

Effect of Spirulina Platensis on Oxidative Stress Induced by Gamma Radiation and H. pylori Infected Rats

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Abstract: Oxidative stress may be defined as “a state where oxidative forces exceed the antioxidant systems due to loss of the balance between them.” Oxidative stress is a phenomenon associated with pathogenesis mechanisms of several diseases including atherosclerosis, neurodegenerative diseases, such as Alzheimer’s, Parkinson’s disease, cancer, diabetes mellitus and an inflammatory diseases.

Gastritis represents an inflammation of the stomach lining in response to injury. *Helicobacter pylori* (*H. pylori*) often play an important role in the pathogenesis of gastritis, peptic ulcer, and probably also gastric cancer. Reactive oxygen species (ROS) produced by this bacterium and/ or gamma radiation may be one of the crucial factors whereby oxidative stress can play a role in the pathogenesis of ulcer disease. *Spirulina platensis* (*Sp*) is a microscopic and filamentous cyanobacterium that contains essential amino acids, fatty acids, vitamins, minerals, polysaccharides and antioxidant compound. Our objective in this study was to evaluate the possible gastroprotective effect of *Spirulina platensis* against *H.pylori* and/or gamma radiation induced oxidative stress in albino rats. In the present experiment about 36 albino Wister rats were used and sub divided into 6 groups of 6 rats each: group 1, untreated rats (control group); group 2, animals received only *Spirulina* (15mg/kg b.w.); group 3, irradiated group animals were exposed to 5Gy whole body gamma radiation as a single shot dose; group 4 animals were infected practically with *H.pylori* bacterium once daily for one week then continuous with distil water for 30 days; group 5, rats were giving orally *Spirulina* (15 mg/kg b.w.) for 30 days then exposed to 5 Gy gamma radiation as a single dose shot; group 6, rats were given orally *Spirulina* (15mg/kg b.w.) for 30 days then infected with *H. pylori* daily in the morning for one week. Our results showed that animals infected with *H.pylori* and/or exposed whole-body gamma radiation significantly increase tumour necrosis factor (TNF- α), interleukin- β (IL-1 β), interleukin-8 (IL-8), measured gastric mucosal myeloperoxidase (MPO) and malondialdehyde (MDA) joined with significant decrease in glutathione (GSH), superoxide dismutase SOD and catalase (CAT) activity. In contrast, there was no significant change in plasma levels of gastrin after radiation exposure but its levels was increased in *H. pylori* infected rats. Oral pretreatment with *Spirulina platensis* considerable amelioration the previous mentioned anti-inflammatory and antioxidant parameter. It could be concluded that, the antioxidant- responsiveness mediated by *Spirulina* may be anticipated to have biological significance in eliminating reactive free radical and improve the change in, which may be responsible for pathogenesis of *H.pylori*-associated mucosal damage. Consequently, *Spirulina platensis* may be used for better gastroprotective effect from *H. pylori* infection as well as to reduce the (ROS) activity produced from exposed to gamma radiation.

Keywords: Oxidative stress, ROS, *Helicobacter pylori*, *Spirulina platensis*, gastritis.

1. INTRODUCTION

The close association between oxidative stress and lifestyle-related diseases has become well known. Oxidative stress is defined as a “state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them”. It not only causes hazardous events such as lipid peroxidation and oxidative DNA damage, but also physiologic adaptation phenomena and regulation of intracellular signal transduction.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are product of normal cellular metabolism (Valko et al., 2007). Over production of ROS is a harmful process that can be an important mediator of damage to cellular structures, including lipids, membranes, proteins and DNA. Most cell damage caused by ionizing radiation is also mediated by ROS generated from interaction between

radiation and water molecule in the cells (Valko et al., 2007) and (Flora SJ 2007). (biomarker in serum MPO..). Since ROS and NOS are highly reactive, the approach most often employed in the study of oxidative stress is assessing the presence and quantity of the biomarkers of lipids (e.g malonaldehyde), nitrosive biomarkers (e.g nitric oxide), antioxidant biomarkers (e.g glutathione, catalase), ant-inflammatory biomarkers (e.g neutrophil/ myeloperoxide) [1,4,5] (biomarker in serum MPO..).

