The Possible Impact of *Spirulina* and *Chlorella* on some Hematological and Biochemical Aspects in Irradiated Rats


* Radiation Microbiology Department, National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority
** Radiation Biology Department, National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority
* Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt.

ABSTRACT

Background: Patients undergoing radiotherapy may suffer from many harmful effects including gut microbiota injury, therefore, the use of exogenous probiotics is suggested to reduce these effects. Aim: The present study aimed to determine the possible protective effect of *Spirulina platensis* and *Chlorella vulgaris* against radiation-induced hematological and biochemical disturbances in male albino rats. Materials and Methods: Spirulina or chlorella was administered orally at a dose level of 300 mg/kg body weight daily for one week before and one week after the exposure to gamma radiation (6Gy, single dose). Results: Whole-body gamma-irradiation induced a significant decrease in total leukocyte count, red blood cell count, hemoglobin concentration, hematocrit value and platelets count, compared to their corresponding values of the control group. A significant increase in alanine aminotransferase (ALT) activity, marker of liver damage, and a non-significant increase in urea and creatinine levels, markers of kidney function was observed. Spirulina or chlorella administration pre- and post-irradiation has significantly attenuated the radiation-induced disturbances in the studied parameters. Conclusion: Oral supplementation of spirulina or chlorella may afford protection against radiation-induced detrimental effect and may preserve the tissue integrity and the functions of different organs in the body.

1. INTRODUCTION

Ionizing radiations are widely used for the treatment of cancer. Although irradiation is targeted at malignant tissues, the surrounding normal tissues may be affected. The hematopoietic system is highly sensitive to ionizing radiation, with an associated decrease in circulating blood cells and hemoglobin concentration that may further cause anemia, bleeding, and declined immune function [1]. Exposure of animals to ionizing radiation causes a series of physiological changes known as acute radiation syndrome, which is dependent on the exposure dose and may lead to death. The damage to the hematopoietic system is a major factor in mortality following acute radiation exposure.

Ionizing radiation has been shown to interact with the biological molecules in the different organs of the body producing reactive oxygen species (ROS). Hence, an over-production of ROS leads to uncontrolled chain reactions, resulting in various pathological conditions that may include disorders in the function of different organs of the body, especially the liver and kidney. The radiation-induced injury of the liver and kidney results in cell degeneration, apoptosis & necrosis, disordered enzyme activity, metabolic disorders, and functional failure of these organs [2,3].

Microalgae have been widely used as novel sources of bioactive substances. Along with this trend, the possibility of replacing synthetic preservatives with natural ones is receiving much attention. In general, microalgae are rich in various phytochemicals like carotenoids, phycocyanin, phenolics, amino acids, polyunsaturated fatty acids, and sulphated polysaccharides [4,5]. These compounds are providing excellent various biological actions including, antioxidant, antimicrobial, anti-viral, anti-tumoral, anti-inflammatory, and anti-allergy effects [6,7]. Some microalgae such as *Chlorella vulgaris* and *Spirulina platensis* have been used in several areas in nutraceutical, pharmaceutical, cosmetics, nutrition, and functional quality of foods [8]. In 2006, World Health Organization has described *Spirulina* as one of the
greatest super-foods on earth serving as an example of the potential of microalgae.

This study was designed to investigate the effect of $\gamma$-irradiation on some aspects of the haemopoietic system which is known to be a relatively radiosensitive biological target and some biochemical aspects linked to liver and kidney function, as well as to investigate the possible impact of supplementation of *Spirulina platensis* or *Chlorella vulgaris* in modulating the damaging effect induced by exposure to $\gamma$-radiation.

2. MATERIAL AND METHODS

2.1. Experimental animals

Adult male Wistar rats were used (weighting 130-160 gm). Animals were obtained from the animal house that belongs to the National Centre for Radiation Research and Technology (NCRRT) Cairo, Egypt. Animals were kept in good ventilation conditions and had free access to water and standard pellet concentrated diet and were adapted in specially designed cage 5 rats per cage and a 12-12 dark-light cycle and under normal pressure and temperature conditions. All experimental procedures were carried out according to the Ethics Committee of the NCCRT, Cairo, Egypt (The approval No: 37A / 21).

2.2. Radiation Facility

Whole-body $\gamma$- irradiation was performed at the NCRRT, Cairo, Egypt using a ventilated Canadian $^{137}$Cs Gamma Cell-40 at a dose rate of 0.39Gy/min. Rats were exposed to 6 Gy.

