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STUDY OF THE HEALTH INDICATORS AND LIVER ENZYMES CHANGES IN WHITE RATS

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Abstract

This study was conducted with the aim of reducing the toxic and negative effects of lead. Feeding rat's different concentrations of medicinal plants (mint and parsley) powders are more efficient achieving the desired goal of the study**.** This process aims to produce an unconventional and widespread treatment for all people. A significant negative effect was observed on a group of variables through this study, such as the lead percentage in the blood increased from (4.5 µg / dl) to (292.68µg / dl). While the mint worked to reduce this rate (64.62 µg / dl at 2 % and 90.09 µg / dl at 4%) and parsley also had a positive effect in reducing the ratio of lead in rats blood to (94.14 µg / dl at 2%) and (95.58 at 4%). The effect of lead on a group of liver enzymes Alkaline Phosphatase was also studied. Rats feeding on lead led to an increase in the ratio of this enzyme from the normal level (73.8 IU / L) to a high level (197.8 IU / L), while the medicinal plants used within the current study From this effect, the mint worked to limit this rise to (Mint with 2% concentration 82.1 IU / L). As for the other mint concentration and parsley concentrations, it converged in the positive effect in reducing the lead effect to (Parsley with 2% concentration 88.4 IU / L) (Parsley with 4% concentration 86.2 IU / L), (Mint with 4% concentration 115 IU / L), As for (aspartate aminotransferase), lead had a negative effect on this enzyme, leading to a high level in the blood (lead (positive control) 242.8 U / l) compared to the standard group fodd (negative control) 91 U / l), so we find it through The current study shows that the best plants with a concentration of (Mint with 2% concentration 131.8 U / l) from the talk of reducing the effect of negative lead on this enzyme follows it in terms of preference (Parsley with 4% concentration 123.8 U / l), (Parsley with 2% concentration 121.6 U / l) and (Mint with 4% concentration 106.8 U / l). Also, the effect of these plant concentrations has been studied in reducing the negative effect of lead on the liver enzyme Alanine Transaminase where the effect of lead was evident by raising the concentration of this enzyme in the blood Rats from (negative control) 36 U / l) to (lead (positive control) 144 U / l), while the best treatment in terms of the positive effect on the enzyme (Mint with 2% concentration 38.2 U / l) came after it where I preferred the results (Parsley with 4% concentration 46 U / l), (Mint with 4% concentration 46.8 U / l), (Parsley with 2% concentration 48 U / l), respectively. The results of the study also indicated the negative effects on the weights of the internal organs of the rats (the subject of the study), as they led to an increase in the weight of the liver and kidneys and a decrease in the weight of the testicles. Mint with a concentration of (2%) worked to reduce the weight gain of the liver and kidneys resulting from lead poisoning. It also worked to reduce weight loss in the testicles, as it is closest to the normal weights of rat that were not fed lead, followed by the other concentration of mint (4%) Parsley with all concentrations (2% - 4%) in terms of preference, respectively. We conclude from the current study that the best treatment for lead poisoning among the variables studied is mint with a concentration (2%). Where mint (2%) distinguished in all results from the studies concentrations.