H. pylori infection has been associated with generation of reactive oxygen species (ROS), which leads to oxidative stress (OS) in the gastric mucosa (Naito and Yoshikawa (2002). (3). *H.pylori* induces infiltration and activation of neutrophils, which produce inflammatory mediators that include ROS (Ernst 1999)(4). These mediators impart OS on the gastric epithelium in the immediate vicinity (*H.pylori* and oxidative stress). The interaction of *H pylori* with the surface mucosa results in the release of proinflammatory cytokine interleukin (IL)-8, which leads to recruitment of polymorphonuclear cells and may begin the entire inflammatory process. High levels of cytokines, particularly tumor necrosis factor- α (TNF- α) and multiple interleukins (e.g, IL-6, IL-8, IL-10), are detected in the gastric mucosa infected with *H. pylori* (Zalewska-Ziob et al., 2009)

The current anti *H. pylori* therapy is punctuated with adverse drug reactions such as hepatotoxicity and renal failure (Hentschel et al.,1993). It has been found that the infection persists even after completion of the therapy due to resistance to the antibiotic drug regimens leading to concomitant administration of three to four drugs.

In recent years, there have been considerable efforts to search for naturally occurring substances for intervention of several diseases. Many components from medicinal or dietary plants have been identified to possess potential properties. The term 'nutraceutical' was coined from 'nutrition' and 'pharmaceutical' and was originally defined as 'a food that provides medical or health benefits, including prevention and/or treatment of a disease' (Kalra, 2003).

In the last 30 years, Spirulina – the nutraceutical food has become widely available as a food ingredient (Parry , 2008). Spirulina platensis (SP) is a unicellular cyanobacterium, with high nutritional value and with wide range of medicine applications. It is the best whole food source of protein, beta-carotene, gamma linolenic acid, B-Vitamins, minerals, chlorophyll, sulpho-lipids, glycolipids, super oxide dismutase, enzymes and trace element (Parry , 2008). It contains very potent naturally occurring antioxidant and free radical scavenging agents. Besides the free radical scavenging and antioxidant activity, spirulina and its active constituent; C-phycoyanin exhibit anti inflammatory, neuroprotective, hepatoprotective , immunomodulatory and anti cancer activities (Batha et al.,2008) and (Reddy et al.,2000) . Furthermore, Spirulina has been reported to ameliorate organ toxicities induced by heavy metals(El-Desoky et al., 2013) Increased interest in Spirulina is based on the fact that, they are believed to be non toxic, bio-available and provide significant multiorgan protection against many drugs and chemicals induced toxic assaults

(Lu et al., 2010) (Tsuchihashi et al. 1987) found that an intake of *Spirulina* at 5% of the diet increased the population of *Lactobacillus* in the caecum of rats by 3 times over a control group of rats not fed *Spirulina*.¹⁴*Lactobacillus* is believed to have three functions: to improve digestion and absorption of foods, to protect from infection, and to stimulate the immune system .

From the previous, we focused the present study on the antioxidant effect of natural product Spirulina on *H.pylori* and gamma radiation exposed male albino rats.

2. MATERIALS AND METHODS

Animals

Thirty six female albino Wister rats (*Rattus Norwegicus*), (weighing 120–150 g) were obtained from the animal farm of the Egyptian Holding Company for Biological Products and Vaccines, Egypt. Upon arrival, the animals were allowed to acclimatize for 1 week before starting the experiment. Animals were kept under standard conditions and were allowed free access to a standard requirement diet and water ad libitum. Animals were kept under a controlled lighting condition (light: dark, 13 h-11 h). The animals' treatment protocol was approved by the Animal Care Committee of the National Center for Radiation Research and Technolog(NCRRRT), Cairo, Egypt.

Irradiation

Whole-body gamma-irradiation was performed at the National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt, using (cesium-137) Gamma Cell-40 biological irradiator. Animals were irradiated at an acute single dose level of 5Gy delivered at a dose rate of 0.012 Gy/s.

Chemicals

Spirulina platensis was purchased from Wisma DXN company- Malaysia (No 213 Lebuhraya Sultan Abdul Halim, 05400 Alor Star Kedah Darul Aman) in a powder form . It was suspended in water and administered orally to rats at a dose of 15mg/kg body weight according to experimental design.

