Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
Original Research

Dandelion-enriched diet of mothers alleviates lead-induced damages in liver of newborn rats

M. Gargouri[1,2*], C. Magné[2], I. Ben Amara[4], H. Ben Saad[3], A. El Feki[1]

[1] Laboratory of Animal Ecophysiology, Faculty of Sciences, University of Sfax, 3038 Sfax, Tunisia
[2] EA 2219 Géoarchitecture, Faculty of Sciences, University of Western Brittany, 6 Avenue V. Le Gorgeu, CS 93837, 29238 Brest Cedex 3, France
[3] Laboratory of Pharmacology, Faculty of Medicine, University of Sfax, 3029 Sfax, Tunisia
[4] Higher Institute of Biotechnology, University of Sfax, 3000 Sfax, Tunisia

Correspondence to: manele.gargouri@gmail.com

Received August 10, 2016; **Accepted** February 20, 2017; **Published** February 28, 2017

Doi: http://dx.doi.org/10.14715/cmb/2017.63.2.10

Abstract: Lead (Pb) is a highly toxic metal present in the environment. It causes disturbances of several functions, including hematologic, renal, reproductive and nervous ones. Preventive or curative use of medicinal plants against these disorders may be a promising and safe therapeutic strategy. This study evaluated the hepatic toxic effects of prenatal exposure to lead in rats and the possible protective effect of dandelion (*Taraxacum officinale*) added to the diet. Female rats were given a normal diet (control) or a diet enriched with dandelion (treated). In addition, lead acetate was administered to half of the rats through drinking water from the 5th day of gestation until the 14th day postpartum. Lead toxicity was evaluated in their offspring by measuring body and liver weights, plasma biochemical parameters, liver damage, as well as protein content and activities of antioxidant enzymes in the liver tissues. Lead poisoning of mothers caused lead deposition in blood and stomach of their pups as well as hepatic tissue damages. Moreover, significant decreases in liver weight and protein content were found. Lead treatment caused oxidative stress and marked changes in the activity of antioxidant enzymes. However, no damages or biochemical changes were observed in puppies from the rats co-treated with lead and dandelion. These results indicate that supplementation of pregnant and lactating rats with dandelion protects their offspring against lead poisoning, likely through reduction of oxidative stress and liver damages.

Key words: Antioxidant enzymes; Lead poisoning; Liver; Neonate rats; Oxidative stress; *Taraxacum officinale*.

Introduction

Heavy metals are environmental pollutants with dangerous effects on human health due to their wide usage in many industrial branches. As a consequence, they are highly present in the air, water and soils (1). Among these metals, lead is one of the most common in our environment. Impact of this heavy metal on public health is well recognized, especially during the period of growth and development (2,3). Following absorption by the organism, lead (Pb) is distributed in soft tissues (blood, liver, etc…), then is rapidly stored in the backbone.

Prenatal life is considered as the most delicate stage of human development, because of the high degree of cell division and differentiation of the fetus. During that period, placenta acts as a selective barrier allowing the translocation of nutrients and oxygen to the fetus, while preventing the passage of other substances (4). As a consequence, at birth, maternal and neonatal blood lead levels are highly correlated (5). After birth, the newborn is further contaminated by lead contained in the milk during lactation, which represents approximately 25 to 30% of total lead ingested by mother (6,7).

The liver plays a vital role in detoxification and metabolism of toxic substances. Therefore, once overloaded, it is no longer capable of neutralizing toxic substances and digestive, endocrine, cardiovascular and immune systems will be intoxicated (8,9). Among soft tissues, the liver generally has the highest lead concentration (10), where it induces hepatotoxicity (11). 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 antioxidant enzymes (12). Indeed, in both young and adult rats, lead treatment was found to cause alteration in expression levels of various antioxidant enzymes in the liver, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and guanylate cyclase (13,14). Although the toxicity of lead has been largely studied in humans and adult rats, its role in generating oxidative stress in the liver of progeny has not been reported hitherto.

During the last years, major interest has been granted to medicinal plants for their effective contribution to the improvement and prevention of oxidative diseases including cardiovascular diseases, cancers and aging. These plants can produce alternative compounds to synthetic molecules with proven serious side effects, for applications in food, pharmaceutical and cosmetic industries. Therefore, the identification of plant products or alternative medicines preventing or scavenging ROS production would help protect from oxidative damages. Accordingly, some bioactive compounds found in plants have been demonstrated to protect cells from oxidative stress by preventing the formation of free radicals or by

detoxifying free radicals, resulting in the prevention of a variety of pathophysiological processes (15).

Among the potentially antioxidant plants, dandelion (*Taraxacum officinale* L., Asteraceae) is an edible plant traditionally used in folk medicine because of its anti-diabetic, choleretic, and diuretic properties (16). Recent studies have provided evidence that it can also reduce inflammation and the risk of tumor (17). However, the potent effect of dandelion on lead-induced hepatoxicity has not been described. Therefore, the purpose of this study was to investigate (i) the hepatotoxicity of lead on newborns from female rats intoxicated during late pregnancy and early postnatal periods, and (ii) the potent hepato-protective action of dandelion.

Materials and Methods

Animals

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 14/10 h of darkness/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 procedures were carried out according to the general guidelines on the use of living animals in scientific investigations (18) and approved by the Ethical Committee of the Faculty of Science of Sfax.

Plants

Dandelion microspheres (ref. TADL060206) were supplied by PARACHIMIC Company, Sfax, Tunisia.

Chemicals

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).

