International Journal of Applied Science and Engineering Research
Vol. 5 No. 1 (January-June, 2020)

Received: 23rd June 2019 Revised: 24th August 2019 Accepted: 01st November 2019

Evaluation of the effect of Spirulina against Gamma irradiation induced oxidative stress and tissue injury in rats

Rehab Makhlouf, Ibrahim Makhlouf
Radiation Health Research Department-National Center for Radiation Research and Technology – Atomic Energy Authority, Cairo, Egypt

Abstract: Many antioxidants have been investigated as hepato-protectors against ionizing radiation induced injury since they reduce the oxidative effect of the reactive oxygen species on normal cells. *Spirulina* is a potent scavenger of a variety of free radicals. The aim of this study was to investigate the radio-protective effect of *Spirulina* algae *a*gainst oxidative stress and tissue injury caused by gamma radiation. Rats were irradiated at two doses of 2 and 4Gy from cesium-137 source. Ten days prior to irradiation, animals received *Spirulina* daily (300mg/kg body weight i.p.). In the irradiated animal group, the oxidative stress markers malondialdehyde (MDA) was significantly increased in the liver, while a marked decrease in hepatic contents of DNA, and glutathione (GSH).The level of cholesterol, triglyceride (TG), low density lipoprotein (LDL), as well as activitiy of aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT), were significantly increased in sera of the irradiated rats. This was coupled with a decreased serum level of high-density lipoprotein (HDL) and total serum protein by irradiation. The administration of *Spirulina* alone daily for 10 days caused a significant decrease in MDA and produced significant elevation of liver GSH. Moreover, a significant decrease occurred in cholesterol, and TG level with no change in serum LDL or HDL levels in serum. Treatment of rats with *Spirulina* for 10 days before acute irradiation significantly abolished radiation induced elevation in liver MDA level and significantly maintained hepatic GSH content and CAT activity close to the control values. Pre-irradiation treatment of rats with *Spirulina* showed a significantly higher hepatic DNA content compared to that of irradiated rats. The level of cholesterol, TG, HDL, LDL as well as the activity of AST, ALT, and GGT in serum was significantly ameliorated when *Spirulina* was injected before irradiation. In conclusion, the increase in oxidative stress markers and the concomitant change in antioxidant levels indicate the role of oxidative stress in radiation-induced tissue damage. It was concluded that administration of *Spirulina* algae possess a radio protective capacity against ionizing-radiation induced oxidative stress and organ injury.

Keywords: Spirulina, radiation, radioprotection, antioxidants, oxidative stress, liver.

1. Introduction

Exposure to ionizing radiation represents a genuine, increasing threat to mankind and our environment. The steadily increasing applications of radiation in clinical practice, industrial and agricultural activities, on top of residual radio-activity resulting from nuclear test explosions, have a measurable impact contributing to possible radiation hazards in humans. Control of radiation hazards is considered as one of the most important challenges in order to protect our lives from radiation damage (Edrees et al., 2008) . Ionizing radiations interact with biological systems through free radicals generated by water radiolysis. This indirect action plays an important role in the induction of oxidative stress leading to cellular damage and organ

*Corresponding author (e-mail: Rehab.2000@hotmail.com)

dysfunction (Berroud et al., 1996). The deleterious effects of ionizing radiation in biological systems are mainly mediated through the generation of reactive oxygen species (ROS) in cells as a result of water radiolysis (Kamat et al., 2000). Among them, particularly, the highly damaging hydroxyl radical ($OH^{\cdot}$) can cause injury by reacting with biomolecules (Breen and Murphy, 1995; Jagetia et al., 2003). ROS and oxidative stress may contribute to radiation-induced cytotoxicity and to metabolic and morphologic changes in animals and humans during radiotherapy, experimentation, or even space flight (Fang et al., 2002).

Against oxidative stress, cells are equipped with several natural enzymatic and non enzymatic antioxidant defenses (Halliwell, 1992). The exposure to ionizing radiation leads to depletion of these endogenous antioxidants (Koc et al., 2003a, b) and ultimately to the development of systemic disease. Irradiation up to 14.4 Gy caused marked decrease in serum enzymes and its pineal biosynthesis 3 and 5 days after radiation exposure (Ahlersova et al., 1998). In addition, total antioxidant capacity of plasma was reduced in patients exposed to whole body irradiation for the purpose of reducing tumor growth. Consequently, the cellular antioxidant capacity is decreased and the organs become more susceptible to the deleterious effects of ROS (Karbownik and Reiter, 2000).

Spirulina is a blue-green microalgae has a spiral cellular structure, and has an extraordinary capacity to survive under conditions that are much too harsh for other algae. Habitats with extensive *Spirulina* growth include the Pacific Ocean near Japan and Hawaii, large fresh water lakes in Africa, North America, Mexico, and South America. Two species of *Spirulina* that are most commonly used in nuritional supplements are *Spirulina platensis* and *Spirulina maxima* (Khan et al.,2005) . *Spirulina*, contains large amounts of protein (70% dry weight)(Dillon, and Phan 1993), carotenoid (4000 mg/kg) (Kapoor,R.and Mehta,1993) (*omega*-3 and *omega*-6 polyunsaturated fatty acids γ –linolenic acid (GLA), sulfolipids, glycolipids, polysaccharides, provitamins; vitamin A (Annapurna et al 1991) vitamin E, (Mitchell, et al.,1990) various B vitamins; and minerals, including calcium, iron, magnesium, manganese, potassium, zinc,(Khan et al., 2005) and selenium (Cases, J. et al., 2002). It is, therefore, a potential therapeutic agent for treating oxidative stress-induced diseases (Bhat, and Madyastha 2000). Recent findings concerning the antioxidant, anti-inflammatory, immune -enhancing, antiviral, anticancer, and hepato -protection properties of *Spirulina*, with reference to the potential advantages of *Spirulina* as a regular nutritious supplement in the prevention of various disorders that are associated with oxidative stress, inflammation, cancer, and liver-malfunctioning diseases.

