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Histological and histochemical changes in liver of gamma-irradiated rats and the possible protective role of *Aphanizomenon flos-aquae* (AFA)

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Abstract

Exposure to ionizing radiation represents a genuine increasing threat to mankind and our environment. *Aphanizomenon flos-aquae* (AFA) is a blue-green microalgal species which has antioxidant properties.**The Aim of the work:** this study aimed to elucidate the possible radioprotective effect of *Aphanizomenon flos-aquae* (AFA) on liver of irradiated adult male rats using biochemical parameters, histopathology and quantitative histochemistry. **Matrerial and methods**: the current experiment was carried out on 48 adult male albino rats (*Rattus rattus*). Rats were randomly and equally categorized into four groups: 1) Group C: control rats left without treatment; 2) Group R: rats were exposed to 4Gy of gamma-radiation as a single dose; 3) Group AFA: rats were treated orally with 94.5mg/kg body weight/ day AFA for 3 weeks and 4) Group AFA+R: rats were administrated AFA for a period of one week before and three weeks after irradiation. The experimental rats were sacrificed after 5 and 21 days post-irradiation. **Results**: exposed to gamma radiation showed many biochemical changes which included a significant increase in serum ALAT, ASAT ,ALP activities and MDA in the liver tissues. Many histopathological and histochemical changes were observed in the liver tissue, such as corrugated and ruptured endothelial lining of the central vein which contained hemolysed blood cells, numerous vacuolated hepatocytes with increased signs of karyolysis and pyknosis in nuclei of hepatocytes, highly dilated and congested hepatic portal vein, numerous hemorrhagic areas and distorted bile ducts. Highly increased collagen fibers were also observed after gamma irradiation in the liver tissue. In addition, irradiated group induced a significant increase in amyloid β-protein, while a significant decrease in PAS+ve materials, total protein and total DNA content was detected. Supplementation with AFA showed a trend toward lowering incidence of hepatic histopathological and histochemical changes induced by γ-radiation.

Conclusion: according to the results obtained in the current study using *Aphanizomenon flos- aquae*as a natural agent showed a strong radioprotective role.

Key words: gamma rays,ionizing radiation, *Aphanizomenon flos-aquae*(AFA),liver, rats.

1 Introduction

Owing to the progressive development in all fields of science and technology in the world there are a huge number of various sources of radiation. Those include: space exploration, mobile communications, development of new technologies in medicine,development of nuclear weapons and the increase in the nuclear industry and power that led to a serious threat to the environment and human health **(Nakamura *et al*., 2012)**.Ionizing radiations induce similar damage at the cellular level. Gamma rays and neutrons are more affecting, causing diffused damage via the body (e.g.radiation sickness, cell death due to damaged DNA, increased cancer incidence) rather than burns. The most striking biological damaging forms of gamma radiation occur in gamma ray window, between 3 and 10 MeV **(Bock, 2008)**. Whole body gamma-irradiation of animals at the sub lethal and lethal dose levels affected the metabolism of different organs and exhibited a series of physiological and biochemical disturbances in various biological tissues **(Mohammed, 2010)**.Radiation-induced liver diseases may occur in human with normal liver function, causing hepatomegaly and mild increase in alkaline phosphatase concentration and this may develop fibrosis, cirrhosis and finally liver failure **(Shadad *et al*., 2013).** Ionizing radiation develops harmful effects on the organisms and due to the cosmopolitan use of radiation in therapy, diagnosis and industry, pharmacological intervention could be most potent policy to protect human or ameliorates the bad effect of these radiation **(Kumar and Tiku, 2016)**.Ionizing radiation is one of the environmental pollutants that may contribute to liver dysfunction due to its oxidative stress. Gamma exposure of animals significantly

decreased the activity of glutathione oxidase and superoxide dismutase as well as enhanced the lipid peroxidation in liver. These effects were concomitant to severe histopathological alterations in liver cells (**Gheriany and Awwad,2017**)

Blue–green algae (BGA) provide a good applicable source for health beneficial foods and drug industry(**Schaap *et al*., 2012**). The most common BGA, *Spirulina platensis*(SP) and *Aphanizomenon flos-aquae*(AFA) were found to have antioxidant activity(**Venkatesan *et al*., 2012**),hypolipidemic and anti-inflammatory properties(**Yang *et al*., 2011; El-Depsi, 2016).** Those together with *Chlorella* sp. are commercially distributed as organic algae dietary supplements. They have significant amounts of protein, lipid, carotenoids,chlorophyll, vitamins, minerals and unique pigments. They may also have potent probiotic components that strength health(**Singh *et al*., 2005; Wu *et al*., 2012).***Aphanizomenon flos-aquae*** (AFA), is fresh water unicellular BGA that spontaneously grows in a German lake(Upper Klamath) and that is used as a nutrient-dense food source and for its health-increasing properties **(Pugh and Pasco, 2001).** AFA is a pivotal source of the blue photosynthetic pigment phycocyanin (PC), which is a potent antioxidant, free radical scavenger and cyclooxygenase-2 inhibitor and thus have the potential to minimize inflammation **(Scoglio *et al*., 2014; Li *et al*., 2016)**. AFA is an exceptional source of carotenoids (more than 240 retinol equivalents per gram). Beta-carotene as well as other carotenoids are powerful antioxidants which help in the protection of cancer and cardiovascular diseases **(Khuantrairong and Traichaiyaporn, 2012)**.Moreover, AFA is rich in protein (63-69% dry weight), vitamin B_{12} and other biologically-active components in addition to a high concentration of α-linolenic acid (18:3n3), which at a concentration of 10-15%, in rat diet, represents a good source of polyunsaturated(n-3) fatty acids **(Fastner *et al*., 2015).**BGA may induce the liberation of antioxidant enzymes with no harmful side effects on both kidney and liver and improves the hematological parameters**(El-Malawany *et al*., 2014).**Liver was chosen, in the current investigation, as a biological indicator reflecting the pathogenesis of irradiation since it is a main metabolic and detoxicant organ highly-sensitive to environmental pollutants. In addition, the present study investigated the possible radioprotective role of AFA against the deleterious effects of gamma radiations in liver tissue.

2 - Material and Methods

A total of forty eight male albino rats (*Rattus rattus*) weighing 180-200 gm, purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo-Helwan, Egypt), were used as experimental animals in this work. The animals were kept in the laboratory for 2 weeks before the experimental work for acclimatization and they were housed in especially designed cages, 6 rats in each, with controlled air, temperature and relative humidity. Animals were fed standard rodent pellets. Food and water were made available *ad-libitum* throughout the whole experimental period. All animal's procedure were consistent with the guidelines of Ethics by Public Health Guide for Care and Use of Laboratory Animals (**National Research Council, 1996**).

Gamma-irradiation procedure:

Irradiation process was performed using Gamma Cell-40 achieved by Egypt's National Center for Radiation Research and Technology (NCRRT), Cairo. This gamma source is a caesium-137 irradiation unit produced by Atomic Energy of Canada Limited. The unit provides means for uniform Gamma-irradiation of small animals or biological samples while providing complete protection for operating personnel. The dose rate was 0.62 Gy/min. at the time of the experiment.

Aphanizomenon flos- aquae **(AFA-Klamath) administration:**

AFA-Klamath capsules (350 mg) obtained from German Egyptian Pharmaceutical Company. Capsules were opened and dissolved in distilled water. The drug was administrated orally by gastric tube at a dose of 94.5 mg/kg body weight/day for 21days. The dose for the rat was calculated according to the Paget's formula on the basis of the human dose **(Paget and Barns, 1964)**.

Experimental design:

Forty eight of the experimental animals were divided into 4 groups. These groups were;

1) Group C:control rats normal healthy rats left without any treatment.

2) Group R: rats were exposed to single dose of 4Gy of γ-radiation.

3) Group AFA: rats were treated orally with 94.5mg/kg body weight/ day AFA for 3 weeks.

4) Group AFA+R: rats were administrated with 94.5mg/kg body weight/day of AFA extract for a period of one week before and three weeks after irradiation.

The experimental rats were sacrificed after 5 and 21 days post-irradiation.

Biochemical assays

The activities of serum alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were assayed by the kinetic method using available commercial kits (Spin react, Spain) according to Young and Friedman (2001). The levels of alkaline phosphatase (ALP) in serum were assayed by the method of Schumann et al.(2002) according to the International Federation of Clinical Chemistry (IFCC).Levels of lipid peroxidation (LPO) in liver tissues were determined according to the method of **Yoshioka *et al.* (1979).**

The histological and histochemical studies

Animals of control and treated groups were sacrificed after five and twenty one days post-irradiation, then livers were immediately excised and fixed in 10% neutral formalin for 24 hours,dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Sections were cut at 5µ thickness and stained by haematoxylin and eosin according to the method of **Bancroft and Gamble (2002)**. Collagen fibres were stained by Mallory's trichrome stain **(Pears, 1977).** Polysaccharides were detected using periodic acid Schiff's (PAS) reaction and DNA was detected by using Feulgen reaction**(Drury and Wallington, 1980)**. Total proteins were detected by

mercuric bromophenol blue method **(Maziaet *al.*, 1953).** Amyloid-β proteins were visualised by Congo red technique **(Valle, 1986).**

Quantitative histochemical analysis

The optical density of histochemical stained sections in liver for carbohydrates, total protein, Amyloid-β protein and total DNA content of control and treated groups was recorded using IPWIN 32 image analysis software.

Statistical analysis

Statistical analyses of data were carried out using analyses of variance (ANOVA) according to **Snedecor and Cochran (1980)**, processed and analyzed using the SPSS software (Statistical Analysis for Social Science, Version 8).Data were presented as mean ± SE and P ≤0.05 was considered statistically significant.

3. Results

Biochemical results

Serum alanine aminotransferase activity:

The rats exposed to γ-radiation exhibited a significant increase in the mean value of serum ALAT which reached to 58.00 ± 6.37 and 54.33 ± 3.83 u/l after 5 and 21 days of treatment respectively as compared to the control group.

On the other hand, drenching AFA to the rats induced non- significant decrease in the mean value of serum ALAT which reached -2.92 and -2.1% on the 5th and 21th day post the treatment respectively as compared to the control group.

