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Prophylactic effect of Spirulina platensis on radiation-induced thyroid disorders and alteration of reproductive hormones in female albino rats

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► ***Original article***

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ABSTRACT

Background: Ionizing-radiation induces oxidative stress and thyroid toxicity. Thyroid function disorders have a great impact on fertility in both sexes. ***Materials and Methods***: Forty female rats were divided into four groups. Control, Spirulina-treated (300 mg/kg); given orally for 15 days, γ-irradiated; given (5 Gy whole body γ-rays) and Spirulina+irradiated; given Spirulina for 15 days before irradiation. Animals were sacrificed the 3rd day post-irradiation. The level of the oxidant/antioxidant markers: Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) was evaluated. In addition, caspase-3 activity was measured as apoptotic marker and comet assay to detect DNA-damage. Serum thyroid stimulating hormone (TSH), triiodothyronine (T3) and thyroxine (T4) were determined to evaluate the thyroid function alterations. Also, analysis of reproductive hormones; follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2) and progesterone (P4) was detected. ***Results:*** Whole body γ-irradiation-induced oxidative stress, denoted by significant decreases of antioxidant markers and an increase in MDA content. The activity of caspase-3 was significantly increased and comet assay revealed DNA damage. Also, serum level of TSH was significantly increased, while T3, and T4, significantly decreased in irradiated rats. Moreover, the reproductive hormones showed significant decreases. Spirulina treatment has significantly attenuated oxidative stress in thyroid tissues, decreased caspase-3 activity and ameliorated DNA damage, concomitant with significant amelioration in the levels of thyroid and reproductive hormones. ***Conclusion:*** Spirulina may alleviate γ-rays-induced thyroid damage and play a significant role in the regulation of thyroid and reproductive hormones in female rats.

Keywords: *Spirulina platensis, thyroid, gamma radiation, caspase-3, comet assay.*

INTRODUCTION

The thyroid gland is a butterfly shaped organ with two lobes connected by an isthmus which is located in the lower part of the neck. It is among the larger endocrine glands typically weighing (10–30) grams in individuals with sufficient iodine intake. It is required for normal growth and maturation of the body, increases oxygen consumption, and helps regulate lipid and carbohydrate metabolism. The gland produces and stores T3 and T4 hormones which are essential for physiological mammalian development and are well known to play fundamental roles in the cardiovascular, nervous, immune and reproductive systems [1]. Previous studies have found that thyrotoxicosis and hypothyroidism can disturb the menstrual cycle and ovulation [2]. Furthermore, thyroid hormones play an essential role in follicular development in the ovary of postnatal and immature rats [3]. Nevertheless, the mechanisms by which thyroid hormones affect reproductive capacity are poorly understood, and various

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factors may participate alone or in combination [4].

Radiation therapy is widely used in the management of head and neck tumors, lymphomas and malignancies of the central nervous system. Although the thyroid gland is not usually the radiation target, its exposure during radiotherapy especially of the head and neck tumors, is often unavoidable. Hypothyroidism after radiotherapy for head and neck cancer was first reported in the 1960s [5]. Since then, many publications have described radiotherapy-induced thyroid disorders such as hypothyroidism, thyroiditis, Graves' disease, adenoma, and carcinoma [6]. The main risk of the radiation exposure of cells arises from the formation of reactive oxygen species (ROS) and the damage of cellular components such as DNA, cell membranes or cell organelles leading to loss function or even cell death [7]. Also it has been shown that the radiation-susceptibility of the thyroid was increased with an elevated level of TSH in rats [8]. So far no drug has been shown to prevent radiation-induced thyroid damage [9].

Oxidative stress is defined as a disturbance in the balance between the production of ROS and the body's antioxidant defense capacity [10]. Compelling evidence demonstrated that oxidative stress plays a critical role in the normal functioning of the female reproductive system [11], and in the pathogenesis of female infertility [3]. ROS react with multiple cellular components, such as DNA, RNA and proteins, resulting in apoptosis, senescence or necrosis [12]. Apoptosis occurs under the direction of an active, intracellular death program that can be stimulated or inhibited by environmental agents such as irradiation, chemotherapeutic drugs, and ROS [13]. By activating caspases, ROS may initiate the propagation of a series of reactions that ultimately trigger apoptosis [14].