2. Materials and methods

2.1 Chemicals

All reagents were of the highest purity available. *Spirulina* powder was purchased from DXN company in Malaysia. All chemicals for measurement of antioxidants, lipid peroxidation, and chemical for biochemical analysis were purchased from Sigma Chemical Co. (St. Louis,MO).

2.2 Animals

Male Westar rats weighing 150–180 g , were used in the present experiments. Experimental animals were housed in cages with free access to drinking water and diet and maintained in the animal care facility throughout the duration of the experiment. The animals were kept at 22–24˚C with the 12 h light/dark cycle. Animal husbandry and experimentation were consistent with the Public Health Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and in accordance with protocols approved by

the local experimental animal ethics committee.

3. Experimental Design

Animals were divided into four groups, each group containing six rats. Animals in group 1 were served as control group and no treatment was given to these rats. Animals in group 2 were given *Spirulina* daily by gavages for 45 consecutive days at dose level of 300 mg/kg dissolved in water (Simsek et al., 2009). Group 3 gamma irradiated rats, the animals were divided into two subgroup : Six rats were whole body irradiated at dose level of 2Gy while other six rats were exposed to the dose of 4Gy radiation. Animals in group 4 were given *Spirulina* (300 mg/kg) by gavages for 10 consecutive days before radiation exposure and continued up to 45 days after radiation exposure

3.1 Irradiation

Animals were placed in a specially designed well-ventilated acrylic container and the whole body of the animals were exposed to either 2 or 4 Gy, given at a dose rate of 0.84 Gy/min from the biological irradiator gamma cell-40, cesium-137 source (Atomic Energy Agency, Canada), belonging to National Center for Radiation Research and Technology (NCRRT), Cairo.

3.2 Collection samples

Animals were sacrificed at the end of the experment (12 h after over night fasting). Blood samples were collected and put into chilled non-heparinized tubes, which were centrifuged at 3000 rpm for 10 min at 4 ºC. The sera were frozen at -20 ºC for the following measurements. The serum levels of cholesterol, triglyceride (TG), high density lipoprotein(HDL), low density lipoprotein (LDH) and total protein, as well as the activitiy of alanine aminotransferase (ALT) , aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma- glutamyltransferase (GGT), were estimated using kits from bio-Merieux, France. HDL and LDL were separated from serum by precipitation of lipoproteins of lower densities with polyethylene glycol (PEG 20 000, Fluka, Switzerland), a method originally developed for separation of HDL, and cholesterol was determined in lipoprotein fractions.A tissue sample from a known portion of the liver was accurately weighed and homogenized (Potter-Elvehjem) in a 10-fold volume of ice cold (20mM) tris-HCl buffer, pH 7.4. The homogenates were subjected to the following biochemical analysis. Lipid peroxidation product, malondialdehyde (MDA), was measured by thiobarbituric acid assay, which is based on MDA reaction with thiobarbituric acid to give thiobarbituric acid reactive substances (TBARS), a red species that absorbs at 535nm (Ohkawa et al., 1979).The GSH content was determined by using spectrophotometer at 412nm using 5, 50-dithiobis-2-nitobenzoic acid (Beutler, 1982). Catalase (CAT) activity was assayed as suggested by Bock et al. (1980). The method is based on the rate of H2O2 degradation by the action of CAT contained in the examined samples. DNA content was assayed by quantitative determination of nucleic acids through the reaction of its sugar components with diphenylamine reagent (Burton, 1956; Thoresen et al., 1983).

3.3 Statistical Analysis

Statistical significance of the data was analyzed using Student's t-test. Data are shown as means ± SD and the level for statistical significance was $P<0.05$ Snedecor and Cochran (1980).

4. Results and discussion

The administration of *Spirulina* (300 mg/kg) alone for 45 days caused significant decreases in TBARS paralleled with significant elevations of GSH content activity in the liver. Hepatic CAT activity as well as

DNA contents were not changed after *Spirulina* treatment (Table 1). Exposure of rats to gamma- radiation resulted in a significant increase in lipid peroxidation, as measured by the formation of TBARS in the rat liver after whole body γ-irradiation (Table 1). Such an increase occurred at the two doses examined. The lower dose (2 Gy) caused the 44% increase, while the higher dose (4 Gy) was effective in further increasing (97%) the extent of lipid peroxidation (Table 1). The formation of TBARS at 2 and 4Gy was quite considerable and therefore protective effect of *Spirulina* was examined at these doses. Daily treatment with *Spirulina* for 10 days before acute irradiation significantly abolished these radiation-induced elevations in TBARS level in the liver (Table 1).

