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HEMATOLOGICAL AND HISTOPATHOLOGICAL EVALUATION OF *Anabaena oryzae* SOS13 PHYCOCYANIN IN WISTAR ALBINO RATS

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ABSTRACT

The safety of phycocyanin pigment extracted from *Anabaena oryzae* SOS13 was assessed by administration to Wistar Albino rats as one single dose or repeated doses (0, 50, 100 and 200 mg/kg body wt/day) over 28 days. Acute toxicity assay indicated the safety of *Anabaena* phycocyanin with the No-Observed-Adverse-Effect-Level (NOAEL) up to 5,000 mg/kg body wt. The changes in body or organ weight in *Anabaena* phycocyanin receiving rats during 28 days were within normal range of the control. The levels of serum protein and A/G ratio did not indicate any inflammatory or liver impairment and rather referred to ameliorated protein metabolism. SDS-PAGE confirmed that *Anabaena* phycocyanin have not any toxic influence on the expression of serum proteins or immunity system. The levels of serum glucose in the rats receiving *Anabaena* phycocyanin did not indicate any toxic effects. The levels of serum urea and creatinine in the phycocyanin receiving rats indicated not only the lack of renal toxic effects but also an ameliorating action on renal performance. *Anabaena* phycocyanin treatments were associated with insignificant increases in the level of hematological parameters revealed the absence of adverse effects on mature circulating cells, hemoglobin synthesis, white blood cell (WBC) synthesis, blood coagulation and homeostasis.

Key words: *Anabaena oryzae*, phycocyanin, acute toxicity, sub-acute toxicity.

INTRODUCTION

Phycocyanin is one of the major pigment constituents of cyanobacteria, used in many countries as dietary supplement whose nutritional and therapeutic values have been very well documented (Kay, 1991; Bockow, 1998). The pigment has a single visible absorption maximum between 615 and 620 nm and a fluorescence emission maximum at ~650 nm. Its molecular weight is between 70,000 and 110,000 Daltons. Phycocyanin is composed of two subunits, α and β, which occur in equal numbers, but the exact number of α and β pairs which make up the molecule may vary among the species (Romay *et al.,* 2003). SDS-PAGE for two subunits of phycocyanin extracted of *Anabaena oryzae* SOS13 indicated the presence of two main bands correspond to low molecular weights between 15 and 25 kD (Sitohy *et al.,* 2015). Phycocyanin extracted from *Calothrix* spp defined the molecular weight of the two comparable SDS-PAGE bands as 17 and 21 kD each while the estimated molecular mass of native purified phycocyanin was 114 kD (Santiago-Santos *et al.,* 2004). Phycocyanin from *Anabaena oryzae* SOS13 has α and β subunit with an isoelectric point near pH 6 (Sitohy *et al.,* 2015). Phycocyanins are increasingly required for technological application based on their multiple functionality (*e.g.;* antioxidant, antimicrobial, food colorant). *Anabaena* is potentially efficient and economical bio-source for phycocyanin based

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on and its ubiquity in nature, its ability to fix atmospheric nitrogen and its simple requirements (Sitohy *et al.,* 2015).

Numerous animal toxicological studies have been performed on phycocyanin by various institutions around the world. These studies include acute, subchronic, chronic, mutagenic, teratogenic / developmental toxicity, carcinogenic, and multiple generational/ reproduction studies. Naidu *et al.,* (1999) studied acute and sub chronic dose of phycocyanin extracted from *Spirulina platensis* on both sexes of albino rats. They found that the phycocyanin at high concentrations- 0.25 to 5.0 g/kg body weight (*W/W*) did not induce either any symptoms of toxicity or mortality of the animals. Feeding phycocyanin at low concentrations (0.5 to 4.0 g phycocyanin/kg diet) for 14 weeks did not affect food intake or body weight gain of phycocyanin-fad., rats compared to control animals. Terminal values on absolute and relative weights of vital organs, hematology and serum enzymes did not reveal any differences between phycocyanin-fed and control groups. Although the effective dosage range of phycocyanin in various animal models was 25-300 mg/kg body weight, the safety of the phycobiliprotein is good. The measured LD_{50} values were estimated to be greater than 3 g/kg for rats and mice. No mortality was induced even at the highest dose of phycocyanin tested (3 g/kg body weight). However, further preclinical pharmacological and toxicological studies are required to determine the safety of phycocyanin as potential drug. Also pharmacokinetic studies and of metabolism must be performed to the phycobiliprotein as previous stages to clinical trials (Romay *et al.,* 2003).

Anabaena phycocyanin was reported as a natural product extracted from a newly identified Egyptian cyanobacterial strain; *Anabaena oryzae* SOS13 with prospective food applications (Salama *et al.,* 2015). It is a potential candidate to be a good alternative and competitive to *Spirulina* phycocyanin. The safety of *Spirulina* phycocyanin has already been established (Naidu *et al.,* 1999; Mitra *et al.,* 2015) while the safety of *Anabaena* phycocyanin needs to be thoroughly examined, assessed and declared before any further food or pharmaceutical applications. So, the objective of the current study is to assess the safety of phycocyanin extracted from *Anabaena oryzae* SOS13 when administered by gavage to Wistar Albino rats as one single dose or repeated doses over 28 days. Acute and subacute toxicity potential of phycocyanin was investigated to give the safety profile of this new phycocyanin.

