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Original Article

Protective Effect of Spirulina Against Irradiation Induced Liver Injury in Adult Albino Rat Rats

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ABSTRACT

Background and Objective: Spirulina is a nutritional supplement that has a variety of pharmacological properties, such as antioxidant and anti-inflammatory effects. This study aimed to investigate the protective effect of Spirulina against liver tissue injury induced by gamma irradiation.

Materials and Methods: Thirty two adult male albino rats were divided into 4 groups each of which included 8 rats: (1) control group, (2) irradiation group: exposed to gamma irradiation in a dose of 8 Gy for 15 min, (3) Spirulina low dose treated (SPL) group which received Spirulina 300 mg/kg b.w. by gavage once/day for 3 weeks prior to irradiation and (4) Spirulina high dose treated (SPH) group which received Spirulina 1000 mg/kg b.w. by gavage once/day for 3 weeks before irradiation. Blood samples were collected to determine levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA) and plasma total proteins, in addition to liver tissue from all groups to be examined histologically by light microscope and transmission electron microscope.

Results: The obtained results showed that plasma levels of ALT and AST were significantly reduced in rats treated with low dose of Spirulina prior to irradiation when compared to irradiation group together with significant rise of plasma total proteins and histological improvement of liver tissue, however, Spirulina high dose treated group did not show any signs of improvement compared to irradiation group. It could be concluded that low dose Spirulina treatment has a hepatoprotective effect against irradiation, whereas high dose treatment may have a hepatotoxic effect that needs further investigations.

Key Words: Spirulina, Hepatoprotective, Irradiation

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INTRODUCTION

Exposure to ionizing radiation is considered to be an increasing threat to mankind. The effects of ionizing radiation in biological systems are mainly mediated through the generation of reactive oxygen species (ROS) in cells as a result of water radiolysis *(Kamat et al., 2000)*. ROS and oxidative stress may contribute to radiation-induced cytotoxicity and to metabolic and morphologic changes in animals and humans during radiotherapy *(Fang et al., 2002)*. The exposure to ionizing radiation leads to depletion of the endogenous antioxidants *(Koc et al., 2003 a, b)*. When the cellular antioxidant capacity is decreased, the organs become more susceptible to the deleterious effects of ROS *(Karbownik and Reiter, 2000)*. Thus, control of radiation hazards is considered as one of the most important challenges in order to protect human lives from radiation damage *(Edrees et al., 2008)*.

Many antioxidants have been investigated as hepato-protectors against ionizing radiation induced injury since they reduce the oxidative effect of the ROS on normal cells.

Spirulina, a blue-green algae, grows as microscopic, corkscrew-shaped multicellular filaments which is now classified as a distinct genus, Arthrospora (A. plantensis is found in Africa and Asia, and A. maxima is found in Central America) *(Kulshreshtha et al., 2008)*. Currently, Spirulina is actively marketed by numerous companies as a nutritional supplement *(Khan et al., 2005)*.

Phycocyanin is a biliprotein pigment of Spirulina which has a variety of pharmacological properties, such as antioxidant, anti-inflammatory, neuro and hepato-protective effects *(Romay et al.,*

2003). In an animal study, Spirulina was shown to modulate radiation induced hematological and biochemical alterations *(Verma et al., 2006)*. Immune cells are highly radiosensitive and are considered as good indicator for the biological effects of ionizing radiation. Spirulina was shown to correct the immune cell parameters in a study with children exposed to prolonged low dose radiation *(Loseva, 1999)*. In addition, Spirulina can elevate the activity of all the antioxidant related enzymes *(Dasgupta et al., 2001)*.

Clinical trials had investigated Spirulina's potential but they were few to support its effects *(Karkos et al., 2011)*. Therefore, this study aimed to investigate the protective effect of different doses of Spirulina against radiation induced damage of liver cells.

MATERIAL AND METHODS

This research was approved by the Committee of Animal Research Ethics (CARE), Faculty of Medicine, Ain Shams University.

Animals

In the present study, 32 adult male albino rats weighing 180 -220 gm were obtained from and bred in the Animal House of Medical Research Center at Faculty of Medicine, Ain-Shams University. The animals were housed in plastic cages 7 days prior to study for acclimatization. They were maintained on a normal 12 h light and dark cycle, at a temperature of 25–27◦ c and allowed free access to commercial rat chow and water ad libitum. All rats were kept under the same circumstances throughout the experiment. All procedures were done according to ethical guidelines for animal use in research.

Chemicals

Spirulina was purchased as SPRU-60, Spirulina sachets (Nile Gate, Egypt). The sachet contains 3 grams. Each sachet was dissolved in 50 ml of distilled water and the dose was calculated according to the body weight in both SPL and SPH.

Irradiation

Rats were placed in a specially designed well-ventilated acrylic container and the whole body of the animals were exposed to 8 Gy from the biological irradiator gamma cell-40, cesium-137 source (Atomic Energy Agency, Canada), belonging to Nuclear Energy Commission, Nasr City, Cairo.

Experimental Design

The animals were allocated into 4 groups, each of 8 animals.

Group I (control group): They received distilled water in equivalent doses by gavage for 3 weeks.

