Hepatoprotective effect of *spirulina platensis* on diethylnitrosamine-injected mice

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A B S T R A C T

The present study was designed to determine the protective effects of *S. platensis* against oxidative damage and hepatotoxicity induced by DEN in mice. Animals were divided into five groups (24 mice per group). Group one was used as a control. Group two was administered 100 mg DEN / kg body weight/ once for 20 weeks. Group three was administered 100 mg DEN/kg body weight / once for 16 weeks then received *S. platensis* (500 mg/kg body weight) for 4 weeks. Group four was administered 100 mg DEN/kg body weight / once for 16 weeks then received *S. platensis* (1000 mg/kg body weight) for 4 weeks. Group five was received *S. platensis* (500 mg/kg body weight) for 4 weeks then administered 100 mg DEN/kg body weight / once for 20 weeks. Serum was separated and used directly for determents of AST, ALT, GGT, total bilirubin, direct bilirubin and alkaline phosphatase. Liver samples were taken for histopathological examination. Also, antioxidant enzymes including CAT, SOD and GPx and hepatic lipid peroxidation including MDA were examined in liver tissue. The results revealed that in *S. platensis* treated groups there were significant decrease of serum AST, ALT, ALP, T. Bili, D. Bil and GGT. Furthermore, there was significant increase of antioxidant enzymes in hepatic tissue. Higher dose of *S. platensis* increased the improvements of all parameters. These results revealed that *S. platensis* has a strong antioxidant and anti-hepatotoxic effects in mice treated with DEN.

Keywords: antioxidant, liver enzymes, *S. platensis*, DEN

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1. INTRODUCTION

Diethylnitrosamine (DEN), an N-nitroso alkyl compound, is a representative compound of the nitrosamine family and described as an effective hepatotoxin in experimental animals producing toxicity after repeated administration (Jose et al., 1998). DEN induces oxidative stress possibly due to the generation of reactive oxygen species (ROS) which are capable of initiating peroxidative damage to the cell (Bansal et al., 2005). DEN is biotransformed by mixed-function cytochrome P 450 dependent mono-oxidase systems and its metabolic activation is reported to be responsible for the onset of the toxic effects (Zimmerman, 1993). Medicinal plants play a key role in the health care. Natural products, especially plants in folk medicine with an anecdotal history of positive effects against liver diseases or other organs, are considered an alternative therapeutic approach (Abdel-Hameed et al., 2014). Due to lack of scientific-based pharmacological data for most of the herbal treatment of liver diseases (Stickel and Schuppan, 2007), hence, there is an ever increasing need for safe hepatoprotective agent (Abdel-Hameed, 2014). *S. platensis* is blue-green algae due to the presence of both chlorophyll (green) and phycocyanin (blue) pigments in its cellular structure. *S. platensis* is rich in proteins, carbohydrates, polyunsaturated fatty acids, sterols and some more vital elements such as calcium, iron, zinc, magnesium, manganese and selenium. It is a natural source of vitamin B_{12}, vitamin E, ascorbic acid, tocopherols and a whole spectrum of natural mixed carotene and xanthophylls phytopigments (Tantawy, 2015, Zhang, 2011). *S. platensis* shows immunomodulation effects, anti-inflammatory activity, antioxidant effects, anticancer effects and anti-viral effects (Pankaj and Varma, 2013, Yigit, 2016, Kim, 2010, Kepekçi, et al., 2013, Karadeniz, 2009, Ibrahim, and Abdel-Daim, 2015).

Consequently, the aim of the present study was to investigate the antioxidant and hepatoprotective effects of *S. platensis* against diethylnitrosamine-induced hepatotoxicity and oxidative stress in mice.

2. MATERIALS AND METHODS:

2.1. Experimental animal rats;

120 male albino mice weighting about 25-29 g. Mice were obtained from laboratory animal center, Faculty of Veterinary Medicine, Benha University. Animals were housed in separate metal cages, fresh and clean drinking water was supplied *ad libtium*. Mice were left for 7 days for acclimatization before the beginning of the experiment. Mice were kept at a constant environmental and nutritional condition throughout the period of the experiment.

2.2 Experimental chemical substances:

Diethylnitrosamine [N-Nitrosodiethylamine, ISOPAC®, $C_4H_{10}N_2O$, Mol.Wt:102.14, d:0.95 g/ml (lit)] obtained from Sigma-aldrich CO., 3050 Spruce Street, St. Louis, Sigma-aldrich Chemie Gmbh and administrated intraperitoneal at a dose 100 mg/kg body weight once and left for 20 weeks.

S. platensis are obtained from DXN Pharmaceutical Sdn (Bhd, Malaysia) and administrated intragasteric using stomach tube at 2 different doses: a dose of 500 mg / kg body weight once daily for 4 weeks and a dose of 1000 mg / kg body weight once daily for 4 weeks.