Experimental procedure

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 2% dandelion was prepared by mixing plant powder with food pellets in distilled water so as to obtain a homogenous paste (19). That mixture was cut into pellets and allowed to dry before the experiment.

A preliminary study, using dandelion doses up to 2% in the diet did not reveal any toxic effects or oxidative stress in adult females. Higher doses resulted in the occurrence of diarrhoea and reduced growth, but were not lethal to rats.

Treatments

After one week 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 in controlling animals drinking distilled water. The second set was given water containing 6 g/L lead acetate, resulting in an average uptake of 343 mg lead/kg body weight/day (20). Each group was then separated into two subgroups of eight animals. Among the animals not intoxicated with lead, rats belonging to C (control) and D (dandelion) subgroups were given a normal diet and a diet enriched with 2% dandelion, respectively. Similarly, the two subgroups treated with lead acetate were given either a normal diet (Pb) or a diet enriched with dandelion (D Pb). Every female rat was treated from day 5 of gestation to day 14 of lactation, development being strongly sensitive to environmental pollutants during that period (21).

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 (22). During the lactating period, food and water intake were evaluated daily at the same time by measuring food and water remaining in the tanks. All the recorded data were then used to calculate the amounts of lead and dandelion ingested by each lactating dam. All samples were stored at -27°C until analyses.

Organ sampling

On day 14 after delivery, every pup was anesthetized with chloral hydrate by intra-abdominal injection. The body weight of pups was recorded and blood samples were collected in heparin tubes from brachial artery. Plasma samples were drawn from blood by centrifugation at 2500 x *g* for 15 min, then kept at -27°C until analyses. Moreover, stomach contents of suckling pups were sampled and weighed. Milk tinged with blood was not taken, thus avoiding the introduction of blood into the milk collected (23). The livers were drawn, cleaned, and weighed. A fraction of each sample was immediately fixed into Bouin's solution (saturated picric acid added with 37–40% formaldehyde and glacial acetic acid, 75:25:5 v/v) for histological studies. The remaining fraction was 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 -27°C until analyses (19).

Evaluation of lead content

Mineralisation of blood, stomach contents and food 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 an ion assay with a disodium salt solution of ethylene diamine tetraacetic acid (EDTA) at a pH between 12 and 13.

Protein quantification

Protein content was assayed as described by Lowry

et al. (24), using bovine serum albumin as standard.

Determination of antioxidant enzymes activities and lipid peroxidation level

Levels of lipid peroxidation in liver and plasma samples were estimated by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Yagi (25). Superoxide dismutase (SOD) activity was determined in sera and liver homogenates according to the method of Beyer and Fridovich (26). Catalase (CAT) activity was measured using the method of Aebi (27). Changes in absorbance were recorded at 240 nm, and the enzyme activity was calculated in terms of µmoles of H_2O_2 consumed/min/mg of protein. Glutathione-peroxidase (GPx) activity was measured according to the method of Floke and Gunzler (28). The decrease in absorbance at 340 nm was measured, and the enzyme activity was expressed as nmoles of GSH oxidized/min/mg of protein.

Histological analyses

Small pieces of neonate liver were fixed in a Bouin's mixture, embedded in paraffin, and sectioned. The sections were stained with haematoxylin-eosin (29) to examine tissue constitution. Six slides were prepared from each liver. All sections were evaluated semi-quantitatively for the degree of liver injury. Thus, the steatohepatitis calculation system (NAS) was applied to evaluate the steatosis, inflammation, and ballooning in liver tissues (30).

Statistical analysis

The data were analysed using the statistical package program Stat Graphics plus 5.1 (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 of the Pb groups, including reduced activity and increasing weakness.

Effects of treatments on liver tissues

Liver histological study was used to determine the effects of lead on liver tissues and the potent protective effect of dandelion on lead-induced injury. Histopathological examination of control group **(Fig. 1(A, A1))** showed normal architecture and appearance of the central vein with a radiating pattern of cell plates that were normal in shape and size. The exposure of rats to Pb induced degenerative signs, including necrotic cells, leucocyte infiltration, and hepatocyte vacuolization **(Fig. 1(B, B1))**. The co-administration of dandelion to the Pb-treated group provoked a marked improvement in the hepatocyte structure **(Fig. 1(C, C1))**. The liver histoarchitecture was normal in control rats supplemented with dandelion.

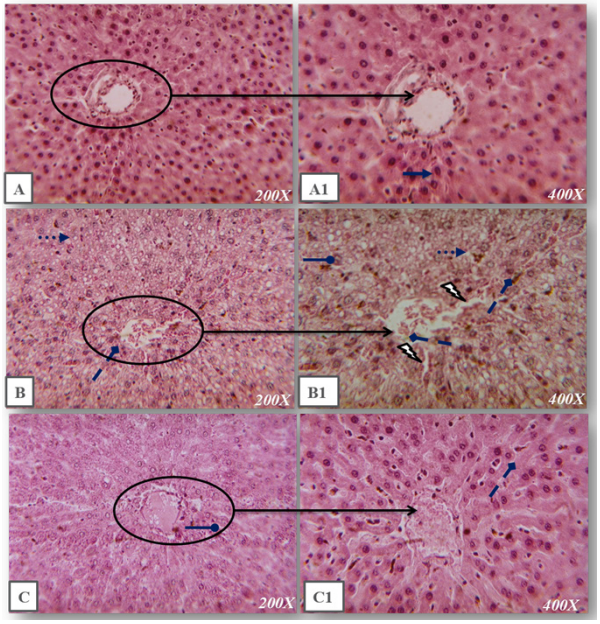

Figure 1. Histological structure of the liver of 14 day-old rats born from control mothers (A, A1), mothers treated with lead acetate (B, B1), mothers intoxicated with lead acetate and fed with dandelion (C, C1) from the 5th day of gestation. Sections were examined by light microscopy (200x, 400x) after haematoxylin–eosin staining. Arrows indicate: Granuloma inflammatory disorders; hepatocytes vacuolization of hepatocytes; necrotic cells; leucocyte infiltrations.