Hepatic GSH content was significantly decreased following irradiation when compared with the control group. The reduction in GSH level observed at 2 Gy, was further enhanced at 4 Gy. On the other hand, *Spirulina* treatment before irradiation markedly maintained hepatic GSH levels close to the control level and showed insignificant changes compared to control and irradiated groups (Table 1). Hepatic CAT activity of both irradiated groups was significantly increased when compared with the control group. In contrast, *Spirulina* pretreatment significantly protected CAT activity in the liver of rats exposed to gamma radiation. The protective action of *Spirulina* on CAT activity was complete in case of rats subjected to 2Gy but not to 4Gy, where it showed activity intermediate between the control and irradiated group levels (Table 1). In 2 and 4Gy-irradiated groups, there were significant decreases in hepatic DNA content. Daily treatment with *Spirulina* for 10 days before irradiation showed significantly higher DNA content in the liver of *Spirulina*-treated+ irradiated groups than irradiated rats (Table1). On its own, *Spirulina* treatment daily for 45 days produced significant decreases in cholesterol, and TG levels, while it did not affect LDL or HDL levels in serum. *Spirulina* also showed insignificant changes in total protein levels as well as ALT and AST activities in serum (Table2). The levels of cholesterol, TG, and LDL in serum were significantly higher in 2 and 4Gy irradiated groups after radiation exposure than those of the control group. Treatment with *Spirulina* before irradiation significantly ameliorated the elevation in the levels of lipid profile (Table 2).

On the other hand, radiation exposure resulted in a significant decrease in HDL level in serum of the irradiated rats. This effect is significantly prevented by daily treatment with *Spirulina* for 10 days before acute irradiation (Table 2). In addition, serum total proteins contents were lower in 2 and 4Gy irradiated groups after radiation exposure than that of the control group. This effect was statistically significant when rats were exposed to the 4Gy dose. Treatment with *Spirulina* before irradiation significantly ameliorated the decline in the contents of serum protein content (Table 2).

The activities of ALT, AST, ALP and GGT in serum showed a significant rise following 2- or 4Gy irradiation doses. The treatment with *Spirulina* before irradiation displayed significant amelioration in the elevation of these enzyme activities in serum (Table 2).

Table 1: Serum activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase(ALP) , Gamma- glutamyl transferase (GGT) and total protein (T. prootein) in various animal groups

	ALT (u/l)	AST (u/l)	ALP (u/l)	GGT (u/l)	T. protein (g/dl)
Control group	23.22±1.52	28.77±1.95	66.20±1.60	40.0±1.50	8.12±0.37
Spirulina group	25.32±1.66	29.80±2.71	65.30±1.00	39.0±1.00	8.53±0.23
Irradiated group 2Gy	41.07±3.98	46.80±1.52	150.03±2.70	65.0±2.00	7.50±0.17

Irradiated group 4Gy	53.50±2.10	59.80±0.53	163.18±1.50	73.0±1.00	6.50±0.21
Spirulina + 2 Gy + Spirulina	39.21±0.30	40.90±2.10	82.21±3.60	45.0±1.60	8.20±0.38
Spirulina + 4 Gy + Spirulina	42.10±0.42	44.50±0.52	109.30±2.70	53.0±1.10	7.30±0.29

Table 2: Serum levels of cholesterol, Triglyceride, HDL, LDL in various animal groups

	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control group	138.60±6.52	130.77±3.95	49.20±0.28	40.0±1.50
Spirulina group	126.32±7.66	81.80±4.71	49.10±0.22	39.0±1.00
Irradiated group 2 Gy	176.07±13.98	162.80±2.52	150.03±2.70	46.90±0.59
Irradiated group 4 Gy	229.50±10.13	267.80±5.53	163.18±1.50	34.80±0.77
Spirulina + 2 Gy + Spirulina	141.21±5.30	145.90±2.10	82.21±3.60	48.00±0.27
Spirulina + 4 Gy + Spirulina	151.10±10.42	188.50±4.52	109.30±2.70	47.90±1.00

Table 3: Hepatic levels of glutathione (GSH), malonaldehyde dismutase (MDA), catalase (CAT) as well as the content of DNA in various animal groups

	GSH (µg/mg tissue)	MDA (ng/g tissue)	CAT (µmol H_2O/min/g)	DNA (mg/g)
Control group	21.60±1.52	86.56±3.62	5.21±0.43	28.1±1.85
Spirulina group	24.32±1.66	70.50±1.18	4.60±0.83	28.1±1.03
Irradiated group 2 Gy	17.07±0.98	198.40±2.52	8.60±0.92	18.5±0.68
Irradiated group 4 Gy	15.50±1.13	382.50±3.53	10.50±0.55	12.8±0.63
Spirulina + 2 Gy + Spirulina	19.21±1.30	118.34±2.10	5.40±0.31	23.7±1.13
Spirulina + 4 Gy + Spirulina	18.10±1.42	296.50±4.52	6.40±0.39	20.3±0.73

Radiations are commonly used in a number of medical and industrial situations; however, their pro-oxidative effects limit their applications. The present study demonstrates that administration of *Spirulina* prior to gamma irradiation protected rats against the oxidative stress and tissue damage produced by sub-lethal doses of gamma radiation. The major forms of cellular damage induced by radiation are DNA damage, lipid peroxidation, and protein oxidation. The present study demonstrates increased concentration of TBARS and nucleic acid in the rat liver, indicating high level of oxidative stress, which markedly enhanced with increasing radiation dose (Table1). Similar observations were reported on radiation-induced oxidative damage in several organs (Bhatia and Manda, 2004; Sener et al., 2003) and mitochondrial

