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Efficacy of the aqueous extract of Siwa dates in protection against the whole body γ irradiation induced damages in mice

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ABSTRACT

Ionizing radiation hydrolyzes H₂O and leads to free radicals production that initiates inflammation and DNA damages. Siwa dates are an important crop in Egypt that has been used as a staple food due to its nutritional value and its antioxidant properties. The study aimed to investigate the radioprotective effects of aqueous extract of Siwa dates in whole body γ -irradiated mice. Animals were divided into four groups: healthy mice, unirradiated mice that received Siwa date aqueous extract, irradiated unprotected mice and irradiated mice that received daily 4 ml/kg Siwa date extract for 14 days prior to irradiation. Twenty four hours post irradiation, blood and liver samples were collected for immunological, biochemical, and histopathological studies. Apoptosis and apoptotic proteins in hepatocytes were measured by flow cytometry techniques. Moreover, the cytokinesis-blocked micronucleus assay was performed to measure DNA damages. Matrix metalloproteinases (MMPs) were evaluated in liver samples by both ELISA and immunohistochemical techniques. The results have shown the improvement in liver histopathological sections and the amelioration in the pro-inflammatory cascade. Siwa date extract allowed DNA protection from the destructive effect of irradiation. In conclusion, Siwa dates were effective in alleviating radiation induced damages such as hepatotoxicity, oxidative stress, inflammation, and DNA damage.

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Siwa dates; matrix metalloproteinase; TNF- α ; IFN- γ ; IL-1 β

1. Introduction

Individuals are continually exposed to different sources, natural or artificial, of ionizing radiation. Human beings are exposed to several sources of ionizing radiation due to the diverse use of radiation in medical diagnosis, industrial application, and cancer therapy (Farid et al., 2020d; Miousse et al., 2017; Zekioglu & Parlar, 2021). Several studies confirmed that radiation exposure, *in vitro* and *in vivo*, has led to a wide range of biological damages that depended not only on the dose but also on the type of radiation (Hill, 2018). Ionizing radiation stimulates the anti- and proliferative pathways which in turn disturb the balance between survival and cell death leading to imbalance in the cell fate decision (Di Maggio et al., 2015). As law as reasonably achievable (ALARA) principle was introduced in 1973; and concentrated on the reduction of exposure duration, increasing the distance from the source of radiation and the improvement in the shielding of individuals. Also, ALARA aimed to reduce the radiation exposure during radiotherapy (Christensen et al., 2014; Mitchel, 2015).

Although radiotherapy has several beneficial effects in minimizing tumors, it leads to sever damages in different body organs such as bone marrow and liver (Khezerloo et al., 2019). Liver is considered an important metabolic organ that control many pathways in

the body and is easily affected by several environmental changes. Many studies reported the incidence of liver cancer (Preston et al., 2007) and fatty livers (Akahoshi et al., 2003) in individuals that survived from Hiroshima and Nagasaki atomic bomb. The radiation-induced liver injuries were attributed to DNA breaks, lipid peroxidation and generation of reactive oxygen species (ROS) (Nakajima et al., 2018). The oxidative stress, which follows ionizing radiation exposure, stimulates the inflammatory cascades producing high levels of TNF- α and IL-1 β , which in turn upregulated matrix metalloproteinase (MMPs). MMPs are a family of endopeptidases that degrade the extracellular matrix (ECM) leading to serious complication in the liver (Tetlow et al., 2001).

These facts raise the need for radioprotective agents to protect healthy tissues from the hazardous effects of radiation exposure. Farid et al. (2020a, 2020b, 2021) reported that radioprotectors have to be easily absorbed and distributed in all body tissues with no side effects to insure its safety when repeatedly used. They, also, added that ROS and reactive nitrogen species (RNS) were the main responsible reasons for radiation-induced damages. Therefore, antioxidants and free radicle scavengers can be an ideal radioprotectors that can limit the initial

radiation induced damages at the molecular level prior to the occurrence of other physiological damages (Farid et al., 2021). Szejka et al. (2016) reported that radioprotective agents alleviated the radionuclide absorption by the stimulation of the free radical scavenging pathways. Several plants and plant derived compounds have been reported to act as radioprotective agents. This was achieved through the scavenging of free radical, anti-inflammation, enhancement of DNA repair, and hematopoietic cells regeneration (Kamran et al., 2016; Mun et al., 2018).

Date palm (*Phoenix dactylifera* L.), usually grows in the arid regions, and is considered an important crop that contributes in raising the economic situation of the inhabitant in these regions (El-Gazzar et al., 2009). It contains many vitamins such as vitamin A, B, B12, and C in addition to many elements such as iron, phosphorus and potassium (Mansouri et al., 2005). Siwa date palm is a well-known fruit in Egypt that contains many bioactive compounds, minerals, and vitamins. Different parts of Siwa date have been widely used in traditional medicine for the treatment of nervous disorder, inflammatory diseases, and cancer. Several studies reported that the date extract has antioxidant, anti-inflammatory and antimicrobial properties (Komarasamy & Sekaran, 2012).

The main target of this study was to investigate the radioprotective effects of Siwa date aqueous extract in whole body γ -irradiated mice. The bioactive components, vitamin C, and other elements in Siwa date scavenged free radicals leading to the reduction in the inflammatory cascade and protection from DNA damages.

2. Materials and methods

2.1. Characterization of Siwa dates

Measurements were performed in three samples and results were expressed as mean \pm SD.

2.1.1. Chemical composition

The moisture, ash, crude fibers, total lipid, protein, and carbohydrates of Siwa dates were determined according to the official methods of the association of official analytical chemist (AOAC, 2005).

Moisture: Briefly, Siwa dates were washed in tap water and then dried in air. Siwa date fruits were deseeded, chopped, and homogenized for analysis. The method depends on measuring water mass in a known sample mass prior and after water evaporation. Three grams of homogenized fruits were dried for 3 h at 105°C. The dried fruits were weighted and moisture was evaluated by the formula:

$$\text{Moisture \%} = (W_1 - W_2) \times 100 / W_1$$

Where W_1 is the weight of sample (g) before drying and W_2 is the weight of sample (g) after drying.

Ash content: Five grams of the processed Siwa date fruits were measured into a previously weighed porcelain crucible. The sample was burnt to ashes at 550°C until it become completely ashed. This was followed by cooling in a desiccator and weighing. The weight of ash was calculated as a percentage of the weight of sample analyzed by the formula:

$$\text{Ash \%} = (W_2 - W_1) \times 100 / \text{Weight of sample}$$

Where, W_1 is the weight (g) of empty crucible and W_2 is the weight of crucible with ash.

Crude fibers: Five grams of the processed Siwa date fruits were boiled in 150 ml of H_2SO_4 solution (1.25%) for 30 min under reflux. In several portions of hot water, the boiled sample was washed using a two-fold cloth to trap the particles. The sample was boiled again in 150 ml of NaOH (1.25%) for 30 min under reflux. The boiled sample was washed again in hot water followed by drying. Dried sample was transferred to a previously weighed crucible and heated at 105°C in an oven to reach a constant weight. Sample was burnt to ashes, and the crude fiber percentage was calculated from:

$$\text{Crude fiber \%} = (W_1 - W_2) \times 100 / \text{Weight of sample}$$

Where, W_1 is the weight of crucible with sample after washing, boiling and drying; and W_2 is the weight of crucible with sample of ash.

Carbohydrates: Forty five ml of the Siwa date extract was diluted to 450 ml with distilled H_2O . One ml of the diluted filtrate was pipetted into a test tube; while 1 ml of H_2O was pipetted into another test tube as a blank and 1 ml of glucose was pipetted into a test tube as a standard. Five ml of freshly prepared anthrone (0.10%) were added to each test tube followed by shaking. Test tubes were heated in a water bath (30°C) for 12 min followed by cooling to room temperature. The absorbance was read at 630 nm against the blank by a spectrophotometer. Total available carbohydrate as a percentage of glucose was calculated from:

$$\text{Glucose \%} = 25A_1 \times 100 / WXA_2$$

Where, W is the weight of sample (g), A_1 is the absorbance of diluted sample and A_2 is the absorbance of diluted standard.