Groups of rats treated with AFA and exposed to γ-radiation showed non-significant decrease in serum ALAT after5 days of exposure as compared to the control group. While, a non-significant increase in serum ALAT was observed after 21 days post γ-irradiation.

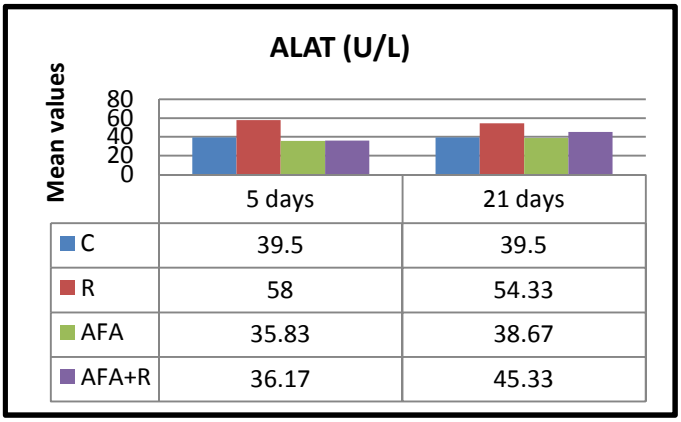


Fig.1: effect of radiation and/ or AFA on serum ALAT (U/L) of the control and all the treated groups of adult male albino rats.

Serum aspartate aminotransferase activity:

The present results showed a highly significant increase in the mean value of serum ASAT on the 5th and 21th day in γ-irradiated rats. This increase was 194.17 ± 7.85 and 202.00 ± 10.28 U/L, respectively compared to the control group (169.50 ± 7.27 U/L). The percentage of increase was 14.55 and 19.17%, respectively.

Conversely, drenching AFA to the rats induced non-significant increase in the mean value of serum ASAT after 5 and 21 days post the treatment as compared to the control group.

Consequently, irradiated rats treated with AFA exhibited non-significant increase in the mean value of serum ASAT after 5 and 21 days post irradiation. The percentage of increase was 0.48 and 5.41% as compared to the control group after 5and 21 days of γ-irradiation respectively.

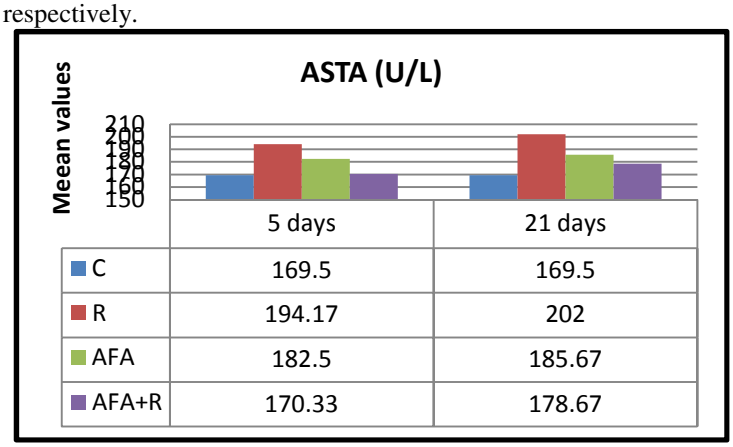


Fig. 2: effect of radiation and/ or AFA on serum ASAT (U/L) of the control and all the treated groups of adult male albino rats.

Serum alkaline phosphatase activity:

The present results showed a highly significant increase ($p < 0.01$)in serum alkaline phosphatase level in γ-irradiated rats as compared to the control group they reached 365.67±10.62and 352.67 ±8.41 U/L, on the 5th and 21th day post-treatment respectively. The decrement percentage was about -24.51 and - 20.09%, respectively.

Administration of AFA resulted in non-significant decrease in the serum alkaline phosphatase as compared to the control group during the experimental periods.

On the other hand, γ-irradiated rats that orally received AFA for 5 days showed a significant decrease in the mean value of serum alkaline phosphatase which reached 247.33 ± 1.96 U/L. While, a non-significant decrease in serum ALP was observed after 21 days post irradiation.

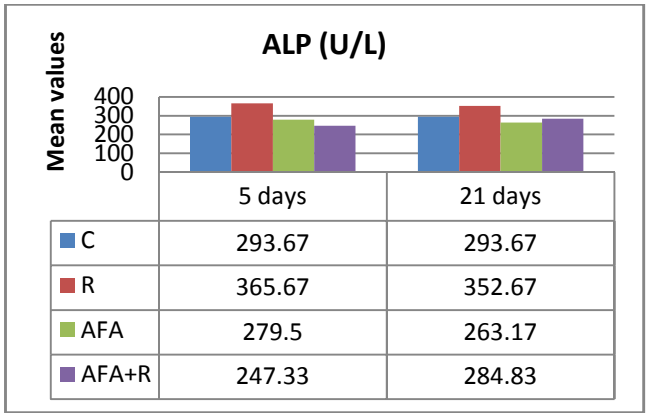


Fig.3: effect of radiation and/ or AFA on serum ALP (U/L) of the control and all the treated groups of adult male albino rats.

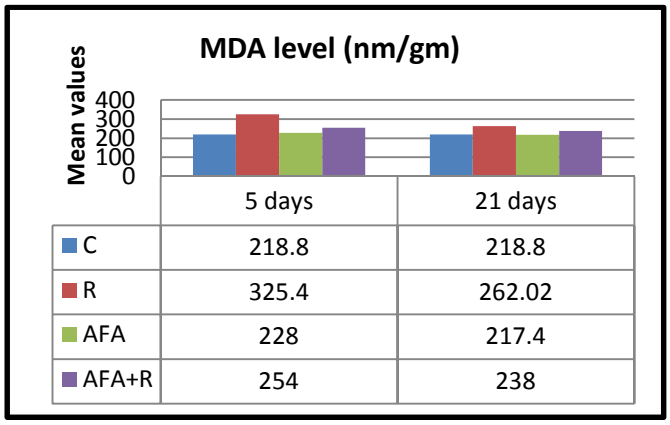
Malondialdehyed (MDA) levels in the liver tissue:

Exposure of rats to whole body γ-radiation induced a very highly significant increase (p<0.00l) in the mean value of MDA level which reached 325.4 ± 4.55 and 262 ± 6.3after the 5th and 21th days post-irradiation respectively.

Meanwhile, treatment with AFA exhibited non significant change in the mean value of MDA which reached 228 ± 3.4 and 217.4 ± 2.9 after 5th and 21th day post the treatment respectively in compare with control group.

Irradiated rats treated with AFA exhibited non significant increase in mean value of MDA which amount 238 ± 6.2 on the 21th day post-irradiation, these data pointed out to ameliorative effect of AFA.

On the other hand, irradiated rats treated with AFA exhibited a significant increase which amounted 16.08% on day five post-irradiation.



	5 days	21 days
C	218.8	218.8
R	325.4	262.02
AFA	228	217.4
AFA+R	254	238

Fig.4: effect of radiation and/ or AFA on hepatic malondialdehyde levels (nm/mg) of the control and all the treated groups of adult male albino rats.

Histopathological observations:

Control group (C). Figs. 5&6 showed typical hepatic lobules with cords of hepatocytes which are radiating from the central vein and separated by the hepatic sinusoids. The hepatocytes have eosinophilic cytoplasm, large rounded nuclei and prominent nucleoli; some cells of them contain double nuclei. The sinusoids are lined with endothelial cells and scattered phagocytic Kupffer cells. The portal area contains a branch of the hepatic portal vein, branch of the hepatic artery and bile ducts. Collagen fibres are supporting walls of hepatocytes, blood vessels and sinusoidal spaces (**Fig. 7**).

Irradiated group (R). Examination of the liver tissue five days post- irradiation showed many drastic changes in the central and portal areas **(Figs. 8-10)**. These changes include: lymphocytic infiltration around the corrugated wall of the central vein which contained hemolysed blood cells, numerous vacuolated hepatocytes with increased signs of karyolysis and pyknosis in nuclei of the hepatocytes, highly distorted portal areas which contained: elongated, dilated and corrugated walls of the hepatic portal veins with hemolysed blood cells inside them, highly distorted walls of bile ducts and delaminated endothelial lining of the hepatic portal vein which contained enlarged nuclei , highly vacuolated hepatocytes in the portal areas, fibrotic areas in and around the portal areas, numerous bleeding areas and numerous hemorrhagic areas, degenerated areas which contained debris of degenerated hepatocytes. In the second group (after twenty one days post- irradiation), liver tissue showed delaminated and ruptured endothelial lining of the central vein with hemolysed blood cells inside it, increased proliferation (hyperplasia) in walls of the bile ducts, hemorrhagic areas in between hepatocytes which were surrounded by lots of lymphocytes, numerous vacuolated hepatocytes which contained pyknotic or karyolytic nuclei and increased Kupffer cells **(Figs. 12-13)**.

Mallory's trichrome stain showed highly increased collagen fibers after 5 and 21 days of gamma irradiation especially in the congested blood vessels, blood sinusoids and around the portal areas **(Figs. 11 and 14)**.

AFA - treated groups

Normal appearance of liver tissue of AFA groups (5 and 21 days post-treatment) was detected in **figs. 15 and 16** with normal distribution of collagen fibres around the hepatocytes, the central vein, in the portal area and in the blood sinusoids**(Figs. 17, 18)**.

AFA+R treated group

On the other hand, rats treated with AFA parallel with radiation exposure after 5 days of γ- radiation showed well developed central areas with highly increased Kupffer cells and increased lymphocytic infiltration in and around the portal areas. Few hepatocytes showed vacuolation**(Figs. 19 ,20)** with somewhat normal distribution of collagen fibres in the central areas **(Fig. 23)**.

In the second group (after twenty one days post-irradiation), liver sections showed well developed architecture of the central and portal areas, but the hepatic portal veins were still congested with highly increased lymphocytic infiltration in and around the portal areas **(Figs. 21,22)** with somewhat normal distribution of collagen fibres in the portal areas **(Fig. 24)**.

Quantitative histochemical measurements

PAS positive materials

Fig. 25 represented deeply stained PAS +ve materials in the central and portal areas of the liver tissue of a control rat.

