Egypt. J. Rad. Sci. Applic. **Vol. 33,** No.1, pp. 9 -18 (2020)

Egyptian Journal of Radiation Sciences and Applications
http://ejrsa.journals.ekb.eg/

هيئة الطاقة الذرية
EGYPTIAN ATOMIC ENERGY AUTHORITY

Effect of *Spirulina platensis* against Thyroid Disorders Associated with Liver Dysfunction and Dyslipidaemia in Irradiated Rats

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SPIRULINA platensis (SP) is a nutritional supplement that has a variety of pharmacological properties and it is considered one of the good nutritional supplements for human and animal feed worldwide. Also, exposure to different forms of radiations is almost unavoidable in daily life, especially during radiotherapy. This study aims at evaluating the effect of SP against thyroid dysfunction associated with alteration in liver functions and lipid profile in gamma-irradiated male albino rats. Male rats were divided into four groups 1-Control, 2-Spirulina, rats were given SP via gavages 300mg/kg/day for 15days, 3-Radiation, whole body γ-irradiated rats at a dose of 5Gy, 4-Spirulina+ Radiation, rats were given SP for 15days before irradiation at a dose of 5Gy. The thyroid tissues of the irradiated rats revealed a significant decrease in the activity of the antioxidant enzymes, accompanied by a significant increase in the level of lipid peroxidation end-product malondialdehyde (MDA) indicating radiation-induced oxidative stress. Furthermore, the activity of the apoptotic marker caspase-3 was significantly increased. Also, serum level of thyroid hormones, triiodothyronine (T3) and thyroxine (T4) showed a significant decrease while the thyroid stimulating hormone showed an increase. Thyroid dysfunction was accompanied by alteration in liver function enzymes and a state of hyperlipidaemia, SP treatment has significantly decreased oxidative stress and caspase-3 activity in thyroid tissues, which was accompanied by a significant improvement in the level of thyroid hormones, liver functions and lipid profile. SP may be a useful adjunct to attenuate thyroid disorders associated with liver dysfunction and dyslipidaemia in irradiated rats by its free radical scavenging and potent antioxidant activity.

Keywords: Hypothyroidism, Lipids, Liver, Radiation, *Spirulina platensis,* Thyroid disorders.

Introduction

The thyroid gland is the site for the synthesis of the thyroid hormones, two iodine containing amine hormones derived from the amino acid tyrosine. The hypothalamus secretes the thyroid releasing hormone (TRH), which stimulates the anterior pituitary to secrete the thyroid stimulating hormone (TSH), which stimulates the thyroid follicular cells to release T4 (80%) and T3 (20%) (Khanam, 2017). Although T4 is secreted at much higher levels (110nmol/ day), than T3 (10nmol/ day), it requires de-iodination and conversion to T3 in peripheral organs such as the liver and kidney to become biologically active (Malik & Hodgson, 2002). Iodine, selenium and iron play an important role in thyroid hormone synthesis (Sharma et al., 2014).

The evidence has demonstrated that thyroid hormones regulate a variety of pathways that are involved in the metabolism of carbohydrates, lipids and proteins. Lipids are known to control cellular functions and homeostasis. Liver plays

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Received 21/2/ 2020 ; Accepted 26/4/ 2020
DOI: 10.21608/ejrsa.2020.24376.1094

a crucial role in lipid breakdown, synthesis and transportation, and thus an abnormal lipid profile is expected in persons with severe liver dysfunction. In the liver, thyroid hormones regulate cholesterol homeostasis, bile acid synthesis and fatty acid metabolism (Mullur et al., 2014). Notably, hyperthyroidism induces a hyper-metabolic state characterized by reduced cholesterol levels, increased lipolysis and gluconeogenesis, whereas hypothyroidism is characterized by increased cholesterol levels, reduced lipolysis and gluconeogenesis (Cicatiello et al., 2018).

Exposure to different forms of radiations is almost unavoidable in daily life, from radiation encountered in the environment, and especially during radiotherapy. Radiations cause oxidative stress and generate free radicals including the highly reactive hydroxyl radicals, and reactive oxygen species (ROS) such as hydrogen peroxide. Excess cellular levels of free radicals and ROS cause damage to cellular components such as proteins, nucleic acids, lipids, cell membranes or cell organelles that causes the morphological and physiological changes in the cells leading to loss of function or even cell death processes such as apoptosis (Li et al., 2014; Stasiołek et al., 2017).