The cyanobacteria; *Spirulina platensis,* is a filamentous blue-green alga belonging to the *Oscillatoriaceae* family that is generally found in tropical and subtropical regions in warm alkaline water. It is characterized by high nutritional value as high protein content (60–70% by dry weight), vitamins, amino acids, gamma-linoleic acid, and minerals [15]. The activity of Spirulina was related to the active biliprotein phycocyanin, which has significant antioxidant and radical scavenging properties, offering protection against oxidative stress [16]. It has been reported that Spirulina protected and normalized the increase of mast cells of female rat's ovaries from toxic impact of many chemicals and toxicants [17-19]. Due to its nutrient profile, Spirulina may be beneficial for certain thyroid conditions like hypothyroidism [20]. Therefore, the present study is designed to evaluate the protective properties of *Spirulina platensis* against gamma radiation-induced oxidative stress, DNA damage and increased caspase activity in the thyroid tissues of female rats associated to variations in the level of thyroid and reproductive hormones.

MATERIALS AND METHODS

Animals

All animal procedures were carried out in accordance with the Ethics Committee of the National Center and conformed to the "Guide for the care and use of Laboratory Animals" published by the US National Institute of Health (NIH publication No. 85-23, 1996). Female albino rats, 2 months old obtained from the Egyptian Holding Company for Biological Products and vaccines were used as experimental animals. The rats were housed in cages and maintained under standard conditions of ventilations, temperature (22-24^{o}C), humidity and lighting (light/dark: 13 h/11 h). Food and water were available *ad libitum*.

Radiation processing

Whole body gamma-irradiation of female rats was performed by the exposure of animals to an acute single dose of 5-Gy using a ^{137}Cs Gamma-cell 40 source (Atomic Energy of Canada Ltd, Ottawa, Ontario, Canada), belonging to National Center for Radiation Research and Technology (NCRRT) at a dose rate of 0.42Gy/ minute at the time of experimentation. The dose was selected to induce significant biochemical changes in order to evaluate the protective role of *Spirulina platensis*.

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Reagents

Spirulina platensis powder was purchased from the DXN Company (Malaysia) suspended in water and administered daily for 15 successive days to female rats by gavages needle at a dose of 300 mg/kg body [21]. All other chemicals used were of the highest purity grade available.

Experimental design

Animal groups: Forty young adult female albino rats (2 months old) were divided into four groups (n=10). Control group; rats received normal food and water during the experimental period, irradiated group; rats were exposed to 5-Gy whole body γ-irradiation, Spirulina-treated group; rats received Spirulina for 15 successive days and Spirulina-treated irradiated group; rats received Spirulina for 15 successive days before irradiation. The last dose of Spirulina was given 60 minutes before irradiation. All rats had free access to tap water and diet.

Blood samples were collected from each rat, to separate the sera for biochemical assays. The thyroid glands were quickly excised and homogenized (10% w/v in cold phosphate buffered saline (PBS) using a Teflon homogenizer (Glass-Col, Terre Haute, Ind., USA). The homogenate was centrifuged at 1000xg for 10 minutes at 4°C by means of a refrigerated centrifuge (K3 Centurion Scientific Ltd, London, UK) and the clear supernatant was stored at −20°C. The supernatant of rat's thyroid gland homogenate was used to determine the activities of SOD, CAT, GSH-Px and MDA content.

Estimation of biochemical parameters

Biochemical analyses of hormones: Triiodothyronine (T3), thyroxine (T4), thyroid-stimulating hormone (TSH), luteinizing hormone (LH) and follicle stimulating hormone (FSH) were quantified in serum according to the manufacturers manual by enzyme linked immunosorbent assay (ELISA) kits (Biovendor company, Catalog No.: ab 108663, 100660, 178664 respectively & RSHAKRFS-010R for FSH and Rshakrlh-010S for LH). Serum concentrations of estradiol (E2) and progesterone (P4) were determined quantitatively using commercial ELIZA kits (MP Diagnostics Cat no. 07138171 and 07DE99881).

Assessment of oxidative stress in thyroid tissues was performed using commercial kits (Diamond Company). Lipid peroxidation was assayed in thyroid homogenates by measurement of malondialdehyde (MDA) formation according to the method of Yoshioka *et al.* [22]. Superoxide dismutase (SOD) activity was determined following the method of Minami and Yoshikawa [23]. Activities of catalase (CAT) and glutathione peroxidase (GSH-Px) were determined according to the methods of Sinha [24] and Flohe and Gunzler [25], respectively.

