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Original Research

Spirulina exhibits hepatoprotective effects against lead induced oxidative injury in newborn rats

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Abstract: Lead is a toxic metal that induces a wide range of biochemical and physiological effects. The present investigation was designed at evaluating the toxic effects of a prenatal exposure to lead of mothers on hepatic tissue of newborn rats, and potent protective effects of spirulina. Female rats were randomly divided into 4 groups which were given a normal diet (control),a diet enriched with *spirulina* (S), lead acetate administered through drinking water (Pb), or a diet enriched with *spirulina* and lead contaminated water (S Pb), respectively. The duration of treatments was from the 5th day of gestation to 14 days postpartum. Lead toxicity was assessed by measuring body and liver weights, blood and stomach lead levels, hepatic DNA, RNA and protein amounts, blood enzyme activities (AST and ALT), as well as lipid peroxidation level and activities of antioxidant enzymes in hepatic tissues of neonates. Lead intoxication of mothers caused reduction of liver weight as well as of hepatic DNA, mRNA and protein levels in newborns. Moreover, oxidative stress and changes in antioxidant enzyme activities were recorded. Conversely, supplementation of mothers with spirulina mitigated these effects induced by lead. These results substantiated the potential hepatoprotective and antioxidant activity of spirulina.

Key words: Antioxidant, DNA and mRNA damage, Lead, Liver, Spirulina supplementation.

Introduction

Toxic effects of chemicals or drugs on animals or humans have been widely reported, some organs being more sensitive than others. Liver is the largest internal organ in the body and plays a vital role in detoxification of harmful substances. It has regulatory effect on many important metabolic functions and is responsible for maintaining homeostasis of the body (1). In the pathophysiology of inflammatory liver diseases, reactive oxygen species play an important role as cytotoxic and signalling mediators (2). The concentration of free radicals is normally lower in the healthy organism, where they are efficiently neutralized or metabolized. Conversely, adverse conditions inducing the production of excessive free radicals may cause liver damage (3).

Lead (Pb) is one of the most widely used metals in industries, so that Pb exposure still remains a widespread problem in many countries. Animals and humans may get exposed to Pb through food or water contamination, air pollution caused by industrial emission and combustion of lead-containing gasoline (4). As lead exposure is generally sub-acute, it produces only subtle clinical symptoms. Therefore, chronic exposure cases are more common than acute toxicity.

Lead, *via* gastro intestinal absorption, is first taken up by the red blood cells, then is distributed to all vascular organs (5). Pathogenesis of lead poisoning is mainly attributed to an induced oxidative stress. Chronic lead exposure is known to disrupt the pro oxidant/antioxidant balance existing in the mammalian cells (6). As a consequence, many of the adverse effects of lead exposure have been attributed to the propensity of lead to induce the production of reactive oxygen species (ROS), DNA damage, and inactivation of anti-oxidant enzymes (7).

As a source of various natural compounds, plants offer alternative healthcare options that are more effective and safe (8). Therefore, increasing interest for phytotherapy research has been paid to the use of medicinal plants with antioxidant activity for protection against heavy metal toxicity (9).

Spirulina (Blue green algae) is a single cell alga which grows in fresh water and has a simple structure but a complex composition. It is a concentrated source of food containing nutraceutical, antioxidant and probiotic properties. Its high level in the blue photosynthetic pigmented protein C-phycocyanin confers to the alga strong antioxidant and anti-inflammatory activities. Moreover, spirulina possesses a number of biological properties, like prevention of anemia (10), inhibition of herpes simplex infection (11), reduction in HIV replication velocity (12), stimulation of antibody production, prevention of neoplastic cell proliferation (13), hepatoprotection (14). Besides, it has antimutagen, antiviral, immune enhancing, cardio protective and anticancer properties (15). Recently, it attracted attention due to its ability to stimulate mineral absorption and its effects on intestinal microflora (16).

The present investigation was designed to examine possible action of Spirulina against hepatic intoxication

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induced by lead in newborn rats, which may pave the way for the possibility to use the microalga for therapeutic purpose.

Materials and Methods

Reagents

All reagents used in the present study were of analytical grade. Lead (in the acetate form) was obtained from SD Fine Chemicals, Bhoisar, Mumbai, India. 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB), L-Glutathione (reduced form), and all current chemicals were purchased from Sigma Chemical Co., (St. Louis, MO, USA).

Algae

Spirulina (*Arthrospira platensis*) variety Lenor (in powder form) was obtained from the University of Liege in Belgium.