2.3. Experimental design:

Mice were divided into 5 main groups: Group I: Consists of 24 mice injected with normal saline and saved as control. Group II: included 24 mice injected with single dose of DEN (100 mg/kg body weight) I.P. and left till the end of the 20 weeks. Group III: included 24 mice injected with single dose of DEN (100 mg/kg body weight) (Afzal et al., 2012) I.P. and left till the end of the 16 weeks followed by oral dose of *S. platensis* at 500 mg/kg b.w (Tantawy 2015) daily for four weeks. Group IV: 24 mice were received single dose of DEN (100 mg/kg body weight) I.P. and left till the end of the 16 weeks followed by oral dose of *S. platensis* at 1000 mg/kg b.w daily for four weeks. Group V: 24 mice received oral dose of *S. platensis* at 500 mg/kg b.w daily for four weeks followed by single dose of DEN (100 mg/kg body weight) I.P. and left till the end of 20 weeks without *S. platensis*.

2.4. Sampling:

Blood samples and liver tissues were collected after 16, 18 and 20 weeks from DEN treatment. A-Blood samples were collected into plain centrifuge tubes to separate serum for estimation of Transaminase (L-alanine and L-aspartate) (Burtis and Bruns, 2014), Alkaline phosphatase (ALP) (Tietz et al., 1983), gamma-glutamyl transferase (GGT) (Szasz et al., 1974) and bilirubin (T.Bil, D.Bil) (Burtis et al., 2012).

B-Liver specimen: At the end of the 16, 18 and 20 weeks, mice were scarified to collect liver tissue. Liver specimens were divided into two portions, the first specimen was rapidly washed with saline to avoid drying, weighted and processed for determination of super oxide dismutase (SOD) (Marklund and Marklund, 1974), glutathione peroxidase (GPx) (Paglia and Valentine, 1967), catalase (Aebi, 1984) and malondialdehyde (MDA) (Okhawa et al., 1979). The second portion was used for histopathological examination. Liver from mice of different groups were fixed in 10% neutral formalin solution, dehydrated in graded alcohol and embedded in paraffin. Fine sections obtained were mounted on glass slides and counter-stained with Hematoxylin Eosin (H&E) for light microscopic analyses according to the method of Banchroft and Gamble, (2008).

2.5. Statistical analysis:

The obtained data were analyzed with the statistical software package *SPSS* for Windows (version 11.0; SPSS Inc., Chicago, USA). Statistical analysis was carried out with one way ANOVA test (Snedecor and Cochran, 1982).

2. RESULTS

The results presented in (Tables 1, 2) revealed that mice injected with DEN induced liver toxicity caused significant increase in serum ALT, AST, ALP, GGT activities and T. Bili and D.Bili Levels after 16 weeks when compared with control mice. Treatment with *S. platensis* treatment (500 mg/kg b.w) showed significant decrease of their levels. Increasing dose of *S. platensis* (1000 mg/kg b.w) at 18 and 20 weeks showed highly significant decrease of hepatic enzymes when compared to DEN-intoxicated mice and improve them near to control group. Pretreatment with *S. Platensis* (500 mg/kg b.w) before DEN administration maintain hepatic enzymes levels close to normal levels.

The results printed in (Tables 3) showed that in DEN injection to mice caused significant decrease in the activity of the liver antioxidant enzymes CAT, SOD and GPx and significant increase of MDA concentration after 16 weeks when compared with control. *S. platensis* in groups III and IV alleviated the decrease in GPx, Catalase and SOD of the liver as compared to DEN-intoxicated mice indicating its antioxidant activity. Prophylactic treatment by *S. platensis* before DEN

intoxication prevents toxic effect of DEN and maintains levels of GR, Catalase and SOD within normal levels. Also, MDA levels revealed significant decrease in *S. platensis* treated groups.

The pathological findings were observed in DEN intoxicated group were focal coagulative necrosis in diffuse manner all over the hepatic parenchyma associated with sever congestion in the central and portal veins, alteration of hepatic architecture and increased mitotic index. Furthermore, macrocytic dysplasia of hepatocytes with infiltrations with lymphocytes and plasma cells were observed. Also, dysplastic hepatocytes were observed with enlarged nuclei (karyomegaly) and multiple nucleoli in the liver sections of mice treated with DEN were shown (Photo. 1, 2&3). Treatment with *S. platensis* decrease toxic effects of DEN and showed mild dilatation and congestion were noticed in the central veins with and few inflammatory cells infiltration in the portal area (Photo. 4&5). Higher dose of *S. platensis* revealed more improvement and increased protection of liver tissue (Photo. 6&7). Pretreatment with *S. platensis* before DEN intoxication revealed mild dilatation and congestion in the central veins with diffuse kupffer cells proliferation between hepatocytes (Photo. 8).

Table (1): Liver enzymes in control, DEN induced, DEN+500 mg/kg *S.p,* DEN+1000 mg/kg *S.p* and protection groups after 16, 18 and 20 weeks.