Exposure of rats to 4 Gy of gamma radiation (R) represented a highly decreased mean value of PAS +ve materials (0.211 & 0.204 after 5 days or 21 days of γ-irradiation respectively) in hepatocytes of the central and portal areas of the liver tissue, but they were increased in walls of the hepatic portal veins, walls of bile ducts, arterial walls, in the thickened wall of the central vein, in the hemolysed RBCs inside the hepatic portal vein and the central vein after 5 days **(Fig.26)** or 21 days **(Fig. 27)** of γ-radiation exposure.

Treatment with *Aphanizomenon flos-aquae* (AFA) showed non significant increase in mean value of PAS +ve materials (0.28 & 0.271 after 5 and 21 days of the treatment respectively) in the liver tissues **(Figs. 28 , 29)**.

Treatment of experimental animals by AFA followed by γ-irradiation represented non significant change in the mean values of PAS +ve materials which reached 0.278 & 0.265 after 5 and 21 days of γ- irradiation respectively in the liver tissues **(Figs. 30 ,31)**.

Total proteins

Moderately stained total protein in the liver tissue of a control rat was realized in **Fig. 33**, but walls of the blood vessels were deeply stained.

Exposure of rats to 4 Gy of gamma radiation (R) represented a significant decrease in mean value of total protein (0.217 & 0.2 after 5 days or 21 days of γ- irradiation respectively) in most hepatocytes of liver tissue, but they increased in the thickened walls of the blood vessels and bile ducts after 5 days **(Fig. 34)** or 21 days **(Fig. 35)** of γ- irradiation. Notice: deeply stained blood cells inside the blood vessels after 5 days.

Treatment with *Aphanizomenon flos-aquae* (AFA) showed a non significant increase in mean value of total protein (0.273 & 0.282 after 5 and 21 days of γ- irradiation respectively) in the central and portal areas of liver tissue after 5 days **(Fig.36)** or 21 days **(Fig.37)** of the treatment.

Treatment of experimental animals with AFA followed by γ- irradiation represented a non significant change in the mean values of total protein which reached 0.265& 0.29 after 5 and 21 days of γ- irradiation respectively in the liver tissue **(Figs. 38,39)**.

Amyloid–β protein

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Fig.41 showed faintly stained amyloid protein in liver tissue of a control group.

Irradiated group exhibited a significant increase in mean value of amyloid–β protein content which reached 1.18 & 93 after 5 days **(Fig.42)** or 21 days **(Fig.43)** of γ- irradiation respectively in the liver tissue relative to the control group all over the experimental periods. This increase was observed in some hepatocytes of the central and portal areas and in the hemolysed RBCs inside the hepatic portal veins and the central veins.

While rats administrated AFA alone showed non significant decrease in mean value of amyloid–β protein content (0.36 & 0.38 after 5 and 21 days of treatment respectively) in the liver tissue **(Figs.44, 45)**.

Treatment of experimental animals by AFA followed by γ- irradiation represented non significant change in the mean values of amyloid–β protein content which reached 0.44 & 0.38 after 5 and 21 days of γ- irradiation respectively in the liver tissue **(Figs.46,47)**.

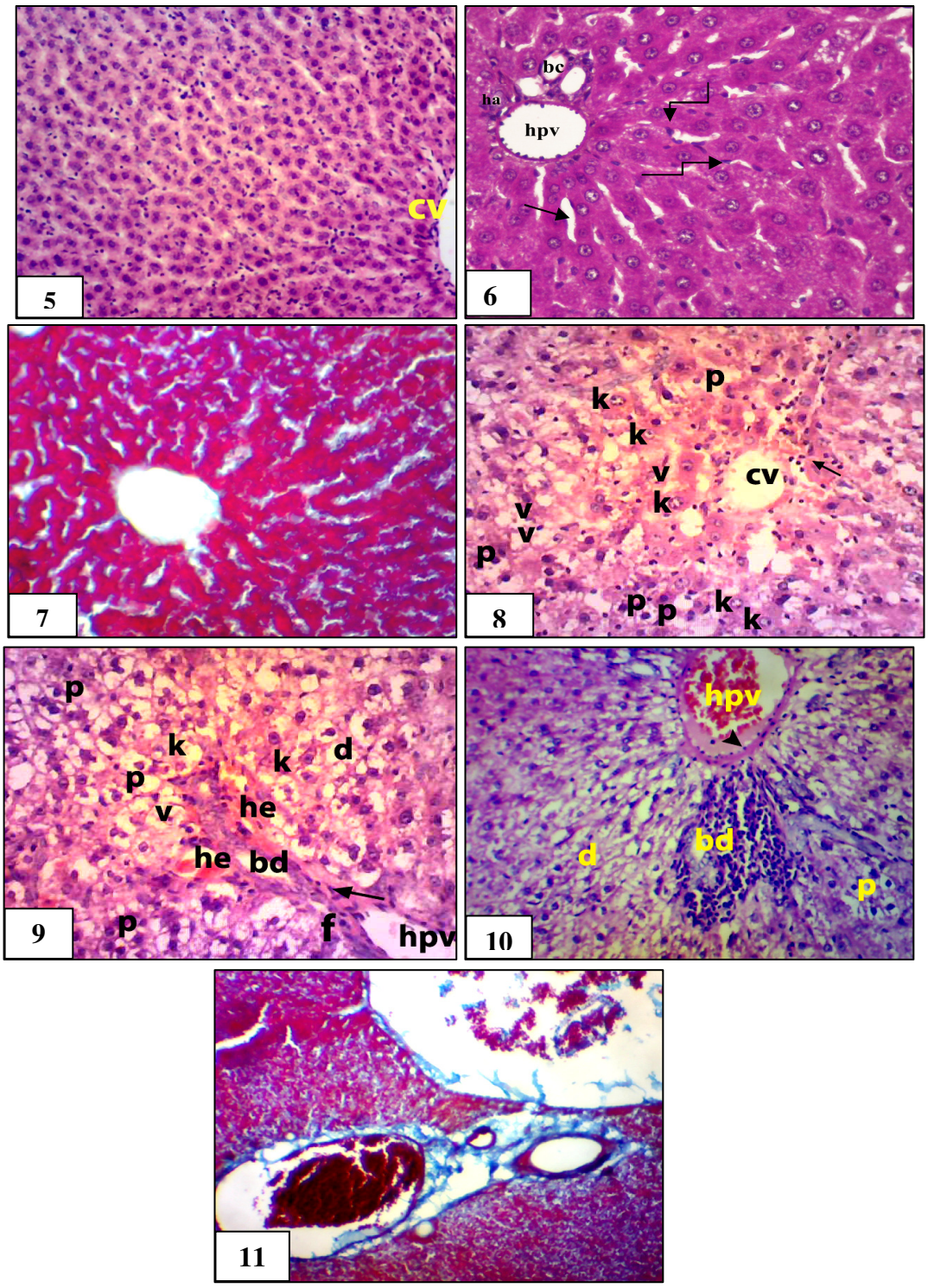
Total DNA content

The liver tissue of a control rat showing moderately stained DNA materials in nuclei of hepatocytes, Kupffer cells and nuclei of endothelial lining of the blood vessels **(Fig.49)**

The liver tissue of γ- irradiated rats showed a significant decrease in mean value of DNA +ve materials (0.24 & 0.22 after 5 days or 21 days of γ- irradiation respectively) in the liver tissue **(Figs.50, 51)**.

Meanwhile, Treatment with AFA showed a non significant increase in mean value of DNA materials which reached 0.33 & 0.31 after 5 and 21 days of treatment respectively in liver tissue **(Figs. 52 - 53)**.

Irradiated rats administrated AFA recorded non significant decrease in the mean values of DNA materials which reached 0.318 & 0.30 after 5 and 21 days of γ- irradiation respectively in liver tissue **(Figs. 54 - 55)**.



Figs. 5-24: photomicrographs of sections in liver tissue of the control and treated groups

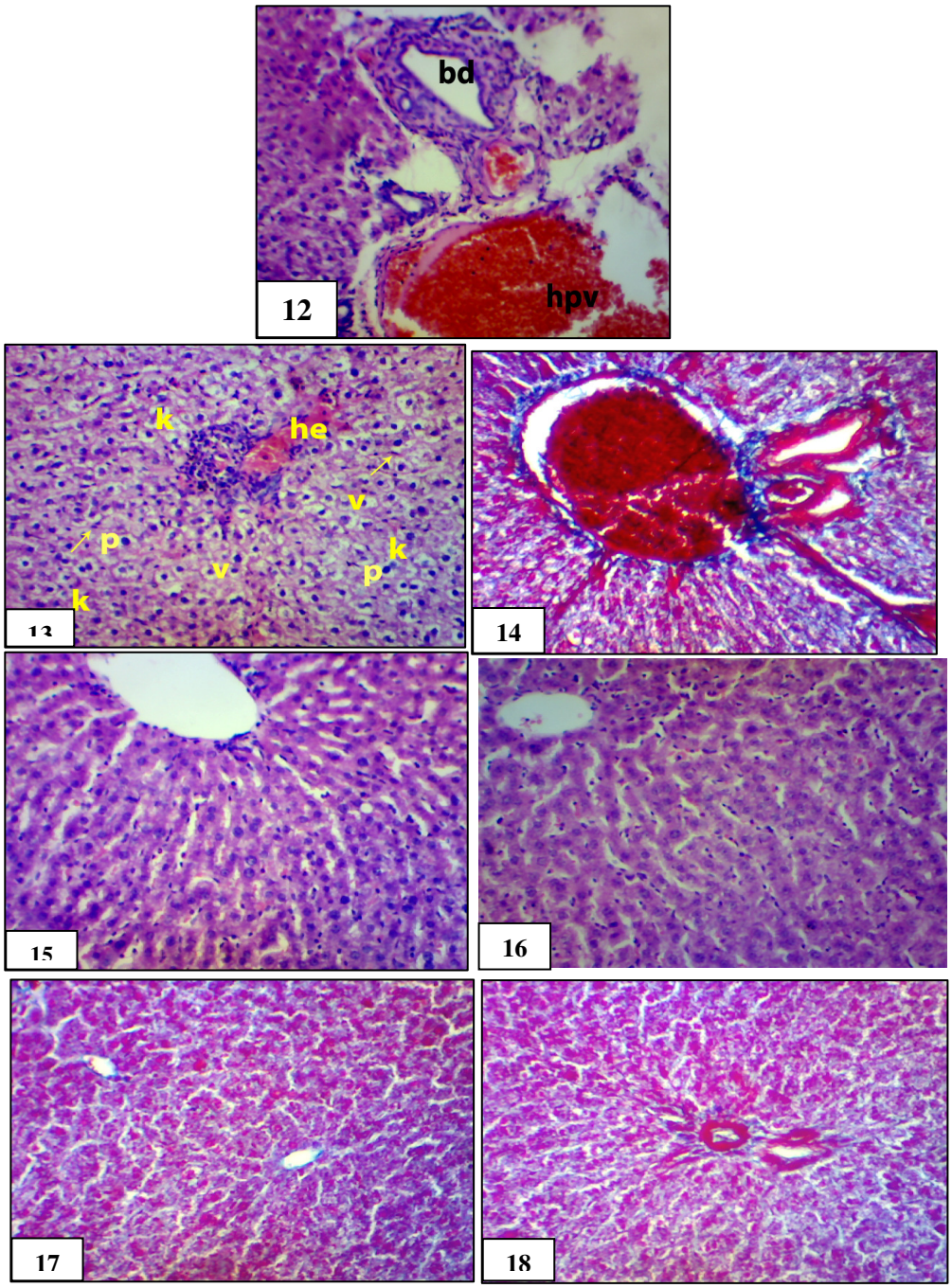
Figs. 5,6: control rats showing normal structure of liver tissue. Notice: the central vein (cv), cords of hepatocytes radiating from it and separated from each other by blood sinusoids(arrow) with many Kupffer cells (corrugated arrow). The portal area contains a branch of the hepatic portal vein (hpv), branch of the hepatic artery (ha) and bile ducts (bc). (H& E X200)

