J. Food and Dairy Sci., Mansoura Univ., Vol. 10 (4): 79 - 84, 2019

Effectiveness Protection of Soy Flour and Spirulina on Lead Toxicity

Nadra S. Y. Hassan[1]; A. B. Elsayed[2] and Amany A. Salem[3]

[1] **Experimental Kitchen Research Unit, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt**

[2] **Algal Biotechnology Unit, Fertilization Technology Department, National Research Centre, Dokki- Giza, Egypt**

[3] **Department of Special food and nutrition. Food Technology Research Institute, Agricultural Research Center, Giza, Egypt**

ABSTRACT

Lead considered as one of the most risk toxicity of environmental contamination. It causes an increment accumulation in food especially plant food. Also, soy flour is widely used, inexpensive and a highly nutritional source of protein. So, this investigation aimed to study the effect of soy flour (as plant protein source) enriched by spirulina against lead toxicity by using male and female rats. Results: Additional lead acetate in drink water caused a decreasing in hematological tests, blood Fe, Ca and Zn contents. While, spirulina improved status of hemoglobin level and blood Fe content. Whereas, the rats fed on soy flour groups resulted in a high shear force and bone Pb content compared with rats fed on combine with soy flour and Spirulina. Conclusions: Do not depend on plant protein only against lead exposure. Must be combine with animal protein specially milk and milk products.

Keywords: Spirulina platensis, soy flour, lead toxicity, rat,

INTRODUCTION

The most toxic of heavy metals is lead and it has a wide distribution for its great usefulness. Several studies have linked health problems with high concentrations of industrial minerals, elements from contaminated rivers, and carelessness of the public service of water (Atibu, 2013). Lead is heavy metal which has toxic effect for living beings even in a very low amount, electrical power sources and is used frequently in dye, plastic and fertilizer industry (Jensen *et al*., 2001). Lead (Pb) causes damage to the erythrocyte membrane resulting in hemolysis or a decrease of blood Fe level which might be decrease concentration of hemoglobin and hematocrit value (Ancheva *et al.*, 2003).

Lead is absorbed through digestive and respiratory tracts, and skin. After absorption into the blood, about 99% of lead is bound to erythrocytes and the remaining 1 percentage stay in plasma to be carried to other tissues (Howard *et al*, 1973). Lead has many interferes with a variety of body processes and has toxic to the body systems including cardiovascular, reproductive, hematopoietic, gastrointestinal and nervous systems, renal functions and release of glutamate [(Patocka and Cerny, 2003), (Kosnett, 2006) and (Xu *et al.*, 2006)].

Changes in the composition of red blood cell (RBCs) membrane proteins and lipids, which inhibit haemoglobine synthesis, insufficient erythrocyte production, and reduce red cell, survive (Ancheva *et al.*, 2003).

Spirulina familiar for 3.5 billion years in the earth as the oldest life formula is a type of cyanobacterium. Spirulina (blue-green algae) is consumed, as human food because of it is high protein and nutritive value. Beside high contents of vitamins, minerals, essential fatty acids and antioxidant pigment such as carotenoids and antioxidant enzymes (Premkumar *et al.*, 2001).

Spirulina is wide-ranging biological activities and ant oxidative, anti-inflammatory, anti- mutagenic, antiviral, cardio protective, anticancer properties (Mohan, *et al*., 2006) and immune enhancing (Simsek, *et al*., 2007). The additional of Spirulina caused an increasing the hemoglobin concentration, red blood cells (RBC) and white blood cell (WBC) counts, and erythropoiesis during chemotherapy (Zhang *et al.*, 2001).

According to, (Hodis *et al.*, 2011) who reported that soy flour (SF) supplementation as a primary preventive therapy remains unexplored. The same authors mentored that isoflavone soy flour (ISF) also reduced subclinical atherosclerosis assessed as carotid artery intima-media thickness progression. Health benefits of soy may be attributed to isoflavone content. For example genistein, the major isoflavone in soy has been shown to improve cardiovascular health by modulating lipid profile and improving endothelial functions in postmenopausal women (Squadrito, *et al*., 2003).

The aim of this study was investigated the protection effects of soy flour and spirulina on hematological tests and some bone mineral contents induced lead toxicity in rats

MATERIALS AND METHODS

Materials:-

Soybean flour was obtained from Food Technology Research Institute, Agricultural Research. Spirulina was obtained from Algal Biotechnology unit –National Research Center. Sugar, and corn oil were obtained from a local market in Giza, Egypt. Casein (Protein ≥85%), cellules, salt mixture, vitamins mixture and choline chloride, nitric acid and peroxide hydrogen were punched from El-Gomhoira, Cairo, Egypt. Kits which were used to determine plasma minerals were punched from Gamma-Tread Company, Cairo Egypt.

Methods

Biological study:

Sixty adult 30 animals Male and 30 Female Sprague –Dawley rats aged 8 weeks old (110±5g) were purchased from the laboratory Animal Department, Food Technology Research Institute, Agricultural. Research center Giza, Egypt. Animals were housed in plastic cages and fed on basal died as observed by (Reeve *et al*, 1993) till weighted 162.00±3 g for male and 142.00±3 g for female). Then, modified percent of protein to (20%) as according by (Barltrop and Khoo, 1975). Also, provided water *ad libitum* for one week as adaptation period. The animal room Temperature was maintained at 21 C ±2°C with lighting 12 h and relative air humidity from 40 % to 60. Lead (pb) groups (G2 to G5) received experimental diets and lead acetate (2 g/L) dissolved in water.

