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# Spirulina Platensis Microalgae Protects against Diethyl Nitrosamine Carcinogenic Effect on Female Albino Rats

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**Key words:**
Cancer-diethylnitrosamine-doxorubicin- spirulina-lung- kidney.

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## Abstract

Although there are many strategies for the treatment of cancer, chemoprevention seems to be the best strategy for lowering the incidence of this disease. The aim of the present study was to investigate the ability of Spirulina Platensis to prevent initiation of carcinogenesis induced by diethylnitrosamine (DENA) which is a potent initiator and carcinogen. Female Wistar albino rats were divided into eight groups. Group 1: received normal saline. Group 2: given Spirulina only. Group 3 injected with DENA. Group 4: treated with Spirulina and DENA. Group 5: received Doxorubicin. Group 6: Spirulina and Doxorubicin. Group 7: received DENA and Doxorubicin. Group 8: treated with Spirulina, DENA and Doxorubicin. Results obtained showed that Groups treated with DENA, Doxorubicin alone or in combination led to significant increase in plasma liver functions tests: Aspartate Transaminase (AST), Alanine Transaminase (ALT), Gamma Glutamyl Transpeptidase (GGT) and Total Bilirubin (T.BIL) and showed increased tumor marker (AFP). Parameters measured in lung and kidney tissues in groups treated with DENA and DOX alone or in combination showed elevation of lipid peroxides (MDA) and Catalase enzyme activity (CAT) and showed significant decrease in Total protein content (T.P.) and tissue antioxidants like Glutathione *S*-Transferase (GST), Total Thiol and Total Antioxidant Capacity (TAC). Histopathological examination of the lung and kidney showed the loss of the normal architecture in DENA and Doxorubicin treated groups. On the other hand, supplementation with Spirulina has attenuated these biochemical and histopathological alterations induced by DENA and Doxorubicin and improved the antioxidant parameters. Data from this study suggested that Spirulina prevents lipid peroxidation, lung and kidney cell damage, protects the antioxidant system against DENA-induced carcinogenesis and could protect against oxidative stress created by Doxorubicin.

## 1. INTRODUCTION

Diethylnitrosamine (DENA, N-Nitrosodiethylamine) a potent carcinogen, is known to cause perturbations in the nuclear enzymes involved in DNA repair/replication (Bhosale *et al.*, 2002). Nitrate and nitrite are added to meat and fish for the purpose of preservation, as colour fixatives and as flavoring. Ingestion of nitrite and nitrate can result in the endogenous formation of nitroso compounds, particularly in the presence of nitrosatable precursors, such as primary amines, in the acidic condition of the stomach (Lin *et al.*, 2002). It has been suggested that DENA, after its metabolic activation produces the pro-mutagenic adducts, $O^6$-ethyl deoxy guanosine and $0^4$ and $O^6$-ethyl deoxy thymidine in liver that may cause carcinogenic effects (Verna *et al.*, 1996).

One approach to control cancer is chemoprevention, by definition it is the means of cancer management in which the occurrence of the disease can be entirely prevented, slowed, or reversed substantially by the administration of one or more nontoxic naturally occurring and/or synthetic agent called anticarcinogen (Ghafar *et al*., 2010).

*Spirulina platensis* is a blue-green alga (Cyanobacterium, family Oscillatoriaceae). It gains more attention because of its nutritional and various medicinal properties (Sharma *et al.*, 2007 ). Spirulina is the only blue-green alga commercially cultivated for food use. Its nutritional value derived from its high protein content (Simpore *et al.,* 2006) rich source of vitamins, mainly vitamin B12 and pro-vitamin A, minerals, especially iron, and γ-linolenic acid (Habib *et al*., 2008). Several studies have reported that Spirulina can prevent or inhibit cancers in animals (Mohan *et al.*, 2006). *In vitro* and animal studies have suggested that Spirulina possesses antiviral effects (Hernandez-Corona *et al.*, 2002). Spirulina is a powerful stimulant for the immune system, as shown in animal experiments, by increasing the phagocytic and natural killer activities (Qureshi and Ali, 1996). Moreover, hypocholesterolemic effects have been reported in some studies (Mazokopakis *et al.*, 2014). Several reports have indicated that Spirulina has a protective effect against many toxicants including mercury (Sharma *et al.*, 2007 ) and copper toxicity (James *et al.*, 2009).

The aim of the present study was to evaluate the antioxidant, radical scavenging, and chemoprevention effect of Spirulina *in vivo via* studying its protective effects alone or in combination with chemotherapy Doxorubicin *via* estimation of antioxidants levels in lung and kidney tissues and their histopathological examination.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals:

Spirulina platensis (dry powder) was obtained from the National Center for Research, Eldoki,Cairo, Doxorubicin was purchased from PHARMACIA company, ITALY. DENA and the other fine chemicals obtained from Sigma Chemical Co., U.S.A. Rat AFP Elisa chemicals from TSZ scientific LLC. All other chemicals and reagents were of analytical grade.

### 2.2. Animals

The experiment was performed on 72 female *Albino* rats weighing 180 g (± 20 g) obtained from Faculty of Veterinary medicine, Cairo University, Egypt. The rats were housed in the laboratory for one week before the experimental work and maintained on the standard diet and water available in the animal house of Zoology Department Faculty of Science, Tanta University. Temperature maintained at 23 ± 2 °C with a relative humidity of 55 ± 5% and at a 12:12 h light dark cycle. All the experiments were done in compliance with guiding principles in the care and use of laboratory animal.

The rats were randomly and equally divided into eight groups (9 animals each).

### 2.3. Experimental design

**Group 1(G1)**: Negative control group
Rats were injected with saline (i.p) for 60 days.

**Group 2(G2)**: spirulina group only
Rats were given spirulina powder dissolved in normal saline in the dose 800 mg/kg b.w for 60 consecutive days by oral gavage (Chamorro-Cevallos *et al*., 2008).

**Group 3(G3)**: DENA group only (Induction of hepatocellular carcinoma, HCC)
Rats were injected with single dose of diethylnitrosamine dissolved in saline (i.p) in the dose of 200 mg/kg b.w (Al-Rejaie *et al.*, 2009).
And after two weeks they were injected with activating single dose of carbon tetrachloride (CCl4) dissolved in olive oil in the dose of 2 ml /kg b.w. (i.p)
Euthenization of rats was performed after 60 days from DENA injection.

