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Effect of soybean on bone and gonad hormones in lead intoxicated rats

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The protective and treated soybean dietary against lead toxicity in male rats was investigated in the bones and the hormones of the reproductive system. Sixty adult male rats were divided into the following groups: Group I: 30 rats fed on standard casein were divided into three equal subgroups; Group Ia: control casein, Group Ib: (casein-protected) rats fed on casein before lead intoxication, Group Ic: (casein-treated) rats fed on casein after lead intoxication; Group II: 30 rats fed on standard soybean. These rats were also divided into three equal subgroups; Group IIa: control soybean, Group IIb: (soybean-protected) rats fed on soybean before lead intoxication, Group IIc: (soybean-treated) rats fed on soybean after lead intoxication. The results revealed that the accumulation of lead in the soft tissues (liver, kidney and testis) was much greater in the protective groups than in the curative ones. There was a significant increase in the femoral bone mass density (BMD) in the soybean protected group when compared with either the soybean-treated or the casein-protected group. Serum testosterone significantly increased in the soybean-treated group compared with the soybean-protected group. Serum luteinizing hormone (LH) did not change between groups. It is concluded that the soybean diet ameliorated bone and testis intoxicated with lead.

Key words: lead intoxication, rats, soybean, gonad hormones, bones.

INTRODUCTION

Lead (Pb) is an environmental pollutant and a metabolic poison with a variety of toxic effects and induces many biological effects to animals and humans (Bagchi & Preuss, 2005). The main targets of lead toxicity are the red blood cells (Goering, 1993), the central and peripheral nervous system (Viala, 1998) and the kidney (Goyer, 1989). Lead may also cause alterations in the reproductive system. Exposure to lead of females increased incidences of sterility, miscarriage, premature delivery and infant mortality in humans and animals (Taupeau *et al*., 2001). Lead also caused impaired male reproductive capacity, prostatic hyperplasia, inhibition of spermatogenesis, testicular degeneration, etc. (Nriagu, 1988; Batra *et al*., 1998). It has been reported that in adult males intoxicated with lead, this metal acts both at the hypothalamic-pituitary-gonadal axis and the gonadal sites to disrupt the reproductive physiology and behavior (Klein *et al*., 1994; Wadi & Ahmad, 1999).

Soybean contains not only estrogenic isoflavones, but also other substances such as coumestans and lignans (Kurzer & Xu, 1997; Hutabarat *et al*., 2000). All these substances are referred to as phytoestrogens, because they are derived from plants and possess a biological activity similar to estrogens. Soybean products are thought to be beneficial in preventing osteoporosis, because they contain estrogenic isoflavones such as genistein, daidzein and their glycans, each of which has been shown to have bone-sparing effects on rats, mice and humans (Knight & Eden, 1996; Alekel *et al*., 2000; Ye *et al*., 2003).

There is evidence that estrogen plays an important role in skeletal tissue in males as well as females.

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Soybean compounds (phytoestrogens such as genistein) act on bones by exhibiting cooperative effects on bone mass and by preventing androgen deficiency-induced bone loss in mice (Wu *et al*., 2003).

The objective of the present study is to examine the effects of dietary soybean on male rat bone and reproductive system intoxicated with lead.

MATERIALS AND METHODS

Experimental animals

Sixty adult males of Sprague Dawley rats, aged about three months, weighing 165-240 g, were purchased from the breeding unit of the Egyptian Organization for Biological Products and Vaccines (Abbassia, Cairo). The rats were housed in steel mesh cages and maintained for one week acclimatization period on commercial pellet diet and drinking water *ad libitum*. Rats were maintained under standard animal house conditions (12:12 hrs light-dark and 21 ± 1 °C temperature). Rats were randomly divided into two major groups:

- Group I: (casein group) including 30 rats. Rats were fed on casein diet (NRC, 1995) *ad libitum* for nine weeks. They were randomly divided into three equal subgroups: Group Ia: casein control group; Group Ib: (casein-protected group). Rats were injected intra-peritoneally (i.p.) with 8 mg kg^{-1} body weight lead acetate trihydrate extrapure (Merck, Darmstadt, Germany) from the 5th week to the 9th week. This treatment was given four days per week for five weeks; Group Ic: (casein-treated group). Rats were injected i.p. with lead from the 1st week to the 5th week.
- Group II: (soybean group) including 30 rats. Rats were fed on soybean for nine weeks. They were also divided into three equal subgroups: Group IIa: soybean control group; Group IIb: (soybean-protected group). Rats were injected i.p. with 8 mg kg^{-1} body weight lead acetate from the 5th week to the 9th week, four days per week; Group IIc: (soybean-treated group). Rats were injected i.p. with lead acetate from the 1st week to the 5th week. The casein and soybean control subgroups were injected with an equivalent dose of sodium acetate. The dose of lead acetate was given according to Thoreux-Manlay *et al*. (1995). At the end of the experiment (nine weeks) rats were sacrificed under anaesthesia with diethyl-ether. Blood and tissues (testis, liver, kidney and femoral bones) were collected.

Blood was divided into two parts; the first part was collected on heparinized solution for lead determination, while the second part was left without anticoagulant for serum separation. Blood, serum and tissues were stored at –20 °C until analysis. A part of testis was collected in 10% formaline for histopathological examination.

The serum luteinizing hormone (LH) was assayed by using a commercial kit supplied by Coat-A-Count LH IRMA, Los Angeles, USA (Odell *et al*., 1967). Serum testosterone was assayed by using a commercial kit supplied by the Diagnostic Systems Laboratories (DSL), Texas, USA (De Lacerda *et al*., 1973). Blood lead levels were assayed by using atomic absorption (Wittmers *et al*., 1981).

Assay of lead in different tissues

The lead in liver, kidney and testis was detected by atomic absorption spectroscopy from nitric acid and perchloric acid extracts using the method by Einarsson & Lindstedt (1969).

Bone analysis

Bone mass density (BMD) was determined according to Archimedes' principle (Kalu *et al*., 1991). Bone lead level was determined by atomic spectroscopy after femur was heated for six hours at 500 °C in a muffle furnace oven. Dry ash was dissolved in 2 N HCl then lead was determined by atomic absorption spectroscopy (Wittmers *et al*., 1981). Calcium (Schmidt-Gayk *et al*., 1997) and phosphorus (Goldenberg & Fernandez, 1966) in the dry ash were determined by using a commercial kit that was supplied by Bicon Co., Germany.

Histological examination

Fresh pieces, randomly cut from the testis of male rats were placed in Zenker's fixative for 24 hrs and embedded in paraffin wax. Sections, 5 to 8 μm thick, were cut and stained with haematoxylin and eosin (Scheuer & Chalk, 1986).

Statistical analysis

Means, standard errors (s.e.) and p values were calculated using the Statistical Package for Social Sciences (SPSS, version 13.0). Student's test was used to compare mean values among groups (p values were considered significant at $p < 0.05$).

RESULTS

The body weight of control rats fed on casein or soybean diet was increased by 38% and 52%, respectively (Table 1). The lead levels in the control rats that were fed on either casein or soybean in different tissues (bone, testis, liver and kidney) were lower than the detected limit (< 1 nmol). The highest mean lead concentration was in femur. The lead levels of all groups increased in the order: bone, kidney, liver and testis. The amount of lead in the rat soft tissues of the casein or soybean protected groups was significantly higher that that in the rats fed on either casein or soybean (Fig. 1).

Femoral bone mass density (BMD) was significantly decreased in the rats exposed to lead before or after casein or soybean diet compared with the controls. On the other hand, femoral BMD in the rats fed on soybean before lead intoxication (protective group), was significantly increased when compared with the group fed on soybean after lead intoxication (treated groups). Femoral calcium and phosphorus levels showed a significant decrease in the rats (exposed to lead) fed on soybean either protected or treated compared with control rats fed on soybean (Table 2).