Food preparation

Standard diet provided to the rats consisted of pellets containing a mixture of wheat, alfalfa, soybean, vitamins and minerals. Alternatively, a diet enriched with plants was prepared by mixing plant powder with food pellets in distilled water so as to obtain a homogenous paste. That mixture was cut into pellets and allowed to dry before starting the experiment.

A preliminary study, using different plant doses in the diet (i.e. 0 to 15%, w/w), did not reveal any toxic effects or oxidative stress in adult females treated with *spirulina* at doses up to 5%. Higher doses resulted in the occurrence of toxicity, diarrhoea and reduced growth, but were not lethal to rats.

Animals and treatments

Wistar rats weighing 170 to 180 g were obtained from the "Central Pharmacy of Tunis" (SIPHAT). They were kept in cages in a breeding farm at a temperature of 21±1°C with alternating periods of 14 h darkness / 10 h illumination and a relative humidity around 40%. All animals had free access to drinking water. The basic food consisted of 15% protein industrial pellets provided by the Industrial Society of Concentrate (SICO, Sfax, Tunisia).

The experimental procedure was carried out according to the general guidelines on the use of living animals in scientific investigations (17) and approved by the Ethical Committee of the Faculty of Science of Sfax.

After one week of acclimatisation in the laboratory conditions, adult females were placed with males on the proestrus night and the presence of spermatozoa in the vaginal smear was noted as day 0. Pregnant females were individually housed in plastic cages in a temperature-controlled nursery (22–24°C).

Thirty two pregnant rats were randomized into two sets of 16 rats. The first set consisted of control animals drinking distilled water. The second set was given water containing 6 g/L lead acetate, resulting in an average uptake of 343.6 mg lead/kg body weight/day (18). Each group was then separated into two subgroups of eight animals. Among the animals not intoxicated with lead, rats belonging to C (control) and S (spirulina) subgroups were given a normal diet and a diet enriched with 5% spirulina. Similarly, two subgroups treated with lead acetate were given either a normal diet (Pb) or a diet enriched with spirulina (SPb). All groups were treated from day 5 of gestation to day 14 of lactation, brain development being strongly sensitive to environmental pollutants during that period (19).

At birth, pups from each mother were weighed and each litter was reduced to eight pups (4 males and 4 females) in order to maximise lactation performance (20). During the lactating period, the dams' food and water intake was measured daily at the same time: the daily amount of ingested diet was calculated as the difference between the weight of feed placed in the food bin (D1) and that remaining the day after (D2). All the recorded data were then used to calculate the daily average feed intake over the whole experiment. Using that method, quantities of Pb and S ingested by each lactating dam were calculated from water and diet intake. Stomach contents of suckling rats were sampled and weighed. All samples were stored at -20°C until analysis.

Organ sampling

On day 14 after delivery, pups (control and treated rats) were anesthetized with chloral hydrate by intra-abdominal injection. The body weights of pups were recorded and blood samples were collected in heparin tubes by brachial artery. Plasma samples were drawn from blood after centrifugation at 2500 x *g* for 15 min. They were kept at -20°C until analysis. The liver were drawn, cleaned, and weighed. Some samples were homogenised (1:2, w/v) in 50 mM Tris buffer (pH 7.4) containing 150 mM NaCl using an Ultra-Turrax device. Homogenates were centrifuged at 5000 x *g* for 25 min at 4°C and aliquots of supernatant were kept at -30°C until analyses.

On day 14 of lactation at 8:00 am, litters were separated from mothers. Stomach contents of suckling rats were taken and weighed. All samples were stored at -20°C until analysis. Milk tinged with blood was not taken, thus avoiding the introduction of blood into the milk collected (21).

Evaluation of lead content

Mineralisation of blood, stomach contents and pellets was carried out at 200°C in Kjeldahl tubes in the presence of a nitric acid/perchloric acid (2:1 v/v) mixture. Lead contents were then determined using a fast sequential atomic absorption spectrometer (220 FSAA, Varian). Accordingly, no lead was detected in food pellets. Calcium contents in the stomach were determined using ion assay with a disodium salt solution of ethylene diaminetetraacetic acid (EDTA) at pH between 12 and 13.

Biochemical assays

ALT and AST analyses

At the end of experiment, fasting blood samples were withdrawn from the retro-orbital vein of each animal using a glass capillary tube after fasting period of 12 h. The blood samples were allowed to coagulate and then centrifuged at 3000 rpm for 20 min. Plasmatic activities of transaminases, *i.e.* alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were determined colorimetrically using commercial reagent kits (Biomaghreb, Ariana, Tunisia. Ref 20053; 20057).

Protein quantification

Protein content was assayed as described by Lowry et al. (22), using bovine serum albumin as standard.

