Research Article

The Protective Role of *Ulva lactuca* Against Genotoxic and Biochemical Effects Induced by γ-Irradiation in Rats

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ABSTRACT

Radiation hazards are considered as one of the most serious challenges that frighteningly threaten our lives. This study aimed to evaluate the antioxidant properties of aqueous and ethanolic extracts of the green alga *Ulva lactuca* against the different genotoxic and biochemical damages induced by γ-irradiation in rats. The experimental model was divided into 4 groups: group I "control" included the non-irradiated rats, group II "Irradiated group" included rats exposed to 4 Gy(^{137}Cs) whole body γ-irradiation, group III composed of rats treated with an aqueous extract of *U. lactuca* 100 mg/kg body weight for 15 days before 4 Gy of γ-irradiation, and finally group IV included rats treated with the ethanolic extract *U. lactuca* 100 mg/kg body weight for 15 days before 4 Gy of γ-irradiation. The results revealed that these algal extracts noticeably eliminated the harmful effects of oxidative stress induced by exposure to γ-irradiation through decreasing the produced levels of H_2O_2 in hepatic tissue. Furthermore, they decreased the incidence of micronucleated PCE in bone marrow cells and apoptotic DNA damage. According to the apoptotic gene expressions, the ethanolic extract of *U. lactuca* appears to be more efficient in down-regulations of apoptotic genes *Bax* and *Caspase-3* than the aqueous one. Total phenolics, flavonoids and sulphated polysaccharides, well-known in order Ulvales, are thought to be the main drivers responsible for this potent antioxidant defense mechanism. The widely-distributed green alga *U. lactuca* seems to be a promising antioxidant and anti-apoptotic tool to overcome the lethal effects of γ-irradiation.

Keywords: *Ulva lactuca*, Antioxidant, Hepatic toxicity, Gene expression, Apoptosis.

INTRODUCTION

Exposure to ionizing radiation leads to generation of free radicals, through radiolysis of water, which interact randomly with a range of intracellular biomolecules and resulted in induction of an oxidative stress causing fatal organ deterioration and cellular damage[1]. In general, it is well-know that ionizing radiation is responsible for depletion of naturally-occurring enzymatic and non-enzymatic antioxidant defenses inside the cells[2,3] and processing of systemic diseases.[4]

At the molecular level, ionizing radiation induces different kinds of cellular DNA damage (i.e., base damage; single or double strand breaks)[5] and generates clusters of reactive oxygen species (ROS) that attack DNA molecules[6,7]. Furthermore, this lesion produces genomic instability and a series of cellular consequences as gene mutation, induction of chromosome aberrations, carcinogenesis and cell death[8,9]. According to the gene expression studies, different target genes such as *Caspases*, *p21*, *bcl-2* and *Bax* are activated by more than one pathway which controlling apoptotic processes and growth inhibition[10].

Many antioxidants have been investigated to act as hepato-protectors and to decline the harmful oxidative effects induced by the ROS, formed via the ionizing radiation processes, on the normal animal cells[4]. In the last years, there is a remarkable tendency to widely use the marine algal extracts as ROS-scavenging products in bio-treatment of many diseases where they act as free-radical scavengers and antioxidants due to their specific and functional bioactive compounds[11-15]. Lee[16] reported that marine algae have numerous health-prompting effects. In addition, it have been reported that synthetic antioxidants, e.g. Butylated hydroxyanisole, are mainly implicated in carcinogenesis and liver damage[17]. The cosmopolitan green alga *Ulva lactuca* Linnaeus is one of the aforementioned algal group and usually proliferating in eutrophicated coastal waters. The bioaccumulation of minerals and heavy metals in *U. lactuca*, particularly manganese, lead, copper and cadmium, don't allow being widely-applied for human consumption or as an ingredient in some food preparations[19]. However, *U. lactuca* is mainly characterized by the presence of unique highly-charged sulphated polysaccharides known as Ulvan[18,19]. This polysaccharide displays many pharmaceutical characteristics of potential interest for diverse applications[20,21,22]. In general, the cell wall polysaccharides of order Ulvales constitute about 38-54% of the dry algal matter[23]. The present study was conducted to investigate the curative effects of the aqueous and ethanolic extracts of the green alga *U. lactuca* against the different genotoxic and biochemical effects induced by γ-irradiation in rats.