The NAS calculation system applied to semi-quantitative evaluation of inflammation and ballooning in the liver tissue confirmed all previous observations **(Fig. 2)**.

Effects of treatments on growth and body weight

Supplementation of control mothers with dandelion

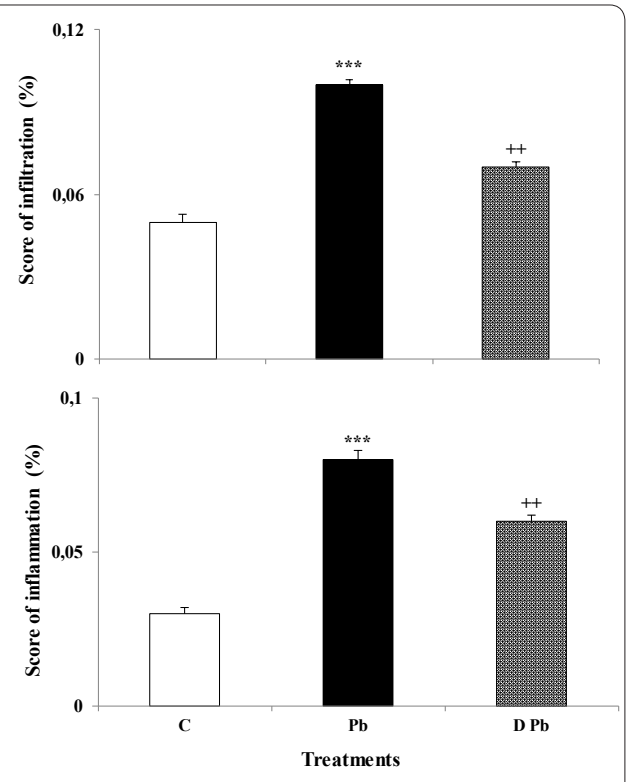

Figure 2. Histological NAS scores of liver tissues of the different set of animals, given as means ± SE of 12 determinations. Means not sharing the same letters within a row are significantly different ($P < 0.05$).

Table 1. Effect of lead exposure and/or dandelion consumption by 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 2% of dandelion in feed (D) or (D 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	D Pb	D	C	Pb	D Pb	D	C	Pb	D Pb	D
Food consumption (g/day)	37.78±4.25	28.40±2.45*	36.71±5.05^{+}	35.18±2.50	-	-	-	-	-	-	-	-
Water intake (mL/day)	70.18±7.85	91.26±5.19*	82.98±9.96^{+}	69.59±3.68	-	-	-	-	-	-	-	-
Body weight (g)	180.70±5.51	167.53±2.94*	179.50±9.71^{+}	197.50±7.74	19.51±0.21	17.62±0.47**	18.00±0.15^{+}	19.22±0.44	18.72±0.29	16.93±0.36*	17.54±0.25^{+}	18.31±0.41

Values are expressed as means ± SD of 8 determinations (mothers) or 4 determinations (pups) in each group.

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

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

had no effect *per se* on their food or water consumption, or on body weight of neonates **(Table 1)**. However, it increased mother body weight (+10% as compared to control). Conversely, lead exposure of mothers caused a significant decrease in the consumption of food (-24.8%) and an increase in water drinking (+30%) ($P < 0.05$). These observations were accompanied with a reduced body weight in mothers and their offspring, compared with the control. Co-treatment with dandelion maintained normal food and water consumptions by mothers, as well as body weight of their pups.

Effects of treatments on lead concentration in neonate blood

Lead concentration in blood of intoxicated pups aged 14 days exhibited 8- and 7-fold increases in male and female pups, respectively, compared to controls **(Table 2)**. However, supplementation of mothers with dandelion upon lead exposure reduced significantly (-40%, $P < 0.01$) the accumulation of lead in the blood circulation of their offspring. The addition of dandelion to the diet of control mothers had no effect *per se* on the blood lead level in pups.

Table 2. Lead concentration in blood of 14 day-old pups from control mothers (C) or mothers treated with 6 g/L of Pb through drinking water (Pb) and 2% dandelion (D) or (D Pb) in feed, from the 5th day of pregnancy until day 14 after delivery.

Treatments	Blood lead concentration (µg/mL)	
	Males (*n*=4)	Females (*n*=4)
C	0.19±0.01	0.24±0.02
Pb	1.59±0.13***	1.78±0.33***
D Pb	0.92±0.20**,++	1.02±0.19**,++
D	0.18±0.01	0.23±0.01

Values are expressed as means ± SD of 4 determinations in each group.

Significant differences between groups were mentioned as follows:

Pb or (D Pb) group compared to control (C): $^{*}P < 0.05$;$^{**}P < 0.01$;$^{***}P < 0.001$.

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

Effects of treatments on lead and calcium levels in neonate stomach

Lead concentration in the stomach of intoxicated pups aged 14 days exhibited a 5-fold increase, compared to that in control **(Table 3)**. Moreover, lead caused a marked change in the mineral content of neonate stomach, especially calcium content which dropped by 78% and 60% in male and female puppies, respectively. Conversely, diet supplementation of lead-intoxicated mothers with dandelion caused a strong decrease of Pb level and parallel increase of Ca level in pup's stomachic content ($P < 0.001$), as compared to Pb group. The addition of dandelion to the diet of control mothers had no effect *per se* on the gastric lead or calcium level in neonate.