	Period (week)	Groups				
		Group1	Group 2	Group 3	Group 4	Group5
ALT (U/L)	16 W	58.04±1.33^{a}	167.31±2.97^{cd}	161.51±1.55^{c}	166.87±1.64^{d}	67.33±1.49^{b}
	18 W	59.69±0.66^{a}	165.48±1.97^{e}	123.42±1.27^{d}	98.04±2.11^{c}	72.97±2.36 ^{b}
	20 W	59.48±0.57^{a}	170.91±2.10^{e}	99.11±1.33^{d}	77.73±1.05^{c}	71.98±0.83^{b}
AST (U/L)	16 W	80.69±0.74^{a}	199.68±2.95^{c}	206.90±1.83^{c}	209.38±1.81^{d}	92.88±1.27^{b}
	18 W	79.61±1.16^{a}	201.45±2.05^{e}	158.40±1.64^{d}	125.51±1.96^{c}	96.39±1.72^{b}
	20 W	80.04±0.63^{a}	198.75±1.56^{e}	129.01±0.84^{d}	96.08±1.40^{b}	99.62±1.13^{c}
ALP (U/L)	16 W	50.34±0.77^{a}	89.11±1.05^{c}	91.37±0.82^{c}	90.46±0.91^{c}	54.04±1.21^{b}
	18 W	50.22±0.83^{a}	90.58±1.41^{e}	79.03±1.10^{d}	66.46±1.12^{c}	55.78±0.78^{b}
	20 W	50.69±0.54^{a}	91.45±1.63^{e}	67.45±0.94^{d}	56.58±0.81^{b}	58.55±0.61^{c}
GGT (U/L)	16 W	43.86±1.72^{a}	92.34±2.12^{b}	89.32±1.13^{b}	90.97±1.55^{b}	47.99±2.03^{a}
	18 W	41.95±1.84^{a}	91.72±1.61^{d}	78.66±1.50^{c}	65.99±2.06^{b}	46.08±1.85^{a}
	20 W	42.51±1.73^{a}	90.94±2.13^{d}	67.96±1.86^{c}	53.43±1.47^{b}	45.93±1.64^{a}

Table (2): Total bilirubin and direct bilirubin in control, DEN induced, DEN+500 mg/kg *S.p,* DEN+1000 mg/kg *S.p* and protection groups after 16, 18 and 20 weeks.

	Period (week)	Groups				
		Group1	Group 2	Group 3	Group 4	Group5
T. Bili (mg/dl)	16 W	1.08±0.04^{a}	2.13±0.07^{c}	1.98±0.04^{c}	2.00±0.04^{c}	1.37±0.03^{b}
	18 W	1.03±0.05^{a}	2.15±0.04^{d}	1.77±0.02^{c}	1.52±0.01^{b}	1.51±0.01^{b}
	20 W	1.12±0.04^{a}	2.08±0.09^{d}	1.55±0.02^{c}	1.41±0.01^{b}	1.49±0.02^{c}
D. Bili (mg/dl)	16 W	0.30±0.01^{a}	1.22±0.07^{c}	1.09±0.04^{c}	1.12±0.05^{c}	0.54±0.03^{b}
	18 W	0.29±0.01^{a}	1.17±0.05^{e}	0.80±0.02^{d}	0.61±0.02^{c}	0.50±0.01^{b}
	20 W	0.30±0.01^{a}	1.08±0.03^{d}	0.66±0.01^{c}	0.51±0.01^{b}	0.49±0.01^{b}

Table (3): Antioxidant enzymes (SOD, GPx and catalase) and MDA in control, DEN induced, DEN+500 mg/kg *S.p,* DEN+1000 mg/kg *S.p* and protection groups after 16, 18 and 20 weeks.

	Period (week)	Groups				
		Group1	Group 2	Group 3	Group 4	Group5
SOD (u/g)	16 W	271.32±5.14^{c}	201.27±11.27^{a}	198.35±15.76^{a}	211.84±9.31^{a}	247.15±8.34^{b}
	18 W	295.81±8.22^{c}	213.65±8.31^{a}	220.16±6.81^{a}	241.77±5.19^{b}	255.09±11.61^{b}
	20 W	288.03±6.81^{d}	207.13±9.44^{a}	239.61±4.54^{b}	263.18±6.55^{c}	250.16±7.28^{c}
Catalase (u/g)	16 W	537.25±6.23^{c}	331.15±10.12^{a}	352.39±9.40^{a}	347.61±6.22^{a}	445.21±8.73^{b}
	18 W	541.89±5.97^{d}	360.27±8.43^{a}	389.62±6.38^{b}	438.12±5.32^{c}	452.30±6.02^{c}
	20 W	542.37±8.19^{e}	344.95±9.11^{a}	482.61±5.15^{c}	501.97±4.85^{d}	439.68±8.04^{b}
GR (u/g)	16 W	3017.52±36.21^{c}	1415.27±6.33^{a}	1402.18±9.23^{a}	1423.75±8.03^{a}	2715.36±12.28^{b}
	18 W	2981.64±21.84^{e}	1426.15±8.21^{a}	1875.31±11.51^{b}	2015.27±15.17^{c}	2699.75±10.12^{d}
	20 W	3164.27±30.91^{e}	1397.29±7.83^{a}	2163.39±11.73^{b}	2521.93±9.77^{c}	2728.21±9.36^{d}
MDA (Mmol/g)	16 W	193.71±8.71^{a}	372.16±4.21^{c}	369.02±5.14^{c}	375.14±7.36^{c}	216.13±2.66^{b}
	18 W	187.31±6.52^{a}	361.25±5.74^{e}	321.48±3.27^{d}	297.08±2.16^{c}	210.75±4.69^{b}
	20 W	195.02±9.23^{a}	368.51±7.09^{e}	280.31±3.11^{d}	251.17±1.97^{c}	224.16±3.89^{b}

