Effect of soybean on some biochemical markers and histopathological changes in lead intoxicated rats.

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ABSTRACT

The protective and treatment effect of dietary soybean against lead toxicity induced changes in the bones and the hormones of the reproductive system in male rats were investigated, Seventy two adult male rats were divided into the following groups:Group1:36 rats fed on soya bean free diet were divided into three equal sub groups:Group1a:control soya bean free diet (-ve), Group1b:rats fed on soya bean free diet after lead intoxicated (rats injected with lead acetate(8mg/body weight) from 1st week to 4th week , Group1c: rats fed on soya bean free diet before lead intoxicated (rats injected with lead acetate (8mg/body weight) from 5th week to 8th week),Group2:36 rats fed on soya bean diet were divided into three equal sub groups:Group2a:control soya bean diet (+ve), Group2b:rats fed on soya bean diet after lead intoxicated (rats injected with lead acetate(8mg/body weight) from 1st week to 4th week), Group2c: rats fed on soya bean diet before lead intoxicated((rats injected with lead acetate(8mg/body weight) from 5th week to 8th week). The results revealed that there was a significant increase in AST, ALT, Urea, Creatinine and testosterone in rats fed on soya bean free diet before lead intoxicated, accompanied with a significant decrease in Calcium, inorganic Phosphorus and Luteinizing hormone(LH) after 4th week when compared with control (-ve) group . Also there was a significant increase in AST, ALT, Urea, Creatinine, testosterone in rats fed on soya bean diet before lead intoxicated, accompanied with a significant decrease in Calcium, inorganic Phosphorus and Luteinizing hormone (LH) when compared with control (+ve) group. On the other hand, there was a significant increase in AST, ALT, Urea, Creatinine and Testosterone in rats fed on soya bean free diet before lead intoxicated accompanied with a significant decrease in inorganic Phosphorus, Calcium and Luteinizing hormone (LH), when compared with control (-ve) group. Also there was a significant increase in AST, ALT, Urea, Creatinine and Testosterone in rats fed on soya bean before lead intoxicated accompanied with significant decrease in inorganic Phosphorus, Calcium and Luteinizing hormone (LH), when compared with control (+ve) group. It concluded that the soya bean diet ameliorated bone and testis intoxicated with lead.

Keywords: lead intoxication, rats, soybean, gonad hormones, bone.

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1. INTRODUCTION

Lead (Pb) is an environmental pollutant and a metabolic poison with a variety of toxic effects and induces many biological changes to animals and humans *(*Bagchi and 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. Moreover, Lead 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 and

Ahmad, 1999).Soybean contains not only estrogenic isoflavones, but also other substances such as coumestans and lignans (Kurzer and 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 and Eden, 1996, Alekel et al., 2000 and Ye et al., 2003). There is evidence that estrogen plays an important role in skeletal tissue in males. 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 this study is to investigate the effect of dietary soybean meal on bone and reproductive system in male rats intoxicated with lead. Also histological studies of testis, bone, liver and kidney were also investigated.

2. Materials and Methods

2.1. Animals

A total number of 72 adult male albino rat, weighting 180- 220 gm were used in this experimental investigation of this study. Rats were obtained from the laboratory animals research center, Faculty of Veterinary Medicine, Moshtohor, Benha University.

Animals were housed in separate metal cages, fresh and clean drinking water was supplied ad-libtium. Rats were kept at a constant environmental and nutritional condition throughout the period of experiment. The animals were left for 15 days for acclimatization before the beginning of the experiment.

2.2. Chemical and drugs:

Lead acetate has molecular weight 379.33. Each one gram of lead acetate 72% contains 521 mg of lead. It was provided by AL-NASR chemical CO.; Egypt was used for rat toxicity in this experiment. Lead acetate was prepared by dissolved 1gm from lead acetate powder in 500 ml saline solution. Each rat within the target group injected intra-peritoneally with 1ml of the prepared solution which is equivalent to a dosage rate of 8mg/kg body weight. This treatment was given four days per week (Moustafa et al., 2007).