MATERIALS AND METHODS

Test Material

Anabaena oryzae SOS13 strain used in the present study was previously isolated from rice fields in Sharkia Governorate, Egypt and identified based on morphological and physiological characteristics (Salem *et al.,* 2011 and Ali 2012). This strain was fully identified using sequences of 16S ribosomal RNA gene and has been deposited in Gene Bank (Salama *et al.,* 2015). The nitrogen free medium ($BG^0$11) used to cultivate the pure culture of these strains.

Phycocyanin was extracted from the fresh *Anabaena* strain biomass using the methods of Sarada *et al.* (1999) with some modified by Salama *et al.* (2015). Thirty day grown *Anabaena* cells were harvested by centrifugation at 3000 xg for 5 min (Jouan, MR 1822, France) at 20°C. Cell pellets were separated and washed with 1M Tris–HCl buffer (pH 8.1). One volume of washed cell mass was re-suspended in five volumes of the same buffer and treated for the extraction of phycocyanin using the freeze-thaw method (freezing at -50°C and thawing at 25°C). The obtained suspension was centrifuged at 5000 xg for 10 minutes to separate the supernatant (the crude extract) which was kept in the refrigerator. The pigment absorption was measured at 615 and 652 µm by a JENWAY–England 6405 UV/VIS Spectrophotometer against 0.05 M phosphate buffer as blank. The procedure of Soni *et al.* (2006) was used for the purification of cyanobacterial phycocyanin (C-PC).

Experimental Animals

Adult healthy male and female white albino rats (*Rattus norvegicus*), Wistar strain (160 ± 10 g, body weight and 130 ± 10 g, body weight, respectively) were obtained from the Organization of Biological Products and Vaccine (Helwan Farm, Cairo, Egypt) and

housed in plastic cages in groups of 5 animals/cage. The experimental animals were allowed to acclimatize under the laboratory conditions (temperature of 22 ± 3°C; relative humidity 30 – 70% and normal light/dark cycle) for 2 weeks at least prior the experiment. They were provided with balanced pelleted diet (23 % protein) and tap water *adlibitum* throughout the adaptation and experimental period. The following animal studies were approved by the Medical Research Ethics Committee in the Faculty of Medicine, Zagazig University and conformed to NIH regulations.

Acute Toxicity Evaluation

According to OECD guideline for testing of chemicals, OECD TG 420 (OECD, 2001a), fifteen male Wistar Albino rats were divided randomly into three groups, 5 rats each. The first group received 2 ml distilled water free from any external treatment (control). The groups 2 and 3 received one acute dose of phycocyanin of 2000 and 5000 mg/kg body weight, respectively. Each group was force-fed using stomach tube with the different specified doses dissolved in distilled water (2 mL). All rats were kept under observation for 24 hr and symptoms of toxicity or mortality were recorded. Animals that were still alive were observed daily for behavioral and body weight changes for 14 days. The acute oral LD_{50} values were calculated using the software (Bio-Stat 2008 professional 5.5.0 – LD_{50}).

Subacute Toxicity Evaluation

According to OECD TG 420 (OECD, 2001b), forty male rats were divided into 4 groups of 10 rats each. Each group was intubated orally by force-feeding for 28 days using stomach tube with different doses of phycocyanin as follows: Group 1: received the same amount of distilled water by gavage without any external treatment and served as control. Group 2 received phycocyanin at doses of 50 mg/kg body weight/day. Group 3 received phycocyanin at doses of 100 mg/kg body weight/day. Group 4 received phycocyanin at doses of 200 mg/kg body weight/day. On the other hand, forty female rats were divided into 4 groups of 10 rats each. Each group was intubated orally by force-feeding for 28 days using stomach tube with different doses of phycocyanin as follows: Group 5 received the same amount of distilled water by gavage without any external treatment and served as control. Group 6 received phycocyanin at doses of 50 mg/kg body weight/day. Group 7 received phycocyanin at doses of 100 mg/kg body weight/day. Group 8 received phycocyanin at doses of 200 mg/kg body weight/day. All animals including control animals were supplied with balanced pelleted diet (23% protein) and tap water *ad libitum* during the testing periods. Rat body weight was recorded daily. Amounts of protein equivalent to those added in the form of the test articles were deduced from the protein component I the diets to keep them in the same levels for all groups. Rat body weight was recorded daily. On day 28^{th} (end of treatments), all the experimental animals were weighed, anesthetized with ether and sacrificed by cervical dislocation.

Body Weight and Organ Weights

Daily body weight of rats was recorded, and then the weight gains were calculated to investigate the effect of tested phycocyanin on body mass during the experimental period. Body weight gains (g) equal Final body weight (g) – (minus) Initial body weight (g).

Biochemical Analysis

Blood samples were collected into clean, dry and labeled Eppendorf tubes containing heparin as anticoagulant (7.5 IU) for clinico-biochemical tests. The serum was assayed *in vivo* for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total proteins, albumin and globulin, glucose and urea and creatinine.

Hematology

Blood samples were collected into clean, dry and labeled Eppendorf tubes containing heparin as anticoagulant (7.5 I.U.) for hematological analysis including white blood cell (WBC), red blood cell (RBC), platelet counts (PLT), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were carried out using the SYSMEX hematology auto analyzer (Japan).

SDS-PAGE of Serum Protein

SDS–PAGE of serum proteins was performed on a discontinuous buffered system according to the method of Laemmli (1970). An aliquot of serum (10 µl) was mixed with 70 µl of SDS-sample buffer (1 M Tris–HCl, 50% glycerol, 10% SDS, 10% β-mercaptoethanol, 0.1% Bromophenol blue, pH 6.8), heated at 96 ºC for 3 min and a 10 µl aliquot from the final mixture was submitted to electrophoresis.