Experimental Design

After the adaptation period a week the rats were randomly divided into 5 groups and 5 subgroups (A for male and B for female) each subgroup contains 6 rats individual as a follows;

Group (1): Rats fed on basal diet and drink tap water (as negative control).

Group (2): Rats fed on basal diet and lead acetate (2 g/L) dissolved in water (as positive control).

Group (3): Rats fed on basal diet with spirulina (0.3%) of diet and lead acetate (2 g/L) dissolved in water.

Group (4): Rats fed on soybean flour and lead acetate (2 g/L) dissolved in water.

Group (5): Rats fed on soybean flour with spirulina (0.3%) of diet and lead acetate (2 g/L) dissolved in water. The experimental diets as a shown in Table (1).

Table 1. Different ingredients used in experimental diets (g/100g)

Ingredients	G1	G2*	G3*	G4*	G5*
Corn starch	46.29	46.29	46.29	30.8	30.5
Casein	23.5	23.5	23.5	--	--
Sugar	10	10	10	10	10
Corn oil	10	10	10	7.33	7.33
Salt Mix	4	4	4	4	4
Vitamin mix	1	1	1	1	1
Choline chloride	0.2	0.2	0.2	0.2	0.2
Cellules	5	5	5	5	5
Spirulina	--	--	0.3	--	0.3
Soybean flour**	--	--	--	41.67	41.67

*** Protein as 20% of diet **Drink water contained lead acetate (2g/L)**
***** DM of soybean flour was (48g/100g weight)**

Blood Samples

The body weight was recorded weekly. Blood samples were collected after 30 and 60 days of experimental period. At the 60th day, the animals were weighted and anaesthetized by diethyl ether. Blood samples were collected from eye plexuses into two heparinized tube to obtain the whole blood, one of them subjected to measure the hematological profile immediately and the other subjected to analysis some blood minerals as described by (Yang *et al*., 2006). The carcasses were dissected and bones were cut off, washed, weighed and determined some mineral contents.

Biochemical Assay:

Blood hemoglobin (g/dl), hematocrit value (%), red blood cells (x106/µl), white blood cells (x103/µl) and differential white cell count (x103/µl) platelets (x103/µl), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration, (MCHC) were determined in whole blood by Coulter counter M4 (30) system according to the method of (Moser *et al*., 2001).

Blood minerals

Whole blood samples (0.5ml) was digested by nitric acid and hydrogen peroxide. Then dilution to 10 ml by di-ons-water to measurement mineral contents Pb, Fe, Zn, and Ca.

Bone analysis

Bone minerals (pb, Ca, Fe, P and Zn) were determined according to the methods outline in (AOAC, 2000). Bone length and thickness of each femur were measured using a venier Caliper according to the method of (Arjmand *et al*, 1996). Shear force was measured as according by (Bourne, 2002); the probe speed was 1 mm s-1. The values are expressed Newton (N).

Statistical Analysis:

Statistical analyses were using SPSS Program (version 19). Data were expressed as means± SEM and the Statistical analysis was per formed using one-way analysis of variance followed by Duncan's tests (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

This study reveals that, soy flour as plant protein with Spirulina showed a protective effect against lead acetate contamination. The decreasing in lymphocyte, RBC and WBC counts, MCHC, HGB and PLT were observed after at 30 and 60 ($p<0.05$) for positive controls in both male and female. Results in Tables (2 and 3) showed that, the effectiveness of soy flour enriched spirulina on blood picture for male and female adult rats which feeding for 60 days. Generally, lead acetate was added with the concentration of (2g/L) in drinking water caused to decrease for all hematological tests. In additional of Spirulina, amounted with (0.3%) in group (3) caused an increase in hemoglobin level in compared to positive control group. While, the replacement of casein by soy flour decreased the hemoglobin level in group (4). Moreover, the mixture between soy flour and spirulina caused decreases in the hemoglobin. These results were showed in both male and female rats. Results showed also a highly significant differences between with negative and positive control groups at ($p<0.05$).

Spirulina platensis has a positive effect on the metabolism of iron and hemoglobin in rats that they were absorbed with a high level of heavy metals such as Pb, Ca and Hg (Chojnacka *et al.,* 2005). According (Jeyaprakash and Chinnaswamy, 2005) reported that, supplementation by *Spirulina platensis* could exert a protective effect against Cd and Pb induced destruction of RBCs. Also, in the same study, *Spirulina platensis* administration decreased the toxic effects of Cd and Pb on the hematological values and has a protective role for anemia. Previous studies have already suggested that, supplementation by spirulina weight by useful in adjuvant of anemia caused by lead toxicities (Simsek *et al.,* 2009). And, (Rahman *et al.,* 2012) reported that, treatment with Spirulina was effective in lead – induced toxicity and restore altered values of hematological and biochemical parameters. Moreover, interaction of lead with hemi biosynthesis has been related to inhibition of cytoplasmic and mitochondrial enzymes (ATSDR, 1993) and the decreasing in the activity of the main enzymes in heme biosynthesis due to defects in iron metabolism as said by Calderan-Salinas *et al.,* (1993).

