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Olive leaf extract effects and lead acetate accumulation on hematological parameters in adult rabbits

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تأثيرات مستخلص أوراق الزيتون وتراكم أسيتات الرصاص على المؤشرات الدموية في الأرانب البالغة

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Abstract:
Lead (Pb) is identified as a toxic heavy metal at high concentrations, where the global blood level for Pb intoxication is 10 µg/dl. This study aimed to assess the effect of olive leaf extract against the accumulation of lead acetate in adult rabbits on hematological parameters and weight. Experiments were conducted in a special farm in eastern Libya; therefore, sixteen adult rabbits were allocated into 4 groups with 4 in each: (G1) control untreated, (G2) supplied with 40 mg/kg body weight (BWT) of lead acetate (PbAc), (G3) supplied with 200 mg/kg BWT of olive leaf extract (OLE), and (G4) supplied with PbAc + OLE with the previous concentrations. The results of the study showed that after two months of various treatments to the animal groups under study, despite the negative effect of lead acetate accumulation on the animals' weights and hematological parameters such as haemoglobin concentration (Hb), red blood cell count (RBCs) and white blood cell count (WBCs) compared to the control group, the use of the aqueous extract of olive leaves had a positive effect, as it improved the animals' weights and all blood parameters when dosed alone and also reduced the negative effects of lead acetate accumulation.

Keywords: lead acetate, rabbits, haematological, olive leaf extract, Hb, RBCs, WBCs.

الملخص:
يُعد الرصاص (Pb) معدن من المعادن الثقيلة السامة عند التركيزات العالية، حيث يبلغ مستوى الرصاص في الدم الذي يُسبب التسمم 10 ميكروجرام/ديسيلتر. هدفت هذه الدراسة إلى تقييم تأثير مستخلص أوراق الزيتون على تراكم أسيتات (خلات) الرصاص في الأرانب البالغة، وذلك من خلال دراسة المؤشرات الدموية والتغير في وزن الأرانب. أُجريت التجارب في مزرعة خاصة في شرق ليبيا؛ حيث تم توزيع ستة عشر أرنبًا بالغًا على أربع مجموعات، بواقع أربعة أرانب في كل مجموعة: (المجموعة الأولى) مجموعة ضابطة غير معالجة، (المجموعة الثانية) مجموعة مُعالجة بـ 40 ملجم/كجم من وزن الجسم من أسيتات الرصاص(PbAc) ، (المجموعة الثالثة) مجموعة مُعالجة بـ 200 ملجم/كجم من وزن الجسم من مستخلص أوراق الزيتون(OLE) ، و(المجموعة الرابعة) مجموعة مُعالجة بـ PbAc + OLE بالتركيزات المذكورة سابقًا. أظهرت نتائج الدراسة أنه بعد شهرين من تطبيق المعاملات المختلفة على مجموعات الحيوانات قيد الدراسة، وعلى الرغم من التأثير السلبي لتراكم أسيتات الرصاص على أوزان الحيوانات ومعايير الدم، مثل تركيز الهيموجلوبين (Hb) وعدد كريات الدم الحمراء (RBCs) وعدد كريات الدم البيضاء(WBCs) ، مقارنةً بالمجموعة الضابطة، إلا أن استخدام

المستخلص المائي لأوراق الزيتون كان له تأثير إيجابي، حيث حسّن أوزان الحيوانات وجميع معايير الدم عند استخدامه بمفرده، كما قلل من الآثار السلبية لتراكم أسيتات الرصاص.

الكلمات المفتاحية: أسيتات الرصاص، الأرانب، الدم، مستخلص أوراق الزيتون، الهيموجلوبين، كريات الدم الحمراء، كريات الدم البيضاء.

Introduction:

Heavy metals (HMs) are hazardous to human health, are among the harmful elements that pollute the air, land, food, and water because of engineering, traffic, agriculture, and garbage. The most prevalent heavy metals that can damage humans, plants, animals, and fish include lead (Pb), mercury, cadmium, arsenic, and chromium. Furthermore, these HMs have a variety of harmful effects on diverse tissues. Even at low concentrations, heavy metals (HMs) have neurotoxic and carcinogenic effects on people, making them the most dangerous environmental contaminants (Melebary, and Elnaggar, 2023). According to WHO, 2011 Pb exposure is thought to be the cause of 0.6% of diseases worldwide, particularly in developing nations. Globally Pb was the reason for about 540 thousand deaths during 2016 (Mu *et al*., 2019). This is because lead is utilized in numerous industrial operations, such as the production of fuel, metal products, ammunition, batteries, lead-based paints and coatings, X-ray shielding devices, toys, and cosmetics (Nag and Cummins, 2022). Lead (Pb) has been identified as a toxic element at high concentrations, and its use has been connected to widespread environmental harm and health problems globally (Li *et al*., 2019). Lead is a cumulative toxin that can harm the kidneys, the neurological system, and blood circulation, especially in young children and fetuses (Guo et al., 2018). The kidney, liver, brain, and bones all contain lead (Zwolak *et al*., 2019). It may eventually accumulate in teeth and bones due to long-term human exposure. Additionally, by inducing organ tissues to undergo apoptosis, lead may influence children's brain and intellectual development (Mani *et al*., 2019). The integument may absorb Pb, but it is primarily absorbed from the respiratory and digestive system. It can lead to neurological, pulmonary, renal, immunological, hematological, cardiovascular, skeletal, embryonic, and reproductive diseases because it disrupts the equilibrium of oxidant-antioxidant agents and induces inflammation in different tissues (Usman *et al*., 2022; Melebary, and Elnaggar, 2023).

Nearly all the world's plant species have strong antioxidant capabilities, and two-thirds are thought to have therapeutic use. Because they are effective, have fewer side effects, and contain phytochemical ingredients that effectively cure a wide range of illnesses, medicinal plants have many uses. Effective medicinal plants are used to treat certain illnesses, preventing or reducing infections; the demand for natural products has skyrocketed worldwide (Usman *et al*., 2022). Approximately 85% of people in developing countries are treated with folk medicine, especially herbal remedies. The detrimental effects of synthetic drugs are the primary driving force for these requests. Consequently, the importance of medicinal herbs has increased significantly. Despite the lengthy history of using plant medicine in conventional treatment systems, identifying active herbal ingredients can lead to new potential therapeutic uses and the production of natural medicines. Medicinal herbs are utilized for disease prevention and health maintenance in addition to adjuvant illness therapy (Melebary, and Elnaggar, 2023).

Recently, the use of natural resources to enhance livestock health has received increased attention. Antioxidant agents are in high demand since chemical preparations might be hazardous (Abd El-Hack *et al*., 2022; Arif *et al*. 2022). Antioxidants can be found naturally in plant products like medicine and aromatic plants. Olive trees *Olea europea* L. are among the most important of these plants, which play a preventive and therapeutic role for many diseases, whether through the oil of their fruits or the extracts from their leaves, due to the healthy and protective elements they contain against many diseases (Ahmed *et al*., 2021). Olive tree *Olea europea* L. was the primary use for many centuries due to its significant economic and nutritional value, the oil from the fruits of. However, olive tree leaves have gained attention in recent decades due to their significant therapeutic value and their usage as antioxidants, antibacterials, anti-inflammatory agents, and disease prevention agents (Mao et al., 2019; Abugomaa, and Elbadawy, 2020).

According to Wang *et al*., 2013 olive leaf extract can shield lead-poisoned mice's blood, spleen, and hippocampus because of their antioxidant properties. Additionally, olive leaf extract can prevent lead-induced brain damage it boosts antioxidant capacity and lowers apoptosis. Many studies have been reported that olive leaf extract protects against stroke and brain damage, as well as neurotoxicity brought on by oxidative stress and neurodegenerative illnesses (Chiaino *et al*., 2020; Castejón *et al*., 2020). Olive leaf extract has been the subject of additional research because of its antioxidant and anti-inflammatory qualities. OLE may help prevent obesity and the immune inflammation that goes along with it by lowering weight gain and adipose tissues, enhancing metabolic processes, and preventing

the induction of inflammatory mediators (Vezza *et al*., 2019; De Cicco *et al*., 2020). Many other studies reported that it was linked between lowering the risk of coronary heart disease and OLE, where it supports cardiovascular health because it lowers dangerous cholesterol, controls blood pressure, and improves atherosclerosis (Olmez *et al*., 2015; Efentakis *et al*., 2015; Lockyer *et al*., 2012).

The experiments were conducted to study the role and effect of aqueous olive leaf extract against to lead acetate accumulation toxicity effects on hematological parameters of adult rabbits.