Group II (irradiation group): They were exposed to a single whole body gamma irradiation in a dose of 8 Gy for 15 minutes *(Shirazi et al., 2013)*.

Group III (Spirulina low dose group: SPL): They received Spirulina 300mg/kg b.w. by gavage once/day *(Bashandy et al., 2011)* for 3 weeks then they were exposed to a single whole body gamma irradiation in a dose of 8 Gy for 15 minutes.

Group IV (Spirulina high dose group: SPH): They received Spirulina 1000 mg/kg b.w. by gavage once/day *(Abdel-Daim et al., 2013)* for 3 weeks, then the animals were exposed to a single whole body gamma irradiation in a dose of 8 Gy for 15 minutes.

At the end of the experiment, 30 days from irradiation, All rats were anesthetized by ether inhalation and the following procedures were done:

Biochemical studies

Blood samples from all animals were obtained directly from the heart before sacrification. Blood samples were collected and put into chilled non-heparinized tubes, which were centrifuged at 3000 rpm for 10 min at 4º c. The sera were frozen at -20º c for determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total protein levels by using commercially available kits. As a product of lipid peroxidation, plasma malondialdehyde level (MDA) was determined according to the technique of *Esterbauer and Cheeseman (1990),* the results being expressed in µmol/ml.

Histological studies

Liver were rapidly dissected from all the animal groups and the specimens were immediately processed for histological and ultrastructural studies.

Light microscopic study

Liver samples were fixed in 10% neutral formalin and processed for preparation of paraffin blocks. Six µm thickness sections were stained with haematoxylin and eosin (H & E) and Masson's trichrome *(Bancroft and Gamble, 2002)* and examined under Olympus light microscope equipped with an automatic photo micrographic camera system (Olympus, E330 Live View digital FLR camera).

Transmission electron microscopic study

For ultrastructural examination, liver samples were cut into small pieces of about 13- mm in size and immediately fixed in 2.5% glutaraldehyde for 48 hours. The specimens were then washed in phosphate buffer (pH 7.2). The specimens were post fixed in 1% osmium tetraoxide for 2 hours, dehydrated in ascending grades of ethyl alcohol, cleared in propylene oxide and finally embedded in spur premix at 60◦ c for 48 hours *(Hayat, 1989)*. Semithin sections of 1–2 µm were cut using ultramicrotome, stained with toluidine blue, then examined by the Olympus microscope. Ultrathin sections (60–90 nm thick) were cut, mounted on copper grids and double stained with uranyl acetate and lead citrate *(Weakley, 1981)*. The grids were examined and photographed with Philips transmission electron microscope in the Electron-microscope Unit at the Anatomy Department, Ain-Shams University and Military Medical Academy.

Statistical analysis

All data were expressed as means ± SE and statistically analyzed using SPSS (Statical Package for Social Science) version 16.0 for Windows (SPSS Inc, Chicago, IL). Statistical significance of differences among different study groups was evaluated by one-way analysis of variance (ANOVA) ($P \leq 0.05$).

RESULTS

Biochemical parameters of liver function

In irradiation group, there is significant elevation in ALT, AST and plasma MDA levels while there is significant reduction in total plasma protein when compared to control group. In SPL group, there is significant reduction in ALT, AST and plasma MDA levels in addition to significant elevation in total plasma protein when compared to irradiation group .However there is significant elevation in plasma MDA level when compared to control group .In SPH group, there is significant elevation in ALT, AST and plasma MDA levels and significant reduction in total plasma protein when compared to control group (Table 1).

Histological results

Light microscopic examination of liver sections of control group showed hepatocytes arranged in the form of cords branching and radiating from the central vein to the periphery of the lobules. The hepatocytes were polygonal cells having moderately acidophilic granular cytoplasm and rounded vesicular nuclei. The cells were separated by blood sinusoids, which were lined by flat endothelial cells (Figs. 1 & 2). Masson trichrome stain revealed minimal collagen fibers content around the central vein and in the portal tract (Fig. 3). In irradiation group, the liver showed signs of degeneration; most of hepatocytes were vacuolated while others show ballooning degeneration with pyknotic nuclei. Congested portal vein and dilatation of the central vein and blood sinusoids were also seen (Figs. 4& 5). Marked increase in collagen fibers deposition in between the liver cords, in the portal tracts and around the central vein was detected in Masson stained sections (Fig. 6).

Examination of SPL group revealed restoration of hepatic architecture with apparent decrease in the signs of degeneration; most of hepatocytes appeared normal except few with deeply acidophilic cytoplasm and pyknotic nucleus (Figs. 7 & 8). Minimal collagen fibres were seen in between the liver cords, in the portal

areas and around the central vein (Fig. 9). In SPH group, signs of inflammation were noticed in the form of mononuclear cellular leucocytic infiltration around central vein, peri-portal tract, and in-between the cords of hepatocytes. Dilated central vein and hypertrophied Kuppfer cells were also present (Figs. 10 & 11). Increased collagen fibers in relation to the control group was seen (Fig. 12).