***Keywords*:** Lead (Pb) toxicity; Mint; Parsley; Rat liver; Rat kidneys; Rat testicles.

Introduction

Among all the problems that surround people and animals alike in our time is the various problems of pollution, whether due to the large industrial growth and the dependence on modern technology or because of the nature of his practicing work, this problem has a direct with the human's health. The problem of pollution with heavy metals is one of the most important and most serious of these problems (Mohajer *et al.,* 2019; Bi *et al.,* 2020; Goretti *et al.,* 2020). People are exposed to this type of pollution directly or indirectly, whether through food (Hashemi, 2018) or through the surrounding environment. Many studies indicate a contamination of soil, plant (Alam *et al.,* 2019; Sun *et al.,* 2019), water (Gorlachev *et al.,* 2019), marine organisms (Hao *et al.,* 2019) and air (Olawoyin *et al.,* 2019). During studies and investigations on the seriousness of these minerals, we chose lead as a second most dangerous when counting heavy metals (Łukomska *et al.,* 2017), its effects on the human health in many aspects, we would also to point out that it is more dangerous for children compared to adults including its effect on the nervous system like encephalopathy, coma, convulsions , Glycogen metabolism impaired memory, concentration, learning and reduced IQ levels (Baranowska-Bosiacka *et al.,* 2017). Alzheimer's disease, glucose intolerance, anemia, breast cancer, osteocalcin and cardiac arrest, the pb is the main problem main cause of all of these aforementioned diseases occurrence (Goher *et al.,* 2019). Also, the anisokaryosis, hydropic degeneration, cytoplasmic inclusions and swelling, nuclear vesiculation binucleation, reduction in glycogen content causative to it is Pb element (García-Niño *et al.,* 2014). As we evident that the Pb toxicity mechanism it is by reducing the antioxidant defense system of cells via depleting GSH, inhibiting sulfhydryl dependent enzymes or activity of antioxidant enzymes. Numerous research studies have been shown that supplying external antioxidants has an important role in significantly reducing the risk of heavy metals as it reduces the possibility of causing oxidative damage, as García indicated in a study on the protective effect of curcumin (García-Niño *et al.,* 2014). Winiarska-Mieczan (2015) mentioned in his study that green and black tea, white tea, and red tea have different effects to prevent the negative effects of lead. Mint has many uses, including herbal mint tea or its addition to many different foods, as spices, condiments, food additives because of their distinct fragrance also its one of the oils producing plants species (Biswas *et al.,* 2018; Asghari *et al.,* 2018). The aim of this study was to explore mint and parsley as potential functional food ingredients that have positive effects in reducing the negative effects of lead on the liver, kidneys, testes and some related blood variables.

Materials and Methods

Preparation of Medicinal plants

Peppermint was obtained from local markets in Najaf, Iraq. The stems (which accounted as 60% of the total weight of the plant) were removed. Mint leaves washed with distilled water. The perforated aluminum foil (5.50 m×7.50 m) was placed on the floor of the room. The mint leaves were spread in a thin layer and left at a controlled temperature (20 °C) for a week to confirm the final drought status. The dried leaves were powdered by milling in batches. After grinding, the samples were placed at room temperature for treatment. The two trials with different amounts of (mint and parsley) added were prepared as follows: 2-4% (120-240 g) powder was mixed with regular food for rats with a percentage of 96-98 % (5,760 -5,880 g) and added with distilled water (8.8 liters) to turn food into a paste. The mixture was transferred to a special room prepared for drying. The dough was formed in the form of longitudinal fingers (to reshape the basic form of food) in rectangular trays. Dough was cut, stirred, and left to dry for 96 h. After drying, the diet was provided to the experimental laboratory animals at 200 g per week. For parsley, the same method of mint was used.

Lead source

All groups were supplied water with lead at 0.4 (CH_3COO) $2Pb.3H_2O$ Mol.-Gew.379.34 E. Merck, Darmstadt, Germany, (Gąssowska *et al.,* 2016), except for the first group negative control by adding lead acetate (4.6 g/1000 ml) and after mixing the mixture is given for the rats. Acutely in fact, the rats were divided into six groups. The first one (T1) were supplied with regular food and distilled water, while all of the other five groups were supplied water with lead at 0.4% $(CH_3COO)2Pb.3H_2O$. The (T_2) supplied water with lead at 0.4% without any medicinal plant treatments, (T_3) provided with 4% mint in addition to regular food also (T_6) but with 2% mint while the T_4 and T_5 were provided with Parsley (4% and 2%) in addition to regular food.