During radiotherapy treatment of head and neck tumours, it is expected that the thyroid gland may be affected. The free radicals generated by radiation may attack different macromolecules in the thyroid causing changes in their structure and functions, which may result in hypo- or hyperthyroidism. Hypothyroidism after radiotherapy for head and neck cancers was first reported in the 1960s (Markson & Flatman, 1965). Since then, many publications have described radiation-induced thyroid disorders such as hypothyroidism, thyroiditis, Graves' disease, adenoma, and carcinoma (Jereczek-Fossa et al., 2004). Cells are equipped with several enzymatic and non-enzymatic antioxidants including the enzyme superoxide dismutase (SOD), which catalyses the reduction of superoxide anion to H_2O_2, the latter is broken down by catalase (CAT) to oxygen and water, as well as by glutathione peroxidase (GSH-Px) in the presence of an adequate amount of reduced glutathione (GSH) (Sun et al., 1998). However, exposure to radiation leads to depletion of these endogenous antioxidants and ultimately to the development of radiation damage, and thus the control of radiation hazards is considered one of the most important challenges.

SP is a filamentous blue-green alga belonging to the *Oscillatoriaceae* family with a spiral cellular structure, and an extraordinary capacity to survive under harsh conditions. SP is considered an excellent nutritional supplement with many health benefits, it is rich in protein, carotenoid (β-carotene), polyunsaturated fatty acids, glycolipids, polysaccharides, vitamins A**,** E, B, iodine, calcium, magnesium, manganese, potassium, zinc, and iron (Hoseini et al., 2013). SP is also a good candidate for selenium enrichment (Chen et al., 2005). Furthermore, SP contains also phycocyanin, a powerful antioxidant which gives spirulina its rich green colour (Lissi et al., 2000). SP is well documented for its clinical importance in diabetes, hypertension, and cancer (Palaniswamy & Veluchamy, 2018) besides its antioxidant, immune-modulating, anti-microbial (Finamore et al., 2017) and radio-protective properties (Ibrahim, 2012, 2014). Administration of SP has been found to lower the heart damage caused by chemotherapy (Khan et al., 2005). In view of the above, this work aims at investigating the protective effect of SP on thyroid and liver disorders and dyslipidaemia in γ-irradiated albino rats.

Materials and Methods

Experimental animals

Male albino rats *Sprague-Dawley* (10± 2 weeks old; 120± 10g) were obtained from the Egyptian Holding Company for Biological Products and Vaccines, Cairo, Egypt. The experimental animals were kept under standard conditions of ventilation, temperature, humidity and lighting (light/dark: 13h/11h) with free access to standard food pellets and water *ad libitum*. Animals were left to acclimatize one week before experimental analyses which was performed in the morning (11:00± 1h AM). The study was approved by the Central Scientific Publishing Committee, Egyptian Atomic Energy Authority, RF-158, 2016 and conformed the Guide for the care and use of Laboratory Animals published by the National Institutes of Health (NIH) publication No. 85–23, revised 1996).

Gamma-irradiation procedure

Animals were placed in a specially designed well-ventilated acrylic container and the whole body of the animals were exposed to 5Gy, given at a dose rate of 0.42Gy/min using a ^{137}Cs, Gamma-Cell-40 source (Atomic Energy of Canada Ltd, Ottawa, Ontario, Canada) belonging to NCRRT, Atomic Energy Authority, Cairo, Egypt.

Spirulina platensis treatment

SP powder was purchased from DXN Company (No 213, Lebuhraya Sultan Abdul Halim 05400 Alor Star Kedah Darul Aman-Malaysia). The powder was suspended in distilled water and administered orally to rats by gavages (300mg/kg body weight) for 15 successive days (Simsek et al., 2009).

Animal groups

Animals were divided into four groups of 6 rats each and treated in parallel. 1-Control group, rats received distilled water during15 days; 2-Spirulina group, rats received SP for 15 days, 3-Radiation group, rats received distilled water 15 days before whole body γ-irradiation with 5Gy; 4-Spirulina+ Radiation group, rats received SP 15 days before whole body γ-irradiation at a dose of 5Gy.