By transmission electron microscope, examination of control group showed cords of hepatocytes contained euchromatic nuclei with regular outline and prominent nucleolus and patches of heterochromatin, the cytoplasm contained numerous mitochondria, multiple arrays of rough endoplasmic reticulum, and dispersed electron-dense glycogen granules. Blood sinusoids lined by Kuppfer cells and bile canaliculi could be seen in between hepatocytes (Figs. 13 & 14). Examination of irradiation group revealed deposition of collagen fibers in peri-sinusoidal space and loss of the chromatin pattern of the nucleus. The cytoplasm showed loss of most of the organelles, degenerated mitochondria, dilated cisternae of rER, and dispersed glycogen with areas of dissolution of the cytoplasm giving it the mouth eaten appearance (Figs. 15 & 16). In SPL group, the normal ultrastructure of the hepatocytes was preserved with regular outline and increased content of glycogen granules; some cells were binucleated and contained euchromatic nuclei. (Figs. 17 & 18). In SPH group, most of the hepatocytes were vacuolated with scanty glycogen content. Mast cells were present in between the hepatocytes

Table 1: Serum activity of ALT, AST, total protein, and MDA in various animal groups.

Animal Groups	**ALT** (IU/L)	**AST** (IU/L)	**Total proteins** (gm/dl)	**Plasma MDA** (µmol/ml)
Group I: control group	17.8 0.46±	188.13 ±20.66	5.68 ±0.11	109.97 ±8.02
Group II: Irradiation group	592.61[a] 29.48 ±	408.14[a] ±20.26	3.79[a] ±0.07	287.61[a] ±24.18
Group III: SPL Spirulina Low dose group	55.32[b] ±2.92	170.34[b] ±7.53	5.1 [b] ±0.22	182.28[a, b] ±15.03
Group IV: SPH Spirulina High dose group	542.26[a] ±20.16	378.71[a] ±19.61	3.98[a] ±0.11	299.8[a] ±10.61

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, MDA: Malondialdehyde.
Data are expressed as means ± SE.
a: Significance when compared to the control group (P ≤ 0.05).
b: Significance when compared to the irradiation group (P ≤ 0.05).

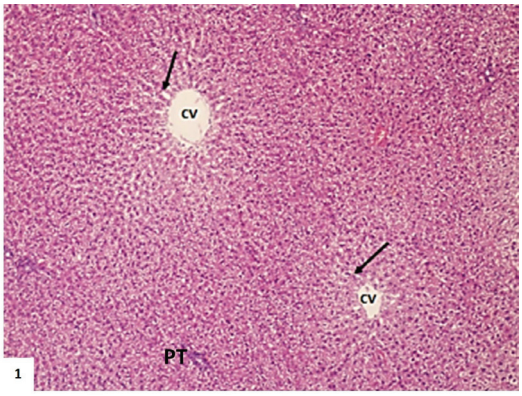

Fig. 1: A photomicrograph of liver tissue of **Control group** showing cords of hepatocytes radiating from the central vein (CV) and are separated by sinusoids (arrow), and portal tract appears at the periphery (PT). H&E X100

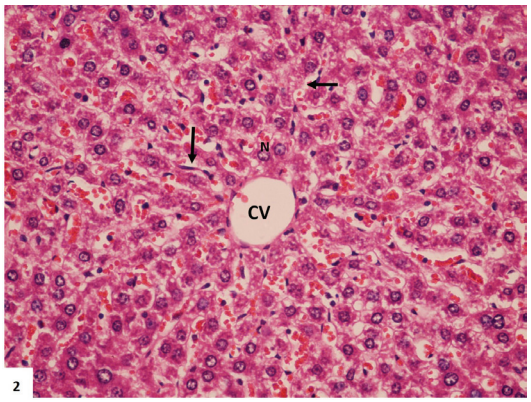

Fig. 2: A photomicrograph of liver tissue of **Control group** showing the hepatocytes are polygonal in shape, separated by sinusoids (arrow), having moderately acidophilic granular cytoplasm and rounded vesicular nuclei (N). H&E X400

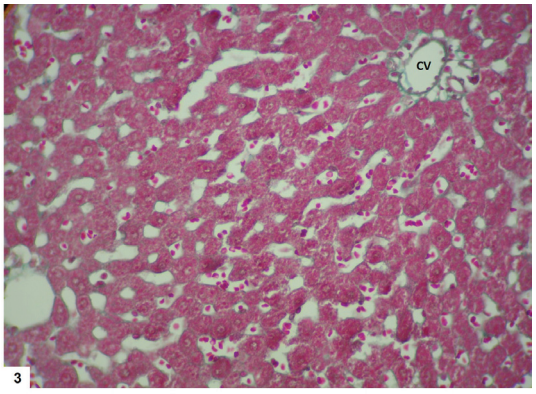

Fig. 3: A Photomicrograph of hepatic tissue of Control group showing scanty collagen fibers surrounding the central vein (CV). Masson Trichrome X400