2.3. Culturing of microalgae:

The microalgae species *C. vulgaris* and *S. platensis* were obtained from the Algal Culture Collection at Al-Azhar University (ACCAZ). *C. vulgaris* isolate was grown on BG11 medium [9] and *S. platensis* was on Zarrouk’s medium [10]. With exposure to fluorescent light and aeration with 12/12 dark-light cycle at a temperature of 30°C for two weeks. Both algal species were harvested and then oven-dried at 50°C to obtain the biomass powder. The *S. platensis* and *C. vulgaris* in powder form were suspended in distilled water to feed rats orally.

2.4. Experimental Design

After one-week accommodation, the animals were divided into 6 equal groups ($n = 8$ rats per group): 1- Control (C): animals of this group were kept normally as a control group, 2- *S. platensis* (SP): Rats were orally supplemented with *S. platensis* (300 mg/kg) daily for two weeks, 3- *C. vulgaris* (CH): Rats were orally supplemented with *C. vulgaris* (300 mg/kg) daily for two weeks 4- Radiation (R): Rats were whole-body exposed to $\gamma$- radiation (6 Gy) without being fed on algae, 5- *S. platensis* + Radiation (SP+R): Rats were supplemented with *S. platensis* for one week before and one week after exposure to $\gamma$- radiation, and 6- *C. vulgaris* + Radiation (CH+R): Rats were supplemented with *C. vulgaris* daily for one week before and one week after exposure to $\gamma$- radiation. At the end of the experiment (one week after irradiation or one day after the last dose of the treatment) the animals were anesthetized with an intraperitoneal injection of pentobarbital, 60 mg/kg according to Shekarforoush et al[11]. The blood was collected for further hematological and biochemical analysis.

2.5. Hematological and biochemical analysis

Intracardiac blood samples were collected in two vials. The first vial containing ethylene diamine tetra-acetic acid as an anticoagulant was used for hematological analysis. The total number of leukocytes, erythrocytes count, platelets count, hematocrit (Hct) %, and hemoglobin (Hb) concentration were estimated by a blood cell counter (Diamond). The second vial does not contain any anticoagulants, allow standing for 30 min at room temperature then centrifuged at 3000 rpm for 15 min and serum was separated for biochemical analysis. Serum transaminases (ALT and AST) were determined according to Reitman and Frankel [12]. Urea and creatinine levels were determined according to Henry [13] and Patton and Crouch [14] respectively.

2.6. Statistical analysis

To determine the significance between treatments (fixed factor with six levels: C, R, SP, CH, SP+R, and CH+R), one-way analysis of variance (ANOVA) was used, and the significance was tested at $\alpha = 0.05$. Normality of data was checked with Shapiro-Wilk’s test and equal variance within treatments was analyzed by Levene’s Median test to check for the ANOVA Assumptions. Results that did not meet the assumptions were transformed before running the ANOVA test. When a significance between treatments was revealed, pairwise multiple comparisons were performed using Tukey’s pairwise test.

3-RESULTS

The protective effect of *S. platensis* and *C. vulgaris* against gamma irradiation was estimated based on some hematological and biochemical aspects in irradiated rats. The results presented in Fig. 1&2 showed non-significant changes in all studied parameters upon supplementation of *S. platensis* and *C. vulgaris* to
normal rats compared to their normal control counterparts.

Whole-body irradiation at dose level of 6Gy resulted in a significant decrease in the total leukocytic count (the percentage change amounted to 91.5 %) compared to the control value. *S. platensis* and *C. vulgaris* supplementation for one week before and one week after exposure to γ- radiation induced insignificant increase (P < 0.05) in total leukocytic count compared to the corresponding value of the irradiated group, and was still significantly lower than the normal control value, Fig. 1(A).

Irradiation of rats resulted in a significant decrease in the red blood cell count (P < 0.05), the percentage change amounted to 23%. Supplementation with *S. platensis* or *C. vulgaris* for one week before and one week after exposure to γ- radiation induced a significant increase (P < 0.05) in the red blood cell count by 11.4% and 19%, respectively compared to the corresponding values of the irradiated group, Fig. 1(B).

Whole-body irradiation Fig. 1(C) resulted in a significant decrease in the hemoglobin content (the percentage change amounted to 29.8%). Supplementation with *S. platensis* and *C. vulgaris* showed a significant increase in the hemoglobin content compared to the corresponding values of the irradiated group (the percentage change was 7.7% and 19.4%, respectively).

Whole-body irradiation Fig. 1(E) resulted in a significant decrease in the platelets content (the percentage change amounted to 93.8%). Supplementation with *S. platensis* and *C. vulgaris* induced amelioration of these changes compared to the corresponding value of the irradiated group (the percentage change was 55.6 % and 65.7 %, respectively).