Protein content: It was measured according to the Kjeldahl method where half gram of Siwa date was mixed with eight to ten cm^3 of concentrated sulfuric acid in a Kjeldahl flask. The solution was digested, in a fume cupboard, until it became colorless. Ten cm^3 of sodium hydroxide (40%) were used for distillation. The condenser tip was immersed into a conical flask containing five cm^3 of boric acid (4%) in a mixed indicator until the boric acid solution became green. Titration was accomplished with hydrochloric acid (0.01 M) till the solution became red.

Total lipids: Two grams of pulverized sample were used to determine the total lipid. Lipid was extracted from the sample by petroleum ether in a Soxhlet extractor at 60–80°C for 5 h.

2.1.2. Quantitative measurements of phytochemicals

Phenols: It was measured by the Folin-Ciocalteu reagent. Briefly, Twenty μl of Siwa date extract were added to 300 μl of Na_2CO_3 solution (20%), 1.16 ml of distilled water and 100 μl of Folin–Ciocalteu reagent, respectively. The calibration curve was done with gallic acid standard solutions (0.01 to 0.05 mg/ml) where measures were determined by a UV spectrophotometer at 760 nm.

Flavonoids: Half ml of Siwa date extract was mixed with 1.5 ml of methanol, 0.1 ml of potassium acetate (10%) and 2.8 ml of distilled water; followed by incubation at room temperature for 30 min. The absorbance was measured by a UV spectrophotometer at 415 nm.

Tannin: Measurement was based on the tannins' ability to react with vanillin producing a red color in the presence of mineral acid. One gram of Siwa date fruits was extracted with 20 mL of HCl (1%) in methanol for 20 min in a water bath (30°C) followed by centrifugation. One ml of the supernatant was added to 5 ml vanillin (0.5%)/HCL (2%) solution and left for 20 min. The absorbance was measured at 500 nm by UV spectrophotometer.

Chlorophyll and carotenoids content: Half gram of Siwa date fruits was homogenized with the addition of 5 to 10 ml of acetone (80%) for extraction. Chlorophyll a, b, and carotenoids were measured by a UV spectrophotometer at 662, 644, and 470 nm, respectively (Yang et al., 1998).

$$\text{Chlorophyll a (chl a)} = 9.784 \times A_{662} - 0.990 \times A_{644}$$

$$\text{Chlorophyll b (chl b)} = 21.426 \times A_{644} - 4.650 \times A_{662}$$

$$\text{Carotenoids} = 1000 A_{470} - 2.27(\text{chl a}) - 81.4 (\text{chl b})/227$$

Sugar content: It was determined according to Zhang et al. (2015). Where, five grams of Siwa date fruits were homogenized with 25 ml of deionized water and left overnight at room temperature. Samples were sonicated and centrifuged for 15 min at 4000 rpm followed by the filtration of supernatant by 0.2 μm nylon membrane. Sugar content was determined by HPLC (smart line, Knauer®, Germany).

Elements' concentration: It was measured using Inductively Coupled Plasma (ICP-AES), Thermo Sci. model: iCAP6000 series.

Vitamin C: It was determined by the titrimetric methods according to Bessey and King (1933) and the laboratory methods of Jayaraman (1981), respectively. One hundred grams of Siwa date fruits were homogenized with 50 ml of distilled water followed by straining and washing with 10 ml of distilled water; and then the extracted solution was completed to 100 ml. Twenty ml

of the solution were mixed with 150 ml of distilled water and 1 ml of starch. The solution was titrated with 0.005 mol/l iodine where the end point was identified as trace of dark blue color appearance.

Vitamin A: determination was accomplished using a diode array detector (DAD) with analytical wavelengths at 320 nm.

2.2. Preparation of Siwa date aqueous extract

Fresh Siwa date fruits were purchased from Agriculture Research Center, Giza, Egypt. The fruit flesh was separated, manually, from the seed then dried at room temperature. An electric stainless steel blender was used in grinding the dried fruits into powder. Water extract of Siwa date was prepared by mixing the grinded fruits with distilled water by the ratio 1:3. The mixture was kept at 4°C for 48 h (Al-Qarawi et al., 2005).

2.3. Animals

Six-week-old male BALB/c mice weighing 20–25 g were purchased from Theodor Bilharz Research Institute, Giza, Egypt. All animals were bred and maintained under specific pathogen free conditions with standard diet (24% protein, 4% fat and about 4–5% fiber) and water provided *ad libitum* in the animal house, Faculty of Science, Cairo University. Animals were maintained under controlled temperature ($21 \pm 2^\circ\text{C}$) and on 12/12 h dark/light cycle. All experimental procedures were complied with the guidelines of the Animal Research Reporting of In Vivo Experiments (ARRIVE) and carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

2.4. Experimental design

Forty mice were distributed into four groups (10 per group): Group I: healthy control mice (C), group II: unirradiated mice that received Siwa date aqueous extract for 14 consecutive days (S), group III: irradiated unprotected mice (I), group IV: irradiated mice that received Siwa date extract for 14 consecutive days prior to irradiation (IS). Siwa date extract were administered to mice at a dose of 4 ml/kg according to Al-Qarawi et al. (2005) and Ahmed et al. (2008). Twenty four hours post irradiation; animals were anesthetized with pentobarbital (80 mg/kg) (Farid et al., 2020c). Blood samples were collected, by cardiac puncture, in heparinized tubes and centrifuged at 500 g for 10 min.

2.5. Irradiation protocol

Animals were subjected to whole body γ -radiation using a Gamma Cell-40 Carloirradiator, ^{137}Cs source. Rats were irradiated at an acute single dose level of 6 Gy that was

delivered at a dose rate of 0.713 cGy/s. Animals were starved for 12 h before radiation exposure and were anesthetized by a combination of Ketamine 50 mg/kg, Xylazine 5 mg/kg and Thiopental sodium 50 mg/kg (Sigma-Aldrich, St. Louis, MO, USA). Irradiation pie shaped cage was used to prevent mice mobility and ensure equal irradiation dose. The pie cage was sanitized between uses.

2.6. Isolation of lymphocytes

Heparinized blood samples were diluted (1:1) in Hanks' balanced salt solution (HBSS), and then overlaid onto Ficoll paque by the ratio 1:3. After centrifugation, the buffy coat was collected and washed three times by HBSS followed by centrifugation. Finally, the cells were counted and suspended in culture plates (1×10^6 /ml) (Farid et al., 2020a,b).

2.7. Cytokinesis-blocked micronucleus assay (CBMN)

According to Fenech (2007), CBMN was performed by blocking cell division at the cytokinesis stage. Phytohemagglutinin (PHA) was used to induce the mitotic division of lymphocytes, where 10 μ l PHA was added to 750 μ l lymphocyte culture. Cultures were incubated in 5% CO₂ humidified incubator for 44 h. Fifty six μ l cytochalasin B (60 μ g/ml) was added on the top of cultures followed by incubation for further 72 h. After incubation, cells were collected by centrifugation and fixed with 3 methanol: 1 acetic acid/ 1% formaldehyde; then dropped onto glass slide and stained with 4% Giemsa. Precautions: triple cultures were setup for each sample; and 1000 cells were scored to determine the frequency of one, two and three micronuclei (MN) in addition to nucleoplasmic bridges (NPBs).

2.8. Immunological measurements

Levels of pro-inflammatory cytokines (TNF- α , IFN- γ and IL-1 β) were measured in cell culture supernatants and in liver homogenates using mouse TNF- α (ab208348, abcam, USA), IFN- γ (# BMS606, Invitrogen Thermo Fisher Scientific, USA) and IL-1 β (ab197742, abcam, USA) ELISA kits, respectively, according to the manufacturer's instructions.