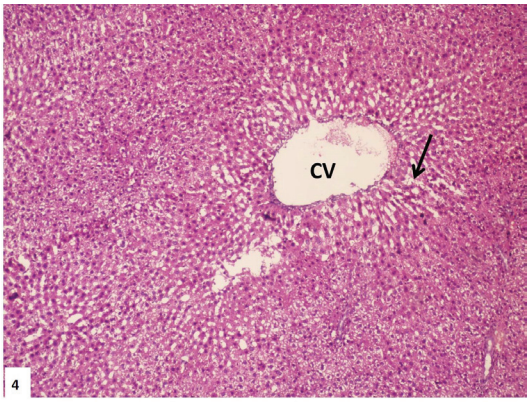

Fig. 4: A photomicrograph of liver tissue of Irradiation group showing dilated central vein (CV) and dilated blood sinusoids (↑). H&E X100

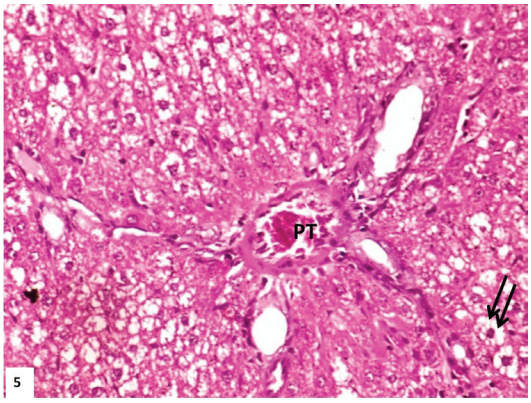

Fig. 5: A photomicrograph of liver tissue of Irradiation group showing congested portal vein, most of hepatocytes are vacuolated and other hepatocytes show ballooning degeneration (double arrow) with pyknotic nuclei. H&E X400

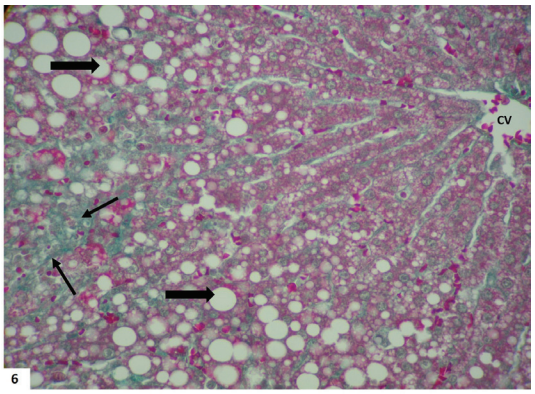

Fig. 6: A Photomicrograph of hepatic tissue of irradiation group showing increase numerous collagen fibers (thin arrows) in between the hepatic cords and surrounding the central veins (CV), also Some hepatocytes show microvesicular steatosis (thick arrows). Masson Trichrome X400

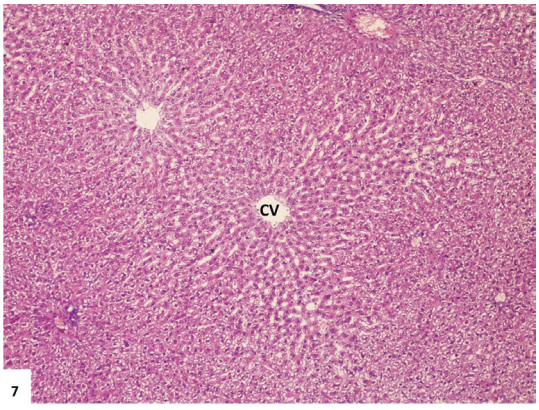

Fig. 7: A photomicrograph of liver tissue of SPL group showing cords of hepatocytes arranged around central vein (CV). H&E X100

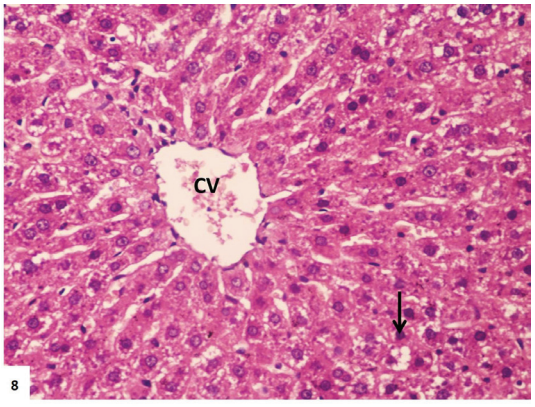

Fig. 8: A photomicrograph of liver tissue of SPL group showing normal appearance of many hepatocytes except few with deeply acidophilic cytoplasm and pyknotic nuclei (arrow). H&E X400

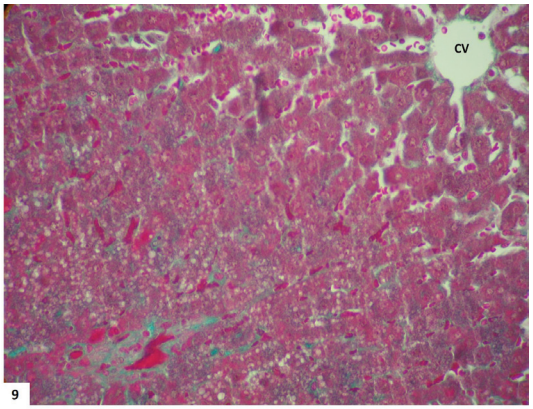

Fig. 9: A Photomicrograph of hepatic tissue of SPL group showing the amount of collagen fibers are comparable to that of the control group. Central vein (CV). Masson Trichrome X400

