



Spirulina platensis protects against L-methionine induced hyperhomocysteinemia by abrogation of oxidative stress markers, hyperhomocysteine and inflammation in rats

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ABSTRACT

Spirulina platensis have therapeutic functions such as antioxidant, anti-bacterial, antiviral, anticancer, anti-inflammatory, anti-allergic and anti-diabetic. The purpose of this study was to evaluate the protective effect of spirulina platensis on L-methionine induced hyperhomocysteinemia (HHcy) in rats. Thirty male albino rats were divided into three equal groups. Group I (control normal): rats administered distilled water. Group II (hyperhomocysteinemic group (HHcy): rats received L-methionine (1.7 gm/kg body weight/day) orally for continuous 8 weeks. Group III (HHcy + spirulina platensis): rats received spirulina (1.5 gm/kg body weight/day) orally for 4 weeks after induction of hyperhomocysteinemia. The obtained results showed significant increase in serum homocysteine, lipids profile (total cholesterol and triacylglycerols), liver marker enzymes (ALT, AST and ALP), liver L-MDA and pro-inflammatory cytokines (TNF- α and IL-8) gene expression level in hyperhomocysteinemic (HHcy) rats. However, liver SOD activity and GSH concentration were markedly decreased. Spirulina treatment to L-methionine induced HHcy in rats caused significant improvement of all previous parameters towards its normal ranges. These results suggested that, spirulina treatment exerts a protective effect on HHcy by reduction of oxidative stress marker, inflammation and hyperhomocysteinemia in rats through free radical scavenging and anti-inflammatory activities as well as regenerating endogenous antioxidant defense system mechanisms.

Keywords: L-methionine, hyperhomocysteinemia, spirulina, oxidative stress.

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1. INTRODUCTION

Homocysteine (Hcy) is an intermediate sulfhydryl-containing amino acid derived from methionine. Hcy has two fates: remethylation to methionine (with the ease of methionine synthase enzyme) or transsulfuration to cysteine (with cystathionine- β -synthase, enzyme (CBS) (Huang et al. 2007). The elevated levels of Hcy, termed as hyperhomocysteinemia (HHcy), is associated with a higher risk of neurovascular diseases. The causes of HHcy are mainly genetic

deficiencies in the enzymes (cystathionine b synthase (CBS) and methylene tetrahydrofolate reductase (MTHFR) responsible for the remethylation or transsulfuration of Hcy and nutritional (B6, B12, choline, and folate) deficiencies of vitamins serving as cofactors for the enzymes. (Beard and Bearden2011; Dayal and Lentz, 2008). There has been an indication towards a significant correlation between hyperhomocysteinemia and cardiovascular disease and its complications such as heart attacks and strokes. It is believed that, hyperhomocysteinemia leads to

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endothelial cell damage, reduction in the flexibility of vessels, and alters the process of hemostasis.

Moreover, hyperhomocysteinemia may lead to an enhancement of the adverse effects of risk factors like hypertension, smoking, lipid and lipoprotein metabolism, as well as promotion of the development of inflammation (Baszczuk and Kopczynski, 2014).

Methionine is besides cysteine one of the two sulfur-containing proteinogenic amino acids and is essential for life. In organisms it can serve as precursor of cysteine. Due to the sulfur, responsible for disulfide bonds, which stabilize proteins tertiary structures, cysteine are mainly present in structural proteins such as collagen or keratin in skin, hair, feathers, and nails respectively. The highest methionine content of about 5 % can be found in albumins, especially egg albumin, which belongs to the water-soluble proteins (globulins). Methionine exists in two isomers, L- and D-methionine, of which the L-form predominates in nature (Goodson et al.; 2012).

The increased production of reactive oxygen species (ROS) caused by HHcy may induce the subsequent oxidation of proteins, lipids and nucleic acids and can lead to the endothelial dysfunction and damage to the vessel wall, followed by platelet activation and thrombus formation, accumulation of oxidized biomolecules alters the biological functions of many cellular pathways (Zou and Banerjee, 2005). Hcy acts as a potent oxidizing agent of -SH groups by reactive species production, such as superoxide anion (O₂⁻) and hydrogen peroxide (H₂O₂), mainly during its auto-oxidation (Faraci and Lentz, 2004).

Spirulina is a free-floating, microscopic, filamentous blue-green alga, grows naturally in alkaline lakes, many studies are focusing on the potential antioxidant activity of spirulina (Sotiroudis and Sotiroudis, 2013). Spirulina strongly induces antioxidant enzyme activity,

helps to prevent lipid peroxidation and DNA damage, and scavenges free radicals (Abdelkhalek et al., 2015). The post-treatment with spirulina reduces the activity of liver marker enzymes such as serum ALT and AST in rats. Also, spirulina is rich in β -carotene and the bioavailability is as good as the pure β -carotene, vitamin E and vitamin C and selenium (Simsek et al., 2009). The anti-inflammatory properties of spirulina treatment significantly reduced the elevated levels of pro-inflammatory cytokines, tumor necrosis factor- α (TNF- α) and interleukin-8 (IL-8), observed in the lead treated rats group. This effect might be attributed to reduction in oxidative stress (El-Tantawy, 2015). Also, spirulina has hepatoprotective properties through decreasing of liver lipids profile and lipo-peroxidation products. Moreover, spirulina prevents the development of fatty liver, and its antioxidant properties seem to mediate such a protective effect, indicated by the reduction of MDA well as the elevation of reduced glutathione (GSH) and SOD levels in liver tissue (Karadeniz et al., 2009). Accordingly, this study was undertaken to evaluate the possible beneficial effect of spirulina against deleterious effect of hyperhomocysteinemia in adult male rats through investigation of homocysteine, lipid profile, liver marker enzymes, biomarkers of oxidative stress and pro-inflammatory cytokines gene expression.

