Immunostimulant effects of dietary *Spirulina platensis* on diethylnitrosamine - injected mice.

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A B S T R A C T

The present study was designed to study the effect of *S. platensis* on blood parameters, immune system, metabolic functions of liver and NF-κB p65 expression in liver tissue against hepatotoxicity induced by diethylnitrosamine (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 once for 20 weeks. Group three was administered 100 mg DEN/kg once for 16 weeks then received *S. platensis* (500 mg/kg) for 4 weeks. Group four was administered 100 mg DEN/kg once for 16 weeks then received *S. platensis* (1000 mg/kg) for 4 weeks. Group five was received *S. platensis* (500 mg/kg) for 4 weeks then administered 100 mg DEN/kg once for 20 weeks. Whole blood samples were collected on EDTA for hematology. Serum was separated for biochemical determents and immunological parameters. Liver samples were taken for immunohistochemical examination of NF-κB p65 expression in hepatic tissue. The results revealed that in *S. platensis* treated groups there were significant decrease of serum cholesterol, triglycerides and glucose. Also, anti-inflammatory cytokines (IL-4 and IL10) were decrease significantly in *S. platensis* treated groups. On the other hand; there was significant increase of total protein, albumin and proinflammatory cytokines (TNF-α and IL-6). Furthermore, *S. platensis* revealed significant decrease of NF-κB p65 expression in hepatic tissue which induced by DEN. Higher dose of *S. platensis* increased the improvements of all parameters. These results revealed that *S. platensis* has a strong anti-hepatotoxic and immune supplement effects in mice treated with DEN.

Key words: TNF-α, IL-6, *Spirulina platensis*, diethylnitrosamine, mice

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1. INTRODUCTION

The liver is a vital organ; plays major role in metabolism including regulation of glycogen storage, plasma proteins synthesis, hormones production and detoxification (El-sharkawy and Mann, 2007). Furthermore, the liver is a unique immunological site in which antigen-rich blood from the gastrointestinal tract is pressed through a network of sinusoids and scanned by antigen presenting cells and lymphocytes. The liver's lymphocyte population plays critical roles in first line immune defense against invading pathogens and modulation of liver injury (Visentainer et al., 2003). Nuclear factor-kappa B (NF-κB) is a transcriptional regulator of genes involved in immunity, inflammatory response, cell fate and function. Recent attention has focused on the pathophysiological role of NF-κB in the diseased liver ((El-sharkawy and Mann, 2007)). Injury-induced activation of hepatic NF-κB is observed in a variety of nonparenchymal and parenchymal liver cells indicating that NF-κB plays a central role in coordinating the inflammatory and wound healing response by stimulating gene transcription in multiple key cellular players. Kupffer cells (the liver's resident macrophages) display powerful NF-κB activation in response to liver injury by alcohol, carbon tetrachloride, endotoxin and cholestasis resulting in production and secretion of proinflammatory cytokines (including the hepatomitogens TNF-α and IL-6) that are strongly implicated as promoters of fibrosis and HCC (Visentainer et al., 2003). Spirulina platensis (S. platensis) (a blue-green algae) has proven to be a rich source of bioactive compounds of potential medicinal interest. Various algae extracts have received increased attention due to their potent pharmacological effects, particularly as vivo antimicrobial (Soltani et al., 2012), immunological (Hassan, 2016, Saker et al., 2004, Cheng, 1994, Muga, 2014, Pankaj, and Varma, 2013,

Soltani, 2012, Simsek, 2009, Simsek, 2007) and antitumoral activities (Iwata, 1990, Lee et al., 2003, Ku, 2013).

Consequently, the aim of the present study was to investigate the immunomodulatory effects of *S. platensis* against diethylnitrosamine-induced hepatotoxicity by following up the level changes of CBC, IL-4, IL-10, IL-6 and TNF-α in mice. In addition, NF-κB expression in hepatic tissue was examined

2. Materials and Methods:

2.1. Experimental animal rats:

This experiment was conducted on 120 male albino mice weighting about 25-29 g. Mice were obtained from laboratory animal center, Faculty of Veterinary Medicine, Benha University. They were housed in separate metal cages, fresh and clean drinking water was supplied *ad libtium*. Before the beginning of the experiment, mice were left for 7 days for acclimatization. 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 (Afzal et al., 2012).