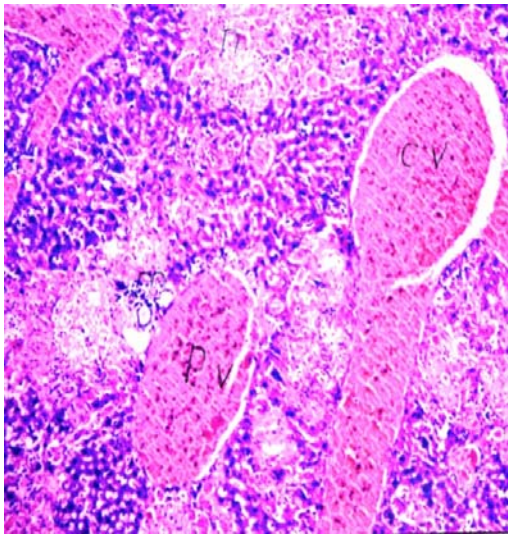

Photo 1: Liver of mice administrated 100 mg/kg DEN for 16 weeks showing focal coagulative necrosis (n) in hepatic parenchyma with sever congestion in the central (cv) and portal veins (pv) and few inflammatory cells infiltration (m). H&E (× 16).

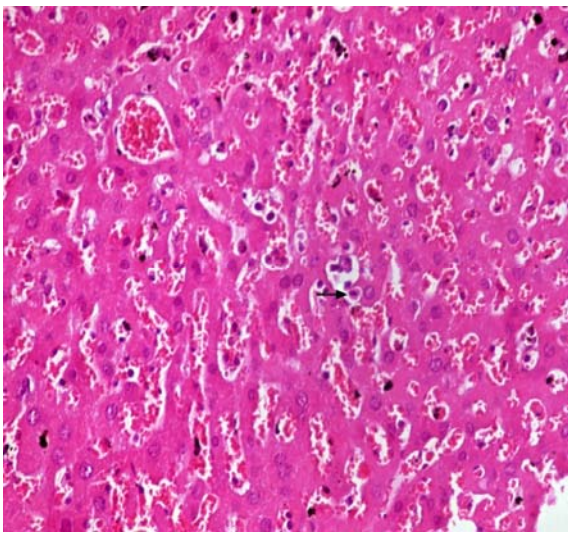

Photo 2: Liver of mice administrated 100 mg/kg DEN for 16 weeks showing alteration of hepatic architecture and increased mitotic index (arrow). H& E (× 40) .

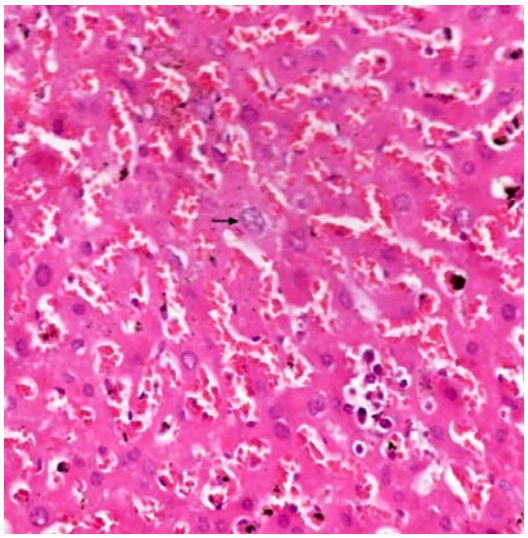

Photo 3: Liver of mice administrated 100 mg/kg DEN for 16 weeks showing dysplastic hepatocytes with enlarged nuclei (karyomegaly) and multiple nucleoli. Macrocytic dysplasia of hepatocytes (arrow) with infiltrations with lymphocytes and plasma cells. H&E (× 40).

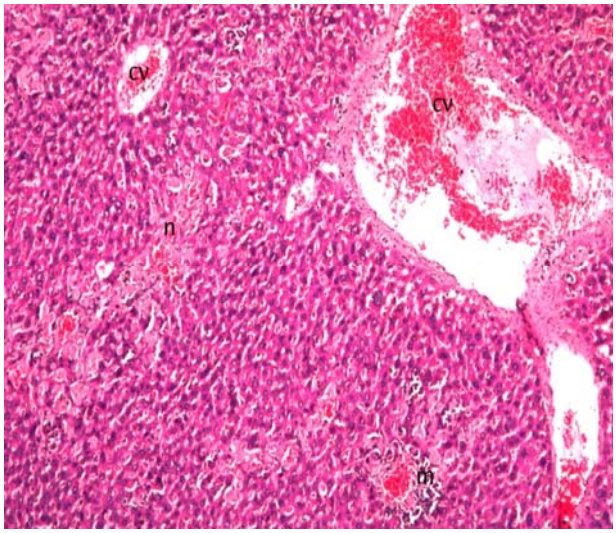

Photo 4: Liver of mice intoxicated by DEN then treated with *S. platensis* (500 mg/kg) at 18 w showing moderate dilatation and congestion were noticed in the central veins (cv) with focal necrosis in the hepatocytes (n) and inflammatory cells infiltration in the portal area (m). H&E (×16).

