Research Article

AL- ANBAR JOURNAL OF VETERINARY SCIENCES

Vol. 14 Issue:2, (2021) ISSN: P-1999:6527 E-2707:0603

Determination of the Toxic Dose of Experimental Lead Poisoning for Male Goats in Fallujah City, Iraq

Omar Attalla Fahad*[1] and Mawlood Abbas Ali Al-Graibawi[2]

[1]Dept. of Internal Medicine &Preventive, College of Veterinary Medicine, University of Fallujah, Iraq.

[2]Dept. of Internal Medicine &Preventive, College of Veterinary Medicine, University of Baghdad, Iraq

Corresponding Author: dr.omarattalla@uofallujah.edu.iq

Doi: https://doi.org/10.37940/AJVS.2021.14.2.7

Received: 2/7/2021 Revised: 14/10/2021 Accepted:18/10/2021

Abstract

This study aimed to induce lead poisoning experimentally in male goats to determine the toxic dose and investigate it is effects on hematological parameters, and the functions of the liver and kidney. The experiment was performed on 15 male goats, aged between 3 - 5 months with a mean weighing 13±0.65 kg. Goats were divided into five equal groups, the first represented the control group given tap water, while the other groups were given orally (by stomach tube) 70,100,200 and 400 mg/kg B.W. of lead acetate respectively, for 5 days. Blood was collected weekly for 4 weeks to estimate the concentrations of lead, hematological and biochemical analysis. The results indicated a significantly (P≤0.05) increase of lead(0.738±0.07ppm), only in goats which received 400 mg Pb/kg B.W of lead with symptoms included: depression, dullness, anemia, muscle twitching, staggering, and teeth grinding, with significant reduction in erythrocyte count, packed cell volume, and hemoglobin 10.391±0.41*10^6/ml, 25.5±0.55%, and 8.30±0.19g/dl respectively, compared with the control ones. Also, increase in total white blood cell count to 9.098±0.08*10^3/ml, neutrophils 39.07±0.93%, monocytes 1.88±0.07%, and eosinophils 4.82±0.05%. The same group results showed significant elevations in the activities of liver enzymes; ALT 59.9±0.20u/l, AST 243±1.3u/l, in addition, the serum levels of creatinine and urea were also increased indicating renal frailer 1.96±0.05 mg/dl and 29.78±0.34 mg/dl respectively. In summary, this is the first study that proved the toxic dose of lead poisoning for male goats in Iraq and estimate their hazardous results on the hematological and chemobiological analyses on goats.

Keywards: Goats, Hematological Parameters, Lead Poisoning, Lead Toxic Does.

تحديد الجرعة السامة للتسمم بالرصاص تجريبيا لذكور الماعز في مدينة الفلوجة -العراق.

الخلاصة

هدفت الدراسة إلى إحداث التسمم التجريبي بخلات الرصاص في ذكور الماعز لتحديد الجرعة السامة ومعرفة تأثيرها على المؤشرات الدموية ووظائف الكبد والكلى. أجريت التجربة على 15 من ذكور الماعز تراوحت أعمارهم بين 3 - 5 شهور ومعدل اوزانهم 0.65 ±13 كجم. قسمت الماعز إلى خمس مجموعات متساوية ، المجموعة الأولى مجموعة السيطرة أعطيت ماء للشرب فقط ، بينما أعطيت المجموعات الأخرى عن طريق الفم 200.100.70 و 400 ملجم / كجم من وزن الجسم خلات الرصاص لمدة 5 أيام. تم جمع الدم أسبوعيا لمدة 4 أسابيع لتقدير تركيز الرصاص و قيم الدم و القيم الكيموحيوية. أشارت النتائج إلى أن الماعز التي تناولت 400 ملجم / كجم من أسيتات الرصاص فقط بلغت مستويات سمية 0.738±0.07 جزء في المليون ، مع ظهور اعراض مثل الاكتئاب والبلادة وفقدان الشهية وفقر الدم وارتعاش العضلات والترنح, بالإضافة الى انخفاض كبير في عدد كريات الدم الحمراء ، وحجم الخلايا المرصوصة ، والهيموغلوبين 10.391* 10^6 / مل ، 25.5 % ، و 8.30 جم / ديسيلتر على التوالي ، مقارنة مع مجموعة الحيوانات السليمة.وكانت هناك زيادة معنوية في عدد كريات الدم البيضاء 9.098*10^3/مل, العدلات 39.07%,الخلايا الوحيدة 1.88%, والحمضات 4.82 % . أظهرت النتائج ارتفاعا معنويا في نشاط أنزيمات الكبد ALT 59.9 u/l, AST 243 u/l ، بالإضافة إلى زيادة مستويات الكرياتينين واليوريا في الدم مما يشير إلى عجز كلوي 1.96مجم / ديسيلتر ، 29.78مجم / ديسيلترعلى التوالي.باختصار ، هذه هي الدراسة الأولى في العراق التي أثبتت الجرعة السامة للتسمم بالرصاص لذكور الماعز وتقدير نتائجها الخطيرة على قيم الدم والقيم الكيموحيوية للماعز.