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 (Tantawy 2015) once daily for 4 weeks and a dose of 1000 mg / kg body weight once daily for 4 weeks.

2.3. Experimental design:

The animals were divided into five groups, each group containing twenty four mice. Animals in group 1 were used as control group and no treatment was given to these rats. Animals in group 2 were injected with single dose of DEN (100 mg/kg body weight) I.P. and left till the end of the 20 weeks. Group 3 was injected (I.P) with single dose of 100 mg/kg DEN and left till the end of the 16 weeks followed by oral gavage of *S. platensis* at 500 mg/kg b.w daily for four weeks. Animals in group 4 were injected (I.P) with single dose of 100 mg/kg DEN and left till the end of the 16 weeks followed by oral gavage of *S. platensis* at 1000 mg/kg b.w daily for four weeks. Animals in group 5 were given *S. platensis* (500 mg/kg) by gavage for 4 weeks before DEN administration and continued up to 20 weeks after DEN treatment.

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 EDTA and plain centrifuge tubes. EDTA tubes used for determination of red blood cell count (RBCs) (Feldman et al., 2000), hemoglobin concentration (Hb%) (D'Armour et al., 1965), hematocrit (HCT%) (Barbra, 1988), mean corpuscular volume (MCV) (Feldman et al., 2000) and mean corpuscular hemoglobin concentration (MCHC) (Feldman et al., 2000). Serum was separated for biochemical analysis of cholesterol (Roeschlau et al., 1974), triglycerides (Fossati and Prencipe, 1982), total protein (Henry et al., 1974), albumin (Doumas et al., (1971) and glucose (Kunst et al., 1984). Also, Proinflammatory cytokines (TNF-α and IL-6) and anti-inflammatory cytokines (IL-4 and IL-10) in serum were measured according to the method of Visentainer et al., (2003).

B-Liver specimen: At the end of the 16, 18 and 20 weeks and 6th week, mice were scarified to collect liver tissue used for immunohisochemical examination of NF-kB expression in hepatic tissue according to method (Punsawad et al., 2013).

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).

3. RESULTS:

The results presented in (Tables 1) revealed significant decrease of RBCs, Hb, HCT and MCHC while MCV showed significant increase in mice injected with DEN when compared to control. Treatment with *S. platensis* (500 mg/kg b.w) showed significant increase of RBCs, Hb, HCT and MCHC and significant increase of MCV. Increasing dose of *S. platensis* (1000 mg/kg b.w) at 18 and 20 weeks showed highly significant improvement of all parameters when compared to DEN-intoxicated mice and became them near to control group. Pretreatment with S. Platensis (500 mg/kg b.w) before DEN administration maintain blood parameters close to normal levels.

The data prefect in table 2 showed a significant increase in serum cholesterol, triglycerides and glucose levels while total protein and albumin levels revealed significant decrease in in DEN injected mice. S. platensis treated mice at a dose of 500mg/kg showed a significant reduction

of cholesterol, triglycerides and glucose levels and
significant increase of total protein and albumin