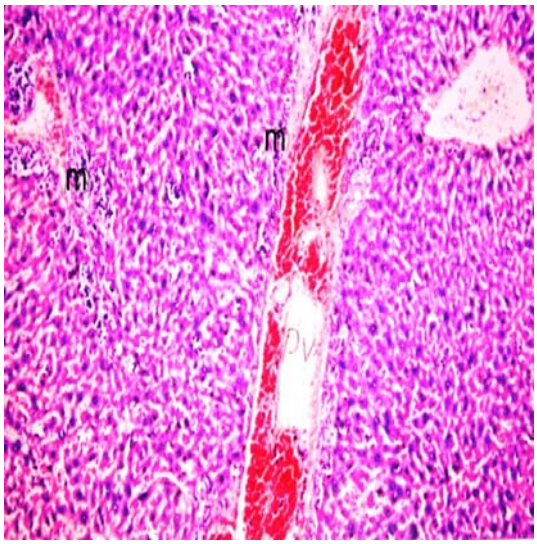

Photo 5: Liver of mice intoxicated by DEN then treated with *S. platensis* (500 mg/kg) at 20 weeks showing mild congestion in the portal veins (pv) with few inflammatory cells (m) infiltration in the portal area. H&E (×16).

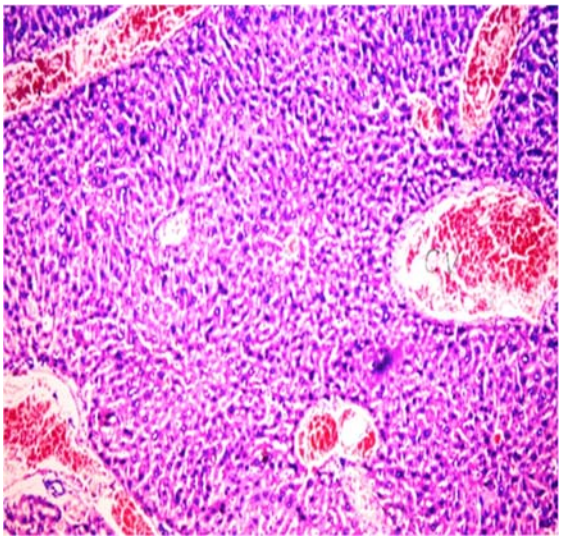

Photo 6: Liver of mice intoxicated by DEN then treated with *S. platensis* (1000 mg/kg) at 18 weeks showing mild dilatation and congestion in the central veins (cv). H&E (× 16).

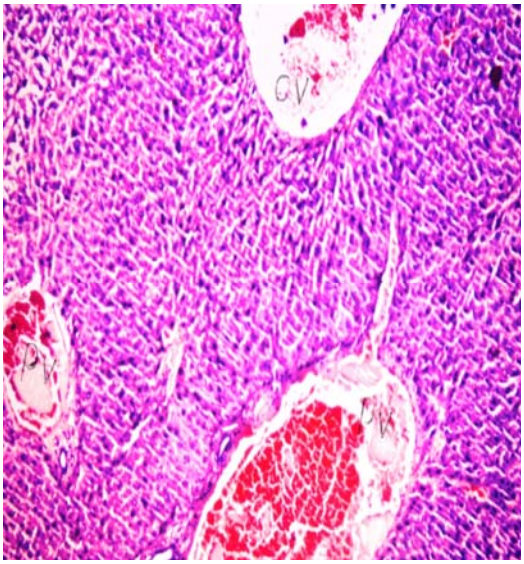

Photo 7: Liver of mice intoxicated by DEN then treated with S. platensis (1000 mg/kg) at 20 weeks showing congestion in both central veins (cv) and portal veins (pv). (× 16).

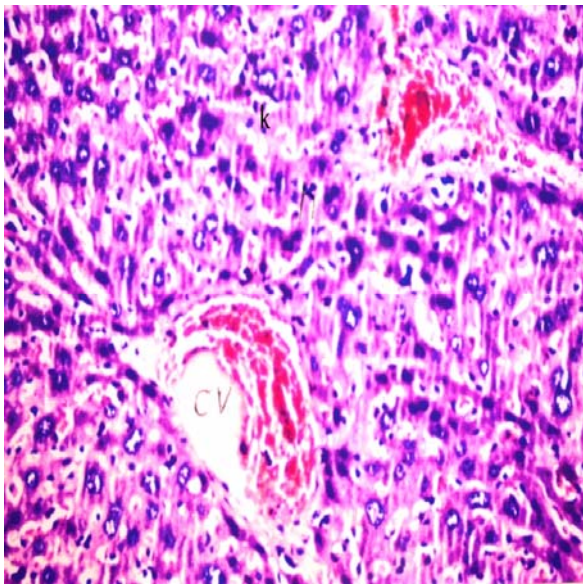

Photo 8: Liver of mice treated with S. platensis before DEN intoxication showed mild dilatation and congestion in the central veins (cv) with diffuse kupffer cells proliferation (k) between hepatocytes. H&E (× 40).