Concerning to white blood cells (WBCs), this study resulted in an increasing amount of white blood cells (WBCs). This increasing is mainly due to an increase in monocyte counts. This result with the line of (Mugahi *et al.,* 2003) who found that, total leukocyte count had increased, and might be due to an increase also in neutrophil and monocyte counts. Yagminas *et al.,* (1990) who reported that leukocytosis due to that lead-induced inflammation. The present study showed that rats which fed on soy flour resulted in decrease in monocytes cells in compared to negative controls. This result is agreement parcel with Soung *et al.,* (2006) who reported that, soy intake with the amount of (25g/day) for a year caused decrease in monocytes for female.

Table 2. Effect of lead acetate (2g/L) and soy flour on hematological determinants for male rats.

Groups	RBCs ($X10^6/\mu l$)	HGB (g/dl)	HCT (%)	MCH (pg)	MCHC (g/dl)	MCV (fl)	PLT ($X10^3/\mu l$)	WBCs ($X10^3/\mu l$)	Differential white cell count ($X10^3/\mu l$) Lymphocyte	Monocytes	Segment
					Blood picture after 30 days						
G1	6.08^{ab}	13.33^{a}	39.53^{a}	21.93^{a}	33.66^{a}	65.03^{a}	714.66^{ab}	12.43^{a}	8.93^{a}	1.50^{a}	2.00^{a}
G2	5.65^{b}	12.23^{ab}	36.80^{ab}	21.66^{a}	33.23^{a}	65.16^{a}	609.66^{b}	12.82^{a}	9.94^{a}	1.50^{a}	1.82^{a}
G3	7.04^{a}	14.16^{a}	38.86^{a}	20.06^{a}	33.86^{a}	59.23^{b}	556.66^{b}	10.40^{a}	6.59^{a}	1.15^{a}	2.65^{a}
G4	6.83^{a}	11.06^{b}	38.00^{ab}	16.46^{b}	30.23^{b}	54.43^{c}	672.66^{ab}	11.87^{a}	8.22^{a}	1.43^{a}	2.21^{a}
G5	6.67^{a}	10.76^{b}	35.50^{b}	15.73^{b}	30.26^{b}	51.90^{c}	889.00^{a}	7.48^{a}	5.64^{a}	0.16^{b}	0.03^{b}
					Blood picture after 60 days						
G1	6.51^{a}	14.00^{a}	44.00^{a}	28.00^{a}	33.00^{a}	83.66^{a}	763.33^{a}	12.51^{b}	8.13^{b}	0.94^{a}	1.88^{c}
G2	4.51^{b}	12.56^{a}	37.33^{b}	27.33^{a}	32.33^{a}	82.33^{a}	591.60^{b}	10.48^{c}	7.40^{c}	0.78^{a}	3.21^{a}
G3	4.70^{b}	14.10^{a}	33.33^{c}	27.33^{a}	32.66^{a}	84.00^{a}	515.67^{c}	9.53^{d}	5.40^{e}	0.51^{a}	1.81^{b}
G4	4.11^{b}	11.73^{a}	33.61^{c}	27.00^{a}	32.33^{a}	81.33^{a}	369.00^{e}	14.93^{a}	8.80^{a}	0.80^{a}	3.14^{b}
G5	4.10^{b}	11.63^{a}	30.67^{c}	26.00^{a}	31.66^{a}	81.00^{a}	464.66^{d}	9.74^{d}	6.13^{d}	0.36^{a}	2.67^{a}

*** Each value in a column followed by the same letter is not significantly different at ($p \geq 0.05$)**

**** Control (-) G1; Control (+) G2; Control (+) plus 0.3% spirulina G3; Soy flour G4; Soy flour plus 0.3% spirulina G5**

Table 3. Effect of lead acetate (2g/L) and soy flour on hematological determinants for female rats