Effects of treatments on liver weight and oxidative status

Effects of treatments on liver weight

Lead administration in pregnant and lactating mothers resulted in a significant decrease of the liver weight in 14 day-old pups ($P < 0.05$) **(Table 4)**. However, the offspring from lead-intoxicated mothers supplemented with dandelion exhibited normal liver weight. Addition of dandelion to the diet of control mothers had no effect

Table 3. Concentration of lead and calcium in stomachic content of 14 day-old pups from control mothers (C) or mothers treated with 6 g/L of Pb through drinking water (Pb) and 2% dandelion (D) or (D Pb) in feed, from the 5th day of pregnancy until day 14 after delivery.

Treatments	Stomach content (µg/g)			
	Males (*n*=4)		Females (*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**
D Pb	2.54±0.16*,++	2.55±0.19**,++	1.70±0.17++	3.61±0.44*,++
D	1.54±0.20	4.23±0.56	1.73±0.23	5.01±1.04

Values are expressed as means ± SD of 4 determinations in each group.

Significant differences between two groups are mentioned as follows:

Pb or (D Pb) group compared to control:$^{*}P < 0.05$; $^{**}P < 0.01$;$^{***}P < 0.001$.

(D 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 pups from control mothers (C) or mothers treated with 6 g/L of Pb through drinking water (Pb) and 2% of dandelion (D) or (D Pb) in feed from the 5th day of pregnancy until day 14 after delivery.

Treatments	Absolute weight (g)		Protein content (mg/g of organ)	
	Males	Females	Males	Females
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***
D Pb	0.60±0.02+	0.59±0.02+	24.26±2.85*,++	33.90±5.29+++
D	0.61±0.01	0.58±0.02	32.40±4.41	33.87±4.00

Values are expressed as means ± SD of 4 determinations in each group.

Significant differences between two groups are mentioned as follows:

Pb or (D Pb) group compared to control:$^{*}P < 0.05$; $^{**}P < 0.01$;$^{***}P < 0.001$.

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

Table 5. TBARS level and activities of CAT, SOD and GPx in the liver of 14 day-old pups from control mothers (C) or mothers treated with 6 g/L of lead (Pb) through drinking water and 2% dandelion (D) or (D Pb) in feed, from the 5th day of pregnancy until day 14 after delivery.

Parameters	Males				Females			
	C	Pb	D Pb	D	C	Pb	D Pb	D
TBARS	0.79±0.19	11.33±2.94***	1.70±0.11$^{*,+++}$	0.90±0.11	2.37±0.31	19.26±10.99***	1.18±0.48^{+++}	1.78±0.11
SOD	32.15±2.30	17.32±1.87***	25.54±2.60$^{*,++}$	30.53±1.59	33.55±5.42	16.61±2.97***	26.00±1.08^{+++}	27.28±3.37
CAT	76.99±5.83	43.31±2.38*	73.23±0.73^{+++}	75.22±4.97	78.86±8.91	51.75±6.44*	72.17±2.80^{++}	76.07±7.29
GPx	20.68±2.57	2.83±0.63***	17.60±3.95^{+++}	19.59±3.24	29.80±1.09	2.30±0.23***	17.44±1.70$^{*,+++}$	28.48±0.98

Results are expressed as mean ± SD of 4 determinations in each group.

TBARS: nmol/mg protein, **Activity of superoxide dismutase (SOD)**: U/mg protein, **Activity of catalase (CAT)**: µmol H_2O_2/min/mg protein, **Activity of glutathione peroxidase (GPx)**: nmol GSH/min/mg protein.

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

per se on the weight of newborn liver.

Effects of treatments on liver protein content

Lead treatment of mothers caused a strong decrease of the hepatic protein level in their offspring **(Table 4)**. The average decreases in protein contents were 70% and 82% in male and female neonates, respectively, compared to control pups. The addition of dandelion to the diet of mothers upon lead exposure markedly reduced such changes in their offspring, which exhibited protein levels similar to those of control.

Effects of treatments on lipid peroxidation levels in liver

Hepatic TBARS production in the offspring varied greatly depending on mother treatment. Lead intoxication of mothers resulted in 14- and 8-fold increases of TBARS concentration in the liver of male and female pups, respectively, as compared to control **(Table 5)**. However, dandelion supplementation of the lead-poisoned mothers significantly reduced the peroxidation level in liver of their offspring *($P < 0.001$)*, reaching control levels. That dandelion effect (TBARS decrease) was particularly marked in female neonates, compared to males. The addition of dandelion to the diet of control mothers had no effect *per se* on the peroxidation level in the liver of their offspring.

Effects of treatments on antioxidant enzyme activities in liver

Dandelion supplementation had no effect *per se* on the hepatic level of antioxidant enzyme activities in control newborns. Lead administration to mothers caused a strong decrease in hepatic SOD activity in male and female pups (by 46% and 50%, respectively) **(Table 5)**. The addition of plant (dandelion) to the diet of mothers reduced significantly that change in the neonate's liver ($P < 0.001$).

Similarly, catalase activity decreased significantly in the liver of male and female pups (-44% and -34%, respectively) upon lead exposure **(Table 5)**. Dandelion supplementation of mothers kept hepatic catalase activity close to control value in their offspring.

A strong decrease in GPx activity was observed in the liver of male and female pups from lead-treated mothers (7- and 13-fold decreases, respectively, $P < 0.001$). Here again, addition of dandelion to the diet of mothers maintained GPx activity close to control values in their offspring.