Fig. 7: control rats showing normal distribution of collagen fibers in central vein area. (Mallory's trichrome stain X 200)

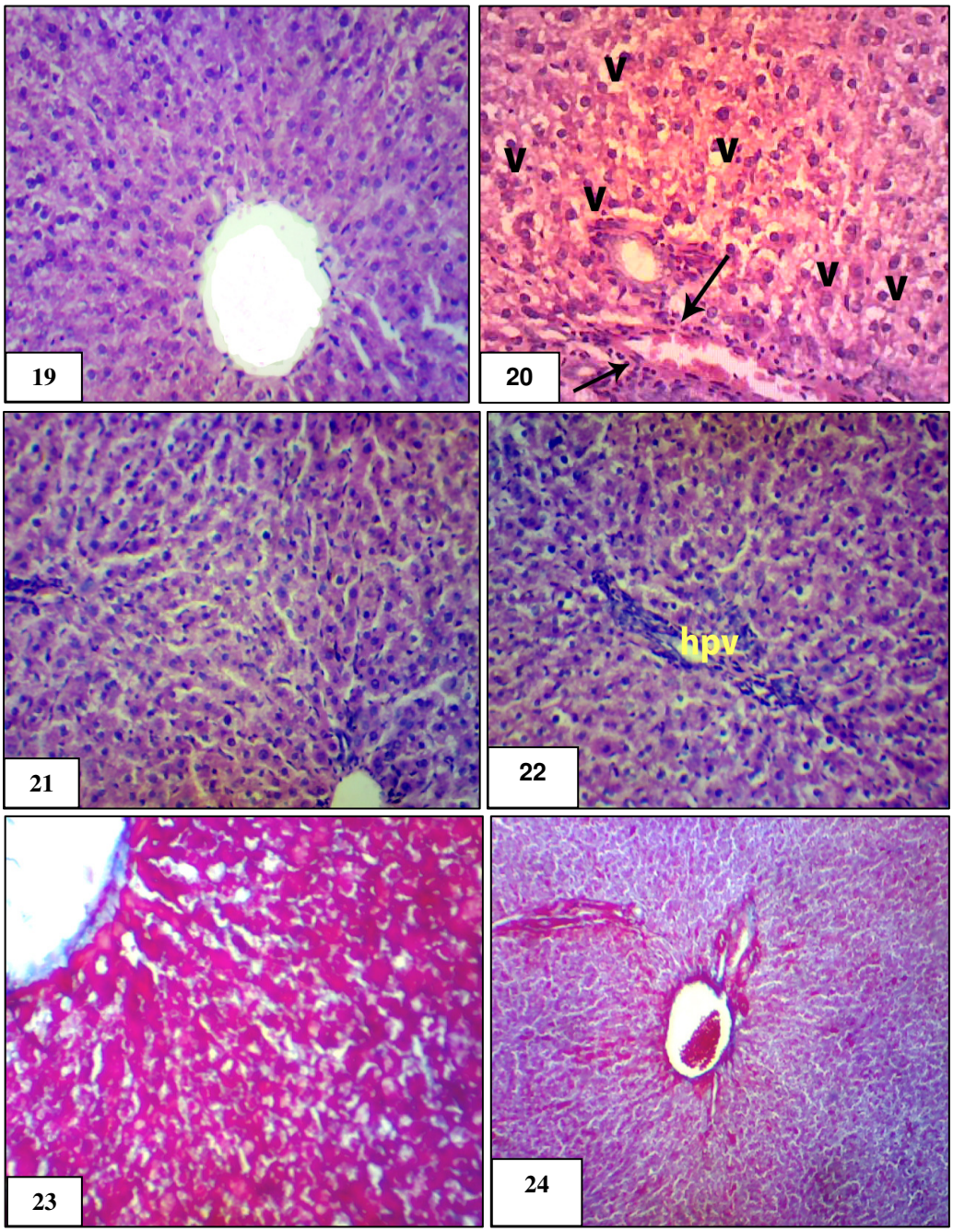
Fig.8: irradiated rats after 5 days showing highly elongated and corrugated wall of the central vein (cv), lymphocytic infiltration around it ( ), it contains hemolysed blood cells with numerous vacuolated hepatocytes (v), nuclei of hepatocytes show pyknosis (p) and karyolysis (k). (H & E X200)

Figs. 9,10: irradiated rats after 5days showing highly distorted portal areas which contain elongated, dilated and corrugated walls of the hepatic portal veins (hpv) with hemolysed blood cells inside them, highly vacuolated hepatocytes in the portal area (v), fibrotic areas in and around the portal areas (f), numerous pyknotic (p) or karyolitic (k) nuclei, numerous hemorrhagic areas (he), highly distorted walls of the bile ducts (bd), numerous degenerated areas which contain debris of degenerated hepatocytes (d), enlarged nuclei of the endothelial lining of the hepatic portal vein (►).H & E X200)

Fig. 11: irradiated rats after 5days showing increased collagen fibres inside the highly dilated hepatic portal vein, in the detached endothelial linings of it, in walls of the bile ducts with numerous scattered collagen fibres in between hepatocytes of the liver. (Mallory's trichrome stain X 100)



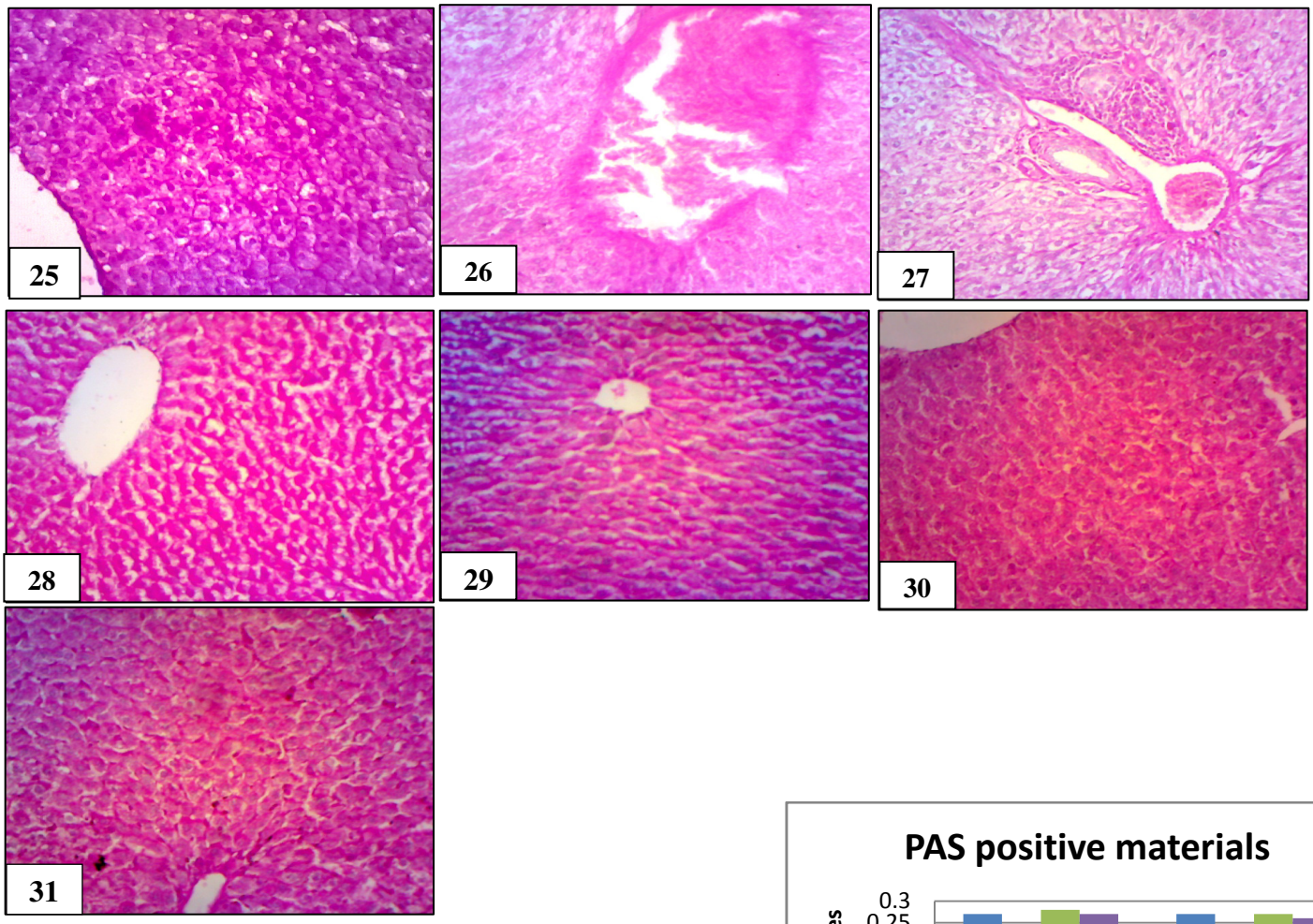
Figs. 12-14 : irradiated rats after 21days showing :
Fig.12: highly dilated, corrugated and ruptured walls of the congested hepatic portal veins **(hpv)** which contain hemolysed blood cells, increased proliferation (hyperplasia) in walls of the bile ducts **(bd)**. **(H&E X 200)**
Fig. 13:hemorrhagic area **(he)** in between hepatocytes which is surrounded by lots of lymphocytes, numerous vacuolated hepatocytes with pyknotic**(p)** or karyolytic**(k)** nuclei and increased Kupffer cells (↗). **(H&E X 200)**
Fig. 14:increased collagen fibres inside the highly dilated hepatic portal vein and in between hepatocytes of the liver tissue. **(Mallory's trichrome stain X 200)**
Figs. 15, 16:AFA treated rats after 5 and 21 days of treatment showing: almost normal structure of liver tissue. **(H&E X 200)**
Figs. 17, 18:AFA treated rats after 5 and 21 days of treatment showing normal appearance of collagen fibres in the liver tissue. **(Mallory's trichrome stain X 100)**



