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Protective Effect of *Spirulina platensis* Against Lead Toxication in Rats

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Abstract: It is known that *spirulina* is rich from the point of view of vitamin, mineral, essential fatty acids and antioxidants such as caretinoids. In this study, the changes on oxidant and antioxidant system occured by *spirulina* and protective effect of *spirulina* against lipid peroxidation in lead toxication in rats are examined. Forty adult female wistar albino rats were divided into four experimental groups: control, *spirulina*, lead and *spirulina* + lead-treated. At the end of the experimental period (1 month), animals in all four groups were fasted for 12 h and blood samples were taken for the determination malondialdehyde (MDA), reduced glutathione (GSH), ascorbic acid, nitrate and nitrite levels. There was a statistically significant difference between the groups for all parameters except for serum ascorbic acid. It is determined that there was a crucial increase in the level of GSH belonging to *spirulina* treated group when compared with control group, lead treated group and *spirulina* + lead treated group ($p<0,05$). It was observed that the lecvel of MDA which is an important sign of lipid peroxidation for lead treated group is statistically crucial when compared with control group and *spirulina* treated group when compared with the other groups and this increase is statistically important ($p<0.05$). Morover, it is seen that there is an increase in the level of nitrate and nitrite for *spirulina* treated group when compared with the other groups and this increase is statistically important ($p<0.05$). Consequently, it is indicated that lipid peroxidation occured an animals treated by lead is prevented by the future of antioxidant of *spirulina platensis* and therefore it assists the level of antioxidants in circulation.

Key words: *Spirulina platensis*, lead, oxidant, antioxidant, rat

INTRODUCTION

Lead toxication known for many years is a health problem. Animates are exposed to lead by mainly food, drinking water and inhalation. After taking lead, as a result of preventing the effectivenes of enzyme, calcium canals and biochemical events due to sulphidrile groups, they make toxic effect in organism[1]. In the studies done recent years, it is stated that lead is closely related with the increase in activity of reactive oxygen types and also lead causes occuring abnormalites such as hypertension, lipid peroxidation and prevention of synthesis[2].

Spirulina platensis known for 3,5 billion years in the earth as the oldest life formula is a type of cyanobacterium. Spirulina known for blue-green alg is consumed as human food because of its high protein and nutritive value. It is known that spirulina is rich from the point of view of vitamin, mineral, essential fatty acids and antioxidan pigments such as caretinoids. At the same time, these are rich from the point of antioxidant enzymes[3].

Metals, especially transition metals, act as catalysts in the oxidative reactions of biological macromolecules; thus metal toxicities might be associated with oxidative tissue damage. Exogenous chemical as lead might produce highly Reactive Oxygen Species (ROS), which are capable of oxidizing biomolecules, resulting in tissue damage and cell death. ROS include a number of chemically reactive molecules derived from oxygen such as hydrogen peroxide (H_2O_2), superoxide (O_2•-) and hydroxyl radical (OH•). ROS are formed and degraded by all aerobic organisms and can readily react with most biomolecules including proteins lipids and lipoproteins and DNA[4]. Lipid peroxidation is a well-established mechanism of cellular injury and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds, which are the most abundant malondialdehyde (MDA). Therefore, measurement of MDA is widely used as an indicator of

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lipid peroxidation. Increased levels of lipid peroxidation products have been associated with a variety of diseases in both humans and model systems. Aerobic organisms are protected against free radicals by antioxidant defense systems. Antioxidants include endogenously synthesized compounds such as reduced glutathione and as well as exogenous substances such as vitamin C[5,6]. In this study, effect of spirulina against oxidant and antioxidants and also protective effect of spirulina against lipid peroxidation are examined through lead toxication occuring rats.

MATERIALS AND METHODS

Animals: Forty adult inbred female Wistar albino rats (n=10×4) weighing about 300 g were obtained from the Laboratory of Animal Science, Medical School, Atatürk University, Erzurum, Turkey. The animals were given standard rat pellets and tap water ad libitum. The rats were housed in individual cages (360×200×190 mm), each containing 2 or 3 animals, 15 days before the start of the experiments. All animals were housed in stainless cages under standard laboratory conditions (light period 07.00 am to 8.00 pm h, 21±2°C, relative humidity 55%) and received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health.