2.9. Biochemical analysis

Malondialdehyde (MDA) was measured by lipid peroxidation (MDA) assay kit (MBS741034, MyBioSource, USA). The antioxidant enzymes levels were measured through determining the activities of superoxide dismutase (SOD; MBS034842, MyBioSource, USA), catalase (CAT; MBS704962, MyBioSource, USA) and glutathione (GSH; MBS026635, MyBioSource, USA).

2.10. Flow cytometry technique

Hepatocyte cell cultures were prepared according to Shen et al. (2012) under sterile conditions. Briefly, collagenase buffer was perfused in the portal vein of anesthetized mice. After perfusion, liver was dissected and cells were isolated and suspended in William's complete medium then filtered through 100 μ m nylon filter and cultured. Annexin-V-FITC/PI apoptosis detection kit (ab14085, abcam, USA) was used to investigate the apoptosis level in hepatocytes. For measuring pro-apoptotic proteins [Bax (MA5-14,003, Thermo Fisher Scientific, USA), caspase 8 (ab32125, abcam, USA), caspase 3 [Clone C92-605, RUO, 559,341 (BD Biosciences, USA)], p53 (ab90363, abcam, USA)] and the anti-apoptotic protein Bcl2 [10C4, 11-6992-42 (Thermo Fisher Scientific, USA)]; hepatocytes were permeabilized by saponin (0.5% v/v in PBS, pH 7.4).

2.11. Measurements of Matrix metalloproteinase (MMP) 9 and Tissue inhibitor matrix metalloproteinase (TIMP) 1

Liver samples were homogenized in cold Tris-HCl buffer (0.1 M, pH 7.4) for preparation of 10% homogenate followed by the centrifugation at 4°C. MMP 9 and TIMP 1 were measured using mouse MMP 9 (MBS2886011, MyBioSource, USA) and TIMP 1 (ab196265, abcam, USA) ELISA kits, respectively.

2.12. Histopathological and immunohistochemical analysis

Liver sections were fixed in 10% buffered formalin, for 24 h, followed by dehydration in descending concentration of alcohol, infiltration, and staining with Hematoxylin and Eosin stain. MMP 9 and TIMP 1 were evaluated through immunohistochemical staining; briefly, liver sections (4 microns) were deparaffinized and rehydrated. Slides were immersed in H₂O₂ (3%) for 15 min to inactivate the endogenous peroxidase; followed by blocking for another 15 min. Primary antibodies [mouse anti-MMP9 (SAB5200294, MERCK, USA) and mouse TIMP-1 antibody (AF980, R&D system, USA)] were added to the slides followed by one hour incubation at room temperature. Secondary biotinylated anti-mouse antibody (21,538-M, MERCK, USA) was applied to slides for half an hour; followed by slides incubation with avidin/biotin mixture for another half an hour. Slides were incubated for 10 min with 3,3'-Diaminobenzidine (DAB). Slides were examined by DSX1000 Digital Microscope (Olympus, Japan).

2.13. Statistical analysis

All data were expressed as mean \pm standard deviation (SD). The differences between the treated and control groups were compared by t-test. All statistical analyses

were performed using (SPSS Inc., Chicago, IL, USA); where P value < 0.05 was considered statistically significant.

3. Results

3.1. Characterization of Siwa date

The results showed that Siwa dates contained fructose, glucose and sucrose (17.8, 21.2, and 5.1 g/100 g, respectively); and other elements as iron and zinc (Table 1). It contained 11.4% moisture, 4.6% crude fibers and 4.2% ash. Total carbohydrates, protein, amino acid and lipids were 78.7%, 4.5, 4.6, and 0.1 g/

100 g, respectively. The bioactive compounds were composed of phenols, flavonoids, tannins, carotenoids, chlorophyll A and B (Table 1). Vitamins A and C concentrations were 0.9 and 0.8 mg%, respectively.

3.2. Cytokinesis-blocked micronucleus assay (CBMN)

Irradiated group III showed a significant decrease in the number of mononucleated cells and a significant increase in number of binucleated cells (Figure 1a); these were accompanied with elevations in the

Table 1. Characterization of Siwa date.

Sugar (g/100 g)	Chemical composition	Phytochemicals (g/100 g)	Elements						
			%		ppm				
Fructose	17.8 ± 1.2	Moisture (%)	11.4 ± 3.1	T. phenols	1.5 ± 0.1	Ca	0.04 ± 0.005	Zn	2.4 ± 0.2
Glucose	21.2 ± 2.4	Ash (%)	4.2 ± 0.7	T. flavonoids	0.1 ± 0.1	P	0.02 ± 0.001	B	3.0 ± 0.1
Sucrose	5.1 ± 0.9	T. carbohydrates (%)	78.7 ± 3.3	T. tannins	0.3 ± 0.2	Mg	0.05 ± 0.002	Fe	6.1 ± 0.6
Maltose	ND	T. lipids (g/100 g)	0.1 ± 0.1	T. carotenoids	169.7 ± 1.6	Na	0.5 ± 0.3	Se	ND
		T. protein (g/100 g)	4.5 ± 0.6	Chlorophyll A	0.8 ± 0.2	K	0.2 ± 0.1	Cu	ND
		Crude fiber (%)	4.6 ± 0.4	Chlorophyll B	1.4 ± 0.3	Vitamins (mg%)			
				T. amino acids	5.8 ± 1.1	Vit. A	0.9 ± 0.01		
						Vit. C	0.8 ± 0.02		