Figs. 19, 20:AFA + Rtreated rats after 5 days post- irradiation showing well developed central area with highly increased Kupffer cells , increased lymphocytic infiltration in and around the portal area (↗) and few hepatocytes show vacuolation(**v**). **(H&E X 200)**

Figs. 21,22: AFA + R **treated rats after 21** days showing well developed architecture of liver tissue, but the hepatic portal vein **(hpv)** is still congested with highly increased lymphocytes. **(H&E X 200)**

Figs. 23,24: photomicrographs showing slightly increased collagen fibres in the central and portal areas of liver tissue of groups AFA+R after 5 and 21 days post- irradiation respectively. **(Mallory's trichrome stain X 200)**



Figs. 25-31: photomicrographs showing distribution of PAS +ve materials in the liver tissue of the control and treated groups after 5 and 21 days of irradiation. **(PAS X 100).**

Fig. 25: control rats showing deeply stained PAS +ve materials in the central area.
Figs. 26 , 27: irradiated rats showing faintly stained PAS +ve materials in the central area after5 and 21 days respectively.
Figs. 28 , 29: AFA **treated rats** showing almost moderately stained PAS +ve materials after 5 and 21 days respectively.
Figs.30 , 31: AFA+R **treated rats** showing almost moderately stained PAS +ve materials in the central area after5 and 21 days respectively.

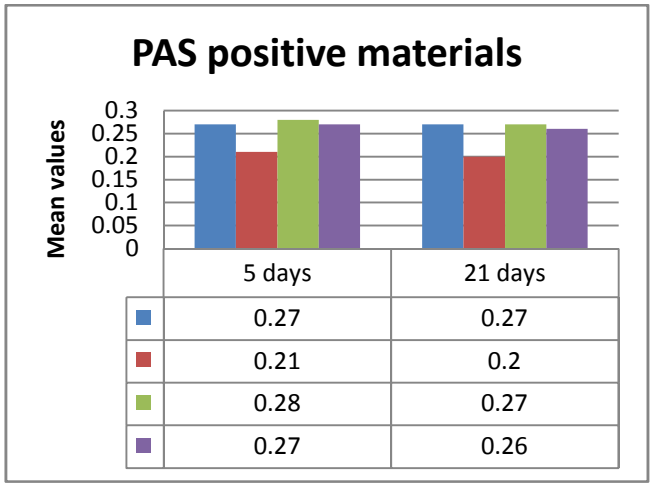
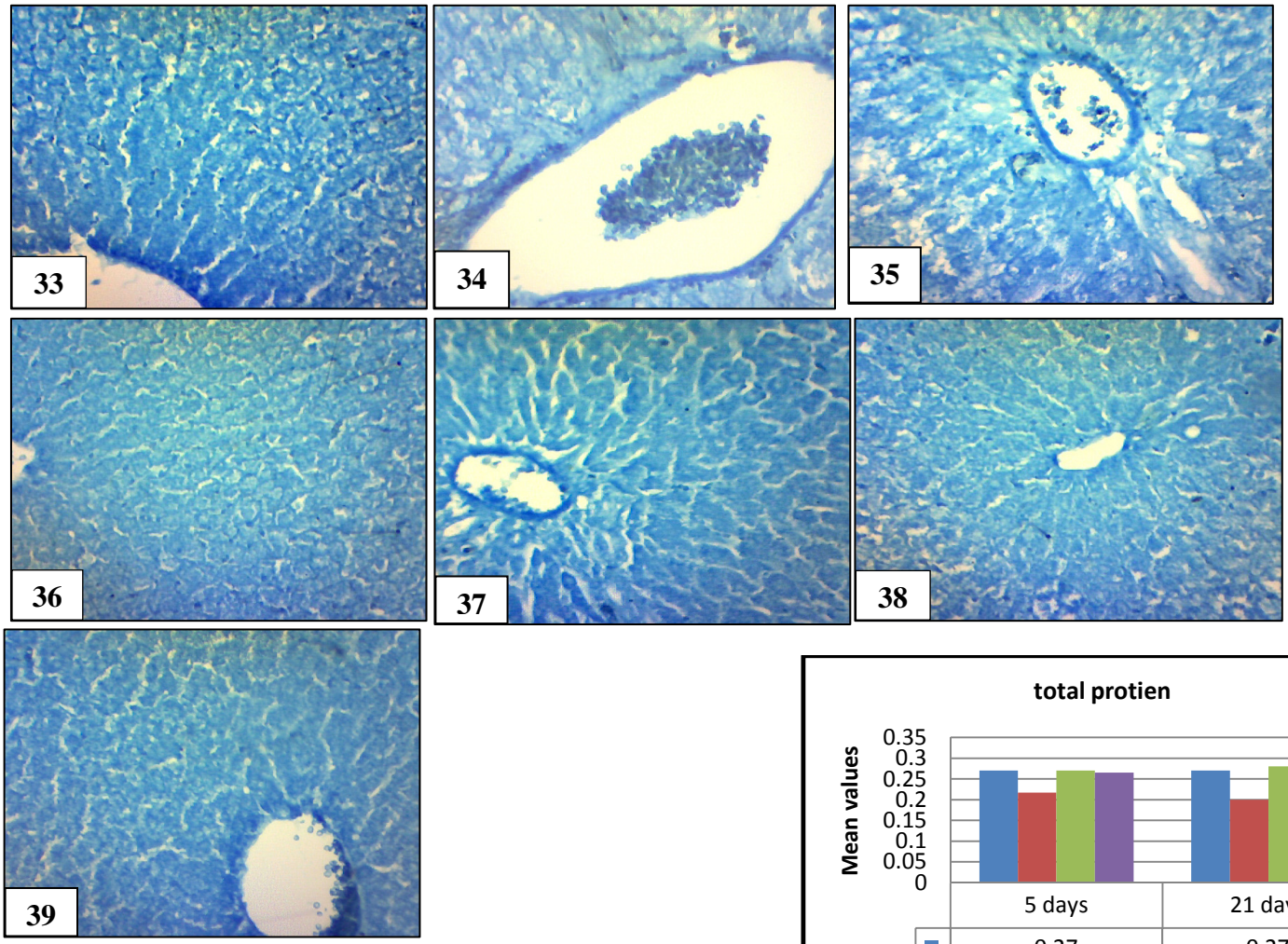


Fig. 32: effect of radiation and/ or AFA on PAS +ve materials in the liver of adult male albino rats.



Figs. 33-39: photomicrographs showing distribution of total protein in liver tissue of the control and treated groups after 5 and 21days of irradiation **(Bromophenol blue X 100).**

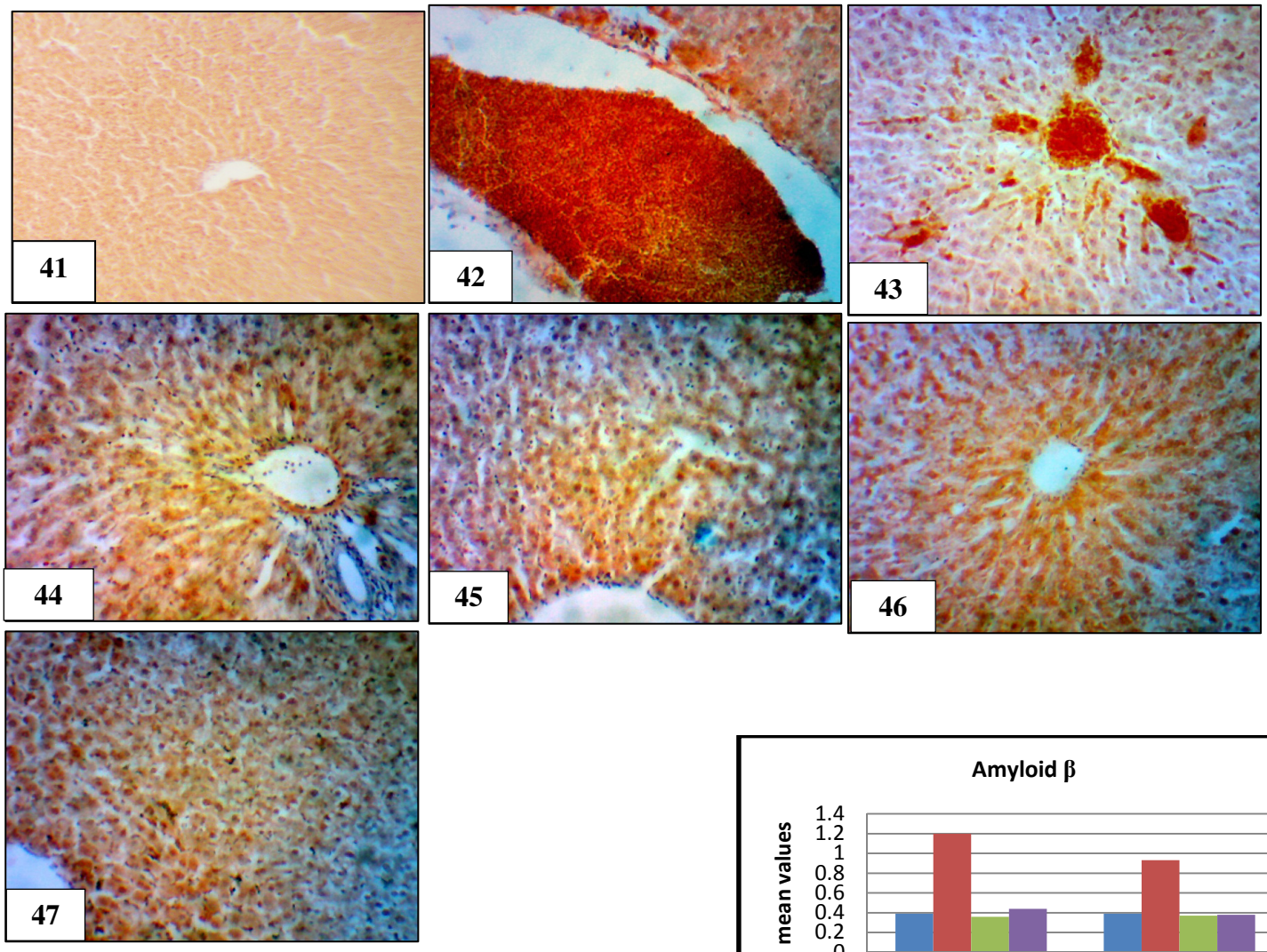
Fig 33: control rats showing moderately stained total protein in the central area of liver tissue, but walls of the blood vessels are deeply stained. .

