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RESEARCH ARTICLE

Effect of blue green algae on some biochemical and hematological markers in mice

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Abstract

Blue-green algae (BGA) are among the most primitive living organisms on Earth. The present work was conducted to assess the effect of BGA (*Aphanizomenon flos-aquae*) on some biochemical and hematological markers in male albino mice. Results revealed that oral administration of 100mg/kg of BGA for 15 consecutive days led to a very high significant elevation ($P<0.0001$) in hepatic catalase (CAT), superoxide dismutase (SOD), glutathione-S-transferase (GST) and glutathione peroxidase (GPX) activities as compared to those of the normal controls. In contrast, the level of hepatic lipid peroxidation, measured as malondialdehyde (MDA) was very high significantly decreased ($P<0.0001$) in mice treated with BGA. Meanwhile, the treatment of mice with BGA for 15 days did not exhibit a toxic effect on the liver and kidney. Also, oral administration of BGA for 15 days significantly increased the number of RBCs ($P<0.05$) and very high increased ($P<0.0001$) in the values of RBCs, Hb, PCV and platelets as compared to those of the controls. In conclusion, BGA can induce the generation of antioxidant enzymes with no harmful side effect on both liver and kidney and helps in improvement of the hematological markers.

Introduction

Blue-green algae (BGA), also known as cyanobacteria, among the phylum of bacteria that utilize photosynthesis to obtain energy. They are technically classified as bacteria but share properties with bacteria (Schaap et al., 2012). They have been used as a source of nutrients and in folk medicine by humans in Asian, African and South American countries (Johnson et al., 2008). Studies have reported various health benefits of BGA, including immune functions, anti-inflammatory, anti-bacterial, anti-viral, anti-cancer, hypocholesterolemic, hypotriglyceridemic and antioxidant properties properties (Kumar et al., 2003). Also, the study of Venkatesen and his colleagues revealed that the most common BGA, *Spirulina platensis* (SP) and *Aphanizomenon flos-aquae* (AFA) were found to have antioxidant (Venkatesan et al., 2012), anti-inflammatory and hypolipidemic properties (Tiniakos et al., 2010 and Yang et al., 2011). *Aphanizomenon flos-aquae* (AFA), fresh water unicellular blue-green algae, that spontaneously grow in Upper Klamath Lake, Germany; it is consumed as a nutrient-dense food source and for its health-enhancing properties (Pugh and Pasco, 2001)

Antioxidants are the primary line of defense against reactive oxygen species (ROS). Superoxide dismutase (SOD) and catalase (CAT) are the key antioxidative enzymes that provide protective defense against ROS. The glutathione S-transferase (GST) belongs to a family of enzymes comprises a long list of cytosolic, mitochondrial and microsomal proteins that are capable of multiple reactions with a multitude of substrates. It is well known that GST and glutathione peroxidase (GPX) play important roles in the detoxification of toxic metabolites (Hubatsch et al., 1998).

Liver and kidney have vital activities in the body that their enzymes served as important biomarkers for different cell organelles and any defect of them will be reflected in their enzyme activities (Das et al., 2012). The haematological parameters have been considered as diagnostic indices of pathological conditions in animals (Lahr et al., 1993). BGA supplements diet with highly rich nutrients. So, the increase in RBC, Hb, and PCV might be due to the effect of BGA on blood-forming organ (Venkatesan et al., 2012), while the increase of WBC count can be

correlated with an increased in antibody production (Joshi et al., 2002). The target of the current study is to investigate the potential effect of BGA supplement on some biochemical and hematological markers of normal mice.

Materials and methods

Materials

Blue green algae (BGA) tablet (250 mg) (*Aphanizomenon flos-aquae*) was obtained from German Pharmaceutical Industries (Life Blau-Green Alge, Hergestellt, Deutschland). Blue green algae tablet was ground and suspended in 10 ml of distilled water immediately before use.