Histo-Pathological Examination

The organs of randomly selected three rats from each group were subjected to histo-pathological examination. Vital organs such as kidney and liver were excised, examined grossly and subsequently fixed in 15% formalin saline. The fixed tissues were processed by dehydration in a series of graded ethanol concentrations, cleared with xylol and embedded in paraffin blocks. Sections of 4 µ thickness were obtained and stained by Hematoxilen - Eosin stain (H and E) for the histo-pathological analysis under light microscope (model OLYMPUS CX 41) at 400 or 1200 x magnification (Humason, 1979).

Statistical Analysis

All data were subject to statistical analysis by one–way ANOVA test (Gad, 2001) using SPSS software for Windows version 10. A probability of $P \leq 0.05$; $P \leq 0.01$; $p \leq 0.001$ was considered as the level of significance unless stated otherwise. Statistical significant differences between all treatments were carried by least significant differences method (LSD).

RESULTS AND DISCUSSION

Acute Toxicity

Three groups of Wistar Albino rats received *Anabaena* phycocyanin at three levels; 0, 2000 or 5000 mg/kg body weight by force-feeding and kept under experimental conditions and continuous observation for the following 14 days. No mortality was recorded for 14 days in the experimental animals following single gavage administration of two levels of *Anabaena* phycocyanin (2000 and 5000 mg/kg body wt/day (data not shown). Twenty four hours after dosing, signs of overt toxicity, abnormal breathing, impaired movements, *etc*. were totally absent in all tested groups (receiving 0.0, 2000 and 5000 mg phycocyanin/ kg body wt/day). All through the next 14 days, the experimental animals of the three groups did not show any signs of adverse effects of these treatments. The changes in body weight did not differ significantly ($p \geq 0.05$) among rat groups and the observed weight gains were in each rat group in the same range 16–18 g/rat. Hence the derived NOAEL (No Observed Adverse Effect Level) for a single dose is likely greater than 5,000 mg/kg body weight.

Absence of mortality after single administration of very high doses (2000 and 5000 mg /kg body wt/day) of *Anabaena* phycocyanin may indicate its safety. Based on the absence of any signs of abnormality during 14 days after the single dose administration *Anabaena* phycocyanin was associated with NOAEL up to 5000 mg/kg body wt.

Subacute Toxicity

Body and organ weight

The body weight increased with time in all the experimental animal groups (Fig. 1). No reductions in body weight were recorded in any group receiving phycocyanin. Conversely, the evolution of body weight over 28 days in phycocyanin-administered groups witnessed relatively higher increases than in control. The increase in body weight in the control male group after 28 days reached 81% of the original weight compared to 94, 90 and 88% for the rat groups receiving 50, 100 and 200 mg phycocyanin (5 times per week), respectively. Similarly, the control female group exhibited a relative increase in body over 28 days amounting to 58 % of the starting weight compared to 71, 69 and 82% in the rat female groups receiving 50, 100 and 200 phycocyanin (5 times per week), respectively. The absence of weight body impairment in the rat groups receiving phycocyanin, either male or female, will exclude phycocyanin-associated toxicity. Under similar conditions, toxic substances, *e.g.* lead acetate engender body weight reduction amounting to 40-44% of the control (Ibrahim *et al.,* 2012). Furthermore, the relatively higher increases in body weight may suggest some beneficial and anabolic effect of the tested substance.

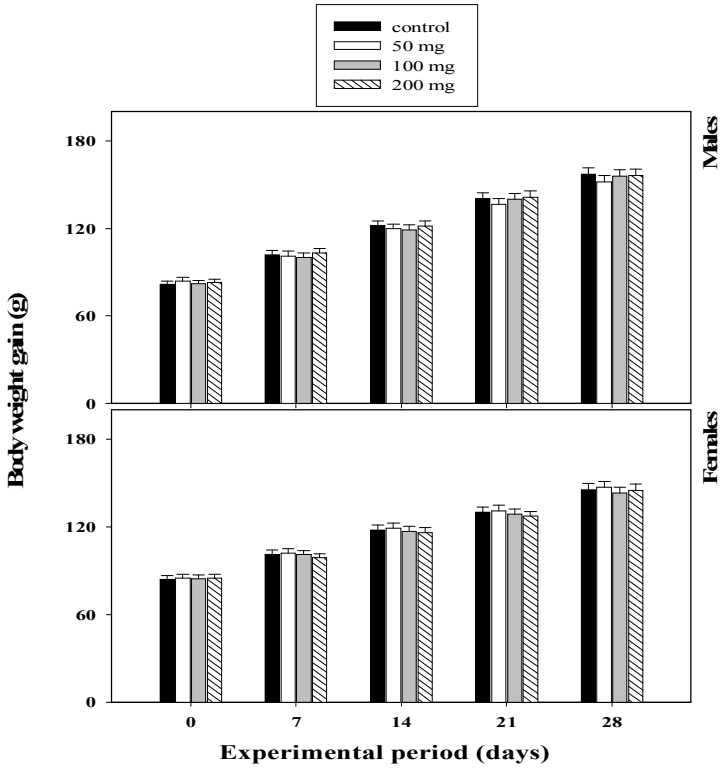

Fig. 1. Rat body weight gain (g) after receiving three different doses of phycocyanin (50, 100 and 200 mg/kg body weight) during 28 days (5 daily doses/week) as compared to control

The changes in organ weights of Albino rats administered different doses of phycocyanin (Table 1) were either slight or absent. For kidney and liver there were significant differences between the two first doses and control. All changes in spleen weight were insignificant in male. The lung weight did not significantly change in female animals but it increased significantly in male animals receiving high doses of phycocyanin. This trend applies to the sex organs. Mostly insignificant changes in organ weight were observed in heart and brain, except slight significant increases with high doses of phycocyanin in male animals. The same result applies to the female brain weight. The slight changes in organ weight either significant or insignificant refer to the absence of toxic effect. Normally toxic substances induce significant increases in organ weight associated probably with fat deposition in the affected organs (Ibrahim *et al.,* 2012).