The estimation of caspase-3 activity in thyroid cell lysate was performed according to the manufacture of R&D Systems kits, (Catalog Number: BF3100) based on the detection of the chromophore p-nitroanilide (pNA) after cleavage from the labeled substrate DEVD-pNA (acetyl - Asp - Glu - Val - Asp - p - nitroanilide). The cleavage of the peptide by the caspase releases the chromophore pNA, the level of caspase enzymatic activity in the cell lysate is directly proportional to the color reaction. The pNA was quantified at 405 nm using a T60 UV/ VIS spectrophotometer (PG instruments, London, UK).

The analysis of DNA damage in thyroid tissues by the comet assay was carried out according to Singh *et al.* [26]. Images of comet analysis were obtained using a JENOPTIK camera (Germany), equipped with an UV filter block connected to a fluorescent microscope (Delta Optical, Poland). Slides were scored using image analysis system to assess the quantitative and qualitative extent of DNA damage in the cells by measuring the tail length and percentage of DNA migrated. Finally, the program calculates tail moment. The comets tails lengths were measured from the middle of the nucleus to the end of the tail. Measurements were made for approximately 50cells per analyzed slide after image archiving. A quantitative measure of the DNA damage and the difference between cells were estimated on the basis of percentages of damage in cell's tail of control versus irradiated one.

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Statistical analysis

Data were analyzed using one-way analysis of variance ANOVA. The results obtained were expressed as mean± standard error (SE), followed by post hoc Tukey's test for multiple comparison of mean. The difference was considered significant when (P-value is <0.05).

RESULTS

The results obtained showed a significant increase (P<0.05) in serum TSH and a significant decrease (P<0.05) in the level of the thyroid hormones T4 and T3 in irradiated rats in comparison to control group. Interestingly, the decrease of T3 and T4 and the increase of TSH were significantly lower (P<0.05) in the irradiated animals treated with Spirulina before irradiation compared to their relative levels in irradiated rats. The administration of Spirulina to normal rats had no significant effect (P>0.05) on the level of serum TSH, T4 and T3 compared to control group (table 1).

Values are expressed as Means± SE, (*n*=10). Values between brackets show percentage of change from control. NS: not significant vs the control group; [a]: Significant vs the control group at p < 0.05 level, [b]: Significant vs the irradiated group at p < 0.05 level.

With respect to the effect of radiation on the reproductive female hormones the results revealed a significant decrease (P<0.05) in the levels of serum FSH, LH, E2 and P4 in the irradiated group in comparison to the control group. The decrease was significantly (P<0.05) ameliorated in Spirulina-treated irradiated rats. The administration of Spirulina to normal rats had no significant effect (P>0.05) on the level of reproductive female hormones compared to control rats (table 2).

Values are expressed as Means± SE, (*n*=10). Values between brackets show percentage of change from control. NS: not significant vs the control group; [a]: Significant vs the control group at p < 0.05 level, [b]: Significant vs the irradiated group at p < 0.05 level.

The effect of radiation on the antioxidant status of thyroid gland revealed a significant decrease (P<0.05) in SOD, CAT and GSH-Px activities. The decrease of antioxidant activity was accompanied by a significant increase (P<0.05) in the content of MDA, compared to the control group. The administration of Spirulina pre-irradiation has significantly ameliorated the radiation-induced oxidative stress. Additionally, there was almost no significant difference (P>0.05) between the Spirulina-treated group and the control group (table 3).

Values are expressed as Means± SE, (*n*=10). Values between brackets show percentage of change from control. NS: not significant vs the control group; [a]: Significant vs the control group at p < 0.05 level, [b]: Significant vs the irradiated group at p < 0.05 level.

A significant elevation (P<0.05) in caspase-3 activity was recorded in irradiated rats compared to control rats, which was significantly ameliorated in Spirulina-treated irradiated rats. There was no significant difference (P>0.05) between Spirulina-treated group and the control group (table 4).

Values are expressed as Means± SE, (*n*=10). Values between brackets show percentage of change from control. NS: not significant vs the control group; [a]: Significant vs the control group at p < 0.05 level, [b]: Significant vs the irradiated group at p < 0.05 level.

Table 1. Effect of Spirulina platensis on the level of thyroid stimulating hormone (TSH), tri-iodothyronine (T3), and thyroxine (T4) in the serum of female albino rats.