Biochemical analysis

Chemicals and kits, used in the current study, were obtained from Sigma-Aldrich, St Louis, MO, USA unless mentioned in the text. The tissues were homogenized using a Teflon-glass homogenizer (Glass-Col, Terre Haute, Ind., USA). The samples were centrifuged by the use of a refrigerated centrifuge (K3 Centurion Scientific Ltd, London, UK) and the absorbance was measured using aT60 UV/VIS spectrophotometer (PG instruments, London, UK). The animals were sacrificed by decapitation three days after irradiation. The blood was collected and sera separated for the determination of thyroid hormones, liver functions and lipid profile. The thyroid gland was quickly excised. A homogenate (10% weight/ volume) was prepared in 0.1M phosphate buffer (pH, 7.5) containing 0.15M KCl and was centrifuged at 1000xg for 15min in cold centrifuge and the clear supernatant was used for the assessment of oxidative stress and determination of the activity of the apoptotic marker caspase-3. Oxidative stress in thyroid tissues was assessed using commercial kits (Diamond Company) by the measurement of the lipid peroxidation MDA according to Yoshioka et al. (1979) and SOD, CAT and GSH-Px activities after Minami & Yoshikawa (1979), Sinha (1972), and Flohe & Gunzler (1984), respectively. The activity of the apoptotic marker caspase-3 in thyroid cell lysate was determined by the use of R&D Systems kits (Catalog Number: BF3100) according to the manufacturer's instructions and based on the detection of the chromophore p-nitroanilide (pNA) at 405 nm (T60 UV/VIS spectrophotometer, PG instruments, London, UK) after cleavage from the labeled substrate DEVD-pNA (acetyl-As-Glu-Val-Asp-pNA). Caspase activity is directly proportional to the color reaction. Determination of the serum level of the thyroid hormones T3, T4, and TSH was carried out according to the manufacturer's manual by enzyme linked immunosorbent assay (ELISA) kits (Biovendor company; Catalog No: ab 108663, 100660, 178664, respectively). Liver function was estimated by the determination of **g**amma-glutamyl transferase (GGT) activity in the serum using GGT Activity Colorimetric Assay Kit Cat. No. MAK089 from Sigma-Aldrich according to the method of Rosalki (1975). The activity of aspartate transaminase (AST) and alanine amino transaminase (ALT) was determined in the serum using Assay Kits (Biodiagnostic, Egypt) based on the method of Reitman & Frankel (1957), and alkaline phosphatase activity (ALP) was determined in the serum using assay Kit (Biodiagnostic, Egypt) according to the method of Belfield & Goldberg (1971). Assessment of the serum lipid profile was carried out by the determination of the level of triglycerides (TG) according to Fossati & Prencipe (1982), total cholesterol (TC) and high-density lipoprotein-cholesterol (HDL-C) according to Richmond (1973). Low-density lipoprotein-cholesterol (LDL-C) was calculated according to an online calculator (https://www.empr.com/home/tools/medical-calculators/ldl-cholesterol-calculator/)

Statistical analysis

The results demonstrated in Table 1reveal that radiation has induced a significant decrease ($P< 0.05$) in the activity of serum antioxidant enzymes accompanied by a significant increase ($P< 0.05$) in the thyroid tissue content of caspase-3 and MDA, compared to the control group.

Results

The results demonstrated in Table 1 reveal that radiation has induced a significant decrease ($P< 0.05$) in the activity of antioxidant enzymes accompanied by a significant increase ($P< 0.05$) in the content of MDA, compared to the control group.

TABLE 1. Effects of SP and/ or γ-rays on SOD, CAT, GSH-Px, Caspase-3 activities and MDA levels in different rat groups.