membranes (Kamat et al., 2000). Ionizing radiation generates ROS as a result of water radiolysis. In actively metabolizing cells, there is considerable water apart from the target macromolecules. These ROS can induce oxidative damage to vital cellular molecules and structures including DNA, lipids, proteins, and membranes (Cadet et al., 2004; Esterbauer, 1996). Products of lipid peroxidation such as MDA and 4NHE (4-hydroxynonenal) have the ability to interact with and alter macromolecules, possibly resulting in diseases (Box and Maccubbin, 1997; Petersen and Doorn, 2004). Oxidative damage to proteins, as assessed by formation of carbonyl groups is a highly damaging event, and may occur in the absence of lipid peroxidation (Dean et al., 1997; Stadtman and Berlett,1997). Thus, modification of lipids and proteins by ROS is implicated in the etiology of radiation-induced physiological disorders and diseases. The present results show that low-energy γ-radiation from cesium-137 source produced a significant oxidative damage in rats after whole body exposure. It is reported that whole-body exposure of rats to high energy radiation from Co-60 causes tissue damage in several organs, as assessed by increased lipid peroxidation, 2, 12, and 72 h after irradiation (Koc et al., 2003a; Sener et al., 2003, 2004). Thus, radiation-induced damage might result in adverse health effects within hours to weeks or delayed effects observable many months after exposure (Vijayalaxmi et al., 2004).Recently, emerging evidence suggests that oxidative stress is possibly involved in the pathology of some diseases and other inborn errors of lipid and protein metabolism (Onody et al., 2003). Significant increase in the levels of serum lipid profile and LDL are demonstrated post radiation exposure of rats, possibly as a result of liver injury (Table2). These changes are in agreement with previous studies on Syrian hamsters (Feurgard et al., 1999) and on mice (Agrawal et al., 2001). This indicates that ionizing-radiation-induced oxidative stress which might altered hepatic lipid metabolism and serum lipoproteins. It seems that there is an association between radiation-induced oxidative stress and elevated levels of lipid fractions and LDL (Onody et al., 2003). This association is similarly observed in other conditions characterized by increased oxidative stress (Baynes, 1991; El-Missiry et al., 2004; Zwirska-Korczala et al., 2003). Therefore, it is suggested that oxidative stress might be an important determinant of altered lipid metabolism due to radiation exposure.

The above mentioned effects were accompanied by elevated activity of ALT, AST and ALP and increased level of total serum proteins of irradiated rats with cesium- 137 source, indicating occurrence of liver injury. These conclusion are in accordance with those of other studies (Bhatia and Manda, 2004) using high-energy radiation from cobalt source. Therefore, it is proposed that oxidative stress is linked to the organ damage following exposure to ionizing radiation. It is hypothesized that if the oxidative stress is involved in the origin of tissue damage, then successful antioxidant treatment should delay or prevent the onset of that damage (Halliwell and Whiteman, 2004). The present result demonstrate that *Spirulina* treatment of rats before irradiation induced protection against oxidative stress, evidenced by decreased TBARS and production in the liver compared to the irradiated non-treated rats. The protective role of *Spirulina* may be attributed to the presence of β-carotene (Luxia et al., 1996), enzyme superoxide dismutase or selenium (Henrikson, 1989) and blue pigment phycocyanin (Bhat and Madyastha, 2001). Luxia et al.(1996) reported that β-carotene of *Spirulina* may reduce cell damage, especially the damage to DNA molecules, thus playing a role in the repair of damaged liver cells. It also prevented the loss of DNA from rat liver.

This interpretation seems to be in accordance with that of recent studies in mouse bone marrow- cells, *spirulina* extract reduced the number of micronuclei from oxidative damage. Since 70% of cellular damage produced by ionizing radiation is due to ºOH formed from water radiolysis (Ward, 1988), the protective action of *Spirulina* might be attributed to its ability to scavenge this damaging radical (Wu et al., 2005). Ionizing radiation causes oxidative damage to tissues within an extremely short period, and possible

protection against it would require the rapid transfer of antioxidants to the sensitive sites in cells. At this point, *Spirulina* seems unique among cellular antioxidants because of its physical and chemical properties; it can easily cross biological membranes and reach the cytosol, nucleus, and cellular compartments (Matsuo et al., 1989).The effect of *Spirulina* in maintaining normal hepatic functions is well documented (Dhu et al., 2004). The decrease in protein level may be due to inhibition of amino acid transporter (Brookes and Kristt, 1989) or RNA synthesis (Sarafian and Verity, 1983). The protective action of *Spirulina* against lipid and protein oxidation as a factor of modifying membrane organization may also be related to *Spirulina*'s ability to scavenge the oxidation-initiating agents, which are produced during the oxidation of proteins and lipids. Since membrane functions and structure are influenced by proteins in membranes and radiation is known to damage thiol proteins (Stacey and Kappus, 1982), it is possible that the protective action of *Spirulina* against membrane damage may be related partially to the ability of *Spirulina* to prevent protein damage. Changes in membrane structure and fluidity due to ROS reactivity after irradiation of rats could also be attributed to graded alterations in the lipid bi-layer environment (Karbownik and Reiter, 2000). Such alterations result in the disruption of membrane potential and enhanced permeability hence altered cellular function. It seems that treatment with *Spirulina* before irradiation protects against these changes. This is indicated by a significant reduction in radiation-induced hepatic injury because the biochemical parameters as well as the hepatic function tests approached control levels (Table2).These data imply that *Spirulina* offer radioprotection at molecular and biochemical levels by protecting membranes, from breaking and consequent alteration of biochemical status of cellular functions.