Table (1): RBCs, Hb, HCT and MCHC 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				
		Group 1	Group 2	Group 3	Group 4	Group 5
Hb (g/dl)	16 W	14.22±0.05^{c}	9.44±0.98^{a}	10.51±0.14^{a}	10.52±0.17^{a}	12.69±0.15^{b}
	18 W	14.29±0.09^{d}	10.12±0.66^{a}	12.06±0.17^{b}	12.76±0.12^{c}	12.67±0.14^{c}
	20 W	14.45±0.13^{e}	10.87±0.82^{a}	12.75±0.11^{b}	13.96±0.06^{d}	12.94±0.15^{c}
RBCs ($\times10^{6}$/µl)	16 W	4.07±0.29^{b}	3.19±0.21^{a}	3.37±0.08^{a}	3.48±0.11^{a}	4.22±0.04^{b}
	18 W	4.72±0.08^{d}	3.55±0.07^{a}	3.77±0.06^{b}	4.14±0.04^{c}	4.16±0.03^{c}
	20 W	4.80±0.06^{e}	3.40±0.16^{a}	4.06±0.02^{b}	4.51±0.03^{d}	4.26±0.01^{c}
HCT (%)	16 W	42.11±1.12^{c}	30.91±0.45^{a}	30.14±1.57^{a}	31.70±0.65^{a}	38.64±0.47^{b}
	18 W	42.77±0.47^{d}	31.24±0.82^{a}	34.07±0.41^{b}	35.61±0.19^{b}	37.44±0.35^{c}
	20 W	41.82±0.50^{d}	31.08±1.17^{a}	37.73±0.47^{b}	39.31±0.43^{c}	39.43±0.41^{c}
MCV (fl)	16 W	90.71±2.36^{a}	98.16±1.79^{b}	101.84±1.46^{b}	99.51±1.05^{b}	91.60±1.27^{a}
	18 W	90.76±1.35^{a}	99.25±0.86^{d}	96.83±0.37^{c}	94.43±0.51^{b}	91.24±0.85^{a}
	20 W	90.41±0.44^{a}	100.34±1.15^{d}	94.44±0.27^{c}	92.88±0.14^{b}	91.77±0.49^{a}
MCHC (%)	16 W	33.81±0.46^{b}	29.98±0.50^{a}	30.47±0.43^{a}	30.72±0.26^{a}	32.87±0.59^{b}
	18 W	33.49±0.42^{d}	30.17±0.61^{a}	31.19±0.22^{b}	32.08±0.11^{c}	32.48±0.23^{cd}
	20 W	33.95±0.43^{c}	30.62±0.44^{a}	32.15±0.19^{b}	32.73±0.27^{b}	32.47±0.38^{b}

Table (2): T. protein, albumin, T. cholesterol, Triglycerides and glucose 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				
		Group 1	Group 2	Group 3	Group 4	Group 5
T. protein (g/dl)	16 W	4.97±0.09^{c}	3.69±0.05^{a}	3.74±0.04^{a}	3.78±0.03^{a}	4.56±0.05^{b}
	18 W	4.96±0.09^{e}	3.71±0.06^{a}	4.01±0.02^{b}	4.34±0.04^{c}	4.62±0.04^{d}
	20 W	4.96±0.09^{d}	3.75±0.04^{a}	4.22±0.02^{b}	4.64±0.02^{c}	4.56±0.02^{c}
Albumin (g/dl)	16 W	3.01±0.05^{c}	2.13±0.06^{a}	2.08±0.03^{a}	2.10±0.02^{a}	2.79±0.04^{b}
	18 W	3.04±0.04^{e}	2.09±0.05^{a}	2.29±0.01^{b}	2.52±0.02^{c}	2.82±0.02^{d}
	20 W	3.09±0.04^{e}	2.15±0.07^{a}	2.44±0.01^{b}	2.72±0.01^{c}	2.83±0.03^{d}
Cholesterol (mg/dl)	16 W	64.65±1.70^{a}	100.41±2.01^{c}	98.68±1.27^{c}	95.94±1.75^{c}	70.97±1.67^{b}
	18 W	67.02±1.35^{a}	99.17±1.45^{d}	79.93±1.62^{c}	74.70±1.44^{b}	71.70±1.08^{b}
	20 W	3.09±0.04^{e}	2.15±0.07^{a}	2.44±0.01^{b}	2.72±0.01^{c}	2.83±0.03^{d}
Triglyceride (mg/dl)	16 W	78.36±1.58^{a}	120.74±1.95^{c}	115.99±2.43^{c}	117.85±1.86^{c}	83.66±1.73^{b}
	18 W	75.77±1.67^{a}	119.21±2.17^{e}	102.74±1.39^{d}	90.96±2.57^{c}	80.56±1.35^{b}
	20 W	74.55±1.60^{a}	117.35±2.40^{d}	90.76±1.59^{c}	81.77±1.74^{b}	80.82±1.41^{b}
Glucose (mg/dl)	16 W	84.52±1.29^{a}	125.18±1.91^{c}	122.73±1.78^{c}	123.32±1.54^{c}	89.56±1.41^{b}
	18 W	83.17±1.71^{a}	123.24±2.05^{e}	112.17±1.51^{d}	106.36±1.30^{c}	90.37±1.39^{b}
	20 W	81.25±1.36^{a}	122.57±1.88^{d}	104.90±1.02^{c}	93.66±2.02^{b}	90.15±2.11^{b}