2.3. Experimental design:

After acclimatization to the laboratory conditions, the animals were randomly divided into six groups (12rats each) placed in individual cages and classified as follow: Group 1: soya bean free diet control (-ve) (SBFC). Group2: soya bean free diet injected with lead acetate from 1st week to 4th week (SBF treated group). Group 3: soya bean free diet injected with lead acetate from 5th week to 8th week (SBF protected group). Group 4: soya bean diet control (+ve) (SBC). Group 5: soya bean diet injected with lead acetate from 1st week to 4th week (SB treated group). Group 6: soya bean diet injected with lead acetate from 5th week to 8th week (SB protected group).

2.4. Sampling:

Blood samples for serum separate was collected at the end of 4th w and 8th w. Samples were collected from the venous plexus located at the medial canthus of the eye by means of capillary tubes. The collected blood was allowed to clot at room temperature for an hour, and then refrigerated for further an hour for clot retraction. Clear sera were separated by centrifugation at 3000 r.p.m for 10 minutes and then collected in Eppendorf tubes using automatic micropipettes. Serum samples were kept in deep freezer (-20˚ c) for analysis of the following biochemical parameters: AST, ALT, Urea, Creatinine, Testosterone, LH, Calcium and inorganic phosphorus.

2.5. Biochemical analysis:

Serum Alanine aminotransferase, Aspartate aminotransferase, Urea, Craetinine, Testosterone, Luteinizing hormone, Calcium, phosphorus were determined according to the methods described by Murrary (1984a), Murrary (1984b), Kaplan et al (1984), Murrary (1984c), Delacerda et al (1973), Odell et al (1967), Schmidt Gayk et al (1997), Goldenberg and Fernandez (1966), respectively.

2.6. Histopathological examination:

Specimens (liver, kidney, testis, bone) were collected from dead and after sacrificing rats then preserved in neutral buffer formalin solution (10%) for histopathological examination. Specimens were collected after 4, 8 weeks from starting doses. After proper fixation, the specimens dehydrated in different ascending grades of ethyl alcohol, cleared in xylene, embedded in paraffin, thin sectioning at 5µm stained by hematoxyline and eosin stains examination according to(Banchroft et al, 1996).

2.7. Statistical analysis

The results were expressed as mean ± SEM of 12 rats per group and statistical significance was evaluated by ANOVA using SPSS (version 22) program followed by the post hoc test, least significant difference (LSD). Values were considered statistically significant when $p > 0.05$

3. RESULTS

3.1. Biochemical parameters

The obtained data in table (1) revealed a significant increase in AST, ALT, Urea, Creatinine and testosterone in soya bean free diet –treated group, accompanied with significant decrease in Calcium, Phosphorus and LH when compared with control (-ve) group. Pretreatment with soya bean in soya bean treated group in rats resulted in significant increase in AL, AST, Phosphorus, Urea, Creatinine and Testosterone, accompanied with significant decrease in Calcium and LH, in comparison with control (+ve)) group. The obtained data in table (2) revealed a significant increase in AST, ALT, Urea, Creatinine and testosterone in soya bean free diet – protected group, accompanied with significant decrease in Calcium, Phosphorus and LH when compared with control (-ve) group. Pretreatment with soya bean in soya bean protected group in rats resulted in significant increase in AST, ALT, Urea, Creatinine and Testosterone, accompanied with significant decrease in Phosphorus, Calcium and LH in comparison with control (+ve) group.

3.2. Histopathological examination

Rats fed on soya bean free diet and treated with lead acetate from 1- 4 weeks. After 4 weeks: The examined livers showed fatty degeneration of the hepatocytes were commonly seen in centrilobular zones of hepatic lobules (figure1).The examined kidneys showed renal glomeruli exhibited multifocal mesangial/endothelial cell necrosis of glomerular tufts characterized by shrinkage of the tuft with hypereosinophilic cytoplasm and pyknotic nuclei (figure3).The testis of the treated rats revealed the interstitium was moderately expanded by homogenous eosinophilic material (edema) admixed with small numbers of mononuclear inflammatory cells (figure 5). The bones of the treated rats revealed early osteoporotic changes characterized by secondary trabeculae were discontinuous with sharp angular borders (figure 7).