Discussion

Lead is a ubiquitous environmental and industrial pollutant that has been detected in nearly all phases of environment and biological system. Its persistence in human and animal tissues has often been associated with considerable health risks (31). In particular, pregnant women, offspring and young children are highly vulnerable upon lead exposure (32). The occupational or accidental exposure to lead affects different organs and tissues, including the respiratory tract, skin, kidney, liver, etc... Our experimental study consisted of chronic exposure of female rats, from gestation until the 14th day of lactation, to 0.6% lead in drinking water. Such intoxication was accompanied with a marked decrease in food consumption by mothers, which in turn reduced their body weight. Similarly, we observed growth retardation in intoxicated young rats, which is likely the result of a limitation of milk absorption by the offspring (32). These results are consistent with previous studies performed with lead or other heavy metals (33,34). The magnitude of these effects appeared to be strongly dependent on the developmental period in which exposure takes place (35). They support the hypothesis that perinatal and postnatal exposures of rats to lead delays the growth of their pups, which may be a consequence of transfer of Pb through both placenta and milk. Besides, although T. *officinale* did not stimulate food or water consumption by mothers, growth activation was recorded upon dandelion supplementation. This could be due to vitamins and iron contents of the plant, which help preventing anemia (36).

In humans, a pregnant woman can transfer her body burden of lead to the growing fetus, as there is no placental barrier for a heavy metal like lead (37). Thus, intoxication may occur during the first months of life through breast-feeding. Indeed, a number of contaminants in breast milk could represent a risk for children (38,39,40). In our study, we found high levels of lead (exceeding 1.5 mg/L) in young rat blood, confirming that offspring exposed to lead from the prenatal period may exhibit elevated blood Pb level, even higher than that of their mother (41,42). Such an observation supports the notion that infants absorb and retain more Pb than older animals (43,44).

Milk is a major source of calcium and is recom-

mended as a prophylactic remedy against lead toxicity, since calcium increases Pb uptake from digestive tract (45). Interestingly, in our study, antiparallel variations of Ca and Pb were found in neonate stomach depending on the treatments. Such reverse trends between the metal and calcium has not been documented hitherto, and its cause remains to be elucidated. Only recent reports have suggested that lead inhibits some regulatory actions of calcium on cell function (46, 47). Exhibiting a similar chemical structure to calcium, the metal would compete for membrane transport systems (48) and have a high affinity for intracellular calcium sites and calcium-regulating processes. As a consequence, it alters intracellular calcium homeostasis and receptor-mediated signal transduction pathways, which contribute to its toxicity (48).

Once absorbed by the mothers, lead is distributed by the blood toward the soft tissues, including the liver which is a key organ in the metabolism and detoxification of xenobiotics. As a consequence, quite high levels of lead are found in the liver (44), and toxicity may occur (49). Our results showed that lead caused significant liver atrophy and drop in hepatic protein level in the pups. Such observation is likely the result of protein synthesis impairment in the hepatocytes, like it has been reported earlier (50). Along, it was accompanied with severe histological injuries in the progeny, including cell structure damage, hepatocellular necrosis and leukocyte infiltration. These results are in agreement with those of Campana et al. (51) and El Heni et al. (52), confirming that late pregnancy and early postnatal are critical periods when pup development is particularly vulnerable to lead toxicity.

Our study shows that supplementation of healthy females with dandelion had no effect *per se* on hepatic functions in either mothers or their offspring. It confirms that dandelion root extract itself does not have any harmful effects on hepatic function (53) and could be considered as safe when added at 2% in the diet. Even, our data showed that ingestion of dandelion by mother rats upon lead intoxication prevents the adverse effects of Pb on neonate liver. This hepatoprotective effect may be related to the presence of antioxidant compounds in root microspheres of *T. officinale* (54), including terpenes and phenolic compounds such as flavonoids (quercetin, luteolin, and luteolin-7-O-glucoside), chicoric acid, chlorogenic acid, and chrysoeriol (16,55). Accordingly, our results suggest that dandelion protects liver tissues against lead-induced damages, in particular oxidative stress and lipid peroxidation, thanks to its flavonoids (56).

In order to understand the mechanism by which dandelion supplementation ameliorates lead-induced toxicity, we evaluated markers of oxidative stress, including thiobarbituric acid reactive substances (TBARS, marker of peroxidation level) and antioxidative enzyme activities. Accumulating evidences have shown that lead causes oxidative stress by inducing the generation of reactive oxygen species (ROS) and weakening the antioxidant defence system of cells (57-58).Thus, lead contamination caused an elevation of lipid peroxidation rate in the newborn liver, confirming the high sensitivity of liver at late pregnancy and early postpartum (59,34). Many studies have reported that lead exposure induces oxidative stress in liver (51-56). Conversely, the suppression of lipid peroxidation in hepatocyte membranes and the alleviation of oxidative stress by antioxidants are key mechanisms for hepatoprotection against lead-induced toxicity (60). On that point, lipid peroxidation was decreased in diabetic rats following dandelion water extract administration (61). These authors attribute the protective effect of the plant to the presence of luteolin, luteolin-7-O-glucoside and polyphenols, which are well-documented antioxidants (62,63).