Serum LH, testosterone level and testosterone/LH ratio in the rats fed on casein before lead intoxication were diminished by 8.2%, 83.5% and 81.3% respectively, when compared with the control rats fed on casein (Table 3, Fig. 3). However, serum testosterone level was reduced by 54% in the rats fed on soybean. Serum testosterone/LH ratio significantly decreased, whereas serum LH level did not show any changes compared with the control rats fed on soybean.

TABLE 1. Body weights of rats fed on casein and soybean before and after lead intoxication

Parameters / Groups	Body weight (g) At start	Body weight (g) At sacrifice	Increase rate of body weight * (%)
Group Ia (control casein)			
n	10	10	
Range	170-225	237-300	
Mean ± s.e.	193.6 ± 5.6	267.0 ± 5.9	38
Group Ib (casein-protected)			
n	10	9	
Range	165-230	158-215	
Mean ± s.e.	189.1 ± 5.8	188.3 ± 7.3	–0.42
Group Ic (casein-treated)			
n	10	9	
Range	170-220	196-254	
Mean ± s.e.	184.0 ± 4.9	221.0 ± 7.4	20
Group IIa (control soybean)			
n	10	10	
Range	165-210	245-325	
Mean ± s.e.	189.2 ± 5.3	288.0 ± 7.7	52
Group IIb (soybean-protected)			
n	10	9	
Range	175-230	167-215	
Mean ± s.e.	206.0 ± 5.9	204.0 ± 5.5	–0.97
Group IIc (soybean-treated)			
n	10	8	
Range	170-240	180-262	
Mean ± s.e.	212.7 ± 8.1	221.0 ± 10.3	3.9

*: change in weight between start and end of the study
n = number of cases, s.e. = standard error

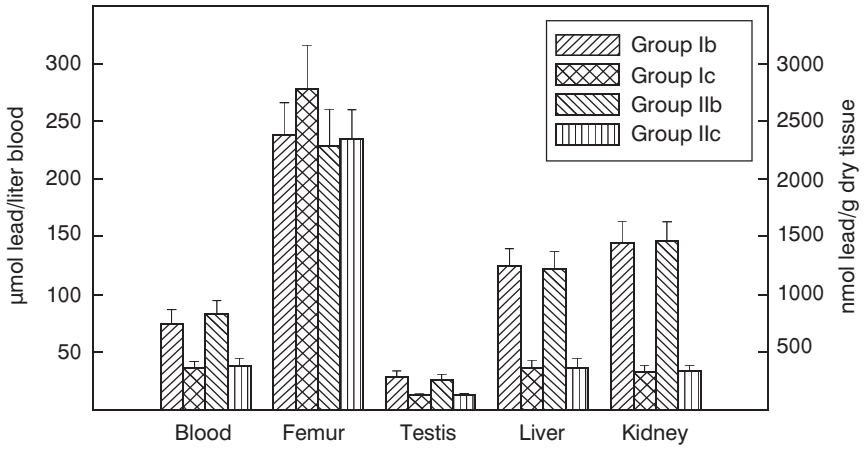

FIG. 1. Blood lead level and lead distribution in different tissues of rats fed with casein or soybean before and after exposure to lead acetate.

TABLE 2. Femoral bone mass density (BMD), femoral calcium and phosphorus levels in rats fed on casein and soybean before and after lead intoxication