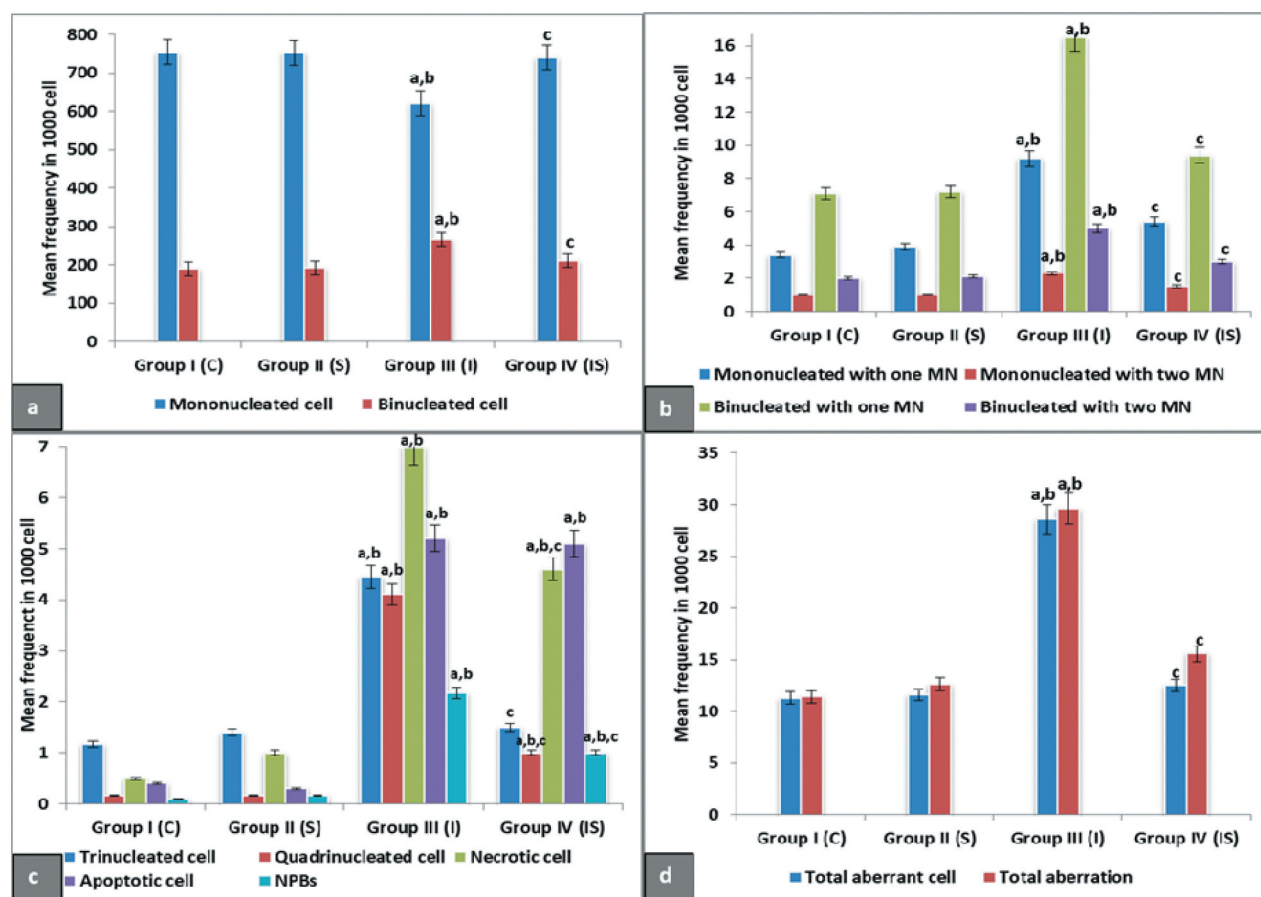


Figure 1. CBMN assay results of mice lymphocytes' cultures in all experimental groups; a) number of mon- and binucleated cells, b) number of cells with one or two MN, c) number of NPBs, tri-, quadrinucleated, necrotic and apoptotic cells and d) number of total aberrant cells and total aberration. where a represented significance when compared to control group I, b represented significance when compared to Siwa date extract administrated unirradiated group II and c represented significance when compared to unprotected irradiated group III ($p < 0.05$). Group I: healthy control mice (C), group II: unirradiated mice that received Siwa date aqueous extract (S), group III: irradiated unprotected mice (I), group IV: irradiated mice that received Siwa date extract for 14 days prior to irradiation (IS).

frequencies of cells with one and two MN (Figure 1b, $p < 0.05$). Numbers of NPBs, trinucleated, quadrinucleated, necrotic, apoptotic, and total aberrant cells were significantly elevated after irradiation exposure in group II (Figure 1b, c). Group IV, administrated with Siwa date extract for 14 days before radiation exposure, revealed a decrease in the strength of radiation consequences when compared to group II. Where, (Figure 1) showed an elevation in the number of mononucleated cells together with the reduction in numbers of binucleated, trinucleated, quadrinucleated, and necrotic cells in group IV. However, no significant change was observed in the number of apoptotic cells between irradiated group II and Siwa date extract administrated irradiated group IV. (Table

2) showed the results of CBMN and was submitted as a supplementary material.

3.3. Immunological measurements

Radiation exposure significantly elevated the levels of pro-inflammatory cytokines (TNF- α , IFN- γ and IL- β) in group III when compared to control group I and Siwa date extract administrated unirradiated group II in both lymphocytes cultures and liver samples (Figure 2). Administration of group IV with date extract for 14 days prior to radiation exposure significantly reduced the levels of cytokines; as there was not any significant difference in their levels between group IV and both control groups (I and II). (Table 3) showed the

Table 2. Cytokinesis-blocked micronucleus assay (CBMN) assay results of mice lymphocytes' cultures in all experimental groups.

	Group I (C)	Group II (S)	group III (I)	group IV (IS)
Mononucleated	754.2 \pm 11.5	752.6 \pm 6.2	679.8 \pm 13.6 ^{ab}	715.6 \pm 11.2 ^c
Mononucleated + one MN	3.4 \pm 0.9	3.9 \pm 2.1	9.2 \pm 4.1 ^{ab}	5.4 \pm 3.1 ^c
Mononucleated + Two MN	0	0	0.5 \pm 0.2 ^{ab}	0.2 \pm 0.2 ^c
Mononucleated + Three MN	0	0	0	0
Binucleated	189.4 \pm 9.5	191.7 \pm 4.5	265.7 \pm 14.3 ^{ab}	255.4 \pm 4.3 ^c
Binucleated + one MN	7.1 \pm 1.2	7.2 \pm 2.1	16.5 \pm 3.5 ^{ab}	9.4 \pm 8.8 ^c
Binucleated +Two MN	0.17 \pm 0.1	0.17 \pm 0.1	0.5 \pm 0.3 ^{ab}	0.17 \pm 0.1 ^c
Binucleated +Three MN	0	0	0	0
Trinucleated	1.17 \pm 0.1	1.4 \pm 0.3	4.45 \pm 0.2 ^{ab}	1.5 \pm 0.2 ^c
Quadrinucleated	0.17 \pm 0.1	0.17 \pm 0.1	4.1 \pm 0.6 ^{ab}	0.17 \pm 0.1 ^{abc}
Necrotic cell	0.17 \pm 0.1	0.17 \pm 0.1	9.7 \pm 1.6 ^{ab}	4.6 \pm 3.3 ^{abc}
Apoptotic cell	0.17 \pm 0.1	0.17 \pm 0.1	5.2 \pm 2.9 ^{ab}	5.1 \pm 0.4 ^{ab}
NPBs	0	0.17 \pm 0.1	1.17 \pm 0.7 ^{ab}	0.33 \pm 0.3 ^{abc}
Total aberrant cell	11.3 \pm 0.6	11.6 \pm 0.7	28.6 \pm 7.1 ^{ab}	12.5 \pm 0.2 ^c
Total aberration	11.4 \pm 0.4	12.6 \pm 1.3	29.6 \pm 3.1 ^{ab}	15.6 \pm 1.1 ^c

Results were expressed as mean \pm SD, where ^a represented the significance when compared to control group I, ^b represented the significance when compared to Siwa date extract administrated unirradiated group II and ^c represented the significance when compared to unprotected irradiated group III ($p < 0.05$). Group I: healthy control mice (C), group II: unirradiated mice that received Siwa date aqueous extract (S), group III: irradiated unprotected mice (I), group IV: irradiated mice that received Siwa date extract for 14 days prior to irradiation (IS). MN: micronucleus and NPBs: nucleoplasmic bridges.

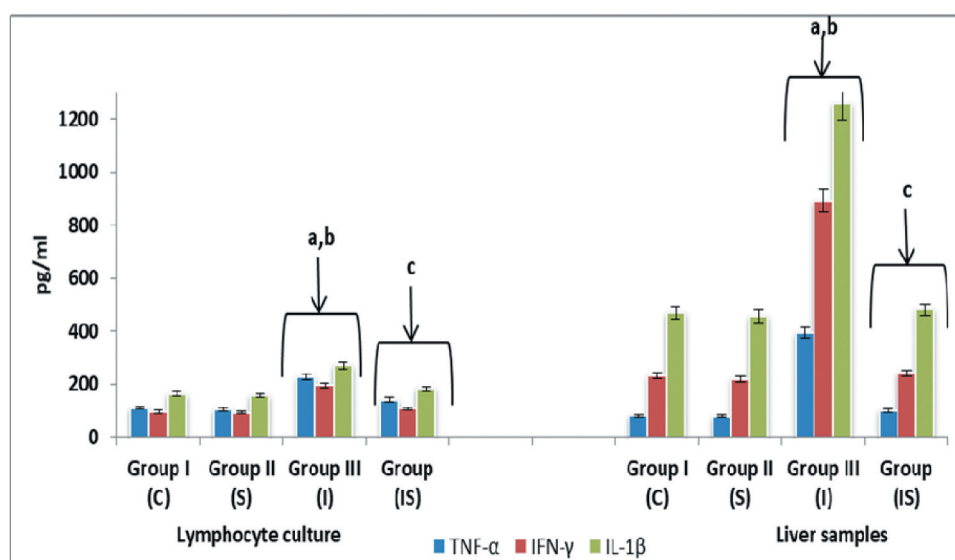


Figure 2. Levels of pro-inflammatory cytokines (TNF- α , IFN- γ and IL- β) in mice blood lymphocytes cultures and liver samples of all experimental groups; where a represented significance when compared to control group I, b represented significance when compared to Siwa date extract administrated unirradiated group II and c represented significance when compared to unprotected irradiated group III ($p < 0.05$). Group I: healthy control mice (C), group II: unirradiated mice that received Siwa date aqueous extract (S), group III: irradiated unprotected mice (I), group IV: irradiated mice that received Siwa date extract for 14 days prior to irradiation (IS).

