The Possible Radio Protective Role of *Aphanizomenon Flos-Aquae* (AFA) on Heart of The Adult Male Albino Rats

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ABSTRACT

Aim of the work: this study demonstrated the drastic effects of ionizing radiation on the heart tissue of the adult male albino rats and the possible radio protective role of AFA extract.

Material and Methods: a total of forty-eight mature male albino rats (*Rattus rattus*) weighing 180-200g were fed on standard rodent pellets. Rats were equally categorized into four groups; each group contained 12 rats. These groups were: 1-untreated normal control rats (C); 2- rats exposed to 4Gy of gamma-radiation (Irradiated group **R**) and then they were sacrificed on days 5 and 21 (**R_1& R_2** groups respectively) post-irradiation; 3- rats were treated orally with 94.5mg/kg body weight/day of AFA extract for 3 weeks (AFA group) and then they were sacrificed on 5 and 21 days (**A_1& A_2** groups respectively) post- treatment; 4- rats were treated orally with 94.5mg/kg body weight/day of AFA extract for a period of one week before irradiation and three weeks after irradiation (AFA-irradiated group), then the rats were sacrificed on 5 and 21 days (**AR_1& AR_2** groups respectively) post-irradiation and treatment, then the hearts were immediately excised.

Results: many drastic changes were observed in the cardiac muscle fibres of rats of **R_1** group. These changes included: numerous hemorrhagic areas which contained hemolyzed blood cells and some nuclei of myocardiocytes were pyknotic and others were hypertrophied with destruction in some fibres and widened endomysium. Aggravated changes were also observed in the heart of rats of **R_2** group such as highly elongated and congested cardiac blood vessels which contained hemolyzed blood cells and hemosiderin granules, numerous hemorrhagic areas, necrotic areas and widened endomysium. On the other hand, highly increased collagen fibres and amyloid-β deposits were also noticed in the cardiac tissues of rats of **R_1 and R_2** groups with decreased PAS positive materials, total protein and DNA materials. Normal architecture of the cardiac muscle fibres was detected in the rats of **A_1, A_2** groups with slightly increased collagen fibres and slightly decreased PAS positive materials, total protein, amyloid-β protein and DNA materials. Also, **AR_1, AR_2** groups showed somewhat normal appearance and well developed cardiac muscle fibres, but some dilated endmysium spaces were still detected in the cardiac muscle fibres of rats of group **AR_2**. Furthermore, increased collagen fibres and amyloid-β deposits were realized in the cardiac muscle fibres of rats of AR_1, **AR_2** groups with decreased PAS positive materials, total protein and DNA materials.

Conclusions: AFA showed cardio- protective effect and powerful antioxidant action. So, it can be used under medical supervision as a natural supplement.

Keywords: gamma-radiation, AFA, heart tissue.

INTRODUCTION

The effects of ionizing radiation on biological systems were mainly generated from experimental studies on animals and the radiation accidents. These effects depend on many factors such as radiation type, radiation dose and radio sensitivity of the tissue receiving the radiation and also the volume of tissue exposed [(1)]. Gamma rays are ionizing radiation and thus are biologically hazardous [(2)].

Gamma rays are generally characterized as electromagnetic radiation having the highest frequency and energy and also the shortest wavelength (below about 10 picometers). Due to their high energy content, gamma rays can cause serious damage when absorbed by the living cells [(3)]. Gamma-rays are not stopped by skin; they can induce alteration by interfering with the genetic materials of the cell. DNA double-strand breaks are generally accepted to be the most biologically significant lesions by which ionizing radiation causes cancer and hereditary disease [(4)].

Exposure to high radiation doses to the heart, coronary, carotid and other large arteries received radiation induced tissue damage, resulting in increased risk of circulatory diseases; the underlying biological mechanism is the high level of cell killing, leading to pro-inflammatory effects and micro-vascular damage [(5)]. Exposure to ionizing radiation was associated with histopathological changes. These changes differ in their severity according to the radio sensitivity and response of the individual organs and tissue [(6)]. Clear histopathological changes were observed

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after whole body gamma irradiation in the cardiac muscles of rats. Abnormal structures of cardiac muscles were found as ill-defined shape, necrotic, pyknotic nuclei, severe dilated, widened and inflammated capillaries in the endomysium were also detected [7]. **Pradeep *et al*.** stated that whole-body γ-radiation exposure (5 Gy) of adult rats resulted in cellular damage in the heart tissue of rats of the exposed group when compared to the control group [8]. **Abd El-Azeem** suggested that the low dose of ionizing radiation (6Gy) showed distinct different histological changes in rat's heart. These changes varied from hypertrophy of the cardiac muscle fibres and the disruption of the striation appeared [6].

Blue-green algae (BGA) are known as cyanobacteria, among the phylum of bacteria that utilize photosynthesis to obtain energy [9]. **Lee *et al*.** reported various health benefits of BGA, including immune functions, anti-inflammatory, anti-bacterial, anti-viral, anti-cancer, hypocholesterolemic, hypotriglyceridemic and antioxidant properties [10]. The most common BGA, *Spirulina platensis* (SP) and *Aphanizomenon flos-aquae* (AFA) were found to have antioxidant [11], anti-inflammatory and hypolipidemic properties [12]. BGA are marketed as a health promoting supplement. They are often sold in the form of capsules and drops which are taken daily. AFA, fresh water unicellular blue-green algae spontaneously grow in Upper Klamath Lake; they are consumed as a nutrient-dense food source for their health-enhancing properties [13]. AFA is rich in protein (63-69% dry weight), carotene, vitamin B_{12} and other biologically-active compounds. AFA contains a high concentration of α-linolenic acid, which at a concentration of 10-15% in the rat diet represented an excellent source of n-3 polyunsaturated fatty acids [14].

MATERIALS AND METHODS

Experimental animals, feeding and maintenance

A total of forty eight male albino rats (*Rattus rattus*) weighing 180-200g, purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo-Helwan, Egypt), were used as the experimental animals for the different investigations carried out in this work. The animals were kept in the laboratory for 2 weeks before the experimental work and they were housed in especially designed cages, 6 rats per cage, with controlled air, temperature and relative humidity. The animals were fed standard rodent pellets. Food and water were made available *ad-libitum* throughout the whole experimental period. Animals were acclimatized to the laboratory conditions before starting the experiment. All animal procedures were consistent with the guidelines of Ethics by Public Health Guide for the Care and Use of Laboratory Animals.

Gamma-irradiation procedure

Irradiation process was performed using gamma cell-40 achieved by Egypt's National Center for Radiation Research and Technology (NCRRT), Cairo. The gamma cell–40 is a caesium-137 irradiation unit manufactured 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. A caesium-137 double encapsulated source is housed in each of two cylindrical sliding drawers, one above and one below the sample cavity. The sample drawers can move from the shielded position to the irradiation position by pneumatic cylinders. A plastic sample tray with lid support for use in the sample cavity is provided with the unit. The internal dimensions of the tray are 30.5 cm in diameter by 10.5 cm deep. The sample tray has ventilation holes in its side which align with ventilation pars through the main shield.The dose rate was 0.62 Gy/min at the time of the experiment.