Source of chemicals and *spirulina*: Chemical used in this study lead acetate was purchased from Sigma. The *Spirulina platensis* was provided by the Research Center of Atatürk University, Turkey. All other chemicals were analytical grade and obtained from either Sigma or Merck. Experimental procedure: They were divided into four groups [Group I - control group, Group II - spirulina-treated (S), Group III - lead-treated (Pb) and Group IV - spirulina + lead (SPb)], each containing 10 animals. Group I received normal food and water during the experiment. Group II received normal food and Spirulina (300 mg kg^{-1} dissolved in water. Group III received normal food and 2 g L^{-1} lead acetate dissolved in water. Group IV received normal food along with Spirulina (300 mg kg^{-1}) mixed and 2 g L^{-1} lead acetate dissolved in water

At the end of experiment all groups were sacrificed under ether anaesthsia. Blood samples were collected by cardiac puncture using heparinised syringe. Whole blood was collected into heparinized tubes and whole blood MDA and reduced glutathione (GSH) levels were studied on the same day of admission. Blood was also collected into a polystyrene microtube and after clotting, this was centrifuged at 4000 rpm for 7 min and the serum was removed using EDTA-washed pasteur pipettes. The serum was stored in polystyrene plastic tube at -70°C until the time of analysis. Whool blood MDA (as an important indicator of lipid peroxidation) levels were measured according to a method of Jain[7]. The principle of the method was based on the spectrophotometric measurement of the color that occurred during the reaction of thiobarbituric acid with MDA. Concentration of Thiobarbituric Acid Reactive Substances (TBARS) was calculated by the absorbance coefficient of malondialdehyde-thiobarbituric acid complex and expressed in nmol mL^{-1}. Whole blood GSH concentration also was measured by spectrophotometric method[8]. Serum vitamin C (ascorbic acid) level was determined after derivatization with 2,4-dinitrophenylhydrazine[9]. The concentrations of nitric oxide (nitrate and nitrite) were detected by the methods of Miranda *et al*[10]. Nitrite and nitrate calibration standards were prepared by diluting sodium nitrite and sodium nitrate in pure water. After loading the plate with samples (100 μL), addition of vanadium (III) chloride (100 μL) to each well was rapidly followed by addition of the Griess reagents, sulfanilamide (50 μL) and N-(1-Naphthyl) ethylenediamine dihydrochloride (50 μL). The Griess solutions may also be premixed immediately prior to application to the plate. Nitrite mixed with Griess reagents forms a chromophore from the diazotization of sulfanilamide by acidic nitrite followed by coupling with bicyclic amines, such as N-1-(naphthyl) ethylenediamine. Sample blank values were obtained by substituting diluting medium for Griess reagent. Nitrite was measured in a similar manner except that samples and nitrite standards were only exposed to Griess reagents. The absorbance at 540 nm was read to assess the total level of nitrite and nitrate in all samples[10].

Statistics: All values were expressed as mean±SE Statistical analysis of data was performed using a one-way analysis of variance (ANOVA) and Tukey's posttest. A value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

None of the animals in group died during 30 days of experiment. In this study, the results belonging to the levels of MDA accepted as oxidant and antioxidant, GSH, vitamin C, nitrate and nitrite are shown on Table 1. A significant increase is determined in the level of GSH belonging to spirulina treated group when compared with control group, lead treated group (Pb) and spirulina + lead (SPb) treated group ($p<0,05$).

Table 1: Oxidant and antioxidant effect of spirulina against lead toxication

Groups	GSH (mg dL^{-1})	MDA (nmol mL^{-1})	Ascorbic acid (mg dL^{-1})	Nitrate (mg L^{-1})	Nitrite (mg L^{-1})
Pb (2 g L^{-1})	5.51±1.08^{a}	4.51±0.87^{b}	0.53±0.05^{a}	7.50±0.74^{a}	1.77±0.25^{a}
S (300 mg kg^{-1})	10.24±0.25^{b}	2.16±0.23^{a}	0.71±0.16^{a}	14.84±2.07^{b}	4.89±0.90^{b}
S (300 mg kg^{-1}) + Pb(2 g L^{-1})	8.41±2.54^{a}	2.86±0.32^{ab}	0.56±0.06^{a}	9.01±0.69^{a}	2.27±0.35^{a}
Control	8.24±1.05^{a}	2.53±0.07^{a}	0.57±0.07^{a}	9.29±0.75^{a}	2.27±0.39^{a}