Table (3): Proinflammatory cytokines (TNF-α and IL-6) and anti-inflammatory cytokines (IL-10 and IL-4) 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				
		Group 1	Group 2	Group 3	Group 4	Group 5
TNF-α (Pg/ml)	16 W	17.81±0.52^{a}	50.17±1.13^{c}	46.64±2.18^{c}	48.58±0.70^{c}	23.80±0.40^{b}
	18 W	18.56±0.53^{a}	48.25±0.91^{e}	41.76±0.49^{d}	38.04±0.66^{c}	27.41±0.42^{b}
	20 W	19.05±0.55^{a}	49.70±1.02^{e}	38.02±0.58^{d}	31.11±0.65^{c}	28.57±0.59^{b}
IL-6 (Pg/ml)	16 W	38.58±1.04^{a}	101.23±1.15^{c}	100.97±1.02^{c}	103.89±1.65^{c}	51.22±0.91^{b}
	18 W	40.75±0.91^{a}	105.32±1.28^{e}	85.32±0.74^{d}	71.45±0.70^{c}	57.89±0.79^{b}
	20 W	43.09±1.01^{a}	98.13±1.24^{d}	72.37±0.80^{c}	60.11±0.71^{b}	61.53±0.61^{b}
IL-10 (Pg/ml)	16 W	20.69±0.85^{c}	10.02±0.99^{a}	9.31±0.56^{a}	9.56±0.53^{a}	16.30±0.43^{b}
	18 W	20.46±0.51^{c}	8.79±1.12^{a}	11.32±0.44^{a}	17.29±0.42^{b}	20.94±0.41^{c}
	20 W	23.79±0.62^{d}	9.67±1.28^{a}	13.57±0.60^{b}	20.52±0.59^{c}	21.10±0.46^{c}
IL-4 (Pg/ml)	16 W	58.93±1.26^{c}	25.46±1.27^{a}	27.19±1.01^{a}	29.89±1.66^{a}	56.69±1.27^{b}
	18 W	57.64±1.11^{e}	26.07±1.19^{a}	32.33±0.91^{b}	44.59±1.02^{c}	52.37±0.79^{d}
	20 W	54.80±0.99^{d}	28.16±0.97^{a}	36.61±0.49^{b}	48.05±0.82^{c}	48.69±0.80^{c}

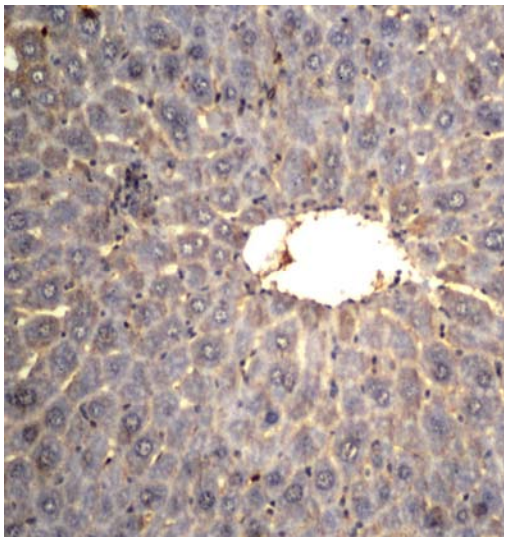

Photo 1: Liver of mice kept as control showing normal hepatocytes and central vein and no expression of NF-κBp65.

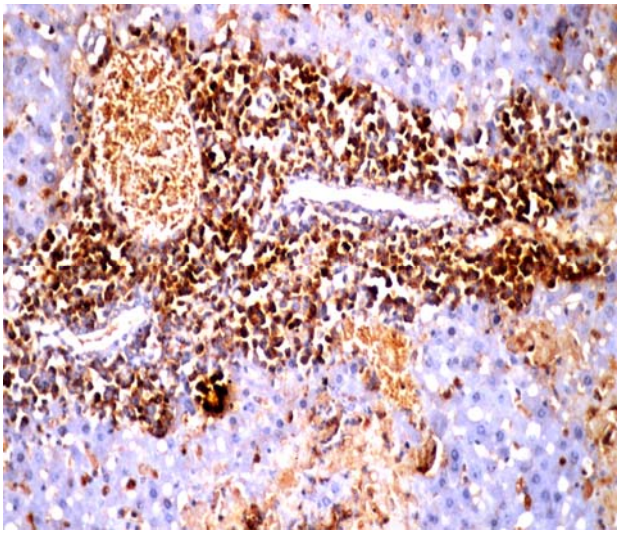

Photo 2: Liver of mice administrated 100 mg/kg DEN for 16 weeks showing sever immunopathological reaction in hepatic tissue. NF-κBp65 expression was significantly increased and localized in the damaged hepatocytes as well as in the inflammatory and kupffer cells