MATERIALS AND METHODS

Algal materials: collection and extraction

The green alga specimens *Ulva lactuca* Linnaeus were collected in April 2009 during a low tide at Al-Qusayr

province (26° 07` N, 34° 13` E), Red Sea, Egypt. The collected specimens were firstly washed well in the field to remove epiphytes and then by distilled water at the lab to be completely free from any debris, salts and sand particles. They were air-dried in shade for two weeks. The dried algal specimens were ground well by a blender to 2 mm or a smaller particle size. Finally, the algal materials were stored in plastic bags at room temperature in a dry dark place till using. The specimens were morphologically identified according to the relevant literature adopted by Aleem[24].

The aqueous and ethanolic *U. lactuca* extracts (1:10 w/v) were prepared according to the method described by Tariq[25] with a little modification. The dried specimens were homogenized with distilled water and ethanol (95%), and then were left overnight at room temperature. After that, the extracts were agitated in an orbital shaker at 120 rpm for 2 h and then were filtered through Whatmann® filter papers No.1. This process was repeated thrice. All the three filtrates were collected and concentrated to dryness on a rotary evaporator at 40 °C (Büchi R-200). The residues were stored at -20 °C in liquid nitrogen till further tests. On using, the algal residues were dissolved in 0.9% NaCl saline solution for the following investigations.

Gamma radiation

Whole-body γ-irradiation was carried out using a cesium (^{137}Cs) source, the Gamma Cell-40 biological irradiator at the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt. The animals were exposed to a single dose of (4 Gy) γ-rays with a dose rate of 0.48 Gy/min.

Experimental animal

Adult male Albino rats of Sprague-Dawley strains each weighing about 150–180 g, were obtained from the animal house of the National Research Center, Egypt. Rats acclimated for a period of one week before the beginning of the experiment. They were maintained in clean, sterile, polypropylene cages at a temperature of 26-28 °C, relative humidity of 60% and 12-hr light/dark cycle. They had been fed with commercial pellet diet and had access to water *Ad libitum.*

Experimental protocol

Rats were allocated into four groups. The experimental model was divided into 4 groups: group I "control" included the non-irradiated rats, group II "Irradiated group" included rats exposed to 4 Gy(^{137}Cs) whole body γ-irradiation, group III composed of rats treated with an aqueous extract of *U. lactuca* 100 mg/kg body weight intraperitonially for 15 days before 4 Gy of γ-irradiation, and finally group IV included rats treated with the ethanolic extract of *U .lactuca* 100 mg/kg body weight intraperitonially for 15 days before 4 Gy of γ-irradiation.

At the end of the experimental period, animals in all groups were fasted overnight, weighed and sacrificed by decapitation. Bone marrow was collected for cytogenetic study. Livers were excised, rinsed with shield saline, weighed and rapidly frozen in liquid nitrogen then stored at -70°C until assayed.

Hydrogen peroxide assay

Liver tissue was homogenized in 5-10 ml cold buffer (50 mM potassium phosphate buffer, pH 7.5, 1 mM EDTA) per gram tissue. The homogenate was centrifuged at 4000 rpm for 15 min at 4°C. The supernatant was removed for assay and store in ice. Liver H_2O_2 levels were assayed by colorimetric method proposed by Aebi[26] using Biodiagnostic Kits according to the manufacturer's instruction and expressed as millimolers per milligrams of tissue.

Micronucleus assay

Immediately after the animals were sacrificed, bone marrow was collected from each animal for the micronucleus assay as described by Schmid[27]. In brief, the femurs were dissected and washed with 1 ml of fetal calf serum, smeared on clean and dry slide, fixed with absolute methanol for 10 min and stained with 5% (v/v) Giemsa stain diluted in phosphate buffer. Two thousand polychromatic erythrocytes (PCEs) were analyzed per animal to ascertain the frequency of micronuclei and the micronucleated cells in the bone marrow of each rat in the different treatment groups.

