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# Potential antioxidative protein-pigment complex *Spirulina platensis* mediated food grade phycocyanin C -Extraction, purification, antioxidative activity and biocompatibility

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Phycocyanin (PC), a photosynthetic pigment produced by cyanobacteria has been gained attention due to its distinct properties such as antioxidant and anti-proliferative. Acute and sub acute toxicity studies were carried out to determine the biocompatibility of the extracted phycocyanin on Wistar rat model. PC was extracted from *Spirulina platensis* biomass by cold maceration followed by successive purification by ammonium sulphate precipitation and gel filtration chromatography. Biocompatibility of the purified phycocyanin was carried out by acute toxicity studies using Wistar rat model. Acute toxicity has been determined by the effect of single oral dose of PC with two different concentrations (250 and 500 mg/Kg) on the body weight, general behaviour, and mortality. In sub-acute treatment. The effect of phycocyanin on the various parameters at the respective concentration as single oral dose daily during 28 days was studied. Cold maceration followed by maceration brought about food grade phycocyanin C (C-PC) which final yield and purity were increased in the successive purification steps. Antioxidative study using DPPH assay reveals the effective free scavenging activity of the phycocynin as concentration-dependent manner. Biocompatibility studies against Wister rat model did not exhibit any harmful effect. Any sign of toxic effect on biochemical, hematological and histopathological parameters was not observed in all the tested animals of treatment groups during the study period which reveals a high level of biocompatibility. The present study suggests the possible utilization of phycocyanin C as an effective pharmaceutical agent.

**Keywords**: Acute Toxicity, Antioxidant, Biocompatibility, Phycocyanin, Pigment, *Spirulina platensis*, Wistar model

Oxidative stress is an important factor in the formation of diverse life-threatening diseases, from cancer to cardiovascular and degenerative diseases[1-3]. In order to protect the body against the consequences of oxidative stress, an efficacious approach has been improving in antioxidant nutrition. In this regard, scientific studies have shown that antioxidants from natural sources have higher bioavailability and therefore higher protective efficacy[4,5]. Recent studies have reported that natural products including plants, animals, and microorganism have been the basis of treatment of various oxidative stress-mediated diseases. Among the sources, algae and algae-based products have gained recent attention due to their high efficacy and best biocompatibility[6].

Phycocyanin C (PC) is a water-soluble, highly fluorescent protein derived from cyanobacteria has a lot of application as food colouring agents, cosmetics, and biomedical research[7,8]. Antioxidant, radical scavenging, anti-inflammatory, anti-cancer, anti-tumour properties of PCs extracted from *Spirulina* has been experimentally proved under laboratory and animal model studies[9-14]. Experimental recent studies reveals phycocyanin from *Spirulina* enhanced curative red blood cells production and immunity in animal model studies[15,17]. Phycobiliproteins are widely used in laboratory tests and immunological assays, due to their properties such as high fluorescence, good storage stability at temperatures between 4 to 10°C, isoelectric point (IP) close to 4.65, making them easily linkable to antibodies and other proteins by conventional techniques without changing its spectral characteristics. It has a high molar absorbance coefficient and emission, oligomeric stability and high photo-stability[18-20]. PCs can be used for the detection of multiple myeloma cells and as a potential therapeutic agent in oxidative stress- induced disease[21].

Because of the potential application of phycocyanin in biotechnology and biomedicine, it is mandatory to determine toxicity assessment tests

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under *in vitro* and *in vivo* condition using standard methods. Unlike *in vitro* toxicity studies, *in vivo* testing is highly preferred to determine the biocompatibility. In order to establish the safety of phycocyanin C, acute and sub-acute toxicity studies using a suitable animal model is essential. The types of toxicity tests which are routinely performed by pharmaceutical manufacturers in the investigation of a new drug involve acute, sub-acute and chronic toxicity. Determination of acute oral toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds[22,23]. In the present work, Wistar rats were selected for studying the acute and sub-acute toxicity of phycocyanin. There are various distinct characters of Wistar rat like physiological, metabolic pathways, anatomical similarities with human, easy maintenance, short life period would suggest the possible utilization in toxicity studies. Evaluation of toxicity of phycocyanin under *in vivo* system using Wistar rat model studied in the present investigation is helpful to confirm biocompatibility and its possible utilization of *Spirulina* mediated PC as an effective and safe nutraceutical agent.

## Materials and Methods

### Algal strain

Extraction of phycocyanin was derived from biomass of laboratory stock culture of *Spirulina platensis* cultivated in BG 11 growth media. Biomass thus obtained after the incubation period of growth was collected and used for further studies.