**Group 4(G4)**: DENA+ spirulina group
Rats were induced for cancer as G3 and treated with spirulina as G2 and treatment began in the same time of DENA injection.

**Group 5(G5)**: Doxorubicin group only
Doxorubicin was administered intraperitoneally (i.p) at the dose of 1mg/kg bw. Rats received five doses of DOX (one dose/week) and injection began at the fourth week of experiment; this therapeutic dose was according to **El-Sayyad *et al.* (2009).**

**Group 6(G6)**: Spirulina+DOX group
Rats were orally injected with spirulina as G2 and in the same time they received doxorubicin injection intraperitoneally (i.p) as G5.

**Group 7(G7)**: DENA +Doxorubicin group

Rats were injected with single dose of diethylnitrosamine and CCl4 as G3 and in the same time they received doxorubicin injection intraperitoneally (i.p) as G5. Euthenization was performed after 60 days from DENA injection.

**Group 8(G8)**: DENA +Spirulina +DOX group
Rats were injected with diethylnitrosamine and CCl4 as in G3 in the same time rats received spirulina as G2 for 60 days and doxorubicin as G5.

**2.4. Tissue collection and preparation**

After the experimental period treatment, rats were fasted overnight, weighted and euthanized. EDTA blood was collected from orbital plexus using heparinized capillary tubes and incubated at room temperature for 10 minutes, centrifuged at 3000 r.p.m for 10 min and the plasma were collected and kept in clean stopper plastic vials at -80°C until estimation of ALT, AST, GGT, Bilirubin and AFP.

AFP Eliza kit was from TSZ scientific LLC (TSZ scientific). We used ALT, AST colorimetric method kits from SPECTRUM according to (Reitman and Frankel, 1957), GGT enzyme level was measured using kinetic kit from VITRO SCIENT according to (Szasz, 1969) and bilirubin was determined using colorimetric method kit from DIAMOND diagnostics according to (Walter, 1970). Measurements were performed following the instructions of the manufactures.

The lung and kidney tissues were immediately isolated, cleaned from tissues adhering matters, washed in ice-cold saline solution, then dried on a filter paper, weighed and frozen at -80°C. The tissues were homogenized (10% W/V) in potassium phosphate buffer (0.01 M pH 7.4) for estimation of glutathione *S*-transferase (GST), catalase (CAT) enzymes activities, total thiol, Total antioxidant capacity (TAC) and Total protein (T.P) content. KCl solution (1.15 M) was used for estimation of malondialdehyde (MDA) using homogenizer (Hettich model EBA 12R, Germany).

Malondialdehyde (MDA) level is one of the terminal products, formed at the time of the decomposition of the polyunsaturated fatty acids mediated by free radicals. MDA was measured by the method of (Lahouel *et al.*, 2004). The protein content in the tissues was determined by the method of (Tsuyosh and James, 1978). Total thiol was measured using DTNB according to the method described by (Sedlak and Lindsay, 1968). TAC was measured using the ferric reducing antioxidant power (FRAP) by the method described by (Benzie and Strain, 1999). GST enzyme activity was estimated through the formation of adduct, due to conjugation of GSH with 1-chloro-2, 4-dinitrobenzene (CDNB) according to the method described by (Habig *et al.*, 1974). CAT enzyme activity was measured by monitoring H2O2 decomposition at 240 nm according to the method described by ( Xu *et al.*, 1997).

**2.5. Histological evaluation**

Histological evaluation was performed on lung and kidney according to Bancroft and Cook (1994).

**2.6. Statistical analysis:**

The data obtained in the experiment were expressed in terms of mean ± SEM. Statistical significance of data variations were assessed by one way analysis of variance (ANOVA) followed by a comparison between different groups using "Tukey-Kramer" multiple comparison t-test, which compare between all groups and showed the significant effect of treatment **(Graph pad Instate software).** A value of $P<0.05$ was considered to be statistically significant.

## 3. RESULTS

Table (1) shows the effect of DENA, DOX and SP administration alone and their combination on the activities of marker enzymes ALT, AST and GGT, Total bilirubin and tumor marker AFP in plasma of all experimental groups.

All groups treated with DENA or DOX alone or in combination showed significant increase in activities of ALT, AST and GGT in plasma ($p<0.001$) compared with both (G1) and (G2).On the other hand, presence of Spirulina has decreased the activity of these enzymes; AST activity was decreased in (G4), (G6) and (G8) significantly ($p<0.001$) compared with (G3), (G5) and (G7) respectively. GGT activity was decreased in (G4) and (G8) significantly ($p<0.001$) compared with (G3) and (G7) respectively. ALT activity was decreased in (G6) significantly ($p<0.001$) compared with (G5).

Bilirubin of (G3), (G5) and (G7) was significantly increased ($p<0.001$) compared with (G1). The protective effect of SP appeared as Bilirubin of (G4) and (G6) decreased significantly ($p<0.01$) than (G3) and (G5) respectively. Also Bilirubin of (G8) was decreased significantly ($p<0.001$) than that of (G7). Bilirubin of (G7) was significantly increased ($p<0.001$) compared with all the other experimental groups showing destructive role of DENA and DOX combination.

AFP level of (G3) and (G7) increased significantly ($p<0.001$) compared with both (G1) and (G2). AFP of (G5) has increased significantly ($p<0.01$) compared with both (G1) and (G2). Presence of Spirulina in (G4) and (G8) decreased the AFP level significantly ($p<0.001$) than that of (G3).

Table (2) shows the effect of DENA, DOX and Spirulina administration on the MDA level, Total protein content, Total Thiol , TAC, GST and catalase activity in Lung tissue of all experimental groups.

MDA level of (G3), (G5) and (G7) showed significant increase ($p<0.001$) compared with (G1) and (G2). Compared with (G2): MDA of (G4) was increased significantly ($p<0.01$), MDA of (G8) increased ($p<0.001$). MDA of (G6) showed significant decrease in MDA level ($p<0.001$) compared with (G5). Addition of SP in (G8) showed significant decrease compared with (G7).

Total protein of (G3), (G5), (G7), (G8) showed significant decrease ($p<0.001$) compared with both (G1) and (G2). (G4) showed significant increase ($p<0.01$) compared with (G3). Also (G6) showed significant increase ($p<0.001$) in total protein compared with (G5). DOX only group was the least in Total protein content.