Oxidative status and antioxidant analyses

Levels of lipid peroxidation in liver and hemolysate were estimated by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Yagi (23). Superoxide dismutase (SOD) activity was determined in serum and liver homogenate according to the method of Beyer and Fridovich (24). Catalase (CAT) activity was measured using the method of Aebi (25)**.** The enzyme activity was calculated in terms of µmoles H_2O_2 consumed/min/mg of protein. Glutathione-peroxidase (GPX) activity was measured according to the method of Flohe and Gunzler (26). The enzyme activity was expressed as nmoles of GSH oxidized/min/mg of protein.

DNA and RNA quantifications

Total DNA and mRNA were isolated from 100 mg of liver tissue, according to the method of Chomczynski and Sacchi (27). Each sample was measured at a wavelength of 260 nm and total DNA content was expressed in µg/g of organ (28).

Statistical analysis

The data were analysed using the statistical package program Stat Graphics plus5.1 (stats graphics). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (FLSD) test as a post hoc test for comparison between groups. Differences were considered significant at different levels ($P < 0.05$, $P < 0.01$, $P < 0.001$).

Results

Death or abortion was not observed in any experimental groups during the treatment period (21 days). However, few clinical signs were noted in suckling pups, including reduced activity and increasing weakness.

Effects of treatments on food consumption and growth

Lead exposure of mothers caused a decrease in the consumption of food (-24.8%) and increase in drinking water (+30%), and was accompanied with a loss in body weight of 14 day-old rats, compared with the controls **(Table 1)**. Co-treatment with spirulina improved food and water consumptions by mothers, which reached normal values. Moreover, the addition of spirulina in the food of pregnant and lactating mothers resulted in highly significant increase in their body weight (+10.6%) and that of 14 day-old males and females as compared to control. Supplementation of spirulina to the diet of control mothers had no effect per se on the body weight of newborn (data not shown).

Effects of treatments on lead concentration in neonate blood

Blood Pb levels were measured in control and intoxicated groups **(Table 2)**. Lead concentration in blood of intoxicated pups aged 14 days exhibited 8.2- and 7.5-fold increases compared to that in control males and females, respectively. Moreover, supplementation of the diet of lead-intoxicated mothers with spirulina reduced significantly (by 50%, $P < 0.01$) the accumulation of

Table 2. Lead concentration in the blood of 14 day-old rats from control (C) or treated mothers with 6 g/L of lead acetate (Pb) and 5% of spirulina in feed (S Pb), from the 5thday of pregnancy until day 14 after delivery.

Treatments	Blood lead concentration (µg/mL)	
	Male (*n*=4)	Female (*n*=4)
C	0.192±0.011	0.237±0.025
Pb	1.590±0.131***	1.785±0.333***
S Pb	0.809±0.140$^{**,++}$	0.899±0.130$^{**,++}$

Values are expressed as means ± SD.

Significant differences between groups were mentioned as follows: Pb or (S Pb) group compared to control (C): $^{*}P < 0.05$;$^{**}P < 0.01$;$^{***}P < 0.001$.

(S Pb) group compared to Pb group: $^{+}P < 0.05$; $^{++}P < 0.01$; $^{+++}P < 0.001$.

Table 1. Effect of lead exposure and/or spirulina consumption of mother rats on their body weight and that of their offspring. Daily food consumption, water intake and body weight of control (C) or treated mothers with 6 g/L lead acetate (Pb) and with 5% of spirulina in feed (S Pb) from day 5 of pregnancy to day 14 after delivery.

Parameters	Mothers (n=8)			Offspring (n=8)					
				Males (n=4)			Females (n=4)		
	C	Pb	S Pb	C	Pb	S Pb	C	Pb	S Pb
Food consumption (g/day/dam)	37.78±4.25	28.40±2.45*	34.85±1.62^{+}	-	-	-	-	-	-
Water intake (mL/day/dam)	70.18±7.85	91.26±5.19*	76.81±5.28^{+}	-	-	-	-	-	-
Body weight (g)	180.70±5.51	167.53±2.94*	171.91±5.44	19.51±0.21	17.62±0.47**	18.77±0.47^{+}	18.72±0.29	16.93±0.360*	18.47±0.57^{+}

Values are expressed as means ± SD.

Significant differences between groups were mentioned as follows: Pb or (S Pb) group compared to control (C): $^{*}P < 0.05$;$^{**}P < 0.01$; $^{***}P < 0.001$.

(S Pb) group compared to Pb group: $^{+}P < 0.05$; $^{++}P < 0.01$; $^{+++}P < 0.001$.

lead in the blood circulation of neonates. Addition of spirulina to the diet of control mothers had no effect per se on the blood lead level of newborns (data not shown).