Isolation of *Helicobacter pylori*

An internal 1 biopsy from a 55-years old patient, endoscopically diagnosed as having *H. pylori* infection, was streaked into blood agar plates and incubated 3-days under micro-anaerophilic conditions at 37°C. Small gray colonies were selected and stained with gram stained to verify the morphology. Colonies with gram negative rods or coccoid form were tested by the rapid urease test (Cristensen's urea test: Remel Inc., Kansas,USA). *H.pylori* express gram negative morphology and positive reaction for urease test. The suspension of the bacteria strain was gently rinsed with sterile physiological saline, placed in vials, then 3ml 20% glycerol in brucella broth were added and maintained at -70°C.

Experimental Design

The experimental 36 rats were divided into six groups, six rats per each.

Group 1: control group: rats received oral distilled water.

Group 2: Spirulina group: rats received only Spirulina orally (15mg/b.w) for 30 consecutive days.

Group 3: irradiated group: rats received orally distilled water for 30 days then subjected to 5Gy whole body gamma irradiation as a single shot dose.

Group 4: *H.pylori* infected rats: rats received 0.5 ml of *H.pylori* brucella broth, containing 2×10^7 cFu/ml, daily in the morning for 1 week then left for 4 weeks.

Group 5: Spirulina irradiated group: rats were given orally Spirulina (15mg/b.w) or 30 days followed by irradiated at a dose level of 5 Gy.

Group 6: Spirulina and *H.pylori* infected rats: : rats were given orally Spirulina (15mg/b.w) for 30 days and then received 0.5 ml of *H.pylori* brucella broth, containing 2×10^7 cFu/ml, daily in the morning for 1 week then left for 4 weeks.

3. SAMPLING

At the end of each treatment period, rats were fasted over night. Blood was collected and centrifuged at $3,000 \times$ r.p.m for 15 min; serum was collected, divided into aliquots and stored at -20°C for determination of serum gastrin, TNF- α and IL-8. Rats were then decapitated and stomachs were dissected out, cut along the greater curvature; The inner mucosal surface of stomach was washed with normal saline and dried with filter paper for determination of MPO, MDA, CAT, SOD.

4. BIOCHEMICAL ANALYSIS

Determination of *H. pylori* infection status: Rapid urease test: the rapid urease test solution was prepared by dissolving 10 grams of urea in 100ml millipore water followed by autoclaving. 0.002 grams of phenol red was added into the solution as indicator. The pH of the solution was adjusted to 6. The isolated pyloric tissue was immediately immersed in RUT solution (Vaira et al.,2007). The change of color from yellow to red within 10 minutes indicated the presence of *H. pylori*.

Determination of gastrin level: serum gastrin was determined by competitive immunoassay technique using DRG rat gastrin kit (DRG International, Inc., USA), following the manufactory's instruction.

Determination of TNF alpha: TNF- α was quantified in blood using ELISA kit BioSource International, Camarillo, CA, USA. The assay employed the sandwich enzyme immunoassay technique and values were expressed as pg/ml.

Determination of IL-1 β : was performed by ELISA technique (BioSource International, Camarillo, CA, USA) according to the manufacturer's instruction.

Detection of serum IL-8: since IL-8 is a major proinflammatory cytokine in relation to *H. pylori* infection-related gastric mucosal inflammation, the IL-8 contents in serum samples with or without *H. pylori* infection were evaluated. IL-8 concentrations were determined using an enzyme-linked immunosorbent assay (MyBioSource ELISA kits) as instructions described.

Determination of myeloperoxidase (MPO) activity: the myeloperoxidase assay was performed according to (Krawisz et al.,1984). Briefly, The mucosa was scrapped to remove the mucus layer using a glass slide and the mucosal scrapings were homogenized in a solution containing 0.5% hexadecyltrimethylammonium bromide dissolved in 50 mM potassium phosphate buffer (pH 6), before sonication in an ice bath for 10 seconds. The homogenates were freeze-thawed three times, repeating the sonication after which they were centrifuged for 15 min at 20,000 \times g. The level of MPO activity was measured spectrophotometrically. 0.1 ml of the supernatant was mixed with 2.9 ml of 50 mM phosphate buffer, pH 6.0, containing 0.167 mg/ml O-dianisidine dihydrochloride and 0.0005% hydrogen peroxide and incubate for 10 min. The change in absorbance at 460 nm was measured spectrophotometrically using horseradish peroxide as a standard and expressed as MPO unit/g protein.

