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Original Research Article

The Protective Role of Date Pits Extract *Phoenix dactylifera* on the Function of the Male Reproductive System of Rats Treated with Lead Acetate

Huda Saber Al-Hamdany[1]*

[1]Department of Biology, College of Education for Pure Science, University of Mosul, Iraq

***Corresponding Author:** Huda Saber Al-Hamdany
Department of Biology, College of Education for Pure Science, University of Mosul, Iraq

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Abstract: ***Aim***: Evaluation of the toxicological effect of lead acetate (50 mg/kg of body weight) on some functions of the male reproductive system and evaluating its treatment with aqueous extract of date pits (200 mg/kg of body weight) for (20) rats for a period (30) days. The study included four groups: the first group was the control, the second group was treated with lead acetate, the third group was treated with aqueous extract, and fourth group was treated with lead acetate and aqueous extract, Body weight was measured before and after the end of the experimental periods, the weights of the testes and epididymis after dissection, and blood was drawn from them for the purpose of measuring the hormones FSH, LH, and numbers of live and dead sperm in each group, the results showed a decrease in the average weights of the body, testes and epididymis, a and concentration of both hormones and the number of live sperm, and an increase in the percentage of dead and deformed sperm in the second group compared to the control group. As for the third group, the results showed an opposite effect to the second group. As for the fourth, the toxic effect of lead acetate is less when it is dosed to rats with the extract, we conclude from this that lead acetate has a clear toxic effect and causes oxidative stress when rats are exposed to it alone. This effect is reduced when rats are exposed to lead acetate and an aqueous extract.

Keywords: Sperm, Lead Acetate, Rats, Oxidative Stress.

INTRODUCTION

The cells of living organisms are exposed to various types of environmental pollutants, most of which are harmful to these organisms in most stages of their growth and development, and among these pollutants is lead [1]. It is one of the most abundant heavy metals, and human exposure to it is abundant due to its used in lead acid batteries, coloring agents, paints, smelters, and printing presses and is metallically alloyed as shielding material. It is a toxic metal acting various organs and developing fetus [2].

Studies have shown that exposure to 3.5 mg of it for several months results in lead poisoning and the formation of free radicals, which affects the DNA membranes. In addition, the mitochondria, which changes its function, causes oxidative stress and reduces the defense mechanism [3], and affects the respiratory system (lung), the circulatory system (blood and blood vessels), the reproductive system (testes, sperm), and the nervous system [4].

The date palm (*Phoenix dactylifera*), which originates in the Middle East, plays an important role in the economic and social life of the world's population (5). The dates nuclei have multiple uses, including a food source because they are rich in protein, fat, and dietary fiber, in addition to containing antioxidants such as phenolic compounds, including (flavonoids, phenolic acids and other compounds, they are also used as a food source (feed) for sheep, cows and poultry [6]. In the food industry, date pits have been utilized experimentally to preserve and enhance the stability during the shelf life of beef burgers and could improve the composition of bioactive compounds (fiber and phenolic content) of such foods

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[7] Phytochemically, date pits are a rich source of polyphenols and flavonoids [8]. Pharmacologically, date pits in cells, animals, or humans possess antioxidant [9], anti-inflammatory antidiabetic, and antibacterial properties [10].

MATERIALS AND WORKING METHODS

Preparation of the Plant Extract

The aqueous extract of date plant nuclei was prepared according to the modified method of [11], as the nuclei were obtained first, then cleaned and washed by tap water, then dried for 2-3 days at room temperature and then ground by an electric grinder, after which a sifting process was carried out by means of a sieve whose holes diameter is (2.36) mm. Then, 50 g of plant powder is taken and mixed with 1 liter of distilled water, kept for 12 hours in the refrigerator for the purpose of soaking, then the mixture is filtered through several layers of gauze. It is filtered again by a Buechner funnel using filter papers (Whatmann No-1) with an emptying Vacuum device to get rid of non-pulverized parts and fibers. Thus, the crude aqueous extract was obtained and then stored in glass bottles with tight lids in the refrigerator at a temperature of 4°C until used in the study.