2. MATERIAL AND METHODS

2.1. Experimental animals:

Thirty white male albino rats of 10-12 weeks old age and average body weight 150-200 g were used in this study. Rats were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The rats were fed on constant ration and fresh, clean drinking water was supplied ad-libitum. All rats

were acclimatized for minimum period of 15 days prior to the beginning of study.

2.2. Chemicals and antioxidant:

The antioxidant and chemicals used in the present study were:

a- L-methionine; 63-68-3; -2-Amino-4-(methylthio)butanoic acid; L-(-)-Methionine was Purchased from El Gomhouria Company for Trading Chemicals and Medical Appliances, Egypt. Methionine was dissolved in in 1M HCL freshly prepared and orally administered at a dose (1.7 g/kg body weight/day) for 8 weeks (Sain *et al.*, 2011).

b- Spirulina: spirulina platensis powder was purchased from (Laboratory of Algal Technology at Zagazig University). It was dissolved in distilled water and administered orally to rats at a dose level of (1500 mg/kg b.wt) once daily for 4 weeks (Xiaoli *et al.*, 2008).

c- Other chemicals used in this study were of the highest purified grades available purchased from El Gomhouria Company for Trading Chemicals and Medical Appliances, Egypt.

2.3. Experimental design:

After acclimatization to the laboratory conditions, the animals were randomly divided into three groups (10 rats each) placed in individual cages and classified as follow:

Group I (normal control group): Rats received no drugs, served as control non-treated for all experimental groups.

Group II (L-Methionine induced hyperhomocysteinemia (HHcy): rats received L-methionine (1.7 g/kg b.wt/ day) orally for continuous 8 weeks.

Group III (HHcy + Spirulina treated group) rats received Spirulina orally at a dose of (1.5 gm/kg body weight/day) for 4

weeks after induction of hyperhomocysteinemia.

2.4. Sampling:

2.4.1. Blood samples:

Blood samples were collected by ocular vein puncture from all animal groups after overnight fasting in dry, clean tubes and allowed to clot for 30 minutes and serum was separated by centrifugation at 3000 r.p.m for 15 minute. The serum was taken by automatic pipette and received in dry sterile tubes, then kept in deep freeze at -20 °C until use for subsequent biochemical analysis. All sera were analyzed for determination of the following parameters: Hcy, Total cholesterol, Triacylglycerols, AST, ALT and ALP.

2.4.2. Tissue samples:

2.4.2.1. Liver tissue for biochemical analysis:

About 0.5 g of liver tissue specimen was taken from each group of rats after had been sacrificed. The specimens were immediately removed and washed several times with saline and blotted between two damp filter papers, weighed and stored at -20°C for subsequent biochemical analyses.

2.4.2.2. Preparation of liver tissue homogenate:

Briefly, liver tissues were cut, weighed and minced into small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH 7.4) to make 10 % homogenates. The homogenates were centrifuged at 6000 r.p.m for 15 minutes at 4°C then the resultant supernatant was used for the determination of the following parameters: L-MDA and SOD.

About 0.2 gm of liver tissues were minced into small pieces homogenized with a glass homogenizer in 0.4 ml of 25% metaphosphoric acid (MPA) (ref. No.:

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253-433-4, Sigma-Aldrich, Germany), then 1.4 mL of distilled water was added, mixed and incubated for 1 hour and centrifuged for 10 min at 3,000 r.p.m then the clear supernatant was removed and used for determination of GSH concentration.

2.4.2.3. Liver tissue for molecular gene expression:

About 0.5 gm of liver tissue put in eppendorf tubes and were immediately kept in liquid nitrogen and stored at -80°C till RNA extraction for determination of TNF α and IL-8 gene expression level.

2.5. Biochemical analysis

Serum Hcy was determined according to the method described by Rat homocysteine (Hcy) ELISA kit (My Bio Source, Cat# MBS703069), Total cholesterol, Triacylglycerols, ALT, AST and ALP were determined according to the method described by Ellefson and Caraway, (1976) and Stein, (1987), Schumann et al., (2002) and EL-Aaser and EL-Merzabani (1975) respectively. Liver tissue L-MDA, SOD and GSH were determined according to the method described by Mesbah et al., (2004), Kakkar et al., (1984), and Patterson and Lazarow, (1955), respectively. Moreover, the mRNA expression level of TNF- α and IL-8 was determined by real-time quantitative polymerase chain reaction (real-time qPCR) analysis in liver of rats. Target gene was normalized with β -actin by used the $2^{-\Delta\Delta\text{Ct}}$ method (Livak and Schmittgen, 2001).

2.6. Statistical analysis:

The results were expressed as mean \pm SE using SPSS (13.0 software, 2009) program. The data were analyzed using one-way ANOVA to determine the statistical significance of differences among groups. Duncan's test was used for

making a multiple comparison among the groups for testing the inter-grouping homogeneity. Values were considered statistically significant when $p < 0.05$.

