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ORIGINAL ARTICLES

The Protective Effects Of Whey Protein And Spirulina Against CCl_4-Induced Erythrocyte Damage In Rats

[1]Khaled G. Abdel-Wahhab, [1]Fathia A. Mannaa, [2]Mosaad A. Abdel-Wahhab

[1]Medical Physiology Department, [2]Food Toxicology & Contaminants Departments, National Research Center, Dokki, Cairo, Egypt.

ABSTRACT

The present study was undertaken to evaluate the antioxidant potential of whey protein concentrate (WPC) and/or Spirulina against erythrocytes toxicity in rats. Animals were treated orally for 30 days as follow: the control group; CCl_4-treated group and the groups treated with Spirulina and/or WPC alone or plus CCl_4. The results revealed that CCL_4 induced a significant decrease in total hemoglobin (Hb) and Oxy-Hb contents accompanied with a significant increase in Met-Hb, Carboxy-Hb and Sulf-Hb fractions. CCL_4 also increased MDA and decreased Met-Hb reductase, catalase and glutathione-S-transferase activities in erythrocytes. In addition, CCl_4 induced a marked elevation in the autoxidation rate of oxyhemoglobin. The correlation coefficient analysis revealed significant negative correlation between the erythrocytic enzymes activity and hepatic MDA level. Total Hb and Oxy-Hb contents also correlated negatively while Met-Hb and Carboxy-Hb correlated positively with the hepatic MDA level. Treatment with WPC and Spirulina resulted in a significant improvement in most of the tested parameters and succeeded to restore their values towards the normal values of the control. In conclusion, the protective effects of WPC and Spirulina against CCl_4-induced erythrocyte toxicity may be attributed to its antioxidant and free radical scavenging activities due to its higher contents of antioxidant components.

Key words: *Spirulina*; whey protein; oxidative stress; erythrocytes; CCl_4

Introduction

Reactive oxygen species are thought to be important in the pathogenesis of various human diseases. They generated endogenously under physiological and pathological conditions and upon exposure to exogenous challenge (Barry, 1991). Active oxygen molecules such as the superoxide radical play an important role in inflammation process after intoxication by carbon tetrachloride (CCl_4) (Slater and Sawyer, 1971). CCl_4 has been studied extensively as a model of xenobiotic-induced lipid peroxidation and toxicity. It is known to be metabolized by cytochrome P450 to reactive intermediates (e.g., trichloromethyl radical) that induce cell injury (Recknagel *et al*., 1989; Comporti, 1989). Erythrocytes represent one of the target cells that are damaged by these reactive metabolites (Schulze and Kappus, 1980). The damage to erythrocytes membranes by CCl_4, is evidenced by the increased amount of lipid peroxidation products, the increased membrane fluidity, and the reduced activities of membrane-bound enzymes (Damodara Reddy and Venkaiah, 1984). Many antioxidant compounds, naturally occurring from different sources, have been identified as free radical or active oxygen scavengers.

Whey protein concentrates (WPC) are a heterogeneous group of proteins (β-lactoglobulin, α-lactalbumin, serum albumin and immunoglobulins) obtained in milk after casein precipitation (Sukkar and Bounous, 2004). WPC contains substances, such as hormones, growth factors (insulin-like growth factors, transforming growth factor-β, platelet derived growth factor) and cytokines, which can have an important physiological role (Sukkar and Bounous, 2004). According to Parodi (1998), WPC contains also carbohydrate (4% lactose) and 5% lipids (approximately 25% fatty acids and 25% phospholipids). Several studies have reported that whey protein has antioxidant activity, owing to the abundance of cysteine or the presence of glutamylcysteine which facilitate glutathione (GSH) synthesis (Bounous and Gold, 1991; Lands *et al*., 1999; Micke *et al*., 2001). So, whey protein may be a therapeutic tool for oxidative-stress-associated diseases (Balbis *et al*., 2009).