Parameters	Control	Spirulina	Radiation	Spirulina+ IRR
SOD (U/ mg protein)	100.00 ± 10.00	102.00 ± 5.00	70.00 ± 7.00[a]	85.00 ± 8.00[ab]
CAT (U/ mg protein)	15.00 ± 1.50	14.10 ± 1.60	10.80 ± 2.10[a]	12.90 ± 2.00[ab]
GSH-Px (U/ mg protein)	50.10 ± 3.00	51.00 ± 4.20	35.10 ± 3.00[a]	41.40 ± 2.20[ab]
Caspase-3 (μmol/ mg protein)	0.40 ± 0.06	0.43 ± 0.10	0.68 ± 0.09[a]	0.56 ± 0.05[ab]
MDA (nmol/ mg protein)	80.00 ± 10.00	78.00 ± 11.00	120.00 ± 11.00[a]	100.00 ± 12.00[ab]

- Values are expressed as Mean± Standard Deviation (n=6).
- [a]: Significant vs the control group, [b]: Significant vs the radiation group ($P < 0.05$).
- Radiation= IRR.

The administration of SP pre-irradiation has significantly ameliorated the radiation induced oxidative stress. The results presented in Table 2 reveal that the irradiated rats 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 comparison to the control group. SP treatment has significantly decreased the elevation of serum TSH, and elevated the levels of T4 and T3, compared with the irradiated rats.

The data presented in Table 3 show a significant increase in serum TC, TG, and LDL-C and a significant decrease in HDL-C levels in irradiated rats when compared to the normal control group. SP treatment has significantly improved the lipid profile.

The results presented in Table 4 reveal that the irradiated rats showed significant increases in serum ALT, AST, GGT and ALP activities when compared with the normal control group. On the other hand, SP treatment of the irradiated male rats caused significant decreases in elevated serum ALT, AST, GGT and ALP activities when compared with the irradiated group.

TABLE 2. Effects of SP and/ or γ-rays on TSH, T3, and T4 levels in different rat groups.

Parameters	Control	Spirulina	Radiation	Spirulina+ IRR
TSH (μIU/ ml)	0.95 ± 0.05	1.00 ± 0.09	2.00 ± 1.00[a]	1.33 ± 1.22[ab]
T3 (ng/ dl)	0.85 ± 0.10	0.86 ± 0.11	0.34 ± 0.09[a]	0.60 ± 0.12[ab]
T4 (ng/ dl)	10.00 ± 3.00	10.10 ± 2.25	3.00 ± 4.00[a]	6.05 ± 3.50[ab]

Legends as in Table 1.

TABLE 3. Effects of SP and/ or γ-rays on serum TC, TG, LDL-C and HDL-C levels in different rat groups.

Parameters	Control	Spirulina	Radiation	Spirulina+ IRR
TC (mg/ dl)	100.00 ±14.00	105.00 ± 12.00	165.00 ± 15.00[a]	130.00 ± 10.00[ab]
TG (mg/ dl)	90.00 ± 9.00	92.00 ± 10.00	180.00 ± 11.00[a]	120.00 ± 15.00[ab]
LDL-C (mg/dl)	50.10 ± 6.10	53.60 ± 5.20	106.00 ± 10.00[a]	77.90 ± 5.90[ab]
HDL-C (mg/dl)	31.90 ± 4.80	33.00 ± 4.00	23.00 ± 5.00[a]	28.10 ± 5.20[ab]

Legends as in Table 1.

TABLE 4. Effects of SP and/ or γ-rays on serum ALT, AST, GGT and ALP activities in different rat groups.

Parameters	Control	Spirulina	Radiation	Spirulina+ IRR
AST (U/ L)	20.00 ± 1.00	19.90 ± 1.70	60.00 ± 2.00[a]	35.00 ± 1.90[ab]
ALT (U/ L)	25.20 ± 2.00	24.90 ± 1.50	54.00 ± 1.00[a]	35.40 ± 1.90[ab]
GGT (U/ L)	30.50 ± 3.00	30.00± 1.50	65.00 ± 2.00[a]	45.20 ± 3.00[ab]
ALP (U/ L)	90.00 ± 10.00	89.00 ± 12.00	150.00 ± 13.00[a]	120.00 ± 10.00[ab]

Legends as in Table 1.

Discussion

Radiation damage due to free radicals is an enormous challenge for human safety. Accumulated evidence has demonstrated that oxidative stress in different tissues is the underlying mechanism of radiation damage. The results of extensive studies have demonstrated that the thyroid gland is a radiosensitive organ (Khan et al., 2014) and that the exposure to ionizing radiation may induce thyroid disorders (Zhai et al., 2017).