Apoptotic DNA fragmentation analysis

Apoptotic changes in animals were evaluated colorimetrically by staining the DNA in hepatic tissues with Diphenylamine (DPA) according to the method adopted by Ray[28] with some modifications and detected at wavelength 600 nm. The percentage of DNA fragmentation was taken as the ratio of DNA in the supernatant to total amount of DNA in pellet and supernatant.

Molecular genetics assays

RNA Extraction

Total RNA from liver tissues was extracted using TRIzol® Reagent (cat#15596-026, Invitrogen, Germany), following manufacturer's instructions and recovered in 100 µL diethylpyrocarbonate (DEPC)-treated water by passing the solution a few times through a pipette tip. An aliquot of total RNA was diluted in RNase free water and set aside to estimate RNA quantity and integrity. The remaining sample was stored at -80°C until gene expression analysis. The concentration and purity of the RNA samples were determined using a Nano-Drop® (ND-1000 Spectro-photometer, Nano-Drop Technologies Inc, Delaware, USA).

Purity of total RNA was assessed by the 260/280 nm ratio which was between 1.8 and 2.1. Additionally, integrity assured with ethidium bromide-stain analysis of 28S and 18S bands by formaldehyde-containing agarose gel electrophoresis.

Reverse Transcription (RT) Reaction

Reverse transcription into complementary DNA was performed on 5 µg of total RNA using random primers in a final volume of 75 µl using RT-PCR kit RevertAidTM First Strand cDNA Synthesis Kit (MBI Fermentas, Germany), according to the manufacturer's instructions.

Real-time PCR (RT-PCR)

Real-time RT-PCR was performed using the Applied Biosystems StepOne™ Instrument. Intron-spanning gene-specific primers that were short enough to ensure optimum amplification chosen from published references (Table 1). For real time quantitative PCR, 2ul of first-strand cDNA in a total volume of 25 ml containing 12.5 ml 2x SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) and 1 µl of each primer (10 pmol) and 8.5 µl RNase free water. For each gene of interest, negative and positive controls were included.

Thermal cycling conditions for glyceraldehyde-3-phosphate dehydrogenase (*GADPH*), *Bax* and *Caspase* genes initial denaturation at 94°C for 5 minutes followed by denaturation at 94°C for 30 seconds was done. Amplification was carried out using 35 cycles with an annealing temperature at 58°C for *GAPDH* for 30 seconds, followed by extension at 72°C for 1 minute and a final extension at 72°C for 8 minutes. For *Bax* gene, 30 cycles of amplification with annealing at 56°C for 30 seconds, followed by extension at 72°C for 1 minute and a final extension at 72 °C for 8 minutes. For *Caspase-3* gene, 28 cycles of amplification with annealing for 15 sec at 55 °C, and primer extension for 45 sec at 72°C and a final extension at 72 °C for 8 minutes.

Calculation of Gene Expression

For each sample, a melting curve was generated after completion of amplification and analyzed in comparison to the positive and negative controls. Each PCR reaction was performed in triplicate and the average threshold cycle (CT) was used for the relative quantity (RQ) calculation after normalization to GAPDH. Mean cycle threshold (CT) values of duplicate samples were used for analysis. Relative expression of studied genes was calculated using the comparative CT method. All values were normalized to the *GAPDH* genes according to Livak and Thomas[32] as follows:

ΔCT (test) = CT(target, test) – CT(reference, test),

ΔCT (calibrator) = CT(target, calibrator) – CT(reference, calibrator),

ΔΔCT = ΔCT test sample – ΔCT calibrator sample

The relative expression was calculated by $2^{-\Delta\Delta CT}$

Statistical analysis

The results were expressed as means ± standard error (SE). All data were computerized using the Statistical Package for Social Sciences (SPSS, version 11). The results were statistically analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) for comparison between the different treatment groups. Statistical significance was set at $P < 0.05$. Percentage of protection of cellular DNA was calculated using the formula given below according to Nair and Menon[33]:

$$\% \text{ Protection} = \frac{(P_i - P_u) - (P_t - P_u)}{(P_i - P_u)}$$

Here "Pi" represents the value of a parameter of the irradiated group, "Pu" represents the value of the parameter of the non-irradiated control group and "Pt" stands for the value of the parameter of each extracted *Ulva lactuca*-irradiated group.