Total Thiol content of (G3), (G5) and (G7) showed significant decrease ($p<0.001$) compared with both (G1) and (G2). Presence of SP in (G6) showed significant increase in total thiol ($p<0.05$) compared with (G5). Also SP in (G8) showed significant increase ($p<0.01$) compared with (G5), ($p<0.05$) when compared with (G7).

TAC of (G3), (G5) and (G7) showed significant decrease ($p<0.001$) when compared with (G2). TAC of (G4), (G6), (G8) showed less significant decrease ($p<0.01$) compared with (G2). TAC of (G5) was the most affected group which showed significant decrease ($p<0.001$) compared with both (G1) and (G2).

GST activity of (G3), (G5) and (G7) showed significant decrease ($p<0.001$) compared with (G1). The decrease in GST activity of (G4), (G6), (G8) was with less significance ($p<0.01$) when compared also with (G1). Compared with (G2): GST of (G3), (G5) was decreased significantly ($p<0.05$), and GST of (G7) decreased by ($p<0.01$).

Catalase activity in lung tissues was significantly increased in (G5) ($p<0.05$) compared with (G2). The most affected group was (G7) which showed the highest increase in CAT activity with significance of ($p<0.001$) compared with (G1) and (G2). (G7) also showed significant decrease ($p<0.01$) compared with (G4) and showed significant decrease ($p<0.05$) compared with (G3). Groups treated with SP did not show any significant change in CAT activity compared with control group (G1).

Table (3) shows the effect of DENA, DOX and SP administration on the MDA level, Total protein content, Total Thiol, TAC, GST and catalase activity in Kidney tissue of all experimental groups.

MDA concentration of (G3) and (G7) was significantly increased ($p<0.001$) when compared with (G2), ($p<0.05$) compared with (G1). MDA concentration of (G5) was significantly increased ($p<0.01$) Compared with (G2). MDA of (G8) was significantly increased ($p<0.05$) Compared with (G2).

Total protein in kidney tissues of (G3), (G5) and (G7) decreased significantly ($p<0.001$) than both of (G1) and (G2). Total protein of (G4), (G6) and (G8) decreased significantly ($p<0.01$) when compared with both (G1) and (G2).

Total Thiol of (G3) was significantly decreased ($p<0.001$) compared with (G2) and ($p<0.01$) compared with (G1). G5 and G7 showed significant decrease ($p<0.05$) compared with (G2).

TAC of (G7) and (G8) showed significant decrease ($p<0.05$) compared with (G1). TAC of (G7) decreased significantly ($p<0.01$) Compared with (G2). TAC of (G8) decreased significantly ($p<0.05$) when compared with (G2).

Compared with (G2): GST activity of (G3) and (G7) significantly decreased ($p<0.01$), GST activity of (G5) significantly decreased ($p<0.05$).

Catalase activity of (G3), (G5) and (G7) showed significant increase ($p<0.001$) compared with (G2) and ($p<0.01$) when compared with (G1). CAT activity of (G5) and (G7) was significantly increased ($p<0.05$) when compared with (G4). Here we did not observe any significant change in CAT activity in groups treated with SP compared with control group (G1).

**Table (1).** Changes in the level of ALT, AST, GGT (U/l) enzymes, Bilirubin total (mg/dl) and AFP (ng/ml) in plasma of female albino rats treated with saline G1(control),G2(SP), G3(HCC), G4(HCC+SP), G5(DOX),G6(DOX+SP), G7(HCC+DOX) and G8(HCC+DOX+SP)

| Groups<br>Organs | **Group I** Neg control | **Group II** spirulina | **Group III** DENA | **Group IV** DENA+ spirulina | **Group V** DOXO | **Group VI** DOXO+ Spirulina | **Group VII** DENA+ DOXO | **Group VIII** DENA+ DOXO+ Spirulina |
|---|---|---|---|---|---|---|---|---|
| **ALT U/l** | 11.48±0.34$^{a}$ | 11.19±0.42$^{d}$ | 19.74±0.69$^{adgi}$ | 16.53±0.29$^{adj}$ | 28.19±1.57$^{adgjm}$ | 22.89±0.51$^{adjm}$ | 23.45±0.59$^{adijm}$ | 21.11±0.55$^{adjm}$ |
| **AST U/l** | 93.71±1.14$^{a}$ | 37.17±1.74$^{ad}$ | 127±2.91$^{adg}$ | 109.45±2.02$^{adgjk}$ | 111.61±1.77$^{adgm}$ | 98.91±1.9$^{dgkmp}$ | 133.7±1.81$^{adjmps}$ | 112.75±2.06$^{adgps}$ |
| **GGT U/l** | 4.68±0.29$^{ac}$ | 3.005±0.13$^{cd}$ | 13.1±0.42$^{adg}$ | 9.53±0.19$^{adg\ jk}$ | 11.68±0.42$^{adkmn}$ | 9.43±0.31$^{adgnp}$ | 14.31±0.63$^{adjmps}$ | 10.39±0.29$^{adgs}$ |
| **Bilirubin total mg/dl** | 0.172±0.016$^{abc}$ | 0.079±0.007$^{ad}$ | 0.29±0.015$^{adghi}$ | 0.225±0.006$^{cdhjk}$ | 0.29±0.011$^{adkmn}$ | 0.231±0.01$^{cdhnp}$ | 0.372±0.014$^{adgjmps}$ | 0.243±0.007$^{bdis}$ |
| **AFP ng/ml** | 178±8.17$^{abc}$ | 183±6.89$^{de}$ | 277±10.9$^{adgh}$ | 207±10.9$^{gl}$ | 228±9.40$^{beh}$ | 206±2.34$^{gr}$ | 243±5.61$^{adlr}$ | 216±3.73$^{cg}$ |

Values are expressed as Mean ±SEM; n=9 The significance of difference was analysed by one-way ANOVA and Tukey test using computer program GraphPad InStat. ANOVA was significant at p<0.05. a,d,g,j,m,p,s (p<0.001) b,e,h,k,n,q,t (p<0. 01) c,f,I,l,o,r,u (p<0.05)

**Table (2).** level of MDA (nmol/g), Total protein( mg/g) total thiol (mM/g), TAC (µmol/g) GST and CAT activity (mol/min/g) in the lung tissues in female albino rats treated with saline G1(control),G2(SP), G3(HCC), G4(HCC+SP), G5(DOX),G6(DOX+SP), G7(HCC+DOX) and G8(HCC+DOX+SP).