Aphanizomenonflos- aquae (AFA) administration

AFA-Klamath capsules (350 mg) were purchased from the German Egyptian Pharmaceutical Company. AFA 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 3 weeks. The dose for the rat was calculated according to the **Paget's** formula on the basis of the human dose**.**

Experimental design

48 of the experimental adult male Albino rats were categorized into 4 groups.

Group 1 (control group): untreated control rats (**C**).

Group 2 (irradiated group): rats exposed to 4Gy of gamma-radiation and then were sacrificed at the 5 and 21 days ($\mathbf{R_1}$& $\mathbf{R_2}$ groups respectively) post-irradiation.

Group 3 (AFA group): rats were treated orally with 94.5mg/kg body weight/day of **AFA** extract for 3 weeks and then they were sacrificed at 5 and 21 days ($\mathbf{A_1}$& $\mathbf{A_2}$ groups respectively) post-treatment.

Group 4 (AFA-irradiated group): rats were treated orally with 94.5mg/kg body weight/day of AFA extract for a period of one week before and three weeks after irradiation. Then the rats were sacrificed at 5 and 21 days (AR_1& AR_2 groups respectively) post-irradiation and treatment.

Histological and histochemical Techniques

The animals of the control and treated groups were sacrificed after five and twenty one days post-irradiation, then the hearts were immediately excised and fixed in 10% neutral formalin for 24 hours followed by dehydration in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Sections were then cut at 5µ thickness and stained by hematoxylin and eosin stain according to the method of **Bancroft and Gamble**. Collagen fibres were stained by using Mallory's trichrome stain according to the method of **Pears**. Polysaccharides were detected by using periodic acid Schiff's (PAS) reagent **(Drury and Wallington** method). Total proteins were detected by using mercuric bromophenol blue method **(Mazia *et al*.** method). DNA was detected by using Feulgen reaction **(Drury and Wallington** method). Amyloid-β protein was detected by Congo red technique according to the method of **Valle**.

Statistical analysis

Statistical analyses were performed and the data were analyzed by using the SPSS software (Statistical Analysis for Social Science, Version 8). Significant differences between treatment means were determined by student t-test. Data were presented as mean ± SD and $P \leq 0.05$ was considered statistically significant.

Laboratory facilities

Facilities of this study, histological, histochemical and quantitative image analyses were performed in the Department of Zoology, Faculty of Science, Al-Azhar University (Girls branch).

RESULTS

The histopathological and histochemical results of the heart

The control group (C)

Normal histological pattern of the heart of a control male rat was observed in **fig.1.** Well developed cardiac muscle fibres and endomysiumin between them were noticed with thin collagen fibres which supported the cardiac muscle fibres and the endomysium (**Fig.2**).

The histopathological results of the heart

Fig. 1 showed many drastic changes in the cardiac muscle fibresof rats of R_1 group. These changes included: numerous hemorrhagic areas which contained hemolyzed blood cells and some nuclei of myocardiocytes were pyknotic and others were hypertrophied with destruction in some fibres and widened endomysium. Aggravated changes were also observed in the heart of rats of R_2 group such as highly elongated and congested cardiac blood vessels which contained hemolyzed blood cells and hemosiderin granules, numerous hemorrhagic areas, necrotic areas and widened endomysium. On the other hand, highly increased collagen fibres in the cardiac muscle fibres with numerous brightly red stained hemorrhagic and fibrotic areas were also noticed in cardiac tissue of rats of R_1 and R_2 groups (**Fig. 2**).

Fig.1 showed normal architecture of the cardiac muscle fibres of rats of A_1, A_2 groups with slightly increased collagen fibres in the cardiac muscle fibres (**Fig. 2**). Also, AR_1, AR_2 groups showed somewhat normal appearance and well developed cardiac muscle fibres, but some dilated endmysium spaces were still detected in the cardiac muscle fibres of rats of group AR_2 (**Fig.1**). Furthermore, increased collagen fibres were realized in the cardiac muscle fibres of rats of AR_1, AR_2 groups with few brightly red stained hemorrhagic areas in the cardiac muscle fibres of rats of AR_2 group (**Fig.2**).

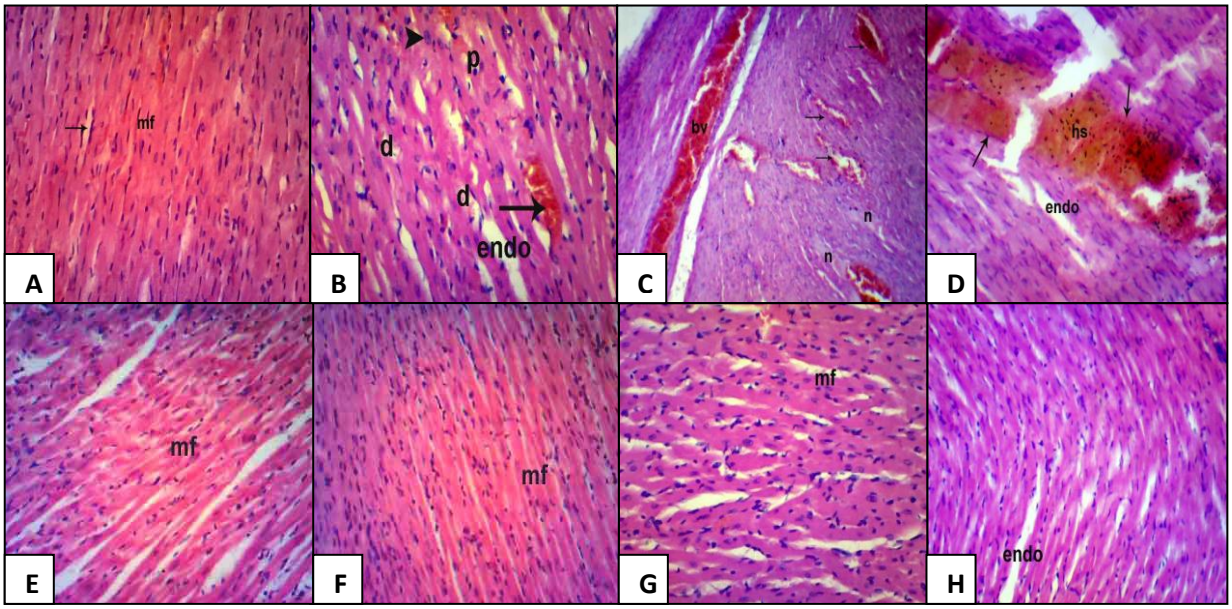

Fig. 1. (A-H) Photomicrographs of sections stained with haematoxylin and eosin showing cardiac tissues of rats: **(A)** well developed control group with cardiac muscle fibres (mf) and endomysium (↗) in between them; **(B)** R_1 group has numerous hemorrhagic areas (↗), some nuclei of myocardiocytes are pyknotic (p) and others are hypertrophied (►) with destruction in some fibres (d) and widened endomysium (endo); **(C, D)** R_2 group has highly elongated and congested cardiac blood vessel (bv) which contains hemolyzed blood cells and hemosiderin granules (hs), numerous hemorrhagic areas (↗), necrotic areas (n) and widened endomysium (endo)**; (E, F)** normal architecture of cardiac muscle fibres (mf) of $\mathbf{A_1}$, $\mathbf{A_2}$ groups respectively**; (G)** normal appearance of cardiac muscle fibres (mf) of a rat of $\mathbf{AR_1}$ group**; (H)** well developed cardiac tissue of a rat of $\mathbf{AR_2}$ group, but some dilated endmysium spaces (endo) are still detected. **(A,B,E,F,G,H X200& C, D X100)**