Spirulina platensis (*Cyanobacterium*; Family-*Oscillatoriaceae*) is a traditional food of some Mexican and African people. It is a planktonic blue-green algae found in alkaline water of volcanic lakes. *Spirulina* has 62% amino acid content and is the world's richest natural source of vitamin B_{12} and contains a whole spectrum of natural mixed carotene and xanthophyll phytopigments (Pińero Estrada *et al*., 2001). *Spirulina platensis* contains also high levels of protein, minerals, polyunsaturated fatty acids and has been reported to have

Corresponding Author: Mosaad A. Abdel-Wahhab, Food Toxicology & Contaminants Department National Research Dokki, Cairo, Egypt.
E-mail: mosaad_abdelwahhab@yahoo.com

pharmaceutical potential (Morist *et al*., 2001; Li *et al*., 2003). Several reports have shown that the protean extract of *S. platensis* is a potent radical scavenger, has chelating ability and inhibits microsomal lipid peroxidation (Pińero Estrada *et al*., 2001; Bermejo *et al*., 2008). Moreover, many studies have reported that *S. platensis* exhibited hepatoprotective (Torres-Duran *et al*., 1999), antioxidant, radical scavenging, antiarthritic and anti-inflammatory properties, as demonstrated by both *in vitro* and *in vivo* experimental models (Romay *et al*., 1998; Reddy *et al*., 2003). The present study was undertaken to evaluate the antioxidant potential of WPC and Spirulina and their combination against the erythrocytes toxicity induced by CCl_4 in rats.

Materials and Methods

Chemicals:

Carbon tetrachloride (CCl_4), perchloric acid and trichloroacetic acid (extra pure 99%) were obtained from SISCO Research Laboratories PVT LTD (Mumbai, India). Thiobarbituric acid was obtained from MERCK (Darmstadt, Germany). Other solvents and chemicals used were either analar or of analytical grade unless otherwise specified.

Materials:

Whey protein (WPC80): Concentrated whey powder containing 80% proteins was purchased from Davisco Foods International, Inc. (Eden Prairie, MN, USA).

Spirulina algae: Food-grade Spirulina microalgae powder was obtained from Bluebio (Yantai) Biopharmaceutical Co., Ltd. (Sichuan, China).

Animals and treatments:

Adult Sprague Dawley male rats weighing 100-120 g were used. The animals had free access to tap water and laboratory diet (160.4 g protein, 36.3 g fat, 41 g fiber per kilogram and 12.08 MJ of metabolizable energy). Animals were housed in filter-top polycarbonate cages in a room free from any source of chemical contamination, artificially illuminated and thermally controlled, at the Animal House Laboratory, National Research Center, Dokki (Cairo, Egypt). All animals received humane care in compliance with the guidelines of the animal care and use committee of the National Research Center, Dokki, Egypt.

After an acclimatization period of 1 week, the animals were divided into eight groups (8 rats each) and treated orally for 30 days as follows: Group (1) the control group; Group (2) received the aqueous solution of Spirulina (0.5 mL/rat); Group (3) received the aqueous solution of WPC (0.5 mL/rat); Group (4) received *Spirulina* plus WPC; Group (5) received CCl_4 in olive oil in a single daily dose of 100 mg/kg B.W.; Group (6) received CCl_4 and *Spirulina*; Group (7) received CCl_4 and WPC and Group (8) received CCl_4 and *Spirulina* plus WPC mixture. The oral doses of WPC and *Spirulina* were prepared according to Gad *et al*. (2011). One hundred milligrams of WPC or Spirulina and their combination (1:1) was dissolved in 1mL of distilled water to obtain a concentration of 100 mg/mL. The daily oral dose was 0.5 mL per rat for each aqueous solution.

Blood and tissue sampling:

At the end of the experimental period, animals were fasted overnight, and following diethyl ether anesthesia, blood samples withdrawn from the retroorbital venous plexus into clean tubes containing heparin. Part of the whole blood was used immediately for the determination of hemoglobin and its derivatives and the other part was used for hemolysate preparation. After blood collection, all animals were rapidly killed and liver tissues were dissected and immediately homogenized in phosphate buffer (pH 7.4) to give 20% w/v homogenate (Lin *et al*., 1998). This homogenate was centrifuged at 1700 rpm and 4 °C for 10 min; the supernatant was stored at -70 °C until analysis. The supernatant was used for the determination of malondialdehyde (MDA) and total antioxidant capacity (TAC) levels.