The results in Fig.2 (A) revealed that exposure to ionizing radiation resulted in a significant elevation in serum ALT activity, compared to their values of the control group. Oral supplementation with *S. platensis* and *C. vulgaris* has ameliorated this change.

The results in Fig.2 (B, C, D) indicated that exposure to ionizing radiation (6 Gy) resulted in a non-significant increase in serum AST, urea and creatinine levels as compared to their values of the control group.
Exposure to ionizing radiation causes damage to cells directly by ionization of cellular targets and indirectly through reactive oxygen species (ROS), which leads to disruption of membrane lipids, disturbance of cellular function, and thus damage to various organs of the body [2,15]. On the other hand, there has recently been an increasing interest in finding safe biological agents to control radiation hazards. Natural products are considered a vital source of protection against ionizing radiation-induced cellular damage. Much of attention was directed to Prebiotics and probiotics, due to their ability to reduce free radicals’ formation [16]. *C. vulgaris* and *S. platensis* are microalgae, which are rich in various phytochemicals like carotenoids, phycocyanin, phenolics, amino acids, polyunsaturated fatty acids, and sulphated polysaccharides [4, 5]. Microalgal biomolecules have remarkable potential antioxidant, anticoagulant, radioprotective, anticancer, antiviral, and antiallergic properties [17, 18, and 19]. Polysaccharides have been found to interact with ionizing radiation by reacting with the ROS produced by the ionization of water and other molecules [20].

In the present study, we investigate the possible radioprotective effect of *C. vulgaris* or *S. platensis* against radiation induced hematological disturbances associated with some biochemical disorders linked to liver and kidney damage.

The present data demonstrated that whole-body irradiation (6 Gy) induced a significant decrease in the total leukocytes, red blood cells, platelets, hemoglobin content, and hematocrit values compared to the corresponding values of the control group. These results are in line with those of Dong et al [1] who reported that whole body irradiation at the same dose can cause injury in hematopoiesis and can result in peripheral blood cell cytopenia. Previously, it was observed that whole body irradiation with single doses of several grays, destroyed so many of the radiosensitive hematopoietic tissue cells in such a short time and with so marked a subsequent

Fig. (2): Mean of biochemical parameters values detected within each treatment group. Capital letters represent the significance between treatments. Error bars represent standard error (±SE).
reduction in the number of blood cells, especially leukocytes and platelets [21]. Leukocytes and platelets are both sensitive to ionizing radiation and dropped markedly in irradiated mice at the 7th day after exposure to different doses of ionizing radiation. This drop in circulating cells means that their release from the bone marrow has stopped due to the disturbance in the hematopoietic function of the bone marrow [21]. In view of the higher radio sensitivity and the shorter life span of leukocytes in the peripheral blood in comparison with red cells, the leukocytic count drops drastically in a short period of time. Evidently, the total leukocytic count reflects more clearly the changes in blood as a result of irradiation.

The marked decrease in red blood cell count, hemoglobin content and hematocrit value 7 days after irradiation agree with those of Mahmoud et al [22] who reported that gamma radiation (4Gy) induced significant decrease in red blood cell count, hemoglobin content and hematocrit (%) 1 and 3 weeks after irradiation. The decrease in red blood cell count is mainly due to a drop in their production, increased their destruction, loss of erythrocytes through damaged and increased permeable capillaries and later hemorrhage associated with thrombocytopenia. It could be concluded that erythrocytopenia develops to a still lesser degree after irradiation because of the much longer circulation time of the erythrocytes as compared with leukocytes and platelets and because of the earlier recovery of erythropoiesis. The decrease in the hemoglobin content may be due to disturbance in the structure of the hemoglobin molecule and increase in its viscosity and conductivity [23].

This reduction in the numbers of circulating blood cells of various kinds after irradiation, is largely a reflection of the effects of radiation on their radiosensitive precursor cells in hematopoietic tissues, the kinetic of hematopoietic cell maturation, and the circulation times and survival times of the blood cells [24].