At sufficiently high radiation doses, GSH becomes depleted, leaving highly reactive ROS, beyond the immediate and normal needs of the cell, to react with critical cellular biomolecules and cause tissue damage. The concentration of intracellular GSH, therefore, is the key determinant of the extent of radiation-induced hepatic injury. Thus, interest has been focused on *Spirulina*, which acts as an antioxidant and is capable of stimulating GSH synthesis. *Spirulina* is demonstrated to increase hepatic GSH content, and hence to inhibit formation of extracellular and intracellular ROS (Dasgupta, et al.2001) . It is also proposed that prevention of GSH depletion is the most efficient method of direct protection against radiation- induced oxidative toxicity. GSH depletion is prevented when *Spirulina* was administered before irradiation (Table 1). Thus, *Spirulina* has an important role in maintaining this crucial antioxidant in the liver and increasing the antioxidant capacity of hepatocytes. CAT is an inducible enzyme, decomposes H2O2, and is involved in the antioxidant defense mechanisms of mammalian cells. Thus it is an index of increased H2O2 production (Meneghini, 1997). This might explain the higher CAT activity demonstrated in the livers of irradiated rats compared to that of controls. Although it is probable that antioxidant enzymes provide important protection from radiation exposure (Sun et al., 1998), the proper balance of the enzymes, in specific cells and in the whole organism, required for maximum radioprotection is far from clear. For example, a large increase in manganese superoxide dismutase activity in some model systems may have a radio sensitizing effect rather than a radio protective effect (Scott et al., 1989), which is probably related to the inability of the cell to cope with over production of H2O2 or ºOH (Weiss et al., 2003). The activity of CAT was maintained within control level by pretreatment of rats with *Spirulina*, indicating controlled H2O2 generation in the liver or its scavenging directly by *Spirulina* (Xue et al., 2010). This effect might result in reduced accumulation of lipid and protein oxidation products and ameliorated oxidative stress in the liver and presumably other tissues.

Like cholesterol, TG, LDL, and free radicals are also risk factors that tend to damage arteries, leading to heart disease. In the current study, administration of *Spirulina* to rats was found to reduce serum cholesterol, TG, and LDL levels in serum, indicating modulation of cholesterol and lipid metabolism in cell

(Ray, 1991). The hypocholesterolemic effect of *Spirulina* on rabbits fed on diet enriched with cholesterol has also been reported (Colla et al., 2008). According to Hill and McQueen (1997), HDL-C has known to be protective against the development of atherosclerosis. There is an inverse relationship between HDL-C concentration and the incidence of coronary heart disease. HDL reverse cholesterol transport whereby cholesterol synthesized is returned to the liver for reuse or re-excretion into the bile resulting in a decrease of cholesterol level. Moreover, the hypotriglyceridemic effect of *Spirulina* may be through its effect on the increased the activity of lipase (Iwata et al., 1990). The presence of antioxidant compounds like phycocyanin and β-carotene, linolenic acid and sulfated polysaccharide in *Spirulina* could be the cause of the properties and action of *Spirulina* on the decrease of plasma lipids levels. According to Nagaoka et al. (2005), phycocyanin cause hypocholesterolemic activity in rats. They hypothesized that phycocyanin binds to bile acids in the jejunum, this binding affects the micellar solubility of cholesterol and then suppresses cholesterol absorption. Seo et al. (2004) reported that β- carotene reduced the elevation of cholesterol and triglycerides of diabetic rats. Both sulfated polysaccharides and linolenic acid showed hypolipidemic effect (Godard et al., 2009;Serougne et al., 2004). Moreover, Kim et al. (2004) found that feeding of rats with linolenic acid rich oil lowers plasma triacylglycerol and inhibits hepatic fatty acids synthesis which may result in a hypolipidemic effect, and control hyperlipidaemia (Mary et al., 2002) .

The hypocholesterolemic effect of *Spirulina* in humans has been reported by reducing serum cholesterol (4.5%), triacylglycerol and LDL were observed when *Spirulina* (4.2 g/day) was added for 8 weeks to the diet of thirty Japanese males with high cholesterol, mild hypertension, and hyperlipidemia (Khan et al., 2006). Becker et al. (1986) evaluated clinical and biochemical outcomes following the application of *Spirulina* to treat obesity. They found weight loss accompanied by reduced cholesterol level. This might potentiate anti -atherogenic effects of antioxidants including *Spirulina* . Therefore, the present data suggest that supplementation with *Spirulina* appears to be hypolipidemic which might potentate its beneficial use before radiation exposure.

The present study also demonstrated a significant increase in serum GGT activity after exposure ofrats to ionizing radiation. GGT is a key enzyme in the catabolism of GSH (Djavaheri-Mergny et al., 2002; Lee et al., 2002). Recently, it has been reported that the extracellular cleavage of GSH by GGT induces the production of ROS, suggesting that GGT plays a pro-oxidant role (Lee et al., 2004a). Therefore, the present results suggest that serum GGT might be one of the enzymes related to oxidative stress after exposure to γ-irradiation. These associations suggest that the net result of high serum GGT activity was the increased, rather than decreased oxidative stress and damage. GGT is inversely associated with antioxidants (Brown et al., 1998; Kilanczyk and Bryszewska, 2003), while *Spirulina* treatment stimulates several antioxidants that raise the total antioxidant capacity of the body (Gini and Muraleedhara 2010). Thus, besides its direct effect, *Spirulina* may protect against oxidative stress by modulating GGT level in serum by acting as antioxidant and working cooperatively with other antioxidants.

5. Conclusion

Spirulina properties and its constituents give the impression of being a radioprotective agent against gamma irradiation induced oxidative stress. This might be through stimulating GSH, modulating serum GGT activity and exerting hypolipidemic impact. Thus, supplementation with *Spirulina* may have a benefit for safe application of radiation technology in medicine and industry.