Hemolysate preparation:

Hemolysate was prepared according to Silva *et al*. (2000). Briefly, whole blood was centrifuged at 3000 rpm for 15 minutes, then the buffy coat was removed, and the packed red cells were washed three times with physiological saline. The washed cells were lysed by suspending in hypotonic phosphate buffer and centrifuged at 7000 rpm for 30 minutes. The resulting pellet is the erythrocyte membrane, and the supernatant represents the hemolysate. The hemolysate obtained was further used for MDA and enzymatic assays.

Determination of autoxidation rate of oxyhemoglobin:

Autoxidation rate of oxyhemoglobin was determined following the method described by Mansounri and Winterhalter (1973).

Determination of total hemoglobin concentration:

Spectrophotometic determination of total hemoglobin (Hb) concentration in whole blood was carried out using a kit purchased from Biodiagnostics Co. (Cairo, Egypt).

Determination of hemoglobin derivatives of different ligands as % of total hemoglobin:

Methemoglobin (Met-Hb) level was determined in blood sample using the method described by Evelyn and Malloy (1938). Oxyhemoglobin (Oxy-Hb), sulfhemoglobin (Sulf-Hb) and carboxyhemoglobin (Carboxy-Hb) levels in the blood sample were determined spectrophotometrically according to the method described by Van Kampen and Zulstra (1965).

Erythrocytic MDA and enzymatic assays:

Colorimetric determination of MDA in the hemolysate was carried out using kits purchased from Biodiagnostic Co (Cairo, Egypt). The activity of methemoglobin reductase (Met-HbR) was estimated in the hemolysate by assessing the spectrophotometrically rate of NADH-oxidation using the chemical method described by Hegesh *et al*. (1968). Glutation-S-transferase (GST) and catalase (CAT) activities were measured in the hemolysate according to the instruction manual of kits obtained from Biodiagnostic Co. (Cairo, Egypt).

Determination of hepatic MDA and TAC:

The determination of hepatic MDA as an end product of lipid peroxidation was carried out by using perchloric acid, trichloroacetic acid and thiobarbituric acid following the chemical method described by Ruiz-Larrea *et al*. (1994). TAC was measured colorimetrically using a kit purchased from Biodiagnostics Co. (Cairo, Egypt).

Statistical analysis:

All data were statistically analyzed using one way analysis of variance (ANOVA). Correlation analysis was also performed. All analyses were performed using statistical analysis system (SAS) program software; copyright (c) 1998 by SAS Institute Inc. (Cary, NC, USA). The significance of the differences among treatment groups was determined using Tukey test (Steel and Torrie, 1980). All statements of significance were based on probability of $P \leq 0.05$.

Results:

The effects of CCl_4, Spirulina and WPC on autoxidation rate of oxyhemoglobin are depicted in figure (1). CCl_4 induced a marked elevation in the autoxidation rate of oxyhemoglobin. Rats treated with Spirulina or WPC alone or in combination showed insignificant changes in the autoxidation rate of oxyhemoglobin compared to the control group. Animals treated with CCl_4 plus WPC, Spirulina, or WPC- Spirulina mixture showed a significant decrease in autoxidation rate compared to those treated with CCl_4 alone. The combined treatment with Spirulina offered the more improvement in the autoxidation rate of oxyhemoglobin.

Date presented in Table (1) shows that CCl_4 administration resulted in significant decreases in total Hb and Oxy-Hb values concomitant with significant increases in Met-Hb, Carboxy-Hb and Sulf-Hb values compared to control while; administration of Spirulina or WPC or their combination had no significant effect on the hemoglobin or its derivatives. The administration of Spirulina or WPC to the intoxicated animals produced insignificant increase in total Hb level compared with those treated with CCl_4 alone, whereas the administration of Spirulina-WPC mixture succeeded in ameliorating significantly the CCl_4-induced reduction in total Hb level. At the same time, all other hemoglobin derivatives in the intoxicated animals were improved significantly by Spirulina and/or WPC.