Liver functions

The levels of liver enzymes, *i.e.*; alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) in Albino rates receiving different doses of *Anabaena oryzae* phycocyanin (Table 2) were slightly and significantly reduced by the dose of application. These reductions are more evident with the two high levels of phycocyanin namely 100 and 200 mg.

Since the elevation of serum aminotransferases (AST and ALT) is taken as an indicator of hepatotoxicity (Govindwar and Dalvi, 1990; Roy *et al.,* 2010; Ibrahim *et al.,* 2012), the obtained reduction in results apparently refer to the absence of hepatotoxicity, *i.e.* absence of damage or necrosis in the hepatocytes or impairment to liver function in the phycocyanin receiving groups. The slight observed reductions in AST and ALT may rather indicate some amelioration in the liver function and performance in the phycocyanin receiving groups probably as a result of the antioxidant potential of phycocyanin. In conclusion, phycocyanin is not only free of toxic hazards but it may also have a protective effect on liver functions either in male or female animals.

Table 1. Organs weight (g) of Albino rats receiving different doses of *Anabaena oryzae* Phycocyanin during 28 days (five doses/week)

Phycocyanin (mg)	Liver	Kidney	Spleen	Lung	Heart	Brain	Sex organ
	G						
	Male						
0.0	5.75±0.23^{b}	1.39±0.08^{b}	0.56±0.008^{a}	0.44±0.01^{b}	0.71±0.002^{a}	1.05±0.02^{a}	2.95±0.04^{b}
50	5.33±0.32^{a}	1.30±0.1^{a}	0.54±0.006^{a}	0.31±0.007^{a}	0.65±0.005^{a}	1.04±0.05^{a}	0.46±0.05^{a}
100	5.86±0.21^{b}	1.42±0.09^{b}	0.61±0.005^{a}	1.01±0.008^{c}	0.73^{a}±0.005^{b}	1.13^{a}±0.01^{b}	3.12±0.02^{c}
200	6.48±0.12^{c}	1.53±0.05^{c}	0.57±0.004^{a}	1.08±0.032^{d}	0.82±0.003^{b}	1.16±0.03^{b}	3.19±0.13^{c}
	Female						
0.0	5.56±0.17^{b}	1.30±0.03^{b}	0.52±0.006^{b}	0.41±0.005^{a}	0.64±0.003^{b}	0.96±0.01^{a}	0.13±0.008^{a}
50	5.30±0.2^{a}	1.24±0.02^{a}	0.42±0.004^{a}	0.35±0.004^{a}	0.57±0.002^{a}	0.90±0.02^{a}	0.37±0.005^{a}
100	5.61±0.11^{b}	1.32±0.06^{b}	0.54±0.005^{b}	0.44±0.007^{a}	0.65±0.005^{b}	1.00±0.01^{b}	0.11±0.006^{a}
200	5.50±0.21^{b}	1.30±0.04^{b}	0.51±0.002^{b}	0.44±0.003^{a}	0.63±0.004^{b}	1.00±0.01^{b}	0.12±0.002^{a}

Table 2. Alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) in Albino rats receiving different doses of *Anabaena oryzae* phycocyanin

Phycocyanin (mg)	ALT	AST	ALP
	IU/L		
	Male		
0.0	35.06± 1.7 b	55.6± 2.0 c	123.3± 2.7c
50	34.73± 1.4 b	51.0± 1.2 b	116.3± 1.7b
100	34.07± 0.7 a	47.3± 1.5 a	111.0± 1.5b
200	33.67± 0.9 a	43.0± 1.2 a	104.0± 1.0a
	Female		
0.0	33.23± 1.4 a	54.6± 4.6 c	207.0± 4.0 c
50	32.73± 1.4 a	48.3± 0.9 bc	195.0± 1.2 b
100	32.33± 1.2 a	41.3± 0.9 b	157.3± 4.9 a
200	31.87± 0.8 a	31.3± 2.8 a	147.3± 3.3 a

Serum proteins

Total serum protein in all phycocyanin receiving rats are generally within normal levels for male and female Albino rats (Table 3), ranging from 6.6 to 7.5 and from 5.8 to 6.5 g/ dL in male and female rats, respectively. These values were significantly higher than control referring to a rather beneficial effect of phycocyanin on liver function. The general metabolism as toxicity is normally associated with a decrease in the total serum protein (Roy *et al.,* 2010). However, the increases in total serum proteins were smaller in female than in male animals. The shown slight insignificant increases in A/G ratio in rats receiving phycocyanin do not indicate any adverse effect on immune system since both albumin and globulin were increased by the treatment. But the increase in albumin synthesis was relatively and slightly higher than the increase in globulin, *i.e.* globulin production was not negatively affected by the treatment. Conversely, the enhanced production of both albumin and globulin refer to good liver function and protein metabolism. The slightly increased or decreased levels of A/G ratio may indicate the absence of any possible hazard on liver and kidney and that the biological systems of the treated animals are within safety limits. Moreover, these changes in A/G ratio do not indicate any emergent diseases in the phycocyanin treated rats. The normal levels of total serum proteins and A/G ratios for the rats receiving different doses of phycocyanins refer to the absence of immune toxicity. Alternatively since this A/G ratio did not show any significant decrease it can be concluded that phycocyanin treatments were not associated with any inflammatory or liver impairment (http:// labtestsonline. org/ understanding/analytes/tp/tab/test/). The changes in A/G ratio are slighter than those observed by Moon *et al.* (2014) in testing some antiviral vaccines.