Animals and study design

Twenty healthy adult male Swiss albino mice (25 ± 2g) were purchased from the Egyptian Organization for Biological Products and Vaccines, Cairo, Egypt, and maintained at the animal room of Zoology Department, Faculty of Science, Menufiya University. The animals were handled under standard laboratory conditions with a 12-h light/dark cycle at a temperature of 25 ± 2^{o}C, and they had free access to standard food and water. The experimental protocol followed the international principles for Laboratory Animal Use and Care as approved by animal use ethical committee, Faculty of Science, Menufiya University. Animals were randomly divided into two groups, 10 mice each; group I animals were orally administrated with 100 mg/kg of blue green algae suspension daily for 15 consecutive days (Kuriakose and Kurup, 2010). Group II animals served as controls. All animals were maintained on normal chow and water *ad libitium*.

At the end of experiment, peripheral blood was collected and serum was separated by centrifugation at 3000 rpm for 5 minutes and kept at -20ºC until use. Livers were removed and rinsed with physiological saline. A half gram of liver was weighed and mechanically homogenized by using electrical homogenizer (Potter-Elvehjem) in a 10-fold volume of ice-cold 20mM tris-HCl buffer, pH 7.4, (Sigma, USA). The homogenate was divided into aliquoted and kept at -70°C.

Measurement of hepatic malondialdehyde (MDA) concentration

The level lipid peroxidation in liver homogenate was estimated colorimetrically by measuring malondialdehyde (MDA) using the method of Ohkawa et al. (1979) and by following the manufacturer's procedure (Biodiagnostics, Egypt).

Estimation of hepatic superoxide dismutase activity

The activity of hepatic superoxide dismutase was estimated according to the procedure of Nishikimi et al. (1972) and by following the manufacturer's procedure (Biodiagnostics, Egypt).

Estimation of hepatic catalase activity

The activity of catalase was estimated in liver homogenate according to the method of Aebi (1984) and by following to the manufacturer's procedure (Biodiagnostics, Egypt).

Estimation of hepatic glutathione peroxidase activity

The measurement of glutathione-S-transferase (GST) activity in liver homogenate was spectrophotometrically assayed by using 1-chloro-2-4-dinitrobenzene (CDNB) as described by Habig et al. (1967) and according to the manufacturer's procedure (Biodiagnostics, Egypt).

Estimation of hepatic glutathione-S transferase activity

Glutathione peroxidase (GPX) activity was estimated in liver homogenate according to the method described by Peglia and Valentine (1967) and by following to the manufacturer's procedure (Biodiagnostics, Egypt).

Liver function tests

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum were estimated according to the method described by Reitman and Frankel (1957). Also, the activity of serum alkaline phosphatase (ALP) was measured colorimetricaly according to the method of Kind and King (1954). Serum total protein (TP) was determined by a colorimetric method using bovine serum albumin as standard as described by Jacobs and his colleagues (Jacobs et al., 1964). The level of serum albumin (Alb) was determined by using the method of Baure (1982). Hepatic gamma-glutamyl transferase (GGT) activity in the liver tissue homogenate was determined colorimetrically according to the method of Bergmeyer et al. (1987).

Kidney function tests

The level of serum creatinine was measured colorimetrically as described by Slot (1965) and blood urea nitrogen (BUN) was measured according to the method described by Mather and Roland (Mather and Roland, 1969).

Hematological parameters

Blood samples used for hematological analysis were collected into EDTA-containing tubes. Erythrocytes (RBCs), total leucocytes count (WBCs), hemoglobin (Hb), hematocrit value (PCV) and platelet values were determined using the cell counter (ADVIA 60/Cell Dyne counting, ABOTT1800, Ireland) (Monreal et al., 1993).

Statistical analysis

The data were presented as mean ± standard deviation. The significance of the difference between the means was compared using the paired Student's t- test (Sokal and Rohlf 1981). The level of significance was accepted at $P<0.05$, high significant at $P<0.001$ and very high significant at $P<0.0001$.