| Groups<br>Organs | **Group I** Neg control | **Group II** spirulina | **Group III** DENA | **Group IV** DENA+ spirulina | **Group V** DOXO | **Group VI** DOXO+ Spirulina | **Group VII** DENA+ DOXO | **Group VIII** DENA+ DOXO+ Spirulina |
|---|---|---|---|---|---|---|---|---|
| MDA **nmol/g** | 34.7±1.47$^{a}$ | 26.94±2.67$^{de}$ | 60.78±3.23$^{adh}$ | 46.92±2.87$^{el}$ | 62.31±1.45$^{adlm}$ | 40.2±3.96$^{hmp}$ | 64.69±6.74$^{adlpu}$ | 49.15±0.83$^{du}$ |
| Total protein **mg/g** | 60.72±0.49$^{abc}$ | 60.72±0.57$^{def}$ | 55.12±0.55$^{adgh}$ | 57.9±0.30$^{behj}$ | 53.96±0.48$^{adjmno}$ | 58.43±0.15$^{cfgm}$ | 56.39±0.72$^{ado}$ | 57±0.31$^{adn}$ |
| Total thiol **mM/g** | 20.32±0.74$^{abc}$ | 20.86±0.57$^{de}$ | 12.18±1.34$^{ad}$ | 15.19±0.89$^{bdkl}$ | 10.41±0.86$^{adkno}$ | 15.02±0.66$^{bdo}$ | 11.12±0.74$^{adlu}$ | 15.65±1.06$^{ceu}$ |
| TAC **µmol/g** | 5.26±0.20$^{abc}$ | 5.39±0.25$^{de}$ | 4.05±0.18$^{bd}$ | 4.28±0.16$^{ce}$ | 3.84±0.28$^{ad}$ | 4.17±0.17$^{be}$ | 4.01±0.10$^{bd}$ | 4.11±0.16$^{be}$ |
| GST **mol/min/g** | 0.98±0.12$^{ab}$ | 0.86±0.04$^{ef}$ | 0.61±0.05$^{af}$ | 0.71±0.01$^{b}$ | 0.63±0.23$^{af}$ | 0.72±0.1$^{b}$ | 0.59±0.06$^{ae}$ | 0.70±0.07$^{b}$ |
| Catalase **mol/min/g** | 0.155±0.005$^{a}$ | 0.149±0.009$^{df}$ | 0.174±0.006$^{i}$ | 0.166±0.008$^{k}$ | 0.198±0.010$^{f}$ | 0.184±0.013 | 0.218±0.010$^{adik}$ | 0.190±0.009 |

Values are expressed as mean ±SEM; n=9 The significance of difference was analysed by one-way ANOVA and Tukey test using computer program GraphPad InStat. ANOVA was significant at p<0.05. a,d,g,j,m,p,s (p<0.001) b,e,h,k,n,q,t (p<0. 01) c,f,I,l,o,r,u (p<0.05)

**Table (3).** level of MDA (nmol/g), Total protein( mg/g) total thiol (mM/g), TAC (µmol/g) GST and CAT activity (mol/min/g) in the Kidney tissues in female albino rats treated with saline G1(control),G2(SP), G3(HCC), G4(HCC+SP), G5(DOX),G6(DOX+SP), G7(HCC+DOX) and G8(HCC+DOX+SP).

| Groups<br>Organs | **Group I** Neg control | **Group II** spirulina | **Group III** DENA | **Group IV** DENA+ spirulina | **Group V** DOXO | **Group VI** DOXO+ Spirulina | **Group VII** DENA+ DOXO | **Group VIII** DENA+ DOXO+ Spirulina |
|---|---|---|---|---|---|---|---|---|
| MDA **nmol/g** | 256.6±5.85$^{c}$ | 243±8.12$^{def}$ | 292.7±7.05$^{cd}$ | 276.5±3.35 | 288.3±11.46$^{e}$ | 259±7.23$^{r}$ | 294.3±10.76$^{cdr}$ | 280.4±4.15$^{f}$ |
| Total protein **mg/g** | 90.01±0.339$^{ab}$ | 90.07±0.818$^{de}$ | 85.56±0.709$^{ad}$ | 86.96±0.541$^{be}$ | 86.1±0.227$^{ad}$ | 86.98±0.366$^{be}$ | 84.49±0.711$^{ad}$ | 86.56±0.554$^{be}$ |
| Total thiol **mM/g** | 36.42±1.29$^{bc}$ | 37.03±1.04$^{df}$ | 28.96±1.83$^{bd}$ | 33.33±0.79 | 31.40±1.48$^{f}$ | 34.38±0.97 | 30.58±1.22$^{cf}$ | 32.28±0.90 |
| TAC **µmol/g** | 10.07±0.28$^{c}$ | 10.28±0.26$^{ef}$ | 8.79±0.29 | 9.09±0.29 | 9.17±0.36 | 9.29±0.29 | 8.3±0.35$^{ce}$ | 8.54±0.47$^{cf}$ |
| GST **mol/min/g** | 0.51±0.035$^{c}$ | 0.53±0.024$^{ef}$ | 0.36±0.028$^{ce}$ | 0.44±0.029 | 0.38±0.026$^{f}$ | 0.44±0.025 | 0.37±0.032$^{ce}$ | 0.42±0.032 |
| Catalase **mol/min/g** | 0.820±0.027$^{b}$ | 0.710±0.038$^{d}$ | 1.155±0.076$^{bd}$ | 0.900±0.065$^{l}$ | 1.194±0.089$^{bdl}$ | 0.949±0.052 | 1.187±0.055$^{bdl}$ | 0.970±0.069 |

Values are expressed as mean ±SEM; n=9 The significance of difference was analysed by one-way ANOVA and Tukey test (compare all vs. control and vs. each group) using computer program GraphPad InStat. ANOVA was significant at $p<0.05$. a,d,g,j,m,p,s ($p<0.001$) b,e,h,k,n,q,t ($p<0.01$) c,f,I,l,o,r,u ($p<0.05$)