The results presented in table (2) show that CCl_4 induced significant decreases in erythrocytic Met-HbR, CAT and GST activities while it significantly increased erythrocytic MDA level as compared to control. Administration of Spirulina, WPC or WPC plus Spirulina generally resulted in a significant protection of these parameters against the oxidative damage of CCl_4.

The correlation between the erythrocytes measurements and the levels of hepatic MDA and TAC in CCl_4 intoxicated rats are depicted in Table (3). The obtained data revealed that hepatic MDA showed a negative correlation with the antioxidant enzymes in the erythrocytes (Met-HbR, CAT and GST). Hepatic MDA correlated also negatively with total Hb and Oxy-Hb while it correlated positively with erythrocytic MDA, MetHb, Sulf-Hb and Carboxy-Hb. The vice versa has occurred with the hepatic TAC.

Discussion:

Several activities of the antioxidants are mediated by inhibition of reactive oxygen species (ROS), which are generated during the oxidative burst. Thus, the usefulness of antioxidants in protecting cellular components against oxidative stress is well established (Mohan *et al*., 2006). In most cells, mitochondria are major source of ROS (Johnson *et al*., 2005). Despite their lack of mitochondria, ROS are continuously produced in the erythrocytes due to the high O_2 tension in arterial blood and their abundant heme iron content (Baynes, 2005).

The source of ROS in erythrocytes is the oxygen carrier protein heamoglobin, oxyhemoglobin (Oxy-Hb) that undergoes autoxidation to produce $O_2^{\bullet-}$ (Johnson *et al*., 2005). Oxy-Hb undergoes a slow autooxidation, producing $O_2^{\bullet-}$, which yields hydrogen peroxide (H_2O_2). Therefore, Hgb is constantly exposed to an intracellular flux of H_2O_2 as well as to an extracellular flux, due to the high permeability of this metabolite. Exposure of Oxy-Hb to H_2O_2 leads to oxidative modifications that have been proposed as selective signals for proteolysis in erythrocytes (Giulivi and Davies, 2001). Occasional reduction of O_2 to $O_2^{\bullet-}$ is accompanied by oxidation of Oxy-Hb to methemoglobin (Met-Hb), a rust brown-colored protein that does not bind or transport O_2 (Johnson *et al*., 2005).

In the present study, we evaluated the protective effects of WPC and Spirulina and their combination against erythrocytes damage induced by CCl_4 in rats. The results revealed that CCl_4 intoxication caused a significant increase in the autoxidation rate of oxyhemoglobin to methemoglobin, which indicated that CCl_4 induced oxidative stress on red blood cells similar to that reported in previous studies (Damodara Reddy and Venkaiah, 1984; Kumaravelu *et al*., 1996) through the increased production of MDA in erythrocytes. Alternatively, Muriel and Mourella (1990) and Mourella and Teresa (1991) reported that the erythrocytes membrane alterations and the loss of functional integrity precede the onset of CCl_4 -induced liver cirrhosis.

CCl_4 is metabolized by a drug-metabolizing enzyme system (Cytochrom P-450) in the hepatic cell into trichloromethyl free radical ($CCl_3\bullet$) which either bind covalently with lipoproteins or reacts with oxygen to form a trichloromethylperoxy radical $(CCl_3OO\bullet)^-$, a much more reactive radical than $CCl_3\bullet$ (Packer *et al*., 1978). These free radicals attack microsomal lipids leading to their peroxidation and also covalently bind to microsomal lipids and proteins. This results in the generation of reactive oxygen species (ROS), which includes the superoxide anion O_2, H_2O_2 and the hydroxyl radical (Packer *et al*., 1978; El Denshary *et al*., 2012).