4. DISCUSSION

In the present study, *S. platensis* were examined for treatment of liver toxicity and significant increase in serum ALT, AST, ALP and GGT activities and T. Bili level were observed in mice injected with DEN when compared with control. This obtained result was nearly similar to the previously reported data by Duan et al., (2014). Also, results come in accordance with Hemieda et al., (2016) who recorded that DEN induces hepatotoxicity indicating hepatocellular damage and impairment of liver functions and subsequent fall in the tissue might be due to the leakage of these cytosolic enzymes into the circulatory system resulting from hepatocellular damage (Duan et al., 2014). Moreover, Rezaie et al., 2014) demonstrated that DEN, one of the most important environmental hepatotoxin and carcinogens, has been suggested to generate ROS resulting in oxidative stress and cellular injury. After being metabolized by cytochrome p450, DEN generates highly reactive free radicals and initiates lipid peroxidation of the cell membrane of the endoplasmic reticulum and causes a chain reaction. Produced ROS can cause oxidative damage in DNA, proteins and lipids (Vitaglione et al., 2004).

Treatment with *S. platensis* lowered the elevated levels of AST, ALT, ALP, GGT activities and T. Bili and D. Bili levels in mice treated with DEN. *S. platensis* stabilizes the hepatocyte membrane by preventing lipid peroxidation, ameliorating the activities of the antioxidant enzymes, inhibition of the inflammation and the radical scavenging activity due to increased amounts of phycocyanine and phenolic compounds and the antioxidant capacity of *S. platensis* (Kepekçi et al., 2013). Furthermore, Luxia et al. (1996) reported that β-carotene of *S. platensis* may reduce cell damage especially to DNA molecules thus playing the role in the repair of regeneration process of damaged liver cells. These findings were in agreement with Tantawy, (2015) and Yigit et al., (2016).

Diethylnitrosamine intoxication causes a significant increase in lipid hydroperoxide and hepatic MDA levels. The observed increase in peroxide levels in mice treated with DEN may indicate oxidative stress which affects liver organelles. Lipid peroxides that accumulate due to peroxidation of lipids are known to be harmful to cells and tissues (Bansal et al., 2005). In contrast, *S. platensis* reduces the oxidative stress produced by DEN via its antioxidant components, phycocyanin and β-carotene (Guan et al., 2009) or via reduction of lipid peroxidation (Karadeniz et

al., 2009). β-carotene of *S. platensis* may scavenge free radicals and reduces lipid peroxidation (Guan et al., 2009). Selenium-containing allophycocyanin (Se-APC), furthermore, inhibited 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH)-induced intracellular ROS production and MDA accumulation (Zhang et al., 2011).

Regarding to antioxidant enzymes, intraperitoneal injection of DEN in mice exhibited a significant decrease in the activity of hepatic GR, Catalase and SOD levels as compared to normal control. The principal toxic effects of DEN involve the complete disruption of the antioxidant defense mechanism of the liver. The decline in these enzyme activities could also be due to reduction in their biosynthesis or their excessive utilization in trapping the free radicals generated (Pradeep et al., 2010). These are in agreement with Poojari et al., (2010) and Jayakumar et al., (2012).

On the other hand, treatment of DEN intoxicated mice with *S. platensis* caused a significant increase in the activity of the antioxidant enzymes GPx, CAT and SOD with control. *S. platensis* exerts its protective effect against DEN directly through its antioxidant properties or indirectly through maintaining the hepatic GR, Catalase and SOD levels. The protective effect of *S. platensis* against oxidative stress has been largely attributed to their high content of C-PC, a water soluble phycobiliprotein in *S. platensis* (Romay et al., 2003). Whereas C-PC prevented DNA damage and scavenged hydroxyl and peroxylradicals (Bhat and Madyastha, 2000). Also, Selenium-containing allophycocyanin (Se-APC) extracted from selenium-enriched *S. platensis* inhibited 2,20-azobis-2-methylpropanimidamide, dihydrochloride (AAPH)-induced oxidative hemolysis. Furthermore, another ingredient of *S. platensis*, β-carotene, has been reported to have antioxidant and anti-inflammatory activities. β-carotene inhibited the production of nitric oxide and prostaglandin E(2) and suppressed the expression of inducible nitric oxide synthase (iNOS) and cyclooxygeanase-2 (COX-2) (Schafer et al., 2002). *S. platensis* also significantly decreased thiobarbituric acid reactive substance (TBARS) values, an index of lipid peroxidation and oxidative stress, in plasma, liver, kidney and heart and reduced oxidative stress induced DNA damage in lymphocytes (Kim et al., 2010). These results are in agreement with Aita, (2014) and Tantawy, (2015).