Serum electrophoretic pattern

Serum electrophoretic patterns of Albino rats receiving different levels of *Anabaena* phycocyanin are shown in Fig. 2 as compared to control. The results reveal very similar electrophoretic patterns among the treatments and control, indicating primarily the absence of any major change in serum proteins or associated phenomena. No differences can be observed with raising the dose of phycocyanin up to 200 mg/rat/day (5 days/week for 4 consecutive weeks). Measuring the intensities of the revealed bands indicate that, albumin bands were generally enhanced by the phycocynin treatments. The bands representing globulins were either similar between control and phycocyanin treatments or (α1 and β-globulin) or slightly enhanced (α2 and γ-globulin) without clear concentration effect. It can be concluded that phycocyanin, at the used doses (50, 100 and 200 mg/rat/day) have not any toxic influence on the expression of serum proteins. It can be concluded that phycocyanin has an anabolic effect on the synthesis of serum albumin while the relatively slight increases in some globulin fractions (α2 and γ-globulin) may not refer to any serious inflammation or immunological reaction. Absence of any reducing effects on serum globulin may add some evidence of the absence of toxic effect on the immunity system. The particular stability or slight increases in the level of γ--globulin may also indicate an ordinary unaffected production of antibodies and the safety of immune system.

The specific profile of the globulins manifested by protein electrophoresis indicates the four major globulin groups: gamma globulins, beta globulins, alpha-2 globulins, and alpha-1 globulins. Gamma globulin, which is immunologically active and composed predominantly of antibodies of IgG type, was not negatively affected. Consequently it can be concluded that antibody synthesis is within its normal status and has not been negatively affected with the ingestion of phycocyanin. The overall distribution of the different serum proteins in phycocyanin-receiving rats did not show any deviation from the pattern of the control group confirming the previous conclusion that phycocyanin ingestion did not engender considerable changes in the protein metabolism or immunity system. The present conclusions that tested treatments do not exert toxic effects based on normal electrophoretic pattern and the absence of negative effects on albumin fractions are in line with Hashem *et al.* (2010).

Table 3. Serum albumin and fractions in Albino rats receiving different doses of *Anabaena oryzae* phycocyanin for 28 days

Phycocyanin (mg)	Serum Protein	A (Serum Albumin)	G (Serum Globulin)	A/G ratio
	g/dL			
	Male			
0.0	6.6± 0.12 a	3.67± 0.22 a	2.90± 0.13 a	1.26± 0.05 a
50	6.8± 0.06 a	3.87± 0.09ab	2.93± 0.10ab	1.32± 0.04 a
100	7.2± 0.06 b	4.03± 0.12 b	3.17± 0.12 b	1.27± 0.04 a
200	7.5± 0.12 b	4.33 ± 0.09 b	3.17± 0.15 b	1.36± 0.06 a
	Female			
0.0	5.6± 0.06 a	3.63± 0.09 a	1.97± 0.08 a	1.84± 0.10 a
50	5.8± 0.06 b	3.70± 0.06 ab	2.10± 0.11ab	1.76± 0.08 a
100	6.2± 0.06 c	3.90± 0.06 bc	2.30± 0.13 c	1.69± 0.08 a
200	6.5± 0.06 d	4.07± 0.09 c	2.43± 0.13 c	1.67± 0.07 a