Acknowledgement

Authors acknowledge the sources that are used to prepare the data of the article and also authors confirm that there is no conflict of interest in the study.

6. References

1. Ahlersova, E., Pastorova, B., Kassayova, M., Ahlers, I., Smajda, B., 1998. Reduce pineal melatonin biosynthesis in fractionally irradiated rats, Physiology Research. 47 (2), 133–136.
2. Annapurna,V.V., Deosthale,Y.G., and Bamji, M.S., 1991. Spirulina as a source of vitamin A. Plant Foods Human Nutrition. 41, 125.
3. Baynes, J.W., 1991. Role of oxidative stress in the development of complication in diabetes. Diabetes. 40, 405–412.
4. Becker, E.W. et al., 1986. Clinical and biochemical evaluations of the algae Spirulina with regard to its application in the treatment of obesity: a double-blind cross-over study. Nutritional supplement review. 33, 565.
5. Berroud A, Le-Roy A & Voisinp 1996. Membrane oxidative damage induced by ionizing radiation detected by fluorescence polarization, Radiation Environmental Biophysics. 35, 289-295.
6. Beutler, E., 1982. Red cell metabolism. In: Butler, E. (Ed.), A Manual of Biochemical Methods. Grune and Stratton, New York, 137.
7. Bhat VB, Madyastha KM., 2001. Scavenging of peroxynitrite by phycocyanin phycocyanobilin from Spirulina platensis: protection against oxidative damage to DNA. Biochemistry, Biophysics Research Communication. 285, 262-266.
8. Bhat, V. B., and Madyastha, K. M., 2000. C-Phycocyanin: a potent peroxyl radical scavenger in vivo and in vitro. Biochemistry, Biophysics Research Communication. 18, 275(1), 20–25.
9. Bhat, V.B. and Madyastha, K.M., 2000. C-Phycocyanin: a potent peroxyl radical scavenger in vivo and in vitro, Biochemistry, Biophysics Research Communication. 275, 20.
10. Bhatia, A.L., Manda, K., 2004. Study on pre-treatment of melatonin against radiation-induced oxidative stress in mice. Environmental Toxicology and Pharmacology. 18, 13–20.
11. Box, H.C., Maccubbin, A.E., 1997. Lipid peroxidation and DNA damage. Nutrition. 13(10), 920–921.
12. Petersen, D.R., Doorn, J.A., 2004. Reactions of 4-hydroxynonenal with proteins and cellular targets. Free Radical Biology and Medicine. 37 (7), 937–945.
13. Breen, A.P., Murphy, J.A., 1995. Reactions of oxyradicals with DNA. Free Radical Biology and Medicine .18, 1033–1077.
14. Brookes N, Kristt DA 1989. Inhibition of amino acid transport and protein synthesis by HgCl2 and methyl mercury in astrocytes : Selectivity and reversibility. Journal of Neurology and Chemistry. 53, 1228-1237.
15. Brown, K.E., Kinter, M.T., Oberley, T.D., Freeman, M.L., Frierson, H.F., Ridnour, L.A., Tao, Y., Oberley, L.W., Spitz, D.R., 1998. Enhanced g-glutamyl transpeptidase expression and selective loss of Cu–Zn superoxide dismutase in hepatic iron overload. Free Radical Biology and Medicine. 24 (4), 545–555.
16. Burton, K., 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic. Biochemical journal. 62(2):315-23.
17. Cadet, J., Bellon, S., Douki, T., Frelon, S., Gasparutto, D., Muller, E., Pouget, J.P., Ravanat, J.L., Romieu, A., Sauvaigo, S., 2004. Radiation-induced DNA damage: formation, measurement, and biochemical features. Journal of Environmental, Pathology, Toxicology and Oncology. 23(1), 33–43.