Besides, antioxidant enzyme activities were measured in the liver of pups to determine how dandelion affects hepatic antioxidant defenses. Our results clearly demonstrated that SOD, CAT and GPx activities decreased upon lead intoxication, indicating that lead exposure induced oxidative stress by lowering hepatic antioxidant defenses. These enzymes are important agents of living organism defense against oxidative stress, scavenging or decomposing highly toxic ROS (64,65). Several studies have shown that Pb can alter antioxidant enzymes because of its high affinity for functional sulfhydryl (SH) groups in these enzymes (65,66). Moreover, the induction of inflammatory process in hepatic tissue following lead chronic exposure was reported (67,68), and the authors suggested that Pb would interact with antioxidant defense, leading to ROS generation. GPx, CAT and SOD depend on various essential trace elements for proper molecular structure and activity, so these antioxidant enzymes are potential targets for lead toxicity (66). This might be largely due to an overwhelming oxidative modification of the enzyme proteins by the ROS.

On the other hand, our observations revealed that ingestion of *T. officinale* prevented the impairment of antioxidant defense system in progeny liver challenged with lead, as indicated by the restoration of enzyme activities upon co-treatment with lead and dandelion. As far as we know, such protective effect on antioxidant enzymes is not clearly elucidated. Some flavonoids identified in dandelion may act as ROS scavengers to protect against oxidative stress, as reported for luteolin and luteolin-7-O-glucoside (17). Along, flavonoid treatments have been shown to increase catalase mRNA expression (69), and catalase and GPx activities (70). Research is currently under way to identify the structure of the hepatoprotectants in dandelion root against lead-induced toxicity.

Dramatic damages caused to the liver by lead consumption are strongly associated with oxidative stress (lipid peroxidation, generation of ROS). Dandelion supplementation to the diet attenuates liver disturbances induced by lead, as evidenced by a recovery of normal hepatic antioxidant status and lipid peroxidation. It is assumed that the antioxidant action of microsphere extract from the root of *T. officinale* would be responsible for improving the oxidative status during the lead poisoning. Therefore, the development of nutritional supplementation using this plant may be useful to protect the liver against lead-induced damage, and further study on the role of this edible plant on human subjects should be addressed to confirm its potential.

Acknowledgements

The authors thank Dr Xavier Dauvergne for his help in statistical analysis of data. The present work was supported by DGRST grant (Direction Générale de la Recherche Scientifique et Technique-Tunisie (Appui à la Recherche Universitaire de base UR/13 ES-73).

References

1.Boguszewska A, Pasternak K. Cadmium-influence on biochemical processes of the human organism. Ann Univ Marie Curie Sklodowska Med 2004; 59: 519–523.

2.Gardella C. Lead exposure in pregnancy: a review of the literature and argument for routine prenatal screening. Obstet Gynecol Surv 2001; 56: 231–238.

3.Wang L, Wang Z, Liu J. Protective effect of N-acetylcysteine on experimental chronic lead nephrotoxicity in immature female rats. Human Exp Toxicol 2010; 29: 581–591.

4.Lewis RM, Brooks S, Crocker IP, Glazier J, Hanson MA, Johnstone ED, Panitchob N, Please CP, Sibley, CP, Widdows KL, Sengers BG. Review: Modelling placental amino acid transfer - From transporters to placental function. Placenta 2013; 34: 46-51.

5.Goyer RA. Transplacental transport of lead. Environ Health Perspect 1990; 89: 101–105.

6.Palminger Hallen I, Jonsson S, Karlsson MO, Oskarsson A. Kinetic observations in neonatal mice exposed to lead via milk. Toxicol Appl Pharmacol 1996; 140: 13–18.

7.Venkatesh T, Vasuki SK. Future of lead chelation, distribution and treatment. *JKIMSU* 2012; 1: 6-23.

8.Conterato GM, Augusti PR, Somacal S, Einsfeld L, Sobieski R, Torres JR, Emanuelli T. Effect of lead acetate on cytosolic thioredoxin reductase activity and oxidative stress parameters in rat kidneys. Basic Clin Pharmacol Toxicol 2007; 101: 96–100.

9.Ponce-Canchihuaman JC, Pérez-Méndez O, Hernandez-Munoz R, Torres-Duran PV, Juarez-Oropeza MA. Protective effects of Spirulina maxima on hyperlipidemia and oxidative-stress induced by lead acetate in the liver and kidney. Lipids Health Dis 2010; 9: 35–41.

10. Gerhardsson L, Englyst V, Lundstrom N, Nordberg G, Sandberg S, Steinvall F. Lead in tissues of deceased lead smelter workers. J Trace Elements Med Biol 1995; 9: 136–143.

11. Ibrahim NM, Eweis EA, El-Beltagi HS, Abdel-Mobdy YE . Effect of lead acetate toxicity on experimental male albino rat. Asian Pac J Trop Biomed 2012; 2: 41–46.

12. Gurer-Orhan H, Sabir HU, Ozgune H. Correlation between clinical indicators of lead poisoning and oxidative stress parameters in controls and lead-exposed workers. Toxicology 2004; 195: 147–154.

13. Farmand F, Ehdaie A, Roberts CK, Sindhu RK. Lead-induced dysregulation of superoxide dismutases, catalase, glutathione peroxidase, and guanylate cyclase. Environ Res 2005; 98: 33–39.

14. Berrahal AA, Nehdia A, Hajjaji N, Gharbi N, Fazâa S. Antioxidant enzymes activities and bilirubin level in adult rat treated with lead. C R Biol 2007; 330: 581–588.

15. Mates JM, Sanchez-Jimenez FM. Role of reactive oxygen species in apoptosis: implications for cancer therapy. Int J Biochem Cell Biol 2000; 32: 157–170.

16. Schütz K, Carle R, Schieber A. Taraxacum – a review on its phytochemical and pharmacological profile. J Ethnopharmacol 2006; 107: 313–323.