Rats fed on soya bean free diet and treated with lead acetate from 5- 8 weeks. After 8 weeks: The microscopical examination of the livers revealed The bile ducts exhibited hyperplasia of biliary epithelium and mild periductal lymphocytic cellular infiltration (figure2). The examined kidneys showed the cortical interstitium was markedly expanded by increased clear space (edema) admixed with lymphocytes and macrophages (figure4). The testis showed congested blood vessels and interstitial

capillaries. The interstitium was expanded by eosinophilic material (edema) infiltrated by few numbers of mononuclear inflammatory cells (figure6). Multifocal, moderate numbers of seminiferous tubules were distorted by marked degeneration or disorganization and exfoliation of germ cells accompanied by reduced spermatogenesis and absence of spermatozoa in the lumen of the tubules (figure6). The bones of the treated rats revealed mild osteoporotic changes.

Rats fed on soya bean diet and treated with lead acetate from 1- 4 weeks. After 4 weeks: The livers of the treated rats showed congestion of the central veins and portal blood vessels. Diffusely, vacuolar and hydropic degeneration of the hepatocytes (figure8). The examined kidneys showed marked pathological changes particularly in renal cortex. Diffusely, glomeruli had one or more of the following changes: expansion of the mesangium by abundant eosinophilic homogenous to fibrillar material, and erythrocytes (figure11). The testes showed congested blood vessels and interstitial edema admixed with small numbers of lymphocytes, macrophages and degenerated neutrophils. Multifocally, testicular degeneration of moderate numbers of seminiferous tubules characterized by cytoplasmic vacuolation and accompanied by reduced spermatogenesis was observed. The bones of the treated rats revealed osteoporotic changes characterized by thin irregular trabeculae projected into the metaphyseal marrow cavity and sharp angular borders of primary and secondary trabeculae. After 8 weeks: The examined kidneys showed marked pathological changes. In the cortex, there was moderate glomerular loss; occasionally, glomerular tufts exhibited mesangial/endothelial cell necrosis with hyper-eosinophilic cytoplasm and pyknotic nuclei, while other tufts were markedly atrophied or completely destructed with presence of eosinophilic remnants in uriniferous space (figure10). The testis showed similar changes to that observed after 4weeks, characterized by interstitial edema and testicular degeneration of some seminiferous tubules. The bones of the treated rats revealed mild osteoporotic changes.

Rats fed on soya bean diet and treated with lead acetate from 5- 8 weeks. After 4 weeks: No histopathological changes were detected in the examined organs of these rats. After 8 weeks: The examined livers showed marked congestion of the central veins and portal blood vessels. Multifocal, vacuolar and hydropic degeneration of the hepatocytes were commonly seen in periportal zones of hepatic lobules. The degenerated hepatocytes were characterized by swollen pale vacuolated cytoplasm (figure 9). The examined kidneys showed degenerative changes of their lining epithelium of renal tubules in cortex characterized by vacuolar and hydropic degeneration (figure12). Rarely, tubules were mildly ectatic and lined by attenuated epithelium. The testis showed congested blood vessels and interstitial edema. Multifocal, moderate numbers of seminiferous tubules were distorted by moderate degeneration and destruction of germ cells accompanied by reduced spermatogenesis and absence of spermatozoa in the lumen of the affected tubules. The bones of the treated rats revealed early osteoporotic changes characterized by irregular trabeculae projected into the metaphyseal marrow cavity (figure13). Primary and secondary trabeculae were discontinuous with sharp angular borders.

1. DISCUSSION:

The obtained data from tables (1,2) revealed that, rats fed on soya bean free diet after lead intoxication exhibited a significant increase in ALT, AST activity after 4 and 8 weeks as compared with control(–ve) group. Also rats fed on soya bean diet before lead intoxication exhibited significant decrease in AST and ALT activities after 8 weeks as compared with G5.