Parameters / Groups	BMD (g cm^{-3} bone volume)	Calcium (mg g^{-1} dry ash)	Phosphorus (mg g^{-1} dry ash)
Group Ia (control casein)			
Range	1.43-1.66	313-494	131-242
Mean ± s.e.	1.53 ± 0.02	382.0 ± 15.6	202.0 ± 10.1
Group Ib (casein-protected)			
Range	1.18-1.56	246-442	108-219
Mean ± s.e.	1.37 ± 0.04	365.0 ± 20.8	170.4 ± 11.3
$p^{(a)}$	< 0.05	ns	< 0.05
Group Ic (casein-treated)			
Range	1.27-1.52	222-412	116-211
Mean ± s.e.	1.34 ± 0.03	308.0 ± 22.0	163.0 ± 10.6
$p^{(a)}$	< 0.001	< 0.05	< 0.02
$p^{(b)}$	ns	ns	ns
Group IIa (control soybean)			
Range	1.45-1.72	359-512	161-251
Mean ± s.e.	1.57 ± 0.02	413.0 ± 12.8	206.0 ± 9.1
$p^{(a)}$	ns	ns	ns
Group IIb (soybean-protected)			
Range	1.37-1.58	229-416	104-204
Mean ± s.e.	1.47 ± 0.02	347.0 ± 21.5	156.0 ± 10.6
$p^{(c)}$	< 0.01	< 0.05	< 0.01
$p^{(b)}$	< 0.05	ns	ns
Group IIc (soybean-treated)			
Range	1.26-1.51	243-448	106-214
Mean ± s.e.	1.33 ± 0.03	331.0 ± 26.5	160.0 ± 14.9
$p^{(c)}$	< 0.001	< 0.05	< 0.05
$p^{(d)}$	ns	ns	ns
$p^{(e)}$	ns	ns	ns

n = number of cases, $p^{(a)}$ = *vs* control rats fed on casein, $p^{(b)}$ = *vs* rats fed on casein before lead intoxication, $p^{(c)}$ = *vs* control rats fed on soybean, $p^{(d)}$ = *vs* rats fed on casein after lead intoxication, $p^{(e)}$ = *vs* rats fed on soybean before lead intoxication, ns = non-significant, s.e. = standard error

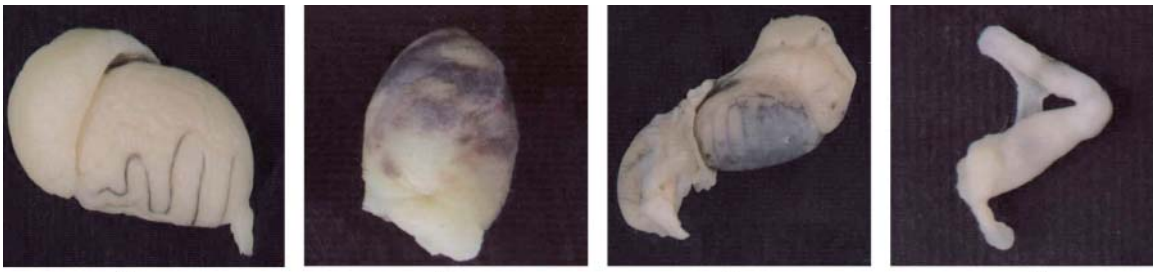

FIG. 2. Testes of group I rats fed on casein: (A) control rat; (B) rat before lead intoxication with a high vascular congestion; (C) rat before lead intoxication with testicular atrophy; (D) rat after lead intoxication with vascular congestion.

TABLE 3. Serum luteinizing hormone (LH) and testosterone levels in rats fed on casein and soybean before and after lead intoxication

Parameters / Groups	LH (ng/100 ml)	Testosterone (ng/100 ml)	Testosterone/LH ratio
Group Ia (control casein)			
Range	26.0-32.0	120-250	3.9-9.1
Mean ± s.e.	28.5 ± 0.7	188.0 ± 13.0	6.7 ± 0.5
Group Ib (casein-protected)			
Range	21.6-30.0	14-76	0.5-0.3
Mean ± s.e.	26.2 ± 0.8	31.0 ± 6.0	1.2 ± 0.3
$p^{(a)}$	< 0.05	< 0.001	< 0.001
Group Ic (casein-treated)			
Range	21.6-30.0	75-150	2.5-7.1
Mean ± s.e.	25.8 ± 0.9	120.0 ± 12.0	4.6 ± 0.4
$p^{(a)}$	< 0.05	< 0.01	< 0.01
$p^{(b)}$	ns	< 0.001	< 0.001
Group IIa (control soybean)			
Range	25.0-31.6	140-300	4.4-12.0
Mean ± s.e.	28.6 ± 0.7	212.0 ± 14.0	7.7 ± 0.7
$p^{(a)}$	<0.05	ns	ns
Group IIb (soybean-protected)			
Range	25.0-29.2	190-1300	0.7-4.9
Mean ± s.e.	27.5 ± 0.5	97.5 ± 15.6	3.5 ± 0.2
$p^{(c)}$	ns	< 0.001	< 0.001
$p^{(b)}$	ns	< 0.001	< 0.001
Group IIc (soybean-treated)			
Range	24.2-30.0	94-240	3.6-8.6
Mean ± s.e.	27.0 ± 0.6	166.0 ± 18.0	6.2 ± 0.7
$p^{(c)}$	ns	< 0.05	ns
$p^{(d)}$	ns	< 0.05	ns
$p^{(e)}$	ns	< 0.05	< 0.01