Figs. 34 , 35: irradiated rats showing faintly stained total protein in most hepatocytes after 5 and 21 days of γ- irradiation respectively. Notice: deeply stained blood cells inside the blood vessels after 5 5days.

Figs. 36, 37: AFA treated rats showing more or less normal distribution of total protein in the hepatocytes after 5 and 21 days of treatment respectively.

Figs. 38 , 39: AFA+R **treated rats** showing almost normal total protein content in hepatocytes after 5 and 21 days of γ- irradiation respectively.

Fig. 40:effect of radiation and/ or AFA on the total protein content in the liver of adult male albino rats.



Figs. 41-47: photomicrographs showing distribution of the amyloid β-protein in the liver tissue of the control and treated groups. **(Congo red stain X 100)**

Fig. 41: control rats showing faintly stained amyloid- β protein in the liver tissue in central areas.

Figs. 42 , 43: irradiated rats showing deeply stained amyloid- β protein in some hepatocytes of the portal and central areas and in the hemolysed RBCs inside veins after 5 and 21 days respectively

Figs.44 , 45: AFA treated rats showing faintly stained amyloid -β protein after 5 and 21days respectively.

Figs. 46 , 47: AFA+R treated rats showing almost moderately stained of amyloid -β protein after 5and 21 days respectively.

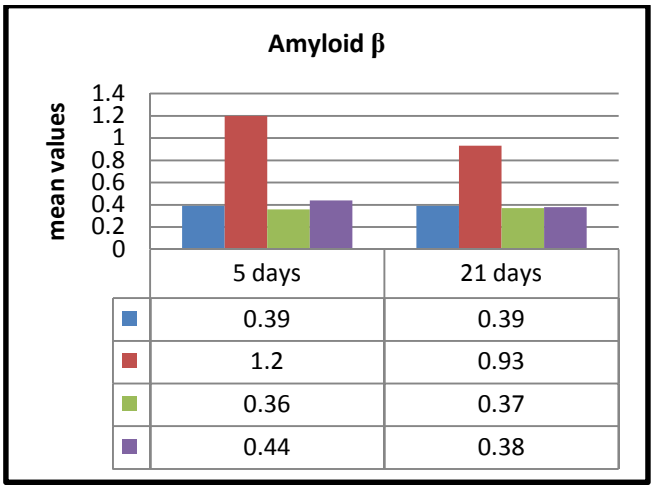
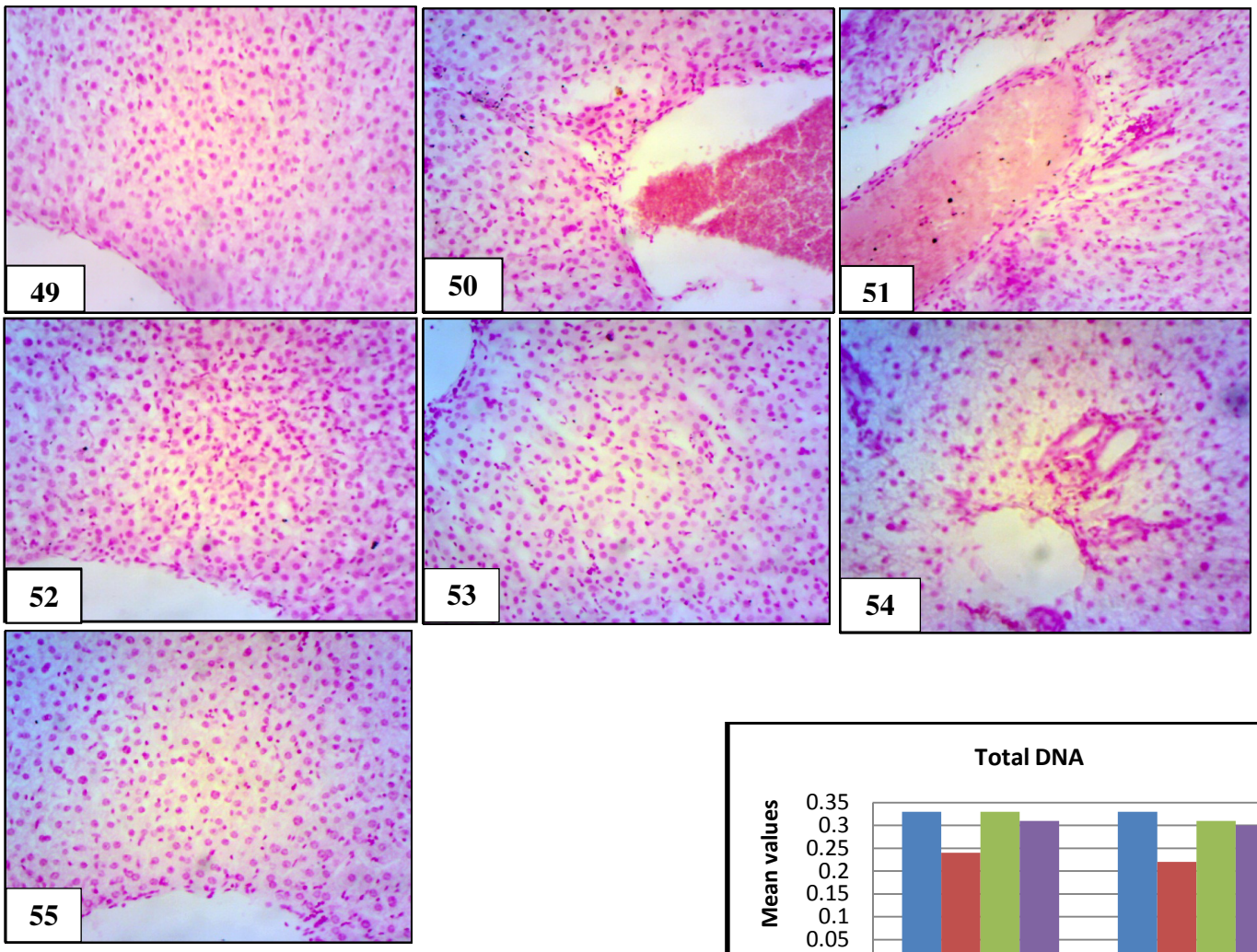


Fig.48: effect of radiation and/ or AFA on the amyloid content in the liver of adult male albino rats.



Figs. 49-55: photomicrographs showing distribution of total DNA content in the liver tissue of the control and treated groups. **(Feulgen stain X 200)**

Fig. 49: control rats showing moderately stained DNA content in nuclei of hepatocytes, Kupffer cells, nuclei of endothelial lining of the blood vessels.

Figs. 50 , 51: irradiated rats showing faintly stained total DNA content in the hepatocytes, but deeply stained DNA content is observed in walls of the blood vessel after 5 and 21 days respectively

Figs.52 , 53: AFA treated rats showing moderately stained DNA content in the liver tissues after 5 and 21days respectively.

Figs. 54, 55: AFA+R treated rats showing almost moderately stained DNA content in the liver tissues after 5 and 21days respectively.

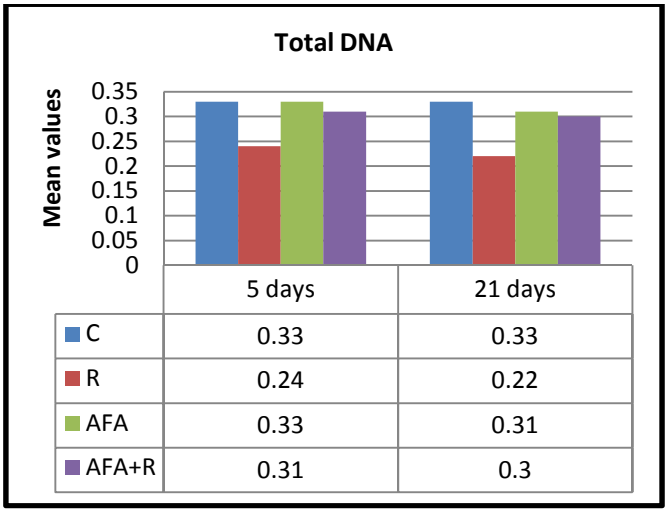


Fig. 56 : effect of radiation and/ or AFA on DNA materials in the liver of adult male albino rats.

4. Discussion

Whole body gamma-exposure of animals at the sub lethal and lethal dose levels affected the metabolism of various organs and induced a series of biochemical and physiological fluctuations in several biological tissues **(Mohammed, 2010)**.