Table 1: Effect of soybean on blood biochemical parameters in lead intoxicated rats after 4 weeks:

	AST U/L	ALT U/L	Calcium mg/dl	phosphorus mg/dl	Urea mg/dl	Creatinine mg/dl	testosterone pg/dl	LH g/dl
Control (-Ve)	146.75 ± 2.66^{bA}	59.50 ± 5.36^{aA}	7.19 ± 0.13^{dB}	8.40 ± 0.28^{cB}	29.49 ± 2.00^{aB}	0.92 ± 0.09^{aA}	5.38 ± 0.23^{aB}	3.15 ± 0.08^{bA}
Soybean free diet-treated Group	197.25 ± 4.87^{cA}	101.50 ± 10.81^{bA}	5.26 ± 0.33^{aB}	5.35 ± 0.29^{aA}	43.25 ± 2.84^{bA}	1.45 ± 0.14^{bA}	10.39 ± 0.45^{bA}	0.59 ± 0.10^{aA}
Soybean free diet-protected Group	146.75 ± 2.66^{bA}	59.50 ± 5.36^{aA}	7.19 ± 0.13^{dB}	8.40 ± 0.28^{cB}	29.49 ± 2.00^{aA}	0.92 ± 0.09^{aA}	5.38 ± 0.23^{aA}	3.15 ± 0.08^{bB}
Control (+ve)	128.75 ± 6.51^{aA}	53.50 ± 6.08^{aA}	6.59 ± 0.24^{cA}	5.63 ± 0.25^{aA}	29.08 ± 0.67^{aB}	1.10 ± 0.10^{aB}	4.55 ± 0.29^{aA}	3.13 ± 0.05^{bA}
Soybean diet-treated Group	187.75 ± 4.89^{cB}	102.00 ± 6.28^{bA}	6.07 ± 0.11^{bA}	6.53 ± 0.14^{bB}	43.75 ± 1.65^{bB}	1.38 ± 0.08^{bB}	11.79 ± 0.60^{bB}	0.79 ± 0.15^{aA}
Soybean diet-protected Group	128.75 ± 6.51^{aA}	53.50 ± 6.08^{aA}	6.59 ± 0.24^{cB}	5.63 ± 0.25^{aA}	29.08 ± 0.67^{aA}	1.10 ± 0.10^{aA}	4.55 ± 0.29^{aA}	3.13 ± 0.05^{bB}

SE: Standard error a, b & c: There is no significant difference (P> 0.05) between any two means, with in the same column have the same superscript letter. A, B & C: There is no significant difference (P> 0.05) between any two means, within the same row have the same superscript letter.

Table 2: Effect of soya bean on blood biochemical parameters in lead intoxicated rats after 8 weeks:

Group	AST U/L	ALT U/L	Calcium mg/dl	phosphorus mg/dl	urea mg/dl	Creatinine mg/dl	testosterone pg/dl	LH g/dl
Control (-Ve)	148.25 ± 3.90^{bA}	66.75 ± 2.17^{aA}	6.92 ± 0.09^{dA}	7.46 ± 0.35^{bcA}	24.00 ± 2.48^{aA}	1.05 ± 0.03^{abB}	4.61 ± 0.34^{aA}	3.05 ± 0.10^{bA}
Soybean free diet-treated Group	211.75 ± 7.39^{cB}	93.75 ± 3.15^{bA}	4.94 ± 0.34^{bA}	5.51 ± 0.49^{abA}	52.75 ± 4.87^{cB}	1.98 ± 0.11^{dB}	11.60 ± 1.25^{bB}	0.77 ± 0.06^{aB}
Soybean free diet-protected Group	269.25 ± 18.54^{dB}	127.25 ± 5.81^{dB}	4.01 ± 0.08^{aA}	5.32 ± 0.15^{aA}	64.75 ± 2.95^{dB}	2.51 ± 0.11^{cB}	13.93 ± 1.03^{cB}	0.73 ± 0.05^{aA}
Control (+ve)	131.25 ± 5.07^{aA}	58.50 ± 3.77^{aA}	7.06 ± 0.07^{dB}	8.08 ± 0.06^{cB}	20.75 ± 0.85^{aA}	0.93 ± 0.05^{aA}	5.39 ± 0.29^{aB}	3.02 ± 0.10^{bA}
Soybean diet-treated Group	153.25 ± 3.33^{bA}	$104.50\pm7.14b^{cA}$	6.17 ± 0.34^{cA}	5.94 ± 0.62^{aA}	39.50 ± 0.65^{bA}	1.15 ± 0.03^{bcA}	10.23 ± 0.48^{bA}	0.78 ± 0.16^{aA}
Soybean diet- protected Group	214.00 ± 8.74^{cB}	109.25 ± 5.81^{cB}	5.33 ± 0.26^{bA}	6.03 ± 0.09^{bB}	55.25 ± 2.66^{cB}	1.29 ± 0.06^{cB}	13.03 ± 0.60^{cB}	0.82 ± 0.16^{aA}