RESULTS

Our results in Figure 1 showed that γ-irradiation induced a significant increase in H_2O_2 level with a value of 1.54 ± 0.23 as compared to the liver homogenate in normal control rats 0.53 ± 0.01. Treatments with the aqueous and ethanolic extracts of *U. lactuca* significantly decreased the levels of produced H_2O_2 as compared to the γ-irradiated group with values of 0.81 ± 0.04 and 0.7 ± 0.09, respectively.

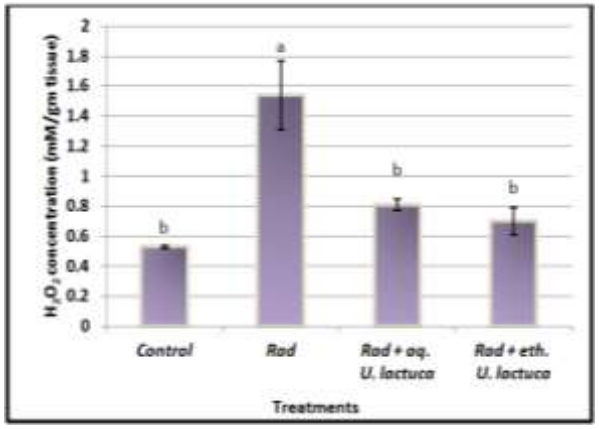

Figure 1: Effects of aqueous (aq.) and ethanolic (eth.) extracts of *Ulva lactuca* on the levels of H_2O_2 produced in hepatic tissue of rats exposed to γ-irradiation. Data are shown as mean ± SEM[a,b] significant at *P<0.05*.

The effects of aqueous and ethanolic extracts of *Ulva lactuca* on γ-irradiation-induced cytogenetic damages in bone marrow cells of male rats utilizing micronucleus assay are presented in Table 2. Micronucleus frequencies observed in polychromatic erythrocytes (MnPCEs) revealed an increase in the incidence of the number of MnPCEs containing one, two, or more than two micronucleus and in the total mean value of MnPCEs rats as compared with the control group (29.00±0.50 vs 7.50±0.29, respectively). Pre-treatments of rats with these algal extracts were found to distinctly decrease the MnPCEs to (15.50 ± 1.71, 13.50 ± 0.96 for the aqueous and ethanolic *U. lactuca* extracts, respectively) when compared to the γ-irradiated group. Moreover, the protection percentage reached 63 % in the *U. lactuca* aqueous extract, and 78 % in the ethanolic one.

The Results of DNA fragmentation are presented in Table 3. The γ-irradiation significantly increased the DNA fragmentation in the hepatic tissue by 21% as compared to the control group (6%). Treatments with the aqueous and ethanolic extracts of *U. lactuca* significantly brought down the levels of DNA damage to be 19.02 % and 15.13%, respectively. In addition, the percent of DNA protection against γ-irradiation using the ethanolic *U. lactuca* extract was in particular higher (43 %) than the aqueous one (18 %).

Table 1: Oligonucleotide primer sequences and the primer references used in real-time PCR assay.

Primers	Sense and antisense	Reference
Bax	5'-AGGATGATTGCTGATGTGGATAC-3' 5'-CACAAAGATGGTCACTGTCTGC-3'	van der Hoeven *et al*[29]
Caspase 3	5'-AAATTCAAGGGACGGGTCAT-3' 5'-ATTGACACAATACACGGGATCTGT-3'	Liu[30]
GAPDH	5-`CAAGGTCATCCATGACAACTTTG-3' 5`-GTCCACCACCCTGTTGCTGTAG-3'	Ahmed[31]

Table 2: Effects of aqueous (aq.) and ethanolic (eth.) extracts of *Ulva lactuca* on the frequency of MnPCEs in the bone marrow cells of rats exposed to γ-irradiation.

Treatments	No of Mn-PCEs/8000PCEs (Mean ± S.E.)	MnPCEs distribution			Protection %
		Mononucleated cells	Binucleated cells	Polynucleated cells	
Control	30 7.50±0.29[c] 116	30	-	-	-
Rad	29.00±0.50[a]	96	16	4	-
Rad+*aq.* *U. lactuca*	62 15.50±1.71[b] 54	58	4	-	63
Rad + eth. *U. lactuca*	13.50±0.96[b]	54	-	-	78

Within each column, means superscript with different letter are significantly different (*P< 0.05*).

