrane stabilizing effect by improving liver function. C-Phycocyanin present in SP, found to be effective against free radical-induced cellular transformation; inhibit cytochrome P450-mediated reactions involved in the formation of reactive metabolites of the hepatotoxins<sup>37</sup>. The antioxidant and hepatoprotective property of SP is due to their presence of selenium, gamma-linolenic acids,  $\beta$ -carotene, xanthophylls, zeaxanthin, echinenone, cryptoxanthin, phycocyanin<sup>19,21,22</sup>, and vitamin E and C working individually or in synergy. SP decreases the amount of Be deposited in the vital organs, it may be due to binding of divalent Be<sup>+2</sup> with the compound present in SP and help in their excretion. This may be due to SP's chlorophyll and phytonutrient contents which shows cleansing property and act as detoxifying agent against the toxic substances<sup>38</sup>.

Be damage to kidney altered normal kidney function by elevation of serum uric acid and creatinine. Administration of beryllium nitrate showed degeneration of glomeruli as well as hypertrophy of epithelial cells of uriniferous tubules. The lumen of uriniferous tubules decreases and results in obstruction of flow of filtrate, thus the excretory material returns back in blood circulation and raise the level of uric acid and creatinine in blood. Post treatment of SP showed protection against Be induced renal injury by maintaining the histoarchitecture of kidney and serum biochemical variables towards normal. In previous intervention, SP elicited

significant nephroprotective activity by decreasing gentamicin induced higher level of lipid peroxidation, creatinine and urea, depleted GSH, SOD activity<sup>39</sup>. Elevation of serum total cholesterol in beryllium intoxication indicated severe lipid peroxidation and altered lipid metabolism. Lipid peroxidation due to ROS increases the supply of non-essential fatty acids, which in turn increases cholesterol level in blood and tissues. Administration of SP reduced the excess lipid peroxidation and prevented the elevated level of cholesterol, showing better free radical scavenging and hypocholesteremic activity of SP. Overall the antioxidant and hepatoprotective effect of SP is owed to their active constituents such as c-phycocyanins, b carotene, vitamins, minerals, proteins, lipids and carbohydrates. Previous study also revealed that C-phycocyanin, the main ingredient of *Spirulina*, reduces the lipid concentrations through scavenging free radicals, inhibiting lipid peroxidation, nicotinamide adenine dinucleotide phosphate oxidase expression, and increasing the activity of lipoprotein lipase, hepatic triglyceride lipase, glycosylated serum protein peroxidase and superoxide dismutase<sup>19</sup>.

Beryllium decreases blood sugar level and interfere with carbohydrate metabolism. Decrease in blood sugar provides a signal for glycogenolysis thus hepatic glycogen decreases<sup>18</sup>. The glucose-6-phosphatase is a SER membrane enzyme involved in gluconeogenesis, decreases in beryllium toxicity; it is due to binding of beryllium ions to it's phosphate group and with key regulatory enzymes of carbohydrate metabolism<sup>16</sup>. Depleted glucose-6-phosphatase activity directly reflects the degradation of SER membrane, observed by ultra-morphology of liver and kidney<sup>14</sup>. Glucose-6-phosphate is produced by breakdown of glycogen, cannot pass the hepatocytes membrane until it is converted to free glucose by the action of glucose-6-phosphatase in liver but due to inhibition of glucose-6-phosphatase activity, excess glucose-6-phosphate enters in to pentose phosphate pathway and compensate the body energy and higher level of glucose-6-phosphate induce G6PDH activity in liver and kidney<sup>10</sup>. Post treatment of SP maintains the glucose-6-phosphatase activity and blood sugar level more towards normal. Previous study reported the metal binding capacity of SP<sup>40</sup> which may also bind Be<sup>+2</sup> (ions) and may prevent binding of Be<sup>+2</sup> to enzymatic active site thus glucose-6-phosphatase activity restored towards normal.



Be increased the activity of acid phosphatase (ACP) in liver and kidney, indicated enhanced tissue catabolism and cellular autophagy leading to tissue damage. Histopathological observations also corroborated this fact with cytoplasmic vacuolation due to degeneration of cellular organelles after Be toxicity. Treatment with SP showed conspicuous hepatorenal protection due to reduction in release of lysosomal enzymes and showed better result in restoration of ACP activity more towards normal. The ALP and ATPase are membrane bound enzymes. Any alteration in membrane lipid leads to change in membrane fluidity, which in turn alters cellular functions mediated by these enzymes. Remarkable depletion in the activities of these enzymes was found after beryllium toxicity.  $\text{Be}^{+2}$  is a potential target for ATP binding and that  $\text{Be}^{+2}$  out competes  $\text{Mg}^{+2}$  for ATP and ADP binding. SP is a rich source of minerals containing magnesium and zinc which may compete with Be for binding to enzymes and help in protection of enzymatic activities. Administration of Be decreased total protein in serum and tissue indicating protein damaging potential of Be which is due to increase in oxidative stress or interference with protein biosynthesis. Administration of SP showed dose dependent recovery may be due to decrease in tissue catabolism by free radical scavenging, and supplementation of essential amino acids which may help in restoration of proteins and maintain enzymatic activities.

Beryllium does not induce DNA damage at these doses; however, Be inhibits the synthesis of nuclear proteins and influences the steps leading to DNA synthesis and selectively interferes with regulatory mechanisms controlling transcriptional events in gene expression<sup>10</sup>. But, it is clear that Be, a bivalent metal interferes with biochemical metabolic pathway, induces oxidative stress and histopathological lesions which lead to hepatorenal abnormalities.

Our results indicate that SP was remarkably effective in amelioration of Be induced oxidative stress could be either directly by inhibiting lipid peroxidation and scavenging free radicals or indirect through the enhancement of the activities of SOD and CAT. These properties could be attributed to the high protein contents, phenolic compounds with essential fatty acid and amino acids, minerals and vitamins, which together acts as antioxidants. Therefore, SP could be used to prevent and treat hepatic and renal diseases especially those induced by oxidative damage.

## Conclusion

The results have demonstrated the antioxidative and hepatorenal protective potential of the blue-green algae *Spirulina platensis* (SP) in powder form as available in market against Beryllium (Be) toxicity by mitigating oxidative stress, decreased Be burden, and maintained hepatorenal function with almost normal histoarchitecture of liver and kidney. Thus, consumption of SP may prove effective in amelioration of Be induced hepatorenal toxicity and it may also useful in prevention and treatment of Be related diseases. Supplementation of SP may also be beneficial in manifestation(s) of other toxic compounds however, further investigation are required.

## Conflict of interest

The authors declare no conflicts of interest.

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