3. RESULTS

The data presented in table (1) showed a significant increase in serum homocysteine, total cholesterol and Triacylglycerols concentrations in L-methionine induced HHcy in rats when compared to normal control group. However, spirulina treatment to HHcy male rats caused a significant decrease in elevated serum homocysteine, total cholesterol and Triacylglycerols concentrations when compared with L-methionine treated group.

The obtained results presented in table (2) revealed that, HHcy rats showed significant increase in serum ALT, AST and ALP activities when compared with normal control group. On the other hand, spirulina treatment to HHcy male rats caused a significant decrease in elevated serum ALT, AST and ALP activities when compared with HHcy group.

The obtained data demonstrated in table (3) revealed that, HHcy rats showed significant increase in liver tissue L-MDA and significant up-regulation of TNF α and IL-8 all over the periods of the experiment when compared to normal control group. However, spirulina treatment to HHcy rats caused a significant decrease in elevated liver tissue L-MDA and a significant down-regulation TNF α and IL-8 gene expression when compared with HHcy group.

The current results presented in table (4) exhibited significant decrease in liver SOD activity and GSH concentration in L-methionine treated rats when compared to

normal control group. Meanwhile, GSH level when compared with HHcy spirulina treatment to HHcy male rats caused a non-significant increase in liver tissue SOD activity and marked increase in group.

Table (1): Effect of spirulina administration on serum homocysteine, total cholesterol and triacylglycerols concentrations in HHcy induced in male rats.

Parameters Exp. groups	Homocysteine (nmol/ml)	Total Cholesterol (mg/dl)	Triacylglycerols (mg/dl)
Group I: Normal control	10.24 ± 0.33 ^c	64.67 ± 4.63 ^b	61.33 ± 2.60 ^b
Group II: (HHcy)	32.35 ± 0.72 ^a	106.0 ± 10.82 ^a	81.0 ± 3.06 ^a
Group III: HHcy + Spirulina	24.11 ± 0.53 ^b	73.33 ± 4.91 ^b	47.33 ± 0.88 ^c

Data are presented as (Mean ± S.E). S.E = Standard error.
Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

Table (2): Effect of spirulina administration on serum ALT, AST, and ALP activities in HHcy induced in male rats.

Parameters Exp. groups	ALT (U/L)	AST(U/L)	ALP(U/L)
Group I: Normal control	20.33 ± 1.45 ^b	22.67 ± 2.60 ^b	143.33 ± 11.14 ^b
Group II: (HHcy)	29.67 ± 2.19 ^a	38.67 ± 1.67 ^a	225.33 ± 17.74 ^a
Group III: HHcy + Spirulina	11.33 ± 2.85 ^c	14.33 ± 1.20 ^c	176.0 ± 14.57 ^b

Data are presented as (Mean ± S.E). S.E = Standard error.
Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

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Table (3): Effect of spirulina administration on liver tissue L-MDA, TNF- α and IL-8 in HHcy induced in male rats.

Parameters Exp. groups	L-MDA (mmol/ g tissue)	Fold change in TNF α gene expression	Fold change in IL-8 gene expression
Group I: Normal control	5.34 \pm 0.11 ^b	1.00 \pm 0.02 ^d	1.00 \pm 0.06 ^d
Group II : (HHcy)	5.16 \pm 0.09 ^b	8.06 \pm 0.31 ^a	9.99 \pm 0.42 ^a
Group III: HHcy+Spirulina	7.71 \pm 0.40 ^a	4.38 \pm 0.12 ^b	4.59 \pm 0.13 ^b

Data are presented as (Mean \pm S.E). S.E = Standard error.

Mean values with different superscript letters in the same column are significantly different at (P \leq 0.05).

Table (4): Effect of spirulina administration on liver tissue SOD activity and GSH concentration in HHcy induced in male rats.

Parameters Exp. groups	SOD (U/g.tissue)	GSH (ng/g.tissue)
Group I: Normal control	47.48 \pm 2.05 ^a	5.06 \pm 0.13 ^b
Group II: (HHcy)	21.75 \pm 1.21 ^b	3.21 \pm 0.21 ^d
Group III: HHcy + Spirulina	30.96 \pm 4.01 ^b	4.40 \pm 0.12 ^c

Data are presented as (Mean \pm S.E). S.E = Standard error.

Mean values with different superscript letters in the same column are significantly different at (P \leq 0.05).

4. DISCUSSION

The obtained results showed that, L-methionine induced HHcy rats showed significant increase in serum homocysteine, total cholesterol and triacylglycerol concentration all over the periods of the experiment when compared to normal control group. These results were nearly similar to (Prasanna and Ashok, 2011) who recorded that, treatment with methionine (1g/kg, p.o.) for 30 days in pathogenic control group rat's elevated level of serum homocysteine, total cholesterol and triglycerides and atherosclerotic index values. Also, (Lan et al., 2011) reported that, L-Methionine in earlier studies has also been demonstrated to induce endothelial dysfunction so that, given high levels of lipids as well as Hcy have been documented to enhance the production of free radicals with subsequent increase in oxidative stress. This increase of homocysteine level due to several disorders, like cardio- and cerebrovascular diseases and neurodegenerative diseases (Maron and Loscalzo, 2009) that affect the central nervous system (CNS), such as Alzheimer's disease (Piazza et al., 2012).