Results

Antioxidant and oxidative status markers

Table (1) illustrates the activities of antioxidant enzymes (CAT, SOD, GST and GPX) and MDA level in the liver homogenate of mice. Results illustrates that the oral administration of normal mice with 100 mg/kg of BGA daily for two weeks resulted in a very high significant elevation in the activities of CAT, SOD, GST and GPX as compared to those of the normal controls ($P<0.0001$). In contrast, the hepatic level of lipid peroxidation was very high significantly decreased in mice treated with BGA when compared with that of the control group ($P<0.0001$).

Table (1): Effect of blue green algae on hepatic antioxidant and oxidative status biomarkers

Parameters	Control	BGA	P
CAT (U/L)	16.9±0.9	25.8±0.5***	<0.0001
SOD (U/g wt tissue)	170±9	197±7.5***	<0.0001
GST (µM/min/g wt tissue)	0.56±0.03	0.86±0.02***	<0.0001
GPX (U/L)	42.9±1.1	56.0±0.7***	<0.0001
MDA (µmol/g wt tissue)	18.9±1.5	16.8±2.0***	<0.0001

Data are expressed as Mean ± SD
(ns) Non significant
(*) Significant difference ($P<0.05$)
(**) High significant difference ($P<0.001$)
(***) Very high significant difference ($P<0.0001$)

Effect of BGA on kidney and liver function

Table (2) shows the activities of ALT, AST, ALP and GGT as well as the levels of total protein and albumin in serum of mice after administration of BGA for 15 two weeks. There is no significant change ($P>0.05$) in the liver enzyme activities of mice treated with 100 mg/kg of BGA for two weeks as compared to those of the normal control group.

Also, table (2) shows the level of blood urea nitrogen (BUN) and creatinine in serum of mice after administration of BGA for two weeks. Results illustrated that there is no significant change ($P>0.05$) in the serum level of BUN and creatinine of mice treated with BGA (100 mg/kg) for 15 days as compared with those of normal control group.

Table (2): Effect of blue green algae on liver and kidney functions

Parameters	Control	BGA	P
ALT (U/L)	29.7±3.7	29.7±3.3^{ns}	>0.05
AST (U/L)	72.5±4.6	73.1±5.4^{ns}	>0.05
ALP (U/L)	29.2±2.0	29.9±2.2^{ns}	>0.05
GGT (mU/dL)	65.9±1.1	65.1±0.9^{ns}	>0.05
TP (g/dL)	5.3±0.3	5.6±0.2^{ns}	>0.05
Alb (g/dL)	3.4±0.1	3.4±0.2^{ns}	>0.05
Creatinine (mg/dL)	0.89±0.03	0.89±0.02^{ns}	>0.05
BUN (mg/dL)	18±0.95	18.1±0.62^{ns}	>0.05

Data are expressed as Mean ± SD
(ns) Non significant
(*) Significant difference (P<0.05)
(**) High significant difference (P<0.001)
(***) Very high significant difference (P<0.0001)

Effect of BGA on some hematological parameters in normal mice

As shown in table (3), there is a significant increase (P<0.05) in the number of RBCs and very high significant increase (P<0.0001) in the values of WBCs, Hb, PCV and platelets in mice treated with 100 mg/kg of BGA daily for two weeks as compared to those of the normal control group (P<0.05).

Table (3): Effect of blue green algae on some hematological parameters

Parameters	Control	BGA	P
RBCs (10^6/µL)	8.4±1.8	9.9±0.3*	<0.05
WBCs (10^3/µL)	2.6±0.25	4.4±0.5***	<0.0001
Hb (g/dL)	10.8±0.3	13±0.2***	<0.0001
PCV (%)	39.4±0.4	46.4±1.4***	<0.0001
Platelets (10^3/µL)	399±3.5	506±24.8***	<0.0001

Data are expressed as Mean ± SD
(ns) Non significant
(*) Significant difference (P<0.05)
(**) High significant difference (P<0.001)
(***) Very high significant difference (P<0.0001)