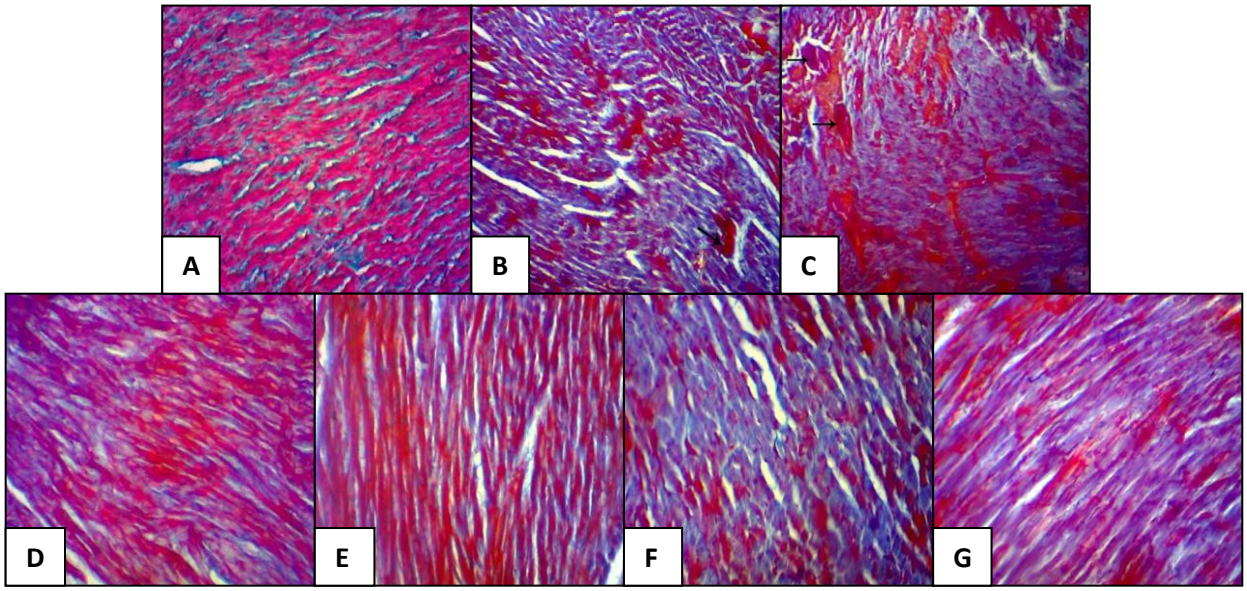

Fig. 2. (A-G) Photomicrographs of sections stained with **Mallory's trichrome stain** showing the distribution of collagen in cardiac tissues of rats: **(A)** thin collagen fibres support the cardiac muscle fibres of a rat of the control group and the endomysium in between them; **(B)** highly increased collagen fibres in the cardiac muscle fibres of rats of $\mathbf{R_1}$ group with brightly red stained hemorrhagic (↗) and fibrotic areas**; (C)** highly increased collagen fibres in the cardiac muscle fibres of a rat of $\mathbf{R_2}$ group with numerous brightly red stained hemorrhagic areas (↗); **(D, E)** slightly increasedcollagen fibres in the cardiac muscle fibres of rats of $\mathbf{A_1}$, $\mathbf{A_2}$ groups respectively; **(F, G)** increased collagen fibres in the cardiac muscle fibres of s of $\mathbf{AR_1}$, $\mathbf{AR_2}$ groups respectively with few brightly red stained hemorrhagic areas in $\mathbf{AR_2}$ group. **(A,D,E,F,G X200& B,C X100)**

The histochemical results of the heart

PAS positive materials

Figs. 3, 4 showed PAS +ve materials in sections of the heart tissue of the control and all experimental groups. The rat of the control group showed moderately stained PAS +ve materials in the cardiac muscle fibres. Exposure of rats to 4 Gy of gamma radiation showed a non significant decreased mean value of the optical density of PAS +ve materials (66.08) in the heart tissue of rats of $\mathbf{R_1}$ group compared to the control group (69.41). Moreover, poorly or negatively stained degenerated areas were also noticed in $\mathbf{R_1}$ group. The cardiac muscle fibres of rats of $\mathbf{R_2}$ group showed a significant decrease in the mean value of the optical density of PAS +ve materials (56.36) compared to the control group. The highly widened endomysium contained moderately to deeply stained PAS +ve materials. Darkly stained hemosiderin granules were also observed. Treatment with AFA showed non significant decrease in the mean values of the optical density of PAS +ve materials (65.55& 66.41in groups $\mathbf{A_1}$& $\mathbf{A_2}$ respectively) in the heart tissue compared to the control group**.** Also, $\mathbf{AR_1}$ group showed non significant decrease in the mean value of the optical density of PAS +ve materials (64.25) compared to the control group.While, $\mathbf{AR_2}$ group showed a significant decrease in the mean value of the optical density of PAS +ve materials which reached 54.01 in the cardiac tissue compared to the control group.