Hyperhomocysteinemia is found in 30% of patients with premature atherosclerosis of carotid and peripheral arteries (Malinow et al., 1993). Up to 40% of patients diagnosed with premature coronary artery disease, peripheral vascular disease, or recurrent venous thrombosis present with HHcy (Brady et al., 2002).

In general, the development and progression of atherosclerosis is considered to be a form of chronic inflammation (Ross et al, 1999). Endothelial dysfunction is the key process promoting inflammatory reactions. In vitro studies have demonstrated that Hcy enhances the production of several pro

inflammatory cytokines indicates that moderate HHcy directly leads to endothelial dysfunction and premature atherogenesis showed significant increase in serum homocysteine, total cholesterol and triacylglycerol concentration (Wang et al., 2000). Elevation in total cholesterol and triacylglycerol concentration was found to be related to HHcy has been suggested to cause impairment of learning as well as memory (Gao et al., 2012). Higher levels of Hcy (Lan et al., 2011) and cholesterol (Yunoki et al., 2011) were suggested to be responsible for development of endothelial dysfunction. L-Methionine in earlier studies has also been demonstrated to induce endothelial dysfunction, so that the increase level of lipids as well as Hcy have been documented to enhance the production of free radicals with subsequent increase in oxidative stress (Lan et al., 2011).

Treatment with spirulina to hyperhomocysteinemic male rats caused a significant decrease in serum Hcy, total cholesterol and triacylglycerol concentration in all over the periods of the experiment. These results were nearly similar to those recorded by Xiaoli et al., (2008) who reported that, treatment with spirulina can effectively reduce the Hcy plasma concentration in HHcy rats, and reduce the oxidative stress induced by HHcy, which has protective effect on HHcy-induced arterial injury. The supplementation of spirulina may reduce the level of homocysteine in hyperhomocysteinemia in an extent better than that of folic acid, The supplementation of spirulina can decrease the level of the oxidative stress, Spirulina can decrease plasma lipid in hyperhomocysteinemia rats, Spirulina can attenuated the arterial lesion, Also, (Farooq et al., 2014) showed a significant results in the use of *Spirulina platensis* against oxidative stress and dyslipidemias at dose (26 mg/Kg) by oral

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gavage three times a week. Moreover, Ble-Castillo et al., (2002) suggested that spirulina has hepato protective properties through decreasing liver lipid profile and lipo peroxidation products. In addition, high gamma-linolenic acid (GLA) content of spirulina has gained the attention, an external food source of GLA such as spirulina, therefore, plays a crucial role in regulating the cholesterol levels (Mani et al., 2008)

Presented findings showed that, HHcy group showed significant increase in serum ALT, AST and ALP activities all over the periods of the experiment when compared to normal control group. These results were nearly similar to those reported by (Cho, 2010) who demonstrated that, the increases in ALT, AST, ALP levels were thought to be due to oxidative stress related to hyperlipidemia. Also, These enzymes are considered to be the markers of organ dysfunction, indicator of cellular damage, cell leakage and the loss of cell membrane integrity in the liver, kidney, heart and other organs (Ramesh et al., 2012). The increase of methionine might lead to liver oxidative stress increment and Hcy itself has the ability to generate potent reactive oxygen species (ROS) when oxidized by highly reactive sulfhydryl group (Yamada et al., 2012).

Spirulina treatment to HHcy male rats group caused a significant decrease in elevated serum ALT, AST and ALP activities when compared with HHcy group. These results were nearly similar to those reported by Hassanen et al., (2015) who reported that, oral administration of water extract of spirulina (1000 mg/kg/b.wt) for six weeks following an acute toxic dose of doxorubicin reduces the hepatotoxicity and attenuates doxorubicin-induced stress. Also, the post-treatment with spirulina reduces the

activity of liver marker enzymes such as serum ALT and AST and ALP in rats. Also, spirulina is rich in β -carotene and the bioavailability is as good as the pure β -carotene, vitamin E and vitamin C and selenium (Simsek et al., 2009). It has been suggested, that the Spirulina extracts could be effective against free radical induced lipid peroxidation which in turn may lead to cellular transformation. Moreover, Spirulina also have protective effects against oxidative stress induced by lead acetate in the liver and kidney of rats (Ponce-Canchihuamanet et al., 2010).

The obtained results demonstrated that, HHcy group had showed significant increase in liver tissue L-MDA and significant up-regulation of TNF α and IL-8 in all over the periods of the experiment when compared to normal control group. Similarly, (da Cunha et al., 2012) reported that, one of the effects of HHcy is an increased lipid peroxidation and protein oxidation. Therefore, we have investigated the effect of chronic HHcy on some parameters of lipid oxidation and oxidative damage of proteins. This increase may be due to homocysteine is a thiol containing amino acid derived from demethylation of dietary methionine, may generate partially reduced ROS that were able to stimulate the lipid peroxidation involved in the atherosclerotic process (Toborek et al., 1995). Also, Hyperhomocysteinemia has recently emerged as an independent risk factor for the development of coronary, cerebrovascular and peripheral arterial occlusive disease (Omenn et al., 1998). Elevated homocysteine promotes atherosclerosis through increased oxidative stress, impaired endothelial function and induction of thrombosis. Oxidative stress was one important mechanism for homocysteine toxicity in neuronal cells (Ho

et al., 2001). Free radicals generated by hyperhomocysteinemia, initiate lipid peroxidation of the membrane bound polyunsaturated fatty acids, leading to the impairment of the structural and functional integrity of the membrane (Ajitha and Rajnarayana, 2001).