Lipid peroxidation product, malondialdehyde (MDA): was measured by thiobarbituric acid (TBARS) assay, which is based on the determination of malondialdehyde (MDA), an end product of lipid peroxidation, which can react with thiobarbituric acid to yield a pink colored complex exhibiting a maximum absorption at 532nm (Yoshioka et al., 1979) .

Assay of glutathione (GSH): the stomach was perfusing intraluminally with 5% sulphosalicylic acid and then homogenized in the same solution (10%). The tissue homogenate was centrifuged for 5 min at 10,000 X g, and the amount of GSH was measured in the supernatant according to Griffith (Griffith, 1980).

Determination of SOD activity: for the determination of activity of superoxide dismutase (SOD), a sample of gastric mucosa was taken, as described above. This method is based on the SOD-mediated increase in the rate of autooxidation of tetrahydrobenzofluorene in aqueous alkaline solution to yield a chromophore with maximum absorbance at 525 nm. This absorbance was measured colorimetrically by spectrophotometer Marcel s300, Warsaw, Poland. Results were expressed as units per gram of tissue (U/g) (Kwiecień et al., 2002).

Statistical analysis

Results were expressed as mean \pm SEM (standard error of the mean). The intergroup variation was measured by one way analysis of variance (ANOVA) followed by Tukey's Multiple comparison test. Statistical significance was considered at $p < 0.05$ (George and William, 1980).

5. RESULTS

The present study showed that *H.pylori* induced gastritis model was evaluated for gastric function, inflammation and neutrophilic infiltration. As observed in table (1), serum gastrin levels showed no statistically significant differences between irradiated and sham animals but there were approximately two fold increase in *H.pylori* gastritis rats after 30 days administration of *H. pylori* brucella broth compared to control rats administrated *H.pylori* free broth ($p < 0.05$). Irradiated group or *H.pylori* treated with Spirulina manifested a significant decrease in gastrin, TNF- α , IL-1 β and IL-8 level after 30 days of treatment as compared to those of irradiated or *H.pylori* infected group, respectively. Animal group treated with Spirulina showed non-significant changes in the levels of serum gastrin, TNF- α , IL-1 β and IL-8 level after 30 days of treatment as compared to the value of control group.

Table (1). Effect of treatment of irradiated and *H. pylori* infected rats with Spirulina on serum Gastrin, Tumor necrosis factor(TNF- α), Interleukin-1 β (IL-1 β) and Interleukin-8 (IL-8)

Groups Parameters	Gastrin 40-169 pg/ml	Serum TNF- α 31-2500 pg/ml	Serum IL-1 β 31.2-2000 pg/ml	Serum IL-8 18.75-1200 pg/ml
Control	109.5 \pm 8.2	32.12 \pm 1.01	32.3 \pm 1.1	21.15 \pm 1.20
Spirulina	107.4 \pm 9.8	31.45 \pm 1.21	31.5 \pm 1.1	20.4 \pm 1.32
Irradiation	111.4 \pm 4.5 ^a	77.34 \pm 5.02 ^a	68.3 \pm 2.3 ^a	44.31 \pm 2.1 ^a
H.Pylori	211.2 \pm 5.6 ^a	98.61 \pm 3.21 ^a	72.6 \pm 1.1 ^a	56.2 \pm 1.1 ^a
Spirulina+Irradiation	112.2 \pm 1.3 ^b	52.45 \pm 2.1 ^b	58.5 \pm 1.6 ^b	32.6 \pm 1.2 ^b
Spirulina+H.pylori	179.6 \pm 1.6 ^c	61.89 \pm 2.60 ^c	62.4 \pm 2.1 ^c	41.2 \pm 2.1 ^c

a: Significant difference from control group.

b: Significant difference from irradiated group.

c: Significant difference from *H.pylori* infected group.