Direct exposure to molecular oxygen and circulating components in the blood and the loss of the de novo synthesizing capacity of new enzyme molecules during maturation put the erythrocytes at high risk of damage by superoxide anion O_2 and H_2O_2 molecules. These reactive molecules are involved in lipid peroxidation (Mengel and Kann, 1966; Gad *et al*., 2011), the oxidation of thiol groups of enzymes (Jacob and Jandl, 1962) and the oxidative degradation and denaturation of Hb (Carrel *et al*., 1975). The denaturated hemoglobin then precipitates and covalently binds to the interior erythrocytes membrane, thus forming Heinz bodies. This process distorts the cell membrane, resulting in increased erythrocytes fragility and hemolysis (Rae, 1999).

In the current study, CCl_4 significantly affect the quantity and function of Hb molecule whereas total Hb and Oxy-Hb contents decreased significantly concomitant with significant increases in Met-Hb, Sulf-Hb and Carboxy-Hb contents. These changes may, therefore, is a consequence of the increase in oxidative stress which caused by free radicals generated during the metabolic degradation of CCl_4 (Makni *et al*., 2012). In erythrocytes under oxidative stress, there is a considerable rise in the level of Met-Hb, which is known to be incapable of reversible oxygen binding (Lukyanenko, 2004). Met-Hb is formed when the ferrous porphyrin complex of Hb is oxidized into the ferric form (Jaffe and Hulquist, 1995). *In vivo*, Met-Hb is predominately reduced by the NADH cytochrome b 5- Met-Hb reductase system, and minor pathway such as the NADPH-dependent Met-Hb reductase (Kennett *et al*., 2005). It was suggested that NADPH concentration may be important in preventing Met-Hb generation. Loss of NADPH and glutathione (GSH) are thought to account for the enhanced rates of Met-Hb generation and lipid peroxidation (Scott *et al*., 1991; Makni *et al*., 2012). The free radicals may also induce configuration changes in Hb molecule and make it susceptible to bind unfavorable ligands other than oxygen such as carbon monoxide (CO) and sulphur (S).

In addition to the inhibition of erythrocytic Met-Hb reductase activity by CCl_4, the oxidative stress of CCl_4 on the erythrocytes in the present work is further confirmed by the significant reduction in erythrocytic GST and CAT activities. Similar reduction in the activities of GST and CAT was obtained by Kumaravelu *et al*. (1996). GSTs are a supergene family of dimeric, enzymes that catalyse the conjugation of the sulfhydryle group of GSH to a variety of electrophiles and metabolites that may cause cell damage (Strange *et al*., 2000; Valko *et al*., 2007). Catalase is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is

found in the erythrocytes and liver. This enzyme decomposes H_2O_2 and protects the tissues from highly reactive hydroxyle radicals (Valko *et al*., 2007). Damodara Reddy and Venkaiah (1984) suggested that the oxidative stress is normally challenged in erythrocyte by cellular antioxidant defenses including reduced glutathione, GPx, and CAT. GPx are important for dealing with the endogenous H_2O_2 produced by Hgb autoxidation, while CAT plays an increasingly important role as the erythrocyte is exposed to increased H_2O_2 flux (Johnson *et al*., 2005). The increased oxidative stress in erythrocytes leads to the exhaustion of the antioxidant capacities which become insufficient to counteract the excessive production of ROS (Fahmy and Hamdi, 2011). These ROS can also inhibit DNA and RNA protein synthesis in liver and therefore affect enzymes synthesis (Sreenivas Rao *et al*., 2004).

In the current study, the treatment with WPC and/or Spirulina could decrease lipid peroxidation in red blood cells that was elevated by CCl_4 and consequently decreased the exhaustion of the antioxidant enzymes. WPC and Spirulina also improved heamoglobine function since they could elevate total Hb and Oxy-Hb contents and decrease Met-Hb formation. These data further confirm the antioxidant potential of WPC and Spirulina. The obtained effect of Spirulina on total Hb in the current study is similar to that obtained by Simsek *et al*. (2009).