Determination of calcium and lead levels in the stomach

Stomachic levels of Pb and Ca were measured in control and intoxicated groups **(Table 3)**. Lead concentration in the stomach of intoxicated pups aged 14 days exhibited 4- and 5-fold increase compared to that in control males and females. Conversely, calcium levels decreased by 78% and 60.3%, respectively, following lead exposure. Diet supplementation of lead-intoxicated mothers with spirulina reduced significantly by 70% in males and 76.7% in females ($P < 0.001$) the accumulation of lead in the stomach of neonates and the concentration of calcium of male and female offspring by 65.1% and 51.1%, respectively, as compared to Pb group.

Effects of treatments on the liver weight and protein content

Lead administration in pregnant and lactating mothers resulted in a highly significant decrease in the liver weight of 14 day-old males (-14%) and females (-15%), as compared to control **(Table 4)**. Conversely, the addition of spirulina to rat diet reduced lead effect on liver weight in both male and female pups and restored control weights. Supplementation of spirulina to the diet of control mothers had no effect *per se* on the weight of newborn liver (data not shown).

Protein level of the liver decreased by 70% and 83% in male and female pups, respectively, following lead exposure of their mothers **(Table 4)**. Conversely, addition of spirulina to the diet of lead-treated rats maintained control protein levels in male neonates.

Effect of treatments on serum transaminase activities

Lead administration to pregnant and lactating mothers caused a significant increase in serum ALT and AST activities of 14 day-old neonates as compared to controls ($P < 0.05$) **(Table 5)**. This effect was more marked with the former activity. Addition of spirulina to the diet of lead-treated mothers strongly reduced serum ALT and AST activities in their offspring ($P < 0.01$).

Effects of treatments on DNA and mRNA levels in the liver

Lead contamination induced a significant decrease in DNA and mRNA contents in the liver of lactating female rats compared with control animals ($P < 0.05$)

Table 3. Concentration of lead and calcium in stomach content of 14 day-old rats from control (C) or treated mothers with 6 g/L lead acetate (Pb) and 5% of spirulina (S Pb) in feed, from day 5 of pregnancy to day 14 after delivery.

Treatments	Stomach content (µg/g of organ)			
	Male (*n*=4)		Female (*n*=4)	
	Lead	Calcium	Lead	Calcium
C	1.23±0.64	4.67±0.91	1.78±0.39	5.09±1.86
Pb	$6.11±1.40^{***}$	$1.04±0.18^{***}$	$8.95±0.38^{***}$	$2.02±0.53^{**}$
S Pb	$1.83±0.42^{**,+++}$	$2.98±0.26^{**,++}$	$2.08±0.27^{**,+++}$	$4.13±0.57^{*,+++}$

Data are means ± SD of 4 determinations in each group.
Significant differences between groups are mentioned as follows:
Pb or (S Pb) group compared to control: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.
(S Pb) group compared to Pb group: $^{+}P < 0.05$; $^{++}P < 0.01$; $^{+++}P < 0.001$.

Table 4. Liver weight and protein content of 14 day-old rats from control (C) or treated mothers with 6 g/L lead acetate (Pb) and 5% of spirulina (S Pb) in feed from day 5 of pregnancy to day 14 after delivery.

Treatments	Absolute weight (g)		Protein content (mg/g organ)	
	Male	Female	Male	Female
C	0.63±0.01	0.61±0.13	31.56±4.19	30.75±5.22
Pb	0.54±0.02**	0.53±0.17**	9.21±2.50***	5.43±2.16***
S Pb	$0.60±0.02^{*,+}$	$0.56±0.02^{*,+}$	$24.26±2.85^{*,++}$	$33.90±5.29^{+++}$

Data are means ± SD of 4 determinations.
Significant differences between two groups are mentioned as follows:
Pb or (S Pb) group compared to control: $^{*}P < 0.05$; $^{**}P < 0.01$; ***$P < 0.001$.
(S Pb) group compared to Pb group: $^{+}P < 0.05$; $^{++}P < 0.01$; $^{+++}P < 0.001$.

Table 5. Plasmatic ALT and AST activities of 14 day-old rats from control mothers (C) or mothers treated with 6 g/L lead acetate (Pb) and 5% spirulina (S Pb) in feed, from day 5 of pregnancy to day 14 after delivery.