Experimental design and data analysis

Thirty healthy male Sprague–Dawley rats (about 90–110 g body weight) were used. All the animals were weighed and observed in individual polypropylene cages (the dimensions of 380 mm × 200 mm × 590 mm). The rats were kept in a room at a temperature of 22 ± 2 °C and light cycle (12 h light/12 h dark) (Abdallah *et al.,* 2009; Tomaszewska *et al.,* 2015). Rat weights ranged from 90-110 g when we started the experiment, the rats were supplied with distilled water and food (standard food) in a way that enables it to reach it freely. The rats were left in the laboratory for 2 weeks for the purpose of habituation to the laboratory conditions until the start of the actual experiment.

Blood tests

First, diethyl ether (inhalation) was used before conducting rat blood tests according to Lee *et al.* (2017). After rats lost consciousness, approximately 5 mL blood samples were extracted. After that, blood samples were divided into two tubes: (z serum Sep clot Activator) tube and EDTA tube. Blood tests were performed at kavosh laboratory as well as at the Iranian National Standardization Organization.

Determination of Alkaline Phosphate:

Liver enzyme was measured by determining ALT (Alanine aminotransferase) by (DGKC) Method Commercial ALAT (GPT) Test basis:

P-Nitrophenyl phosphate $+H_2O \xleftrightarrow{ALP}$ Phosphate +P- Nitrophenol ([b] Thomas, 1998).

AST Determination:

Liver enzyme was measured by determining AST (aspartate aminotransferase) by (IFCC) commercial method ASAT (cot) Test basis:

L-Aspartate +2 -Oxoglutarate $\xleftrightarrow{ASAT}$ L-Glutamate + Oxoglutarate

Oxoglutarate + NADH $+H^+ \xleftrightarrow{MDH}$ L-Malate +NAD (Thomas, 1998).

ALT Determination:

Liver enzyme was measured by determining ALT (Alanine aminotransferase) by (IFCC) Commercial method ALAT (GPT) Test basis:

L-Aspartate +2 -Oxoglutarate $\xleftrightarrow{ASLT}$ L-Glutamate + Pyruvate

Pyruvate + NADH $+H^+ \xleftrightarrow{MDH}$ L-Lactate +NAD (Thomas, 1998).

The internal organs (liver, kidneys, testes)

After completing the experiment, the rats were anesthetized and killed with euthanasia, and then the internal organs weights were determined.

Statistical analysis

A complete random design (CRD) and topical experiments were used to study the interaction between the different concentrations of the medicinal plants involved within this study. Statistically significant differences between the averages were compared by testing the least significant difference (LSD) and the use of general statistics (2012) in the statistical analysis of the data studied.

Results and Discussion

Blood Lead level

The results of the lead effect on increasing the blood Lead level of rats and the response of rats to the different medicinal plants have presented in Fig1. Our results showed high blood lead level approximately (292.68µg/dl **)**in control group compared with rats those feed in regular food only with the lead in water. whereas, regular food group have been showed approximately (4.5 µg/dl**)**. Results of rats feed regular food supplemented with mint in addition to the salts of lead at same time showed a highly positive effect to reduce lead attraction inside the body in both concentrations 2% and 4% (64.62 µg/dl and 90.09 µg/dl) respectively. Tomaszewska *et al.* (2015) mentioned different result of tea effect on negative lead effect on the rat's blood Lead level. Winiarska, (2015) used Poland liquid tea instead of whole Mint powder to study the potential protective effect of green, black, red and white tea infusions against adverse effect of cadmium and lead during chronic exposure. A rat model study showed different result of tea effect on the rat's blood Lead level. Mehana, *et al.* (2012) used Egyptian liquid green tea instead of whole Mint powder to study the effects of green tea extract on lead induced liver toxicity in rats. The results indicated similar to that of the tea effect on the rat's blood Lead level in the current study. However, using additional amount of Mint 4% concentration and Parsley at 2 % concentration in the current study resulted in weaker effect when compared to the Mint at the lower level (2 %) concentration. This is the first study attending the effect of whole Mint powder addition in the rat food to resist lead contaminants Figure1. Rats which feed with toxic lead and at the same time provided by parsley powder showed decrease of lead level in blood compared with rats those subjected to lead toxic without any of medicinal plant. Furthermore, the lower level (2%) of parsley (94.14 µg/dl) gave better in reducing lead intake by the body, whereas, high level 4 % works to reduce the intake of lead in the body but the effect may be less than the concentration of 4% (95.58 µg/dl). This is considering the first study using the parsley powder to reduce the attraction of lead to the body Fig. 1. On other hand, We can refer to use the parsley with low concentration 2% which have a good effect on the rat's blood Lead level when compared to rats feed with Parsley and the same time treated with lead at high level Parsley (4% concentration). All lead concentrations for this study differed with the results of (Dearth *et al.*, 2002), but indicated in part a similar effect of lead on the liver.