Introduction

Lead (Pb) is an extremely toxic, non-disintegrative heavy metal with a bluish-grey color, a molecular weight of 207.2, an atomic number of 82, a melting point of 621.43 F^{O}, and a density of 11.34 g/cm^{3}, it can be shaped easily, formed, and mixed with other metals to form alloys (1). Any domestic animal could be poisoned with Pb, like goats, cattle, horses, sheep, dogs and birds, Pb can be found in neighboring agricultural equipment and garbage dumps, grease, dye, lead shot, and contaminated flora growing near smelters or alongside roadsides (2). Blindness, staggering gait, rolling eyes, colic, uncoordinated attempts to climb obstacles, excessive response to external stimuli, and convulsions, putrid dark diarrhea, head pressing are public signs in affected animals, which death could be occurring (3). Renal failure, abdominal pain, and the nervous and circulatory systems can be affected by rising blood Pb levels, plus hematopoiesis and bone growing (4). Pb on large levels, on the other hand, can cause anemia by interfering with heme formation and causing red cell apoptosis (5).

Materials and Methods

Animals

Fifteen local breed male goats, aged between 3 - 5 months with a mean weighing 13±0.65 kg were obtained from the local market of Al Fallujah city, Iraq. The animals were housed in indoor pens and were fed three times a day with a mixture of alfalfa hay, barley grain, and wheat straw, while freshwater was freely available throughout the study. Prior to the beginning of the experiment, goats were dewormed with Albendazole, animal health status was evaluated by clinical examination (temperature, respiratory rate and pulse rate), and goats were housed and fed in the aforementioned conditions for 14 days to ensure proper acclimation.

Experimental design

The 15 goats were divided into five groups, each with three goats: the control group received only tap water, other groups received 70,100,200, and 400 mg/kg body weight of lead acetate (Sigma, St Louis, MO, USA) as a 1% (w/w) aqueous solution for five days.

Blood collection

Blood samples (5 ml) were collected from the left jugular vein of each goat on days 0, 6, 13, 20, and 27 using heparinized tubes (with heparin anticoagulant) for measuring lead levels and hematological analysis, and plain tubes (without coagulant) for evaluating biochemical parameters of the samples in each group.

Lead Concentrations

Lead levels in collected blood samples were detected by the atomic absorption spectrophotometer(Phoenix-986/USA) at 283.3 nm wavelength, 1 mL of blood was taken and digested in 9 mL of freshly prepared acid mixture 65 % HNO3 and 37 % HCl. The mixture was then gently boiled for 4–5 hours over a water bath (95°C) or until the sample had completely dissolved (6).

Hematological parameters

Red blood cells (RBCs), white blood cells

(WBCs), differential leukocyte count (DLC), haemoglobin (Hb, platelet count and pack cell volume (PCV), were calculated using digital Hematology analyzer of HORIBA-Japan, was used for all of these haematological tests. Besides that, blood specimens were estimated for ESR using Westegren tubes, with blood drawn to mark (0) and the tubes were hung vertically on the rake for 24hrs, and the ESR values were recorded in mm. (7).