Additionally, (Zhang et al., 2011) recorded that, HHcy induced atherosclerosis and atherosclerosis activate further release of cytokine-signaling molecules that recruit more inflammatory cells. The inflammatory cells most involved were monocytes and T-cells that can release MCP-1. The more mature plaques contain dendritic cells, mast cells, B cells, and natural killer T-cells. Several of these cells were activated, and produced inflammatory cytokines like TNF- α . Furthermore, Jablonski et al., (2011) studied that, activation of NF κ B has been demonstrated to induce endothelial dysfunction in HHcy. Also, Poddar et al., (2001) reported that, homocysteine has also been shown to increase expression of IL-8. Moreover, HHcy stimulated the expression of MCP-1, in rats, leading to increased monocyte adhesion to the aortic endothelium. Such an effect may contribute significantly to the development of atherosclerosis by facilitating monocyte/macrophage infiltration into the arterial wall. So that, levels of MCP-1 and IL-8 were significantly higher in HHcy group than in normal group and there were some foam cells and depositions of lipochondria in aortic tunica intima in HHcy group (Wang et al., 2002).

Spirulina treatment to HHcy rats group caused a significant decrease in elevated liver tissue L-MDA and a significant down-regulation TNF α and IL-8 gene expression when compared with HHcy group. These results were nearly similar to the findings of Sharoud, (2015) who indicated that, oral administration of rats with

spirulina (500 mg/kg b.w. daily for 21 days) resulted in a significant decrease in MDA levels in paracetamol induced hepatic injury in rats. The decrease in MDA levels due to the protective effect of spirulina platensis against paracetamol induced oxidative stress could be either direct by inhibiting lipid peroxidation and scavenging free radicals or indirect through the enhancement of the activity superoxide dismutase and the enzymatic free radicals scavengers in the cells. These properties could be attributed to the high levels of antioxidants such as c-phycoyanin, carotenoids, vitamins, minerals, lipids, proteins and carbohydrates, reported in. Also, dietary spirulina enhanced responses of phagocytic activity responses, interleukin (IL-8) expression, and tumor necrosis factor (TNF)- α genes in carp (Watanuki et al., 2006). Moreover, El-Tantawy, (2015) reported that, spirulina treatment significantly reduced the elevated levels of hepatic TNF- α observed in the lead treated rats group. This effect might be attributed to reduction in oxidative stress.

The Presented data showed that, significant decrease in SOD and GSH activity was observed in HHcy rats all over the periods of the experiment when compared to normal control group. These results are nearly similar to (Aparna et al., 2010) who reported that, free radical-scavenging enzyme, such as superoxide dismutase (SOD), are in the first line of cellular defense against oxidative injury, decomposing O₂ and H₂O₂ to prevent formation more reactive hydroxyl radical (HO). These enzymes protect the red blood cells against O₂-and H₂O₂ mediated lipid peroxidation and their lower activities could be related to inactivation of the enzymes by cross-linking or to exhaustion of the enzymes by increased peroxidation. Moreover, (Sharma and Singh, 2011) showed that, L-Methionine treatment has

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induced a rise in superoxide anion in aortic strip as well as TBARS in serum and brain, along with reduction in brain GSH levels in this study, which is a reflection of oxidative stress and is probably one of the major contributing factors in L-methionine, induced endothelial dysfunction. This suggestion was confirmed by the finding of Kim et al., (1998) who observed, that ultra- structural damage and functional impairment of the endothelium associated with elevated Hcy levels were related primarily to the generation of H₂O₂, depletion of myocardial GSH content and diminished G6PDH activity. Additionally, Hyperhomocysteinemia may promote the generation of reactive oxygen species (ROS) such as H₂O₂ and hydroxyl radicals via the auto-oxidation of sulfhydryl (-SH) group or by decreasing the intracellular levels of GSH that are involved in the elimination of free radicals (Heinecke et al., 1987).

Spirulina treatment to HHcy male rats group caused a non-significant increase in liver tissue SOD activity and a significant increase GSH activity all over the period of experiment when compared with HHcy group. These results came in accordance with the recorded data of Hassanen et al., (2015) who reported that, oral administration of spirulina extract for six weeks following an acute toxic dose of doxorubicin in rats, led to an obvious increase in the activity of antioxidant parameters including SOD. Moreover, the C-phycoyanin has an excellent antioxidant property and scavenging free radicals like superoxide and hydroxyl radicals, it correlated with the decline of circulatory antioxidants such as SOD, the generation of ROS, free radicals and decreased GSH levels significantly increased oxidative stress (Nagaraj et al., 2011). The overall effect of depletion of glutathione with concomitant