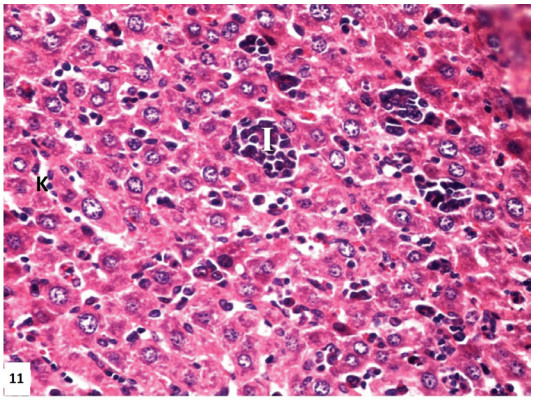

Fig. 11: A photomicrograph of liver tissue of SPH group showing leucocytic infiltration (I) and hypertrophied kuppfer cells (K). H&E X400

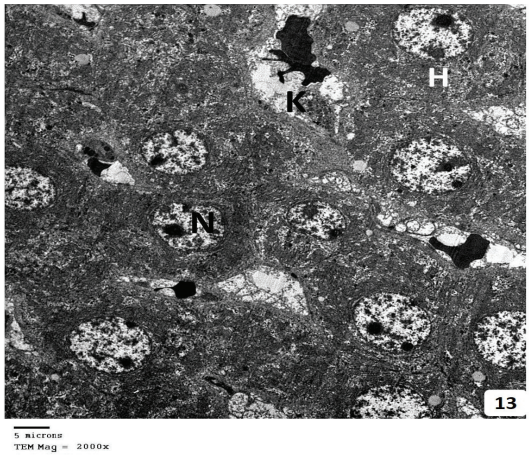

Fig. 13: Transmission electron micrograph of liver cells of control group showing cords of hepatocytes (H) with bile canaliculi in-between and blood sinusoids lined by kuppfer cells (K). hepatocytes contain euchromatic nuclei (N) with regular outline and prominent nucleolus and patches of heterochromatin. TEM Mag X2000

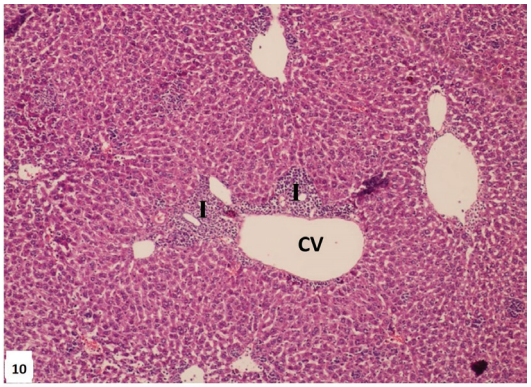

Fig. 10: A photomicrograph of liver tissue of **SPH group** showing dilated central vein (CV), with leucocytic infiltration (I) around central vein, peri-portal tract and in-between the cords of hepatocytes . H&E X100

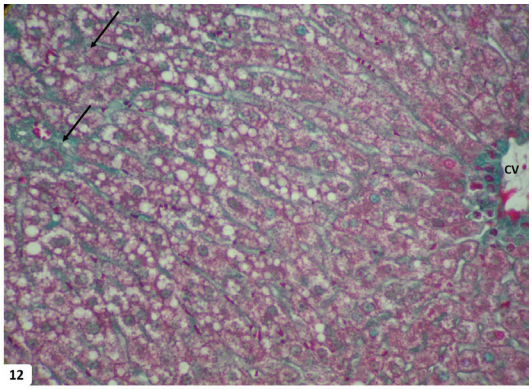

Fig. 12: A Photomicrograph of hepatic tissue of SPH group showing numerous collagen fibers (↑) in between the hepatic cords and surrounding the central veins (CV). Masson Trichrome X400

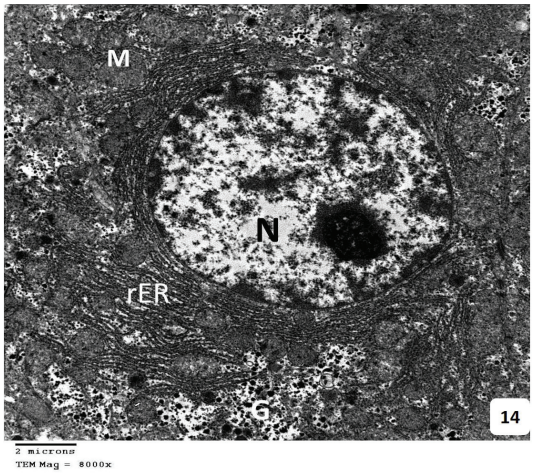

Fig. 14: Transmission electron micrograph of liver cells of control group showing the cytoplasm with numerous mitochondria (M), multiple arrays of rough endoplasmic reticulum (rER) and dispersed electron-dense glycogen granules (G). TEM Mag X8000