The current study also revealed that administration of WPC and Spirulina alleviated CCl_4-induced depletion of total antioxidant capacity content in liver, and consequently reduced oxidative damage in liver cells (data not shown). This may in turn improves liver function and consequently improves the synthesis of the antioxidant enzymes and glutathione required for erythrocytes protection. The correlation analysis performed in the present study, supports this suggestion. This analysis demonstrated a strong correlation between the healthy state of the liver and erythrocytes function. We found significant negative correlation between the erythrocytic enzymes activity and hepatic MDA level. Total Hb and Oxy-Hb contents also correlated negatively while Met-Hb and Carboxy-Hb correlated positively with the hepatic MDA level.

In a previous study (Gad *et al*., 2011), found that WPC and Spirulina are free radical scavengers and able to react with the DPPH radical in a dose-dependent manner. Moreover, the same authors found that the chelating activity from WPC and Spirulina exhibited a strong inhibition of ferrozine-Fe_2 complex formation which indicated the presence of antioxidant compounds that might act as electron donors. WPC and Spirulina contain a number of bioactive compounds which are generally believed to be the active constituents responsible for their antioxidant activity. The major components responsible for the antioxidant activity of the tested materials are including thiol (SH) groups in WPC, total carotenoids, total tocopherol and total phycocyanin in Spirulina (Gad *et al*., 2011).

Several studies have indicated that whey proteins are rich in cysteine and glutamate residues which suggested that their ingestion may contribute to increase the level of free cysteine and consequent production of GSH (Lands *et al*., 1999; Micke *et al*., 2001). GSH, a tripeptide product synthesized from cysteine, glutamate and glycine are low-molecular-weight thiol reductant present in most cells. However, GSH is of major significance in the cellular antioxidant activity of the ‘‘GSH antioxidant system’’ because it participates directly in the destruction of reactive oxygen species and also maintains in a reduced form by ascorbate and a-tocopherol, which also exerts an antioxidant effect (Meister, 1994).

The liver is the major site of GSH synthesis and has the ability to convert the sulphur amino acid methionine to cysteine required for GSH synthesis (Kaplowitz *et al*., 1985; Meister *et al*., 1986). Almost 95% of GSH synthesized in the liver is released in the blood stream, which supplies the extra-hepatic tissues (Kaplowitz *et al*., 1985). GSH is considered the major antioxidant in erythrocytes and protects important proteins such as spectrin, (Carroll *et al*., 2006), supports antioxidant defense and also maintains -SH groups in Hgb and enzymes in the reduced state (Baynes, 2005).

Previous studies on the mechanisms of CCl_4-induced hepatotoxicity have shown that GSH plays a key role in the detoxification of the reactive toxic metabolites of CCl_4 and liver necrosis begins when GSH stores are markedly depleted (Balbis *et al*., 2009). GSH is also important in immune regulation (Barta *et al*., 1991) and cancer prevention in animals (Bounous and Gold, 1991; McIntosh *et al*., 1995) and in helping overcome GSH-deficiency in seropositive and Alzheimer’s disease patients (Madureira *et al*., 2007). On the other hand, Spirulina platensis is gaining more attention because of its nutritional and various medicinal properties. Its nutritional value derives from its high protein (Simpore *et al*., 2006), lipids and carbohydrates (Upasani *et al*., 2003; Gong *et al*., 2005) contents. Spirulina contains phycobilisomes as light-harvesting protein-pigment complexes (Pińero Estrada *et al*., 2001). Phycobilisomes are mainly composed of polypeptides named phycobiliproteins. Phycocyanin and allophycocyanin are considered the more important phycobiliproteins (Bermejo-Besĉs *et al*., 2008). In addition, Spirulina contains vitamin B12, β-carotene, and xanthophyll phytopigments which, together with phycocyanin, seem to be related to its antioxidant activity (Miranda *et al*., 1998; Bhat and Madyastha, 2000; Pińero Estrada *et al*., 2001). Moreover, Spirulina contains some minerals such as selenium, magnesium, manganese and vitamins including alpha tochopherol and alpha lipoic acid (Upasani and Balaraman, 2003; Gong *et al*., 2005), which may strengthens its antioxidant potential and make it more effective than WPC in protecting the erythrocytes from the damaging impact of CCl_4.