increases in lipid peroxide levels provides evidences about the vital role of glutathione during oxidative stress. Some of the active constituents of spirulina such as flavonoids, β -carotene and phycoyanin have been reported to possess strong antioxidant activity and provokes free radical scavenging enzyme system. The significant protection offered by C-phycoyanin demonstrated the radical scavenging activity and its inhibitory effect on lipid peroxidation chain reaction (Abdel-Daim et al., 2013). Similarly (Abdel-Daim et al., 2013) who observed that, increase in circulating lipid peroxides of paracetamol treated animals, correlates with the decline of circulatory antioxidants such as GSH. Additionally, the generation of ROS, free radicals and decreased GSH levels significantly increased oxidative stress; the overall effect of depletion of glutathione with concomitant increases in lipid peroxide level provides (Nagaraj et al., 2011). Moreover, the protective effect of SP against oxidative stress could be either direct by inhibiting lipid peroxidation and scavenging free radicals or indirect through the enhancement of the activity superoxide dismutase; the enzymatic free radical scavengers in the cells. These properties could be attributed to the high levels of antioxidants such as c-phycoyanin, carotenoids, vitamins, minerals, lipids, proteins and carbohydrates, reported in spirulina, therefore, spirulina could be used to prevent and treat hepatic and renal diseases especially those induced by oxidative damage, and C-phycoyanin demonstrated the radical scavenging activity and its inhibitory effect on lipid peroxidation chain reaction (Abdel-Daim et al., 2013).

The present study demonstrated that, administration of spirulina relieved harmful effects caused by exposure to L-methionine

induced HHcy. HHcy affected different organs mainly liver and these occurred through changes in several parameters. L-methionine induced HHcy caused significant increase in serum Hcy, TC, TG, AST, ALT, ALP and liver tissue L- MDA, TNF α and IL-8, however, a significant reduce in liver tissue SOD GSH. Spirulina treatment in HHcy rats relieved all previous parameters towards its normal range with best result after 4 weeks. So, these results confirmed the strong antioxidant, anti-inflammatory effects of spirulina in HHcy.

5. REFERENCES

- Abdel-Daim, M.M., Abuzead, S.M.M., Halawa, S.M. 2013. Protective role of Spirulina platensis against acute deltamethrin-induced toxicity in rats. Plos one., 8: e72991.
- Abdelkhalek, N.K., Ghazy, E.W. Abdel-Daim, M.M. 2015. Pharmacodynamic interaction of Spirulina platensis and deltamethrin in freshwater fish Nile tilapia, Oreochromis niloticus: impact on lipid peroxidation and oxidative stress. Environ Sci Pollut Res Int 22(4):3023–3031.
- Ajitha, M., Rajnarayana, K.2001. Role of oxygen free radicals in Human Disease. Indian Drugs. 38:545–599.
- Aparna, P., Betigeri, A.M., Pasupathi, P.2010. Homocysteine and oxidative stress markers and inflammation in patients with coronary artery disease. Int J Biol Med Res2010; 1: 125-129.
- Baszczuk, A., Kopczynski, Z. 2104. Hyper homocysteinemia in patients with cardiovascular disease [Abstract]. Postepy Hig Med Dosw 68:563-579.
- Beard, R.S., Jr, Bearden, S.E., 2011. Vascular complications of cystathionine beta-synthase deficiency: future directions for homocysteine-to-hydrogen sulfide research. Am J Physiol Heart Circ Physiol 300: H13–H26.
- Ble-Castillo, J.L., Rodriguez-Hernandez, A., Miranda-Zamora, R., Juarez-Oropeza, M.A., Diaz-Zagoya, J.C. 2002. Arthrospira maxima prevents the acute fatty liver induced by the administration of simvasta-tin, ethanol and a hypercholesterolemic diet to mice. Life Sci.; 70:2665-2673.
- Brady, M., Bhatia, M., Christmas, S., Boyd, M.T., Neop-tolemos, J.P., Slavin, J. 2002. Expression of the chemokines MCP-1/JE and cytokine induced neutrophil chemoattractant in early acute pancreatitis. Pancreas, 25: 260-269.
- Cho, H.C. 2010. The association between serum GGT concentration and diabetic peripheral polyneuropathy in type 2 diabetic patients. Korean Diabetes Journal. 34(2): 111-118.
- da Cunha, M. J., da Cunha, A. A., Ferreira, A.G. 2012. Physical exercise reverses glutamate uptake and oxidative stress effects of chronic homocysteine administration in the rat. Int J Dev Neurosci 2012; 30: 69-74.
- Dayal ,S.,Lentz, S.R .2008. Murine models of hyperhomocysteinemia and their vascular phenotypes. Arterioscler Thromb Vasc Biol (28):1596–1605.
- EL-Aaser, A.A., EL-Merzabani, M.M. 1975. Simultaneous determination of 5'-nucleotidase and alkaline activities in serum. Z. Klin. Chem. Klin.Biochem 13, 453.
- Ellefson, R.D. Caraway, W.T. 1976. fundamentals of clinical chemistry.Ed Tietz NW; p506.
- El-Tantawy, W.H. 2015. Antioxidant effects of Spirulina supplement against lead acetate-induced hepatic injury in rats. J Traditional Complem Med. 02.001
- Faraci, F.M., Lentz, S.R.2004. Hyperhomocysteinemia, oxidativestress, and cerebral vascular dysfunction. St.roke 35:345-347.
- Farooq, S.M., Boppana, N.B., Asokan, D., Sekaran, S.D., Shankar, E.M., Ebrahim, A.S. 2014. C-Phycocyanin Confers protection against oxalate-mediated oxidative stress and mitochondrial