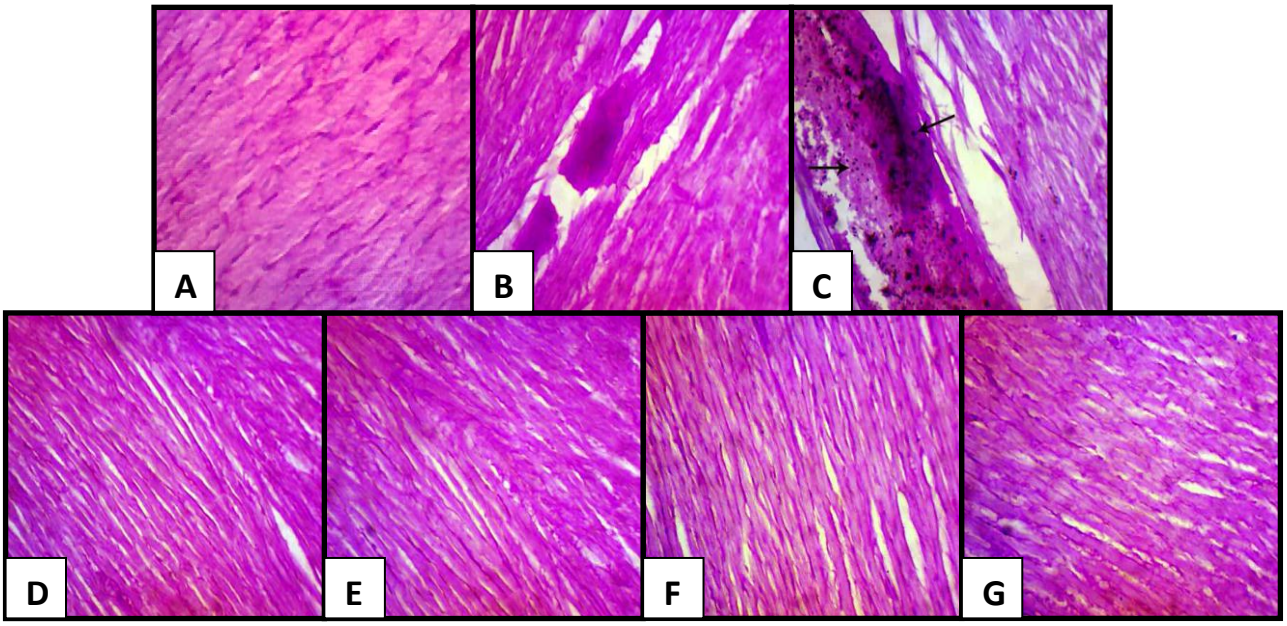

Fig. 3. (A-G) Photomicrographs of sections in the cardiac tissues of the rats showing the distribution of PAS +ve materials: **(A)** moderately stained PAS +ve materials in the cardiac muscle fibres of a rat of the control group; **(B**) decreased PAS +ve materials in the highly widened endomysium of heart tissue of a rat of $\mathbf{R_1}$ group and degenerated areas are poorly or negatively stained**; (C)** reduced staining affinity of PAS +ve materials in the cardiac muscle fibres of a rat of $\mathbf{R_2}$ group, while the highly widened endomysium contained moderately to deeply stained PAS +ve materials with darkly stained hemosiderin granules (↗); **(D, E)** Moderately stained PAS +ve materials in the cardiac tissues of rats of AFA groups $\mathbf{A_1}$, $\mathbf{A_2}$ respectively; **(F)** somewhat normal distribution of PAS +ve materials in the cardiac tissue of a rat of $\mathbf{AR_1}$ group; **(G)** slightly reduced staining affinity of PAS +ve materials in the cardiac tissue of a ratof $\mathbf{AR_2}$ group. **(PAS reaction, A-G X200)**

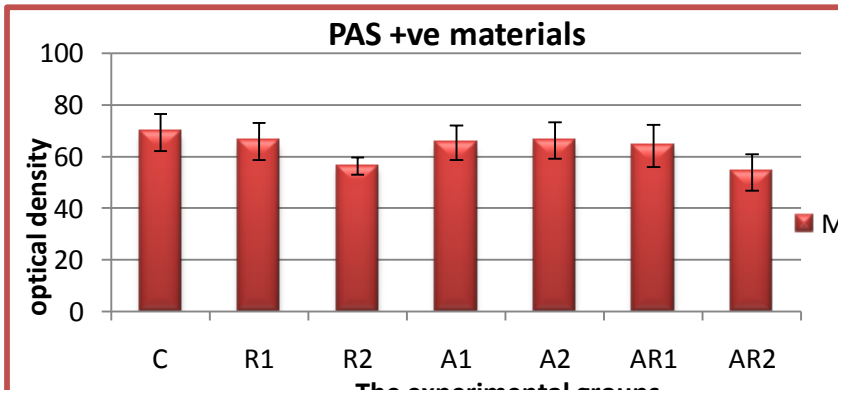

Fig. 4. Histogram showing the optical density values of PAS + materials in the cardiac tissues of the control and different experimental groups of male Albino rats (Each value represents the mean and ± standard deviation with significant difference from the control at $P \leq 0.05$).

Total protein

Figs. 5, 6 showed total protein in sections of the heart tissues of rats of the control and all the experimental groups. Moderately stained total protein was realized in the cardiac muscle fibres of a rat of the control group (**C**). However, cardiac muscle fibres of rats of $\mathbf{R_1}$ group showed a non significant decrease in the mean value of the optical density of total protein which recorded 70. 43 compared to the control group (75.04) with increased staining affinity in walls of the blood vessels and blood cells inside them and in the hemorrhagic areas. Also, rats of $\mathbf{R_2}$ group showed non significant decrease in the mean value of the optical density of total protein which reached 66.98 in the cardiac muscle fibres compared to the control group, but the blood cells in the hemorrhagic areas and in the endomysium showed increased staining affinity**.** Treatment with AFA showed non significant increase in the mean value of the optical density of total protein (78.08) in the cardiac muscle fibres of rats of $\mathbf{A_1}$ group**,** while in $\mathbf{A_2}$ group, the mean value of the optical density recorded 74.42 compared to the control group.

Exposure of the experimental animals with γ-radiation followed by AFA represented a non significant decrease in the mean values of the optical density of total protein in the cardiac muscle fibres which reached 71.19& 72.38 in $\mathbf{AR_1}$, $\mathbf{AR_2}$ respectively compared to the control group.

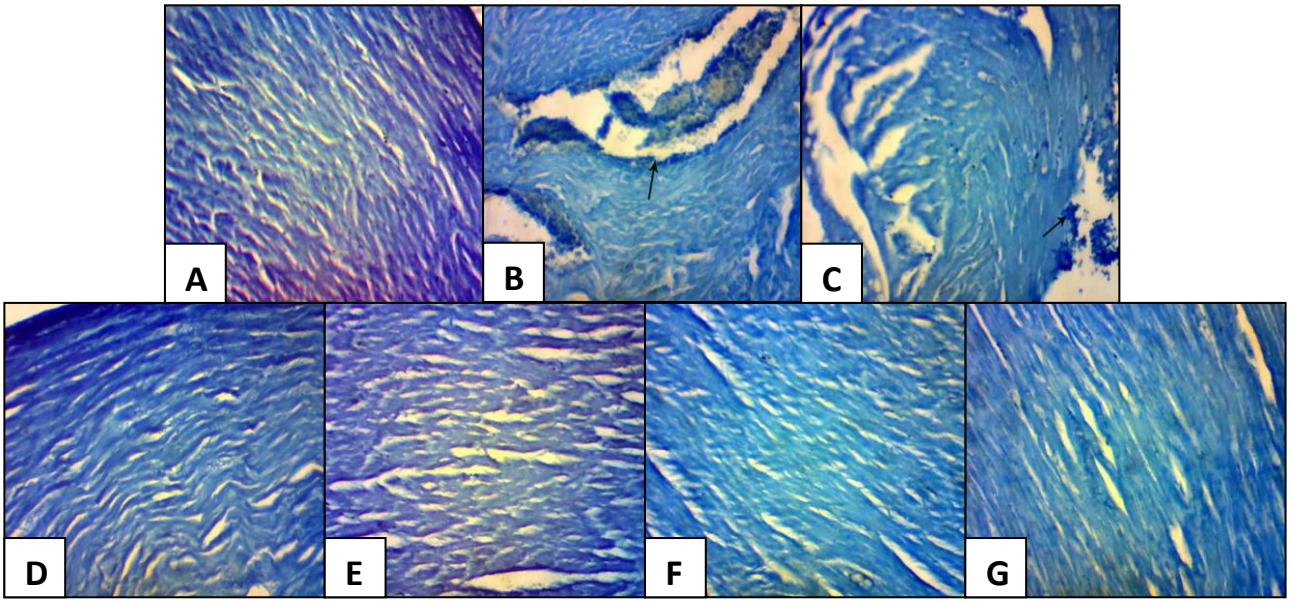

Fig. 5. (A-G) Photomicrographs of sections in the cardiac tissues of the rats showing the distribution of total protein: **(A)** moderately stained total protein in the cardiac muscle fibres of a rat of the control group; **(B, C**) decreased staining affinity of total protein in the cardiac muscle fibres of a rat of R_1, R_2 groups respectively with increased staining affinity in walls of the blood vessels and in the blood cells inside them and also in the hemorrhagic areas (↗); **(D)** increased staining affinity of total protein in the cardiac muscle fibres of a rat of $\mathbf{A_1}$ group; **(E)** normal distribution of total protein in the cardiac muscle fibres of a rat of $\mathbf{A_2}$ group; **(F, G)** somewhat normal distribution of total protein in the cardiac muscle fibres of rats of $\mathbf{AR_1}$, $\mathbf{AR_2}$ groups respectively. **(Mercuric bromophenol blue, A-G X200)**