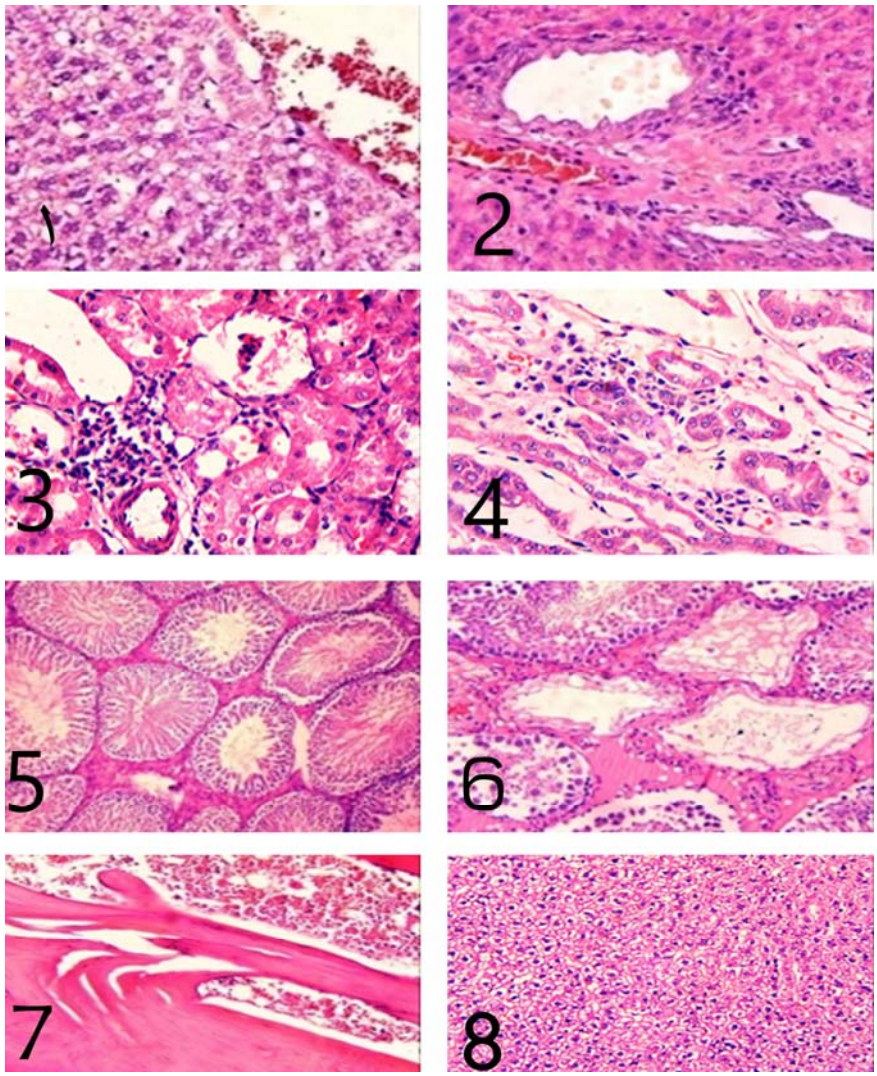

Fig.1: Liver of rat fed on soya bean free diet and treated with lead acetate, 1- 4 weeks, showing fatty degeneration of the hepatocytes in centrilobular zones of hepatic lobules. H&E stain x 400. **Fig.2:** Liver of rat fed on soya bean free diet and treated with lead acetate, 5- 8 weeks, showing hyperplasia of biliary epithelium and mild periductal lymphocytic cellular infiltration H&E stain x 400. **Figure3:** Kidney of rat fed on soya bean free diet and treated with lead acetate, 1- 4 weeks, showing mesangial/endothelial cell necrosis of glomerular tufts characterized by shrinkage of the tuft with hypereosinophilic cytoplasm and pyknotic nuclei. H&E stain x 400. **Figure4:** Kidney of rat fed on soya bean free diet and treated with lead acetate, 5-8 weeks, showing interstitial oedema admixed with lymphocytes and macrophages. H&E stain x 400. **Fig.5:** Testis of rat fed on soya bean free diet and treated with lead acetate, 1- 4 weeks. showing The interstitium was moderately expanded by homogenous eosinophilic material (edema) admixed with small numbers of mononuclear inflammatory cells . H&E stain x 1000**. Fig.6**: Testis of rat fed on soya bean free diet and treated with lead acetate, 5- 8 weeks, showing interstitial oedema and seminiferous tubules distorted by marked degeneration, disorganization and exfoliation of germ cells accompanied by reduced spermatogenesis and absence of spermatozoa in the lumen of the tubules. H&E stain x 200. **Fig.7:** Bone of rat fed on soya bean free diet and treated with lead acetate, 1- 4 weeks, showing osteoporotic changes characterized by discontinuous trabeculae with sharp angular borders. H&E stain x 400. **Figure8:** Liver of rat fed on soya bean diet and treated with lead acetate, 1- 4 weeks,showing diffuse vacuolar and hydropic degeneration o f the hepatocytes. H&E stain x 200.