Groups	RBCs ($X10^6/\mu l$)	HGB (g/dl)	HCT (%)	MCH (pg)	MCHC (g/dl)	MCV (fl)	PLT ($X10^3/\mu l$)	WBCs ($X10^3/\mu l$)	Differential white cell count($X10^3/\mu l$) Lymphocyte	Monocytes	Segment
					Blood picture after 30 days						
G1	5.81ab	13.20^{b}	37.86^{b}	22.73^{b}	34.86^{c}	65.20^{c}	620.33^{ab}	14.51^{a}	13.67^{a}	1.93^{a}	0.73^{a}
G2	6.17^{bc}	12.36^{b}	35.76^{b}	22.20^{b}	32.56^{b}	61.36^{b}	532.66^{ab}	11.35^{ab}	10.59^{a}	1.23^{a}	3.20^{b}
G3	5.49^{a}	12.56^{b}	36.73^{b}	20.80^{b}	34.20^{c}	66.96^{c}	501.00^{a}	11.69^{ab}	8.44^{a}	1.03^{a}	2.21^{ab}
G4	6.59^{c}	10.70^{a}	35.86^{ab}	16.20^{a}	30.46^{a}	53.26^{a}	653.66^{ab}	11.42^{ab}	8.76^{a}	0.93^{a}	1.71^{ab}
G5	6.26^{bc}	9.66^{a}	32.86^{a}	15.46^{a}	29.46^{a}	52.53^{a}	666.00^{b}	9.33^{b}	7.81^{a}	1.19^{a}	1.66^{ab}
					Blood picture after 60 days						
G1	5.81^{b}	13.20^{b}	37.86^{b}	22.73^{a}	34.86^{b}	65.20^{a}	620.33^{a}	14.67^{a}	12.77^{a}	0.67^{a}	2.32^{b}
G2	5.85^{b}	10.81^{a}	46.66^{c}	28.66^{d}	32.00^{a}	83.33^{cd}	445.00^{b}	10.59^{b}	7.05^{c}	0.83^{b}	2.87^{a}
G3	5.73^{b}	14.70^{b}	47.66^{c}	28.00^{cd}	33.00^{ab}	85.00^{d}	376.00^{c}	10.66^{b}	7.48^{b}	0.78^{a}	2.31^{b}
G4	3.92^{a}	11.20^{a}	33.66^{a}	26.33^{bc}	31.66^{a}	81.00^{ab}	382.00^{c}	8.60^{c}	7.32^{bc}	0.63^{a}	1.90^{ab}
G5	3.50^{a}	10.00^{a}	33.66^{a}	25.00^{b}	31.00^{a}	80.00^{b}	426.66^{b}	8.50^{c}	6.30^{d}	0.37^{b}	2.35^{a}

*** Each value in a column followed by the same letter is not significantly different at ($p \geq 0.05$)**

**** Control (-) G1; Control (+) G2; Control (+) plus 0.3% spirulina G3; Soy flour G4; Soy flour plus 0.3% spirulina G5**

Some of blood minerals are presented in Table (4) after 30 and 60 days of experimental period. Generally, Fe, Zn and Pb contents on 60th days were lower than the other after 30th days for both male and female. While, blood Ca was increased for male in compared with female rat groups. The female rats were higher decrease than male. The male rats in group (3) which fed on spirulina with the amount of (0.3%) only to basal diet caused an increasing of Ca, Fe and Zn contents after 60 days both male and female in negative control groups. While, the addition of Spirulina with (0.3%) to soy flour as replacement of casein caused decrease in blood Fe after 60 days of feeding (46.09%) in compared with 30 days of feeding. Meanwhile, the female rats in lead groups were decrease in blood Ca, Fe, Zn and Pb levels. The female rats which fed on soy flour and drinking tap water contained lead acetate with the concentration of (2g/L) had a high decrease with 52.35 and 73.33% in compared to group which fed on soy flour mixture with Spirulina (0.3%) in blood Fe and Zn.

Concerning to blood Pb, Table (4) mentioned in, effect of drinking water which contained lead acetate amounted with (2g/L) and replaced casein by soy flour as source of plant protein on blood Pb. Generally, both male and female rats in negative control groups had the lowest level of blood Pb. Moreover, the results also showed that, blood Pb contents after 60 days was lower than those of male rats after 30 days. These results may be due to accumulate of lead in the bone. While, male rats in groups (4 and 5) fed on soy flour had high levels of blood Pb.

It could be seen from the above results, that male rats which fed on soy flour with spirulina at (0.3%) had more decreasing in blood Pb level than those of male rats fed on soy flour only.

The female rats had a different result as shown in Table (4). The female rats in negative control had the similar results of blood Pb (0.28 ppm). The female rats in G2 and G3 had decrease in blood Pb level after 60 days by (0.79 and 0.27, respectively). Meanwhile, the female rats in groups (G 4 and G5) fed on soy flour enriched spirulina had an increase amount in blood Pb level after 60 days of feeding period by (0.41 and 0.43, respectively).

Lead toxicity is due to its binding with sulfur groups on important protein specially enzymes, hormones and cell receptor; displace some minerals (calcium, zinc, selenium, magnesium and copper) from the body and decreased metabolism of carbohydrate, protein and lipids (ATSDR, 1993).

The present study illustrated that blood Ca and Fe were a significantly increased at ($p<0.05$) in lead groups (3, 4 and 5) compared to negative controls. These results are agreement with (Taha *et al.*, 2013) who found that, Ca and Fe concentrations were significantly increased by lead exposure. Moreover, amount of Ca were increase in rats, which administered with lead acetate in drinking water for 60 days. This might be due to impairment of renal function or inhibitory action of lead on cation transport in tissues (Missoun *et al.,* 2010). In addition, lead has direct effect on osteoblast function including inhibition of active vitamin D_3 stimulated synthesis of osteocalcin (Ronis, *et al* 2001). Also, effect of lead on local regulation of bone cell function via interference with Ca homeostasis and Ca regulated secondary messenger system via disruption of CAMP signals (Pounds *et al.,* 1991).

The increasing of blood Ca and Fe were showed after 30 and 60 days of experimental period. While, the rats in soy flour groups both male and female showed a significantly decrease in whole blood Fe content on 60 days of experimental period (4 and 5). The increasing in Fe levels may be due to progressive destruction of RBCs and increasing fragility of RBCs. This mainly that effects of lead toxicity on the hematopoietic system through restraining the synthesis of hemoglobin by inhibiting various key enzymes involved in the heme synthesis pathway and it reduces the life span of circulating erythrocytes by increasing the fragility of cell membranes (Guidotti *et al.,* 2008). Whereas, our data showed a significantly decreased in HGB, RBCs and whole blood Fe in soy flour groups (both male and female) on 60^{th} days. These results might be due to lead poison which associate the anemia status was occurred. Perhaps as a result of lead induced inhibition of the hem biosynthetic pathway. Although, only 1-3% of lead exists in plasma, it can transfer between blood and mineralizing tissues, so it may be more biologically significant as reported by (Jin *et al.,* 2008).