Table (2). Effect of treatment of irradiated and *H. pylori* infected rats with Spirulina on antioxidant enzyme (Glutathion (GSH) & superoxide dismutase (SOD) , malondialdehyde (TBARS) and myeloperoxidase (MPO) levels

Groups Parameters	TBARS nmol/mg protein	GSH u/mg protein	SOD u/mg protein	MPO u/g protein
Control	1.44 \pm 0.51	3.38 \pm 0.21	6.22 \pm 0.44	104.1 \pm 6.1
Spirulina	1.34 \pm 0.66	3.45 \pm 0.14	6.40 \pm 0.32	102.8 \pm 3.2
Irradiation (5Gy)	2.27 \pm 1.93 ^a	1.40 \pm 0.16 ^a	3.09 \pm 1.57 ^a	198.6 \pm 5.9 ^a
H.pylori	2.50 \pm 1.13 ^a	1.94 \pm 0.15 ^a	2.79 \pm 1.56 ^a	231.5 \pm 7.8 ^a
Spirulin+Irradiation	1.72 \pm 1.2 ^b	2.98 \pm 0.14 ^b	4.91 \pm 1.20 ^b	149.2 \pm 2.4 ^b
Spirulina+H.pylori	1.83 \pm 1.53 ^c	2.63 \pm 0.18 ^c	4.49 \pm 1.51 ^c	202.4 \pm 2.1 ^c

a: Significant difference from control group.

b: Significant difference from irradiated group.

c: Significant difference from *H.pylori* infected group.

As shown in table (2), levels of antioxidant enzyme SOD and GSH were significantly decreased while a significant increase in TBRAS was noticed in the irradiated and *H.pylori* infected groups as compared with control group. Irradiated group or *H.pylori* infected group treated with Spirulina revealed a significant increase in the level of SOD and GSH associated with a significant decrease in TBRAS as compared to those of irradiated group or *H.pylori* infected one, respectively. In gastric mucosa, neutropilic infiltration was assessed by increased MPO activity in irradiated group or in *H.pylori* group after 30 days of infection. Animal group treated with Spirulina showed non-significant changes in the level of SOD, GSH, TBRAS and MPO as compared to the values of control group.

6. DISCUSSION

Helicobacter pylori (*H. pylori*) is spiral –shaped, flagellated, Gram-negative bacterium. It colonizes the stomach of about 50 percent of the world population, especially in the developing countries (Marshall BJ and Warren, 1983, Bruce and Maarros, 2008). It is directly implicated in the dyspepsia, acute and chronic gastritis, peptic ulceration, MALT lymphoma and it is an independent risk factor for gastric adenocarcinoma (Atherton, 2006). It may also be a risk factor for pancreatic including cancer (Trikudanathan et al, 2011). *H.pylori* has been also associated to some extra-gastric diseases including several autoimmune diseases. The prevalence of *H. pylori* infection varies from country to country with large differences between developed and developing countries (Neunert et al, 2011) The epidemiology of *H.pylori* infection in developing countries is characterized by a rapid rate of acquisition of the infection such that approximately 80 percent of the population is infected by the age of 20 (Robinson et al, 2007) because the disease is most often acquired in childhood or when young children are present in the household. Chronic *H. pylori* –associated gastritis per se is asymptomatic

but the initial acquisition of the infection cause acute gastritis with hypochlorhydria which may cause abdominal pain, nausea and vomiting that resolve within a few days (Fischer et al, 2001). Gastric inflammation is highly complex biochemical protective response to the cellular tissue injury. Chronic gastritis is associated with the inflammatory cellular infiltrate predominantly consisting of lymphocyte and plasma cells in gastric mucosa. Many evidences suggest that Helicobacter pylori infection and non steroidal anti- inflammatory drug (NSAID) ingestion are major causative factors. Both are highly implicated in the pathogenesis of gastric mucosal oxidative injury in humans.