### Extraction and purification

Extraction of phycocyanin from *Spirulina platensis* was carried out by cold maceration method followed by sonication[24]. In this method, known quantity of algal biomass which previously dried at 40°C for 12 h was immersed in 100 mL of phosphate buffered saline (pH 7.4) followed by sonication for 2 h. After sonication, the suspension was subjected to freezing at −80°C for 24 h, thawed to room temperature. Centrifugation of the thawed suspension was done at 12000 rpm for 10 min 2 times. Supernatant thus obtained was collected and used as the source of crude phycocyanin and subjected to purification by different methods. Different concentration of ammonium sulphate (25 and 50%) was added to the collected supernatant with continuous stirring. The resulting suspension was centrifuged at 12000 rpm for 30 min and the collected pellet was re-suspended in 0.1 M Tris-HCl buffer followed by dialysis. Dialysis was done using dialysis membrane (Hi-media, India) with the molecular weight cut off l KD. The dialysed material was subjected to column chromatography separation using 10 mM phosphate buffer pre-equilibrated Sephadex G 25 column followed by elution with 10 mM phosphate buffer. Eluents thus, obtained was lyophilized and used for further studies. Characterization of the lyophilized samples was done by UV visible spectroscopy using Shimadzu-1800 spectrophotometer (800-200 nm range) and FTIR analysis. Lyophilized samples were pelletized with KBr and the pelletized spectrum was recorded in the range of 4000-500 $cm^{-1}$ (Bruker Optic GmbH Tensor).

### Antioxidative activity

Antioxidative activity of the purified phycocyanin was done by DPPH free radical assay Shiban *et al*.[25].

### Biocompatibility assessment using wistar rat model

#### *Test animal selection and maintenance*

Biocompatibility assessment of extracted phycocyanin was studied by determination of acute and sub acute toxicity using Wistar rat model according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Rats (CPCSEA) and the protocol was approved by the Institutional Animal Ethics Committee (IAEC No: KMCRET). Healthy male and female Wistar rats were procured from Sri Venkateshwara Enterprises Private Limited, Bangalore, India. Selected animals were maintained in an air conditioned room at 26°C, 35% of relative humidity with 12 h dark cycle. Polypropylene cages were used for housing of the animals with rice husks for bedding andnormal rat chow as feed (procured from Sai Durga animal Feed, Bangalore, India) followed by acclimatization for ten days in the environment prior to experiment.

#### *Experimental design*

Test animals were divided into three groups and each group consisting of six animals. Group-I Control, group II group III were Phycocyanin C treatment categories. Group I received saline, group II and group III received 250 mg/Kg and 500 mg/Kg of phycocyanin C, respectively. Freeze dried form of PC was added in distilled water and completely dissolved for oral administration. The dose was prepared of a required concentration before dosing by dissolving PC in distilled water. Phycocyanin was administered orally to each rat as single dose using a needle fitted

on to a disposable syringe of appropriate size at the following different doses. The concentration was adjusted according to its body weight. The volume was not exceeding 10 mL/Kg body weight. Variability in test volume was minimized by adjusting the concentration to ensure a constant volume at all dose levels.

After administration, the treated animals were observed at least twice daily with the purpose of recording any symptoms of ill health or behavioural changes. Clinical signs of toxicity for 28 days.

Prior to the beginning of treatment and daily, the food intake of each cage was recorded for the period of 28 days and the mean weekly intake per rats was calculated. In addition, the water consumption in each cage was measured daily for a period of 28 days.

#### *Body weight*

The body weight of each rat was recorded one week before the start of treatment and during the course of the treatment on the day of initial, 3$^{rd}$, 7$^{th}$, 10$^{th}$, 14$^{th}$, 17$^{th}$, 20$^{th}$, 24$^{th}$, and 28$^{th}$ days (day of sacrifice). The mean weights for the different groups and sexes were calculated from the individual weights.

### Hematological and biochemical parameters

#### *Blood collection*

Blood was collected through retro-orbital sinus from all the animals of different groups on the 28$^{th}$ day. Animals were fasted overnight prior to the blood collection and the blood was collected in tubes containing Heparin/EDTA as an anticoagulant.

During the 4$^{th}$ week of treatment, samples of blood were withdrawn from the orbital sinus of 6 males from each group, under light ether anesthesia after fasting for 16 h. The blood samples are used to evaluate hematological parameters like hemoglobin (g/L), PCV (Packed cell volume), white blood corpuscles ($\times 10^3$/cmm), red blood corpuscles ($\times 10^3$/cmm), blood plateletcount, lymphocytes, ($\times 10^3$/cmm), neutrophils (%), monocytes, eosinophils, mean platelet volume using auto analyser (Bio-Rad).

#### *Biochemical parameters*

The collected blood samples were centrifuged at 10000 g for 10 min to separate the serum. The separated serum used to evaluate biochemical parameters like total cholesterol, triglycerides, high density lipoprotein (HDL), total bilirubin (direct & in direct), SGOT, SGPT, alkaline phosphatase, creatinine, urea, and uric acid using full automated biochemical analyser (Bio Rod).