Table 4. Effect of lead acetate (2g/L) and soy flour on some blood mineral contents (mg/dl) in male and female during 60 days of feeding.

Blood mineral / Groups	Ca	Fe	Zn	Pb (ppm)	Ca	Fe	Zn	Pb (ppm)
	After30 days				After 60 days			
Male								
G1	68.66^{b}	3.33^{c}	0.22^{c}	0.15^{d}	81.33^{a}	6.60^{ab}	0.28^{a}	0.02^{c}
G2	73.03^{ab}	7.80^{ab}	0.39^{b}	0.44^{c}	84.29^{a}	7.59^{a}	0.12^{a}	0.07^{c}
G3	70.78^{b}	5.33^{bc}	0.38^{b}	0.51^{c}	83.29^{a}	6.27^{ab}	0.13^{a}	0.12^{b}
G4	75.91^{ab}	9.17^{a}	0.46^{b}	0.79^{b}	83.79^{a}	5.20^{bc}	0.28^{a}	0.71^{a}
G5	78.33^{a}	7.16^{ab}	0.99^{a}	0.92^{a}	82.93^{a}	3.86^{c}	0.13^{a}	0.35^{ab}
Female								
G1	43.62^{c}	5.64^{c}	0.59^{b}	0.28^{c}	49.62^{a}	7.30^{a}	0.62^{a}	0.28^{c}
G2	67.05^{b}	9.07^{b}	0.83^{ab}	1.02^{a}	51.27^{a}	5.73^{c}	0.34^{b}	0.79^{a}
G3	73.59^{b}	11.05^{a}	0.94^{a}	1.07^{a}	51.64^{a}	6.66^{b}	0.13^{c}	0.27^{c}
G4	74.02^{b}	9.38^{b}	0.90^{a}	0.33^{b}	51.48^{a}	4.47^{d}	0.14^{c}	0.41^{b}
G5	91.29^{a}	9.35^{b}	0.82^{ab}	0.41^{b}	51.44^{a}	4.75^{d}	0.22^{c}	0.43^{b}

***Each column followed by the same letter is not significantly different at (*p* ≥0.05)**

**** Control (-) G1; Control (+) G2; Control (+) plus 0.3% spirulina G3; Soy flour G4; Soy flour plus 0.3% spirulina G5**

Concerning blood Zn, our results showed increase in blood Zn after 30 days of the experimental period. While, blood Zn was decreased on 60^{th} days of feeding. Simons *et al.,* (1995) reported that, the Pb could be binding about 20 times higher than Zn. On the other hand, the decrease of Zn concentration after lead exposure might be due to the imbalance of metabolism produced by enzyme, which is necessary for many metabolic processes (Ibrahim *et al.,* 2012). In addition, Pb poisoning can decrease the absorption rate and biologic availability of Zn in the body, mainly because of their competition for binding to the sulfhydryl (-SH) group site (S) in different enzymes and tissues as reported by (Telisman, 1995). Moreover, Krajčovičová-Kudláčková *et al*. (2006) who found that, a high significantly cadmium concentration (as heavy metal) in blood which was measure in vegetarian group when compared to non-vegetarian group. The highest cadmium content was found in vegan subgroup which depends on plant only and that value decreased with increasing animal food consumption (which added dairy products and egg or fish and poultry). Cadmium risk for who consumes the significantly high amounts of whole grain products, grain sprouts and oil seeds.

Results in Table (5) showed that, the effect of lead acetate (2g/L) in drinking and fed on soy flour on bone measurements for male and female adult rats. All groups fed on soy flour without spirulina showed decrease of weight, height and thickness of femur bone compared to control groups. While, the same group showed increase of bone shear force. Using of soy flour without spirulina (0.3%) in group (5) was improved shear force in compared with using of soy flour with Spirulina (0.3%) in group (4) and negative controls, too.

The present study showed that, there were a significantly decrease in bone weight, length and thickness for soy flour animal groups. But, no significant changes were observed in length in negative and positive control groups. This result is in the line with (Bhardwaj and Rai, 2016).

Table 5. Effect of lead acetate (2g/L) and soy flour on femur bone measurements after 60 days in both of male and female rats.