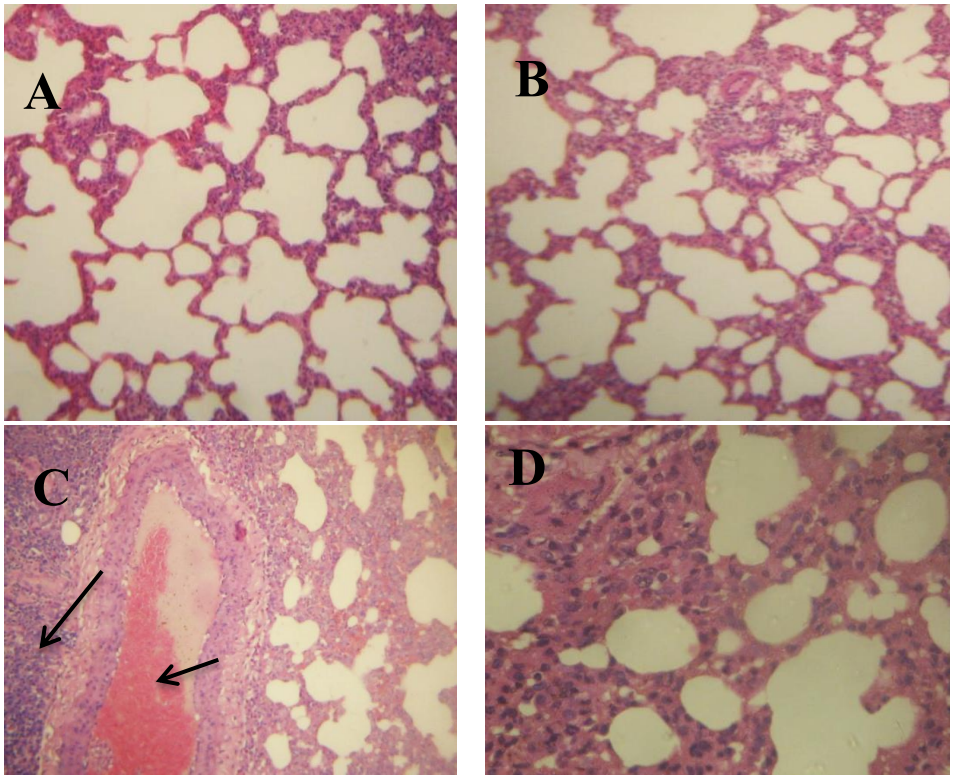



**Fig. (1 A, B, C, D): A** photomicrographs shows Lung section of Control group showing normal spongy histological structure and architecture of the lung with alveoli (H&E X100). **B** photomicrographs shows Lung section of Spirulina group showing normal architecture (H&E X100). **C** photomicrographs shows Lung section of HCC group showing markedly thickened inter alveolar septa and heavy infiltration of inflammatory cells, mainly lymphocytes were observed (H&E X100). **D** photomicrographs shows Lung section of HCC+ Spirulina group showing Mild thickened inter alveolar septa and moderate infiltration of inflammatory cells and light congested blood capillaries were observed (H&E X200).

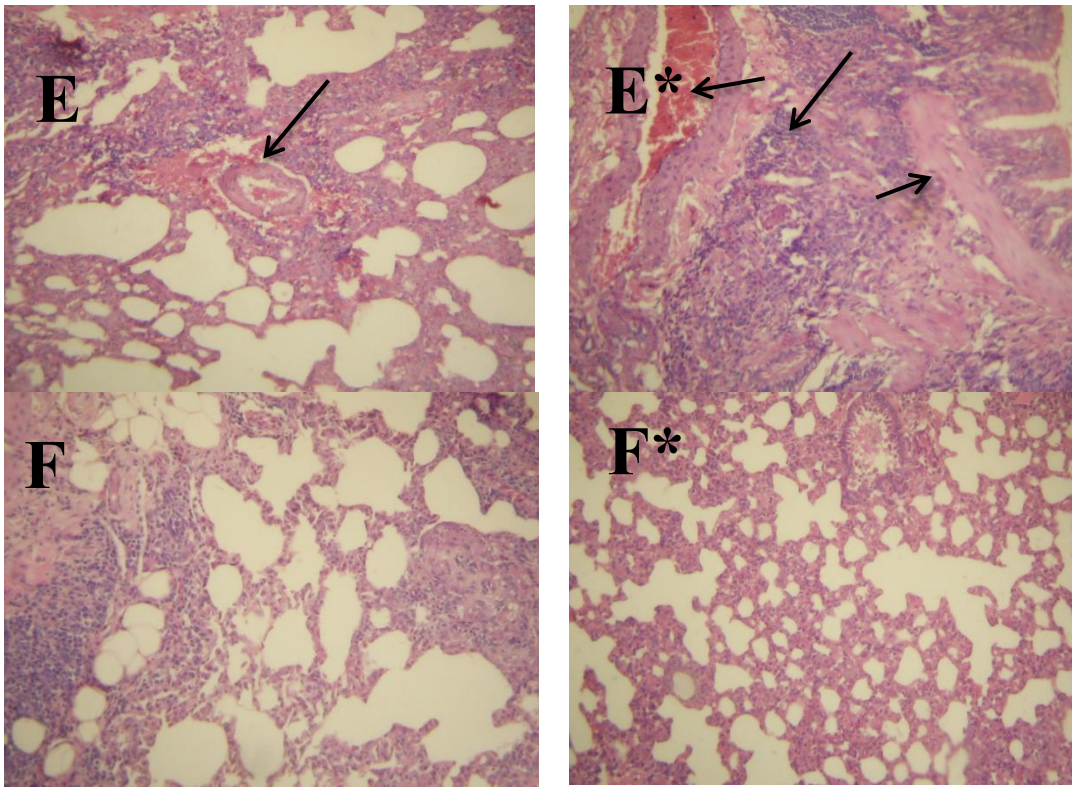
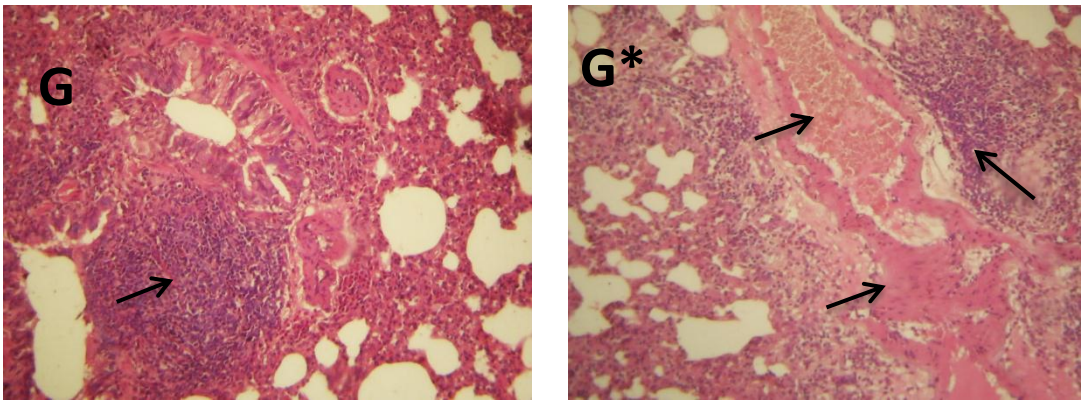