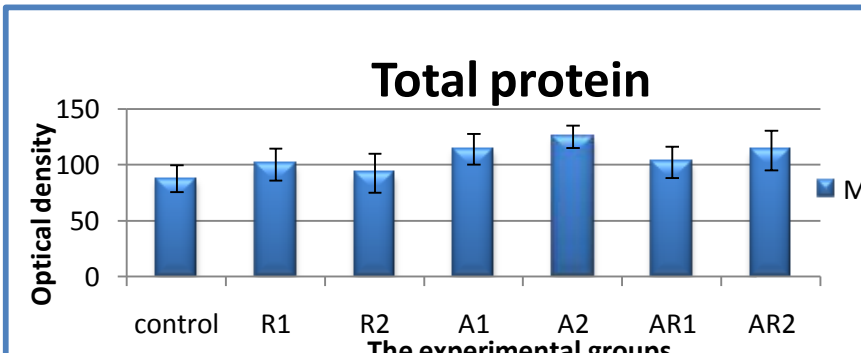

Fig. 6. Histogram showing the optical density values of total protein in the cardiac tissues of the control and different experimental groups of male Albino rats (Each value represents the mean and ± standard deviation with significant difference from the control at $P \leq 0.05$).

Amyloid–β protein

Figs. 7, 8 showed amyloid–β protein content in sections of the heart tissue of the control and all the experimental groups. The cardiac tissue of a rat of the control group showed faintly stained amyloid-β protein. While, the irradiated groups exhibited highly significant increase in the mean values of the optical density of amyloid–β depositions which reached 148.92&141.13 in groups **R_1**, **R_2** respectively relative to the control group (69.58) all over the experimental periods with less stained endomysium spaces of cardiac muscle fibres of rats of **R_1** group.

Rats administrated AFA alone showed non significant decrease in the mean values of the optical density of amyloid–β protein content (67.16& 68.81) in groups **A_1**, **A_2** respectively in the cardiac tissue compared to the control group. The cardiac tissues of rats of **AR_1** and **AR_2** groups represented non significant increase in the mean values of the optical density of amyloid-β accumulations which reached 73.12& 71.45 respectively compared to the control group.

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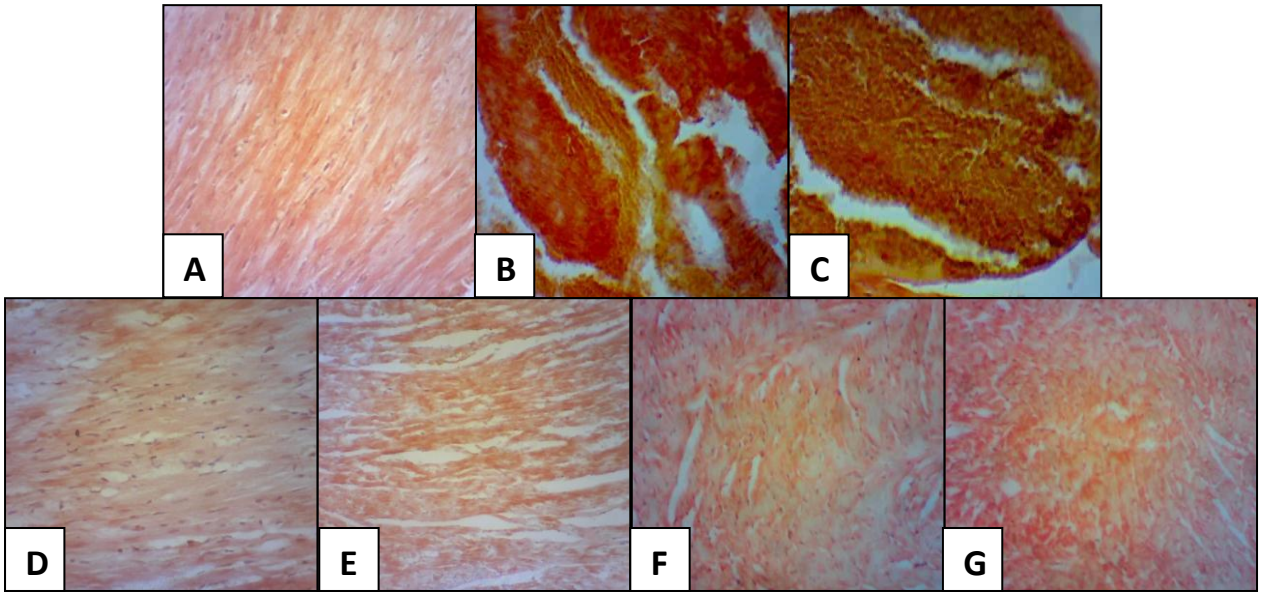

Fig. 7. (A-G) Photomicrographs of sections in the cardiac tissues of the rats showing the depositions of amyloid-β protein: **(A)** faintly stained amyloid-β protein in the cardiac tissue of a rat of the control group; **(B, C)** highly increased amyloid-β depositions in the cardiac tissue of a rat of **R_1**, **R_2** groups respectively; **(D, E)** pale stained amyloid-β protein in the cardiac tissue of rats of **A_1**, **A_2** groups; **(F, G)** slightly increased amyloid-β accumulations in some areas of the cardiac tissue of rats of **AR_1**, **AR_2** groups respectively. **(Congo red stain, A-G X200)**

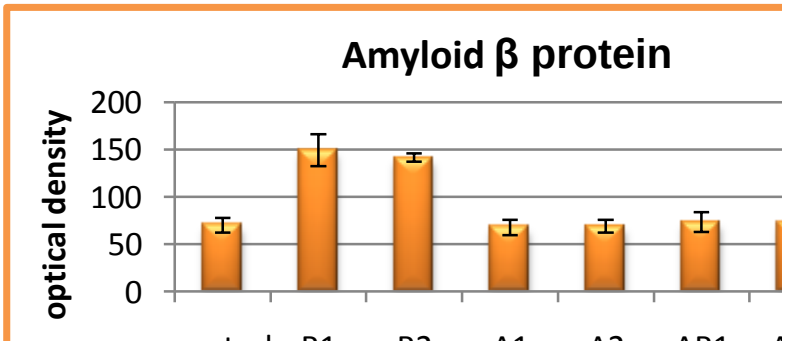

Fig. 8. Histogram showing the optical density values of amyloid-β protein in the cardiac tissues of the control and different experimental groups of male Albino rats (Each value represents the mean and ± standard deviation with significant difference from the control at $P \leq 0.05$).