Lead Acetate: Rats were treated orally (50 mg/kg of body weight) of lead acetate by tube feeding for 30 days [12].

Animals Used

In this current study [20], adult white rats of the strain (Sprague dawey) were used, aged [2, 3], months and in good health condition. Their weight ranged between (200-220 g). They were obtained from the College of Veterinary Medicine / University of Dohuk, placed in plastic cages with metal mesh lids for breeding rats with dimensions (25*19*21) cm, and attention was paid to the cleanliness and sterilization of cages from time to time, as well as changing the sawdust in each week. The rats were also given water and ration of 24.5% barley, 30% wheat, 22.5% corn, 5.2% soybeans, 4.5% table salt, 0.13% limestone, 7.22 animal protein concentrate, edible oil at an amount of 5.7%, 1% of powder milk, and 50 gm of an anti-fungal substance. All animals were left for a period of two weeks for the purpose of removing the effects of shock during transfer to the kidney and acclimatization under standard laboratory conditions in terms of ventilation and temperature, which were around 26±2°C, and a natural light cycle (12 hours light and 12 hours of darkness) with water given throughout the experiment period [13].

Experience Design

The animals were divided into four groups; each group comprises (5 rats) with close weights. The doses used for the materials in the experiment were as follows for a period of 30 days:

1. The control group was treated with drinking water + food.
2. The second group treated with (50 mg/kg of body weight) of lead acetate by oral administration
3. The third group was fed with (200 mg/kg of body weight) of date pits extract by oral administration
4. The fourth group was treated with lead acetate + and aqueous extract of date pits

Calculation of Body Weight and Organs

All rats were weighed by a special animal weighing scale (Seca, Germany) at the start of the experiment before treatment, and then weighed at the end of the specified periods.

The rats were anesthetized with chloroform, dissected after withdrawing the required blood from each animal, then the parts to be studied were separated, the gained or lost weights were weighed using a sensitive electronic scale (Seca, Sartorius, Germany).

Calculation of the Percentages of Live, Abnormal and Dead (Abnormal) Sperm

All rats were anesthetized after the end of the time specified for each experiment including the rats that died during the experiments after being anesthetized using chloroform [14].

Rats were transferred to the autopsy dish, then its front and rear limbs are fixed with precise pins. Their stomachs are opened with sharp scissors at the bottom of the abdominal skin area, by making a longitudinal incision starting from the back area to the front area. Then towards the extremities, Then, the outer parts of the skin were removed, the testes and epididymis were extracted after removing all the connective tissues and related fatty materials, placed in a petri dish containing physiological solution (0.9% NaCl), and the seminal fluid was obtained from the epididymis based on the Method of [15], by squeezing the contents of the tail of the epididymis after cutting it into a clean, dry watch bottle, then placing a small drop of semen on a clean, dry glass slide, and adding a drop of Eosin-Nigrosine dye to it. The drops were mixed and left for half a minute. A semen swab was prepared. All the used glass slides were placed after drying in the incubator at 37°C (Karl-Klob, Memmert, Germany). After the swab was completely dry, it was examined with the objective lens with a magnification power of 40X [16], then the percentages of live sperms (that did not take the dye) and the percentages of abnormalities were calculated in 100 sperms from each slide. The percentages were extracted according to the (17) using the following equation:

Percentage of live sperm = (dyed sperm number/ drawn sperm count) *100
As for the Percentage of natural sperm = the number of natural sperm/ the number of sperm from the calculated number *100

Obtaining Blood Samples

After the end of the specified period of the experiment, they were anesthetized by chloroform, and blood samples were obtained by cardiac stabbing. Approximately 4-5 ml of blood was collected and placed in test tubes. They were left for about a quarter of an hour in a water bath at 37 °C, and then the blood was separated by a centrifuge at a speed of 3000 cycle/minute to obtain the blood serum. The serum that was placed in special test tubes (Alappendorf) was separated. Then, it was preserved at -20 C) until the measurement of the hormone FSH, LH) was used according to the circulars of the American manufacturer based on the principle of antibody and antigen using a special kit from Biolabo.