The initial response to H. pylori infection is an interaction of the host epithelial cells with the bacteria (Correa, 1988) and had been reported to be implicated in various gastrointestinal diseases, such as gastric ulcer, adenocarcinoma and lymphoproliferative disorders (Uemura et al., 2001). However, the pathogenetic mechanisms of chronic infection with H. pylori and gastric ulcer are yet to be full determined (Rad et al., 2004). H. pylori-infected gastric mucosa showed infiltration of polymorphonuclear leukocytes, lymphocytes, monocytes and plasma cells in the lamina propria, and intraepithelial severe neutrophil infiltration (Fan et al., 1996). The later well documented to correlate mucosal damage due to the effects of various cytokines, free radicals, and monochloramine (Karttunen, 1991). Moreover, H. pylori-induced inflammation is implicated in the development of mucosal damage and is characterized by strong granulocytic and lymphocytic infiltration (Rad et al., 2004). These changes would accelerate apoptosis and proliferation in the mucosal layer (Ohkura et al., 2003). In addition, it had been reported that H. pylori infection induced a three-fold increase in the serum gastrin concentration but was without effect on the thickness of the oxyntic mucosa (Zhao et al., 2003). Gastrin is gastrointestinal (GI) peptides that is mainly produced in the stomach. The most gastrin is produced in endocrine cells (G cells) of the gastric antrum [6]. The mechanisms of radiation-induced GI tract injury are complicated. The relationship between alterations of plasma GI peptides and symptoms and signs of the radiation sickness has not been previously well described

(Sakdhisapol et al., 2008) .Since the gastric mucosa is relatively more radioresistant than the duodenum and other parts of small bowels such as the jejunum and ileum, the lack of significant changes in plasma levels of gastrin after irradiation were not surprising and this was in agreement with the present study as there were no significant change in serum gastrin between irradiated group and control group, table (1).

H. pylori infection induces an inflammatory response that is also oxidative. The gastric epithelium and the bacteria induce production of interleukin-8 (IL-8) that contributes to the generation of great amounts of toxic reactive oxygen species (ROS), with marked infiltration of inflammatory cells and can elicit induction of interleukin-1 β (IL-1 β), interleukin -6 (IL-6), IL-8, IL-12, tumor necrosis factor- α (TNF- α) and interferon- γ (INF- γ) (Marshall et al, 1985) and these were contribute with the present study shown in table (1), the level of (TNF- α) , (IL-1 β) and serum(IL-8) were elevated in H. pylori infected group and irradiated one in comparison to control. The inflammatory response induced during H. pylori infection does not appear to confer protective immunity and the resulting oxidative burst caused by phagocytic cells can damage gastric tissues (Graham et al, 2004) (Disction of H.Pylori and strategy...). Wilson et.al, 1996 showed that the gastric mucosal levels of proinflammatory cytokines, such as TNF- α , IL-1 β , IL-6 and IL-8, were significantly higher in H. pylori positive patients than in H. pylori negative patients. Crabtree *et al.*, showed that increased gastric mucosal production of TNF- α and IL-6 was associated with H. pylori gastritis. Moreover, they implied that inflammatory cytokines generated locally within the gastric mucosa can be relevant to the gastric physiology of H. pylori infection. In 2006, Prabjone *et al.*, investigated the effects of chronic H. pylori infection on serum TNF- α level in rats. They found a significant increase in serum TNF- α in the H. pylori infected groups compared with the control groups.

Table (1) shows the activity of serum gastrin, TNF- α , IL-1 β and IL-8. There was a significant increase in these parameters in both H.pylori infected group or irradiated rats when compared with controls. The administration of Spirulina to the treated rats tended to bring the values of these parameters to normal levels.

C-phycoyanine (C-PC) , a biliprotien found in the blue green algae such as Spirulina platensis, may affect the function of mast cells that are an important resource for synthesis and release of prostaglandine D2, leukotrienes, ROS and cytokines such as TNF- α . IN addition, TNF- α plays an

early and crucial role for the subsequent inflammatory response through stimulating the production of cyclooxygenase (COX) product and IL-8 that induce local production of sympathetic amines, thus inhibition of TNF- α formation by (C-PC) in carrageen an injected paws may contribute to its antihyperalgestic activity (Mohamed, 2012).

Table (2) shows the activity level of SOD,GSH,TBARS and MPO. There was a significant increase in the level of TBARS and MPO while a significant decrease in the level of SOD and GSH was noticed in the H.pylori infected group or irradiated one in comparison with control rat. Animal groups treated with Spirulina tended to bring the results significantly towards the normal levels (Mohamed, 2012).There substantial evidence that oxygen derived free radicals play an important role in the pathogenesis of the injury of various tissues, including the digestive system.SOD and GSH are the important endogenous antioxidant enzyme playing an important role in protecting gastric mucosal tissue against oxidative damage.SOD superoxide radical to form hydrogen peroxide and water (Hemnani and Parihar1989) (40).GSH is also an important inhibitor of free radical mediated lipid peroxidation (Meistor,1983 and Gosavi 2012) .