Material and methods:

Experiment animals and experimental design:

16 adult male rabbits were supplied from a local market in eastern Libya with an average weight of 1800 g, and were divided into 4 equal groups of 4 rabbits each, as follows:

- **Group 1:** Control (G1), rabbits supplied daily with distilled water for two consecutive months.
- **Group 2:** Lead acetate (PbAc)-supplied group (G2): rabbits treated orally with 40 mg/kg BW/day for two months.
- **Group 3:** olive leaf extract (PLE)-supplied group (G3): rabbits in this group were treated orally with 200 mg/kg BW/day of olive leaf extract for two consecutive months.
- **Group 4:** lead acetate + olive leaf extract (PbAc + OLE)- supplied group (G4): rabbits orally administered 200 mg/kg BW/day of olive leaf extract then given 40 mg/kg BW/day PbAc for two consecutive months.

These groups were randomly distributed and kept in sterile, clean metal cages with a 14-hour daily light cycle at ambient temperature (22–25°C). To make sure they were disease-free, they were acclimated for a week before the trial began. Water and a well-balanced daily diet were given to the animals.

The rabbits were also weighed at the beginning and end of the experiment to determine the effect of lead accumulation and the use of an aqueous extract of olive leaves on the rabbits' weight.

Olive leaves extraction:

The process described by Zari and Al-Attar (2011) was used to create the aqueous extract of olive leaves, *Olea europaea* L., where the green leaves of *Olea europaea* L. were gathered from olive trees in Libya's Ain Mara region in November and December of 2024. Ten grams of fresh olive leaves were combined with one liter of cold water in an electric mixer for 15 minutes. Following this, the mixture was centrifuged, and the clear supernatant was carefully collected and stored as the final extract for treatments in a refrigerator.

Lead acetate (PbAc):

Lead acetate was prepared in the laboratory according to Greenwood and Earnshaw (2012) at the Laboratory of Chemistry at Faculty of Science in Derna University, at a concentration of 40 mg/kg. This was done by mixing lead oxide with acetic acid and diluting it with distilled water to reach the required concentration. The mixture was heated with continuous stirring until the lead oxide was completely dissolved and reacted with the acid. After complete dissolution, the solution was allowed to cool to obtain lead acetate crystals.

Blood sample collection:

After two months (60 days) and the experimental period's end, and one day after the last dose of the four treatments, rabbits were fasted for 8 h, rabbits were anesthetized, and blood samples were collected from all 16 animals under study. The samples were placed in vacuum tubes containing anticoagulants (EDTA) to prepare samples for RBCs and WBCs count, Hb concentration.

Hematological parameters assessment:

The RBCs and WBCs count were adopted by Thrall *et al*., 2012 methodology use Hemocytometer, and according to Higgins, 2005 methodology using the Cyanmethemoglobin, Hemoglobin (Hb) concentration was measured.

Statical analysis:

Results are reported as means ± standard error (SE). The differences between means were assessed using one-way analysis of variance (ANOVA) with SPSS (v. 20). Differences between means were also determined using the least significant difference ($LSD_{0.05}$).

Results and discussion:

The effects of lead acetate 40 mg/kg BW, olive leaf extract 200 mg/kg BW, and a mixture on rabbits' growth rate are presented in Table 1, and figure 1. Generally, values of initial body weight were about 1800 gr for all. After 60 days there was an increase in all groups (1, 3, and 4) except the second group, which was treated by lead acetate. The data showed significant differences were observed in rabbits' body weight among treated groups and the control. Final body weight was depressed significantly by 300 gm in group 2 individuals due to lead acetate exposure when compared to the initial body weight of rabbits and by 600 gm compared with the final body weight of control group (untreated group). On the other hand, the average daily weight gain of the untreated rabbits (control) was 5 gm, but the

average daily weight gain of the rabbits reached its maximum in the group treated with olive leaf extract, recording 6.67 gm per day, while the daily gain for the fourth group, which was given lead acetate with olive leaf extract, was 0.17 gm, but it increased by 26.67% compared to the second group (which was treated with lead acetate alone). The results thus illustrate the negative effect of dosing adult male rabbits with lead acetate on their growth rate and weight gain, as well as the positive effect of dosing rabbits with olive leaf extract, which, when used alone, led to an increase in the feed conversion ratio and thus an increase in the rabbits' growth rate compared with other treatments. Also, the use of olive leaf extract for rabbits dosed with lead acetate reduced the negative effects of lead accumulation on the rabbits' weight gain rate.