Animal groups	**TSH** (µIU/ml)	**T3** (ng/dl)	**T4** (µg/dl)
Control	0.930 ± 0.040	0.860 ± 0.140	10.200 ± 1.100
Spirulina	0.980 ± 0.040 [NS] (+5)	0.895 ± 0.144 [NS] (+4)	10.210 ± 1.152 [NS] (--)
Irradiated (5Gy)	1.700 ± 0.26 [a] (+83)	0.438 ± 0.089 [a] (-49)	4.510 ±0.071 [a] (-56)
Spirulina + Irradiated	1.100 ± 0.25 [b] (+18)	0.710 ± 0.120[ab] (-17)	8.100 ± 0.310 [ab] (-21)

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Table 2. Effect of Spirulina platensis on the level of follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2) and progesterone (P4) in the serum of female albino rats. Progesterone ng/ml.

Animal groups	FSH (IU/L)	LH (IU/L)	E2 (pg/ml)	P4 (ng/ml)
Control	0.84 ± 0.070	0.94 ± 0.130	16.25 ± 0.03	43.05 ± 2.22
Spirulina	0.88 ± 0.071 ^{NS} (+5)	0.97 ± 0.131 ^{NS} (+3)	16.85 ± 0.02 ^{NS} (+4)	44.10 ± 3.00 ^{NS} (+2)
Irradiated (5Gy)	0.64 ± 0.073^{a} (-24)	0.53 ± 0.029 ^{a} (-44)	12.02 ± 0.04 ^{a} (-26)	33.00 ± 2.00^{a} (-23)
Spirulina + Irradiated	0.75 ± 0.070 ^{ab} (-11)	0.71 ± 0.032 ^{ab} (-24)	13.75 ± 0.08 ^{ab} (-15)	39.01 ± 1.95^{ab} (-9)

Table 3. Effect of Spirulina platensis on the activity of the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), and the content of the lipid peroxidation product malondialdehyde (MDA) in the thyroid tissue of female albino rats.

Animal groups	SOD (U/mg protein)	CAT (U/mg protein)	GSH-Px (U/mg protein)	MDA (nmole/mg protein)
Control	82.30±0.450	11.22±0.050	56.23±0.054	50.01 ± 2.50
Spirulina	83.20±0.430 ^{NS} (+1)	11.45±0.060 ^{NS} (+4)	57.23±0.055 ^{NS} (+2)	48.90 ± 1.33 ^{NS} (-2)
Irradiated (5Gy)	64.10±0.280 ^{a}(-22)	8.26±0.020 ^{a} (-25)	42.22±0.053 ^{a} (-25)	70.00 ± 3.00 ^{a} (+40)
Spirulina+Irradiated	73.65±0.350 ^{ab}(-11)	9.75±0.050 ^{ab} (-11)	48.22±0.052 ^{ab} (-14)	60.05 ± 2.95^{ab} (+20)

Table 4. Effect of Spirulina platensis on DNA damage in the thyroid tissue of female albino rats.

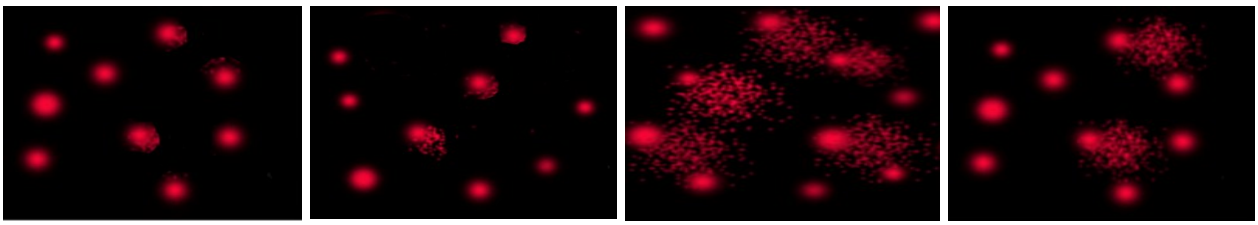
Animal groups	Caspase-3 (µmol/mg protein)
Control	0.30 ± 0.060
Spirulina	0.31 ± 0.135^{NS} (+3)
Irradiated (5Gy)	0.53 ± 0.126^{a} (+77)
Spirulina + Irradiated	0.36 ± 0.081^{ab} (+20)

The results presented in table 5 and figure 1 revealed that the exposure of female rats to gamma-irradiation induced DNA damage in the thyroid tissues. There was an elongation in DNA tails in irradiated rats and the damage was significantly ameliorated by Spirulina treatment.

Values are expressed as Means± SE, (n=10). NS: not significant vs the control group; ^{a}: Significant vs the control group at $p < 0.05$ level, ^{b}: Significant vs the irradiated group at $p < 0.05$ level.