Table 3. Levels of pro-inflammatory cytokines (TNF- α , IFN- γ and IL- β) in mice blood lymphocytes cultures and liver samples of all experimental groups.

	Group I (C)	Group II (S)	Group III (I)	Group (IS)
Lymphocyte cultures				
TNF- α (pg/ml)	110 \pm 2.1	103 \pm 2.1	225 \pm 3.1 ^{ab}	141 \pm 6.4 ^c
IFN- γ (pg/ml)	95 \pm 3.6	92 \pm 2.6	195 \pm 2.5 ^{ab}	106 \pm 2.3 ^c
IL-1 β (pg/ml)	163 \pm 4.1	156 \pm 6.7	270 \pm 11.3 ^{ab}	182 \pm 1.2 ^c
Liver samples				
TNF- α (pg/ml)	81 \pm 9.1	79 \pm 6.6	395 \pm 3.6 ^{ab}	99 \pm 9.7 ^c
IFN- γ (pg/ml)	230 \pm 11.6	219 \pm 5.4	893 \pm 4.4 ^{ab}	241 \pm 2.7 ^c
IL-1 β (pg/ml)	469 \pm 8.7	456 \pm 10.1	1260 \pm 16.7 ^{ab}	480 \pm 6.8 ^c

Results were expressed as mean \pm SD, where ^a represented the significance when compared to control group I, ^b represented the significance when compared to Siwa date extract administrated unirradiated group II and ^c represented the significance when compared to unprotected irradiated group III ($p < 0.05$). Group I: healthy control mice (C), group II: unirradiated mice that received Siwa date aqueous extract (S), group III: irradiated unprotected mice (I), group IV: irradiated mice that received Siwa date extract for 14 days prior to irradiation (IS). TNF- α : tumor necrosis factor α , IFN- γ : interferon γ and IL-1 β : interleukin 1 β .

immunological results and was submitted as a supplementary material.

3.4. Biochemical analysis

Our results showed that radiation exposure, significantly, increased MDA level and decreased the antioxidant enzymes levels (SOD, CAT and GSH) in either lymphocytes culture or liver samples of group III when compared to control group (Figure 3). Siwa date extract administration, in group IV, reduced

MDA level and elevated the antioxidant enzymes levels in a significant way in comparison to irradiated group III. (Table 4) showed the biochemical results and was submitted as a supplementary material.

3.5. Flow cytometry results

Irradiated group III showed a significant decrease in the number of viable hepatocytes; and a significant increase in the number of both necrotic and apoptotic hepatocytes when compared to control group I. This was accompanied with the up regulation of intracellular apoptotic proteins (Bax, P53, caspase-3, and caspase-8) and down regulation of anti-apoptotic protein Bcl2. Siwa date extract administration prior to radiation exposure protected liver tissue from radiation hazards; this was obvious in the reduction of the numbers of necrotic and apoptotic cells together with the down regulation of apoptotic proteins in group IV (Figure 4a, b). (Table 5) showed the flow cytometry results and was submitted as a supplementary material.

3.6. MMP 9 and TIMP 1 levels in liver samples

Irradiated group III showed high levels of MMP 9 and TIMP 1 in liver samples more than those of control group I and Siwa date extract administrated unirradiated group II (Figure 5a, b). On the other hand,

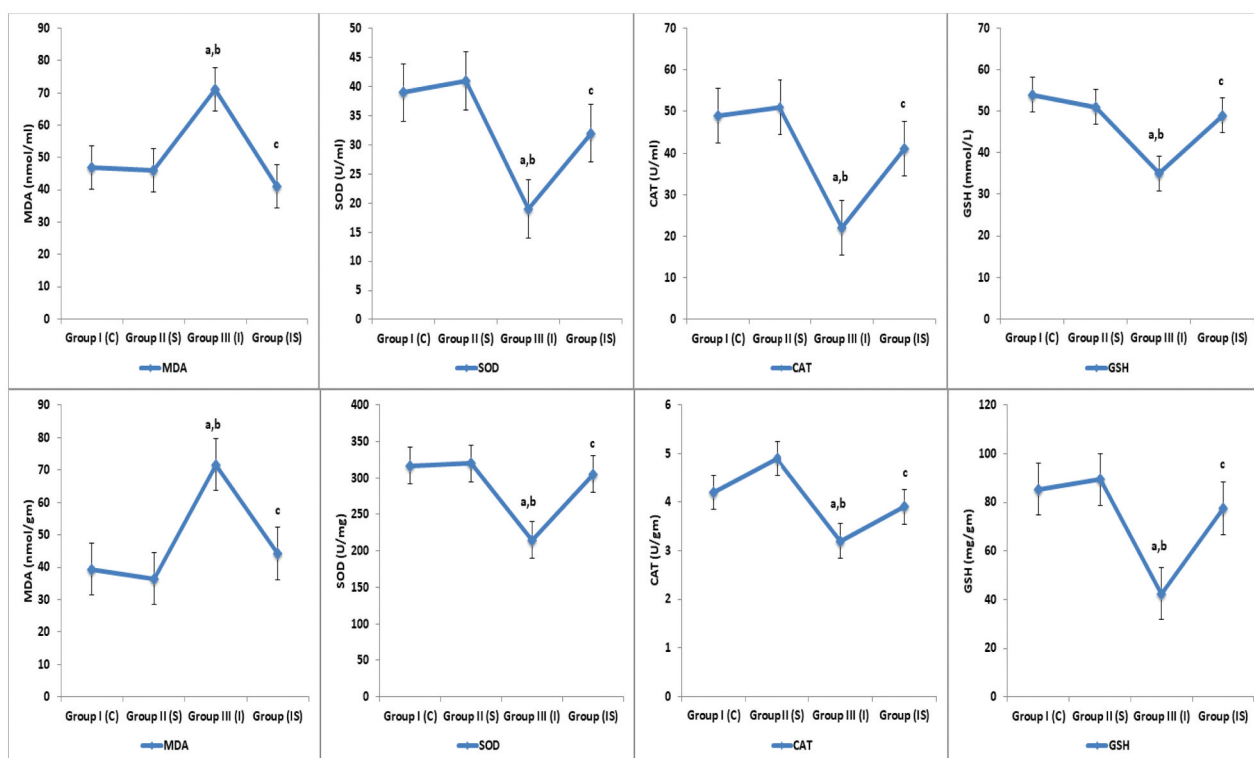


Figure 3. Biochemical analysis of different experimental groups; MDA, SOD, CAT and GSH levels in lymphocytes culture (a-d) and in liver samples (e-h). Where a represented significance when compared to control group I, b represented significance when compared to Siwa date extract administrated unirradiated group II and c represented significance when compared to unprotected irradiated group III ($p < 0.05$). Group I: healthy control mice (C), group II: unirradiated mice that received Siwa date aqueous extract (S), group III: irradiated unprotected mice (I), group IV: irradiated mice that received Siwa date extract for 14 days prior to irradiation (IS).

Table 4. Biochemical analysis of different experimental groups.