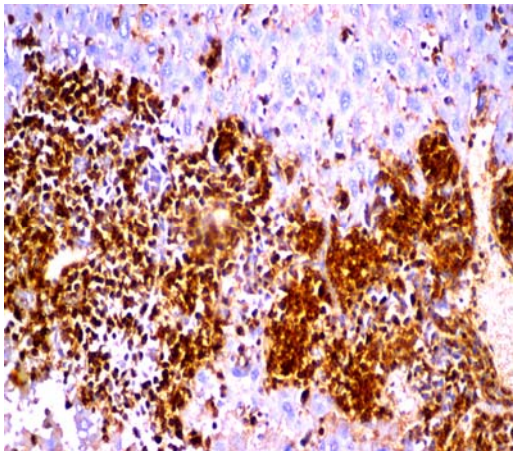

Photo 3: Liver of mice administrated 100 mg/kg DEN for 16 weeks showing sever immune pathological reaction in hepatic tissue. NF-κBp65 expression was significantly increased and localized in the damaged hepatocytes as well as in the inflammatory and kupffer cells.

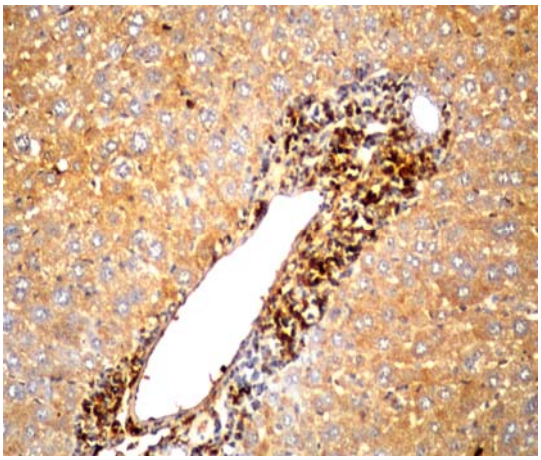

Photo 4: Liver of mice intoxicated by DEN then treated with S. platensis (500 mg/kg) at 18 weeks showing moderate immunopathological reaction in hepatic tissue. NF-κBp65 expression was increased in the damaged hepatocytes as well as in the inflammatory and kupffer cells

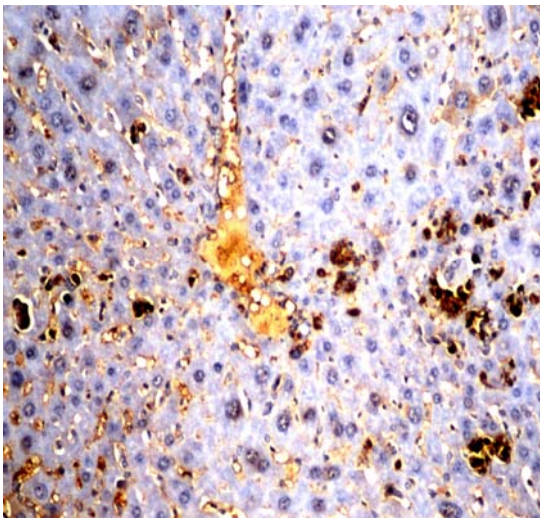

Photo 5: Liver of mice intoxicated by DEN then treated with S. platensis (500 mg/kg) at 20 weeks showed mild immunopathological reaction in hepatic tissue. NF-κBp65 expression was increased slightly and localized in the damaged hepatocytes.