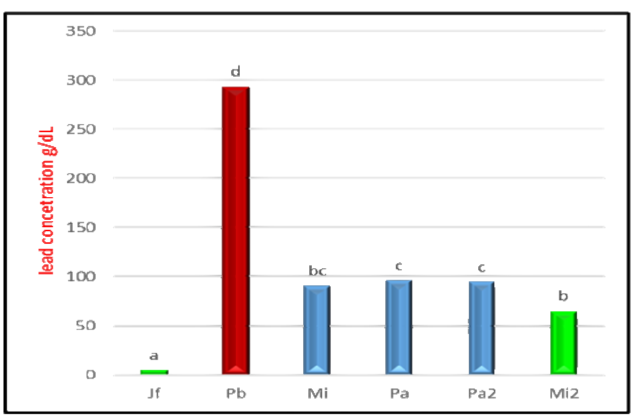


Fig. 1 : The lead concentration of the rats blood. JF = food negative control, Pb =Lead positive control, Mi1 Mint at 4% concentration, Mi2 mint at 2% concentration, Pa1 Parsley at 4 % concentration, Pa 2 Parsley at 2% concentration. In each Colum: (a, b, c). Means indented with different letters are significantly at the specified confident level with significantly by Duncan's multiple range test p > 0.05.

Alkaline Phosphatase

The results of lead effect on increasing the Alkaline Phosphatase protein level of rats and the response of rats to the different medicinal plants have presented in Fig. 2 and Table 1. The results have been showed that a significant lead effect on increasing the blood Alkaline Phosphatase level of rats (lead positive control) (197.8 IU/L) compared to the standard group (negative control) (73.8 IU/L), in addition the results have been shown that using 2% Mint (82.1 IU/L) gave positive highly effect in inhibition the lead effect on increasing the blood Alkaline Phosphatase level in rats. Also, the results of treatment Parsley with 2 % concentration (88.4 IU/L), Parsley with 4 % concentration (86.2 IU/L) and Mint with 4% concentration (115 IU/L) have been shown moderate positive effect in inhibition the lead effect on increasing the blood Alkaline Phosphatase level of rats as shown in Figure 2. Our results showed a significant difference (<.0001).

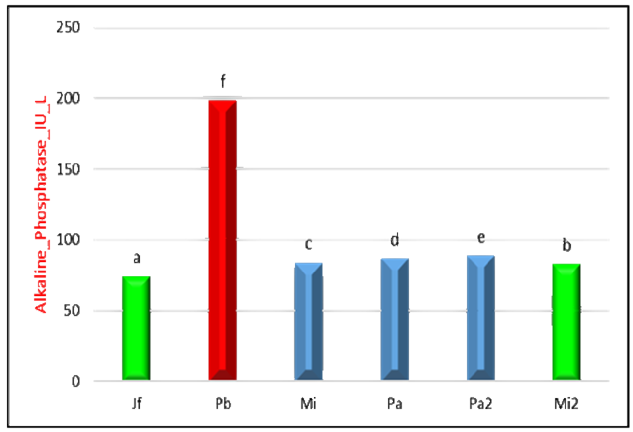


Fig. 2 : Shows the lead effect on the rats blood Alkaline Phosphatase level feed by different medicinal plants.Pa1 (Parsley with 4 % concentration), Pa 2 (Parsley with 2% concentration), JF = fodd(negative control), Pb =Lead (positive control), Mi1 (Mint with 4 % concentration), Mi2 (mint with 2% concentration). In each column Means indented with different letters are significantly at the specified confident level : (a, b, c,d,e). With significantly by Duncan's multiple ranges test (p > 0.05).