### Terminal studies

#### *Sacrifice and macroscopic examination*

On completion of the 4 weeks of treatment, Wistar rats were sacrificed by ether inhalation. A full autopsy was performed on all animals which included an examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities, and their contents both in situ and after evisceration. As the number of animals exceeded the number that could be sacrificed in one day, the autopsies were carried out over three consecutive days at the end of the treatment period.

#### *Organ weights*

After the macroscopic examination, the following organs were weighed after separating the superficial fat: brain, heart, spleen, kidneys, testes, liver, lungs, pancreas and stomach.

#### *Histopathological examination*

The tissues from the respective group of sacrificed animals were immediately fixed in 10% buffered neutral formalin solution. The tissue embedded in molten paraffin with the help of metallic blocks, covered with flexible plastic moulds and kept under freezing plates to allow the paraffin to solidify. Cross sections (5 µm thick) of the fixed tissue were cut using microtome (Leica PM 2125, Germany). These sections were then stained with hematoxylin and eosin method[26] and visualized under a light microscope (Magnus, India) to study the microscopic architecture.

## Results

### Isolation of PC

Phycocyanin was isolated from *Spirulina platensis* algal biomass by cold maceration followed by sonication followed by successive purification methods. Figure 1 shows the extracted phycocyanin

Fig. 1 — Extracted phycocyanin

with bright blue color water-soluble pigment (Fig. 1). The purity of phycocyanin was found to increase in 25 and 50% ammonium sulphate precipitation. Final yield and purity were increased in the column chromatography using Sephadex G-25 column (Fig. 2). Purified phycocyanin fractions thus obtained was characterized by UV-vis spectroscopy and FTIR which reveals the high rate purity of extracted C-PC by showing maximum absorbance at 280/620 nm using a Shimadzu UV-2401 spectrophotometer Lyophilized PC thus obtained reveals $A_{620}/A_{280}$ ratio greater than 0.7, which is considered as food grade (Fig. 3), characteristic absorption peaks at specific wave number coincided with those expected for the peptide. (Fig. 4).

**Antioxidative activity**

Antioxidative activity was studied by DPPH method. The DPPH scavenging activity has been commonly used to detect the antioxidant activity of different samples sources, due to its sensitivity to lower concentrations of active standards from natural sources. The steady radical, DPPH, has a maximum absorbance at 517 nm and could swiftly undergo scavenging by antioxidants. Complex free radical scavenging activities of samples are indicated by lower absorbance at 517 nm. It was found that the highest concentration of PC at 100 µg/mL and 75 µg/mL had the highest percentage of antioxidant activity (Fig. 5).

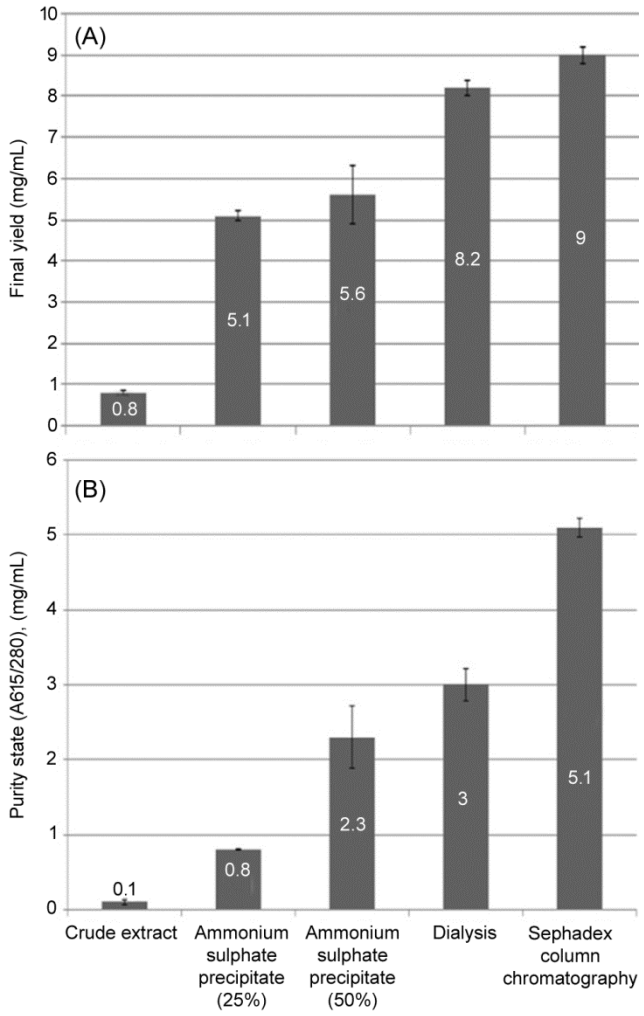


Fig. 2 — Effect of purification methods on the purity and final yield of phycocyanin