Spirulina is cultivated around the world, its nutritional value and potential health benefits attracted its use as a functional food (Belay, 2002). Spirulina is used as a dietary supplement for human beings as well as in aquaculture, aquarium, and poultry industries, and is available in tablets, flake, and powder form (Belay, 2002; Wang et al., 2005).

In the current study, the results obtained showed that whole body exposure of rats to γ–rays at a dose of 5Gy has induced oxidative stress in the thyroid tissues denoted by significant decreases in the activity of the antioxidant enzymes, SOD, CAT and GSH-Px accompanied by a significant increase in the level of the lipid peroxidation end-product MDA. Furthermore, the activity of the apoptotic marker caspase-3 was significantly increased. The increase of MDA may be due to the interaction of hydroxyl radical (•OH) with polyunsaturated fatty acids in cellular membranes. The decrease of antioxidants may result from their utilization to neutralize the excess of free radicals as well as their release to the blood from damaged cell membrane. Protein oxidation may also inhibit the activity of the enzymes.

It is well documented that iodine is a major substrate for the synthesis of thyroid hormones, as ingested iodine is absorbed through the small intestines and transported in the blood to the thyroid. Moreover, a deficiency of selenium (Köhrle, 2013) and iron (Zimmermann & Köhrle, 2002) will also affect thyroid hormones synthesis. In the current study, oxidative stress in thyroid tissues was associated with significant decreases in the levels of T3, and T4, which may be attributed to a decrease in the essential elements required for their synthesis caused by a poor gastrointestinal absorption resulting from radiation-induced damage in the intestinal mucosa (Saada et al., 2010). In this line, Elzaki et al. (2012) and Lin et al. (2018), reported that exposure to ionizing radiation induces iodine deficiency.

SP treatment prior to irradiation significantly reduced the increased levels of irradiation-induced oxidative stress and caspase-3 activity in thyroid tissues, which was accompanied by significant improvement in the level of thyroid hormones. SP contains large amounts of antioxidant compounds including β-carotene and the active biliprotein phycocyanin, which has significant antioxidant and radical scavenging properties (Lissi et al., 2000). The protective role of SP may be attributed to its ability to scavenge free radicals as well as its ability to stimulate the antioxidant capacity of the body (Ibrahim & Abdel-Daim, 2015). The results are in agreement with the findings of Stivala et al. (1996), that β-carotene in SP may attenuate the damage to DNA molecules, which would enhance the regeneration process of liver cells. In addition, the antioxidant protective role of SP could be due to the presence of Phycocyanin (C-phycocyanin and allophycocyanin), a biliprotein pigment which stimulates the antioxidant systems to reduce the early radiation response (Patil et al., 2008; Yoshikawa & Belay, 2008). Therefore, it may play a role in radioprotection of subjects exposed to radiation (Ivanova et al., 2010). Furthermore, deficiency of selenium and zinc is associated with a decrease in the level of thyroid hormones (Betsy et al., 2013; Ibrahim et al., 2016). Thus, due to the abundance of these elements in SP, its supplementation appears beneficial for hypothyroidism (Ashton, 2015).

In the current study, hypothyroidism was accompanied by a significant increase in the level of TG, TC and LDL-C, while HDL-C showed a decrease. The results are in agreement with the findings of Hammad (2018) who reported a correlation between serum lipids and thyroid hormones. Furthermore, the results demonstrate that hypothyroidism is associated with increased cholesterol and triglyceride levels.

In the present study, irradiation has provoked significant increases in serum TC, LDL and TG indicating hyperlipidaemia. Radiation-induced hyperlipidaemia may be attributed to an increase in the activity of enzymes involved in the synthesis of lipids as well as the release of fats from adipose tissues to the blood stream (Said et al., 2004; Alkhalf & Khalifa, 2018).