**Fig. (1 E, E*, F, F*): E, E*** photomicrographs shows Lung section of DOX group showing severe alveolar damage, markedly thickened inter alveolar septa and heavy infiltration of inflammatory cells, associated with thickened wall pulmonary blood vessels. Congestion of blood capillaries and fibrous connective tissue was observed (H&E X100). **F, F*** photomicrographs shows Lung section of DOX + Spirulina group showing mild to moderate alveolar damage. Mild thickened inter alveolar septa and moderate infiltration of inflammatory cells (H&E X100).



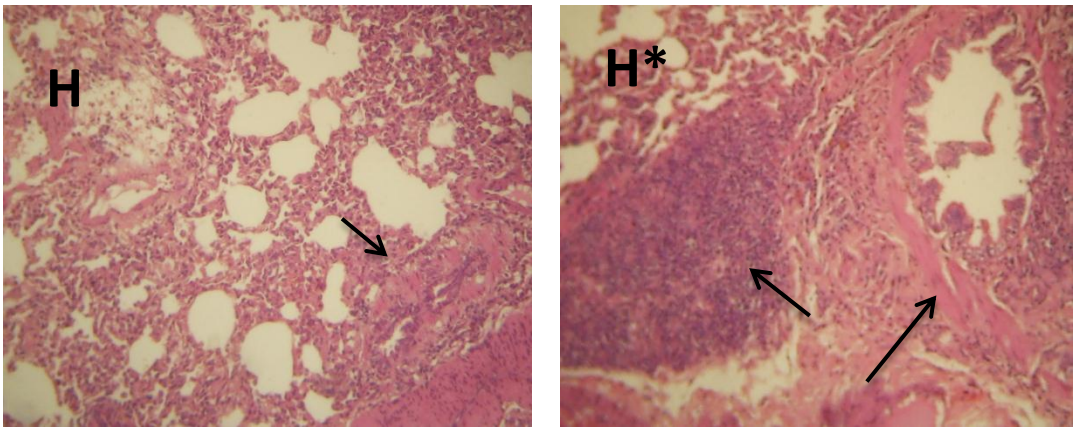



**Fig. (1 G, G*, H, H*): G, G*** photomicrographs shows Lung section of HCC + DOX group showing severe alveolar damage, marked thickness of inter alveolar septa and heavy infiltration of inflammatory cells were observed. Congestion of blood capillaries was also detected. Fibrous connective tissue was also observed (H&E X100). **H, H*** photomicrographs shows Lung section of HCC + DOX + Spirulina group showing evident reduction of the alveolar changes except for thickening of interalveolar septa with mild inflammatory cellular infiltration (H&E X100).

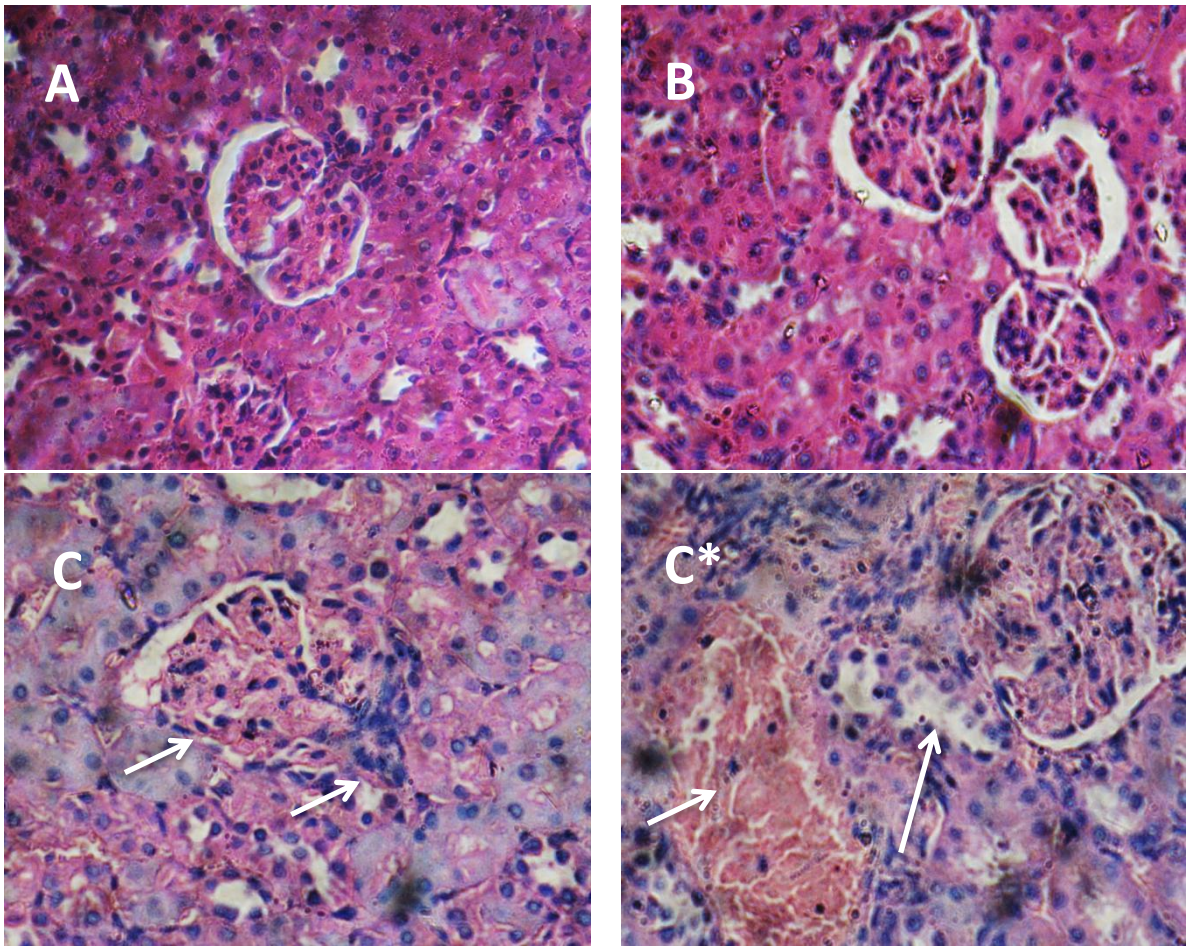



**Fig. (2 A, B, C, C*): A** photomicrograph shows Kidney section of Control group showing normal structures of the renal cortex which comprised renal corpuscles, proximal and distal convoluted tubules (H&E X200). **B** photomicrograph shows Kidney section of Spirulina group showing normal structures of the renal tissue (H&E X200). **C, C*** photomicrographs show Kidney section of HCC group showing congestion of the blood capillaries, inflammatory infiltration, some glomeruli and urinary tubules lost their characteristic configuration and others were atrophied (H&E X200).