**Acute and sub-acute toxicity studies**

Biocompatibility assessment of PC was carried out on Wistar rat models which was confirmed by changes in the various parameters of the tested animal groups. In this toxicity study, the rats were treated with two concentration of phycocyanin as 250 and 500 mg/Kg which did not exhibit any signs of toxicity, behavioural changes, and mortality in the test groups when observed during 28 days of the acute toxicity experimental period. No major sign of changes in behaviour and mortality was observed in all the groups which show the administrated phycocyanin seems to be safe at a dose level of 250 and 500 mg/Kg. Any pharmaceutical drug or compound with a higher than 100 mg/Kg could be considered safe and low toxic[31].This suggests that the administrated phycocyanin is nontoxic in the single oral dose of 250 and 500 mg/Kg. Phycocyanin at both dosages did not induce any toxic effect on organ weight, biochemical, hematological parameters after the 28 days of treatment.

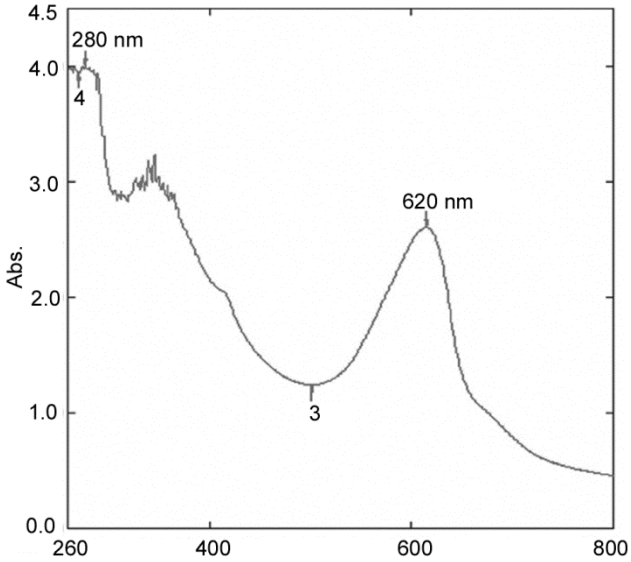


Fig. 3 — UV- Spectra of isolated phycocyanin

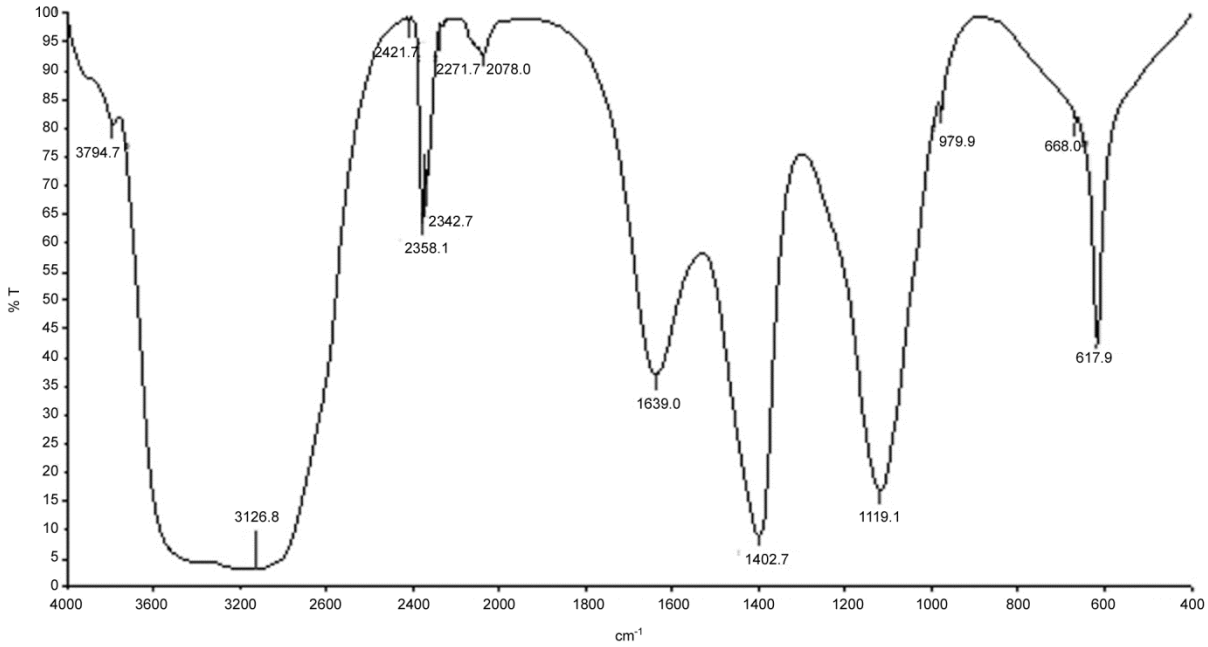


Fig. 4 — FTIR spectra of phycocyanin

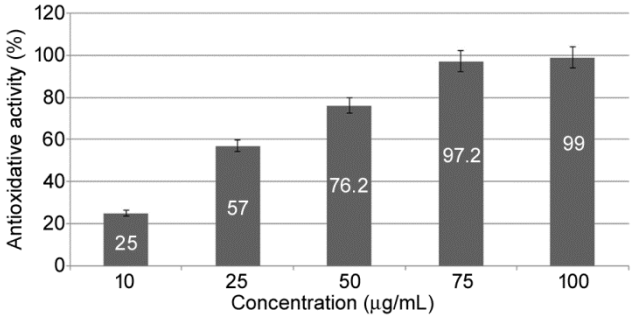


Fig. 5 — Antioxidant activity (%) of phycocyanin

Study on the body weight was studied one week before the start of treatment, and during the course of the treatment Distinct effect on the body weight of tested animals of both sexes at all the tested time periods was not recorded inboth dosages of phycocyanin treatment (Table 1). The absence of a significant difference inthe weight of vital organsas presented in table provides support for the safety of phycocyanin. Acute toxicity test revealed phycocyanin at the both tested concentration was found to non-toxic. No mortality was observed during the whole experiment. During the dosing period and in the last day, the quantity of food and water intake by different dose groups was found to be comparable with the control group. No abnormal deviations were observed. No significant changes were observed in the values of different parameters studied when compared with controls and values obtained were within normal laboratory limits.

Table 1 — Effect of sub-acute doses (28 days) of phycocyanin C on organ weight (physical parameters)

| Organ | Control | Dose (250 mg/Kg) | Dose (500 mg/Kg) |
|---|---|---|---|
| Brain | 1.805 ± 0.181 | 1.770 ± 0.166 | 1.952 ± 0.144 |
| Heart | 0.900 ± 0.113 | 0.851 ± 0.052 | 0.950 ± 0.145 |