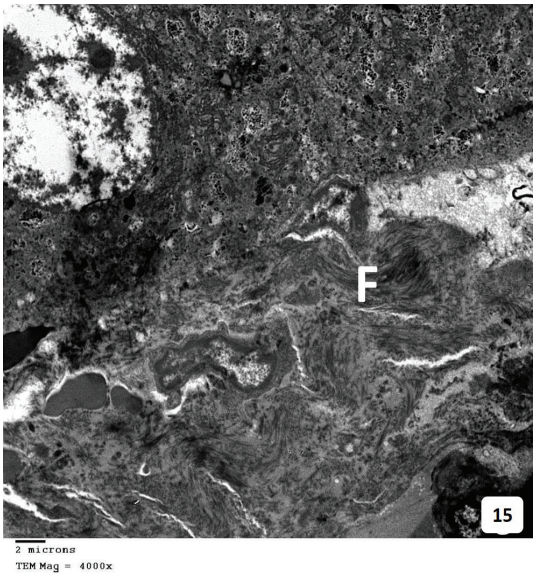

Fig. 15: Transmission electron micrograph of liver cells of irradiation group showing deposition of collagen fibers in peri-sinusoidal space (F).
TEM Mag X4000

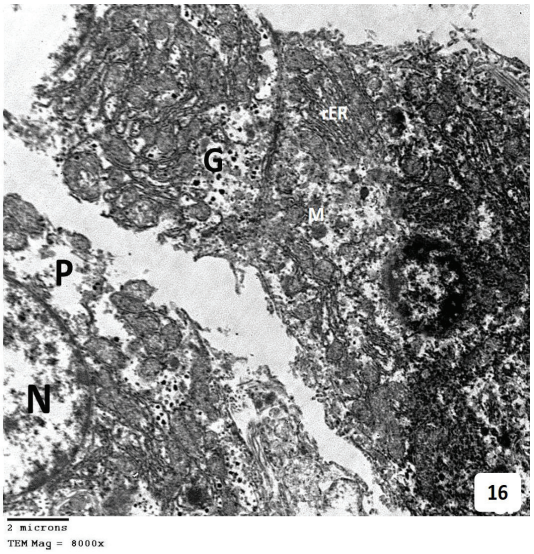

Fig. 16: Transmission electron micrograph of liver cells of irradiation group showing degenerated hepatocytes with dilated rough endoplasmic reticulum (rER), degenerated mitochondria (M), dispersed glycogen (G) and area of dissolution of cytoplasm (P). The nucleus contains dispersed chromatin (N). TEM Mag X8000

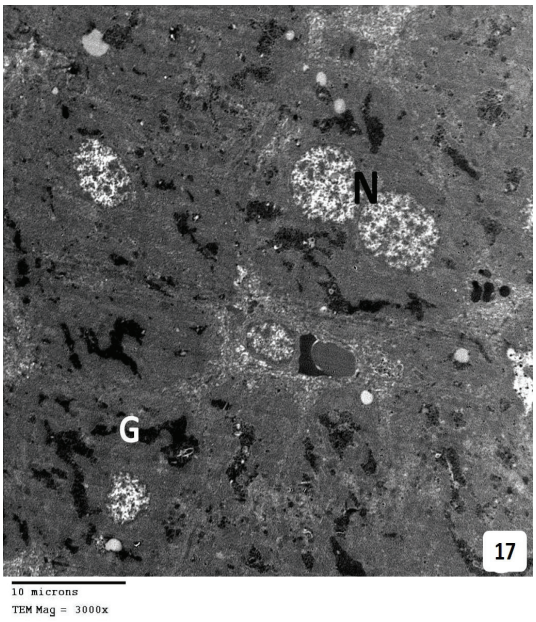

Fig. 17: Transmission electron micrograph of liver cells of SPL group showing apparently normal ultrastructure of hepatocytes with binucleated nucleus(N) and some glycogen granules (G).
TEM Mag X3000

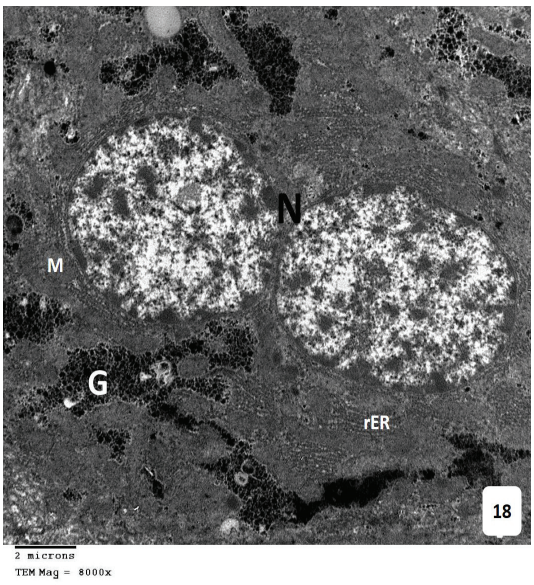

Fig. 18: Transmission electron micrograph of liver cells of SPL group showing binucleated cell and contain euchromatic nuclei (N). The cytoplasm contains numerous mitochondria (M)., rough endoplasmic reticulum (rER), and glycogen granules (G). TEM Mag X8000