Biochemical parameters : Serum specimens were calculated for liver enzyme concentration (AST), (ALP), and (ALT), also creatinine and urea enzyme levels for renal functions, by using available kits (DiaSys, Germany).

Statistical Analysis

To detect the effect of various factors in study parameters, the Statistical Analysis System- SAS (2012) application was used. In this study, a least significant difference –LSD test was used to compare means (8).

Results and discussion

Clinical Signs : Generally, goats that received 70,100 and 200 mg Pb/kg B.W showed no clear clinical signs of toxicity. While fetid and dark diarrhea appeared with other goats that received 400 mg Pb/kg B.W with slight difficulty in swallowing, depression, dullness, anorexia, anemia, muscle twitching, staggering, teeth grinding, salivation, teeth grinding, and blindness, the rectal temperature, respiratory and heart rates were normal at all times. The poisoned goats usually precede demise with recurrent moaning and tenesmus, as well as they die at last. This study's findings were consistent with those of Soliman et al. (2013) who found that lead-intoxicated goats had moderate excitation, ataxia, 10% weight loss compared to control goats, and pale mucous membranes, as well as wasting, loss of appetite, slight dullness, depression, anorexia, fetid diarrhea, anemia, constipation, recumbency, paralysis, and death during the experimental period (9). Haneef et al. (1998) noticed that clinical signs appeared in goats between 25-39 days (10). Siddiqui and Rajurkar (2008) noticed that the animals are simply found down or dead on the ground (2).

Lead Concentrations At the end of the experiment period, goats that received 70,100 and 200 mg Pb/kg B.W had normal Pb concentrations in blood (the normal values of Pb range 0.05-0.25ppm), while other goats which received 400 mg Pb/kg B.W lead acetate recorded toxic values reach 0.738±0.07 ppm (0.159 LSD value) with significantly ($P \leq 0.05$) increased in blood Pb level, compared to the control group as shown in (table -1-), and both of goats died at 28^{th},32^{th} day, so that is the first time recorded the toxic dose of lead poisoning for goats experimentally in Iraq.

This experiment was approved by Davis (1976) who found that the toxic values were started from 400-1.280 mg Pb/kg B.W. was fatal or produced a moribund condition in goats (11). Radostits et al. (2007) considered that 400 mg/kg enough to induce acute lead poisoning in goats (12). The current study correlated with those of Mousa et al. (2002) when they recorded lead concentrations of lead toxicity in goats with a significant

(P≤0.05) increase in blood on days 0, 7 and 14 was 0.09±0.02, 0.76±0.06 and 1.12±0.08 ppm, respectively (13). The current study results were in agreement with Bischoff *et al.* (2010) when they recorded lead levels of lead toxicity more than 0.35 ppm in the blood (14). Muhammad et al. (2017) found the Pb concentration on day 30 was 1.03±0.108 mg/l in serum throughout the experiment in the control group (15). Also, Peterson and Talcott (2013) showed that the diagnosis of lead poisoning is made by measuring whole blood lead levels above 0.3 to 0.35 ppm indicates significant lead exposure and blood levels may not necessarily correlate with the severity of the clinical signs (16).