Furthermore, in the present study oral supplementation of C. vulgaris and S. platensis exhibited improvement in hematological parameters (RBCs, platelets, Hb, hematocrit % and hemoglobin concentration) after irradiation which is probably due to antioxidant properties enabling regeneration of hematopoietic cells and reducing oxidative stress. This result is supported by Abou Gabal et al [25] who found that S. platensis has induced a positive effect on hemopoiesis in mice exposed to carbon tetrachloride. Recently, it was observed that S. platensis enhanced RBCs count and Hb concentration in rats suffering from iron deficiency anemia [26] and in mice exposed to cyclophosphamide-induced bone marrow toxicity [27], suggesting that spirulina could play an important role in the erythropoiesis process. On the same concern, Sayed et al [28] recommended C. vulgaris as a feed supplement to improve haemato-biochemical alterations and oxidative damage induced by polyethylene microplastic toxicity in the African catfish, due to its antioxidant contents, such as chlorophyll, polyphenol, vitamins, and sulfur-containing compounds that have the capacity to scavenge free radicals. On the contrary, the results showed that oral supplementation of C. vulgaris and S. platensis did not improve the leukocyte count. This may be due to the short treatment period or insufficient dose used.

Since the liver is a vital organ involved in detoxifying harmful drugs and chemicals, liver injury may arise from excessive exposure to toxins and can progress to severe liver diseases. Producing toxic intermediate products such as free radicals after exposure to ionizing radiation can induce liver metabolic disturbances. It is well known that ALT and AST are found in serum and organ tissues, especially the liver and are important enzymes in carbohydrates and amino acid metabolism. Injury or diseases affecting the liver causes a release of these enzymes into the blood stream. The results of the present study showed that whole-body irradiation at a dose level of 6 Gy induced a significant increase in the activity of liver marker enzyme, ALT and no significant change in AST. This result come in accordance with that of Abdel-Aziz [3] who reported that exposure to environmental toxins as gamma radiation (6 Gy) and/or carbon tetrachloride induced complex cellular events, including oxidative stress, lipid peroxidation and inflammation that may undergo liver injury and disturbance in its metabolic function. It was reported that the increase in liver enzymes levels in serum reflects an increase in plasma membrane permeability, which may be associated with cell death [29]. Although the increase ALT activity after radiation exposure, AST was not affected, this may be due to the differences in their behavior; AST is less radio sensitive than ALT.

Again, the results of the present study showed that oral supplementation of C. vulgaris or S. platensis after whole body irradiation exhibited inhibition in ALT activity compared to the corresponding value of the irradiated group. This effect may be due to the high quantities of phycocyanin and phenolic compounds and their antioxidant capacities [4, 5].
The fact that *C. vulgaris* or *S. platensis* could ameliorate gamma radiation and chemical toxicants-induced increase in serum ALT activity points to their possible protective effects on the liver, probably mediated through reducing the level of lipid peroxidation, improving antioxidant defense system and suppressing reactive ROS [30,31]. Previously, Wu et al [32] reported that *S. platensis* protective effect may be attributed to its ability to scavenge the oxidation-initiating agents produced during the oxidation of proteins and lipids. Salah El Din et al [33] observed that treatment with a low dose of *S. platensis* (500 mg/ kg body weight) and not a high dose (1000 mg/ kg body weight) prior to irradiation induced protection against oxidative stress, evidenced by improvement of liver function.

The kidney is a vital organ that participates in the detoxification of xenobiotics by releasing them through the urine. Excessive production of reactive oxygen and nitrogen species has been reported as the main mechanism of radiation-induced kidney injury. The results of the present study showed that exposure to ionizing radiation induced non-significant increase in serum concentration of urea and creatinine. This result disagrees with Abdel Aziz [2] who observed a significant increase in urea and creatinine levels eight days after exposure to 6 Gy. This may be due to the differences in animal species, the environmental factors, and the time interval after irradiation. On the other hand, Moussa [34] observed that dietary supplementation with *S. platensis* was renoprotective, preventing deterioration of renal function induced by ionizing radiation due to its potential antioxidant properties. Moreover, *S. platensis* was reported to exhibit a significant protective effect against lead-induced oxidative stress in the kidneys of adult and newborn rats [35, 36]. It also decreased blood pressure, a known accelerant of kidney disease propagation [37]. In addition, *C. vulgaris* was shown to protect against paracetamol-induced renal toxicity and to prevent the disruption of kidney function through their free radical scavenging and antioxidant effects, and thus can be used as a prophylactic agent against paracetamol toxicity [38].

**CONCLUSION**

According to the results obtained in this study, oral supplementation of *C. vulgaris* or *S platensis* may afford protection against radiation-induced detrimental effects and may preserve the tissue integrity and the functions of different organs in the body especially the hematopoietic organs. However, it is suggested to conduct future experiments with increasing the dose of *Chlorella* and *Spirulina* and duration of treatment to observe their effects on the leucocytic count and other organs.

**REFERENCES**