Also, thyroid hormones play a vital role in lipid homeostasis through the regulation of fatty acid β-oxidation, cholesterol transport and clearance (Sinha et al., 2018). The evidence has shown that thyroid dysfunction manifested by hypothyroidism affects lipid metabolism causing hypercholesterolemia, mainly due to damage of LDL receptors associated to a reduction of T3 which modulates cholesterol biosynthesis (Duntas & Brenta, 2012, 2018). Disturbance in lipid profile, hypercholesterolaemia and accumulation of triglycerides increases the risk for cardiovascular and fatty liver diseases (Bayard et al., 2006; Duntas & Brenta, 2018).

Thyroid hormones control cholesterol levels by increasing the expression of hydroxylase, the rate-limiting enzyme that converts cholesterol into bile acids (Goldberg et al., 2012). Furthermore, thyroid hormones control the expression of many of the genes involved in lipogenesis (Wang et al., 2015). Thyroid hormones increase the expression of LDL-C receptors on hepatocytes (Lopez et al., 2007), stimulate the expression of the major apolipoprotein in HDL-C (Malik & Hodgson, 2002), while suppress the expression of the major apolipoprotein in LDL-C, to reduce serum levels of LDL-C (Goldberg et al., 2012). In this context, hypothyroidism has been shown to be associated with a state of hyperlipidaemia (Mullur et al., 2014; Cicatiello et al., 2018).

Sp pre-treatment of the irradiated rats significantly improved the level of thyroid hormones which was accompanied by a significant improvement in the level of lipid profile. The present data suggest that supplementation with SP appears to be hypo-lipidaemic. The hypo-cholesterolaemic effect of SP on rabbits fed on diet enriched with cholesterol has also been reported (Colla et al., 2008). The hypolipidaemic effect of SP may be attributed to the presence of β-carotene (Seo et al., 2004), and linolenic acid (Morise et al., 2004), both of them affect cholesterol synthesis. Furthermore, SP is rich in phycocyanin, which binds to bile acids in the jejunum, and suppresses cholesterol absorption (Nagaoka et al., 2005). Furthermore, in both animals and humans, hypothyroidism was associated with a decline in hepatic lipase activity (Brenta et al., 2016), and thus the decrease of TG may be mediated through increasing the activity of hepatic lipase (Iwata et al., 1990).

In the current study the results revealed that in 5Gy gamma-irradiated rats, the alteration in the level of thyroid hormones was accompanied by alteration in liver functions manifested by significant increases in the activity of AST, ALT, ALP and GGT. SP pre-treatment caused significant decreases in elevated serum ALT, AST, GGT and ALP activities when compared with the irradiated group. This indicates a hepato-protective effect of SP.

The results substantiate the relationship between thyroid hormones and hepatic functions and that hypothyroidism causes hepatic dysfunction (Ajayi & Akhigbe, 2012). The obtained results agree with previous studies where the level of T3 and T4 correlates negatively with the activity of liver enzymes in hypothyroid subjects, while TSH correlates positively (Ajala et al., 2013; Punekar et al., 2018), suggesting that the level of T3, T4, and TSH can be used as prognostic markers for liver injury. SP platensis pre-treatment followed by irradiation significantly improved the level of thyroid hormones, relative to irradiation treatment alone, was accompanied by a significant amelioration in liver enzyme activities. The antioxidant effect of SP is important for its hepato-protective effects (Yoshikawa & Belay, 2008).

In the current study, the results obtained showed that whole body exposure of rats to gamma rays at a dose of 5Gy has induced oxidative stress in thyroid tissues with a simultaneous increase in the activity of the apoptotic marker caspase-3. On the other hand, the administration of SP rich in phycocyanin for 15 days before irradiation has significantly attenuated oxidative stress in thyroid tissues with a concomitant decrease in the activity of caspase-3. The results corroborate with previous findings of Khan et al. (2006) that phycocyanin ameliorates oxidative stress and apoptosis, which may be attributed to the potent antioxidant activity of spirulina, thereby inhibiting mitochondrial production of ROS and subsequent changes in intracellular redox status (El-Tantawy, 2015).

Conclusion

It could be concluded that SP is non-toxic, bio-available and the possible synergistic relationships between its elements may be a useful adjunct to attenuate radiation-induced thyroid disorders, liver dysfunction and hyperlipidaemia. Further studies will be necessary to determine the mechanism(s) of SP action on metabolic parameters.

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