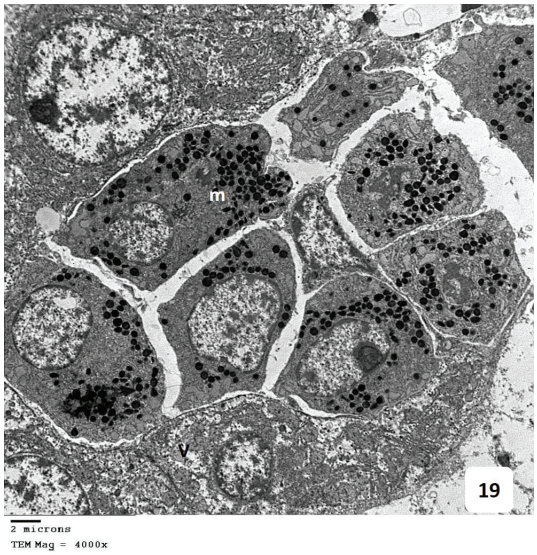

Fig. 19: Transmission electron micrograph of liver cells of SPH group showing mast cells infiltration in-between hepatocytes. Loss of the chromatin pattern of the nucleus and cytoplasmic vacuolization are not iced. TEM Mag X4000

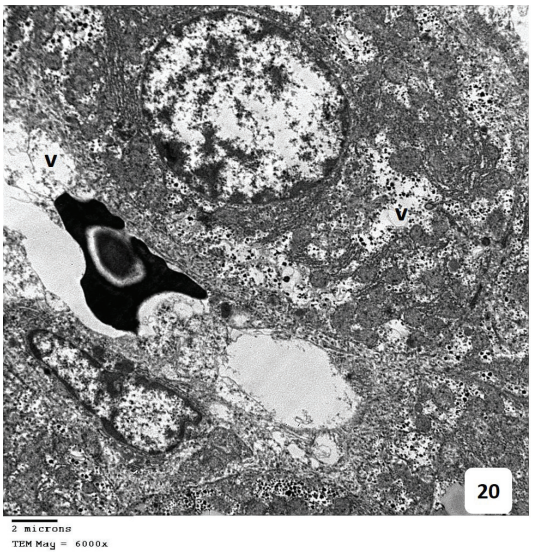

Fig. 20: Transmission electron micrograph of liver cells of SPH group showing Loss of the chromatin pattern of the nucleus and cytoplasmic vacuolization are noticed. TEM Mag X6000

DISCUSSION

Radiation hazards due to free radical generation is an enormous challenge for medical safety. The current study provides an insight for the possible protective role of Spirulina against liver tissue injury and cellular damage induced by gamma radiation.

In the present study, exposure to gamma radiation led to significant increase in the levels of AST & ALT. The increase in aminotransferase activities by radiation could be attributed to hepatocellular damage which leads to an increase in the permeability of cell membranes thereby facilitating the passage of cytoplasmic enzymes outside the cells leading to the increase in the aminotransferase activities in liver and blood serum *(Gaur and Bhatia, 2009)*. Significant decrease in plasma total serum protein after exposure to gamma radiation in the current study also indicates hepatic injury. *Bernheim (2005)* found that, the concentration of total serum protein reflects the functional capacity of the liver.

The observed increase in plasma MDA level could indicate that the oxidative damage reached a level in the tissue that allowed for release of lipid peroxidation products into the blood. The deleterious effects of ionizing radiation in biological systems are mainly mediated through the generation of ROS in cells as a result of water radiolysis *(Kamat et al., 2000)*. These ROS can induce oxidative damage to vital cellular molecules and structures including DNA, lipids, proteins, and membranes. It seems that modification of lipids and proteins by ROS is implicated in the etiology of radiation-induced physiological disorders and diseases *(Cadet et al., 2004)*.

Hepatocellular damage in this study was evident by the cellular vacuolation, tissue lysis, pyknotic nuclei, mitochondrial abnormality, congested blood vessels and evidence of areas of fibrosis. This could be explained according to *Kamat et al. (2000)* who reported that radiation-induced oxidative damage to mitochondrial membrane. Also, *Zavodnik (2003)* reported that exposure to a single whole-body gamma irradiation in rats results in plasma and liver microsomal membrane lipid peroxidation with impairments in the membrane structure and function. ROS and oxidative stress may contribute to radiation-induced cytotoxicity and to metabolic and morphologic changes in animals and humans during radiotherapy *(Fang et al., 2002, Onody et al., 2003)*.