Concerning the biochemical changes recorded in the current study as regards the radiation exposure (4Gy), liver of exposed animals revealed a significant increase in ALT, AST and ALP activities. These results come in agreement with those of **Ramadan *et al.* (2002) ; El-Gabry *et al*. (2003)**and **Muriel (2009)**. who reported that whole body gamma-irradiation induced hepatotoxicity and increased serum ALAT, ASAT as well as Gamma-glutamyl transferase (GGT) activities. This increase in liver enzyme was attributed to the damage of cellular membranes of hepatocytes, which leads to an increase in the permeability of cell membranes and facilitates the passage of these enzymes outside the cells. The recorded elevations could be also due to a hypoxia state in the liver cells **(Kafafy, 2000)** or mitochondrial membrane **(Romero *et al*., 1998).** Other investigators **(El-Masry and Saad, 2005** and **Ammar, 2009)** reported that ionizing radiations induced significant elevations in the physiological and metabolic processes, as well as,disorders in blood biochemical parameters and chain peroxidation.In this respect, **Eshak and Osman (2013)El-Desouky *et al*.(2014)** noticed elevations in ALAT, ASAT and ALP in sera of irradiated (4& 6 Gy) albino rats.This was in parallel with liver cells degeneration, lymphocytic infiltration and necrosis of the hepatic tissues.

In the present study non significant changes in the activities of ALAT, ASAT and ALP were recorded in AFA group and the irradiated groups supplemented with AFA and indicating that supplementation with AFA manifested good ameliorative effects in liver enzymes activities.These results go in parallel with previous ones that revealed a potential hepatoprotective effect of c-phycocyanin in rats with induced hepatitis **(Yan-Fei *et al*., 2007)**. Moreover, the study of **Viswanadha *et al*. (2011)** revealed the hepatoprotective role of BGA against hepatotoxicity induced by 4-nitroquinoline-1-oxide in experimental rats. Also, **Makhlouf and Makhlouf (2012)**.They showed increases in serum ASAT, ALAT, ALP and GGT activities after exposure of rats to 2 and 4Gy of gamma radiation.Treatment with BGA (*Spirulina*) prior to irradiation disclosed a significant amelioration in the activities of these enzyme in serum.The current results, also, in agreement with those described previously by **Sharoud (2015)** who found increases in ALAT, ASAT and ALP in paracetamol treated animal groups compared to the control group, indicating liver injury. Administration of blue green algae (*Spirulina*) at 500 mg/kg body weight, significantly ($p<0.05$) lowered the elevation of these enzymes.

Results of the present study showed a significant increase in MDA level in the liver tissue of rats of the irradiated group when compared to the control. Results of the present study come in agreement with the results of **Song *et al*. (2006)who** reported that mice irradiated at 4.5 Gy gamma rays had a significant increase of MDA levels. and the elevation in MDA levels in liver and kidney of the irradiated rats leading to tissue damage **(Khan *et al*., 2012)** due to presence of membrane rich in polyunsaturated highly oxidizable fatty acids **(Cini *et al*., 1994).** Elevated lipid peroxides in the irradiated rats is quite correlated with the disturbance in the concentration of Na^+ and K^+ as recorded by **Abu-Safi *et al*. (2006)**. In the present study supplementation of AFA caused improvement in the MDA level. These results are supported by the results of the histological and histochemical studies. **El-Malawany *et al*. (2014)**indicated that the oral administration of AFA (100mg/kg) for 15 days increased activities of the antioxidant enzyme like SOD, CAT and GPx together with a decrease in the level of MDA marker for lipid peroxidation in normal mice after treatment with AFA as compared to that of the normal control. The current study showed that administration of AFA to the exposed rats caused good amelioration in the MDA level in the liver tissue.In the present study improvement in these parameters in the exposed group treated with BGA come in agreement with the work which was done by **Makhlouf and Makhlouf (2012)** who found that whole body γ-irradiated rats with two doses 2Gy and 4 Gy for 45 days showed higher levels of MDA. While the treatment of rats with BGA for 10 days before acute irradiation caused a significant decrease in MDA. The protective effect of this extract may be attributed to presence of flavonoid compounds and their antioxidant effects and free radical scavenging properties **(El-Lakkany *et al*., 2011; El-Depsi,2016)**. Antioxidants prevent lipid peroxidation chain reaction in the cell membrane **(Shodehinde and Oboh, 2013)**.Also, the study of **Venkatesan *et al*. (2012)**showed that the most common BGA, *Spirulina platensis* (SP) and *Aphanizomenon flos-aquae (*AFA) were found to have antioxidant activity.

As regards the histopathological changes, in the present study, liver tissue of irradiated rats after 5 and 21 days showed corrugated and ruptured endothelial lining of the central vein which contained hemolysed blood cells, numerous vacuolated hepatocytes with increased signs of pyknosis and karyolysis in nuclei of hepatocytes, highly dilated and congested hepatic portal vein, numerous hemorrhagic areas and destructed bile ducts. These alterations support and confirm the current preceding biochemical changes of liver enzymes. **Abdel Mottaal and Abdel-Maguid(2007) and Nakajima *et al*., (2016)** recorded similar results including dilatation and congestion of blood vessels with increased proliferation of bile ducts in the portal areas post-irradiation with

lymphocytic infiltration between the degenerated hepatocytes. These findings are also supported by the study of **Gokcimen *et al.* (2002)** who reported that exposure of rats to magnetic fields (MF) caused changes in liver tissue such as sinusoidal dilatation, mixed cell infiltrations in the periportal area, necrosis, vacuolar degeneration, congested central veins and stagnant hypoxia. Ionizing radiation is known to induce oxidative stress via the release of ROS resulting in an imbalance in prooxidant and antioxidant status in the cells (**Hahn *et al.*, 1994**). Moreover, radiation induced ill defined hepatic cells, necrosis, dilatation and congestion of the central vein and sinusoids with blood petechea **(Soliman, 2007)**.Hemolysed RBCs were observed by **Attar *et al.* (2007)** who declared that lipid peroxidation led to hemolysis due to penetration of water.Results of the present study also come in agreement with those of **Waer and Shalaby (2012)** who recorded hepatocellular damage of male rats exposed to accumulated dose of 0.5Gy of γ-radiation every 2 days for one month. This damage was represented by dilatation and congestion of central and portal veins with ruptured endothelial lining, lymphocytic infiltration, internal hemorrhage, fragmentation of nuclei with vacuolated cytoplasm, focal pyknotic and necrotic areas**.** Hemorrhage and extravasated blood elements post-radiation exposure were also observed by **Ozguner *et al.* (2006)**.**Abdel–Rahman (2013)** showed distortion in the architecture of hepatic lobules, degeneration of liver cells and lymphocytic infiltration. Liver cells showed necrosis, the nuclei showed pyknosis and karyolysis. They also observed dilation of portal spaces and blood vessels in liver of rats exposed to whole body gamma-radiation at the dose level of 7 Gy.**Topali *et al.* (2015)** disclosed marked hydropic degeneration in the parenchyma, particularly in pericentral regions, vacuolization in the mitochondria, expansion in the endoplasmic reticulum and necrotic hepatocytes in rat pups that were daily-exposed to 900MHz for1h during days 13–21 of pregnancy.

Supplementation of AFA ameliorated the histological pattern of liver of rats exposed to gamma radiation and recorded a radioprotective effect. **Vedi *et al.* (2013)**reported that BGA have multiple liver-protective factors, including amino acids (e.g. methionine, arginine and isoleucine), chelating trace minerals and potent antioxidants, such as phycocyanins and superoxide dismutase (SOD). Antioxidant seems to improve the condition of blood vessels by helping to neutralize the free radical molecules or reactive oxygen species (ROS) that may prevent endothelial cells from releasing nitric oxide. Nitric oxide is responsible for the dilation of blood vessels **(Mietus-Snyder and Malloy, 1998)**. Furthermore, C-PC-rich extract from AFA inhibited peroxyl radical-induced oxidative hemolysis and lipid peroxidation in normal human erythrocytes **(Scoglio *et al.*, 2014).**C-phycocyanin (C-PC) can constitute up to 15% of the dry weight of a blue-green algae harvest and contribute to the antioxidant, neuroprotective, anti-inflammatory and hepatoprotective effects **(Eriksen, 2008)**.C-PC was evaluated as an antioxidant as it was able to scavenge alkoxyl, hydroxyl and peroxyl radicals and inhibited microsomal lipids peroxidation *in vitro* **(Romay *et al.*, 2003; Li *et al.*, 2016)**. Besides, its free radical scavenging effect, C-PC also acts as a selective inhibitor of cytochrome oxidase-2, has hepatoprotective and anti-inflammatory effects**(Reddy *et al.*, 2000)**.Extracts of phycocyanin (the blue pigment) from blue-green algae helped to restore the efficiency of antioxidant defenses, dehydrogenase activity and energy-rich phosphate levels in rats exposed to X-rays(dose of 5 Gy) **(Karpov *et al.*, 2000)**.**Vedi *et al.* (2013)** recorded the same hepatoprotective effect of BGA (*Spirulina fusiformis*) against galactosamine induced hepatotoxicity in mice. The protective efficacy is promising and may be attributed to the presence of various constituents which are present in *Spirulina fusiformis*. **Mohamed *et al.* (2014)** indicated that orally administration of BGA (100 mg/kg BW), alone or combined with praziquantel PZQ (250 mg/kg BW) inhibited the histopathological alterations in the liver of *S. mansoni*-infected mice. Also, **Kuriakose and Kurup (2010)** showed that BGA ameliorated the histopathological pattern of liver in a paracetamol toxicity model. In addition **Xin *et al.* (2007)** reported that C-phycocyanin, has a hepatoprotective effect in experimental hepatitis.The present study showed increased collagen deposition in hepatic tissue of the exposed groups.**Horn *et al.* (1985)** declared that the presence of collagen in the peri sinusoidal spaces might affect the blood supply to liver cells and would reduce the exchange of metabolites, perhaps resulting in hepatocellular dysfunction and necrosis. **Enzan *et al.* (1995)** attributed a similar finding to the activation of myofibroblast- like cells present normally within the hepatic and renal parenchyma. **George *et al.* (2001)** suggested that decreased synthesis of collagenolytic enzymes by the damaged hepatocytes might contribute to further accumulation of collagen. Hepatic stellate cells (HSC) and liver fibroblasts have modulatory roles in inflammatory conditions, based on their capability of cytokine and chemokine production. The hepatic stellate cells store vitamin A, but produce extra cellular matrix and collagen when activated. They are located in the space of Disse between hepatocytes and endothelial cells **(Saile and Ramadori, 2007)**.Hepatic stellate cells play a vital role in fibrogenesis by synthesizing increased amounts of collagen when activated by profibrogenic factors such as oxidant stress **(Ramadori *et al.*, 2008)**.Highly increased collagen fibres was observed in the liver and lung tissues of the pregnant rats and their fetuses exposed to 2Gy gamma rays on day 7 or day 14 of gestation **(Abuo El Naga and Abd Rabou, 2012)**. Increased collagen post-radiation exposure in the different tissues was detected by **El –Salkh(2009)**.In the present study somewhat normal distribution of collagen fibres was demonstrated in the central and portal areas of

liver tissue of AFA &AFA+R groups. **Yang *et al.* (2013)** reported that the anti-inflammatory function of BGA is mediated to decrease the production of pro-inflammatory mediators. BGA can also decrease oxidative stress due to their free radical scavenging activity and inhibition of lipid peroxidation. The work done by **El-Depsi(2016)**showed that administration of AFA to the diabetic rats showed somewhat normal appearance of collagen fibres in the spleen tissue.