Hematological parameters : In this study, the lead acetate decreased RBC count, Hb, and PCV concentrations considerably (P≤0.05), particularly in the group that received 400mg/kg B.W. of lead acetate, which had the lowest values for RBCs 10.391±0.41*10^6/ml, Hb 8.30±0.19g/dl, and PCV 25.5±0.55% as shown in (table-2-), although ESR values were within the normal reference range. The study supposed that Pb toxicity has been to cause a defect in haem synthesis, via an inhibitory effect on globin synthesis, inhibits iron to form haem in red cells (17). Similar, low Hb (8.13±0.14 g/dl) and PCV (32.0±1.4%) values were also reported by Zaki et al. (2010) in Merino sheep (18), and Sellaoui et al. (2016) in Ouled Djellal ewes which were intoxicated with Pb group, there was a decrease of Hb values observed, but no significant change (P>0.05) compared to controls, PCV was varied from 32.2±3.4% before exposure to 28.8±1.3% in the 9th week; the decrease was significant (P<0.01) (19). As seen by Topashka-Ancheva et al. (2003), higher blood Pb levels destruction the red cell membrane, resulting in hemolysis or a decrease in blood iron concentration, which could result in reduced rates of Hb and PCV in animals (20). In agreement with the present study, Sajid et al. (2017) found the Pb treated sheep showed the lowest RBC value at day 30 that was 8.04±0.122 *10^6/µl the mean values of Hb and PCV were recorded in Pb treated group was 10.18±0.360 g/dl and 30.79±0.783% respectively (15). Also, Chandra et al., (2010) described a decline in haemoglobin concentration, red blood cell count, and packed cell volume as a result of lead poisoning, which was produced by both shortening of the erythrocyte lifespan and inhibition of heme synthesis with reticulocytosis, this effect could have been caused by the substitution of the iron site with lead (21). This study, found there were a significant (P<0.05) increase in WBC count 9.098±0.08*10^3/ml, neutrophils 39.07±0.93%, monocytes 1.88±0.07%, and eosinophils 4.82±0.05%, from the control group values especially in the last group which goats received 400mg/kg of lead acetate as shown in (table-3-), the leukocytes count have been increased may be due to the lead-induced inflammation (22,23). In similar, Zaki et al. (2010) reported an increase in WBCs values 10.03+0.49 in agreement with our findings (18). The variable mean values of DLC in the present work have agreed with the findings of

Farkhondeh et al., (2014) who observed a rise in neutrophils (24), and Alabbassi et al. (2017) who observed higher neutrophils and lower eosinophils in Pb toxicity in different studies (25). Muhammad et al. (2017) found at 30 days of sheep poisoning with Pb, neutrophils 24.87±0.492, monocytes 1.5±0.161, and eosinophils 5.07±0.285 (15). However, the possible reason for the difference in monocyte eosinophils, count in our study than (25) and (15) might be due to differences in animal breed or the dose of lead acetate. With disagreement of this study Kar et al. (2015) observed that blood leukocyte, basophil, and lymphocyte counts were reduced, while blood monocyte, neutrophil, and eosinophil counts were significantly larger than normal levels (26). Also, the findings of this study differed from the findings of Muhammad et al. (2017) who found a decrease in WBC count to 6.13±0.371(15).

Biochemical parameters : The present study showed a significant (P≤0.05) increase of ALT, and AST concentrations in goats especially those that were exposed to 400 mg/kg B.W. lead acetate 59.9±0.20 u/l, 243±1.3 u/l respectively, but ALP within the normal range 132.6±0.20u/l as seen in (table-4-). In the present study, the high levels of liver enzymes (ALT, AST and ALP) are attributed to liver injury because they are considered to be the biomarker of hepatotoxicity in live animals, this agreed with the findings of Badiei et al. (2009) who observed the higher ALT and AST concentration in experimentally lead intoxicated Iranian male sheep (27). Similar findings were also reported by (18) in Merino sheep (ALT 63±1.62, AST 173±2.45). Muhammad et al. (2017) recorded after 30 days of giving lead acetate to sheep the ALT, AST were higher than normal values 36.2±1.301, 34.3±1.229 respectively (15). The elevation of levels of ALT often suggested the existence of medical problems such as hepatic damage, viral hepatitis and bile duct problems, so that ALT assessment is commonly used as a way of screening for hepatic problems (28). As per the current findings the lead-exposed group, especially those who received 400 mg/kg B.W. lead acetate had quite a role in a significant increase (P≤0.05) in urea 26.08 (13-28 mg/dl) and creatinine amounts 1.96 (0.7-1.5mg/dl) indicating renal insufficiency (table-5-), the group's rise blood urea and creatinine indicated renal failure resulting from Pb poisoning in this experiment. Also, Haneef et al., (1998) noticed an increase in urea 39.94±2.8 mg/dl and creatinine 2.40±0.24 mg/dl levels (10). Muhammad et al. (2017) noticed after 30 days of giving lead acetate to sheep that urea and creatinine were higher than normal values 33.3±0.666, 1.15±0.061 respectively (15). The impairment of the brush border of epithelial cells making them impermeable to urea and creatinine due to their high content in the blood is a possible mechanism of renal insufficiency produced by Pb poisoning, that with the agreement of (29, 30). Also, a significant (P≤0.05) higher serum urea concentration was observed, which is consistent with the results of (31, 32).