| Kidney left | 0.701 ± 0.044 | 0.754 ± 0.109 | 0.830 ± 0.091a |
| Kidney right | 0.692 ± 0.096 | 0.713 ± 0.086 | 0.817 ± 0.096 |
| Liver | 5.766 ± 0.787 | 6.306 ± 1.325 | 6.674 ± 0.394 |
| Lungs | 1.495 ± 0.263 | 1.369 ± 0.214 | 2.015 ± 0.390a |
| Spleen | 0.951±0.286 | 0.779 ± 0.214 | 0.904 ± 0.202 |

Values are expressed as the mean±SD; Statistical significance (P) calculated by one way ANOVA followed by Dennett's [a]$P < 0.05$ calculated by comparing treated group with control group.

Effect of phycocyanin on hematological parameters were studied using the auto analyser. There were no significant changes were observed in hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), Erythrocyte sedimentation rate (ESR) in all the treated groups as compared to respective control groups (Table 2). There was no significant changes were recorded in biochemical parameters of the animal groups (Table 3). Alkaline phosphatise, bilirubin, total cholesterol, creatinine, urea, SGPT, and SGOT level were not significantly varied in the test groups

Histopathological examination of the tissue section was also supported biocompatibility tissue section

Table 2 — Effect of phycocyanin C on the haematological parameters

| Haematological parameters | 500 mg/Kg Female | 500 mg/Kg Male | 250 mg/Kg Female | 250 mg/Kg Male | Control Female | Control Male |
|---|---|---|---|---|---|---|
| Total RBC count ($1 \times 10^6$ mm$^3$) | 6.19 ± 0.012[c] | 6.18 ± 0.231[c] | 4.18 ± 0.162 | 5.00 ± 1.016 | 4.55 ± 0.404 | 4.33 ± 0.150 |
| Total WBC count ($1 \times 10^3$ mm$^3$) | 11.10 ± 1.270[b] | 12.90 ± 1.501 | 9.50 ± 0.346[c] | 12.35 ± 2.483 | 15.00 ± 0.924 | 13.60 ± 0.577 |
| Haemoglobin (g/L) | 17.30 ± 1.270[a] | 17.00 ± 1.848[a] | 11.70 ± 0.693[b] | 14.00 ± 0.693 | 14.85 ± 0.520 | 14.40 ± 0.462 |
| Lymphocytes (%) | 89.50 ± 0.577 | 87.00 ± 3.464 | 75.50 ± 0.577[c] | 89.00 ± 3.464 | 91.00 ± 1.155 | 90.50 ± 0.577 |
| Mean corpuscular haemoglobin (Picograms/cell) | 20.50 ± 0.577[a] | 21.55 ± 0.173[c] | 19.35 ± 0.289 | 19.95 ± 0.173 | 19.35 ± 0.866 | 17.90 ± 0.693[a] |
| Monocytes (%) | 3.50 ± 0.577 | 4.00 ± 2.309 | 2.50 ± 0.577 | 2.50 ± 0.577 | 2.00 ± 1.155 | 1.50 ± 0.577 |
| Packed cell volume (%) | 45.85 ± 5.485 | 47.30 ± 5.543 | 33.65 ± 1.790[c] | 43.70 ± 2.540 | 46.10 ± 0.231 | 44.45 ± 2.714 |
| Eosinophils (%) | 4.50 ± 0.577[b] | 5.00 ± 1.155[c] | 3.50 ± 0.577 | 4.50 ± 0.577[b] | 2.50 ± 0.577 | 2.00 ± 0.000 |
| Polymorphs (%) | 2.50 ± 0.577 | 4.00 ± 0.000 | 14.50 ± 4.041[c] | 4.00 ± 2.309 | 4.50 ± 1.732 | 4.50 ± 0.577 |