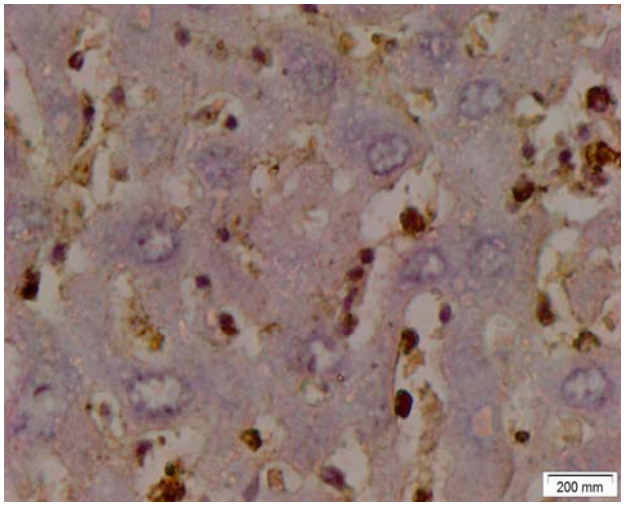

Photo 6: Liver of mice intoxicated by DEN then treated with S. platensis (1000 mg/kg) at 18 weeks showed mild immunopathological reaction in hepatic tissue. NF-κBp65 expression was increased slightly and localized in the damaged hepatocytes.

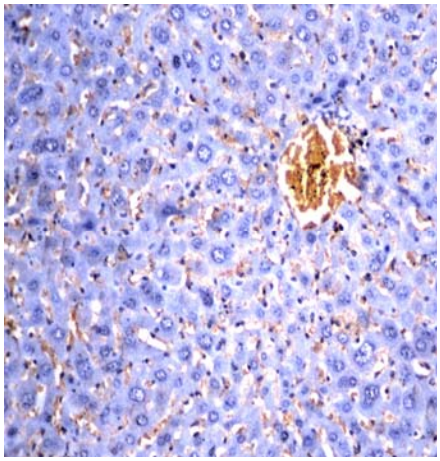

Photo 7: Liver of mice intoxicated by DEN then treated with S. platensis (1000 mg/kg) at 20 weeks showed no immunopathological reaction

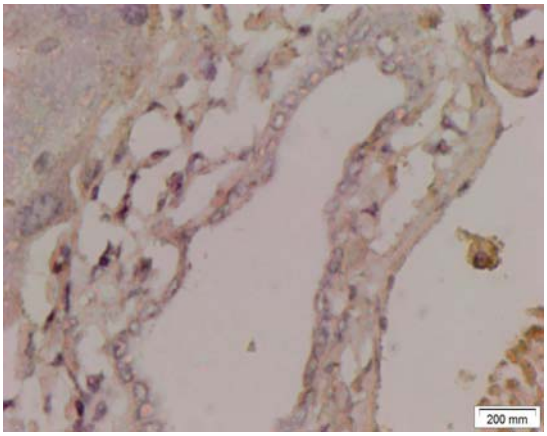

Photo 8: Liver of mice administrated S. p (500 mg/kg) then DEN (100 mg/kg) mild immunopathological reaction in hepatic tissue. NF-κBp65 expression was increased slightly and localized in the damaged hepatocytes as well as in the inflammatory and kupffer cells.

levels as compared with DEN group at 18 and 20 weeks. By time, high dose of S. platensis (1000 mg/kg) showed more improvement of cholesterol, triglycerides, glucose total protein and albumin levels near to normal levels. Preventive dose of *S. platensis* (500 mg/kg) maintain these metabolic parameters of liver close to control levels.

The results printed in (Tables 3) showed that in DEN injection to mice caused significant increase in the activity of proinflammatory cytokines including TNF-α and IL-6 and significant decrease of the anti-inflammatory cytokines including IL-4 and IL-10. Treatment with *S. platensis* (500 mg/kg b.w) revealed significant decrease of TNF-α and IL-6 and significant increase of IL-4 and IL-10 at 18 and 20 weeks. Higher dose of *S. platensis* (1000 mg/kg b.w) at 18 and 20 weeks showed highly significant decrease of proinflammatory cytokines and highly significant increase anti-inflammatory cytokines when compared to DEN-intoxicated mice and became them near to control group.

Immunohistochemical examination of NF-*κ*Bp65 expression of hepatic tissue showed significant increased and localized in the damaged hepatocytes as well as in the inflammatory and kupffer cells in DEN intoxicated mice. Treatment with *S. platensis* (500 mg/kg) at 18 weeks revealed moderate expression of NF-*κ*Bp65 and mild expression at 20 weeks. Higher dose of *S. platensis* (1000 mg/kg) at 18 weeks revealed mild expression while at 20weeks there was no expression of NF-*κ*Bp65 in hepatic tissue. In group treated with *S, platensis* before DEN injection, mild immunopathological reaction was observed in hepatic tissue. NF-*κ*Bp65 expression was slightly increased in the damaged hepatocytes as well as in the inflammatory and kupffer cells.