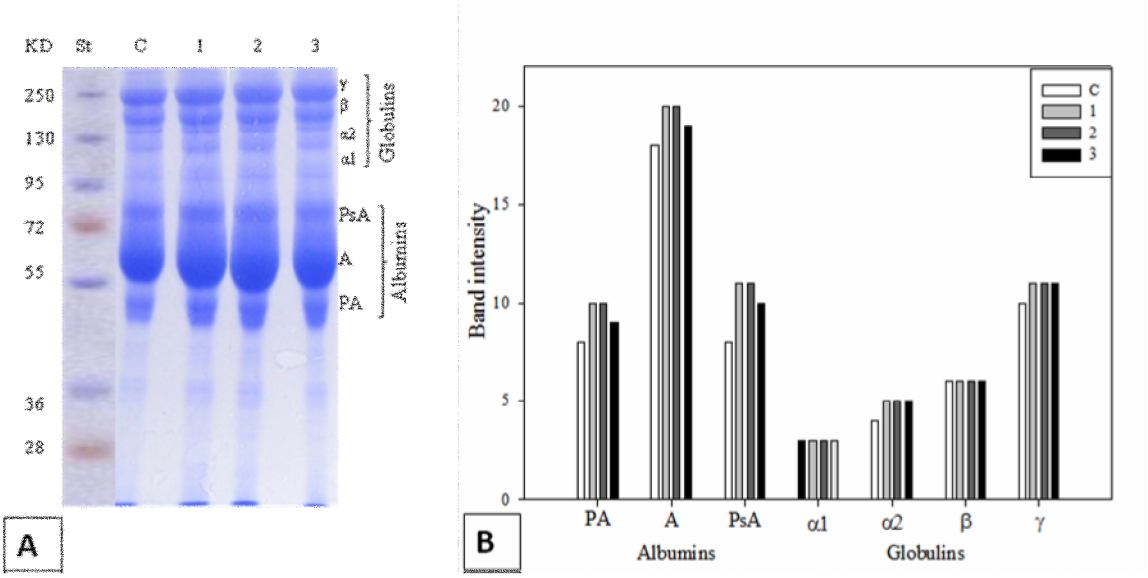

Fig. 2. SDS–PAGE profile (A) and band intensities (B) of serum of male Albino rats receiving *Anabaena oryzae* phycocyanin at different doses (50, 100 and 200 mg/ kg body weight) during 28 days (5 daily doses/week) as compared to control. Lane St: protein molecular weight markers; lane C: control; lanes 1, 2 and 3: phycocyanin at 50, 100 and 200 mg/kg body wt/ day, respectively. G = Globulins, PsA = post-albumin, A = albumin, PA = pre-albumin

Serum glucose and renal parameters

Data in Table 4 indicate that serum glucose levels were slightly and significantly reduced in the male Albino rats receiving high doses of phycocyanin (100-200 mg). The reductions represent 1-4% of the control level corresponding to the high phycocyanin doses. In female Albino rats receiving the high doses of phycocyanin, showed insignificant decreases in serum glucose levels, ranging from 0.7-2.6%. The general overview of this biochemical parameter indicate normal biochemical and physiological status of the rat receiving the different doses of *Anabaena* phycocyanin. The slight reductions (0.7-4%) in serum glucose levels are very far from the changes reported for lead poisoning which were ranging between 21-30% reduction (Ashour *et al.,* 2007). As toxicity is usually associated with abnormal glucose metabolism (Ghorbe *et al.,* 2001) and to adversely affect endocrine function related to glucose metabolism (Baccarelli, 1999), it can be concluded that the current case does not represent toxic effects.

The recorded values of renal parameter (urea and creatinine) indicate either insignificant reductions, for the all doses of phycocynin with male rats. These reductions were in the range of 6-33% for serum urea and 16-32 for serum creatinine. Female rats showed insignificant reductions in serum urea but significant ones with serum creatinine due to all the applied doses (50-200 mg). These reductions were in the range of 9-30 and 19-40% for serum urea and creatinine in female Albino rats receiving phycocyanin, respectively. Toxic renal effects are normally manifested with increases in the level of serum urea (Khalil *et al.,* 1992; Ashour *et al.,* 2007). Ashour *et al.,* (2007) reported that lead poisoning (1000 ppm) was associated with significant increases in urea valued 24-42% after 20-40 days as a result of protein catabolism. It is clear in this study that the results may conversely indicate an anabolic effect on protein metabolism.

Increased creatinine level is taken as suitable prognostic indicator of renal dysfunction in case of toxicity (Oberley *et al.,* 1995; Weaver *et al.,* 2003; Ashour *et al.,* 2007). This study showed a complete opposite trend (decreased creatinine) indicating not only the lack of renal toxic effect but rather a further ameliorating action on renal performance.

Hematological parameters

The hematological parameters for Albino rats receiving pycocyanin at three different doses for 28 days (5 days per week) are shown in Table 5. Insignificant slight changes from control can be seen in the levels of red blood corpuscles (RBC) in both male and female rats receiving three doses of phycocyanin (50,100 and 200 mg phycocyanin per rat, 5 times per week). This may indicate that phycocyanin does not adversely affect the synthesis of RBC and does not consequently pose any associated health hazard. Conversely, *Anabaena* phycocyanin treatments were associated with insignificant increases in the level of hemoglobin in male rats and some significant increases in female rats. These findings may indicate the absence of destruction of mature circulating cells or loss of cells from the circulation by haemorrhage, or reduced RBC production (Nunia *et al.,* 2007).

The levels of Hematocrit (HCT) of the *Anabaena* phycocyanin-administered rats and control are within the normal range for male and female and with insignificant increases, confirming the beneficial effect of *Anabaena* phycocyanin on RBC and HGB in male. It may also confirm the absence of a potential health hazard associated with *Anabaena* phycocyanin administration such as anemia, diet deficiency, leukemia or any other medical condition. The level of mean corpuscular volume (MCV) which is the average volume of red cells had not any significant changes in male rats receiving phycocyanin compared to control, while female rats receiving the same treatment showed slight significant decreases. However all recorded levels are within the normal range, confirming the previous conclusions that *Anabaena* phycocyanin is not associated with any hazard affecting the status of red blood corpuscles. The changes in mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were insignificant and within normal range for phycocyanin-receiveing male or female rats, confirming the previous results and conclusion that the treatment does not adversely affect hemoglobin synthesis.