Values are expressed as the mean±SD; Statistical significance (P) calculated by one way ANOVA followed by Dennett's [a]$P< 0.05$ calculated by comparing treated group with control group.

Table 3 — Effect of phycocyanin C on biochemical parameters

| Biochemical parameter | 500 mg/Kg Female | 500 mg/Kg Male | 250 mg/Kg Female | 250 mg/Kg Male | Control Female | Control Male |
|---|---|---|---|---|---|---|
| Total cholesterol (mg/dL) | 94.35 ± 5.600[c] | 138.40 ± 5.543[c] | 109.90 ± 0.346[b] | 128.90 ± 4.157[a] | 120.50 ± 2.656 | 98.80 ± 0.462[c] |
| Triglycerides (TG) (mg/dL) | 123.80 ± 17.782[c] | 295.90 ± 11.316[c] | 151.05 ± 6.755[b] | 311.20 ± 4.157[c] | 199.90 ± 31.639 | 124.45 ± 0.981[c] |
| High Density Lipoprotein (HDL) (mg/dL) | 46.20 ± 1.155[b] | 56.60 ± 7.275 | 70.00 ± 2.656 | 56.00 ± 8.198 | 60.65 ± 5.023 | 63.95 ± 0.635 |
| Bilirubin Total (mg/dL) | 0.55 ± 0.058[b] | 0.95 ± 0.035 | 0.98 ± 0.029 | 0.85 ± 0.404 | 1.05 ± 0.058 | 0.85 ± 0.058 |
| Bilirubin Direct (mg/L) | 0.16 ± 0.023 | 0.19 ± 0.012 | 0.17 ± 0.023 | 0.20 ± 0.115 | 0.22 ± 0.023 | 0.15 ± 0.006 |
| Bilirubin In direct (mg/L) | 0.43 ± 0.081[b] | 0.85 ± 0.150 | 0.85 ± 0.058 | 0.65 ± 0.289 | 0.85 ± 0.058 | 0.61 ± 0.052 |
| SGOT(U/L) | 47.25 ± 1.212[b] | 60.90 ± 5.196 | 67.80 ± 0.462 | 59.30 ± 18.591 | 76.50 ± 11.201 | 44.75 ± 0.173[c] |
| SGPT(U/L) | 39.00 ± 1.386 | 49.60 ± 11.432[b] | 49.95 ± 0.404[b] | 41.30 ± 5.196 | 30.65 ± 10.104 | 28.55 ± 1.212 |
| ALP(U/L) | 259.40 ± 3.464 | 306.00 ± 30.600 | 277.95 ± 1.328 | 297.90 ± 9.930 | 278.30 ± 16.166 | 198.80 ± 2.771[c] |
| Creatinine (mg/dL) | 0.65 ± 0.058 | 0.75 ± 0.058 | 0.75 ± 0.173 | 0.65 ± 0.173 | 0.60 ± 0.000 | 0.60 ± 0.115 |
| Urea(mg/dL) | 18.80 ± 0.693 | 23.90 ± 0.577 | 21.75 ± 0.404 | 23.05 ± 8.025 | 21.55 ± 1.674 | 23.90 ± 1.848 |
| Uric acid (mg/dL) | 4.25 ± 0.289[a] | 5.45 ± 0.058 | 4.00 ± 0.924[a] | 4.30 ± 0.577[a] | 9.00 ± 4.388 | 5.50 ± 0.577 |

Values are expressed as the mean±SD; Statistical significance (p) calculated by one way ANOVA followed by Dennett's [a]$P< 0.05$ calculated by comparing treated group with control group.

derived from various organs were examined using standard methods. Phycocyanin treatment shows no significant pathology. Tissue sections of heart, lungs, liver, stomach, kidneys, uterus and testis reveal the complete absence of tissue damage at both the dosages tested (Fig. 6). Histopathological examination of heart tissue of both the dosages of phycocyanin treatment group exhibited no morphological changes in myocardium. As in the control group, there is mild edema, focal lymphocytic infiltrates in the stroma. There is no loss of striations or myocyte degeneration or inflammations. Both group I and group II of lungs tissue shows normal alveoli, bronchi, and bronchioles. Blood vessels show congestion and there is mild inflammatory seen in the interstitium. There is no inflammatory infiltrates seen in the alveoli. The Normal lobular architecture was observed in liver tissue of control and phycocyanin treatment groups. Hepatocytes of both control and treatment groups show no significant pathology. Portal traid shows bile duct hyperplasia and mild periportal infiltration. Central vein shows mild congestion. Sinusoids show