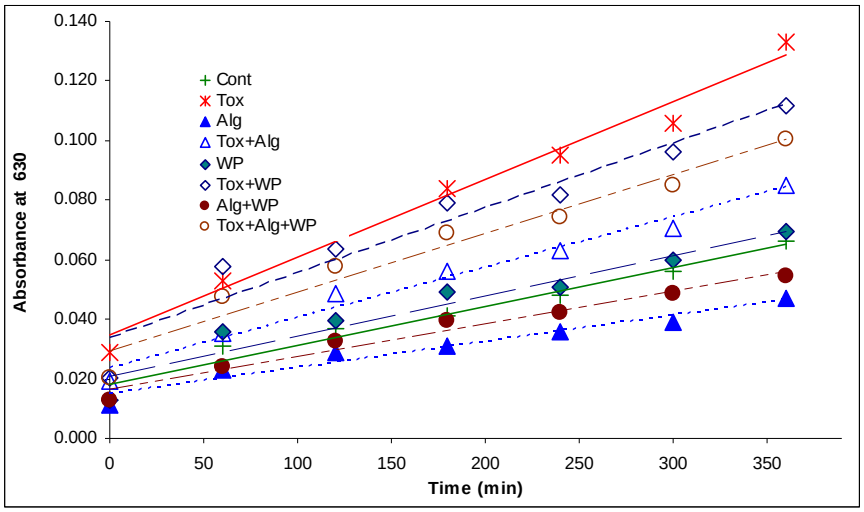

Fig. 1: Effects of Spirolina (Alg) and Whey protein (WP) on the autoxidation rate of oxyhemoglobin in CCl_4 (Tox) - intoxicated rats.

Table 1: Effect of different treatments on the levels of hemoglobin (g/dl) and its derivatives (expressed as % of total hemoglobin) in the blood of CCl_4-intoxicated rats.

	Total Hb	Oxy-Hb	Met-Hb	Carboxy-Hb	Sulf-Hb
	g/dl	%	%	%	%
Control	14.83 ± 0.91^{A}	$96.8 \pm 0.0.43^{A}$	$2.63 \pm 0.0.42^{D}$	0.49 ± 0.14^{B}	0.12 ± 0.017^{A}
Spirulina	14.87 ± 0.59^{A}	97.3 ± 0.36^{A}	2.14 ± 0.30^{D}	0.46 ± 0.11^{B}	0.13 ± 0.016^{A}
WPC	14.97 ± 0.29^{A}	97.1 ± 0.10^{A}	2.31 ± 0.25^{D}	0.46 ± 0.11^{B}	0.14 ± 0.016^{A}
Spirulina + WPC	14.92 ± 0.30^{A}	97.4 ± 0.25^{A}	2.01 ± 0.20^{D}	0.46 ± 0.11^{B}	0.13 ± 0.016^{A}
CCl_4	11.08 ± 0.59^{C}	89.7 ± 0.63^{D}	7.29 ± 0.38^{A}	2.72 ± 0.52^{A}	0.27 ± 0.11^{B}
CCl_4+ Spirulina	13.10 ± 0.44^{ABC}	96.0 ± 0.49^{AB}	3.01 ± 0.37^{CD}	0.93 ± 0.31^{C}	0.11 ± 0.013^{A}
CCl_4+ WPC	12.56 ± 0.55^{BC}	93.6 ± 0.55^{C}	5.10 ± 0.41^{B}	$1.15 \pm 0.0.34^{C}$	0.13 ± 0.016^{A}
CCl_4+ Spirulina + WPC	13.35 ± 0.32^{AB}	94.8 ± 0.40^{B}	3.88 ± 0.27^{CD}	1.24 ± 0.28^{C}	0.12 ± 0.014^{A}

Values are mean ± SE for 8 rats per group.
Within each column, means with different letters are significantly different ($P < 0.05$) using one way (Tukey) ANOVA test.

Table 2: Effect of different treatments on lipid peroxidation products (MDA) and primary enzymatic antioxidants of the erythrocytes of CCl_4-intoxicated rats.