Table 3: Effects of aqueous (aq.) and ethanolic (eth.) extracts of *Ulva lactuca* on the percentage of DNA fragmentation in livers of rats exposed to γ-irradiation.

Treatments	Percentage of DNA Fragmentation Mean ±S.E.	Protection %
Control	6.12±0.45 [c]	-
Rad	21.81±1.24 [a]	-
Rad+*aq.* *U. lactuca*	19.02±1.09 [a]	18
Rad + eth. *U. lactuca*	15.13±1.62 [b]	43

Within each column, means superscript with different letter are significantly different (*P< 0.05*).

Quantitative real-time PCR (qRTPCR) was used to determine apoptotic genes, *Bax* and *Caspase*-3, expression in hepatic tissues of rats exposed to γ-irradiation and possible protective effect of different extracts of *Ulva lactuca*.

All genes presented a single peak in the PCR melting curve revealed the absence of primer-dimer formation during the reaction and specificity of the amplification (data not shown).

The data of the current study represented in Figure 2 revealed that hepatic mRNA levels of *Bax* gene significantly elevated in γ-irradiated group that reached the 3.23±0.5 fold as compared to the control.

Treatments with the different extracts of *U. lactuca* markedly regulated the expressions of *Bax* mRNA, which becomes 2.63±0.38 fold with the aqueous extract and 1.68 ± 0.38 fold in the ethanolic one.

Figure 3 showed up regulation of *Caspase-3* gene level in liver tissues in γ-irradiated group that significantly increased to 4.35 ± 0.35 fold when compared to the normal control group. The expression of *Caspase-3* gene was down regulated significantly in rats treated with the ethanolic extract to 2.9±0.6 fold. However, expression of *Caspase-3* gene in the aqueous extract of *U. lactuca* treated groups (4.14 ± 0.4 fold) remained at almost the same level of γ-irradiated group. These results indicated that the ethanolic extract appears to be more efficient than aqueous extract of *U. lactuca* in down regulation of apoptotic genes in hepatic tissue exposed to γ-irradiation.

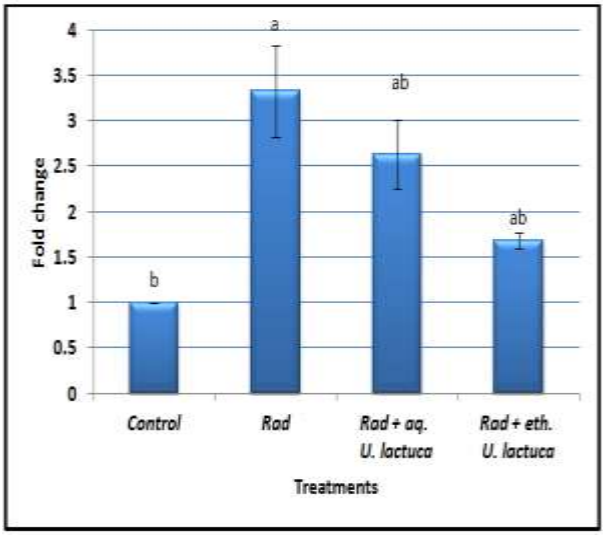

Figure 2: Relative fold change in hepatic *Bax* gene expression profiles of γ- irradiated rats treated with the *Ulva lactuca* extracts. Data are shown as mean ± SEM[a,b] significant at *P<0.05*.

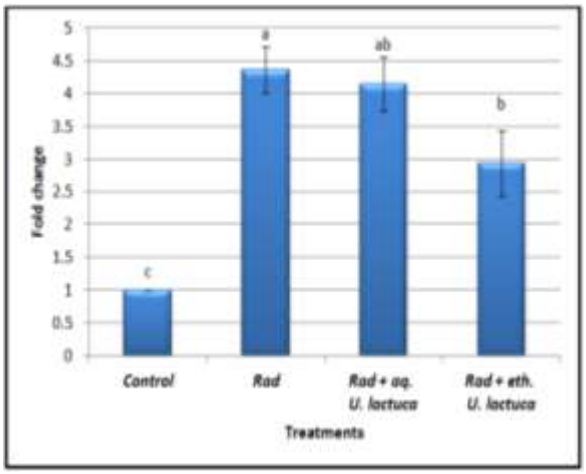

Figure 3: Relative fold change in hepatic *Caspase-3* gene expression profiles of γ- irradiated rats treated with the *Ulva lactuca* extracts. Data are shown as mean ± SEM[a,b,c] significant at *P<0.05*.