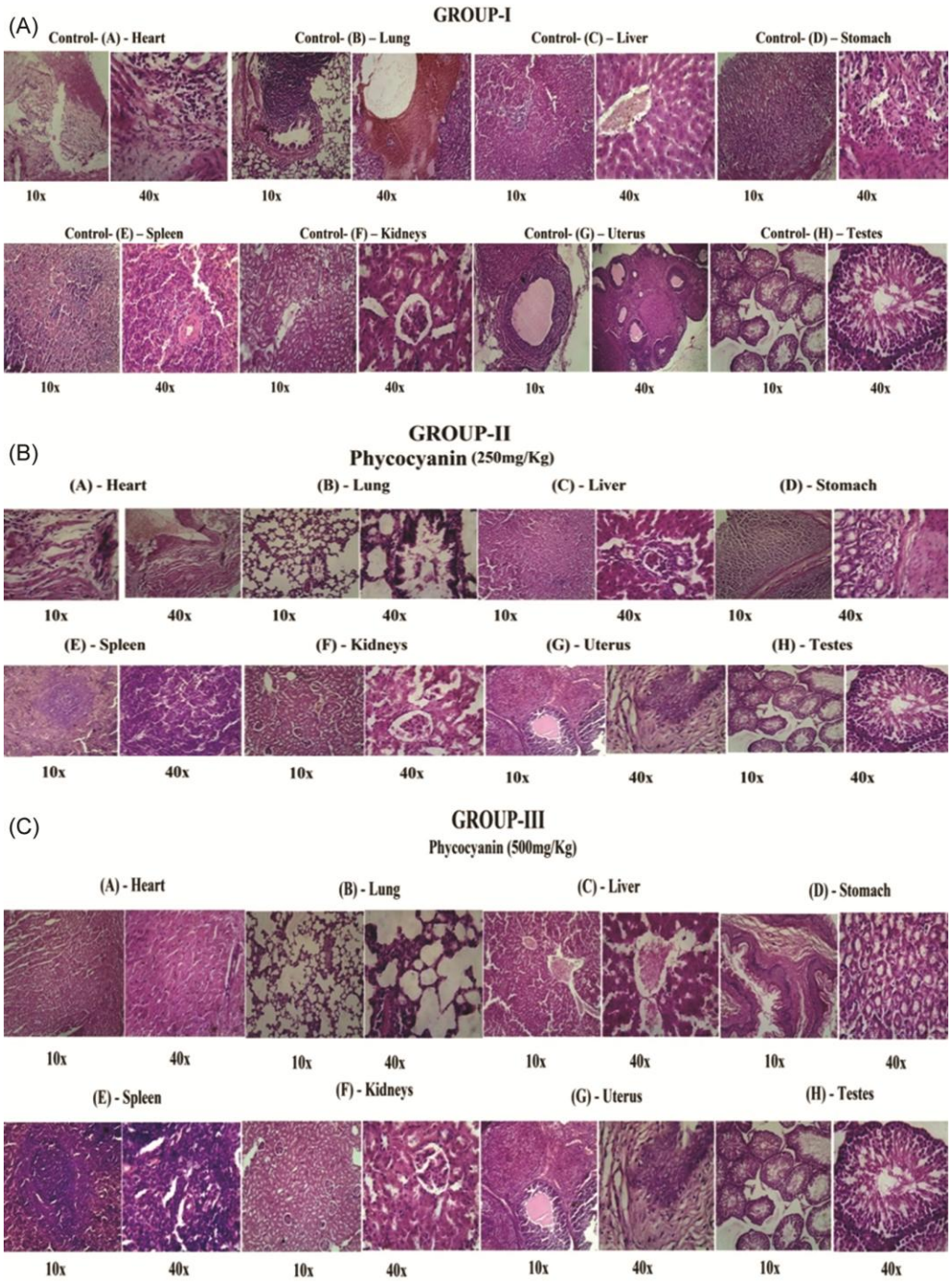


Fig 6 — (A) Histopathological examination of Group I (Control); (B) Histopathological examination of Group II (Phycocyanin 250 mg/Kg) treated animals; and (C) Histopathological examination ofGroup III (Phycocyanin C 500 mg/Kg) treated animals

mild dilatation. There is the presence of pan lobular inflammation composed of lymphocytes. Portal raid shows bile duct hyperplasia and mild periportal infiltration. Central vein and Sinusoids show mild dilatation.