Treatments	Male		Female	
	AST (UI/L)	ALT (UI/L)	AST (UI/L)	ALT (UI/L)
C	167.20±4.64	20.50±0.77	164.56±2.32	22.38±1.32
Pb	$218.11±1.54^{**}$	$53.25±1.29^{***}$	$221.01±1.49^{***}$	$53.43±2.61^{***}$
S Pb	$150.38±3.10^{*,+++}$	$30.61±0.51^{**,+++}$	$158.62±3.09^{+++}$	$29.21±1.89^{*,+++}$

Data are means ± SD of 4 determinations.
Significant differences between two groups are mentioned as follows:
Pb or (S Pb) group compared to control: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.
(S Pb) group compared to Pb group: $^{+}P < 0.05$; $^{++}P < 0.01$; $^{+++}P < 0.001$.

(Fig. 1, 2). Conversely, an increase in DNA and mRNA contents was found in both male and female neonates following spirulina supplementation to the diet of lead-treated mothers.

Effects of treatments on lipid peroxidation levels in liver and blood

TBARS production in liver and blood of the offspring varied greatly depending on mother treatment (**Table 6**). Lead intoxication resulted in a 93% increase of TBARS concentration in liver tissues of neonates as compared to control. That effect was even more marked in blood. Here again, addition of spirulina to the diet of control mothers had no effect *per se* on the lipid peroxidation level in its offspring (data not shown). However, dietary spirulina supplementation to the lead-poisoned mothers significantly reduced the peroxidation level in liver and blood of their neonates towards control levels ($P < 0.05$). That restoring effect was particularly important in male pups, compared to females.

Effects of treatments on antioxidant enzyme activities in liver and blood

Addition of spirulina to the normal diet of control mothers had no effect *per se* on the level of antioxidant enzyme activities in newborns (data not shown). Lead administration to mothers caused a strong increase in SOD activity in the liver of male and female offspring (by 123 fold and 97%, respectively) as compared to control animals (**Table 6**). This feature was significantly reduced upon addition of spirulina to the diet of mothers ($P < 0.05$). Similar observations could be made with SOD activity in the blood of neonates, which increased (by 55% and 71.8% in males and females, respectively) following Pb intoxication of mothers, but significantly decreased ($P < 0.05$) when spirulina was added to the diet.

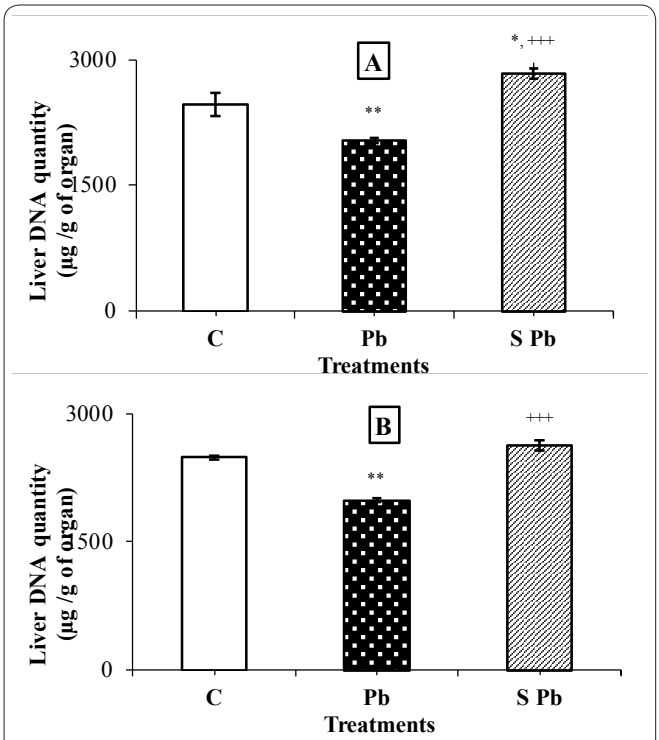

Figure 1. DNA quantity in the liver of 14 day-old rats (A: male; B: female) from control (C) or treated mothers with 6 g/L of lead acetate (Pb) and 5% of spirulina (S Pb) in feed, from day 5 of pregnancy to day 14 after delivery.
Data are means ± SE of eight rats per group.
Significant differences between groups are mentioned as follows:
Pb or (S Pb) group compared to control (C): *P < 0.05; **P < 0.01; ***P < 0.001.
(S Pb) group compared to Pb group: +P < 0.05; ++P < 0.01; +++P < 0.001.