4. DISCUSSION

Regarding to the results of total serum proteins and albumin showed significant decrease in DEN group than control group. These results were ascertained by Afzal et al., (2012) and Duan et al., (2014) who found that there were significant decreases in both total protein and albumin in DEN toxicity which may be due to changes in protein and free amino acids metabolism and their synthesis in liver and reflected the damaging effect of DEN on liver cells. It was found that treatment with S. platensis after DEN administration significantly increase total protein and enhance the albumin synthesis. Our results are in agreement with previous reports (El-Malawany et al., 2015) and Tantawy, 2015) who recorded that there were significant increases in both total protein and albumin. *S. platensis* restored the liver injuries by scavenging the free radicals and preventing inflammation more effectively (Tantawy, 2015). Furthermore, Hassan, (2016) mentioned that *S .platensis* significantly improved levels of total protein, albumin and globulin due to the immunostimulatory action of *S. platensis* that

elevate globulin than albumin this activate liver to synthesis immunoglobulin from globulin especially Ig G.

The present investigation demonstrates that DEN induced significant elevation of cholesterol, triglycerides. Oxidation stress caused by DEN injection significantly increased respectively serum total cholesterol and triglycerides (Naglaa et al., 2015). On contrast, cholesterol and triglycerides of DEN+*S. platensis* groups showed a significant reduction as compared with DEN group. Phycocyanin caused hypocholesterolemic activity as phycocyanin binds to bile acids in the jejunum; this binding affects the micellar solubility of cholesterol and then suppresses cholesterol absorption (Nagaoka et al., 2005). Furthermore, the hypotriglyceridemic effect of *S. platensis* may be through its effect on increase the activity of lipase (Iwata et al., 1990). In our animal study, DEN injection caused significant elevation of glucose level when compared to control mice. DEN induced hepatic parenchymal cell damage which are responsible for the development of hepatogenous insulin resistance (Kawaguchi et al., 2011) and impairment of insulin secretion (Narita et al., 2004). On the other hand, the treatment with *S. platensis* revealed a significant reduction of glucose level as compared with DEN group. Hassan, (2016) reported the benefit role of *S. platensis* in correction of the hyperglycemic picture in rats through help the pancreatic tissue to secrete insulin hormone from β-islets of langerhans or by transporting excess blood glucose to the peripheral tissue and also may be by increasing level of insulin hormone and C-peptide.

Concerning hematological indices, significant decrease in erythrocytic count, HCT% and Hb concentration was recorded in DEN administered rats compared to control one. The previous changes were coupled with increased MCV and decreased MCHC which suggest developing of macrocytic hypochromic anemia. Several mechanisms have been implicated in DEN-induced anemia. DEN induces oxidative stress due to the generation of reactive oxygen species (ROS) which are capable of initiating peroxidative damage to the cell (Bansal et al., 2005). ROS causing oxidative damage in RBCs which result in loss of membrane functions (Sarkar et al., 1995). Furthermore, decreased RBC count and hemoglobin concentration indicate the severity of hepatic damage induced by DEN. Hepatic damage induction causes folic acid, vitamin B_{12} and iron deficiencies producing severe anemia (Kumar and Radhakrishnan, 2014). Also, hepatic damage leads to release of copper which in turn has an oxidative action on red blood cell membrane phospholipids leading to their breakdown (Roberts and Schilsky, 2003).

The previous indices are recouped considerably in rats' concurrently administered *S. platensis* with DEN compared with group of rats administered DEN. The antioxidants effects of *S. platensis* have been instrumental in avoiding oxidative effects of DEN on RBCs membrane (Simsek et al., 2009) and consequently maintain the hematological parameters near normal limits. In addition, Morcos *et al*. (2004) found that phycocyanin (the major pigment constituents of spirulina) stimulates the secretion of erythropoietin and regulates bone marrow stem cell production of red blood cells. Furthermore, *S. platensis* powder which is rich source of iron contributed to the elevated levels of hemoglobin. (Pankaj and Varma, 2013). *S. platensis* improve hemoglobin function by elevating total hemoglobin and Oxyhemoglobin contents and decreasing Met hemoglobin formation (Cheng-Wu et al., 1994).