Table 5. Effect of Spirulina platensis on DNA damage in thyroid cells of female rats in the different groups.

Animal groups	Tail length (µm)	% of DNA migrated	Tail moment
Control	3.40 ± 0.02	0.87 ± 0.90	0.01 ± 0.02
Spirulina	4.20 ± 0.04^{NS}	0.98 ± 0.01 ^{NS}	0.04 ± 0.03 ^{NS}
Irradiated (5Gy)	173.30 ± 18.61^{a}	75.63 ± 7.96 ^{a}	130.30 ± 24.48 ^{a}
Spirulina + Irradiated	58.25 ± 7.50^{ab}	40.77 ± 8.84 ^{ab}	20.64 ± 6.26 ^{ab}



A- Control B- Spirulina C- Irradiated D- Spirulina + Irradiated

Figure 1. Representative comet assay for DNA damage.

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DISCUSSION

Whole body exposure to a high dose of radiation (> 1.0-1.5 Gy) usually induces an acute radiation syndrome or radiation sickness [27]. Accumulated evidence demonstrated that oxidative stress in different tissues is the underlying mechanism of radiation toxicity [28, 29].

The thyroid gland has been considered as a radiosensitive organ [30] and the effect of radiation on the thyroid is well documented [10, 31]. In the current study, whole body exposure of irradiated female albino rats (5Gy), has significantly increased the level of TSH, reduced the synthesis of serum T3 and T4 as well as increased their metabolism verifying that radiation induced hypothyroidism [32, 33,34,35]. Results also showed that irradiated group has significantly decreased the levels of serum FSH and LH. These were consistent with the previous findings of Rezk and Abd El-Azime [36] who observed that exposure of rats to a single shot dose of (4 Gy) gamma irradiation was significantly diminished serum FSH and LH levels, and corroborated that hypothyroidism and elevated serum TSH associated with decreasing of FSH and LH [37]. Both, E2 and P4, were playing essential roles in uterine development and menstrual cycle. In the current study, the decrease of E2 and P4 were concomitant with the decrease of T4 and T3 substantiate that thyroid hormones might influence uterine development by altering the reproductive hormone levels [38]. The results confirm also that hypothyroidism and increased serum TSH are associated with a reduction in the level of E2 [37].

Lipid peroxidation in thyroid tissue demonstrated a significant imbalance between the activity of peroxidation processes and antioxidant defense system [11]. This creates the conditions for the damaging action of peroxidation processes on the thyroid structures and the impact of ROS on pro- and anti- apoptotic targets and mechanisms mediated directly or indirectly through the intracellular redox-dependent signal-conveying systems [39].

In thyroid tissue lysate of irradiated rats, a significantly elevated caspase-3 activity was observed compared to control group. Reactive oxygen intermediates induced by ionizing radiation can trigger mitochondrial pathway to release caspase-activating-factors. Therefore, oxidative stress may play a direct role in radiation-induced apoptosis [14].

In comet assay, the factors that influence the response of living cells to radiation are the DNA repair status, the physiological state of cells, the presence of oxygen and chemicals, as well as pre -and post-irradiation treatments [40]. The protective effect of Spirulina against γ-radiation-induced DNA damage in thyroid cells is in agreement with the finding of Ashton [20] who reported that β-carotene and unique polysaccharides of Spirulina may reduce cell damage especially DNA breaks and enhance cell nucleus enzyme activity. These results may be attributed to enhance repairing of DNA damage.

Spirulina is well documented for its clinical importance in diabetes, hypertension, and cancer [41] besides its antioxidant, immune-modulating, anti-microbial [42] and radio-protective properties [28, 29]. In the current study, the administration of 300 mg / kg of *Spirulina platensis* to normal rats had no significant effect on oxidative stress, DNA and caspase-3 activity in thyroid tissues. Also the investigated serum hormone showed normal levels. On the other hand, Spirulina administration for 15 days before irradiation provided female rats a substantial protection against the harmful effects of radiation. Results indicated that Spirulina was non-toxic, bio-available and provide significant multi-organ protection [43, 44] owing to its free radical scavenging and potent antioxidant activity [45]. Spirulina treatment has significantly attenuated oxidative stress in thyroid tissues, decreased caspase-3 activity and ameliorated DNA damage. This was associated to a significant amelioration in the level of thyroid and reproductive hormones.

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CONCLUSION

Spirulina may alleviate radiation-induced thyroid damage and play a significant role in the regulation of thyroid and reproductive hormones in female rats.

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