Aspartate Transaminase protein level (AST)

The results of lead effect on increasing the Aspartate Transaminase protein level of rats and the response of rats to the different medicinal plants have presented in Fig.3 and Table 1. The results have been shown that a significant lead effect on increasing the blood Aspartate Transaminase level of rats (positive control) (242.8 U/l) compared to the standard group (negative control) (91 U/l), while the results have been showed that mint with 2% concentration (131.8 U/l) have positive highly effect in inhibition the lead effect on increasing the blood Aspartate Transaminase level in rats. Moreover, the results of treatment with Parsley 4% concentration (123.8 U/l), Parsley with 2 % concentration (121.6 U/l) and Mint with 4% concentration (106.8 U/l) showed moderate positive effect in inhibition the lead effect on increasing the blood Aspartate Transaminase level of rats as show in (Figure 3). All the results showed a significant difference (<.0001).

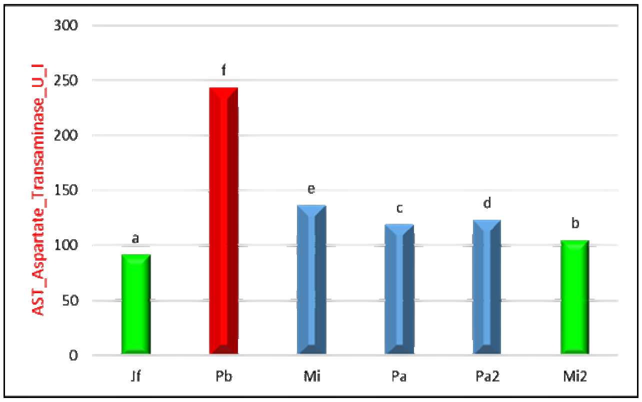


Fig. 3 : Shows the lead effect on the rats blood Aspartate Transaminase. Pa1 (Parsley with 4 % concentration), Pa 2 (Parsley with 2% concentration), JF = fodd (negative control), Pb =Lead (positive control), Mi1 (Mint with 4 % concentration), Mi2 (mint with 2% concentration). In each column Means indented with different letters are significantly at the specified confident level :(a, b, c,d,e). With significantly by Duncan's multiple range test ($p > 0.05$).

Alanine Transaminase protein (ALT)

The results of the lead effect on increasing the Alanine Transaminase protein level of rats and the response of rats to the different medicinal plants have presented in Fig4 and Table 1. The results have been showed that a significant lead effect on increasing the blood Alanine Transaminase level of rats (positive control) (144 U/l) compared to the standard group (negative control) (36 U/l), while the results have been shown that the following treatment Mint with 2% concentration (38.2 U/l) have positive highly effect in inhibition the lead effect on increasing the blood Alanine Transaminase level in rats. The results of treatments with Parsley 4 % concentration (46 U/l), Mint with 4% concentration (46.8 U/l) and Parsley with 2 % concentration (48 U/l) showed moderate positive effect in inhibition the lead effect on increasing the blood Alanine Transaminase level of rats as shown in Figure (4) . All the results showed a significant difference (<.0001).