Table 4. Serum glucose, urea and creatinine in Albino rats receiving different doses of *Anabaena oryzae* phycocyanin for 28 days

Phycocyanin (mg)	Glucose (mg/dL)	Urea (g/dL)	Creatinine (mg/dL)
		Male	
0.0	96.6± 0.88 c	49.0± 6.0 b	0.63± 0.09 b
50	98.6± 0.33 c	42.6± 1.3 ab	0.67± 0.03 b
100	95.6± 0.67 b	39.0± 0.6 ab	0.53± 0.03 ab
200	92.3± 1.12 a	32.6± 1.6 a	0.43± 0.03 a
		Female	
0.0	91.6± 0.9 b	50.3± 8.8 a	0.70± 0.06 b
50	92.0± 0.6 b	45.6± 1.2 a	0.57± 0.03 a
100	91.0± 0.6 b	42.6± 1.5 a	0.47± 0.03 a
200	89.3± 1.2 b	35.3± 1.5 a	0.43± 0.03 a

Table 5. Hematological parameters in Wistar Albino rats receiving three doses of *Anabaena oryzae* phycocyanin during 28 days (5 times/week)

	Phycocynin concentration (mg)			
Parameter	**0.0**	**50**	**100**	**200**
		Male		
RBC ($\times 10^6$ /mL)	6.29± 0.14 a	6.25± 0.10 a	6.37± 0.03 a	6.47± 0.09 a
HGB (%)	15.2± 0.38 a	16.4± 0.82 a	16.8± 0.43 a	16.6± 0.64 a
HCT (%)	44.4± 0.59 a	44.3± 1.19 a	46.7± 0.90 a	47.3± 1.20 a
MCV (FL)	88.3± 0.40 a	89.4± 1.23 a	84.8± 3.98 a	92.2± 1.17 a
MCH (pg)	25.5± 0.27 a	26.2± 0.38 a	27.5± 0.29 a	26.9± 1.12 a
MCHC (g/dL)	33.3± 0.88 a	31.6± 0.31 a	31.2± 0.62 a	32.7± 0.46 a
WBC ($\times 10^3$/ mL)	7.83± 0.38 b	6.33± 0.22 a	7.67± 0.15 b	6.67± 0.19 a
PLT ($\times 10^3$/µL)	240± 6.3 c	238± 4.4 c	220± 14.1 b	221 ± 4.5 a
		Female		
RBC ($\times 10^6$ /mL)	7.37± 0.09 a	6.97± 0.09 a	7.23± 0.23 a	7.30± 0.15 a
HGB (%)	13.4± 0.54 a	13.6± 0.31 ab	14.7± 0.21 bc	15.7± 0.33 c
HCT (%)	36.9± 0.47 a	41.1± 0.47 b	43.3± 0.35 c	44.3± 0.67 c
MCV (FL)	89.7± 1.20 b	84.7± 1.20 a	85.3± 0.67 a	91.7± 0.88 b
MCH (pg)	30.7± 1.85 a	29.3± 1.20 a	31.3± 0.64 a	31.9± 0.98 a
MCHC (g/dL)	31.5± 0.34 a	31.0± 0.1.53 a	32.3± 1.45 a	33.4± 1.05 a
WBC ($\times 10^3$/ mL)	8.67± 0.07 c	8.47± 0.26 bc	7.77± 0.15 ab	7.50± 0.32 a
PLT ($\times 10^3$/µL)	375 ± 3.80 a	369 ± 2.60 a	372 ± 1.70 a	374 ± 4.20 a

RBC: Red blood corpuscles, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, WBC: White blood corpuscles, PLT: Platelets.

Generally the changes in the serum RBC, HCT, HGB, MCV, MCH and MCHC of *Anabaena* phycocyanin-treated Albino rats were either insignificant or slight increases. But there is no evident reducing effect indicating toxic effects. Decreases or significant changes in such parameters are normally correlated with toxic action (Svoboda *et al.,* 2001; Saoudi *et al.,* 2011).

Slight changes were also observed in white blood corpuscles (WBC) in response to the administration of phycocyanin. All changes were slight reductions in phycocyanin level and no increases in this parameter were recorded for any treatment. The absence of any significant elevation in the level of WBC may indicate that *Anabaena* phycocyanin treatments are not associated with any potential inflammatory phenomena. The toxic substances are normally associated with significant increases in the levels of WBC (Celik and Suzek, 2008; Roy *et al.,* 2010). Therefore the absence of similar increases in the level of WBC may mean the absence of toxic potential. This may also indicate that *Anabaena* phycocyanin did not stimulate the formation of WBC as a xenobiotic substance, *i.e.* the animal organism is tolerating the presence of phycocyanin within its biological and metabolic activities.

Platelets level was reduced by the high doses of phycocyanin in males, but females showed only insignificant changes when receiving the test substance. The reductions in male animals amounted about 8% of the original value. However, all platelets values are within normal range. Since the reduced level of platelets is still much higher than the lower limit of normal platelet range, it can be concluded that phycocyanin does not adversely affect platelet functionality. *Anabaena* phycocyanin does not cause either too high platelet count (thrombocytosis) or too low one (thrombocytopenia). So the substance of study is most probably free from associated health hazards. The slight reduction (8%) in platelet level by *Anabaena* phycocyanin high dose (200 mg/rat/day) is relatively very slight when compared to reduction induced by toxic substances (*e.g.* deltamethrin) amounting up to 63% of the control value (Saoudi *et al.,* 2011). It can be concluded that *Anabaena* phycocyanin (50-200 mg/rat/day) does not have potential toxic effect on blood coagulation and blood homeostasis (Abbes *et al.,* 2006).

Histo-pathological examination

Histological slices of rat organs were examined at the end of the experimental period and those of liver and kidney are shown in Fig. 3 as models for the other organs. Liver sections of the rats receiving *Anabaena* phycocyanin showed normal arrangements of central vein and normal liver cords. They are void of any abnormality, necrosis or deformation. The nuclei seem unaffected. Likewise, the kidney sections of the *Anabaena* phycocyanin receiving rats had normal renal tissue with normal renal glomeruli, renal tubules very similar to control.