	Group I (C)	Group II (S)	Group III (I)	Group (IS)
Lymphocyte cultures				
MDA (nmol/ml)	47±2.1	46±0.9	71±2.3 ^{ab}	41±0.8 ^c
SOD (U/ml)	39±1.3	41±0.4	19±0.2 ^{ab}	32±0.6 ^c
CAT (U/ml)	49±4.1	51±0.8	22±0.3 ^{ab}	41±0.1 ^c
GSH (mmol/L)	54±2.3	51±0.6	35±0.7 ^{ab}	49±0.3 ^c
Liver samples				
MDA (nmol/gm)	39.4±1.6	36.5±1.7	71.6±1.7 ^{ab}	44.2±0.9 ^c
SOD (U/mg)	317±1.7	320±6.4	215±6.8 ^{ab}	305±9.1 ^c
CAT (U/gm)	4.2±0.6	4.9±0.4	3.2±0.4 ^{ab}	3.9±5.1 ^c
GSH (mg/gm)	85.4±6.1	89.4±4.7	42.4±0.2 ^{ab}	77.5±4.9 ^c

Results were expressed as mean ± SD, where ^a represented the significance when compared to control group I, ^b represented the significance when compared to Siwa date extract administrated unirradiated group II and ^c represented the significance when compared to unprotected irradiated group III (p < 0.05). Group I: healthy control mice (C), group II: unirradiated mice that received Siwa date aqueous extract (S), group III: irradiated unprotected mice (I), group IV: irradiated mice that received Siwa date extract for 14 days prior to irradiation (IS). MDA: malondialdehyde, SOD: superoxide dismutase, CAT: catalase and GSH: glutathione.

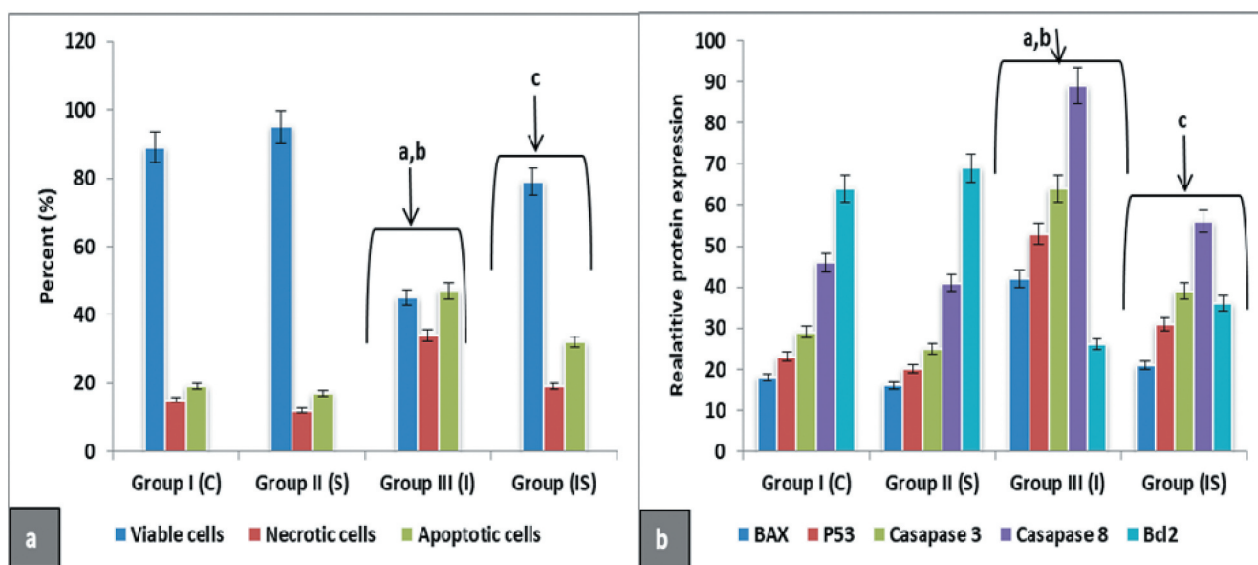


Figure 4. Changes in a) apoptosis level, b) apoptotic intracellular proteins (Bax, P53, caspase-3 and caspase-8) and anti-apoptotic protein Bcl-2 in hepatocytes of different experimental groups. Where a represented significance when compared to control group I, b represented significance when compared to Siwa date extract administrated unirradiated group II and c represented significance when compared to Siwa date extract unprotected irradiated group III (p < 0.05).

group IV showed lower levels of MMP 9 and TIMP 1 when compared to those of group II. (Table 6) showed the levels of MMP 9 and TIMP 1 and was submitted as a supplementary material.

3.7. Histopathological results

Group III showed sever liver damage that was indicated by hepatocyte degeneration and inflammatory cells infiltration (Figure 6c); together with the high expression of both MMP 9 (Figure 6g) and TIMP 1 (Figure 6k). Liver sections from mice administrated with Siwa date extract before radiation exposure, group IV, showed mild hepatocyte degeneration with the reduced expression of both MMP 9 and TIMP 1 when compared to group III.

Table 5. Changes in the levels of apoptosis, apoptotic intracellular proteins (Bax, P53, caspase-3 and caspase-8) and anti-apoptotic protein Bcl-2 in hepatocytes of different experimental groups.

	Group I (C)	Group II (S)	Group III (I)	Group (IS)
Viable cells	89 ± 3.1	95 ± 2.3	45 ± 5.1 ^{ab}	79 ± 0.3 ^c
Necrotic cells	15 ± 7.4	12 ± 1.2	34 ± 5.3 ^{ab}	19 ± 0.7 ^c
Apoptotic cells	19 ± 0.6	17 ± 0.4	47 ± 7.1 ^{ab}	32 ± 0.6 ^c
BAX	18 ± 0.7	16 ± 0.6	42 ± 2.1 ^{ab}	21 ± 0.1 ^c
P53	23 ± 0.9	20 ± 0.7	53 ± 3.4 ^{ab}	31 ± 0.3 ^c
Caspase 3	29 ± 0.3	25 ± 0.1	64 ± 2.6 ^{ab}	39 ± 0.4 ^c
Caspase 8	46 ± 0.4	41 ± 0.2	89 ± 9.7 ^{ab}	56 ± 0.9 ^c
Bcl2	64 ± 1.1	69 ± 0.7	26 ± 0.9 ^{ab}	36 ± 6.4 ^c

Results were expressed as mean ± SD, where ^a represented the significance when compared to control group I, ^b represented the significance when compared to Siwa date extract administrated unirradiated group II and ^c represented the significance when compared to unprotected irradiated group III (p < 0.05). Group I: healthy control mice (C), group II: unirradiated mice that received Siwa date aqueous extract (S), group III: irradiated unprotected mice (I), group IV: irradiated mice that received Siwa date extract for 14 days prior to irradiation (IS).