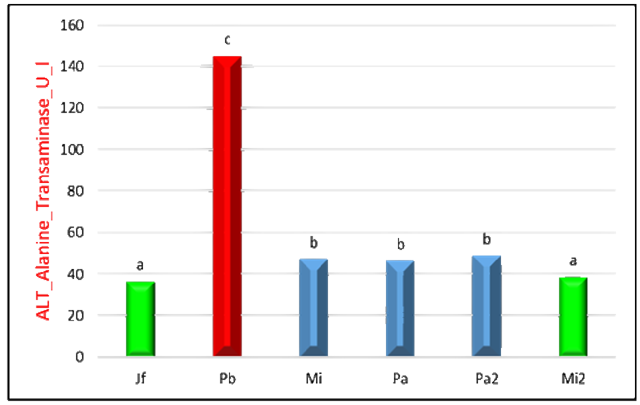


Fig. 4 : Shows the lead effect on the rats blood: Alanine Transaminase level. JF = fodd (negative control), Pb =Lead (positive control), Mi1 (Mint with 4 % concentration), Pa1 (Parsley with 4 % concentration), Pa 2 (Parsley with 2% concentration), Mi2 (mint with 2% concentration). In each column Means indented with different letters is significantly at the specified confident level :(a, b, c). With significantly by Duncan's multiple ranges test ($p > 0.05$).

Table 1 : Shows the lead effect on the rats blood: Blood Lead concentration, Alkaline Phosphatase, AST Aspartate Transaminase, ALT Alanine Transaminase level feed by different medicinal plants.

Treatment		Parameters			
		Blood Lead concentration µg/dl	Alkaline Phosphatase IU/L	AST Aspartate Transaminase U/l	ALT Alanine Transaminase U/l
Jf		$4.5^{a}\pm0$	73.8a±0	$91^{a}\pm0$	$36^{a}\pm0$
Mi2		$64.62^{b}\pm0$	$82.1^{b}\pm0$	$104.2^{b}\pm0$	$38.2^{a}\pm0$
Pa2		$94.14^{c}\pm0$	$88.4^{e}\pm0$	$122.6^{d}\pm0$	$48.6^{b}\pm0$
Mi1		$90.^{bc}\pm0$	$83.3^{c}\pm0$	$136.2^{e}\pm0$	$46.8^{b}\pm0$
Pa1		$95.58^{c}\pm0$	$86.2^{d}\pm0$	$118.8^{c}\pm0$	$46^{b}\pm0$
Pb		$292.68^{d}\pm0$	$197.8^{f}\pm0$	$242.8^{f}\pm0$	$144^{c}\pm0$
P-Value	Treatment	<.0001	<.0001	<.0001	<.0001
	Time	<.0001	<.0001	<.0001	<.0001
	T*Ti	<.0001	<.0001	<.0001	<.0001

Table shows the lead effect on the rats blood: Alkaline Phosphatase, AST Aspartate Transaminase, ALT Alanine Transaminase level. Mi1 (Mint with 4% concentration), Mi2 (mint with 2% concentration), Pa1 (Parsley with 4 % concentration), Pa 2 (Parsley with 2% concentration), (JF = food (negative control), Pb =Lead (positive control). Ti (Time), T (Treatment). In each column Means indented with different letters are significantly at the specified confident level :(a, b, c, d, e, f). With significantly by Duncan's multiple range test ($p > 0.05$).

The internal organs (liver, kidneys, testes):

The effect of lead poisoning on the internal organs and according to the treatment in terms of the effect of the medicinal plant on the possibility of reducing the negative effect of lead on the internal organs was studied:

Liver

The continuous exposure to lead acetate during the trial period led to an effect on the weight of the liver and this is proportional to the results of liver enzymes (AST, ALT, Alkaline Phosphatase) for the same study and these results generally came to some extent similar to the results of the study of Winiarska-Mieczan, 2015). The results of the current study indicated that the mint with (2% concentration) had a great role in reducing the liver weight resulting from liver toxicity with aqueous acetate Lead (6.75 g) compared to the first treatment which is only feed with lead without treatment (12.259g) while the first treatment which is just feed without any lead was 6.423g (Figure 5) and (Table 2). Also the treatment of 4% mint and 4% and 2% Parsley gave somewhat positive results (136.2g, 118.8g and 122.6g) Respectively .