These results are consistent with what Taha *et al*., 2019 reported: that lead accumulation in rabbits led to a decrease in average body weight, compared to the control group. Ahmed *et al*. (2021), also demonstrated the negative effect of lead acetate on the rate of weight gain in mice, which led to a reduction in their final body weight compared to the initial body weight and compared with control group and the use of olive leaf extract. They reported that these effects may be due to the toxic impact of lead acetate on various body organs, which can result in physiological and morphological changes. These changes manifested as abnormal symptoms, including lethargy, loss of appetite, and diarrhea in mice treated with lead acetate, ultimately leading to alterations in body weight.

Our findings are consistent with some research suggesting that PbAc may cause an imbalance in vitamin D and glucose metabolism, as well as inhibiting certain vital enzymes in the synthesis of proteins and nucleic acids, leading to a loss of body weight (Rahman *et al*., 2018). It may also impair normal intestinal absorption of food or the occurrence of intestinal cramps and diarrhea associated with lead poisoning (Ibrahim *et al*., 2012). However, Ponce-Canchihuamán *et al*. (2010) shown that oxidative stress is one of the primary causes of lead's harmful effects, where lead may inhibit the activity of antioxidant enzymes and damage nucleic acids in cell membranes, which may impact the building process and consequently cause weight loss. It may also increase lipid peroxidation and reactive oxygen species in cell membranes, which hinder growth because they interfere with metabolic processes (Dkhil *et al*., 2016).

Conversely, many studies reported the positive effects of olive leaf extract; the findings of Ahmed *et al*. (2021) demonstrated that rats treated with olive leaf extract (OLE) did not experience any adverse effects, such as diarrhea or appetite loss; additionally, OLE decreased inflammatory toxicity and improved body weight. Also, similar results were found by Cazarin *et al*. (2015), who claimed that OLE can improve infections in the membranes lining the digestive system and reduce colitis because these infections impair the absorption of nutrients in the stomach and intestine.

Table (1): Effect of lead acetate, and olive leaves extraction on weight of adult male rabbits (gm)

Body weight gain (gm/ day)	Body weight gain (gm/60 days)	Final body weight (FBW) gm	Initial body weight (IBW) gm	Treatment
5	300	2100 ± 60	1800 ± 50	Control
-5	-300	1500 ± 70	1800 ± 50	Lead acetate (PbAc)
6.67	400	2200 ± 50	1800 ± 60	Olive leaf extract (OLE)
1.67	100	1900 ± 50	1800 ± 40	PbAc + OLE

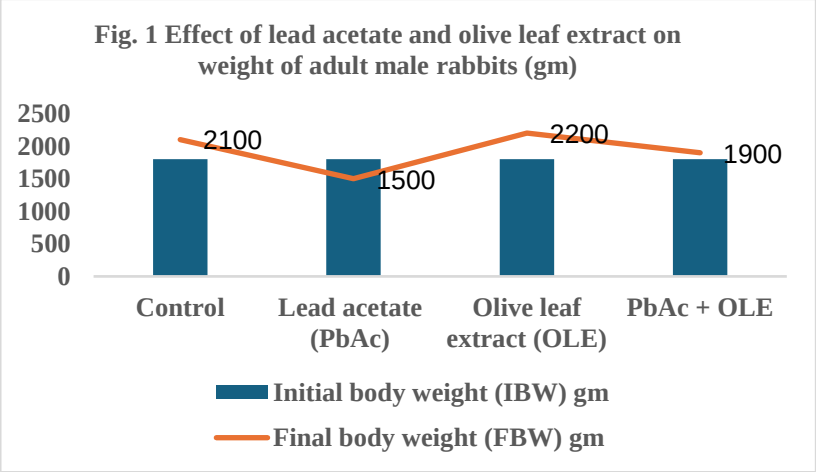

Fig. (1): Effect of lead acetate, and olive leaves extraction on weight of adult male rabbits (kg)