DISCUSSION

Ionizing radiation is characterized by producing a group of reactive oxygen species (ROS) that could be considered as the deleterious outcome of their harmful toxicity, and their association with different oxidative stress responses in many biological cells[34]. Our results showed that whole body γ-irradiation of rats at 4 Gy enhanced the formation of hepatic H_2O_2. Similar results have been reported by Hassan and Ibrahim[35], where they postulated that the increase in H_2O_2 production following exposure to a high dose of γ- rays may be attributed to the overproduction of ROS in the hepatic tissue. H_2O_2 and other toxic oxygen species produced in the cellular compartments induce an oxidative stress and consequently resulting in an acceleration of lipids peroxidation, DNA oxidation, and other oxidative damages including mutagenesis and apoptosis[36,37]. Rats treated with *U. lactuca* extracts distinctly decreased the levels of H_2O_2 compared with those of the radiated group. Moreover, the *U. lactuca* ethanolic extract seems to be more efficient than aqueous extract one. In general, these results could be attributed to the well-known antioxidant and total phenolic characteristics of this green alga to scavenge/or reduce the oxidative damages induced by ionizing γ-radiation. For more details, the antioxidant activities of these compounds could be referred to their superoxide and hydroxyl radicals-scavenging abilities by reducing power of singlet and triplet oxygen molecules[38,39]. In fair agreement with this observation, Abd El-Baky[40] and Abd Elmegeed[41] showed that the crude extracts of *U. lactuca* exhibited remarkable antioxidant and ROS-scavenging activities. Hassan[21] pointed out that the sulfates polysaccharides extract of *U. lactuca* highly ameliorate hepatic enzymatic (catalase, glutathione peroxidase and superoxide dismutase), non-enzymatic (reduced glutathione & total thiol) antioxidant defenses and thiobarbituric acid reactive substances in albino rats.

Genotoxic effects of γ-irradiation can be mediated by direct absorption and generating several chromosomal aberrations (single- and double-strand DNA damage) due to ionization of both the nucleobases and sugars[42] or by indirect effects of hydrogen and hydroxyl radicals produced during water radiolysis, and their interaction with cellular DNA[43]. This study clearly showed that γ-irradiation, as previously reported, induces micronuclei in bone marrow cells of exposed rats[44]. It produced a highly significant increase in the mean frequencies of MnPCE either containing one, two, or more than two MN. Evaluation of the micronuclei formation is widely-used test to assess the genotoxicity, as more than one mechanism may be integrated in the micronuclei formation, either from mitotic spindle damage or chromosome breakage[45]. Balasem and Ali[46] reported that the presence of acentric fragments or complete chromosomes, excluded as separate entities from the main nuclei and had not been incorporated into daughter nuclei at mitosis, lead to micronuclei formation. In general, treatments with *U. lactuca* extracts are markedly

efficient in reducing the number of MnPCE cells with one, two and multiple micronuclei when compared to the concurrent radiation-alone group. It was recorded that ethanolic extract of *U. lactuca* offered higher protection 78% than aqueous one, which offered a protection reached only 63 % to bone-marrow cells of those rats exposed to 4Gy γ-irradiation.

In the present study, it was also recorded that rats exposed to γ-irradiation exhibited the distinct features of apoptosis, including the increase in the percentage of nuclear DNA fragmentation. It is authenticated that the ionizing radiation leads to generation of several ROS, which causes different damages to the cells and genomic instability[47]. Apoptosis is well-known active process of programmed cell death that acts under control of molecular mechanisms and need energy to proceed[48,49]. Different studies showed an increase in the DNA damage after exposure to γ-irradiation[50,51]. Zhang[43] reported that radiation-induced DNA damage and apoptosis in human intestinal epithelial cells. However, treatment with *U. lactuca* extracts significantly reduced the percentage of DNA fragmentation and the protection was around 18 % with aqueous extract and 43% with ethanolic one.