Table (1) lead concentrations in blood of the poisoning goats experimentally (ppm).

Groups	Days of experiment					LSD value
	zero day	sixth day	thirteenth day	twentieth day	twenty-seventh day	
Control	0.061±0.03	0.055±0.05	0.057±0.006	0.063±0.09	0.059±0.01	NS
70mg	0.058±0.02	0.07±0.01	0.083±0.02	0.099±0.06	0.114±0.07 A	0.049
100mg	0.064±0.04	0.088±0.03	0.106±0.03	0.121±0.03	0.136±0.03 A	0.063*
200mg	0.057±0.06	0.103±0.09	0.144±0.05	0.178±0.02	0.203±0.01 A	0.104*
400mg	0.061±0.03	0.295±0.02 D a	0.464±0.07 C a	0.569±0.08 B a	0.738±0.07 A a	0.165 *
LSD value	NS	0.071 *	0.105 *	0.128 *	0.159 *	---
* (P≤0.05), NS: Non-Significant						

The different capital letters refer to significant differences within groups VS control at (P≤0.05). The different small letters refer to significant differences between different groups at (P≤0.05).

Table (2) Erythrogram values of the experimental goats. The different small letters refer to significant differences between different groups at (P≤0.05).

Groups	Hb (8-12g/dl)	PCV (22-38%)	ESR (1-3mm)	RBCs (8-18*10^6/ml)	Platelets 300-600 10^3/µl
Control	10.55±0.28	33±0.58	2.1± 0.02	12.446±0.36	4.08±0.3
70 mg	10.28±0.17	32.8±0.35	2.0± 0.04	12.290±0.25	4.51±0.22
100 mg	9.94±0.04	31.5±0.29	2.2± 0.01	11.978±0.16	4.64±0.31
200 mg	9.67±0.22	31±0.38	2.3± 0.02	11.840±0.20	4.77±0.45
400 mg	8.30±0.19 a	25.5±0.55 a	2.3± 0.03	10.391±0.41 a	6.89±0.34 a
LSD value	1.39 *	7.4 *	NS	1.459 *	1.18 *
* (P≤0.05), NS: Non-Significant					

Table (3) Leukogram values of the experimental goats. The different small letters refer to significant differences between different groups at (P≤0.05).

Groups	WBCs ($4-13*10^3$/ml)	Neutrophils (30-48%)	Lymphocytes (50-70%)	Monocytes (0-4%)	Eosinophils (1-8%)	Basophils (0-1%)
Control	7.691±0.10	31.45±0.17	58.73±1.73	0.20±0.16	2.23±0.06	0.01± 0.03
70 mg	7.964±0.19	31.53 ±0.24	58.41±2.14	0.32±0.12	2.35±0.01	0.03± 0.01
100 mg	8.252 ±0.12	31.70±0.19	57.97±0.29	0.37±0.14	2.42±0.08	0.01 ±0.04
200 mg	8.671±0.20	32.15±0.26	57.86±2.52	0.46±0.26	2.69±0.02	0.01± 0.02
400 mg	9.098±0.08 a	39.07±0.93a	57.69±0.41	1.88±0.07 a	4.82±0.05 a	0.02 ±0.00
LSD value	1.102 *	6.21*	NS	0.71*	1.23*	NS
* (P≤0.05), NS: Non-Significant						

Table (4) liver function biochemical tests of the experimental goats. The different small letters refer to significant differences between different groups at (P≤0.05).