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- dysfunctions in MDCK cells. *Plos One*. 9:1-9.
- Gao, L., Zeng, X.N., Guo, H.M., Wu, X.M., Chen, H.J., Di, R.K., 2012. Cognitive and neurochemical alterations in hyperhomocysteinemic rat. *Neurol Sci* 33(1):39–43.
- Goodson, J.; Thomson, J.; and Helmbrecht, A. 2012. Feeding value of L-methionine versus DL-methionine. *Animal AG Resource Center, Evonik Whitepaper Aug. (28):1596–1605*.
- Hassanen, M.R., Mahfouz, M.K., Farid, A.S., Fadlullah, A.H. 2015. Biochemical effects of spirulina platensis against oxidative stress caused by doxorubicin. *BVMJ*.28(2): 147-154.
- Heinecke, J.W., Rosen, H., Suzuki, L.A., Chait, A. 1987 The role of sulfur-containing amino acids in superoxide production and modification of low density lipoprotein by arterial smooth muscle cells. *J Biol Chem*. 262:10098–103.
- Ho, P.I., Collins, S.C., Dhitavat, S., Ortiz, D., Ashline, D., Rogers. E., 2001. Homocysteine potentiates betaamyloid neurotoxicity: Role of oxidative stress. *J Neurochem*. 78:249–53.
- Huang, Y., Guo, B.J., Wong, R.N.S., Jiang, Y. 2007. Characterization and antioxidant activity of selenium-containing phycocyanin isolated from *Spirulina platensis*. *Food Chem* 100:1137-1143.
- Jablonski, K.L., Chonchol, M., Pierce, G.L., Walker, A.E., Seals, D.R. 2011. 25-Hydroxyvitamin D deficiency is associated with inflammation-linked vascular endothelial dysfunction in middle-aged and older adults. *Hypertension* 57(1):63–9.
- Kakkar, P., Das, B., Viswanathan, P.N. 1984. A modified spectrophotometric assay of superoxide dismutase. *Indian J. Biochem Biophys* 21, 130-132.
- Karadeniz, A., Cemek, M., Simsek, N. 2009. The effects of Panax ginseng and *Spirulina platensis* on hepatotoxicity induced by cadmium in rats. *Ecotoxicol Environ Safety*.,72:231-235.
- Kim, Y., Farhat, M., Myers, A. 1998 Beta estradiol regulation of myocardial glutathione and its role in protection against myocardial stunning in dogs. *J Cardiovasc Pharmacol* 32:457–465.
- Kolling, J., Scherer, E. B., da Cunha, A. A., da Cunha, M., J., Wyse, A.T. 2011. Homocysteine induces oxidative-nitrative stress in heart of rats: prevention by folic acid. *Cardiovasc Toxicol* 11: 67-73.
- Lan, T.H., Xu, Z.W., Wang, Z., Wu, Y.L., Wu, W.K., Tan, H.M. 2011. Ginsenoside Rb1 prevents homocysteine-induced endothelial dysfunction via PI3K/Akt activation and PKC inhibition. *Biochem Pharmacol* 82(2):148–55.
- Livak, K.J., Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT Method. *Methods* 25(4), 402-408.
- Malinow, M.R., Nieto, F.J., Szklo, M., Chambless, L.E., Bond, G. 1993. Elevated plasma homocysteine levels have been defined as an independent risk factor for coronary heart disease. *Circulation* 87: 1107-1113 .
- Mani, U., V., Iyer, U. M., Dhruv, S. A., Mani, I. U., Sharma, K. S. 2008. Therapeutic utility of spirulina. In: Gershwin M. E., Belay A., editors. *Spirulina in Human Nutrition and Health*. New York, NY, USA: CRC press pp. 71–99.
- Maron, B. A., Loscalzo, J. 2009. The treatment of hyperhomocysteinemia. *Annu Rev Med* 60:39–54.
- Mesbah, L., Soraya, B., Narimane, S., Jean, P.F. 2004. protective effect of flavonides against the toxicity of vinblastine cyclophosphamide and paracetamol by inhibition of lipid – peroxydation and increase of liver glutathione. *Haematol* 7 (1), 59-67.
- Nagaraj, S., Arulmurugan, P., Karuppasamy, K., Jayappriyan, K.R., Sundararaj, R.,