Statistical Analysis

Statistical analysis of data and results was carried out using SPSS 11.5 program for Windows. The comparison of means was done using Anova-one way by Analysis of variance. For the variables in the criteria under study for each group separately, using Duncan's test at the level of significance (D<0.05) to compare between different exposure periods [18].

RESULTS AND DISCUSSION

1-Body Weights and Testes and Epididymis Weights

The results table (1) show that the administration of lead acetate to rats with a concentration of (50mg/kg of body weight) for a specific period (30 days) had a clear effect on body weights, testes and epididymis. The results indicate a significant decrease at the level ($P < 0.05$) in those weights in the experimental group compared to the weights of the control group and the other group. The results of our study agree with those conducted by [19], who state that the decrease in lead toxicity is due to the level of antioxidants present in the body as a whole and all organs in particular, which led to the formation of free radicals and thus led to a decrease in body and organs weight (20). While Liu, X *et al*., [21], indicate that the daily dose of lead acetate to rats caused a significant decrease in body weight as well as other parts of the body. It led to damage to the tissues of the testes and the composition of the epididymis. The study also shows that dosing animals with lead acetate for 4 weeks led to a loss of 12.4% of body weight and various organs. This is caused by the animal's loss of appetite for food and consequently losing weight and the accumulation of toxic substances (free radicals) in the organs, which leads to significant damage to the organ and loss of weight (22).The results in table (1) show that the rats dosed with aqueous extract of date nuclei at a concentration (200mg/kg of body weight) and for a specific period (30 days) had a clear effect on body weights, testes and epididymis. The results indicate a significant increase at the level ($P < 0.05$) in those weights in the experimental group compared to their weights for the control group and the other group which works to curb free radicals and toxic substances in the body that hinder the building of cells and thus increase the weight of the body and all other organs (23).The results of this study also show a significant increase in body weights, testes and epididymis in the group dosed with lead acetate and aqueous extract of dates nuclei compared with the group dosed with lead acetate. The results agree with other studies, as they show that dosing animals with cadmium chloride and aqueous extract of dates nuclei led to an increase in animal body weights compared to the weights of animals dosed with cadmium chloride alone, and this effect is caused by the presence of antioxidants, including phenols , as it was found that this extract has a high percentage of phenolic substances and anti-oxidants, including vitamin A and C, which prevent the formation of free radicals, which leads to curbing the destruction of different cells of the body and thus working to increase the weight of organs and consequently the body as a whole [24].

Table 1: Shows the body weights, testes, and epididymis in experimental rats

Parameter Group	Epididymis weight (g/100g of body weight)	Testis weight (g/100gof body weight)	Body weight (g/100gof body weight)
Control	44.1+1.54	72.0+3.43	204.38 + 5.74
Lead acetate	25.8 + 0.20	31.1+ 0.83	134.49 + 4.08
Aqueous extract of date pits	58.6+ 2.23	74.0+3.51	210 + 5.46
Leadacetate+ Aqueous extract of date pits	39.6+1.54	63.2+ 3.07	186 + 2.71

2-The Effect of Hormones

The results of the current study in table (2) show that feeding rats with lead acetate at a concentration (50mg/kg of body weight), and for a specific period (30 days) had a clear effect on the level of FSH, LH. It led to a significant decrease at the level ($P < 0.05$) in the concentration of each of the two hormones in the experimental group compared to the control group and the other group. This may be due to the toxicity of lead, which led to a decrease in the level of reproductive hormones, including androgens in the blood serum. This is an important indicator of reproductive hormones. If a significant decrease was observed in each of the hormones, it explains the mechanism of lead poisoning [5]. Leiva *et*