n = number of cases, $p^{(a)}$ = *vs* control rats fed on casein, $p^{(b)}$ = *vs* rats fed on casein before lead intoxication, $p^{(c)}$ = *vs* control rats fed on soybean, $p^{(d)}$ = *vs* rats fed on casein after lead intoxication, $p^{(e)}$ = *vs* rats fed on soybean before lead intoxication, ns = non-significant, s.e. = standard error

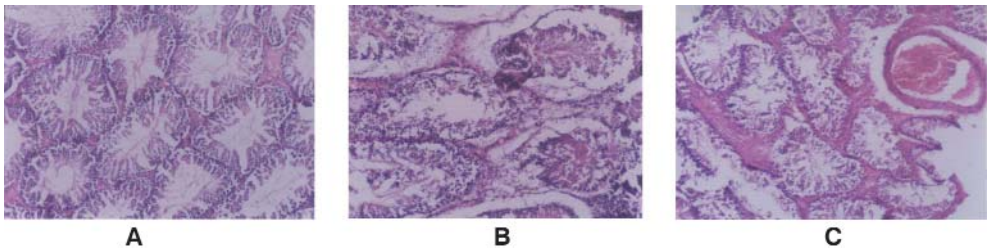

FIG. 3. Seminefrious tubules of group I rats fed on casein: (A) control rat; (B) rat before lead intoxication with interstitial edema; (C) rat after lead intoxication (×800).

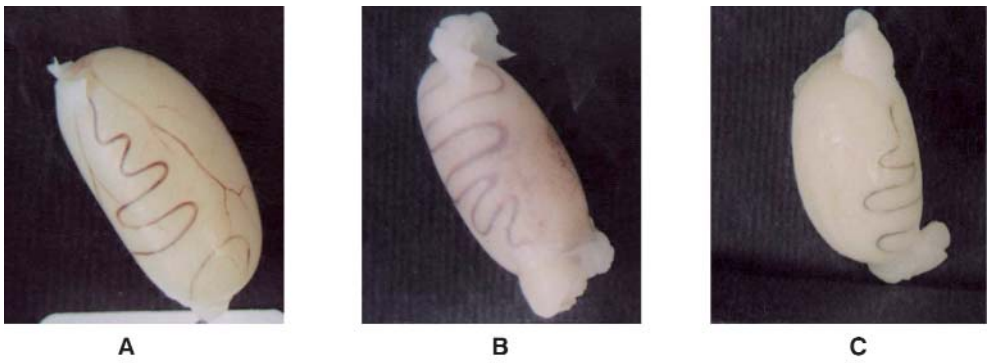

FIG. 4. Testes of group II rats fed on soybean: (A) control rat; (B) rat before lead intoxication with a slight vascular congestion; (C) rat after lead intoxication which appeared as control.