Groups	ALT (15-52 u/l)	ALP (61-283 u/l)	AST (66-230 u/l)
Control	17.2±0.26 d	127.24± 0.25	149.4± 0.29
70 mg	18±0.33	129.2±0.14	153± 0.50
100 mg	18.9±0.09	130±0.09	162.6±1.29
200 mg	24.1±0.45 b	131.8±0.16	173.8±0.46 b
400 mg	59.9±0.20 a	132.6±0.20	243±1.3 a
LSD value	6.6*	NS	22.1*
* (P≤0.05), NS: Non-Significant			

Table (5) kidney function biochemical tests of the experimental goats. The different small letters refer to significant differences between different groups at (P≤0.05).

Groups	Creatinine (0.7-1.5 mg/dl)	Urea (13-28 mg/dl)
Control	0.65 ±0.04	16.17 ±0.14
70 mg	0.73±0.11	16.97±0.03
100 mg	0.82 ±0.14	17.76 ±0.11
200 mg	0.91±0.06 b	18.59±0.21 b
400 mg	1.96±0.05 a	29.78±0.34 a
LSD value	0.24*	2.37*
* (P≤0.05), NS: Non-Significant		

Conclusion

This is the first study that fixed the toxic dose of Pb poisoning in male goats experimentally at Fallujah city, Iraq. With a recording of hazard effect of blood Pb elevation by decreasing RBC, Platelets, PVC and Hb. While ALT, AST, urea and creatinine enzymes concentration were significantly higher ($P \leq 0.05$) increasing than their normal values, referring to the liver and renal disorders.

References

1- World Health Organization (WHO). Action is Needed on Chemicals of Major Public Health Concern. WHO, 2010. 2018-04-20]. http://www. who. int/ipcs/features/10chemicals_en. pdf.

2- Siddiqui MF, Rajurkar GR. Lead-An Emerging threat to livestock. Veterinary world. 2008 Jul 1;1(7):213.

3- Constable PD, Hinchcliff KW, Done SH, Grünberg W. Veterinary medicine-e-book: a textbook of the diseases of cattle, horses, sheep, pigs and goats. Elsevier Health Sciences; 2017.

4- Zadnik T. Lead in topsoil, hay, silage and blood of cows from farms near a former lead mine and current smelting plant before and after installation of filters. Veterinary and human toxicology. 2004 Oct 1;46(5):287-90.

5- Alinejad S, Aaseth J, Abdollahi M, Hassanian-Moghaddam H, Mehrpour O. Clinical aspects of opium adulterated with lead in Iran: a review. Basic & clinical pharmacology & toxicology. 2018 Jan;122(1):56-64.

6- Ang HH, Lee KL. Analysis of mercury in Malaysian herbal preparations.2005.

7- AL-Hadithy AH. The hematological parameters in clinically normal Iraqi local breed goats. The Iraqi Journal of Veterinary Medicine. 2015 24;39(2):12-6.

8- SAS J. Statistical Analysis System, v. 10.0. 2. Cary, North Carolina. USA. 2012.

9- Soliman AM, Abu-Basha EA, Youssef SA, Amer AM, Murphy PA, Hauck CC, Gehring R, Hsu WH. Effect of chronic lead intoxication on the distribution and elimination of amoxicillin in goats. Journal of veterinary science. 2013 Dec 1;14(4):395-403.

10- Haneef SS, Swarup D, Dwivedi SK, Dash PK. Effects of concurrent exposure to lead and cadmium on renal function in goats. Small Ruminant Research. 1998 Jun 1;28(3):257-61.

11- Davis JW, Libke KG, Watson DF, Bibb TL. Experimentally induced lead poisoning in goats: clinical observations and pathologic changes. The Cornell Veterinarian. 1976 Oct 1;66(4):490-7.

12- Radostits, O. M., Gay, C. C., Hinchcliff, K. W., & Constable, P. D. (2007). A textbook of the diseases of cattle, horses, sheep, pigs and goats. *Veterinary medicine*, *10*, 2045-2050.

13- Mousa HM, Al-Qarawi AA, Ali BH, Abdel Rahman HA, ElMougy SA. Effect of lead exposure on the erythrocytic antioxidant levels in goats. Journal of Veterinary Medicine Series A.

2002 Dec;49(10):531-4.

14- Bischoff K, Priest H, Mount-Long A. Animals as sentinels for human lead exposure: a case report. Journal of Medical Toxicology. 2010 Jun;6(2):185-9.