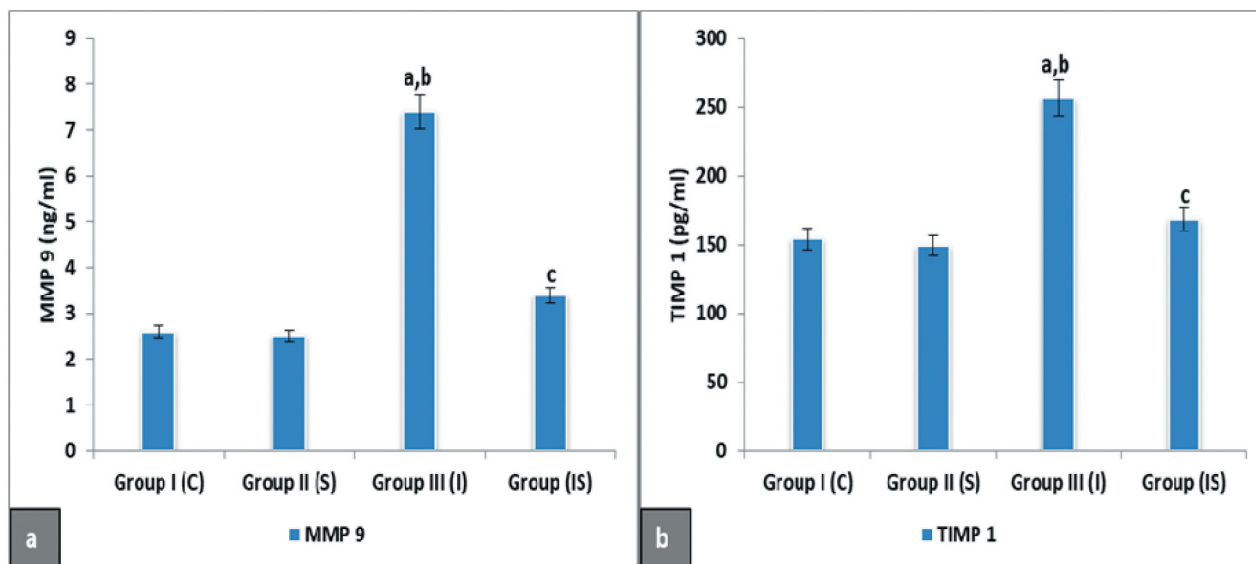


Figure 5. Levels of a) MMP 9 and b) TIMP 1 in liver samples of different experimental groups; where a represented significance when compared to control group I, b represented significance when compared to Siwa date extract administrated unirradiated group II and c represented significance when compared to unprotected irradiated group III ($p < 0.05$). Group I: healthy control mice (C), group II: unirradiated mice that received Siwa date aqueous extract (S), group III: irradiated unprotected mice (I), group IV: irradiated mice that received Siwa date extract for 14 days prior to irradiation (IS).

Table 6. Levels of MMP 9 and TIMP 1 in liver samples of different experimental groups.

	Group I (C)	Group II (S)	Group III (I)	Group IV (IS)
MMP 9 (ng/ml)	2.6 ± 1.7	2.5 ± 2.4	7.4 ± 1.2 ^{ab}	3.4 ± 0.7 ^c
TIMP 1 (pg/ml)	154.3 ± 8.7	149.2 ± 9.7	256.4 ± 12.4 ^{ab}	168.4 ± 11.2 ^c

Results were expressed as mean ± SD, where ^a represented the significance when compared to control group I, ^b represented the significance when compared to Siwa date extract administrated unirradiated group II and ^c represented the significance when compared to unprotected irradiated group III ($p < 0.05$). Group I: healthy control mice (C), group II: unirradiated mice that received Siwa date aqueous extract (S), group III: irradiated unprotected mice (I), group IV: irradiated mice that received Siwa date extract for 14 days prior to irradiation (IS). MMP 9: matrix metalloproteinase 9 and TIMP 1: tissue inhibitor matrix metalloproteinase 1.

4. Discussion

Radiation can be classified into a non-ionizing radiation that has sufficient energy to allow atoms vibration but not for exciting bound electrons; and ionizing radiation that has sufficient energy for atoms ionization (Mu et al., 2018). Generally, the cells contain high percent of water that may reach 75–90% (Mun et al., 2018). Ionizing radiation hydrolyze water molecules forming ROS like hydroxyl ($\cdot\text{OH}$) and hydrogen ($\cdot\text{H}$) ions. These free radicles oxidize the cellular macromolecules such as cell membrane; and are responsible for DNA damage leading to cell necrosis (Singh et al., 2016). The inflammatory response, another consequence of radiation exposure, leads to high production of inflammatory cytokines and chemokines (Son et al., 2019) that damage the different tissues (Hall et al., 2016).

Nowadays, researchers have examined several natural or chemical substances for their radioprotective properties (Dowlath et al., 2021; Farid et al., 2021). Date palm has an important nutritional value due to its vitamins and sugar contents; in addition to its antioxidant properties (AlFaris et al., 2021; Baliga et al., 2011). El-Far et al. (2019) reported the curative effects of dates against cancer, diabetes, bacterial, and fungal infections. Several studies related the therapeutical action of dates to its antioxidant activities (Baliga et al., 2011; El-Far et al., 2019; Khan et al., 2016).

According to our results, Siwa dates contained several bioactive phytochemicals, sugars, iron, zinc, vitamins A and C. These results were in agreement with Parvin (2015) who investigated the active compounds in three Tunisian dates. They reported that the dates contained carbohydrates (51–55%), protein (2%), lipid (0.1–0.7%), vitamin A and C (1.2 and 0.9 mg%, respectively); in addition to 11 elements such as Mg, P, Ca, Zn, and Cu. Mohamed et al. (2014) reported the presence of vitamin A, C and E in Sudanese date palm. The research studies of Mansouri et al. (2005), Hammouda et al. (2013), Hamad et al. (2015), and Hinkaew et al. (2021) reported many phenolic compounds such as gallic, caffeic, dactyliferic, and vanillic in dates. Dates, also, have a flavonoid content that was analyzed into luteolin, quercetin, apigenin, and rutin according to Al-Shahib and Marshall (2003) and Benmeddour et al. (2013).

The present study used CBMN assay to evaluate: 1- chromosomal damage (micronucleus and nucleoplasmic bridges), 2- cells' viability (apoptosis and necrosis) and 3- mitotic event (counting mono-, and binucleated cells). Unprotected irradiated group III showed an