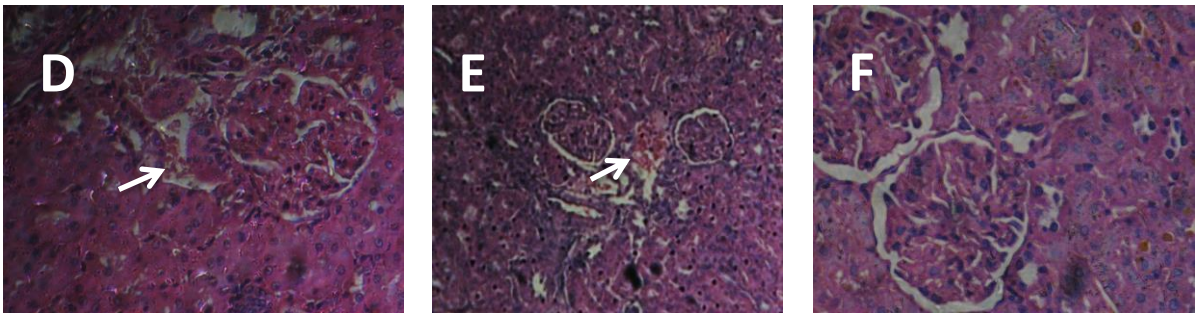



**Fig. (2 D, E, F): D** photomicrograph shows Kidney section of HCC + Spirulina group showing few localized areas were seen with inflammatory infiltration and glomeruli and urinary tubules showed normal architecture (H&E X200). **E** photomicrograph shows Kidney section of DOX group showing severe congestion of the blood capillaries, few inflammatory infiltration was also observed, some glomeruli and urinary tubules lost their characteristic configuration (H&E X100). **F** photomicrograph shows Kidney section of (DOX + Spirulina) group showing very few localized areas were seen with inflammatory infiltration and glomeruli and urinary tubules showed normal architecture (H&E X200).

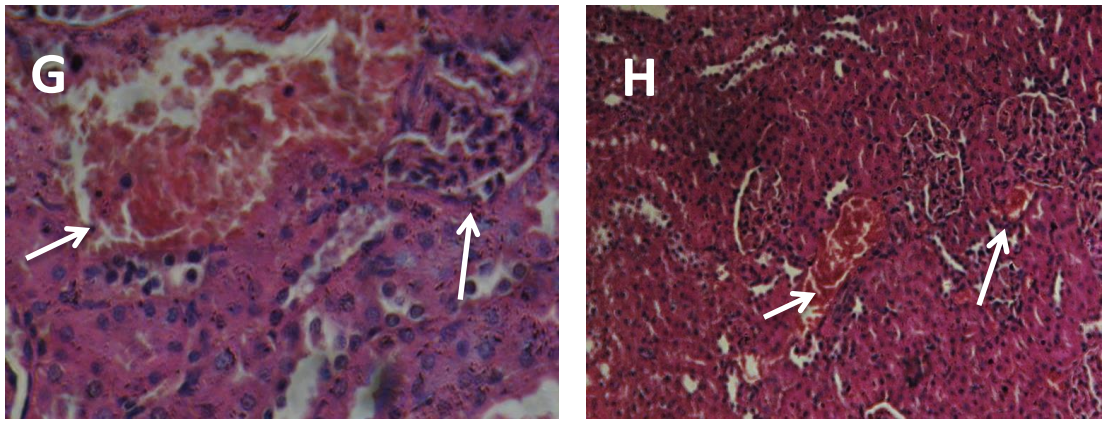



**Fig. (2 G, H): G** photomicrograph shows Kidney section of HCC + DOX group showing severe congestion of the blood capillaries, inflammatory infiltration , some glomeruli and urinary tubules lost their characteristic configuration and others were atrophied (H&E X200). **H** photomicrograph shows Kidney section of HCC + DOX + Spirulina group showing mild congestion of the blood capillaries, few inflammatory infiltrations, most of glomeruli and urinary tubules showed normal characteristic configuration (H&E X100).

## 4-DISCUSSION

The present study was carried out to investigate the chemopreventive effect of spirulina against DENA carcinogenic effect and Doxorubicin chemotherapy on oxidative stress and biochemical alterations in lung and kidney tissues.

Aminotransferases (aspartate transaminases (AST) and alanine transaminases (ALT) are reliable marker enzymes of liver and they are the first enzymes to be used in diagnostic enzymology when liver damage has occurred ( . Because of their intracellular location in the cytosol, toxicity affecting the liver with subsequent breakdown in membrane architecture of the cells leads to their spillage into plasma, and their concentration rises in the latter, Whittby *et al*., 1984).

Gamma-glutamyl transferase (GGT) is a membrane-bound enzyme, which exhibits a tissue specific expression and is modified under various physiologic and pathologic conditions, such as development and carcinogenesis (Yao *et al.*, 2004).

Our study showed that groups treated with DENA or DOX alone or in combination had a significant increase in ALT, AST and GGT. We suggest that this increase was due to the ability of both DENA and DOX to generate free radicals that made damage of the cell membrane.

Results showed that spirulina treated groups had a significant decrease in ALT, AST and GGT levels which could be attributed principally to its antioxidant hepatoprotection activity that decreased the damaged effect of DENA and DOX on tissues, this was in accordance with Jayakumar *et al.* (2012) who showed that DENA injection cause the increase of these enzymes and Walaa *et al.* (2014) who showed the same effect of DOX.