	Met-HbR	CAT	GST	MDA
	nmol/min/mgHb	U/ g Hb	U/ g Hb	nmol/g Hb
Control	0.217 ± 0.13^{A}	433 ± 26.5^{A}	257 ± 15.7^{A}	267 ± 16.4^{B}
Spirulina	0.221 ± 0.05^{A}	435 ± 22.4^{A}	259 ± 13.4^{A}	259 ± 13.4^{B}
WPC	0.219 ± 0.04^{A}	442 ± 22.9^{A}	261 ± 12.4^{A}	256 ± 13.2^{B}
Spirulina + WPC	0.214 ± 0.03^{A}	437 ± 22.7^{A}	257 ± 13.4^{A}	246 ± 12.8^{B}
CCl_4	0.141 ± 0.02^{B}	302 ± 18.4^{B}	185 ± 11.3^{B}	393 ± 24.1^{A}
CCl_4+ Spirulina	0.194 ± 0.04^{A}	415 ± 19.5^{A}	246 ± 12.8^{A}	296 ± 15.4^{B}
CCl_4+WP	0.179 ± 0.05^{A}	353 ± 19.8^{B}	242 ± 12.6^{A}	307 ± 15.9^{B}
CCl_4+ Spirulina + WPC	0.188 ± 0.04^{A}	407 ± 20.0^{A}	260 ± 13.6^{A}	294 ± 15.3^{B}

Values are mean ± SE for 8 rats per group.
Within each column, means with different letters are significantly different ($P < 0.05$) using one way (Tukey) ANOVA test.

Conclusion:

It could be concluded that the supplementation of WPC and Spirulina alleviated antioxidants depletion in erythrocytes and liver, which consequently suppressed oxidative stress and improved erythrocytes and liver functions in CCl_4-intoxicated rats. These data suggested that these agents are potential multiple-protective agents against xenobiotic toxicity.

Table 3: The statistical correlation coefficient (R) between the erythrocytes measurements and the levels of hepatic MDA and TAC in CCl_4-intoxicated rats.

			Erythrocytes									Liver
			Hb	met-Hb	Sulf -Hb	Carboxy-Hb	Oxy-Hb	met-HbR	CAT	GST	MDA	MDA
Liver	TAC	R	0.2990	-0.4081	-0.3976	-0.1367	0.3450	0.40146	0.4969	0.4650	-0.3632	-0.2228
		P	0.0281	0.0022	0.0029	0.3243	0.0106	0.0026	0.0001	0.0004	0.007	0.1053
	MDA	R	-0.7027	0.7477	0.0683	0.8545	-0.8432	-0.5476	-0.4655	-0.31998	0.6698	
		P	<0.0001	<0.0001	0.6237	<0.0001	<0.0001	<0.0001	0.0001	0.0183	<0.001	
Erythrocytes	MDA	R	-0.3552	0.5973	0.2600	0.5972	-0.6410	-0.06174	-0.0276	0.1176		
		P	0.0083	0.0083	0.0576	<0.001	<0.0001	0.6574	0.8431	0.3970		
	GST	R	0.5607	-0.5694	-0.2360	-0.2764	0.5027	0.8255	0.9688			
		P	<0.001	<0.0001	0.0858	0.043	0.0001	<0.0001	<0.0001			
	CAT	R	0.6476	-0.6597	-0.2069	-0.4121	0.6158	0.8823				
		P	<0.0001	<0.0001	0.1333	0.002	<0.0001	<0.0001				
	Met-HbR	R	0.6855	-0.6484	-0.726	-0.4991	0.6409					
		P	0.0001	<0.0001	0.2119	0.0001	<0.0001					
	Oxy-Hb	R	0.7892	-0.9646	-0.1253	-0.8743						
		P	<0.0001	<0.0001	0.3668	0.0001						
	Carboxy-Hb	R	-0.7287	0.7178	-0.0769							
		P	<0.0001	<0.0001	0.5806							
	Sulf -Hb	R	-0.0668	0.1733								
		P	0.6312	0.2102								
	Met-Hb	R	-0.7407									
		P	<0.0001									

Conflict of Interest:

The authors declare that there are no conflicts of interest.

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