The present study demonstrated that treatment with low dose of Spirulina prior

to irradiation induced protection against oxidative stress, evidenced by improvement of liver function and histological appearance of liver tissue. This agreed with *Halliwell and Whiteman (2004)* who stated that the use of antioxidant treatment delay or prevent the damage induced by oxidative stress. Spirulina preadministration at a dose of 300 mg/kg significantly reduced the serum hepatic biomarkers; AST & ALT, while serum total protein level was increased in comparison with radiation group. *Wu et al. (2005)* noted that the protective action of Spirulina might be attributed to its ability to scavenge the oxidation-initiating agents, which are produced during the oxidation of proteins and lipids. The present study showed that liver retained its architecture and the hepatocytes had indistinct borders with no necrosis or fibrosis noticed. The use of Spirulina has been associated with attenuation of fibrosis by the antioxidative activity and decrease in proinflammatory cytokine gene expression. *Luxia et al. (1996)* reported that β-carotene of Spirulina may reduce cell damage, especially the damage to DNA molecules, thus playing a role in the regeneration process of damaged liver cells. In addition, the antioxidant protective role of Spirulina could be due to the presence of Phycocyanin (C-phycocyanin and allophycocyanin) *(Bhat and Madyastha, 2001)*. These Phycocyanin are biliprotein pigments which stimulate the antioxidant enzymatic defense systems to reduce the early radiation response *(Patil et al., 2008, Yoshikawa and Belay, 2008)*. Therefore, it may play a role in the radioprotection of subjects exposed to low doses of radiation *(Ivanova et al., 2010)*.

In the present study, treatment with high dose of Spirulina before irradiation showed increased levels of aminotransferases and decreased plasma total protein level. However, plasma MDA level was insignificantly changed. In addition, histological examination showed dilatation and congestion of hepatic sinusoids with increased number of Kupffer cells and marked inflammatory cells infiltration in rat liver treated with high dose of Spirulina prior to radiation. This was in agreement with the results of *Pugh et al. (2001)* and *Balachandran et al. (2006)*, who reported that, Spirulina caused activation of monocytes and macrophages as well as augmentation of interleukin and interferon production *(Mao et al., 2005)*. Spirulina was found to have a major impact on the immune system by stimulating the NK cells. It also played a role in the activation and mobilization of T and B cells due to its stimulatory effects in the production of cytokines and antibodies *(Ravi et al.,2010)*.

Despite of Spirulina beneficial effects, few side effects and contraindications have been reported with its use. *Babicca et al. (2006)* reported that the cultivation environments of cyanobacteria Spirulina were suitable for the growth of some toxic cyanobacteria species, such as Anabaena, Microcystis and Oscillatoria. These cyanobacteria could produce microcystins (MCs), a group of monocyclic heptapeptides *(Soares et al., 2004),* which cause morphological and functional changes in hepatocytes *(Gulledge et al., 2002)*. Hepatotoxicity has also been reported in a case where Spirulina has been contaminated with microcystins *(Iwasa et al., 2002)*. Other studies suggested that Spirulina has an antioxidant effect *(Wu et al., 2005),* but clinical importance has not been demonstrated *(McCarty, 2007; Deng and Chow, 2010)*.

CONCLUSION

Spirulina in low dose has an antioxidant effect which can protect the liver against radiation hazards. However, high dose of Spirulina may have a harmful effect on the liver which needs further investigations.

CONFLICT OF INTERESTS

The authors have no conflict of interests.

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التأثير الوقائي للسبيرولينا ضد إصابة الكبد المحدثة بالإشعاع في الفأرالأبيض

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ملخص البحث

المقدمة: تعتبر سبيرولينا مكمل غذائى له خواص دوائية مختلفة مثل التأثيرات المضاد للأكسدة و التأثيرات المضادة للالتهاب. و تهدف هذه الدراسة الى التحقق من التأثير الوقائى لسبيرولينا ضد اصابة نسيج الكبد بأشعة جاما.

الطرق: استخدم فى هذا البحث اثنان و ثلاثون فأر أبيض ذكر بالغ تم تقسيمهم الى اربع مجموعات كل منها ثمانية فئران.

1) مجموعة ضابطة.

2) مجموعة اشعاع: تم تعريضها لأشعة جاما بجرعة 8 جراى لمدة 15 دقيقة.

3) مجموعة معالجة بسبيررولينا جرعة منخفضة: تم اعطائها سبيرولينا 300 مج/كج بالفم مره فى اليوم لمدة 3 اسابيع قبل التعرض للأشعاع.

4) مجموعة معالجة بسبيرولينا جرعة عالية: تم اعطائها سبيرولينا 1000 مج/كج بالفم مره فى اليوم لمدة 3 اسابيع قبل التعرض للأشعاع. تم تجميع عينات الدم لمعرفة مستويات الينين امينوترانسفيراز (ALT)، اسبرتات امينوترانسفيراز (AST) ومجموعة بروتينات البلازما، بالأضافة الى تجميع عينات الكبد من جميع المجموعات لفحص الانسجة بالميكرسكوب الضوئى والاليكترونى.

النتائج: اظهرت النتائج الانخفاض الملحوظ لمستويات (ALT) و(AST) فى الفئران المعالجة بجرعة منخفضة من سبيرولينا قبل الاشعاع بالمقارنة بالمجموعة المشعة مع ارتفاع ملحوظ لمجموع بروتينات البلازما و تحسن فى نسيج الكبد بينما وجد ان المجموعة المعالجة بجرعة عالية من سبيرولينا لم تظهر اى علامات تحسن مقارنة بالمجموعة المشعة و من هنا يمكن ان نستخلص ان المعالجة بجرعة منخفضة من سبيرولينا لها تأثير وقائى على الكبد بينما المعالجة بجرعة عالية قد يكون لها تأثير سام على الكبد وهذا يحتاج الى دراسة مستقبلية.