Plasma bilirubin is one of the most sensitive tests employed in the diagnosis of hepatic diseases. It provides useful information on how well the liver is functioning (Ramakrishnan *et al.*, 2007). A significant increase in the level of plasma bilirubin was observed in DENA and DOX treated rats, which may be a result of mass inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged hepatocytes (Rajkapoor *et al.*, 2006). Decrease in plasma bilirubin after treatment with Spirulina indicated its effectiveness in maintaining the normal functional status of the liver. This result agrees with Gad *et al.* (2011) who showed that SP can decrease bilirubin in CCL4 treated rats. Jagan *et al.* (2008) also showed the same effect of DENA on bilirubin level.

High levels AFP are believed to be strongly suggestive of HCC (Endo *et al.*, 1975) because greater than 70% of HCC patients have high plasma concentration of AFP because of the tumor secretion. The increased level of AFP observed in DENA-induced animals is an indication of HCC. DOX treated groups also showed an increase in the AFP level and this agree with results of Walaa *et al.* (2014) which is an indication of DOX toxicity. Spirulina has attenuated these results near the normal level which is indication of the chemoprevention effect of spirulina. This was in accordance with Ismail *et al.* (2009). The potential hepatoprotective role of SP may be associated with its antioxidant constituents such as selenium, chlorophyll, carotene, gamma-linolenic acid, tocopherol, phenolic compounds content and vitamin E and C working individually or in synergy (Torres-Duran *et al.*, 1999 and Garcia-Martinez *et al.*, 2007)

Oxygen radicals react with polyunsaturated fatty acid residues in phospholipids. One of the most abundant carbonyl products of lipid peroxidation is MDA, which reacts with DNA to form adducts. Lipid peroxidation appears to be a major source of endogenous DNA damage in humans that may contribute significantly to the occurrence of cancer and other genetic diseases (Marnett, 2002).

In 0ur study we found significant elevation in level of MDA in groups treated with DENA and DOX but this was reversed in groups treated with spirulina which means that spirulina has protective effect and significantly decreased MDA level due its free radical scavenging activity. Our results agree with Upasani *et al.* (2001) who reported that administration of spirulina reduced the lead induced MDA conjugates in liver, lung and kidney in rats.

ROS generated in cells by toxic oxidative materials make damage of cell membrane which let cellular proteins exposed to damage that decrease the protein synthetic function and increase protein leakage from the cells (Abdel-Wahhab *et al.*, 2007).

In our results the Total protein concentration in DENA and DOX treated groups was significantly decreased compared with control and SP only group and this effect was minimized when we combined Spirulina with DENA or DOX in groups treated with Spirulina. This was in agreement withSharma and Pracheta (2013) who showed a decreased content of kidney total protein after DENA injection. Increased level of Total protein in tissues by Spirulina indicated tissue protective activity, as stimulation of protein synthesis that accelerates the regeneration process.

The total thiol status of the body especially thiol (-SH) groups present on protein are considered as major plasma antioxidants *in vivo* and most of them are present over albumin (Prakash *et al.*, 2004) ultimately behaving as major reducing groups, present in our body fluids. Synthesis of glutathione and cysteine mainly occurs in hepatocytes, whereas most other tissues are supplied with these thiols via sinusoidal efflux into the blood (Peters *et al.*, 2007). In our current study, the significant decrease in the level of total thiols (non-protein thiols and protein thiols) confirms the occurrence of oxidative stress formed due to the electrophiles generated by the toxicity of DENA and DOX. Treatment with Spirulina has restored the level of total thiol significantly near the normal level confirming the Spirulina ability as antioxidant. Our results are in agreement with Sharma and Pracheta (2013) who showed that DENA injection has decreased GSH content in renal tissue and Naura et al. (2007) who showed decreased level of GSH in lung after DENA injection.

It is well known that Total Antioxidant Capacity (TAC) includes enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPX) and macromolecules such as albumin, ceruloplasmin, and ferritin. TAC may provide more relevant biological information compared with that obtained by measurement of its individual components, because it considers the cumulative effect of all antioxidants present in plasma and body fluids (Gad *et al.*, 2011). According to Benzie and Strain (1996) an absolute or relative deficiency of antioxidant defenses may lead to a situation of increased oxidative stress, and this may be associated with both the causes and consequences of a variety of disorders, including coronary heart

disease and cancer. In the present study, TAC was decreased in groups treated with DENA and DOX. As spirulina is a cocktail of potent antioxidants it is not surprising to find that it may act as powerful antioxidant at 800mg/kg to show a significant reversal of DENA and DOX effect on different organs. This agrees with Thaakur and Jyothi (2007) and Gad *et al.* (2011) who studied the effect of Spirulina on the TAC in rats.

GST catalyzes the conjugation of GSH to a variety of electrophilic compounds, including carcinogens, as well as endogenous reactive compounds (Fiala *et al.*, 1976). These conjugates thus become less toxic than the original toxic compound and can be easily excreted from the body.

In our experiments, we have observed a striking decrease in the GST activity in liver (data not shown) and the other organs after DENA and DOX treatment. However, administration of Spirulina to both DENA treated and control animals led to a significant enhancement in the GST activity, which indicated an increased ability to detoxify carcinogens. Our results were compatible with Naura *et al.* (2007) who showed the same effect of DENA on GST in lung. Also, Sharma and Pracheta (2013) has used DENA in induction of renal carcinogenesis and showed the same effect of DENA on GST level.

Catalase is a peroxisomal enzyme catalyzing the breakdown of hydrogen peroxide into oxygen and water. It plays a central role in organismal oxidant defense. CAT is present in almost every organ in humans, particularly in the liver and erythrocytes. It is contained in minor quantities by the brain, heart, and skeletal tissues (Martin *et al.*, 1997).

Our results showed that HCC induced groups and DOX treated groups contained significant elevation of catalase activity and the treatment with spirulina has decreased that effect by reducing catalase activity.

We suggest that CAT activity was increased since the level of hydrogen peroxide production is enlarged in HCC group and it may correspond with the report which showed that some human cancer cell lines produced a large amount of hydrogen peroxide (Ozdemirler *et al.*, 2005).

Our results agree with Gopcevic *et al.* (2013) who showed that catalase increased with colorectal cancer.

## 5- CONCLUSION

The present study demonstrated that Spirulina possesses potent free radical scavenging and antioxidant activities. From the results, it is evident that Spirulina is capable of modulating the levels of MDA, AFP and significantly increases the endogenous antioxidant defense mechanisms in case of DENA-induced carcinogenesis and in case of Doxorubicin chemotherapy treatment.

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