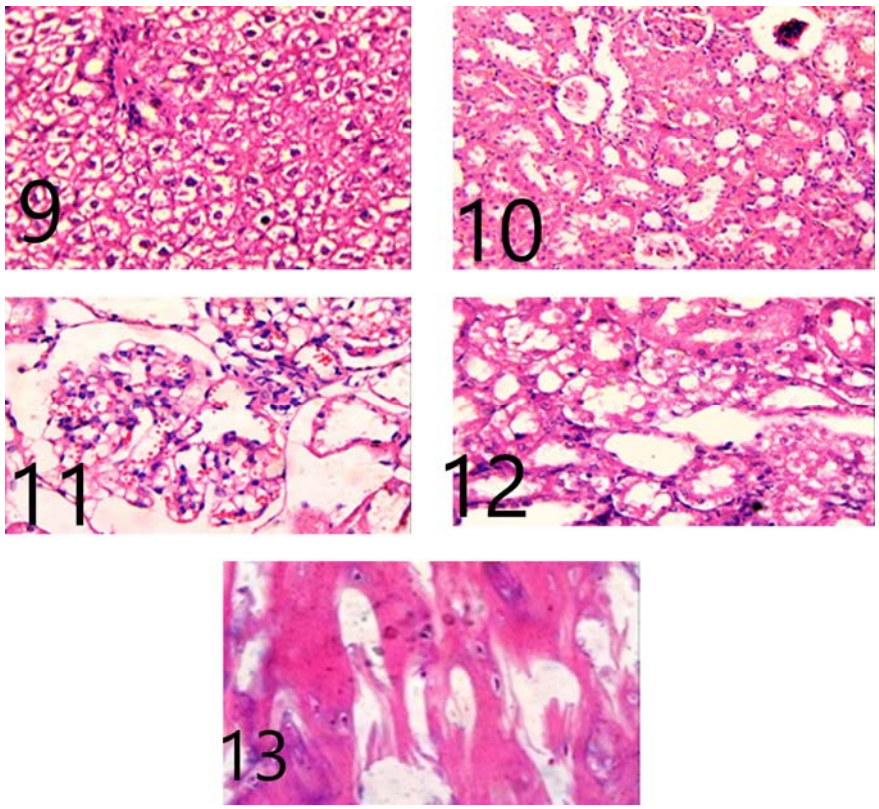

Figure9: Liver of rat fed on soya bean diet and treated with lead acetate, 5 - 8 weeks,showing periportal degenerated hepatocytes characterized by swollen pale vacuolated cytoplasm. H&E stain x 400. **Figure10**: Kidney of rat fed on soya bean diet and treated with lead acetate, 1- 4 weeks, showing mesangial/endothelial cell necrosis of glomerular tufts with hypereosinophilic cytoplasm and pyknotic nuclei. Note also atrophied and destructed tufts with presence of eosinophilic remnants in uriniferous space. H&E stain x 200. **Figure11:** Kidney of rat fed on soya bean diet and treated with lead acetate, 1- 4 weeks, showing expansion of glomerular mesangium by abundant eosinophilic homogenous to fibrillar material, and erythrocytes. H&E stain x 400. **Figure12**: Kidney of rat fed on soya bean diet and treated with lead acetate, 5 - 8 weeks , showing vacuolar and hydropic degeneration of the lining epithelium of renal tubules in cortex. H&E stain x 400. **Fig.13:** Bone of rat fed on soya bean diet and treated with lead acetate, 5 - 8 weeks, showing osteoporotic changes characterized by irregular trabeculae projected into the metaphyseal marrow cavity.

The increase in AST and ALT activities caused by the higher concentration of lead causes mainly liver damage in which free radicals are involved, but low concentration of lead may disturb the normal biochemical process in the hepatobiliary system and lead may precipitate into gallstones (Sipos et al., 2003***)***. More ever serum AST and ALT in adult male rats fed on soya bean before lead intoxication showed a significant decrease compared with G5. The decrease in serum AST and ALT activities caused by nutrient enriched soybean tempeh (NESTE) extract was capable of recovering the damaged hepatocytes to their normal structures. Thus, it can be concluded that NESTE produced through fermentation process was able to enhance hepatoprotective and antioxidant effects in vivo (Yusof et al, 2013**,** ELnattat and ELkady, 2007 and

Danzhou et al, 2012***)***. The obtained data demonstrated from table (2) revealed that, rats fed on soya bean diet after lead intoxication exhibited a significant decrease in urea and creatinine concentrations after 8 weeks as compared with G3. Also, rats fed on soya bean free diet before lead intoxication exhibited a significant increase in serum urea and creatinine after 8 weeks as compared with G1.On the other hand, rats fed on soya bean diet before lead intoxication exhibited a significant decrease in serum urea and creatinine concentrations after 8 weeks as compared with G5.The increase in serum creatinine and urea concentrations caused by increase lead concentration in blood which lead to renal deficiency which is confirmed by a decrease of creatinuria and urinary pH ***(***Groube et al, 2001***)***. The decrease in serum creatinine and urea caused by increase