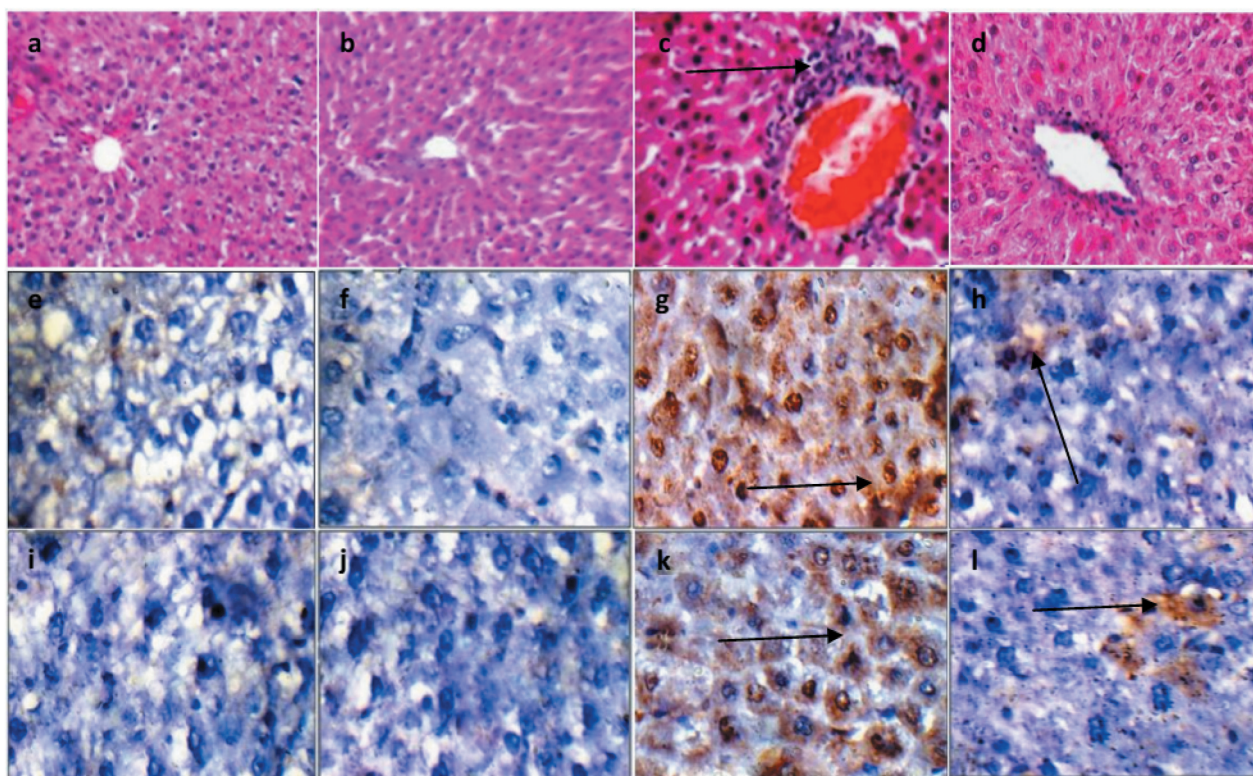


Figure 6. Histopathological liver sections showing a&b] average central vein with hepatocytes arranged in single-cell cords (H&E X400; group I and II, respectively), c] degenerated hepatocytes (arrow) with congested central vein and inflammatory cell infiltration (H&E X400, group III), d] mild vacuolar degeneration of hepatocytes (H&E X400, group IV), e&f] negative MMP 9 reactivity (IHC X400; group I and II, respectively), g] high MMP 9 expression (IHC X400, group III), h] low MMP 9 expression (IHC X400, group IV), i&j] negative TIMP 1 reactivity (IHC X400; group I and II, respectively), k] high TIMP 1 expression (IHC X400, group III) and l] low TIMP 1 expression (IHC X400, group IV). Group I: healthy control mice (C), group II: unirradiated mice that received Siwa date aqueous extract (S), group III: irradiated unprotected mice (I), group IV: irradiated mice that received Siwa date extract for 14 days prior to irradiation (IS).

elevation in the numbers of bi-, tri- and quadrinucleated cells; together with the increase in frequencies of cells with one and two micronucleus and numbers of nucleoplasmic bridges. Radiation significantly affected the lymphocytes viability that was aberrant in the elevation of the numbers of necrotic and apoptotic cells in group III. On the other hand, protected irradiated group IV showed a significant reduction in the numbers of micronucleus, nucleoplasmic bridges, and necrotic cells. These indicated that Siwa date aqueous extract administration reduced the chromosomal damage and increased the cell viability.

Also, unprotected irradiated group III showed a high significant increase in lipid peroxidation, oxidative stress and inflammation. This was evident by the elevations in the levels of MDA and pro-inflammatory cytokines (TNF- α , IL-1 β , and IFN- γ); together with the reduction in the antioxidant enzymes (SOD, CAT, and GSH) activities. MDA is the end product of lipid peroxidation in the cell, where its high level indicates a high level of free radicals with the suppression in the antioxidant defense mechanisms. When the aqueous dates extract was administrated prior to irradiation (group IV), a high significant elevation in levels of SOD, CAT,

and GSH was noticed that led to a significant reduction in levels of MDA and cytokines.

Our results were in agreement with Khezerloo et al. (2019) who found that the extract of date palm seeds protected mice from total body irradiation that was indicated by the decrease in the mortality rate of treated group. Also, Baharara et al. (2015) indicated that pollen of date palm protected male mice from the adverse effects of electromagnetic field. Moreover, Mangood and Kamal (2011) reported that the antioxidant effects of dates extract protected albino rats from radiation induced cardio-toxicity. Date syrup alleviated radiation toxic consequences and has a hepatoprotective effects according to Abou-Zeid et al. (2018). Several research studies reported the hepatoprotective nature of dates against toxicants such as CCL4 and trichloroacetic acid (Ahmed et al., 2008; El Arem et al., 2014; Attia et al., 2016; Saafi et al., 2011). Al-Shwyeh (2019) reported the antioxidant and antimicrobial activities of date palm. Lamia and Mukti (2021) reported the anticancer activity of Bangladeshi wild date palm fruits.

In this study, radiation exposure induced serious damages in liver of unprotected group III. This was

apparent by the high increase in levels of MDA and pro-inflammatory cytokines. Histopathological liver sections showed degenerated hepatocytes with congested central vein and inflammatory cells infiltration. Also, levels of MMP 9 and TIMP 1 were highly elevated in liver tissues of group III when compared to control group. On the other hand, dates extract administrated group IV showed mild vacuolar degeneration of hepatocytes with negative MMP 9 and TIMP 1 reactivity in immune-stained liver sections.

Flow cytometric results showed a significant decrease in the number of viable hepatocytes; and a significant increase in the number of both necrotic and apoptotic hepatocytes in group III. This was accompanied with the up regulation of intracellular apoptotic proteins (Bax, P53, caspase-3, and caspase-8) and down regulation of anti-apoptotic protein Bcl2. Siwa date extract administration prior to radiation exposure protected liver tissue from radiation hazards; this was obvious in the reduction of the numbers of necrotic and apoptotic cells together with the down regulation of apoptotic proteins in group IV.

Although MMPs are a group of endopeptidases that remodel liver ECM in healthy situations, their expression dysregulation contribute to liver damage progression. MMP 9 is involved in ECM degradation, stimulating infiltration of leukocytes and inflammatory cascade leading to liver injury (Geervliet & Bansal, 2020). TIMP 1 is an inhibitory molecule which controls MMPs. In healthy condition, the production of liver ECM is controlled by both MMPs and TIMP. The balance between MMPs and TIMP is disrupted during liver injury (Bedossa & Paradis, 2003). Tetlow et al. (2001) found that several pathological disorders were related to the imbalance in MMP/TIMP ratio that activated MMPs transcription leading to extracellular matrix (ECM) degradation. Al-Dashti et al. (2021) found that consumption of dates has regulated plasma lipid levels, including triglycerides and cholesterol, and reduced oxidative stress and inflammation in animal studies.

Our results indicated that radiation exposure activated the production of pro-inflammatory cytokines in liver of unprotected group III leading to sever inflammation and initiation of leukocytes infiltration. This was in agreement with Patrino et al. (2012) who found an increase in the activities of MMP 2 and 9 in THP 1 cells after exposure to electromagnetic field. Caliskan et al. (2015) reported the increase in levels of MMP 9 and TIMP 1 in chondrosarcoma cells after exposure to pulsed electromagnetic field.

The present work declared that Siwa date aqueous extract alleviated the liver damages induced by whole body γ irradiation. This was indicated by the improvement in liver histopathological sections and amelioration in the pro-inflammatory cascade. The antioxidant capacity, in liver, was improved as evidenced by the

increased activities of antioxidant enzymes and the reduction in liver MDA level. Moreover, Siwa date extract allowed DNA protection from the destructive effect of irradiation as indicated by CBMN assay. The histopathological and immunohistochemical results were congruent with the biochemical and immunological measurements either in serum or liver.

The protective effect of Siwa date was attributed to the presence of Flavonoids, a polyphenol, that have an antioxidant capacity. Flavonoids exerted their actions through free radicals scavenging (Kandaswami & Middleton, 1994). Many studies reported that plant flavonoids protected mice from irradiation hazards (Benkovic et al., 2008; Jagetia, 2003; Shimoi et al., 1996). Also, Siwa dates contained vitamin C and A that have a role in elevating the antioxidant mechanisms (Weiss & Landauer, 2003). Also, Ibrahim et al. (2021) reported the nutritive value of dates and its medicinal activities against inflammation and cancer. Narra et al. (1993) found that vitamin C has got a radioprotector effects from exposure to internal radionuclide Iodine-131. Mozdarani and Nazari (2007) found that vitamin C reduced chromosomal aberration in irradiated mice through free radical scavenging. Al-Jasass et al. (2015) proofed the antioxidant activities of date fruits in many cultivators in United States.

In conclusion, Siwa date was effective in: 1- alleviating radiation induced damages and DNA damage, 2- reduced inflammatory cytokines and oxidative stress, 3- reduced apoptosis in hepatocytes and 4- decreased expression of MMP 9 and TIMP 1. These activities were due to the antioxidant and anti-inflammatory activities of date's phytochemicals like flavonoids. Therefore, it can be used as a supplement during radiotherapy due to its nutritive value and antioxidant effects that will protect normal cells from the destructive consequences of radiation.

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