Toxic effect of phycocyanin on tissue morphology of stomach also revealed the best biocompatibility. Section studied from the stomach shows normal mucosa. Parietal cells and peptic cells are normal in number, distribution, and morphology. Muscularis propria and serosa show no significant pathology. There is no inflammation/granuloma seen in the section studied. The section from lower part of oesophagus shows no structural modification. A similar finding

was also observed in both dosages of phycocyanin treated animals. There is no distinct histopathological effect was recorded in the treatment group. Normal morphological structure of the kidney was not affected in treatment groups. As in the control group, section studied from the kidney shows both cortex and medulla. The glomeruli show normal morphology. Both the tubules are histologically normal. The interstitium shows focal dense lymphocytic in filtrates. Blood vessels show congestion.

Biocompatibility of phycocyanin was also confirmed by no significant histopathic effect on the uterus. Uterus tissue sections of control and treatment groups illustrate the normal morphology of uterus tissue. Section studied from the uterus with cervix shows chronic cervicitis, various stages of ovarian follicle having fluid accumulation in the centre of the follicle (follicular antrum) showing normal development. The fallopian tube also shows normal development. Nontoxic effect of phycocyanin treatment is also supported by the complete absence of histological effect of both the dosages of phycocyanin on the testis. Both control and treatment demonstrated normal seminiferous tubules which confirm the normal spermatogenesis. There is no maturation arrest/atrophy seen.

**Discussion**

Cyanobacterial phycocyanin (PC) is one of the major light-harvesting biliproteins of Cyanobacteria and is of great importance because of its various biological and pharmacological properties. The success of phycocyanin isolation from *Spirulina* dry powder is primarily based on extraction procedure. Cold maceration is one of the best methods of extraction of PC from cyanaobacterial species. In this study, cold maceration method (using water and 0.1 M sodium phosphate buffer, pH 7.0) followed by sonication method was used which yield high stable phycocyanin[24]. Extracted phycocyanin was further purified by various purification techniques like ammonium sulphate precipitation, dialysis and column chromatography separation. For purifying phycocyanin, various precipitating agents are used. Ammonium sulphate is the widely used precipitating agent because of its high rate of precipitation which used to obtain high enriched phycocyanin in the extracted suspension and easier availability[27-30]. In the present study, enrichment of PC in the extracted solution was found be increased in ammonium sulphate precipitation and further increase in purity and yield of was recorded in successive purification steps. Antioxidative activity of purified phycocyanin was studied by DPPH assay which reveals tested phycocyanin brought about a high rate of free radical scavenging activity.

Biocompatibility of extracted phycocyanin was studied on Wister rat model by determination of behavioural changes, mortality, body weight, hematological, biochemical and histopathological parameters of rat model administrated with different dosages of PC. The present study clearly reveals a single oral dose of the phycocyanin showed no mortality and no changes in the body, organ weight of these rats even under higher dosage levels. Scientific reports indicate that decrease or increase in the body or organ weight is due to drug-mediated physiological stress[32]. These results showed that high margin of safety of the phycocyanin. Hematological and biochemical parameters also recorded no distinct effects on the treatment groups. Reports suggest that the bone marrow is responsible for the production of the blood cell and drugs derived from various sources have to affect red blood cell level[33]. In this study, the tested phycocyanin may not have harmful effects on bone marrow function and justify the fact that at all doses ofphycocyanin does not induce anemia and immune suppressive state making it safe. Many studies have confirmed that elevated serum levels of hepatic enzymes, transaminases (SGPT and SGOT) are not a directly linked for liver injury but increase levels are responsible to cause inflammation, cellular leakage and damage of cell membrane to cells in the liver[34,35]. Antioxidative enzymes are the major defense barriers which gives protection against oxidative cell damages[36]. The main target organ for drug or bioactive active compound is liver where exposed to the foreign substances being absorbed in intestines and metabolized to other compounds which may or may not be hepatotoxic to the mice[37]. Phycocyanin treatment did not show any significant rise in hepatic enzymes which suggests the best biocompatibility of the phycocyanin. Histopathological study also supported non-toxic effect of phycocyanin by showing no changes in the tissue morphology of all the tested tissues derived from organs. Nutraceutical therapy values of dietary proteins from the fresh water mussel *Lamellidens marginalis* (Lam.) reported by Haldar *et al*.[38] reveals the functional properties of bioactive peptides as in C-Phycocyanin a bioactive peptide from fresh water algae.

## Conclusion

Phycocyanin extracted from *Spirulina platensis* by cold maceration followed by successive purification brought about food grade phycocyanin C. It seems that PC exhibited less toxicity under *in vivo* studies. There was no obvious toxicity with the treatment of PC on the tested rat animal model. It was found to be safe nutraceutical and antioxidative agent. Further study helps to optimize the conditions for the enhanced production of phycocyanin and its utilization as the nutraceutical agent in the human trails.

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