DNA materials

Figs. 9, 10 showed DNA materials in sections of the heart tissue of the control and all the experimental groups. The cardiac tissue of a control rat showed moderately stained DNA materials in nuclei of myocytes of the control group.The cardiac tissue of γ- irradiated rats showed a non significant decrease in the mean value of the optical density of DNA materials in myocytes of rats of $\mathbf{R_1}$ group (34.99) compared to the control group (47.13), moreover the degenerated areas showed negatively or poorly stained nuclei. While, nuclei of cardiac tissue of rats of $\mathbf{R_2}$ group showed a highly significant decreased mean value of the optical density of DNA materials (22.18) relative to the control group. Diffused staining affinity was detected in the white blood cells inside the large hemorrhagic areas.Treatment with AFA only showed a non significant decrease in the mean values of the optical density of DNA materials in nuclei of the cardiac tissue which reached 45.94& 46.59 in $\mathbf{A_1}$ and $\mathbf{A_2}$ groups respectively compared to the control group. Meanwhile, irradiated rats administrated AFA recorded a non significant decrease in the mean values of the optical density of DNA materials in nuclei of the cardiac tissue which reached 40.75& 41.13 in AR1 and AR2 groups respectively compared to the control group.

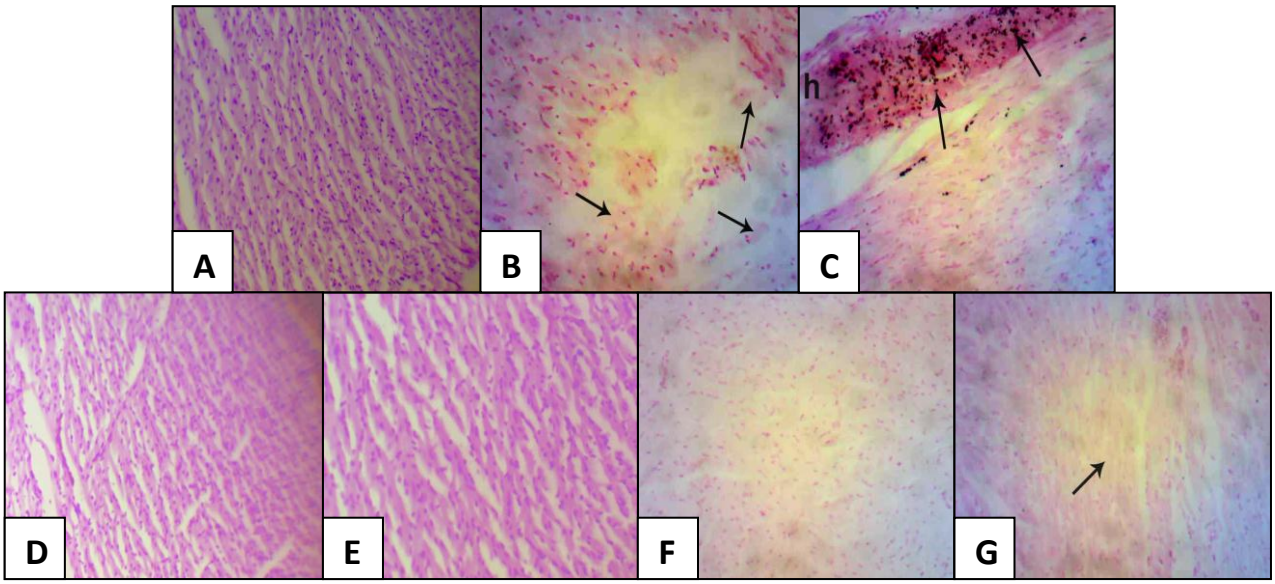

Fig. 9. (A-G) Photomicrographs of sections in the cardiac tissues of the rats showing DNA materials: **(A)** moderately stained DNA materials in nuclei of myocytes in the heart tissue of a rat of control group; **(B)** faintly stained nuclei of myocytes of a rat of $\mathbf{R_1}$ group, but degenerated areas show negatively or poorly stained nuclei (↗); **(C)** highly reduced staining affinity in nuclei of myocytes of a rat of $\mathbf{R_2}$ group with diffused staining affinity in the white blood cells inside the large hemorrhagic area (h) with darkly stained hemosiderin granules (↗); **(D, E)** somewhat normal distribution of DNA materials in heart tissue of rats of $\mathbf{A_1}$, $\mathbf{A_2}$ groups respectively; **(F, G)** slightly reduced staining affinity of DNA materials in the cardiac tissue of rats of $\mathbf{AR_1}$& $\mathbf{AR_2}$ groups respectively. **(Feulgen stain, A-G X200)**

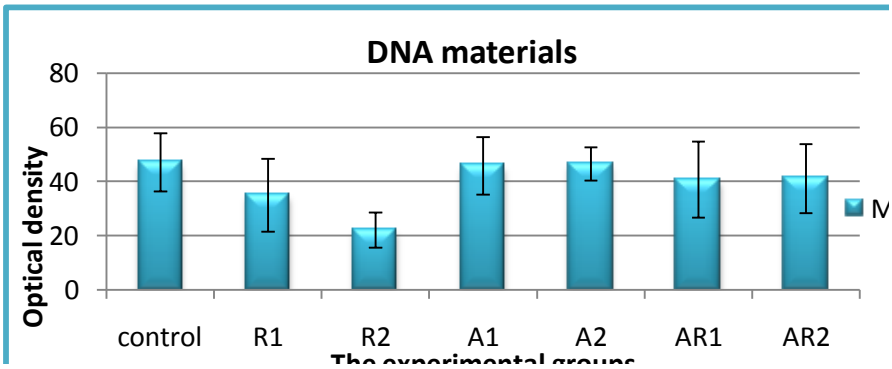

Fig. 10. Histogram showing the optical density values of DNA materials in the cardiac tissues of the control and different experimental groups of male Albino rats (Each value represents the mean and ± standard deviation with significant difference from the control at $P \leq 0.05$).

DISCUSSION

Ionizing radiation produces harmful effects on the organisms and due to the wide spread use of radiation in diagnostic therapy, industry and many purposes, pharmacological intervention may be most potent strategy to protect human or ameliorates the deleterious effects of ionizing radiation [15]. Exposure to ionizing radiation is associated with histopathological changes. These changes differ in their severity according to the radio sensitivity and responses of the individual organs and tissue. The human diet contains an array of natural antioxidants that may contribute to the endogenous antioxidant defense system [6]**.** In the present study, the cardiac tissue of the irradiated rats showed many drastic changes in the cardiac muscle fibres of the rats which were sacrificed after five days post irradiation **(R_1** group**)**. These changes included: numerous hemorrhagic areas which contained hemolysed blood cells, some nuclei of myocardiocytes were pyknotic and others were hypertrophied with destruction in some fibres and widened endomysium. Aggravated changes were observed in the heart of rats of **R_2** group such as: highly elongated and congested cardiac blood vessels which contained hemolysed blood cells and hemosiderin granules, numerous hemorrhagic areas, necrotic areas and widened endomysium. These results were also recorded by several authors [8, 16]**. Abd El Azeem** exposed rats to 6 Gy of ionizing radiation as a fractionated dose of gamma radiation (Radiation doses were fractionated dose levels of 2 Gy each 3 days to reach accumulative dose of 6 Gy) and after 3 days of each exposure rats were sacrificed, except, those left for recovery test one month after the last exposure [6]. The author noticed that there were distinct different histological changes in rat's heart. These changes varied from hypertrophy of the cardiac muscle fibres and disruption of the striation with prominent massive extended hemorrhagic areas. Clear histopathological changes were observed after whole body gamma irradiation (2 Gy every 3 days up to 8 Gy total doses) in the cardiac muscles of rats. Abnormal structures of cardiac muscles were found as ill-defined shape, necrotic, pyknotic nuclei with severe dilated, widened and inflamed capillaries in the endomysium [7]**.**The present results come in accordance with results of **Pradeep *et al.*** who stated that whole-body γ- radiation exposure (5 Gy) of adult rats resulted in cellular damage in the heart tissue of rats of the exposed group when compared to the control group [8]. Also, the present findings come in agreement with those of **Mohamed and Emam** who showed highly widened endomysium and degenerated muscle fibers with loss of striations and bizarre distribution of nuclei in mother's heart of the irradiated pregnant rats that exposed to 2Gy gamma rays in the 7^{th} or 14^{th} day of gestation and sacrificed on day 20 of gestation [16]. Higher degree of histopathological changes was observed in the group that exposed for 14 day. As, highly dilated and elongated wall of the cardiac vein and arteries with disturbed arrangement of nuclei of tunica media and highly widened tunica adventitia. The endomysium became wider than that of the previous groups, which may indicate a considerable degree of cardiac infarction, highly degenerated areas and numerous pyknotic nuclei were detected. These results are also in agreement with several studies [7]. **Eid *et al.*** reported that numerous histopathological and histochemical changes were detected in the heart tissue of adult male albino rats of the irradiated group which were exposed to mobile phone generator radiation (900MHz) for 2hr/day 3days/week for two months. These changes included distorted cardiac muscle fibres with deeply stained nuclei (Pyknotic) and highly thickened and elongated arterial wall which contained hemolysed RBCs with altered collagen fibres, polysaccharides in the cardiac muscle fibres of the exposed group [17]. Highly