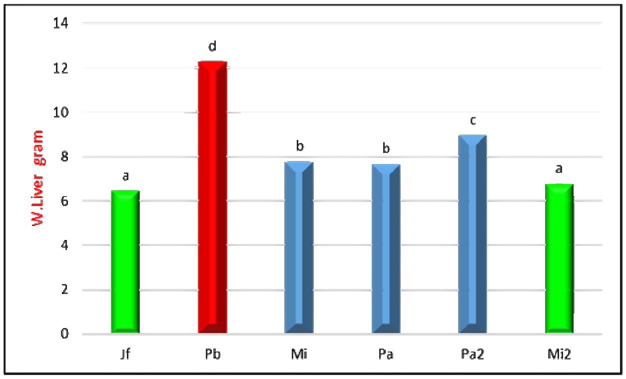


Fig. 5 : Shows the lead effect on the Rat liver weight. JF = food (negative control), Pb =Lead (positive control), Mi1 (Mint with 4 % concentration), Pa1 (Parsley with 4 % concentration), Pa 2 (Parsley with 2% concentration), Mi2 (mint with 2% concentration). In each column Means indented with different letters is significantly at the specified confident level :(a, b, c). With significantly by Duncan's multiple ranges test (p > 0.05).

Testis and Kidney

Kidney: Chronic exposure to lead led to the enlargement of the rat's kidneys which were involved in this experiment, which in turn led to an increase in the weight of kidneys. The results of this study showed a noticeable increase in the weight of the kidneys members of the animals that were provided with lead in water without any treatment T2 (3.48g) while the feeding process for other groups of the same current experiment with medicinal plants under study led to a minimize of the negative effect of lead. The results of the study indicated the role of mint (4% and 2%) in reducing the overweight of the kidneys (2.08g and 2.095g) respectively, Where the weight results of these treatments were approximated within the normal weight of the rats that were provided with normal food (without lead 1.823g). Parsley (4% and 2%) also had a positive effect in reducing the increase caused by lead, as it led to a decrease in the kidney weight of the animals involved in the current study to (2.31g and 2.33g) respectively. This is considering the first study of the effect of lead on kidney weight. Figure 6 and Table 2.

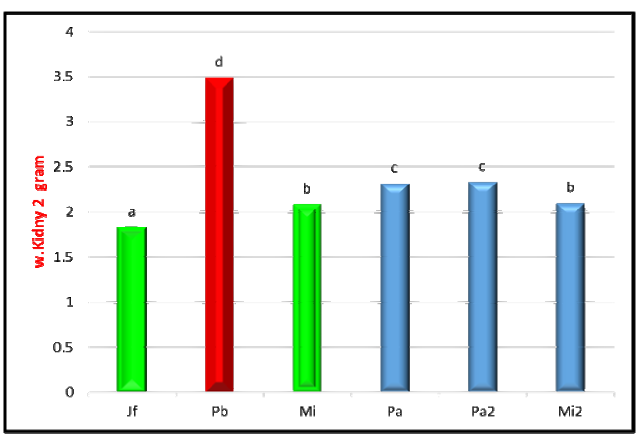


Fig. 8 : shows the lead effect on the Rat Kidney weight. Pa1 (Parsley with 4 % concentration), Pa 2 (Parsley with 2% concentration), Mi2 (mint with 2% concentration), Mi1 (Mint with 4 % concentration), JF = food (negative control), Pb =Lead (positive control). In each column Means indented with different letters is significantly at the specified confident level :(a, b, c). With significantly by Duncan's multiple ranges test (p > 0.05).

Testis: Chronic exposure to lead led to decrease the testicular weight of rat that participated in this experiment. The results of this study showed a marked decrease in the weight of the testicle members of animals that were supplied with lead in water without any treatment T2 (3.249 g). While the feeding process for other groups of the same current experiment with medicinal plants under study led to reducing the negative effects of lead. The results of the study clarified the role of 2% mint in increasing testicular underweight (4.303 g), as the results of these treatments were approximated similar to normal weight for rat that were provided with normal food (without lead) (4,781 g). Mint (4%) and parsley (4% and 2%) also had a positive effect in reducing the weight loss caused by lead, which led to an increase in the testicular weight of animals participating in the present study to (3.542g) and (3.437g and 3.854g) respectively.