Data in table 2 and figure 2 showed the effect of lead acetate and olive leaf extract on hematological parameters (RBCs, WBCs, and Hb) in adult male rabbits. The results of the one-way analysis of variance (ANOVA) showed significant differences in RBC and WBC counts and Hb levels in rabbits treated with lead acetate and olive leaf extract, either alone or in combination. Treatment of rabbits with lead acetate (G2) caused a significant ($p < 0.05$) decrease in RBCs and Hb recorded at 7.06 ± 1.12 (10^6/µL), and 16.63 ± 1.00 (g/dl) respectively and reached 8.2 ± 3.20 (10^6/µL) and 18.17± 1.56 (g/dl) for untreated rabbits (control). On the other hand, there was a significant increase in WBCs when compared with the control, where it increased from 6.18 ± 0.5 (10^3/µL) in untreated rabbits to 11.10 ± 1.89 (103/µL) in the second group, which was treated by lead acetate. These results are consistent with what Ibrahim *et al*. 2012 explanation of the detrimental effects of lead acetate use, which decreased the quantity of red and white blood cells in mice, but conversely led to a decrease in the level of hemoglobin. Ekanem *et al*., 2015, reported that giving mice lead acetate resulted in splenomegaly, a significant change in hemoglobin levels and decrease in packed cell volume (PCV).

Similarly, administering olive leaf extract to rabbits had the same negative effect on the total red blood cell count (RBCs), which decreased to 7.82 ± 0.52 (10^6/µL). Conversely, the white blood cell (RBCs) count increased to 8.56 ± 1.12 (10^3/µL), and tas well as he hemoglobin (Hb) level rose by 0.79 (g/dl) compared to the control, which was 18.96 ± 1.09 (g/dl). Administering a mixture of lead acetate and olive leaf extract to the fourth group (G4) of rabbits resulted in an increase in RBCs count, which was the highest observed among all groups 8.38 ± 0.71. Hb levels also increased compared to group 2 which was treated by lead acetate, although they did not exceed the increase of either treatment alone, registering 17.48 ± 1.72 (g/dl). On the other hand, although the use of the mixture led to a decrease in WBCs count, it was observed that the use of olive leaf extract reduced the negative effect on WBCs count in the individuals of the fourth group treated with lead acetate.

Table (2): Effect of administering an aqueous extract of olive leaves on rabbits suffering from lead acetate poisoning on some hematological parameters

Hb (g/dl)	WBCs (10^3/µL)	RBCs (10^6/µL)	Treatment
18.17± 1.56	6.18 ± 0.5	8.2 ± 3.20	Control
16.63 ± 1.00	11.10 ± 1.89	7.06 ± 1.12	Lead acetate (PbAc)
18.96 ± 1.09	8.56 ± 1.12	7.82 ± 0.52	Olive leaf extract (OLE)
17.48 ± 1.72	8.38 ± 0.71	8.38 ± 0.71	PbAc + OLE

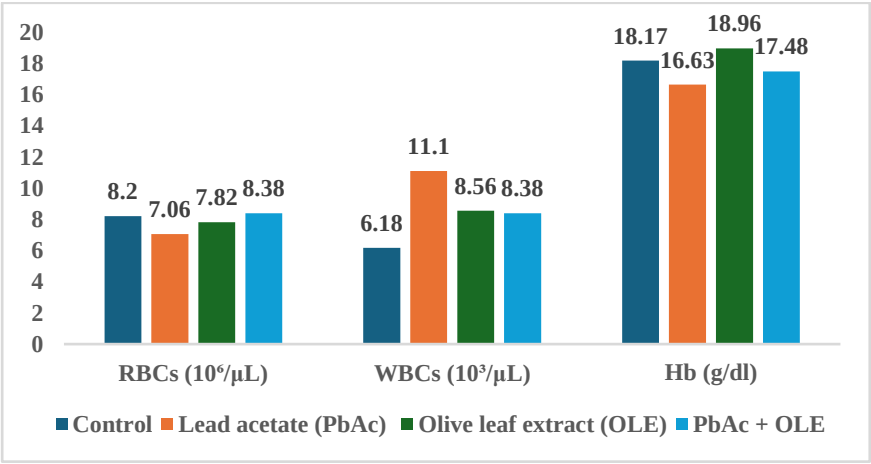

Fig. (2): Effect of administering an aqueous extract of olive leaves on rabbits suffering from lead acetate poisoning on some hematological parameters

Conclusion:

This study suggests that administering an aqueous extract of olive leaves to rabbits suffering from lead acetate poisoning can improve various hematological parameters such as (RBCs, WBCs, and Hb) in experimental rabbits. Also, the use of olive leaf extract reduced the negative effects of lead accumulation on the rabbits' weight gain rate.

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