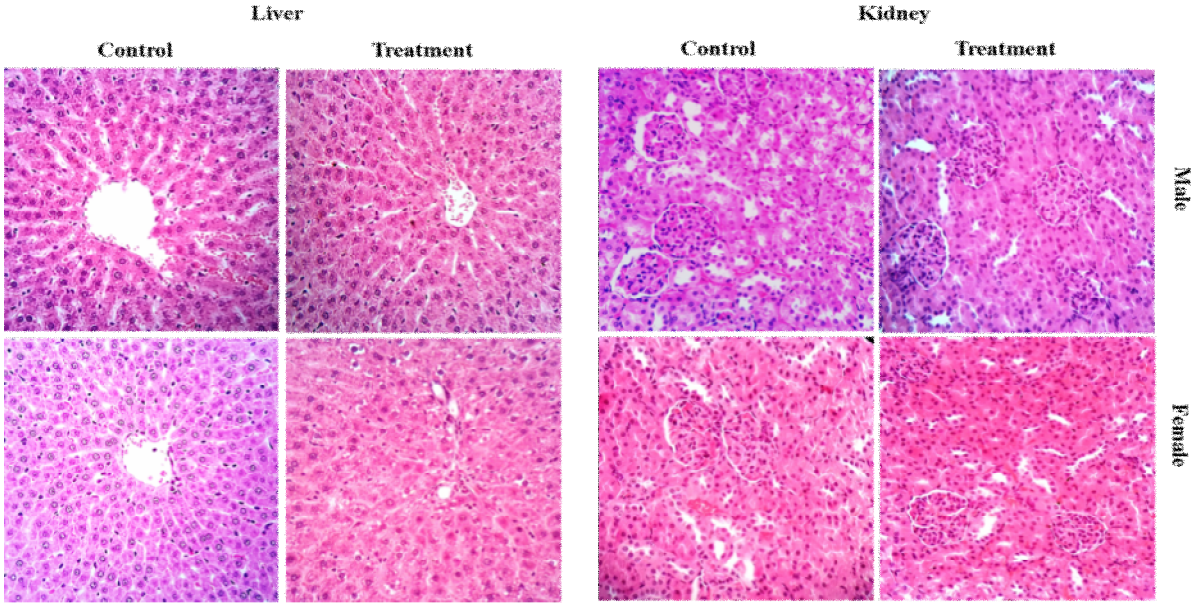

Fig. 3. Histo-pathological assays of the organs of rats receiving different doses of phycocyanin (50, 100 and 200 mg /kg body weight) during 28 days (5 daily doses/week) as compared to control

Toxicity is normally associated with a necrosis and dissolution of the nucleus and nuclear membrane (El Malti *et al.,* 2007). Major alterations in the hepatocytes are caused by toxic substances including different signs of histological deformations including mainly anisokaryosis, nuclear vesiculation, binucleation, cytoplasmic inclusions, cytoplasmic swelling, hydropic degeneration, and necrosis (Jarrar and Taib, 2012). Freedom of abnormalities and deformation and the soundness of nuclei confirm the previous conclusions that Anabaena phycocyanin does not pose toxic hazards on animal organs.

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تقييم تأثير فيكوسيانين الانابينا أوريزا SOS13 على صفات الدم والصفات التشريحية والمرضية فى فئران ويستار البيضاء

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تم تقييم سلامة الفيكوسيانين المستخلص من *Anabaena oryzae* SOS13 بإستخدام تقنية الفئران البيضاء صنف ويستار كجرعة واحدة أو جرعات متكررة (٠، ٥٠ و ١٠٠ و ٢٠٠ ملجرام/كجم من وزن/اليوم) لمدة ٢٨ يوم. وأشارت نتائج تقييم السمية الحادة لهذا الفيكوسيانين أنه ليس له أى تأثير سام حتى معدل ٥٠٠٠ ملجرام/ كجم من وزن الجسم. وكانت التغيرات في وزن الجسم لدى الفئران التى تلقت جرعات من هذا الفيكوسيانين لمدة ٢٨ يوماً فى حدود المعدل الطبيعي مقارنة بالكنترول، لم يشر مستوى البروتين في الدم وكذلك نسبة الالبيومين الى الجلوبيولين إلى وجود التهابات أو قصور فى الكبد بل على العكس أشارت إلى تحسن فى تمثيل البروتين فى الجسم الفئران، أظهرت نتائج SDS-PAGE أن هذا الفيكوسيانين ليس له أي تأثير سام على تعبير بروتينات السيرم أو الجهاز المناعى فى الجسم، كما أظهرت كذلك مستويات الجلوكوز في سيرم دم الفئران التي تلقت هذا الفيكوسيانين عدم وجود أي آثار سامة له، كذلك أشارت مستويات اليوريا والكرياتينين في الدم أن هذا الفيكوسيانين ليس له آثار سامة على الكلى فحسب بل أدى كذلك إلى تحسن فى أداء الكلى فى الجسم، كما أدى إستخدام هذا الفيكوسيانين إلى عدم حدوث زيادة معنوية فى مقاييس الدم التى تشير إلى غياب التأثيرات السالبة على خلايا النقل الناضجة وتخليق الهيموجلوبين وتكوين خلايا الدم البيضاء وتجلط الدم والنزيف.

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