18. Cases, J. et al., 2002. Assessment of selenium bioavailability from high-selenium Spirulina Sub-fractions in selenium-deficient rats, Journal of Agriculture and Food Chemistry. 50, 3867.
19. Colla LM, Muccillo-Baisch ALVieira Costa JA 2008. Spirulina platensis effects on the levels of total cholesterol, HDL and triacylglycerols in rabbits fed with a hypercholesterolemic diet. Brazilian Article in Biology and Technology. 51, 405-411.
20. Dasgupta, T. et al., 2001. Chemomodulation of carcinogen metabolising enzymes, antioxidant profiles and skin and fore stomach papillomagenesis by Spirulina platensis. Molecular Cell and Biochemistry. 226(1–2), 27–38.
21. Dean,R.T.,Fu,S.,Stocker,R.,Davies,M.J.,1997. Biochemistry and pathology of radical mediated protein oxidation. Biochemistry journal. 324, 1–18.
22. Dhu P, Garg ML, Dhawan DK 2004. Protective role of Zinc in nickel induced hepatotoxisty in rats. Chemical biology interaction 150, 199-209.
23. Dillon, J.C. and Phan, P.A., 1993. Spirulina as source of protein in human nutrition, Bulletin d' Institute Oceanographique. 12, 103.
24. Djavaheri-Mergny, M., Accaoui, M.J., Rouillard, D., Wietzerbin, J., 2002. Gamma-glutamyl transpeptidase activity mediates NF-kappa B activation through lipid peroxidation in human leukemia U937 cells. Molecular Cell Biochemistry. 232 (1–2), 103–111.
25. Edrees GMF, El-Kholy WM, El. Habiby EM and El-Sherbiny SA 2008. Protective action of peanut oil in rats exposed to gamma-ray.Begian journal of zoology. 149-153.
26. El-Missiry, M.A., Othman, A.I., Amer, M.A., 2004. L-Arginine ameliorates oxidative stress in alloxan-induced experimental diabetes mellitus. Journal of Application Toxicology. 24, 93–97.
27. Esterbauer, H., 1996. Estimation of peroxidative damage: a critical review. Pathology and Biology. Paris 44, 25–28.biochemical features. Journal of Environmental, Pathology, Toxicology and Oncology. 23(1), 33–43.
28. Feurgard, C., Boehler, N., Ferezou, J., Serougne, C., Aigueperse, J., Gourmelon, P., Lutton, C., Mathe, D., 1999. Ionizing radiation alters hepatic cholesterol metabolism and plasma lipoproteins in Syrian hamster. International Journal of Radiation Biology. 75(6), 757–766.
29. Gershwin M.E.and Amaha Belay 2007. Spirulina in human nutrition and health. 120-121.
30. Gini C. Kuriakose and Muraleedhara G. Kurup, 2010. Hepatoprotective effect of Spirulina lonar on paracetamol induced liver damage in rats. Asian journal biology science. 1(3), 614-623.
31. Godard M, Decorde K, Ventura E, Soteras G., 2009. Polysaccharides from the green alga Ulva rigida improve the antioxidant status and prevent fatty streak lesions in the high cholesterol fed hamster, an animal model of nutritionally-induced atherosclerosis. Food Chemistry. 115, 176-180.
32. Halliwell, B., Whiteman, M., 2004. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Journal of Pharmacology. 142, 231-255.
33. Henrikson R., 1989. Earth food Spirulina. Recolina Ltd. Ronore enterprises Inc., Launa beach, California. 27-65.
34. Hill SA, Mc Queen MJ., 1997. Reverse cholesterol transport-a review of the process and its clinical implications. Clinical Biochemistry. 30, 517-525.
35. Iwata K, Inayama T, Kato T., 1990. Effects of Spirulina platensis on plasma lipoprotein lipase activity in fructose induced hyperlipidemic rats. Journal of Nutritional Science. 36, 165-171.

36. Jagetia, G.C., Rajanikant, G.K., Shaival, K., Rao, M., Baliga, S., 2003. Alteration the glutathione, glutathione peroxidase, superoxide dismutase and lipid peroxidation by ascorbic acid in the skin of mice exposed to fractionated g radiation. Clinical Chemistry.332, 111–121.
37. Kamat, J.P., Boloor, K.K., Devasagayam, T.P.A., Venkatachalam, S.R.,2000.Antioxidant properties of Asparagus racemosus against damage induced by γ-radiation in rat liver mitochondria. Journal Ethnopharmacology. 71, 425–435.
38. Kapoor, R. and Mehta, U., 1993. Utilization of beta-carotene from Spirulina platensis by rats. Plant Foods of Human Nutrition. 43,1.
39. Karadeniz A, Yildirim A, Simsek, N, Kalkan Y, Celebi F 2008. Spirulina platensis protects against gentamicin-induced nephrotoxicity in rats. Phytotherapy Research. 22, 1506-1510.
40. Karbownik, M., Reiter, R.J., 2000. Antioxidative effects of melatonin in protection against cellular damage caused by ionizing radiation. Proceedings of Social Experimental Biology and Medicine. 225, 9–22.
41. Karbownik, M., Reiter, R.J., 2000. Antioxidative effects of melatonin in protection against cellular damage caused by ionizing radiation. Proc. Social Experimental Biology and Medicine. 225, 9–22.
42. Khan, M., 2006. C-Phycocyanin ameliorates doxorubicin-induced oxidative stress and apoptosis in adult rat cardiomycetes, J. Cardiovascular Pharmacology. 47, 9.
43. Khan, Z., Bhadouria, P., and Bisen, P.S., 2005. Nutritional and therapeutic potential of Spirulina. Current Pharmacology and Biotechnology. 6, 373.
44. Kilanczyk, E., Bryszewska, M., 2003. The effect of melatonin on antioxidant enzymes in diabetic skin fibroblasts. Cell Molecular Biology. 8(2), 333–336.
45. Kim HK, Chois S Choi H., 2004. Suppression of hepatic fatty acid synthesis by feeding –linolenic acid rich perilla oil lowers plasma triacylglycerol. Level in rats. Journal of Nutritional Biochemistry. 15, 485-492.
46. Koc, M., Taysi, S., Buyukokuroglu, M.E., Bakan, N., 2003a. The effect of melatonin against oxidative damage during total-body irradiation in rats. Radiation Research. 160, 251–255.
47. Koc, M., Taysi, S., Buyukokuroglu, M.E., Bakan, N.,2003b. Melatonin protects rat liver against irradiation-induced oxidative injury. Journal of Radiation Research. 44, 211–215.
48. Lee, D.H., Blomhoff, R., Jacobs Jr., D., 2004a. Is serum gamma glutamyltransferase a marker of oxidative stress? Free Radical Research. 38 (6), 535–539.
49. Lee, D.H., Ha, M.H., Kim, J.R., Gross, M., Jacobs, D.R., 2002.Gamma-glutamyltransferase, alcohol, and blood pressure: a four year follow up study. Annual Epidemiology. 12, 90–96.
50. Luxia AS, Monica S, Ornella C, Pizzala B, Loura R, Livia B, Anio M, Ennio P, 1996. Effect of B-carotene on cell cycle progression of human fibroblasts. Mutagenesis 17, 2395-2401.
51. Mary, N.K., Shylesh, B.S., Babu, B.H., Padikkala, J., 2002. Antioxidant and hypolipidaemic activity of a herbal formulation—liposem. Indian Journal Experimental. Biology. 40 (8), 901–906.
52. Matsuo T, Kashiwaki Y, Itoo S 1989. Membrane damage caused by exposure to t-butyl hydroperoxide. Phytochemistry 28, 1003-1006.
53. Meneghini, R., 1997. Iron homeostasis, oxidative stress, and DNA damage. Free Radical Biology Medicine. 23(5), 783–792.
54. Mitchell, G.V. et al., 1990. Effects of graded dietary levels of Spirulina maxima on vitaminsA and E in male rats. Journal Nutrition. 120, 1234.