Concerning the histochemical changes of Polysaccharides the present study revealed highly significant decreased polysaccharides in the hepatocytes of the irradiated group. The reduction of PAS+ve materials was noticed by **Saeid *et al.* (2010)** who observed reduction of PAS +ve materials around the central vein of liver of the white rabbits and they reported that EMFs can decrease liver glycogen stores. It can be proposed that decreased glycogen stores noticed in liver sections of the exposed group, more energy was needed to detoxify and overcome EMF induced stress. Thus, the next alternative source of energy to meet the increased energy demand is proteins **(Lehninger *et al*., 2008)**.

The reduction of PAS +ve materials was also noticed by **Eid *et al.* (2015)** who observed a significant decrease of PAS +ve materials in the central and portal areas in liver of adult male albino rats exposed to RF-EMF from mobile phone radiation 900MHz. Reduced glycogen in cells after irradiation may be due to decreased T3 and T4 hormones of the thyroid glands, which decrease entrance of glucose to the cells **(Abuo El Naga and AbdRabou, 2012)**.

Administration of AFA in the present study showed normal distribution of PAS +ve materials in the liver tissue.

Several food grade microalgae, including, *Aphanizomenon flos- aquae, Spirulina platensis* and *Chlorella pyrenoidosa* are also known to contain polysaccharides with potent immune stimulators of human monocytes and macrophages **(Pugh and Pasco, 2001)**.The cell protein wall of AFA is a source of glycogen, used by the liver for energy, which is one reason why people often report an increase in energy once they start eating it. AFA gives the body many nutrients difficult to obtain from other sources. Many people who eat it report that it has helped offset obesity, autism, depression, hypoglycemia, diabetes, ulcers, anemia and many other symptoms of nutritional deficiency **(Cook, 2003)**.
In the present study administration of AFA to the exposed group showed somewhat normal appearance of PAS +ve materials in the liver tissue.

Improvement in polysaccharide content observed in this study in group AFA+R may be due to the antioxidant activity of *Aphanizomenon flos-aquae*. **Lahitova *et al.* (2014)** reported that several blue green algae, including *Aphanizomenon flos-aquae*(AFA) showed protective effects including antioxidant and antibacterial properties, glucose and cholesterol regulatory effects as well as host immune system modulation.

Concerning the histochemical changes of total protein the present results revealed reduced in most hepatocytes of the liver tissue of the irradiated group, but they increased in the thickened walls of the blood vessels and bile ducts with mild staining affinity in the hemolysed blood cells. In this respect, **Kilberg and Nachaus (1978)** found that whole body irradiation of rats led to degeneration of tissue protein. It has been found that ionizing radiation usually inhibits the protein synthesis and the decline may be attributed to degeneration of cellular tissues **(Ali *et al*., 2007; Eid *et al*., 2015)**.This decrease in total protein may be due to highly affected RER, mitochondria and Golgi apparatus with increased lysosomes noted by **Eid and Al-Dossary (2007)** in fetal hepatocytes exposed maternally to EMF radiation. Also, decreased protein content was noted in hepatocytes post-irradiation by **AlGahtani (2006)**.Decreased protein content post-irradiation was realized by **Chen *et al.* (2006)**.They reported that hypo-staining affinity may be due to damaged DNA. Gamma rays cause lesions on template DNA strand which result in impaired gene transcription, therefore the synthesis of functional m-RNA is impaired and this may change the pattern of protein synthesis either by stimulation or by inhibition **(Ali *et al*., 2007)**.Proteins are mainly involved in the architecture of the cell **(Radwan *et al*., 2008).Abdel-Meguid *et al*. (2012)** stated that the decrease in protein could be attributed to the disruption of lysosomal membranes under the effects of different toxicants; thus leading to the liberation of their hydrolytic enzymes in the cytoplasm. Additionally, the presence of hydrolytic enzymes can cause the lysis and dissolution of the target material within the cytoplasm.

Irradiation of animals at 900-1800 MHz resulted in a marked reduction in the total protein content giving weak to moderate reaction in some hippocampal areas **(Mohammed, 2014).**Administration of AFA in the present work showed normal total protein content in the central and portal areas of liver tissue and also showed somewhat normal distribution of total protein in hepatocytes of liver tissue of group AFA+R.

Antioxidants protect biologically important molecules such as lipids, DNA and proteins from oxidative damage and consequently reduce the risk of several chronic diseases **(Myung *et al*., 2013)**.**Devi (1983)** demonstrated the ability of algal diets to stimulate the regeneration of blood serum and liver proteins in rats. Because microalgal protein is composed of shorter and less complex polypeptide chains with an abundance of all essential amino acids it can be more readily utilized at the cellular level. Protein is used to construct, maintain and repair every tissue in our bodies from our teeth, bones, muscles, nerves, glands, heart, blood, skin, liver, hair and everything in between. A lack of protein is mostly

associated with muscular weakness, slow healing and brain chemistry imbalances. Some of the free amino acid peptides found in AFA may be responsible for helping to detoxify our bodies of heavy metals. AFA has been effective in chelating (removing) dangerous, toxic heavy metals such as cadmium, lead and mercury **(GEPSO, 2013)**.

Concerning the histochemical changes of Amyloid- β protein the current study recorded a significant increase in the amyloid–β protein content in hepatocytes of the irradiated animals. Beta-amyloid is a small piece of a larger protein called "amyloid precursor protein" (APP). Although scientists have not yet determined APP's normal function, they have mentioned a great deal about how it appears to work. In its complete form, APP extends from the inside to the outside of brain cells by passing through a fatty membrane around the cell. When APP is activated to do its normal job, it is cut by other proteins into smaller sections that stay inside and outside cells. APP can be cut by several ways. Under some circumstances, one of the pieces produced is beta-amyloid **(Alzheimer's Association, 2007)**.**Moges (2011)** reported that amyloidosis refers to deposition of a particular amyloid protein in different organs and tissues of animals and human. In this form of amyloidosis, the deposited amyloid β protein is derived from serum amyloid-A synthesized in the liver **(Kim *et al.*, 2005)**. **Eid *et al.* (2013)** recorded that slightly increased amyloid β deposits in hepatocytes of the central and portal areas and in the blood cells inside the blood vessels of the liver tissue of the exposed rats to RF-EMF from mobile phone (45min/day) for one month group. The present findings showed normal appearance of amyloid β-protein in the liver tissue of groups AFA & AFA+R all over the experimental period.AFA Klamath is rich in the essential fatty acids such as omega 3 and omega 6 **(Ku *et al.*, 2013)**.Omega 3 fatty acids (FAs) have powerful inhibitory effect against H_2O_2-induced damage in human keratinocytes and fibroblasts **(Phan *et al.*,2001)**. Also, it decrease the oxidized protein in amyloid pathology in Alzheimer transgenic mice **(Lim *et al.*, 2001)**. Omega 3 FAs inhibits oxidative stress and rat intestine damage induced by indomethacin **(Song *et al.*, 2016)**. Results of the present study come in agreement with the work carried by **Nassar *et al.* (2008)** who reported the radioprotective role of the chlorophyll-rich foods, they found that chlorophyll-rich foods can be effective in decreasing the effects of radiation and it doubled the life span of animals exposed to fatal doses of radiation.

Results of the present study showed highly decreased nuclear DNA content in hepatocytes of liver of the irradiated group. These findings were previously reported by **Fouda *et al.* (2009)**who found that at lower doses of irradiation there was no obvious injury, but a number of the cells that survive will have incorrectly repaired DNA damage so that they carry mutations, while at doses high enough, cells may be killed by damage of DNA and other parts of the cell to cause great injury to the body and even rapid death. The harmful effects resulting from ionizing radiation are related in most cases to increased production of free radicals that cause damage to the cellular macromolecules, especially DNA. Thus, a comprehensive approach has been made in the scientific community to identify antioxidant agents for their potential protective effect at the cellular level **(Hosseinimehr, 2010)**.

Administration of AFA alone in the present work showed normal appearance of DNA materials in the liver tissue and the treatment of the exposed group with AFA showed somewhat normal appearance of DNA materials in the liver tissue. The protective role of BGA may be attributed to the presence of β- carotene**(Luxia *et al.*, 1996)** enzyme superoxide dismutase or selenium **(Abd El-Baky *et al.*, 2007)** and blue pigment phycocyanin **(Li *et al.*, 2016).**This interpretation seems to be in accordance with that of studies in mouse bone marrow cells, *Spirulina* extract reduced the number of micronuclei from oxidative damage. Since 70% of cellular damage produced by ionizing radiation is due to ºOH formed from water radiolysis**(Ward, 1988)**, the protective action of *Spirulina* might be attributed to its ability to scavenge this damaging radical**(Farag *et al.*, 2016)**.The present investigation is supported by the work done by **Makhlouf and Makhlouf (2012)** who found that the whole body γ-irradiated rats with two doses 2 and 4 Gy for 45 days showed a marked decrease in hepatic contents of DNA. While, Pre-irradiation treatment of rats with BGA (*Spirulina*) showed a significantly higher hepatic DNA content compared to that of irradiated rats.

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