of calcium in blood and phosphaturia and the increase of these parameters would indicate a renal deficiency and presence of calcium oxalate dehydrate crystals observed in sample of urine of exposed rats. All lead treated rats shown intra nuclear inclusion bodies in kidney proximal tubular (Missoun et al, 2010***)***. The level of creatinine and urea in adult male rats fed on soya bean before and after lead intoxication exhibited a significant decrease because soya bean have beneficial effects in treatment and prevention of chronic kidney disease (Henderson, 2010 and Anderson et al, 1999). The obtained data demonstrated in tables (1, 2) revealed that, rats fed on soya bean free diet after lead intoxication exhibited a significant decrease in Calcium and Phosphorus concentrations after 4and8 weeks as compared with G1. Rats fed on soya bean diet after lead intoxication exhibited a significant increase in Calcium and Phosphorus concentrations after 4 and 8 weeks as compared with G3. Meanwhile, rats fed on soya bean free diet before lead intoxication exhibited a significant decrease in Calcium, phosphorus concentration after 8 weeks compared with G1. Furthermore, rats fed on soya bean diet before lead intoxication exhibited a significant increase in Calcium, Phosphorus concentrations after 8 weeks as compared with G5. The administration of lead to rats resulted in marked decrease in body weight of the rats fed on no soya bean or soya bean diet .previous studies have also reported a ***(***Taupeau et al, 2001) decline in body weight in animals exposed to high concentrations of lead (Rasile et al, 1995***)***. The lead levels of all groups in femur lead accumulation was much greater in the rats fed on no soya bean and soya bean before lead intoxication than after lead intoxication. Lead accumulation in the bone was the highest. The high affinity of lead for bony tissues is consistent with bone lead as indicator of cumulative lead exposure and a marker of chronic lead intoxication (Rasile et al, 1995***)***. 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***)***. Lead alters the metaphyseal and growth plate morphology of bones in lead exposed animals. Lead can be bound to the growth plate morphology of bones in lead exposed animals. Lead can be bound to growth plate cartilage matrix sites normally associated with calcium and phosphorus (Hamilton and O·Flaherty, 1994***)***. It was also showed that femoral BMD in male adult rats fed on soya bean before lead intoxication showed a significant increase when compared with rats fed on soya bean free diet before lead intoxicated. The improvement of bone strength with dietary soya bean was attributed to the efficiency of the intestine to absorb calcium. The conversion of calcium with dietary soya bean compared with other proteins may be due to the reduction in the urinary excretion of calcium (Messina and Messina, 2000***)***.