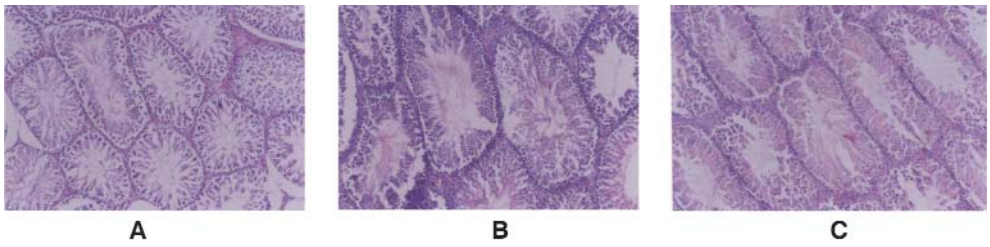

FIG. 5. Seminefrious tubules of group II rats fed on soybean: (A) control rat; (B) rat before lead intoxication with some loss of germinal cells; (C) rat after lead intoxication with a slight damage of germinal cells (×800).

Serum testosterone in rats fed on soybean before lead intoxication (protective group) significantly increased ($p < 0.001$) compared with rats fed on casein before lead intoxication. Also, serum testosterone and testosterone/LH ratio in the protective group significantly increased ($p < 0.05$) compared with the control group.

Five out of nine rats fed on casein before lead intoxication (Group Ib) and three out of nine rats fed on casein after lead intoxication (Group Ic) had a testicular vascular congestion in one pair of testes. Two testes in five rats (Group Ib) had a testicular atrophy in two different rats (Fig. 2). The cross sections of testes in these two groups showed a varying degree of damage of the seminiferous tubules. The interstitial oedema was devoid of mature spermatids (Fig. 3). Six our of nine rats fed on soybean before lead intoxication (Group IIb) and two out of eight rats fed on soybean after lead intoxication had a slight testicular vascular congestion in one pair of testes (Fig. 4). The

cross sections of testes in the Group IIb showed that the seminiferous tubules had lost some of the germinal cells. On the other hand, the cross sections of testes of the Group IIc showed a slight damage of the germinal cells and the organized seminiferous tubules (Fig. 5).

DISCUSSION

Several studies have dealt with the effects of lead exposure on various aspects of health, in particular on the reproductive system and the bones. Often the results from these studies are conflicting and contradictory due, in part, to the use of different animal species (Thoreux-Manlay *et al*., 1995; Wadi & Ahmad, 1999). The severity of lead toxicity appears to be dependent on the age of the animal, the duration of the exposure, the dose administered and the body burden of lead (Pinon-Lataillade *et al*., 1993; Ronis *et al*., 2001).

The consumption of soybean may also have a beneficial effect on the skeletal tissues. A positive effect of soybean was achieved by enhanced bone formation rather than a slowed resorption in ovariectomized rats (Arjmandi *et al*., 1998). Blum *et al*. (2003) have suggested that the mechanism for partial preservation might be different than that known of estrogen including increases in bone formation.

The administration of lead to rats resulted in a marked decrease in body weight of the rats fed on casein or soybean diet. Previous studies have also reported a decline in body weight in animals exposed to high concentrations of lead (Orsi *et al*., 1988; Rasile *et al*., 1995). High lead concentrations have adverse effects on growth and body weight, and influence the behavior via the central nervous system (Hammond & Dietrich, 1990) and the secretion of growth hormones (Huseman *et al*., 1992; Ronis *et al*., 1998).

The lead levels of all groups increased in the order: femur, kidney, liver, testis. Lead accumulation was much greater in the rats fed on casein and soybean before lead intoxication than after lead intoxication except for the bones. Most of the metals became accumulated in the bones and lead concentration in the bones was the highest (Taupeau *et al*., 2001). The high affinity of lead for bony tissues is consistent with bone lead as an indicator of cumulative lead exposure and a marker of chronic lead intoxication (Hu *et al*., 1998). Lead transported to blood was initially absorbed by the soft tissues and deposited in the bones over subsequent years (Donald *et al*., 1986; Taupeau *et al*., 2001).