17. You Y, Yoo S, Yoon HG, Park J, Lee YH, Kim S, Oh KT, Lee J, Cho HY, Jun W. In vitro and in vivo hepatoprotective effects of the aqueous extract from Taraxacum officinale (dandelion) root against alcohol-induced oxidative stress. Food Chem Toxicol 2010; 48: 1632–1637.

18. Council of European Communities. Council instructions about the protection of living animals used in scientific investigations: Off J Eur Communities (JO 86/609/CEE) 1986; 1–18.

19. Gargouri M, Ghorbel-Koubaa F, Bonenfant-Magné M, Magné C, Dauvergne X, Ksouri R, Krichen Y, Abdelly C, El Feki A. Spirulina or dandelion-enriched diet of mothers alleviates lead-induced damages in brain and cerebellum of newborn rats. Food Chem Toxicol 2012; 50: 2303–2310.

20. Ghorbel F, Boujelbene M, Makni-Ayadi F, Guermazi F, Croute F, Soleilhavoup JP, El Feki A. Exploration des effets cytotoxiques du plomb sur la fonction sexuelle endocrine et exocrine chez le rat pubère mâle et femelle. Mise en évidence d'une action apoptotique. C R Biol 2002; 325: 927–940.

21. Bunn TL, Parsons PJ, Kao E, Dietert RR. Exposure to lead during critical windows of embryonic development: differential immunotoxic outcome based on stage of exposure and gender. Toxicol Sci 2001; 64: 57–66.

22. Fisheck KL, Rasmussen LM. Effects of repeated cycles on maternal nutritional status, lactational performance and litter growth in ad libitum fed and chronically. Food J Nutr 1987; 117: 1967–1975.

23. Heil SH, Hungund BL, Zheng ZH, Subramanian MG. Ethanol and lactation: effects on milk lipids and serum constituents. Alcohol 1999; 12: 43-44.

24. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265–275.

25. Yagi K. A simple fluorometric assay for lipoperoxide in blood plasma. Biochem Med 1976; 15: 212– 216.

26. Beyer WF, Fridovich I. Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. Anal Biochem 1987; 161: 559–566.

27. Aebi H. Catalase in vitro. Methods Enzymol 1984; 105: 121-126.

28. Floke L, Gunzler R. Assays of glutathion peroxidase. Methods Enzymol 1984; 105:114–121.

29. Gabe M. In: Masson (Ed.), Techniques Histologiques 1968; 838–841.

30. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005; 41: 1313–1321.

31. Juberg DR, Kleiman CF, Simona CK. Position paper of the American Council on Science and Health: lead and human health. Ecotoxicol Environ Safe 1997; 38: 162–180.

32. Adgate JL, Weisel C, Wang Y, Rhoads GG, Lioy PJ. Lead in house dust: relationships between exposure metrics. Environ Res 1995; 70: 134–147.

33. Bellinger D, Leviton A, Waternaux C, Allred E. Methodological issues in modeling the relationship between low-level lead exposure and infant development: examples from the Boston lead study. Environ Res 1985; 38: 119–129.

34. Garoui EM, Fetoui H, Ayadi Makni F, Boudawara T, Zeghal N. Cobalt chloride induces hepatotoxicity in adult rats and their suckling pups. Exp Toxicol Pathol 2011; 63: 9–15.

35. IARC. Inorganic and Organic Lead Compounds. IARC Monographs on the Evaluation of Carcinogenic Risks to Human. 2006; 87: 519.

36. Tahtamounia LH, Alqurnab NM, Al-Hudhuda MY, Al-Hajjb HA. Dandelion (Taraxacum officinale) decreases male rat fertility in vivo. J Ethnopharmacol 2011; 135: 102–109.

37. Bellinger D, Leviton A, Rabinowitz M, Alfred E, Needleman H, Schoenbaum S. Weight gain and maturity in fetuses exposed to low levels of lead. Environ Res 1991; 54: 151–158.