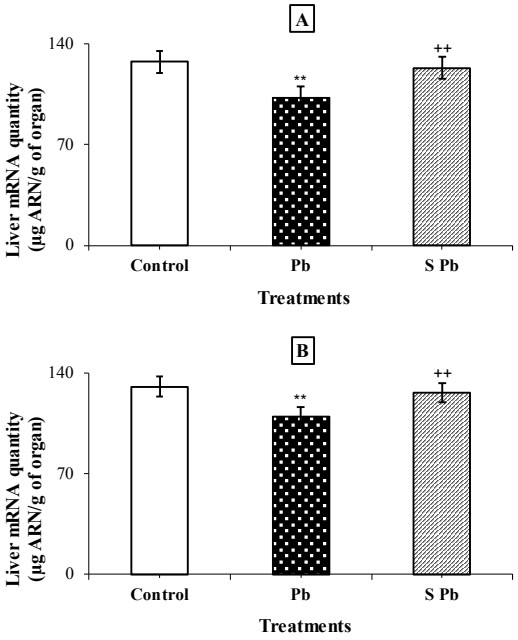

Figure 2. RNA quantity in the liver of 14 day-old rats (A: male; B: female) from control (C) or treated mothers with 6 g/L of lead acetate (Pb) and 5% of spirulina (S Pb) in feed, from day 5 of pregnancy to day 14 after delivery.
Data are means ± SE of eight rats per group.
Significant differences between groups are mentioned as follows:
Pb or (S Pb) group compared to control (C): *P < 0.05; **P < 0.01; ***P < 0.001.
(S Pb) group compared to Pb group: +P < 0.05; ++P < 0.01; +++P < 0.001.

Catalase activity decreased markedly in the liver of male and female offspring (by 43% and 34%, respectively) from lead-intoxicated mothers (**Table 6**). Conversely, catalase activity in the blood of neonate increased following Pb intoxication of mothers ($P < 0.05$). Addition of spirulina to the diet of mothers maintained control level of CAT activity in its offspring, although that effect was less significant in female pups.

Upon lead intoxication of female rats, a 6-and 9-fold increase in GPX activity was observed in the liver of male and female neonates, respectively. Such effect was less marked in blood of intoxicated pups aged 14 days exhibiting a 9% increase compared to that in control animals ($P < 0.05$). Here again, addition of spirulina to the diet of mothers allowed GPX activity in newborn to remain close to control values.

Discussion

Lead (Pb) is one of the most widespread and insidious environmental toxins that has been detected in all phases of environment and biological system. Many investigations have indicated that lead exposure induces a wide range of biochemical and physiological dysfunctions in humans and laboratory animals (29). More pre-

Table 6. TBARS levels and activities of CAT, SOD and GPX in liver and blood of 14 day-old rats from control (C) or treated mothers with 6 g/L lead acetate (Pb) and 5% of spirulina (S Pb) in feed, from day 5 of pregnancy until day 14 after delivery.

Parameters	Male			Female		
	C	Pb	S Pb	C	Pb	S Pb
TBARS						
Liver (*n*=4)	0.78±0.19	11.33±2.94***	0.78±0.11+++	2.37±0.31	33.26±10.99***	2.48±0.47+++
Blood (*n*=4)	0.15±0.01	0.30±0.01**	0.16±0.00+++	0.16±0.01	0.33±0.01***	0.18 ±0.01++
Superoxyde dismutase						
Liver (*n*=4)	17.15±2.29	38.32±4.88***	20.83±4.71++	23.55±5.41	46.61±2,97***	34.31±3.30++
Blood (*n*=4)	16.63±0.46	25.79±0.52 **	13.55±1.42*+++	15.55±0,07	26.72±0,11***	14.79±0,11*+++
Catalase						
Liver (*n*=4)	76.98±5.83	43.30±2.37**	75.79±4.98++	78.86±8.91	51.75±6.44**	71.43±3.37++
Blood (*n*=4)	33.47±2.35	40.40±1.44*	34.31±2.69+	33.36±3.50	45.84±3.21**	37.25±1.63++
Glutathione peroxidase						
Liver (*n*=4)	2.68±0.57	20.83±1.62***	2.26±0.70+++	2.80±1,09	29.30±3.22***	1.40±0.25*+++
Blood (*n*=4)	12.67±0.66	22.68±1.04**	14.47±0.65++	14.78±1.73	22.08±0.49**	14.73±1.22++

Data are means ± SE of 4 determinations.

TBARS: nmol/mg protein; Superoxide dismutase activity: U/mg protein; Catalase activity: mol H_2O_2/min/mg protein; Glutathione peroxidase activity: nmol GSH/min/mg protein.

Significant differences between two groups are mentioned as follows:

Pb or (Pb + S) group compared to control group: *P < 0.05;**P < 0.01; ***P < 0.001.

(Pb + S) group compared to Pb group: +P < 0.05; ++P < 0.01; +++P < 0.001.

cisely, pregnant ladies, infants and young children are mostly affected by lead exposure (30). Most of the environmental exposure occurs through inhaling air containing lead dust, drinking water supplied through leaded pipelines, and consuming processed or stored food (31).