Bone mesermuts / Groups	Weight (g)	Length (cm)	Thickness (cm)	Shear force (N)	Weight (g)	Length (cm)	Thickness (cm)	Shear force (N)
	Male				Female			
G1	0.52^{a}	3.36^{a}	0.39^{a}	10.64^{b}	0.40^{a}	3.40^{a}	0.40^{a}	9.93^{b}
G2	0.48^{a}	3.43^{a}	0.39^{a}	9.78^{b}	0.32^{b}	3.00^{bc}	0.28^{b}	8.67^{b}
G3	0.47^{a}	3.40^{a}	0.32^{bc}	12.09^{ab}	0.39^{a}	3.13^{ab}	0.30^{b}	10.82^{ab}
G4	0.28^{b}	3.03^{b}	0.33^{b}	13.95^{a}	0.28^{bc}	2.76^{c}	0.31^{b}	12.81^{a}
G5	0.27^{b}	2.93^{b}	0.30^{c}	11.78^{ab}	0.23^{c}	2.90^{bc}	0.29^{b}	9.62^{b}

***Each column followed by the same letter is not significantly different at (*p* ≥0.05)**

**** Control (-) G1; Control (+) G2; Control (+) plus 0.3% spirulina G3; Soy flour G4; Soy flour plus 0.3% spirulina G5**

Concerning of femur bone mineral contents Table (6). Results showed that a differences between male and female for bone minerals contents. Positive control groups which treaded by lead acetate at (2g/L) in drinking had decrease in (Ca, P, Fe and Zn) and increase in bone Pb. The male rats fed on soy flour without spirulina (G5) were increase in bone Ca, P, Fe and Zn contents. Using soy flour as diet protein caused an increases in bone Pb. But additional spirulina (0.3%) decreased of accumulate of Pb. The results in the rats in positive control which drinking water contained lead acetate at the amount of (2g/L) had decreased in bone Ca content. The decreasing in Ca concentration may be due to the increase bone resorption (Bhardwaj and Rai, 2016). According, (Ronis *et al.,* 2001) who reported that, the impairment of bone Ca due to the inhibitory action of lead on the osteoblast cells and Vitamin D synthesis for osteocalcin. Moreover, lead is reduce the maleic acid level and the activity of alkaline phosphatase that has been considered as biomarkers of osteoblast’s function (Bhardwaj *et al.,* 2009).

The rats in soy flour groups (G4 and G5; both male and female) had a high level of bone's Pb. These results are agreed with (Rader *et al.,* 1981) who found the femur lead was higher lead level in rats gave daily lead acetate in water than rats in negative control.

Table 6. Effect of lead acetate (2g/L) and soy flour on some femur bone minerals (mg/100g) after 60 days.

Bone mineral	Ca	P	Fe	Zn	Pb	Ca	P	Fe	Zn	Pb
Groups	Male					Female				
G1	65.04^{b}	23.51^{c}	1.11^{c}	2.51^{d}	0.85^{a}	84.68^{b}	41.65^{a}	3.05^{b}	4.62^{b}	1.74^{e}
G2	58.79^{c}	19.19^{d}	0.93^{e}	2.90^{c}	1.37^{b}	72.61^{d}	34.35^{c}	1.65^{e}	3.36^{c}	1.85^{d}
G3	63.12^{b}	19.38^{d}	1.01^{d}	2.25^{e}	1.51^{c}	98.09^{a}	30.70^{d}	4.30^{a}	2.76^{e}	3.98^{c}
G4	70.73^{a}	37.60^{b}	4.82^{a}	3.53^{a}	12.23^{e}	70.50^{e}	32.38^{d}	2.45^{d}	2.90^{d}	12.66^{b}
G5	49.06^{d}	54.49^{a}	4.43^{b}	3.32^{b}	9.50^{d}	78.27^{c}	36.68^{b}	2.70^{c}	4.66^{a}	14.34^{a}

***Each column followed by the same letter is not significantly different at ($p \geq 0.05$)**

**** Control (-) G1; Control (+) G2; Control (+) plus 0.3% spirulina G3; Soy flour G4; Soy flour plus 0.3% spirulina G5**

CONCLUSION

In conclusion our results revealed that could not use plant protein alone against lead toxicity. A high significantly lead concentration in blood which was soy group group when compared to casein group. So, this study concluded must use combination between animal and plant such as spirulina, milk and milk products.

REFERENCES

Agency for toxic substances and disease Registry (ATSDR); 1993. Toxicological Profile for Lead. Update. Prepared by Clement International Corporation under contact No. 205-88-0608 for ATSDR, U.S. Public Health Service, Atlanta, GA.

Ancheva, MT; Metcheva, R and Teodorova, S (2003). Bioaccumulation and damaging action of polymetal industrial dust on laboratory mice *Mus musculus alba.* II. Genetic, cell, and metabolic disturbances, Environ. Res. 92; 152–160.

A.O.A.C. (2000). Official Methods of Analysis of the Association of the Analytical Chemists. 17th ed published by the Association of Official Analytical Chemists. Po Box 540. Benjamin Franklin Station Washington DC. 20044.

Arjmand, BH; Alekel, L; Hollis, BW; Amin, D; Sapuntialcis, AS; Guo, P and Pukereja, SC (1996): Dietary soybean prevents bone loss in an ovariectomzed not model of osteoporosis. J. Nut. 126: 161-167.

Atibu, K.; Devarajan, N. and Thevenon, F. (2013). Concentration of metals in surface water and sediment of Luilu and Musonoie Rivers, Kolwezi-Katanga, Democratic Republic of Congo. Applied Geochemistry, 39, pp. 26–32.

Barltrop, D and Khoo, HE (1975). The influence of nutritional factor on lead absorption. Postgradute Med. J. 51(601): 795 – 800.

Bhardwaj, P and Rai, RV (2016). Effect of lead toxicity on bone calcium content and morphometric parameters. Int. J Res. Med. Sci.; 4(1): 177-180.