55. Nagaoka S, Shimizu K, Kaneko H, Shibayama F et al., 2005. Novel protein C-phycocyanin plays a crucial role in the hypocholesterolemic action of Spirulina platensis concentrates in rats. Journal Nutrition. 135, 2425- 2430.
56. Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Annual Biochemistry. 95,351–358.
57. Onody, A., Csonka, C., Giricz, Z., Ferdinandy, P., 2003. Hyperlipidemia induced by a cholesterol-rich diet leads to enhanced peroxynitrite formation in rat hearts. Cardiovascular Research. 58(3), 663–670.
58. Peter C 2008. Antioxidant potential of Spirulina platensis preparations. Phytotherapy Research. 22, 627-633.
59. Petersen, D.R., Doorn, J.A., 2004. Reactions of 4-hydroxynonenal with proteins and cellular targets. Free Radical Biology and Medicine. 37(7), 937–945
60. Ray RA., 1991. Microalgae as food and supplement, Critical reviews in food science and nutrition., 30, 555-573.
61. Romay, C. et al., 2003. C-Phycocyanin: a biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. Current. Protein Peptide Science. 4(3), 207–216.
62. Romay, C. et al., 1998. Antioxidant and anti-inflammatory properties of c-phycocyanin from blue-green algae. Inflammation Research. 47, 36–41.
63. Sarafian T, Verity MA 1983. Inhibition of RNA and protein synthesis in isolated cerebellar cells by in vitro and in vivo methyl mercury. Molecular Chemistry and Neuropathology. 3, 27-39.
64. Scott, M.D., Meshnick, S.R., Eaton, J.W., 1989. Superoxide dismutase amplifies organismal sensitivity to ionizing radiation. Journal Biology and Chemistry. 264, 2498–2510.
65. Sener, G., Jahovic, N., Tosun, O., Atasoy, B.M., Yegen, B., 2003. Melatonin meliorates ionizing radiation-induced oxidative organ damage in rats. Life Science. 74, 563–572.
66. Seo JS, Lee KS, Jang JH, Quan Z, Yang KM, Burri BJ., 2004. The effect of dietary supplementation of _-carotene on lipid metabolism in streptozotocin-induced diabetic rats. Nutrition Research. 24, 101-1021.
67. Serougne C, Gripois D, Blouquit M-F, Lutton C, Hermier D., 2004. Effects of dietary alpha linolenic acid on cholesterol metabolism in male and female hamsters on the LPN strain. Journal of Nutrition Biochemistry. 15: 51-61.
68. Snedecor, G.W. and Cochran, W.G.., 1980. The Iowa State University Press.
69. Stacey NH, Kappus H 1982. Cellular toxicity and lipid peroxidation in response to mercury. Toxicology Application and Pharmacology. 63, 29-35.
70. Stadtman, E.R., Berlett, B.S., 1997. Reactive oxygen mediated protein oxidation in aging and disease. Chemistry Research and Toxicology.10, 485–490.
71. Sun, J., Chen, Y., Li, M., Ge, Z., 1998. Role of antioxidant enzymes on ionizing radiation resistance. Free Radical Biology and Medicine. 24, 586–593.
72. Thoresen, S.S., Clayton, J.R., Dortch, Q.F., Ahmed, S.L., 1983. A rapid technique for the determination of RNA and DNA in marine phytoplankton. Journal of Plankton Research. 5, 253–261.
73. Vijayalaxmi, Reiter, R.J., Tan, D.X., Herman, T.S., Thomas, C.R., 2004. Melatonin as a radioprotective agent: a review. International Journal of Radiation Oncology. 59, 639–653.

74. Ward, J.F., 1988. DNA damage produced by ionizing radiation in mammalian cells: identities, mechanisms of formation, and reparability. Progress of Nuclear Acid Research. Molecular Biology. 35, 95–125.
75. Weiss, J.F., Michael, R., Landauer, M.R., 2003. Protection against ionizing radiation by antioxidant nutrients and phytochemicals. Toxicology. 189, 1–20.
76. Wu, L., et al., 2005 Antioxidant and ant proliferation activities of spirulina and chorella water extract. Journal of agricultural and food chemistry. (10), 4207- 4212.
77. Xue, F. Miao, J. Zhang, X. Tan, T., 2010. A New Strategy for Lipid Production by Mix Cultivation of Spirulina platensis and Rhodotorula glutinis. Electronic Table of Contents (ETOC) (United Kingdom).
78. Zwirska-Korczala, K., Jochem, J., Rybus-Kalinowska, B., Polaniak, R., Birkner, E., 2003. Assessment of blood superoxide dismutase, glutathione peroxidase activity and malondialdehyde concentration as oxidation status parameters in obese women. Poly Archival of Medical Wewn. 110 (1), 725–731.