Our results clearly demonstrated the anti-genotoxic and anti-apoptotic potentials of *U. lactuca* extracts markedly protected rats from the clastogenic effects and DNA damages from the γ-ionizing radiation and its produced ROS. In accordance with these observations, Bhagavathy and Sumathi[52] demonstrated that green algae are able to decrease the genotoxic effects of carcinogenic agents in human lymphocytes. In addition, green algae have been postulated to enhance the DNA repairing of fish and therefore inhibit fish diseases[53]. This defense mechanism might be due to the flavonoids content present in the green algae[54]. In a similar study, Balaji[55] threw the light on the free radical scavenging effects of *U. reticulata* to reduce the hepatic oxidative stress. In addition, the highly-charged sulphated polysaccharides Ulvan might have a major role in these antioxidant defenses. Sathivel[56] pointed out that *U. lactuca* polysaccharides presented a free radical quenching activity by increasing the functional ability of liver during the oxidative stress induced by D-Galactosamine.

Studying the gene expression has demonstrated the presence of more than one pathway regulating the apoptotic processes and growth inhibition[57]. Two main families of proteins are considered as the key elements on apoptosis process including, Caspase enzymes and Bcl-2 family[58,59]. *Caspase-3* is the most important member of Caspase enzymes family, which plays an effective role in apoptosis[60]. *Bax*, is proapoptotic members in Bcl-2 family that proceed programmed cell death. A specific mechanism by which apoptotic signals converge toward a common death pathway including Caspases perform apoptosis and the Bcl-2 family proteins regulate it[61]. Our study clearly indicated that rats exposed to whole body γ-irradiation showed an over expression of *Bax* and *Caspase-3* genes as compared to the control "non-exposed" rats. Concurrent with our findings, the up-regulation of *Bax* gene was detected in mice liver of 0.5 γ-irradiation mice[35]. Also, a significant elevation was observed in hepatic level of mRNA of *Bax* and *Caspase-3* genes in irradiated rats as compared to the control[10]. Earlier studies have suggested that ROS with their harmful effects on cells are increasingly involved in various cell destiny decisions and signal transduction pathways[62]. Reactive oxygen intermediates induced by ionizing radiation lead to release of apopotogenic mitochondrial mediators including a proapoptotic member of the Bcl-2 family and *Caspase*-activating-factors[63,64]. Therefore, oxidative stress has an effective role in apoptosis induced by radiation[65].

Treatments with *U. lactuca* extracts prior to γ-irradiation decreased the level of apoptotic gene expressions in rats' livers as compared to the irradiated group. In addition, the *U. lactuca* ethanolic extract seems to be more efficient than aqueous one in down-regulation of apoptotic genes, *Bax* and *Caspase-3*, in hepatic tissue. These results could be attributed to the presence of a higher amount of sulphated polysaccharides in the ethanolic extract and they are mainly responsible for its protective defense mechanism than the aqueous one. This result was in consistent with those of Mahmoud and Hussein[66] who revealed that the concomitant treatment with sulphated polysaccharides of *U. lactuca* restored the antioxidant enzyme levels in kidneys near to normal better than the aqueous extract, and exhibited a significant dose dependent protective feature against N-nitrosodiethylamine inducing nephrotoxicity. Qi *et al*[67] also stated that polysaccharides extracted from *U. pertusa* has the ability to increase the defensive function of liver against free radicals. In general, it had been reported that antioxidants affect the gene expressions as well as their scavenging properties on reactive oxygen species in laboratory animals, cultured cells, and humans[68]. This study indicated the ability of *U. lactuca* extracts to protect against γ-irradiation-induced cellular DNA damages through their direct effects on the levels of apoptotic gene expressions.

In conclusion, the green alga *Ulva lactuca*, especially the ethanolic extract, seems to be a promising tool in treatment of harmful oxidative stress of γ-ionizing irradiation on hepatic tissue, DNA damage, and apoptotic gene expressions. However, there are still perspective and outlook chemical analyses on both the aqueous and ethanolic extracts to identify their total phenolics, total flavonoids and antioxidant characterizations.

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