Myeloperoxidase (MPO) is an enzyme existing in the gastric tissue whose expression is enhanced in the inflammatory conditions of the gastric mucosa and is responsible for elevation of oxidative stress³⁸. MPO seems to act on macrophage as deduced by the increased production of cytokines and oxidative burst when macrophages are exposed to MPO or other peroxidases(Lefkowitz et al.,1992) ³⁹. MPO is a key weapon of the innate immune system, providing a first line defense. At the cellular level MPO occurs in the macrophages. In the inflammatory conditions, monocyte infiltration leads to elevation of MPO and hypochlorous acid within the macrophage (Sorg, 2004 and Rodrigues 2002). MPO has been attributed to its unique capacity to produce hypochlorous acid and other toxic agents that create an environment within the phagolysosome neutrophils that inhibits or kills ingested microbes. MPO utilizes H₂O₂ to increase posttranslational modifications of target molecules, following a paradigm utilized by all members of the animal peroxidase family, although the capacity to oxidize Cl⁻ to Cl⁺ at physiologic pH is a property unique to MPO. Concomitant with release of MPO into the phagosome, the NADPH-dependent oxidase of phagocytes is activated to generate the required H₂O₂ for MPO to mediate HOCl (hypochlorous acid) generation (Hansson, 2006).

Usually at inflammatory sites neutrophils precede macrophage infiltration. It appears that macrophages can acquire MPO by engulfing neutrophils or MPO released by these cells at the inflammatory site (Shepherd & Hoidal 1990) and (Leung &Goren 1989) 43-44. Macrophages and neutrophils vary from a resting to a fully activated state. The activation includes the expression of several types of proteins, such as membrane receptors, soluble factors and enzymes. Reactive oxygen (RO) intermediates appear to be important in some signaling pathways involved in phagocyte activation (Remick & Villarete 1969) and (Schreck 1992) 45, 46. Hence, the cellular activity of enzymes that utilize or generate reactive oxygen species (ROS) could reveal the steady state concentration of these species. These mediators lead to necrosis and dysfunction of the gastric mucosa (Baker &Campbell 1990) and (Grisham 1994). The endogenous cellular antioxidants are exhausted which promotes further ulceration of the gastric mucosa (Ohkawa 1979) and (Bradley 1989) 50, 51 .The levels of MPO in the gastric mucosa represent the extent of gastric mucosal damage and regarded as a measure of neutrophil infiltration and oxidative stress (Nishiwaki, 1999) and (Oh et al., 2005) 54,55.(protocol of H.pyl.) Spirulina was also found to significantly reduce the MPO activity in a dose dependent manner as contain several active ingredients notably phycocyanine and β -carotien that have potent antioxidant and anti-inflammatory activities.

With the increasing prevalence of antibiotic-resistant organisms, new strategies to combat infection are being sought. One such strategy is the use of antiadhesive molecules targeting the primary step of infection, i.e. adhesion of the organism to the host (Ofek et al., 2003). Inhibition of *H. pylori* adhesion works on the principle that by blocking bacteria attaching to host tissue there is a lower risk that these bacteria will develop resistance compared with the use of bactericidal compounds (Bavington & Page, 2005). Investigations into algal materials as sources of antiadhesives have become common over the past few years, as many algal species contain polysaccharide (PS) substances with various biological activities at relatively high concentrations. Mun et al., (2007) found that there was a decrease in H. pylori load in the stomach of mice fed with PS or Spirulina even for a short period of 2 h before infection. When treatment with PS or Spirulina was extended to 4

weeks, the reduction in H. pylori load was even more obvious. This may suggest that PS and Spirulina may have a protective effect against the colonization of H. pylori (Spirulina and H.pylori).

In conclusion, the data substantiate the protective effect of Spirulina against oxidative stress induced by exposed to either gamma radiation and / or infected by Helicobacter pylori bacteria. The potential protective of Spirulina may be associated with its antioxidant constituent. In addition, the inhibition of TNF- α and IL- β expression suggest that Spirulina may be potential therapeutic drug for reducing inflammatory interception. Further studies with larger samples and longer duration are required to ascertain the mechanism of Spirulina's actions and its antioxidant and anti-inflammatory capacity.

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