15- Muhammad S, Muhammad Y, Muti-ur-Rehman K, Anjum AA, Syed EU. Effects of experimental lead toxicity on hematology and biochemical parameters in Lohi sheep. International Journal of Agriculture and Biology. 2017;19(6):1409-13.

16- Peterson ME, Talcott PA. Small Animal Toxicology-E-Book. Elsevier Health Sciences; 2013 Aug 7.

17- Ali MA, Quinlan A. Effect of lead on globin synthesis in vitro. American journal of clinical pathology. 1977 Jan 1;67(1):77-9.

18- Zaki MS, Mostafa S, Awad I. Some studies on lead toxicity in Marino sheep. J Am Sci. 2010;6(4):128-31.

19- Sellaoui S, Boufedda N, Boudaoud A, Enriquez B, Mehennaoui S. Effects of Repeated Oral Administration of Lead Combined with Cadmium in Non Lactating Ewes. Pakistan Veterinary Journal. 2016 Oct 1;36(4).

20- Topashka-Ancheva M, Metcheva R, Teodorova S. Bioaccumulation and damaging action of polymetal industrial dust on laboratory mice Mus musculus alba II. Genetic, cell, and metabolic disturbances. Environmental research. 2003 Jun 1;92(2):152-60.

21- Chandra S, Srivastava AK, Kumar S. Pathomorphological and haematological changes in experimentally induced lead toxicity in rats.

22- Yagminas AP, Franklin CA, Villeneuve DC, Gilman AP, Little PB, Valli VE. Subchronic oral toxicity of triethyl lead in the male weanling rat. Clinical, biochemical, hematological, and histopathological effects. Toxicological Sciences. 1990 Oct 1;15(3):580-96.

23- Xintaras C. Impact of lead-contaminated soil on public health. Analysis paper, US Department of health and human services. Public Health Service. ATSDR (Agency for Toxic Substances and Disease Registry), Atlanta, Georgia. 1992.

24- Farkhondeh T, Boskabady MH, Kohi MK, Sadeghi-Hashjin G, Moin M. Lead exposure affects inflammatory mediators, total and differential white blood cells in sensitized guinea pigs during and after sensitization. Drug and chemical toxicology. 2014 Jul 1;37(3):329-35.

25- Alabbassi MG, Hussain SA, Ali SH. Therapeutic effects of melatonin in lead-induced toxicity in rats. Iranian Journal of pharmaceutical Research. 2008; 17:47-54.

26- Kar I, Mukhopadhayay SK, Patra AK, Pradhan S. Metal concentrations and histopathological changes in goats (Capra hircus) reared near an industrial area of West Bengal, India. Archives of environmental contamination and toxicology. 2015 Jul;69(1):32-43.

27- Badiei K, Nikghadam P, Mostaghni K, Zarifi

M. Effect of lead on thyroid function in sheep. Iranian Journal of veterinary research. 2009 Sep 30;10(3):223-7.

28- Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007 Apr;45(4):846-54.

29- Abdel-Wahhab MA, Omara EA, Abdel-Galil MM, Hassan NS, Nada SA, Saeed A, ElSayed MM. Zizyphus spina-christi extract protects against aflatoxin B1-intitiated hepatic carcinogenicity. African Journal of Traditional, Complementary, and Alternative Medicines. 2007;4(3):248.

30- El-Nekeety AA, El-Kady AA, Soliman MS, Hassan NS, Abdel-Wahhab MA. Protective effect of Aquilegia vulgaris (L.) against lead acetate-induced oxidative stress in rats. Food and chemical toxicology. 2009 Sep 1;47(9):2209-15.

31- Goswami K, Gachhui R, Bandopadhyay A. Hepatorenal dysfunctions in lead pollution. Journal of environmental science & engineering. 2005 Jan 1;47(1):75-80.

32- Abd El-Hameed AR, Shalaby SI, Mohamed AH, Sabra HA. Effect of oral administration of lead acetate on some biochemical and hormonal parameters during pregnancy in Baladi Goats. Global Veterinaria. 2008;2(6):301-7.