Bhardwaj, P; Kaur, H and Rai, DV (2009). New insight into the effects of lead modulation on antioxidant defense mechanism and trace element concentration in rat bone. Interdisciplinary Toxicology; 2(1):18-23.

Bourne, M. C. (2002). Food texture and viscosity. Concept and measurement, 2nd Ed. Academic Press, London. Pp. 427.

Calderon-Salinas, V; Hernandez-Luna, C; Maldonado, M and Saenz, D (1993). Mechanisms of the toxic effects of lead. I. Free lead in erythrocytes. J. Expo. Anal. Environ. Epidemiol.; 3 (suppl. 1), 153 - 64.

Chojnacka, K; Chojnacki, A and Gorecka, H (2005). Bio sorption of $Cr^{3+,}$ Cd^{2+} and Cu^{2+} ions by blue-green algae Spirulina sp.: kinetics, equilibrium and the mechanism of the process, Chemosphere 59; 75–84.

Guidotti, TL; McNamara, J and Moses, MS (2008). The interpretation of trace element analysis in body fluids. Indian J Med Res.; 128: 524 – 532.

Hodis, N.; Wendy M and Mack, J. (2011). Isoflavone Soy flour Supplementation and Atherosclerosis Progression in Healthy Postmenopausal Women. *Stroke*.; 42:3168 - 3175.

Howard, D., Stowe, D.V.M., Goyer, R.A., Krigman, M. (1973). Experimental oral lead toxicity in young dogs. Arch. Pathol. 95: 106-116.

Ibrahim, NM; Eweis, EA; El-Beltagi, H and Abdel – Mobdy, TE (2012). Effect of lead acetate toxicity on experimental male albino rats. Asian Pacific Journal of Tropical Biomedicine; 41-46.

Jensen, GS; Ginsberg, DI and Drapeau, C (2001). Blue-green algae as an immuno-enhancer and bio modulator. J. Am. Nutraceutical. Assoc. 3; 24–30.

Jeyaprakash, K and Chinnaswamy, P (2005). Effect of Spirulina and Liv-52 on cadmium induced toxicity in albino rats, Indian J. Exp. Biol. 43; 773–781.

Jin, C; Li, Y; Li, YL; Zou, Y; Zhang, GL; Normura, M and Zhu, GY (2008). Blood lead: Its effect on trace element levels and iron structure in hemoglobin. NIMB Beam Interactions with Materials & Atoms 266; 3607–3613.

Kosnett (2006). Global approach to reducing Lead exposure and poisoning. *Mutation Research.* 659(1-2): 166-175.

Krajčovičová-Kudláčková, M; Ursínyová, M; Mašánová, V; Béderová, A and Valachovičová, M (2006). Cadmium blood concentrations in relation to nutrition. Cent. Eur. J. Publ. Health; 14 (3): 126-129.

Missoun, F; Slimani, M; and Aoues, A (2010). Toxic effect of lead on kidney function in rat Wister. Afric. J of Biochem. Res., 4(2):21-27.

Mohan, I.; Khan, M. ; Shobha, J.; Naidu, M. ; Prayag, A. ; Kuppusamy, P. and Kutala, V. (2006). Protection against cisplatin-induced nephrotoxicity by Spirulina in rats, Cancer Chemother. Pharm. 58: 802–808.

Moser, K; Seelenbinder, F.; McFadden, S.; Adkins, C.; Goshay, M. and Davis, F. (2001). Selecting a new analyzer for the hematology Laboratory. The experience at Ohio Health Hospital. In: Laboratory Hematology: 245-254.

Mugahi, MHN; Heideri, Z; Sagheb, HM and Barbarestani, M (2003). Effect of chronic lead acetate intoxication on blood indices of male adult rat. DARU; 11(4): 147 – 151.

Patocka J,and Cerný K. (2003). Inorganic lead toxicology. *Acta Medica (Hradec Kralove).* 46(2):65-72.

Pounds, JG; Long, GJ and Rosen, JF (1991). Cellular and Molecular toxicity of lead in bone. Environ. Health. Erspect. 91:17-32.

Premkumar, K.: Pachiappan, S: Abraham, S: Santhiya, P.: Gopinath and Ramesh, A (2001). Effect of spirulina fusiformis on cyclophosphamide and mitomycin-C induced genotoxicity and oxidative stress in mice. Fitoterapia. 72:906-911.

Rader, JI; Peeler, JT and Mahaffey, KR (1981). Comparative toxicity and tissue distribution of lead acetate in weaning and adult rats. Envi. Heal. Perspective 42: 187 – 195.

Rahman, MA; Moitry, NF; Alam, M; Yasmin, Z; Debnath, D and Mostofa, M (2012). Effects of Spirulina in Lead Induced Toxicities in Long Evans Rats. J. Environ. Sci. & Natural Resources, 5(1): 79 – 82.

Reeves, PG; Nielen, FH and Fahey GC. (1993). AI-93 purified diets for laboratony rodents: final report of the American Institute of nutrition ADHOC writing Committee on the formulation of the AIN-76a rodent diet. J. Nutr., 123(12); 1939 – 1950.