Histologically, in DEN injected mice the specimen of liver showed focal coagulative necrosis in diffuse manner all over the hepatic parenchyma associated with sever congestion in the central and portal veins, alteration of hepatic architecture and increased mitotic index and macrocytic dysplasia of hepatocytes with infiltrations with lymphocytes and plasma cells. Furthermore, dysplastic hepatocytes were observed with enlarged nuclei (karyomegaly) and multiple nucleoli in the liver sections of mice treated with DEN confirmed the toxic effects. These results came in agreement with Choi et al., (2010) and Rezaie et al., (2014).

Treatment with *S. platensis* reversed DEN induced pathogenic changes in liver caused improvement in liver necrosis with regeneration of hepatocytes became nearly similar to normal liver. Mice treated with *S. platensis* showed mild dilatation and congestion in the central veins with few focal necrosis in the hepatocytes and few inflammatory cells infiltration in the portal area. These results are in accordance with (Tantawy, 2015) who recorded that *S. platensis* restored the liver injuries by scavenging the free radicals and preventing inflammation more effectively. The increased phycocyanine content of *S. platensis* may also be partly involved in the inflammatory processes. It has been recently reported that phycocyanine could block inflammatory infiltration through its anti-inflammatory activities.

Therefore, from the present study it can be concluded that *S. platensis* showed antioxidant and hepatoprotective effects in DEN induced hepatotoxicity, which can be attributed to its nutritive elements and vitamins content. This could serve as a steppingstone towards the discovery of newer safe and effective antitumor agents.

3. REFERENCES:

Abdel-Hameed O.M., Mohamed E. Shehata, M.E., Gabr, F. El-Bagoory, G.F. and Amin, A.G., (2014). Effect of Calotropis Procera extract on thioacetamide induced liver cirrhosis in rats. *Benha Veterinary Medical Journal*, 27(2), 126-139.

Aebi, H., (1984). Catalase in vitro. *Methods in enzymology* 105, 121-126.

Aita, N.A., (2014). Hepatoprotective effect of *spirulina platensis* against aluminum chloride induced liver damage in rats. *Global Veterinaria* 13 (4), 552-559.

Banchroft, J.D. and Gamble, M., (2008). Theory and practice of histological techniques. *Elsevier Health Sciences*. Churchill Living stone, New York, London, San Francisco, Tokyo.

Bansal, A.K., Bansal, M., Sonni, G. and Bhatnagar, D., (2005). Modulation of N-nitrosodiethylamine induced oxidative stress by vitamin E in rat erythrocytes. *Hum Exp Toxicol* 24, 297-302.

Bhat, V.B. and Madyastha, K.M., (2000). C-phycocyanin: a potent peroxylradical scavenger in vivo and in vitro. *Biochem Biophys Res Commun* 275, 20–25.

Burtis, C.A. and Bruns, D.E., (2014). Tietz fundamentals of clinical chemistry and molecular diagnostics. *Elsevier Health Sciences.*

Burtis, C.A., Ashwood, E.R. and Burns, D.E. (2012). Tietz textbook of clinical chemistry and molecular diagnostics. *Elsevier Health Sciences.*

Choi, J.H., Wang, Y.H., Choi, C.Y., Chung, Y.C. and Jeong, H.G., (2010). Anti-fibrotic effects of the anthocyanins isolated from the purple-fleshed sweet potato on hepatic fibrosis induced by dimethylnitrosamine administration in rats. *Food and Chemical Toxicology* 48, 3137–3143.

Duan, X.Y., Pan, Q., Yan, S.Y., Ding, W.J., Fan, J.G. and Qiao, L., (2014). High-saturate-fat diet delays initiation of diethylnitrosamine-induced hepatocellular carcinoma. *BMC Gastroenterology* 14, 195-198.

Guan, X.Y., Zhang, W.J., Zhang, X.W. and Li, Y.X., (2009). A potent antioxidantproperty: fluorescent recombinant alpha-phycocyanin of *Spirulina*. *Appl. Microbiol* 106, 1093-1100.

Hemieda, F.A.E., Serag, H.M., El-Baz, E. and Ramadan, S.M.E., (2016). Therapeutic efficacy of licorice and/or cisplatin against diethylnitrosamine and carbon tetrachloride-induced hepatocellular carcinoma in rats. *J Am Sci* 12(1), 10-19.

Henry, R.J., Cannon, D.C. and Win, J.W., (1974). Method of Protein Determination in Plasma. *Clin.Chem* 20, 1362-1363.

Ibrahim, A.E. and Abdel-Daim, M.M. (2015). Modulating effects of spirulina platensis against tilmicosin-induced cardiotoxicity in mice. *Cell Journal (Yakhteh)* 17(1), 137–144.

Jayakumar, S., Madankumar, A., Asokkumar, S., hakumar, S., Gokuladhas, K., Kamaraj, S., Divya, M.G. and Devaki, T., (2012). Potential preventive effect of carvacrol against diethyl nitrosamine-induced hepatocellular carcinoma in rats. *Mol Cell Biochem* 360, 51–60.

Jose, J.K., Kuttan, R. and Bhattacharaya, R.K., (1998). Effect of emblica officinalis extract on hepatocarcinogenesis and carcinogen metabolism. *J.Clin. Biochem. Nutr* 25, 31-39.