38. Chovancova J, Conka K, Kocan A, Sejakova ZS. PCDD, PCDF,

PCB and PBDE concentrations in breast milk of mothers residing in selected areas of Slovakia. Chemosphere 2011; 83: 1383-1390.
39. EFSA. Scientific Opinion of the EFSA Panel on Contaminants in the Food Chain on Polybrominated Diphenyl Ethers (PBDEs) in Food. EFSA J 2011; 9 (5).
40. Anses. Rapport d'appui scientifique et technique relatif à l'exposition agrégée au plomb : prise en compte des différentes voies d'exposition. (appui à la saisine n°2013-SA-0092). Maisons-Alfort: Anses, 2014 ; pp. 118.
41. Cao J, Xu X, Hylkema MN, Zeng EY, Peter SD, Suk WA, Bergman Å, Huo X . Early-life Exposure to Widespread Environmental Toxicants and Health Risk: A Focus on the Immune and Respiratory Systems. Ann Glob Health 2016; 82: 119-131.
42. Zentner EAL, Rondo HCP, Duran CM, Olivaeira MJ. Relationships of blood lead to calcium, iron, and vitamin C intakes in Brazilian pregnant women. Clin Nutr 2008; 27: 100-104.
43. Gargouri M, Ben Saad H, Ben Amara I, Magné C, El Feki A. Spirulina exhibits hepatoprotective effects against lead induced oxidative injury in newborn rats. Cell Mol Biol 2016; 62: 85-93.
44. EFSA. Scientific Opinion of the EFSA panel on Contaminants in the Food Chain on Lead in Food. EFSA J 2010; 8 (4).
45. James HM, Hilburn ME and Blair JA. Effects of meals and meal times on uptake of lead from the gastrointestinal tract in humans. Human Toxicol 1985; 4: 401-407.
46. Zentner EAL, Rondo HCP, Duran CM, Olivaeira MJ. Relationships of blood lead to calcium, iron, and vitamin C intakes in Brazilian pregnant women. Clin Nutr 2008; 27: 100-104.
47. Gubrelay U, Agnihotri RK, Shrotriya S, Sharma R. Effect of Lead stress on phosphatase activity and reducing power assay of Triticum aestivum. Cell Mol Biol 2015; 61: 57-62.
48. Oliver B, Jörg K. Calcium: Not just another ion. In: R. Hell and R. R. Mendel (eds.), Cell Biology of Metals and Nutrients, Plant Cell Monographs 2010; 17: 54.
49. Mudipalli A. Lead hepatotoxicity and potential health effects. Indian J Med Res 2007; 126: 518–527.
50. Shalan MG, Mostafa MS, Hassouna MM, El-Nabi SE. Amelioration of lead toxicity on rat liver with vitamin C and silymarin supplements. Toxicology 2005; 206: 1–15.
51. Campana O, Sarasquete C, Blasco J. Effect of lead on ALAD activity, metallothionein levels, and lipid peroxidation in blood, kidney, and liver of the toadfish Halobatrachus didactylus. Ecotoxicol Environ Safe 2003; 55: 116–125.
52. El Heni J, Messaoudi I, Hamouda F, Kerkeni A. Protective effects of selenium (Se) and zinc (Zn) on cadmium (Cd) toxicity in the liver and kidney of the rat: histology and Cd accumulation. Food Chem Toxicol 2008; 46: 3522–3527.
53. Park JY, Park CM, Kim JJ, Song YS. Hepatoprotective activity of dandelion (Taraxacum officinale) water extract against D-galactosamine-induced hepatitis in rats. J Korean Soc Food Sci Nutr 2008; 37: 177–183.
54. Dorsch W. Clinical application of extracts of Echinacea purpurea or Echinacea pallida. Critical evaluation of controlled clinical studies. Z Arztl Fortbild 1996; 90: 117–122.
55. Williams CA, Goldstone F, Greenham J. Flavonoids, cinnamic acids and coumarins from the different tissues and medicinal preparations of Taraxacum officinale. Phytochemistry 1996; 42: 121–127.
56. Bechara FJH, Medeiros MGH, Monteiro HP, Hermes Lima M. A free radical hypothesis of lead poisoning and inborn porphyries associated with 5-aminolevulinic acid overload. Quim Nova 1993; 16: 385-392.
57. Flora SJS, Flora G, G. Saxena, Mishra M. Arsenic and lead induced free redical generation and their reversibility following chelation. Cell Mol Biol 2007; 53:26-47.
58. Liu CM, Zheng YL, Lu J, Zhang ZF, Fan SH, Wu DM, Ma JQ. Quercetin protects rat liver against lead-induced oxidative stress and apoptosis. Environ Toxicol Pharmacol 2010; 29: 158–166.
59. Sakr S, Yosry A, Okdah H, Sabah F. Gibberellic A3 induced histological and histochemical alteration in the liver of Albino rats. Sci Asia 2003; 29: 327–331.
60. Czaja MJ. Induction and regulation of hepatocyte apoptosis by oxidative stress. Antioxid Redox Signal 2002; 4: 759–767.
61. Cho SY, Park JY, Park EM, Choi MS, Lee MK, Jeon SM, Jang MK, Kim MJ, Park YB. Alternation of hepatic antioxidant enzyme activities and lipid profile in streptozotocin- induced diabetic rats by supplementation of dandelion water extract. Clin Chim Acta 2002; 317: 109–117.
62. Hagymasi K, Blazovics A, Lugasi A, Kristo S, Feher J, Kery A. In vitro antioxidant evaluation of dandelion (Taraxacum officinale WEB.) water extracts. Acta Aliment 2000; 29: 1–7.
63. Park CM, Cha YS, Youn HJ, Cho CW, Song YS. Amelioration of oxidative stress by dandelion extract through CYP2E1 suppression against acute liver injury induced by carbon tetrachloride in Sprague-Dawley rats. Phytother Res 2010; 24: 1347–1353.
64. Bowler C, Van Montagu M, Inzé D. Superoxide dismutase and stress tolerance. Annu Rev Plant Physiol Plant Mol Biol 1992; 43: 83-116.
65. Salin ML. Toxic oxygen species and protective systems of the chloroplast. Physiol Plant 1987; 72: 681-689.
66. Hsu PC, Guo YL. Antioxidant nutrients and lead toxicity. Toxicology 2002; 180, 33-44.
67. Jarrar BM, Taib NT. Histological and histochemical alterations in the liver induced by lead chronic toxicity. Saudi J Biol Sci 2012; 19: 203-210.
68. Salińska A, Włostowski T, Zambrzycka E. Effect of dietary cadmium and/or lead on histopathological changes in the kidneys and liver of bank voles Myodes glareolus kept in different group densities. Ecotoxicology 2012; 21: 2235–2243.
69. Rohrdanz E, Ohler S, Tran-Thi QH, Kahl R. The phytoestrogen daidzein affects the antioxidant enzyme system of rat hepatoma H4IIE cells. J Nutr 2002; 132: 370–375.
70. Alvarez E, Leiro J, Orallo F. Effect of (-)-epigallocatechin-3-gallate on respiratory burst of rat macrophages. Int Immunopharmacol 2002; 2: 849–855.