widened endomysium and degenerated areas in the cardiac tissue observed in the present study were considered as a reactive change that may be related to the inhibitory effect on the vascular smooth muscles which induced relaxation and consequent vasodilatation. This vasodilatation and increased vascular permeability may lead to loss of fluid from the blood, so the vessels are engorged with blood cells with consequent slowing down of the blood stream which would result in degeneration and necrosis in the cardiac tissues [16]. Also, **Rezk** revealed that the cardiac muscle sections of the irradiated rats showed degenerated cardiac myofibrils, necrosis and appearance of karyolitic and peripheral nuclei, severe dilated, widened and inflamed endomysium capillaries [18]. Irradiation of rats induced formation of structural changes in their aortas, degeneration of the endothelial cell layer of the tunica intima which might be the cause of edema, fibrosis and increase of vascular permeability, as well as degeneration and decreased number of smooth muscle cells of the tunica media of the aorta [19]. Moreover, **Elkady and Mohamed** reported that exposure of rats to whole body gamma irradiation (10 Gy) resulted in numerous histopathological changes such as infiltration, fibrinoid necrosis in the coronary vessel, edema and hyalinization of myocardial muscles [20]. Supplementation of AFA showed normal architecture of the cardiac muscle fibres in A_1, A_2 groups. Treatment of irradiated rats with AFA showed somewhat normal appearance and well developed cardiac muscle fibres in AR_1, AR_2 groups. Treatment with AFA post irradiation indicated the radioprotective effect of AFA and its ability to scavenge free radicals caused by γ-radiation. These results come in agreement with those of **Abdelhafez and kandeal** who reported that supplementation of AFA ameliorated the histological pattern of liver of rats exposed to gamma radiation and recorded radioprotective effect [21]. Also, BGA have attracted attention as health beneficial foods and as source materials for drug development [9]. According to **Fastner *et al.*** AFA contains vitamins A, C, E and K, along with, choline, biotin, niacin, folic acid and pantothenic acid. Added to this diverse vitamins store are minerals like calcium, chloride, chromium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc [14]. **Eid *et al.*** reported that administration of vitamin E to irradiated rats which were exposed to mobile phone generator radiation (900MHz) for 2hr/day 3days/week for two months showed somewhat normal appearance of cardiac muscle fibres [17]. The present study showed highly increased collagen fibres in the cardiac muscle fibres with numerous brightly red stained hemorrhagic and fibrotic areas in cardiac tissue of rats of the irradiated groups (R_1, R_2) which were sacrificed after five and twenty one days post irradiation respectively. These results come in agreement with results of **Mohamed and Emam** who detected increased collagen fibres in most of cardiac muscle tissues of the pregnant rats exposed to 2Gy γ-rays on day 7 or day 14 of gestation when compared to the control group [16].

The present investigation is supported by the work done by **Eid *et al.*** who detected highly increased collagen bundles in the distorted cardiac tissue of the irradiated group which were exposed to mobile phone generator radiation (900MHz) for 2hr/day 3days/week for two months [17]. Also, increased collagen fibres post irradiation were detected in the different tissues as described by several authors [22 , 21]. The present study showed slightly increased collagen fibres in the cardiac muscle fibres of rats of groups which were treated with AFA alone (A_1, A_2 groups) and post irradiation (AR_1, AR_2 groups) which were sacrificed after five and twenty one days respectively. The present results are in line with results of **Abdelhafez and kandeal** who reported that rats which were administrated AFA alone and post irradiation showed somewhat normal distribution of collagen fibres in the central and portal areas of liver tissue [21]. According to **Fastner *et al.*** AFA contains vitamine E [14]. **Eid *et al.*** reported that treatment of rats with vitamine E showed somewhat normal appearance of collagen fibres in the cardiac muscle fibres [17].In the present study, the rats which were exposed to 4 Gy of gamma radiation and sacrificed after five days (R_1 group) showed non significant decrease in the mean value of the optical density of PAS +ve materials which reached 66.08±7.24 compared to the control group (69.41) with slightly increased in the highly widened endomysium of heart tissue. Moreover, poorly or negatively stained degenerated areas were noticed. While, cardiac muscle fibres of rats of R_2 group showed a significant decrease in the mean value of optical density of PAS +ve materials (56.36) compared to the control group, while the highly widened endomysium contained moderately to deeply stained PAS +ve materials. These results come in agreement with results of **Mohamed and Emam** who observed reduced staining affinity of PAS +ve materials in the maternal cardiac tissue of pregnant rats that exposed to 2Gy of γ -rays on day 7 or day 14 of gestation when compared to the control group [16].Results of the present investigation are in a line with results of **Eid *et al.*** who investigated depleted PAS +ve materials in the ruptured cardiac muscle fibres of rats of the irradiated group which

were exposed to mobile phone generator radiation (900MHz) for 2hr/day 3days/week for two months [17]. They added that decreased PAS +ve materials may be due to failure of the tissue to synthesize or store glycogen and may be also a result of degeneration observed in the endomysium. Decreased glycogen content post-irradiation exposure was noticed in studies of many authors [17, 22, 21].

Rats Treated with AFA alone showed non significant decrease in the mean values of optical density of PAS +ve materials which reached 65.55& 66.41 in groups **A_1& A_2** which were sacrificed after five and twenty one days respectively in the cardiac tissues compared to the control group. Also, treatment of irradiated rats with AFA showed non significant decrease in the mean value of optical density of PAS +ve materials which reached 64.25 in **AR_1** group which were sacrificed after five days post irradiation compared to the control group. While, **AR_2** group which were sacrificed after twenty one days post irradiation showed a significant decrease in the mean value of optical density of PAS +ve materials which reached 54.01 in the cardiac tissue compared to the control group. According to **Fastner *et al.*** AFA contains vitamin E [14] and vitamin E administration decreased polysaccharides in the hepatocytes [17].