In our experimental study, exposure of female rats to Pb during late pregnancy and early postnatal periods decreased the body weight of their suckling pups. This could be explained by a decrease of food intake and increase to water by lactating rats (32). In humans, a pregnant lady can transfer her body burden of lead to the growing foetus, as there is no placental barrier for a heavy metal like lead (33). Thus, mother and foetus can be considered a unique system that remains in equilibrium through pregnancy so, at the end of pregnancy, similar lead concentrations have been reported in the blood, bones and soft tissues of both the mother and the child (34). In some cases, offspring from adults exposed to lead during gestation have higher blood Pb level at birth than that of dams sampled at the same time point (19, 33). Accordingly, in our study, we found high levels of lead (exceeding 1.5 mg/L) in young rat blood and stomach contents. However, supplementation with spirulina improved food and water consumptions by mothers, increased their body weight and that of offspring, and decreased blood lead levels. Interestingly, such a stimulating effect of spirulina on food or water consumption has not been reported hitherto. However, growth activation of mothers and their offspring upon spirulina consumption could be due to spirulina composition, since it is a well-known source of natural protein (about 60% digestible proteins) with all amino acids, phyto-nutrients, carbohydrates, vitamins and minerals.

The current study revealed significant changes in the stomach calcium content, of newborn rats upon lead exposure of mothers. In turn, the decrease in stomachic Ca levels in intoxicated animals would contribute to the delay in the growth of these young rats. Recent reports indicate that Pb inhibits some regulatory actions of calcium on cell function (35). On the other hand, it is suggested that lead, exhibiting similar chemical structure to calcium, competes for Ca^{2+} transport systems at the plasma membrane, as well as at endoplasmic reticulum and mitochondria membranes (36). In the same way, the high affinity of lead for intracellular calcium receptor and calcium-regulating processes is responsible for the alteration of intracellular calcium homeostasis and of receptor-mediated signal transduction pathways, thus contributing to its toxicity (36). Therefore, we studied lead storage organs in neonates, to determine their Pb levels and to evaluate the damages caused by lead intoxication.

Once absorbed by the body, lead is distributed by the blood and is found in the soft tissues after a period of approximately 3 to 4 weeks (37). Among the soft tissues, liver is the primary organ site for xenobiotic metabolism. As such, it is one of the major organs involved in the storage, bio-transformation and detoxification of toxic substances (4). In some cases, the metabolic process is accomplished without injury to the liver itself, whereas many inorganic or organic lead compounds are toxic and can cause liver injury (38). The activities of ALT and AST are common bio-indicators of hepatotoxicity (39). In the present study, there was a significant increase of ALT and AST activities in the liver of lead-intoxicated mothers and their offspring, indicating that Pb exposure affects hepatic tissue of both animals. The increased activities of both serum hepatic enzymes (AST, ALT) may be attributed to cellular leakage and loss of functional integrity of cell membrane in the liver, as it has been reported previously (40).

Accumulating evidence has shown that lead causes the generation of reactive oxygen species (ROS), thus inducing oxidative stress, excitotoxicity, damages to DNA, proteins and lipids within cells, and finally tissue injury. Moreover, hepatocyte proliferation and finally subsequent apoptosis in rat livers has been reported (41). In the current study, the hepatic DNA and RNA levels in the offspring dramatically decreased upon lead intoxication. This observation may be related to the previously mentioned effect of lead ingestion on body growth. It may be due to either a direct oxidative dam-

age to DNA, stimulation of nucleic acid decomposition enzymes or inhibition of protein and nucleic acid biosynthesis (42). Conversely, spirulina supplementation of mothers inhibited the decrease of nucleic acid levels in neonate liver. Such hepatoprotective effect may be related to the prevention of oxidative damage of DNA through stabilization of plasma membrane, thereby preserving the structural integrity of the cell, as well as to the repair of hepatic tissue damage caused by lead (43, 44). Accordingly, it has been demonstrated that the blue pigment phycocyanin in spirulina has a specific protective role against DNA degradation, thus reducing lead-induced hepatotoxicity (43).