It is worth to be mentioned that proinflammatory (TNF-α and IL-6)cytokines production is one of the earliest events in many types of liver injury. TNF-α is produced primarily by macrophages in response to bacterial and viral pathogens (Tracey and Cerami, 1994). Also, IL-6 is responsible for the hepatic response to infections or systemic inflammation. Concentrations of IL-6 in serum are increased in situations of chronic liver inflammation including alcoholic hepatitis, HBV and HCV infections, steatohepatitis and conditions that may lead to development of HCC (Abiru et al., 2006). On contrast, anti-inflammatory cytokines (IL-10 and IL-4) inhibits inflammation and limits adverse outcomes in hepatic diseases (Han et al., 2010). Anti-inflammatory cytokines (IL-10 and IL-4) decreases the production and expression of Th1 cells and enhances B cell survival, proliferation and antibody production and blocks NF-κB activity (Mosser and Zhang, 2008, Sokol et al., 2008).

Our investigation revealed significant increased levels of proinflammatory cytokines (TNF-α and IL-6) and significant decrease of anti-inflammatory cytokines IL-10 and IL-4 in DEN-intoxicated mice. DEN exposure promoted production of IL-6 in kupffer cells (resident liver macrophages) in a manner dependent on the Toll-like receptor adaptor protein MyD88**.** Additionally, DEN causes modest accumulation of tumor necrosis factor-alpha mRNA (Naugler et al., 2007).

On the treatment by *S. platensis*, *S. platensis* revealed significant decrease of TNF-α and IL-6 and significant increase of IL-10 and IL-4. S. platensis organic extracts markedly decreased the secretion of proinflammatory cytokines including: IL-6 and TNFα (Ku et al., 2013). γ-linolenic acid

in *S. platensis* can be metabolized to dihomo γ-linolenic acid that undergoes oxidative metabolism by cyclooxygenases and lipoxygenases to produce anti-inflammatory eicosanoids (Fan and Chapkin, 1998). Also, C- phycocyanin suppresses the activation of NF-κB by preventing degradation of cytosolic inhibitor of κB α consequently reducing the production of TNF-α in lipopolysaccharide macrophages (Cherng et al., 2007). Additionally, β-carotene of *S. platensis* inhibited the production of TNF-α through blocking nuclear translocation of NF-κB p65 subunit (Bai et al., 2005) and suppressed the transcription of IL-6 in macrophage cell line stimulated by lipopolysaccharide or interferon-gamma (IFN-γ) (Katsuura et al., 2009). Furthermore, *S. platensis* exhibited its ability in inhibiting inflammation and activation of anti-inflammatory through increasing of IL-10 (Muga and Chao, 2014). S. Platensis was showed to be a moderate inducer of IL-4 production (Panda, 2013). Cherng et al., (2007) mentioned that the anti-inflammatory effect of S. platensis may be attributed to C-phycocyanin, a selective inhibitor of cyclooxygenase-2.

NF-κB is a family of dimeric transcription factors that regulate inflammation, innate and adaptive immunity, wound healing responses, and cell fate and function (El-sharkawy and Mann, 2007). Immunohistochemicl examination in our study revlead sever expression of NF-κB in hepatic tissue in DEN intoxicated mice. NF-κB is activated in HSCs in response to liver injury and stimulates expression of proinflammatory molecules (IL-6, monocyte chemoattractant protein-1, and intercellular adhesion molecule-1) and antiapoptotic factors (growth arrest and DNA damage-inducible gene 45*β*) required for HSC function and survival during the fibrogenic response (El-sharkawy and Mann, 2007). S. platensis treatment decreased NF-κB expression in hepatic tissue. C-phycocyanin suppresses the activation of NF-κB by preventing degradation of cytosolic inhibitor of κB-α consequently reducing the production of TNF-α (Cherng et al., 2007). Also, translocation of NF-κB from cytoplasm to nucleus was inhibited by S. platensis extracts (Ku et al., 2013). β-carotene inhibited the production of nitric oxide and prostaglandin E (2) and suppressed the TNF-α and IL-1β. Such suppression of inflammatory mediators by β-carotene is likely resulted from its inhibition of NF-κB activation through blocking nuclear translocation of NF-κB p65 subunit (Bai et al., 2005).

Therefore, from the present study it can be concluded that *S. platensis* showed immunomodulatory effects and improve metabolic functions of liver in 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.

5. REFERANCES

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