al., [25], show that the level of the two hormones in the blood serum was decreased in the rats treated with lead. It was also shown that FSH decreased by 80%, while LH decreased by 32% in the animals treated with lead. The results also show that the rats dosed with aqueous extract of dates nuclei at a concentration (200mg/kg of body weight) for a specific period (30 days), had a clear effect on the level of FSH, LH, which led to a significant increase ($P < 0.05$) in the concentration of each of the two hormones in the experimental group compared to the control group and the other group as shown in the table (2). The results of our study agree with [26], who show that the aqueous extract of the nuclei contains many antioxidants, which led to a rise in reproductive hormones. It also shows [27], an increase in the level of FSH and LH hormones in the blood serum of animals treated with date kernel extract, as well as an increase in the number of sperm due to the removal of free radicals by the action of the antioxidants that make up the extract. The results of this study indicate a significant increase in the concentration of FSH and LH in the group dosed with lead acetate and aqueous extract of compared with the group dosed with lead acetate alone, and a slight decrease compared to the group dosed with dates nuclei extract alone. The present study reveals that the increase in the concentration of hormones is believed to be due to the removal of free radicals formed by the action of lead acetat.

Table 2: Shows the LH &FSH Levels in experimental rats

Parameter Group	LH mlU/mL	FSH mlU/mL
Control	0.86 + 2.08	0.48 +0.01
Lead acetate	0.08 +0.01	0.21 +0.01
Aqueous extract of date pits	1.50 +0.09	0.53 +0.017
Lead acetate + Aqueous extract of date pits	0.48 +0.01	0.38 +0.08

3- Sperm Count

The results of our current study in table (3) show that rats dosed with lead acetate at a concentration (50mg/kg of body weight) for a specific period (30 days) had a clear effect on the number of live (normal) and dead (abnormal) sperms. The results indicate a significant decrease at the level ($P < 0.05$) in the number of live animals and a rise in dead animals in the experimental group compared to their weights for the control group and the other group. The reason may be the toxicity of lead, which caused a defect in the stages of sperm formation and its incompleteness, and thus its death. The results of this study agree with [28]. As it was shown that lead toxicity reduced the activity of steroidal enzymes, ascorbic acid, and glutathionine content, which caused degenerative changes in the stages of sperm formation and a decrease in the number of live sperms. The studies also show that the treatment of animals with lead acetate for high doses and for long periods led to a significant decrease in the percentage of live sperm and a high percentage of distorted and dead ones in addition to causing damage to Sertoli cells [27]. The study shows that long-term treatment of rats with lead led to degenerations in Leydig cells and a defect in the formation of germ cells due to disorders in the formation of steroids [29]. The results of our study in table (3) show that rats dosed with aqueous extract of dates nuclei at a concentration (200mg/kg of body weight) and for a specific period (30 days) had a clear effect on sperm count. The results indicate a significant increase ($P < 0.05$) in the number of live animals compared to dead animals in the experimental group treated with aqueous extract of dates nuclei compared to the control group and the other experimental group. The reason may be due to the active ingredients of the extract from the antioxidants that enter the stages of sperm formation and that work to curb free radicals and toxic substances that may cause a decrease in the number of sperms and thus their death [30]. The results of our study also show a significant increase in the number of live sperms and a decrease in the number of dead sperms in the group dosed with lead acetate and aqueous extract of dates nuclei compared with the group dosed with lead acetate alone. The results of our study agree with what was stated by [31], that the increase in the number of live sperms due to the aqueous extract of dates nuclei caused the conversion of androgens in the testes to estrogen by the action acomplex of enzymes called (aromatase), which caused an increase in the production of natural sperms. On the other hand, the date kernel extract is rich in antioxidants, including flavonoids, which plays a great role in the formation of free radicals, causing an increase in the activity of sex hormones, which play a role in the stages of sperm formation and a decrease in sperm death and distortion [32].

Table 3: Shows Percentage of live or no sperm from epididymis head% in experimental rats

Parameter Group	Percentage of non-living sperm from epididymis head%	Percentage of live sperm from epididymis head%
Control	6.9 + 8.40	79.8+ 4.31
Lead acetate	9.6+5.83	26.9+4.36
Aqueous extract of date pits	7.43 +6.01	86.0 +2.08
Lead acetate + Aqueous extract of date pits	2.50 +2.36	47.1 +4.46

CONCLUSION

Our study showed that the date pits extract *Phoenix dactylifera* led to an increase in the weights of the body weight and male reproductive organs of rats, as well as an increase in sperm and concentration of the hormone FSH and LH, compared to the group treated with lead acetate, (50 mg/kg of body weight)

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