Statistical difference between the groups showed by different letters on the same column is crucial. GSH, reduced glutathione; MDA, malondialdehyde; ascorbic acid, Vitamin C. All values were expressed as mean ± S.E. Statistical analysis of data was performed using a one-way analysis of variance (ANOVA) and Tukey's posttest

Morover, the levels of both nitrate and nitrite in spirulina treated group are found higher the other groups ($p<0,05$). One of the most important signs of oxidative stress; MDA level is esthablished as the least in spirulina group. There isn't so crucial difference between the groups in terms of the level of vitamin C ($p<0,05$).

Many illnesses occur because of reactions existing in body as a result of harmful effects of free radicals on cells. These toxic metabolits harming cell occur as a result of aerobic oxidation and these increase in pathologic situations. In this kind of situations; taking antioxidant substances decrease the cellular harm in a big rate and it can prevent the ilnesses occuring due to free radicals[11].

In this study , the cahnges in the level of MDA, GSH, vit C (ascorbic acide), nitrate an nitrite are examined to designate the protective effect of *spirulina platensis* on cellular harm being occured by lead in rats. In the studies, it is stated that δ-amino laevulinic acid and free oxygen radicals occur in animals as a result of lead toxication and cellular deaths occur due to these factors[3,12].

GSH is an important cellular antioxidant and plays a major role in protecting cells against oxidative stress. GSH acts as an intracellular redox buffer; for example, intracellular hydrogen peroxide formed under oxidative stress is reduced by glutathione peroxidase with concomitant conversion of GSH to the oxidized form[13]. This effectively detoxifies hydrogen peroxide and protects the cell from oxidative damage. GSH has vital roles such as detoxification of Xenobiotics in organism. It is expressed that the level of GSH decrease in the tissues exposed to lead[11]. In our study, it is also found that the level of GSH for lead-treated group is lower than the other groups. Being high of the level of GSH for spirulina treated group and also being low of the level of GSH for lead + spirulina treated group point that treated spirulina increases the level of GSH. It shows that this situtation is sourced by antioxidant feature of spirulina. At the same time it is provided that spirulina provides decrease in the level of hepatic sitocrom P450 and increase in the level of liver glutation peroxidase; morover it inhibitates chemical mutagen and the effect of carcinogens[14]. It is informed that it decreases degranulation in mast cells on the studies done by *spirulina platensis* and for that reason, it inhibitates anaflactic shock reactions[15].

In the present study, it is observed that MDA damage occured as a result of oxidation damage being happened by lead is prevented by protective effect of spirulina. Occuring δ-amino levulinic acid, free oxygen radicals on animal treated by lead increases lipid peroxidation due to iron[1]. These peroxits cause cellular damage. Peroxites changes structure of membran by restricting the movement of phospholipide and they make cellular damage by easing the occurance of peroxites.

In this study, it is observed that taking lead at lower dosages for along time increase the occurence of free radical in circulation. The knowladge that lead increases occurence of free radical[2] and observing high level of MDA for lead treated group in the presented the study show taht lead causes lipid peroxidation.

It is determined that the level of ascorbic acide was higher for spirulina treated group when compared with the other groups; howewer it is observed that this increase isnt statistically important.

The importance of nitric oxide in antioxidant defence system is known. Although nitric oxide is stable in low concentrations and in absence of oxygen, it isnt stable in the presence of biological enviroment where oxygen and free radicals exist. Nitric oxide in biological system is reduced to oxyhemoproteins NO^{-3}. *In vivo* enviroment, the biggest metabolite of nitric oxide in blood is NO^{-3}[16] and in the present of strong oxidative agent, NO^{-3} occurs by NO^{-2}[17]. In our study it is observed that level of nitrate and nitrite belong in the animals treated spirulina is higher when compared with the other groups. The results designate that spirulina is stimulative for the occurence of nitric oxide and therefore it has supporting effect on antioxidant system.

CONCLUSION

In this presented study, it is proved that lipid peroxidation occured on lead treated animals is prevented by the feature of antioxidant of *spirulina platensis* and for that reason it assists level of antioxidants in circulation.

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