- Vijayanand, N., Rengasamy, R. 2011. Hepatoprotective and antioxidative effects of C-Phycocyanin in CCL4 induced hepatic damage rats. *Academic Journal of Cancer Research* 4: 29-34.
- Omenn, G.S., Beresford, S.A., Motulsky, A.G. 1998. Preventing coronary heart disease: B vitamins and homocysteine. *Circulation*. 97:421-4.
- Patterson, J.W., Lazarow, A. 1955. Determination of glutathione; *Methods of Biochemical Analysis* 2, 259-278.
- Piazza, F., Galimberti, G., Conti, E. 2012. Increased tissue factor pathway inhibitor and homocysteine in Alzheimer's disease. *Neurobiol Aging* 33: 226-233.
- Poddar, R., Sivasubramanian, N., Dibello, P. M., Robinson, K., Jacobsen, D. 2001. Homocysteine induces expression and secretion of monocytes chemoattractant protein-1 and interleukin-8 in human aortic endothelial cells: implications for vascular disease. *Circulation* ;103: 2717-3.
- Ponce-Canchihuaman, J.C., Perez-Mendez, O., Hernandez-Munoz, R., Torres-Durán, P.V., Juárez-Oropeza, M.A. 2010. Protective effects of *Spirulina maxima* on hyperlipidemia and oxidative-stress induced by lead acetate in the liver and kidney. *Lipid in Health and disease* 9: 35.
- Prasanna, G.S., Ashok, P. 2011. Antihyperhomocysteinemic and antihyperlipidemic effect of *Trichilia connaroides* in methionine-induced hyperhomocysteinemic animals. *Indian J Pharmacol* 43(2):203-6.
- Ramesh, T., Kim, S.W., Sung, J.H., Hwang, S.Y., Sohn, S.H., Yoo, S.K., Kim, S.K. 2012. Effect of fermented *Panax ginseng* extract (GINST) on oxidative stress and antioxidant activities in major organs of aged rats. *Experimental Gerontology*. 47(1): 77-84.
- Ross, R. 1999. Atherosclerosis: an inflammatory disease, *N. Engl. J. Med* 340: 115-126.
- Sain, H., Sharma, B., Jaggi, A.S., Singh, N. 2011. Pharmacological investigations on potential of peroxisome proliferator-activated receptor-gamma agonists in hyperhomocysteinemia-induced vascular dementia in rats. *Neuroscience* 192: 322-333.
- Schumann, G., Bonora, R., Ceriotti, F., Féraud, G., Ferrero, C.A., Franck, P.F., Gella, F.J., Hoelzel, W., Jørgensen, P.J., Kanno, T., Kessner, A., Klauke, R., Kristiansen, N., Lessinger, J.M., Linsinger, T.P., Misaki, H., Panteghini, M., Pauwels, J., Schiele, F., Schimmel, H.G., Weidemann, G., Siekmann, L. 2002. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 degrees C. International Federation of Clinical Chemistry and Laboratory Medicine. Part 6. Reference procedure for the measurement of catalytic concentration of gamma-glutamyltransferase. *Clin Chem Lab Med* 40(7), 734-738.
- Sharma, B., Singh, N. 2011. Behavioral and biochemical investigations to explore pharmacological potential of PPAR-gamma agonists in vascular dementia of diabetic rats. *Pharmacol Biochem Behav* 100(2):320-9.
- Sharoud, M.N.M. 2015. Protective effect of *Spirulina* against paracetamol-induced hepatic injury in rats. *Journal of Experimental Biology and Agricultural Sciences*., Volume - 3(1).
- Simsek, N.A., Karadeniz, Y., Kalkan, O.N., Keles, B., Unal, J. 2009. *Spirulina platensis* feeding inhibited the lead- and cadmium-induced anemia and leucopenia in rats. *J Hazard Mater*.164:1304-1309.
- Sotiroudis, T.G., Sotiroudis, G.T. 2013. Health aspects of *Spirulina*(*Arthrospira*) microalga food supplement. *J Serb Chem Soc*. 78(3):395-405.
- Stein, E.A. 1987. lipids, lipoproteins, and apolipoproteins. In NW Tietz, ed. *Fundamentals of clinical chemistry*, 3rd ed. Philadelphia: WB Saunders; 448.

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- Toborek, M., Kopieczna-Grzebieniak, E., Drózd, M., Wieczorek, M. 1995. Increased lipid peroxidation as a mechanism of methionine-induced atherosclerosis in rabbits. *Atherosclerosis*. 115:217–224.
- Wang, G.P., Siow, Y.L., O. K. 2000. Homocysteine stimulates nuclear factor kappaB activity and monocyte chemoattractant protein-1 expression in vascular smooth-muscle cells :possible role for protein kinase C. *Biochem J* 37:107–116.
- Wang, G.P., Woo, C., Sung, F.L., Siow, Y.L., O, K. 2002. Increased monocyte adhesion to aortic endothelium in rats with hyperhomocysteinemia. Role of chemokine and adhesion molecules. *Arterioscler Thromb Vasc Biol* 22 :1777-1783.
- Watanuki, H., Ota, K., Tassakka, A.C., Kato, T., Sakai, M. 2006. Immunostimulant effects of dietary Spirulina platensis on carp, *Cyprinus carpio* *Aquaculture, Res* 258:157–163.
- Xiaoli, S., mei, L., Ni, C., Yulian, D., East, P.D., Zhong, M.H. 2008. Effects of Spirulina on Oxidative Stress and Arterial Injury in Hyperhomocysteinemia Rats *Chinese Medicine*, 03, 129-131.
- Yamada, H., Akahoshi, N., Kamata, S., Hagiya, Y., Hishiki, T., Nagahata, Y., Matsuura, T; Takano, N., Mori, M., Ishizaki, Y., Izumi, T., Kumagai, Y., Kasahara, T., Suematsu, M. Ishii, I., 2012. Methionine excess in diet induces acute lethal hepatitis in mice lacking cystathionine-lyase, an animal model of cystathioninuria. *Free Radic. Biol. Med.* 52 (9), 1716–1726.
- Yunoki, K., Nakamura, K., Miyoshi, T., Enko, K., Kubo, M., Murakami, M, 2011. Impact of hypertriglyceridemia on endothelial dysfunction during statin±ezetimibe therapy in patients with coronary heart disease. *Am J Cardiol* 108(3):333–9.
- Zhang, X., Liu, X., Shang, H., Xu, Y., Qian, M.2011. Monocyte chemoattractant protein-1 induces endothelial cell apoptosis in vitro through a p53-dependent mitochondrial pathway. *Acta Biochim Biophys Sin (Shanghai)*. 43(10):787–795.
- Zou, C.G., Banerjee, R. 2005. Homocysteine and redox signaling. *antioxidant ant Redox Signal* 7: 547-559.