Ronis, MJJ; Aronson, J; Gao, GG; Hogue, W; Skinner, RA; Badger, TM and Lumpkin, CK (2001). Skeletal effect of developmental lead exposure in rats. Toxicol. Sci. 62:321-329.

Simons, TJB (1995). The affinity of human erythrocyte porphobilinogen synthase for Zn^{2+} and Pb^{2+}, Eur. J. Biochem. 234; 178.

Simsek, N; Karadeniz, A; Kalkan, Y; Keles, ON and Unal, B (2009). *Spirulina platensis* feeding inhibited the anemia- and leucopenia-induced lead and cadmium in rats. Journal of Hazardous Materials 164; 1304–1309.

Snedecor GW and Cochran WG (1989). Statistical Methods. The Lowe State University Press. Ames, Lowe.

Soung, Do Y; Patade, A; Khalil; DA; Lucas, EA; Devareddy, L; Greaves, KA and Arjmandi, BH (2006). Soy flour supplementation does not cause limphocytopenia in postmenpausal women. Nutri. J.; 5(12).

Squadrito F, Altavilla D, Crisafulli A, Saitta A, Cucinotta D, Morabito N, D'Anna R, Corrado F, Ruggeri P, Frisina N and Squadrito G (2003). Effect of genistein on endothelial function in postmenopausal women: a randomized, double-blind, controlled study. *Am J Med* 114:470 - 476.

Taha, N; Korshom, M; Mandour, A; Lebdah, M and Aladham, E (2013). Effect of lead toxicity on mineral metabolism and immunological factors in rats. Alexandria Journal of Veterinary Sciences, 39: 64-73.

Telisman, S (1995). Interactions of essential and/or toxic metals and metalloids regarding inter individual differences in susceptibility to various toxicants and chronic diseases in man. Arh. Hig. Rada. Toksiko, l46: 459-476.

Xu HH, Chen ZP and Shen Y (2006). Meta analysis for effect of lead on male reproductive function. *J of Industrial hygiene and occupational disease*. 24(10): 634-36.

Yagminas, A.P., Franklin, C.A., Villeneuve, D.C., Gilman, A.P., Little, P.B., Valli, V.E. (1990). Subchronic oral toxicity of triethyl lead in the male weanling rat. Clinical, biochemical, hematological, and histopathological effects. Fundam. Appl. Toxicol. 15: 580-596

Yang H; Chen S; Chang N; Chang J; Lee M; Tasi P; Fu H; Kao W; Chiang H; Wang H and Hseu Y (2006). Protection from oxidative damage using bidens pilosa extracts in normal human erythrocytes. Food Chem. Toxicol. 44: 1513 – 1521.

Zhang, H.; Lin, A.; Sun, Y. and Deng, Y. (2001). Chemo- and radio-protective effects of polysaccharide of *Spirulina platensis* on hemopoietic system of mice and dogs, Acta. Pharmocol. Sin. 22: 1121–1124.

الحماية الفعالة لدقيق الصويا وطحلب الأسبيرولينا على سمية خلات الرصاص

نادرة سيد يوسف حسن[1]، أبو الخير بدوى السيد[2] و أمانى عبد الغتاح سالم[3]

[1] وحدة بحوث المطبخ التجريبي – معهد بحوث تكنولوجيا الأغذية – مركز البحوث الزراعية- الجيزة- مصر

[2] وحدة البيو كيمياء الطحالب –قسم تكنولوجيا التسميد- المركز القومي للبحوث –الدقي- الجيزة- مصر

[3] قسم الأغذية الخاصة والتغذية- معهد بحوث تكنولوجيا الأغذية- مركز البحوث الزراعية- الجيزة-مصر

الرصاص هو أكثر خطورة فى سمية التلوث البيئي. لأنه يتراكم في المواد الغذائية وخاصة الأغذية النباتية. كذلك نجد أن استخدام دقيق الصويا على نطاق واسع لأنه غير مكلف وكمصدر غذائي عالي البروتين. لذا فهذع الدراسة تهدف إلى دراسة تأثير دقيق الصويا (كمصدر للبروتين النباتي) لوحده او مع إضافة طحلب الأسبيرولينا للحماية من سمية الرصاص باستخدام الفئران (ذكور وإناث). ولقد تم جمع عينات الدم في اليومين الثلاثين والستين من التغذية. كما تم تقييم الدم وبعض المعادن. في نهاية الفترة التجريبية. النتائج: أظهرت النتائج أن خلات الرصاص في مياه الشرب تسببت في انخفاض صورة الدم ومحتوى الدم من الكالسيوم والحديد والزنك. ولكن وجد تحسن باستخدام طحلب الأسبيرولينا فى الهيموجلوبين وكذلك فى محتوى الحديد في الدم. كما أدت التغذية على دقيق الصويا الى صلابة العظم ولكن ارتفع محتوى عظم الفخذ من الرصاص مقارنة بالتغذية على مزيج مع دقيق الصويا وطحلب الأسبيرولينا. وأيضا وجد انخفاض في الوزن والطول. التوصية: لا يجب الأعتماد على البروتين النباتى فقط للحمابة من التلوث بالرصاص. ولكن مع مشاركة والبروتين الحيواني خاصة اللبن ومنتجاته.

الكلمات الدالة: طحلب الاسبيرولينا – دقيق الصويا- خلات الرصاص- الفئران.