Karadeniz, A., Cemek, M. and Simsek, N., (2009). The effects of panax ginseng and *spirulina platensis* on hepatotoxicity induced by cadmium in rats. *Ecotoxicology and Environmental Safety* 72, 231– 235.

Kepekçi, R.A., Polat, S., Çelik, A., Bayat, N. and Saygideger, S.D., (2013). Protective effect of Spirulina platensisenriched in phenolic compounds against hepatotoxicity induced by CCl4. *Food Chem* 141, 1972-1979.

Kim, M., Cheong, S.H., Lee, J.H., Kim, M.J., Sok, D.E. and Kim, M.R., (2010). *Spirulina* improves antioxidant status by reducing oxidative stress in rabbits fed a high-cholesterol diet. *J Med Food* 13, 420–426.

Luxia, A.S., Monica, S., Ornella, C., Pizzala, B., Loura, R., Livia, B., Anio, M. and Ennio, P., (1996). Effect of B-carotene on cell cycle progression of human fibroblasts. *Mutagenesis* 17, 2395-2401.

Marklund, S. and Marklund, G., (1974). Involvement of the superoxide anion radical in the auto-oxidation of pyrogallol and a convenient assay for Superoxide dismutase. *Eur J Biochem* 47, 469-474.

Okhawa, H., Ohishin, N. and Yagi, K., (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95, 351-358.

Paglia, D.E. and Valentine, W.N., (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med.* 70(1), 158-69.

Pankaj, P. and Varma, M.C., (2013). Potential role of *Spirulina platensis* in maintaining

blood parameters in alloxan-induced diabetic mice. *Int J Pharm Pharm Sci* 5(4), 450-456.

Poojari, R., Gupta, S., Maru, G., Khade, B. and Bhagwat, S., (2010). Chemopreventive and hepatoprotective effects of embelin on N-nitrosodiethylamine and carbon tetrachloride induced preneoplasia and toxicity in rat liver. *Asian Pacific Journal of Cancer Prevention* 11, 1015-1020.

Pradeep, K., Mohan, C.V., Gobianand, K. and Karthikeyan, S. (2010). Protective effect of cassia fistula linn. on diethylnitrosamine-induced hepatocellular damage and oxidative stress in ethanol pretreated rats. *Biol Res* 43, 113-125.

Rezaie, A., Pashmforosh, M., Karamallah, M.H., Fazlara. A., Haghighat. N. and Shahriari. A., (2014). Hepatoprotective effect of caffeine on diethyl nitrosamine-induced liver injury in rats. *Bulgarian Journal of veterinary medicine* 17(3), 183-190.

Romay, C., Gonzalez, R., Ledon, N., Remirez, D. and Rimbau, V., (2003). C-phycocyanin; Abiliprotein with antioxidant, anti-inflammatory and neuroprotective effects. *Curr Protein Pept Sci* 4, 207–216.

Schafer, F.Q., Wang, H.P., Kelley, E.E., Cueno, K.L., Martin, S.M. and Buettner, G.R., (2002). Comparing beta-carotene, vitamin E and nitric oxide as membrane antioxidants. *Biol Chem*. 383, 671–681.

Stickel, F. and Schuppan, D., (2007). Herbal medicine in the treatment of liver diseases. Dig Liver Dis 39(4):293-304.

Snedecor, G.W. and Cochran, W.G. (1982). Statistical method.7th ed., Iowa state university press, Ame Iowa, USA.

Szasz, G., Persijn, J.P. and Coll, E., (1974). Kinetic Method for quantitative determination of gammaglutamyl transpeptidase. *Z. Klin. Chem. Klin. Biochem* 12, 228–238.

Tantawy, W.H., (2015). Antioxidant effects of *Spirulina* supplement against lead acetate-induced hepatic injury in rats. *Journal of Traditional and Complementary Medicine.*

Tietz, N.W., Burtis, C.A., Duncan, P. and Ervin, K., (1983). Reference method for measurement of alkaline phosphatase activity in human serum. *Journal of Clinical Chemistry* 29(5), 751–761.

Vitaglione, P., Morisco, F., Caporaso, N., and Fogliano, V., (2004). Dietary antioxidant compounds and liver health. Crit. Rev. Food Sci. Nutr. 44, 575-586.

Yigit, F., Gurel-Gurevin, E., Isbilen-Basok, B., Esener, O., Bilal, T., Keser, O., Altiner, A., Yilmazer, N. and Ikitimur-Armutak, E.I., (2016). Protective effect of *Spirulina platensis* against cell damage and apoptosis in hepatic tissue caused by high fat diet. *Biotech Histochem* 91(3), 182-194.

Zhang, H., Chen, T., Jiang, J., Wong, Y.S., Yang, F. and Zheng, W., (2011). Selenium-containing allophycocyanin purified from selenium enriched Spirulina platensis attenuates AAPH-induced oxidative stress in human erythrocytes through inhibition of ROS generation. *J Agric Food Chem* 59, 8683–8690.

Zimmerman, H.J., (1993). Hepatotoxicity. *Dis Mon* 39, 675-787.