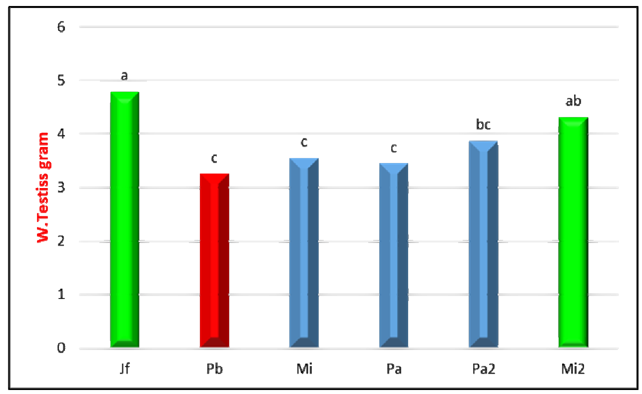


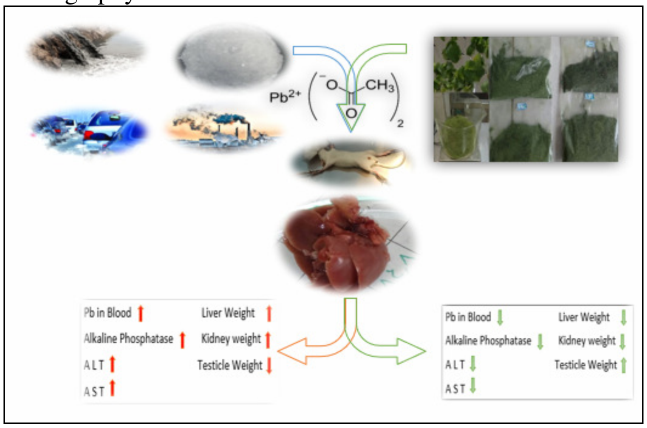
Fig. 7 : Shows the lead effect on the Rat Testis weight. Pa1 (Parsley with 4 % concentration), Pa 2 (Parsley with 2% concentration), Mi2 (mint with 2% concentration), JF = food (negative control), Pb = Lead (positive control), Mi1 (Mint with 4 % concentration). In each column Means indented with different letters is significantly at the specified confident level :(a, b, c). With significantly by Duncan's multiple ranges test (p > 0.05).

Table 2 : Shows the lead effect on the rat's weight of the Liver, Testis and Kidney feed by different medicinal plants.

Treatment		Parameters		
		Liver weight gram	Testis weight gram	Kidney weight gram
Jf		6.423^{a}	4.781^{a}	1.823^{a}
Mi2		6.75^{a}	4.303^{ab}	2.095^{b}
Pa2		8.936^{c}	3.854^{b}	2.33^{c}
Mi1		7.728^{b}	3.542^{c}	2.08^{b}
Pa1		7.633^{b}	3.437^{c}	2.311^{c}
Pb		12.259^{d}	3.249^{c}	3.48^{d}
P-Value	Treatment	<.0001	<.0001	<.0001
	Time	<.0001	<.0001	<.0001
	T*Ti	<.0001	<.0001	<.0001

Mi1 (Mint with 4 % concentration), Mi2 (mint with 2% concentration), Pa1 (Parsley with 4 % concentration), Pa 2 (Parsley with 2% concentration), (JF = food (negative control), Pb =Lead (positive control). Ti (Time), T (Treatment). In each column Means indented with different letters are significantly at the specified confident level: (a,b,c,d,e,f). With significantly by Duncan's multiple ranges test (p > 0.05).

Photography Abstract



Highlights

1. The negative effect of lead on liver enzymes
2. The extent to which the weight of the internal organs was affected by lead

Resist the lead negative impact by different concentrations of mint and parsley

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