The levels of femoral calcium and phosphorus in the adult male rats fed on casein or soybean diets after lead intoxication showed a significant decrease when compared with the controls fed on casein or soybean, respectively. Previous studies have shown that lead precipitates with calcium and phosphorus to form lead hydroxyapatite when lead was injected subcutaneously in soft tissues (McClure, 1980). Lead alters the metaphyseal and growth plate morphology of bones in lead exposed animals. Lead can be bound to the growth plate cartilage matrix sites normally associated with calcium and phosphorus (Hamilton & O'Flaherty, 1994).

The femoral BMD in adult rats exposed to lead and fed on casein or soybean diets was lower than that of the controls. This result is in agreement with that by Bagchi & Preuss (2005) who showed that lead accumulation in rats has caused reduction in BMD compared with rats untreated with lead of similar age. It has been suggested that skeletal growth and quality are under regulation by vitamin D metabolism, pituitary growth hormone, insulin-like growth factor 1 (IGF-1) and sex steroids, all of which have been demonstrated to be suppressed by lead exposure (Camoratto *et al*., 1993; Ronis *et al*., 2001).

It was also showed that femoral BMD in male adult rats fed on soybean before lead intoxication showed a significant increase when compared with male adult rats fed on casein before lead intoxication. The improvement of bone strength with dietary soybean was attributed to the efficiency of the intestine to absorb calcium. The conversion of calcium with dietary soybean compared with other proteins may be due to the reduction in the urinary excretion of calcium (Messina & Messina, 2000).

Our histological study showed changes in the seminiferous tubules and loss of germinal cells in the rats fed on casein before and after lead intoxication. These results are similar to those by Batra *et al*. (1998) who showed that the seminiferous tubules were severely damaged after lead administration with sloughing and detachment of the spermatids. The histological damage may also be attributed to the biochemical alternation. In the present study it was shown that in the rats fed on soybean in the protective and curative groups, the damage of the seminiferous tubules became ameliorated compared with the rats fed on casein before and after lead intoxication.

With regard to serum LH, this was reduced by 8.2% and 9.3% in the rats fed on casein before and

after lead intoxication, whereas the levels of serum LH did not change in the rats fed on soybean in the protective and curative groups. Thoreux-Manlay *et al*. (1995) have reported that chronic administration to rats of high doses of lead reduced the level of plasma LH by 32%. On the other hand, a change in the level of LH has never been observed at lower doses of lead exposure (Kempinas *et al*., 1990; Nathan *et al*., 1992; Pinon-Lataillade *et al*., 1993). Sokol *et al*. (2002) found no significant differences in the serum LH and the gonad releasing hormone (GnRH) levels among animals treated with increasing dose of lead acetate for any period of time.

A significant reduction in serum testosterone levels by 83.5% and 36% was observed in the casein fed rats before and after lead intoxication, respectively. In the rats fed on soybean in the protective and curative groups, there was a significant reduction of this hormone by 54% and 21%, respectively. In agreement with this, a large reduction in plasma and testicular testosterone has been observed by about 80% (Thoreux-Manlay *et al*., 1995), contrary to the findings for lower doses of lead exposure (Nathan *et al*., 1992; Pinon-Lataillade *et al*., 1993), except for those by Sokol and co-workers (Sokol *et al*., 1985; Sokol & Berman, 1991).

A high degree to lead exposure can impair testosterone production that could later have a secondary reproductive consequence such as impairment of spermatogenesis, since testosterone is essential for the maintenance of spermatogenesis.

The large reduction in testosterone concentration is not solely due to the decrease of the level of serum LH, as shown by serum testosterone/LH ration. The impairment of testosterone production may be attributed to a decrease in the number of LH binding sites in the Leydig cells, as previously observed in testicular homogenates of lead-exposed rats (Kempinas *et al*., 1990).

From our study, it can be concluded that an appreciable amount of lead reaches the reproductive organs of the male rat groups. Lead accumulation in bones of rats as the main site of deposition was lower in BMD in the rats fed on casein before and after lead intoxication and ameliorated in the rats fed on soybean in the protective and curative groups. Biochemical and histopathological studies revealed that a testicular damage was caused by lead intoxication. Soybean may provide protection from lead in the bones, as indicated by the high femoral bone mass density.

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