Discussion

The target of this study is to test the potential activity of the natural product BGA on some biochemical and hematological markers in normal mice. Results indicated that the oral administration of BGA (100mg/kg) for 15 days ameliorated the antioxidant capacity in normal mice, where, the activities of hepatic antioxidant enzymes CAT, SOD, GST and GPX were very high significantly increased (P<0.0001) as compared with those of the normal controls. On the other hand, the level of hepatic lipid peroxidation was very high significantly (P<0.0001) reduced in normal mice after treatment with BGA as compared with that of the normal controls. Kuriakose and Kurup (2012) who reported that treatment of the normal rats with BGA (*Aphanizomenon flos-aquae*) significantly increased the activities of CAT, SOD and GST as compared to those of the normal controls. In addition, results of the current

study go in harmony with the report of Scoglio et al (2009) who revealed that AFA has a powerful antioxidant activity. Also, Romey et al. (1998) recorded that BGA exhibit both antioxidant and anti-inflammatory properties.

Liver and kidney have vital activities in the body that their enzymes served as important biomarkers for different cell organelles and any defect in them will be reflected in their enzyme activities (Das et al., 2012). The current study revealed that the liver and kidney functions have not been affected after oral administration of normal mice with 100 mg/kg of BGA daily for two weeks as compared to those of the control group (Table 2).

Results of the current study go in parallelism with previous studies that revealed a potential hepatoprotective effect of c-phycocyanin in rats with induced hepatitis (González et al., 2003 and Yan-Fei et al., 2007). In addition, BGA (*Aphanizomenon flos-aquae*) exhibited a hepatoprotective effect against paracetamol which caused liver damage (Kuriakose and Kurup, 2008 and 2010). Also, these findings are supported by the results of Bjelakovic et al. (2010) who reported that treatment of hepatitis C & B patients with vitamin A, vitamin C and vitamin E led to a depression in the activity of liver enzymes such as ALT, AST and ALP as compared with control group. This action may be due to the antioxidant activity of BGA constituents such as vitamin A, vitamin E and vitamin C (Kay, 1991). Moreover, the study of Viswanadha et al. (2011) revealed the hepatoprotective role of BGA against hepatotoxicity induced by 4-nitroquinoline-1-oxide in experimental rats. Meanwhile, the oral treatment of diabetic mice with 300 mg/kg of BGA led to the amelioration of kidney function as it is significantly reduced the albuminuria (Zheng et al., 2013).

On the other hand, the current study demonstrates that BGA induced the hematological measures of normal mice as shown in table (3). The results illustrated that the RBCs, WBCs, Hb, PCV and Plts were significantly increased in normal mice after treatment with 100 mg/kg of BGA daily for two weeks as compared to those of the normal controls ($P<0.05$). These findings are in agreement with the report of Janes et al. (2001) who reported that BGA exhibited a stimulatory action on the metabolism of iron and hemoglobin in normal rats. Also, previous reports revealed that the levels of Hb, RBC and PCV were significantly increased in normal mice after treatment with BGA (Davis et al., 2003 and Venkatesan et al., 2012). In addition, Simsek and his colleagues showed that the dietary BGA (*S. platensis*) increased the leucocytic count and its subtypes (Simsek et al., 2007). The stimulatory action of BGA on the hematological markers might be attributed to its constituent phycocyanin which stimulates the production of erythropoietin (EPO) hormone resulting in induction of haematopoesis (Henrikson, 1994). Phycocyanin also regulates the production of white blood cells even when bone marrow stem cells are damaged by toxic chemicals or radiation (Sharma and Sharma, 2005).

In conclusion, the present study revealed that BGA supplement ameliorated the antioxidant capacity without exhibiting harmful side effects on liver or kidney function. The hepato- and nephroprotective effect of BGA in mice might be attributed to its ROS scavenging properties. Also, BGA exhibited the ability to induce the hematopoietic progenitor to produce blood cells which have roles in enhancement of immunologic response and in maintaining the healthy condition of the individuals. These findings encourage people to use BGA supplementation to augment their antioxidant capacity and to support immune system against several diseases.

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