The obtained data demonstrated in tables (1,2) revealed that rats fed on soya bean free diet after lead intoxication exhibited a significant decrease in serum LH concentration after 4 and 8 weeks as compared with G1. Rats fed on soya bean free diet before lead intoxication exhibited a significant decrease in serum LH

concentration after 8 weeks as compared with G1.The decrease in the level of serum LH concentration combined with the significant reduction of inter tubular tissue volume in testis indicate impaired Leydig cell function (Manaly et al, 1995*)*.The decrease in level of serum LH concentration reported by Pb alters basal Steroidgenic acute regulatory protein (StAR) synthesis, but does not alter gonadotropin –stimulated StAR synthesis, hence, suggesting the primary action of Pb to suppress Estradiol is through its known action to suppress the serum levels of luteinizing hormone and not due to decreased responsiveness of StAR synthesizing machinery *(*Srivastava et al, 2004*)*. It was also showed that serum LH concentration in male adult rats fed on soya bean before and after lead intoxication showed a non-significant change. The improvement of semen parameters with dietary soya bean, therefore, intrauterine insemination was performed. Also resulted in pregnancy, and a healthy baby weighing 3,300g was born and a possible therapeutic role for phytoestrogens in the treatment of Oligospermia (Casini et al, 2006*)* the consumption phytoestrogen rich diet prevent western diseases such as breast and prostate cancer (Rishi et al, 2002*)*.

The obtained data demonstrated in tables (1,2) revealed that, rats fed on soya bean free diet after lead intoxication exhibited a significant increase in serum testosterone after 4 and 8 weeks as compared with G1. Also rats fed on soya bean free diet before lead intoxication exhibited a significant increase in serum testosterone after 8 weeks as compared with G1. On the other hand, rats fed on soya bean diet before lead intoxication exhibited a significant decrease in serum testosterone after 8 weeks as compared with G5. A significant reduction in serum testosterone levels by 83.5% and 36% was observed in rats fed on soya bean free diet before and after lead intoxication, respectively. In the rats fed on soya bean in the protected and treated 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% (Manlay et al, 1995*)* contrary to the finding for lower doses of lead exposure (Nathan et al, 1992) and (Lataillade et al, 1993*)*, except for those by sokol and co-workers (Sokol et al, 1985, Sokol and Berman, 1991*)*.

2. CONCLUSION

Biochemical and histopathological studies revealed that testis, liver, kidney and bone damage was caused by lead intoxication. Soya bean may provide protection and ameliorated bone and testis from damage. So we recommend that, soya bean should be standardized purified so we can take advantage of its great protection and therapeutic effect in treatment of fertility and osteoporosis without side effect.

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