Results of the present study are in a line with results of **Abdelhafez and kandeal** who reported that administration of AFA to adult male albino rats showed normal distribution of PAS +ve materials in the liver tissue [21]**.** AFA is also known to contain polysaccharides with potent immunostimulators of human monocytes and macrophages [13]**.**

The present study recorded decrease in the mean values of optical density of total protein which reached 70.43 & 66.98 in the cardiac tissue of both irradiated groups **(R_1, R_2)** which were sacrificed after five and twenty one days post irradiation respectively. Decreased total protein in the tissues post exposure to the different types of radiations was noticed by many authors [22, 21]**.** Also, these results come in agreement with the work done by **Mohamed and Emam** who noticed deeply stained total protein in the cardiac muscle fibres, but degenerated cardiac muscle fibres acquired poor staining affinity; widened endomysium contained faintly bromophenl blue stained connective tissue of the irradiated group exposed to 2Gy of γ-rays on day 7 or day 14 of gestation when compared to the control [16].

Rats treated with AFA alone showed non significant increase in the mean value of optical density of total protein which reached 78.08 in the cardiac muscle fibres of rats of **A_1** group which were sacrificed after five days compared to the control group**,** while **A_2** group showed normal appearance of total protein and the mean value of optical density recorded 74.42.

These results come in agreement with the results of **Abdelhafez and kandeal** who reported that rats administrated AFA showed normal total protein content in the central and portal areas of liver tissue [21]**.** AFA contains more protein than any other organism (plant or animal). There are 22 amino acids. *Spirulina* is used in human nutrition because of its high protein content (68%) and its excellent nutritive value [23]**.**Treatment of irradiated rats with AFA represented a non significant decrease in the mean values of optical density of total protein in the cardiac muscle fibres which reached 71.19& 72.38 in **AR_1, AR_2** groups respectively compared to the control group. These results come in agreement with results of **Abdelhafez and kandeal** who revealed that irradiated rats treated with AFA showed somewhat normal distribution of total protein in hepatocytes of liver tissue [21]**. Eid *et al.*** found that treatment of adult male albino rats of the irradiated group which was exposed to mobile phone generator radiation (900MHz) for 2hr/day 3days/week for two months with vitamin E showed somewhat normal appearance of total proteins in the cardiac muscle fibres [17]. Moreover, antioxidants in AFA protect biologically important molecules such as DNA, proteins and lipids from oxidative damage and consequently reduced the risk of several chronic diseases [11, 12, 21]. Also, vitamin E in AFA supplementation played an important role in tissue formation and prevention of damage or oxidation of some tissues. The current study recorded highly significant increased mean values of optical density of amyloid–β depositions which reached 148.92& 141.13 in **R_1, R_2** groups which were sacrificed after five and twenty one days post irradiation respectively relative to the control group (69.58) all over the experimental periods with less stained endomysium spaces of cardiac muscle fibres of rats of **R_1** group. Results of the present study come in agreement with results of **Abdelhafez and kandeal** who found that exposure of adult male albino rats to 4Gy of gamma radiation recorded a significant increase in the amyloid–β protein in hepatocytes of the central and portal areas of the liver tissue [21]**.** Application of Congo red stain recorded slightly increased amyloid-β deposits inside some of the granular cells of hippocampus of the irradiated mice, but those neurons which underwent apoptosis in most of hippocampal areas exhibited a high strong amyloid-β reaction. In the present study rats administrated AFA alone showed non significant decrease in the mean

values of optical density of amyloid–β protein which reached 67.16& 68.81 in groups $\mathbf{A_1}$, $\mathbf{A_2}$ which were sacrificed after five and twenty one days respectively in the cardiac tissue compared to the control group. These results come in agreement with results of **Abdelhafez and kandeal** who reported that rats administrated AFA alone showed normal appearance of amyloid β-protein in the liver tissue [(21)]**.** It seems that use of antioxidant compounds may also have a role in reducing amyloid β-induce toxicity. Results of the present study showed that treatment of irradiated rats with AFA exhibited non significant increase in the mean values of amyloid-β accumulations in the cardiac tissue of rats of $\mathbf{AR_1}$ and $\mathbf{AR_2}$ groups which reached 73.12 & 71.45 respectively compared to the control group. According to **German Egyption Pharmacutical Scintific office,** AFA Klamath contains chlorophyll. Results of the present study come in agreement with the work carried by **Nassar *et al*.** who reported the radioprotective role of the chlorophyll-rich foods; they found that chlorophyll-rich foods may be effective in decreasing the effects of radiation and it doubled the life span of animals exposed to fatal doses of radiation [(24)]**.**The cardiac tissue of γ- irradiated rats showed non significant decrease in the mean value of optical density of DNA materials which reached 34.99 in myocytes of rats of $\mathbf{R_1}$ group compared to the control group (47.13), moreover the degenerated areas showed negatively or poorly stained nuclei. While, rats of $\mathbf{R_2}$ group showed highly significant decrease in the mean value of optical density of DNA materials which reached 22.18 relative to the control group in the nuclei of myocytes. Moreover, diffused staining affinity in white blood cells inside the large hemorrhagic areas was also detected in heart of rats of $\mathbf{R_2}$ group. Results of the present study are in accordance with results of **Abdelhafez and kandeal** who found highly decreased nuclear DNA content in hepatocytes of liver tissue of the irradiated rats which were exposed to 4Gy of gamma radiation [(21)]**.**Similar to the present studies **Dawoud** also observed reduction in DNA content in most hepatocytes of the liver tissue after whole body exposure to 6Gy gamma radiation [(22)]. In the current study, the rats treated with AFA alone showed a non significant decrease in the mean values of optical density of DNA materials in the cardiac tissue which reached 45.94& 46.59 in $\mathbf{A_1}$ and $\mathbf{A_2}$ groups compared to the control group. Also, treatment of irradiated rats with AFA recorded a non significant decrease in the mean values of optical density of DNA materials in the cardiac tissue which reached 40.75& 41.12 in $\mathbf{AR_1}$ and $\mathbf{AR_2}$ groups compared to the control group**.** Similar to the present studies **Abdelhafez and kandeal** reported that administration of AFA alone showed normal appearance of DNA materials in the liver tissue and the treatment of the irradiated group with AFA showed somewhat normal appearance of DNA materials in the liver tissue [(21)]**.** The present results come in agreement with results of **Makhlouf and Makhlouf** who reported that administration of BGA prior to gamma irradiation protected rats against the oxidative stress and tissue damage produced by sub-lethal doses of gamma radiation. The major forms of cellular damage induced by radiation were DNA damage, lipid peroxidation and protein oxidation [(25)].

CONCLUSION

The present study confirmed the deleterious effects of gamma radiation on the heart tissues of the rats as shown in the histopathological and histochemical changes and the radio-protective effects of AFA against radiation exposure. The antioxidant property of the extract may be attributed to the presence of various constituents which are present in AFA. Generally, people must be take natural supplement before and after exposure to gamma radiation to lessen the harmful effect of radiation. AFA can be used under medical supervision as a natural supplement for their antioxidant and radio-protective properties.

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