Lead is a heavy metal that produces oxidative damage in the liver by enhancing lipid peroxidation (45). Lead toxicity induces damages by two separate, although related, pathways: (1) the generation of reactive oxygen species (ROS), including hydroperoxides, singlet oxygen, and hydrogen peroxides, evaluated by MDA levels as the final products of lipid peroxidation, and (2) the direct depletion of antioxidant reserves (46). In the present study, there was a significant increase in MDA levels and concomitant decrease in antioxidant levels in lead-intoxicated newborn. Our results are in agreement with other studies previously reported in adult animals (47). They can be explained by the relatively high content of polyunsaturated fatty acids, which are sensitive to peroxidative damage (48), in liver tissue. Furthermore, our previous studies proved that the lead-induced TBARS production could be alleviated by dietary vitamin supplementation (vitamin C and, to a lesser extent, vitamin E) (5). Accordingly, we found that ingestion of spirulina during lead treatment of mother rats caused a significant decrease in TBARS production (lipid peroxidation) in their offspring, suggesting that active ingredients from the algae exert anti-oxidant action. Such role of spirulina could be attributed to its high content in antioxidant vitamins (A, C and E) or phycocyanins (45, 49). Accordingly, a recent study by Gini and Muraleedhara (50) reported that C-phycocyanin present in spirulina inhibited paracetamol-induced lipid peroxidation in rats liver cells.

Another effective mechanism set up in the body to counter free radical-induced damage proceeds *via* endogenous antioxidant enzymes, including SOD, CAT and GPX. An imbalance between free radicals production and antioxidant defence system generally results in oxidative stress. This further deregulates cellular functions, leading to various pathological signs. However, when the antioxidant defence system is overwhelmed, free radicals may inflict direct oxidative damage to cellular macromolecules, leading to cell death (51). Literature concerning the effects of lead on SOD activity is contradictory: Some studies report an elevated enzymatic activity as an indicator of increased $\bullet O^-$ production (5, 52), whereas other authors observed a decrease in SOD activity (53). Our results confirm those of previous work (5, 52), suggesting a strong ability of liver and blood to defend against free radicals. Noteworthy, SOD in neonate liver and blood recovered normal activity after consumption of spirulina by nursing mothers, which is likely related to the decrease in radical production mentioned above.

CAT was the only antioxidant enzyme whose activity decreased upon lead intoxication, which implies a Fenton reaction-mediated conversion of more H_2O_2 to the ultimate toxicant, OH• (54). Nevertheless, there are contradictory data in the literature concerning the effects of lead on catalase activity. Some studies reported an elevated enzymatic activity which could play a significant role in protecting cells (55), whereas others observed a decrease in catalase activity upon lead intoxication, attributable to the reduced absorption of iron or the inhibition of haem biosynthesis (56). Thus, our results on liver CAT in newborn are in good agreement with the former reports. However, converse results were obtained with blood CAT, so that further investigations are needed to elucidate the role of CAT in offspring following Pb exposure. Noteworthy was the improving effect of dietary spirulina supplementation on catalase activity in the offspring of lead-poisoned mothers. Such observation could be related to the ROS-scavenging activity of natural antioxidants, allowing protection toward oxidative stress, and of antioxidant enzymes. Accordingly, spirulina is a well-known source of antioxidants, including vitamins A and C or the polypeptide pigment phycocyanin (57).

Activity of GPX, another major antioxidant enzyme, in the organism could mitigate and repair the damage caused by ROS. Meanwhile, liver, compared to other soft tissues like brain, lung and kidney, contains relatively low levels of enzymatic and non-enzymatic antioxidants and high amounts of peroxidizable unsaturated lipids, making it more vulnerable to oxidative stress compared to other tissues (58). Our results, showing an increase of GPX activities in liver and blood of neonates upon lead intoxication, could reflect a line of defence under oxidative conditions, as it has been reported previously (59). Interestingly, the simultaneous administration of lead and spirulina to the mothers attenuated the GPX stimulation in the offspring. Such effect may be attributed, at least for spirulina, to the presence of selenium (57). Accordingly, organic selenium reacts with the sulphur amino acids (methionine and cysteine) that are responsible for GSH synthesis, thus decreasing it and, in turn, GPX activity (60).

Overall, our data showed that lead consumption by pregnant and lactating mothers causes dramatic liver damages in their offspring, most of which being associated with oxidative stress (lipid peroxidation, generation of ROS, nucleic acid oxidation, tissue injury). However, the consumption of spirulina by mother rats efficiently prevented the adverse effects of Pb on neonate liver and blood. *Spirulina platensis* is rich in bioactive pigments (chlorophylls, phycocyanin, β carotene), vitamins, minerals (particularly manganese, zinc, copper, iron and calcium), proteins and essential amino acids. Thus, the protective effect of spirulina against lead-induced damages in neonate liver is likely to be attributed to the inhibition of the formation of reactive metabolites and/or to a radical scavenging activity. Therefore, the development of nutritional supplementation using this algae may be useful to protect the liver against lead-induced damage, and further study on on human subjects is now needed to confirm its potential.

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