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Protective effect of olive leaf extract on liver enzymes activity against lead acetate accumulation in rabbits

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Abstract

Lead is a heavy metal known for its toxicity effect, which causes many effects on different organs of the body, and lead acetate is a form of lead in the environment. Due to the health and protective nature of the olive tree, by its elements, against many diseases, the leaf extract of this tree was chosen in our study because it is rich in oleuropein and hydroxytyrosol. The experiment was conducted in a special farm in the Al-Qubbah region in eastern Libya during November and December 2024. This study was to investigate the role of olive leaf (*Olea europea* L.) extract (OLE) against lead acetate (PbAc) effect on liver enzyme activity (AST, ALT, and LDH) in adult male rabbits. Sixteen adult male rabbits were divided into four groups: untreated as a control (G1), treated with PbAc (G2), treated with OLE (G3), and treated with a mixture of PbAc + OLE (G4). The results showed that the use of lead acetate led to an increase in liver enzyme activity compared to the control group. However, the use of an aqueous extract of olive leaves improved enzyme activity, whether used alone or in combination with lead acetate. Based on these results, it can be concluded that olive leaf extract (OLE) has protective and therapeutic effects against the negative impact of lead acetate on the activity of the liver enzymes under study (AST, ALT, and LDH).

Keyword: Lead, Pb, plant extracts, olive, rabbits, AST, ALT, LDH, liver enzymes

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التأثير الوقائي لمستخلص أوراق الزيتون على نشاط إنزيمات الكبد ضد تراكم أسيتات الرصاص في الأرانب

صبرية فتح الله ابوعجيلة
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الملخص

يعد الرصاص أحد أهم العناصر الثقيلة والمعروف بتأثيره السام، الذي يُسبب العديد من الآثار على مختلف أعضاء الجسم، وتعد أسيتات الرصاص أحد أشكاله الموجودة في البيئة. ونظرًا للعديد من فوائد شجرة الزيتون الصحية والوقائية، بفضل عناصرها، ضد العديد من الأمراض، فقد تم اختيار المستخلص المائي لأوراق الزيتون في دراستنا لمحتواها العالي من الأولوروبين والهيدروكسي تيروسول. أُجريت التجربة في مزرعة خاصة بمنطقة القبه بشرق ليبيا خلال شهري نوفمبر وديسمبر 2024. هدفت هذه الدراسة إلي التعرف علي دور المستخلص المائي لأوراق الزيتون علي نشاط إنزيمات الكبد المختلفة ضد تراكم أسيتات الرصاص في ذكور الأرانب البالغة. قُسِّم ستة عشر أرنبًا بالغًا من الذكور إلى أربع مجموعات: مجموعة ضابطة (G1) لم تُعالج، ومجموعة مُعالجة بأسيتات الرصاص (G2)، ومجموعة مُعالجة بمستخلص أوراق الزيتون(G3) ، ومجموعة مُعالجة بمزيج من أسيتات الرصاص ومستخلص أوراق الزيتون .(G4) أظهرت النتائج أن استخدام أسيتات الرصاص أدى إلى زيادة في نشاط إنزيمات الكبد مقارنةً بالمجموعة الضابطة. ومع ذلك، فقد حسّن استخدام المستخلص المائي من أوراق الزيتون نشاط الإنزيمات، سواءً استُخدم بمفرده أو مع أسيتات الرصاص. وبناءً على هذه النتائج، يُمكن الاستنتاج أن مستخلص أوراق الزيتون له تأثيرات وقائية وعلاجية ضد التأثير السلبي لأسيتات الرصاص على نشاط إنزيمات الكبد قيد الدراسةAST ، ALT، وLDH

الكلمات المفتاحية: الرصاص، المستخلصات النباتية، الزيتون، الأرانب، انزيمات الكبد، AST، ALT، LDH

http://www.doi.org/10.62341/habd2579

Introduction

The earth's crust naturally contains metals, and the compositions of these metals varies depending on the location, leading to structural differences in surrounding concentrations. Heavy metals can contaminate the ecosystem and participate in trophic food chain transmission. When these metals get into living things, they mix with proteins, enzymes, and DNA molecules to create extremely stable bio-toxic chemicals that change how the organisms operate normally and prevent them from participating in bioreactions (Mishra *et al*., 2018). Generally, cadmium, lead, mercury, nickel, chromium, arsenic, copper, zinc, and other metals with higher atomic numbers (above 20) and greater densities (5 g/cm^3) are together referred to as heavy metals. These metals are closely linked to issues of biological toxicity and environmental contamination because they have strong inhibitory effects on biodegradation processes (Gupta *et al*. 2014). According to (Yadav *et al*., 2017), heavy metal contamination in the environment (soil, water, and air) may present hazardous toxicological risk and issues for both humans and animals. lethal metal accumulation in the human body has detrimental effects on health, including irregularities in growth and development, cancer, neuromuscular defects, mental disorders, and metabolic activity failure (Chandra *et al*., 2011). Increased risks of kidney and liver failure, infertility and reproductive diseases, cancer, nerve breakdown, leukemia, mental illness, and other toxicity issues result from the occurrence of excess concentrations of metals (Khan *et al*., 2011). Additionally, it harms basic physiological functions and genetic expressions as well as bodily systems and organs such the kidney, liver, reproductive system, neurological system, urinary system, and immune system (Su, 2014). Where these metals such as lead, cadmium, chromium, and arsenic are extremely poisonous and have genotoxic, carcinogenic, and mutagenic effects (Mishra *et al*., 2018).There are numerous uses for lead (Pb), and we can be exposed to it in several ways, such as industries, motor fuel (gasoline), colors, foods stored in metal cans, water pipes, building materials, certain cosmetics, and some toys for kids. Lead remains a concern and danger to human health despite significant attempts to decrease exposure, particularly for children who are more affected than adults. Depending on the duration of exposure, lead can cause acute or chronic toxicity by entering the

http://www.doi.org/10.62341/habd2579

body through the skin, mouth, and nose and harming several internal organs (Ahmed *et al*., 2021). The severity and toxicity of lead exposure are explained by the fact that it damages numerous bodily organs, including the heart, liver, kidneys, brain, and testes, which may raise the death rate (Abdelhamid *et al*., 2020).

The medicinal and aromatic plants that offer a wealth of useful materials for the pharmaceutical industry have attracted a lot of attention on a global scale (Abd *et al*., 2020; Tousson *et al*., 2020). Due to its significant economic and nutritional value, the oil from the fruits of the olive tree *Olea europea* L. was the primary use for many centuries. However, the olive tree's 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 (Abugomaa, and Elbadawy, 2020; Ahmed *et al*., 2021). Olive leaf extract (OLE) has been the subject of numerous studies because of its antioxidant and anti-inflammatory qualities. By lowering weight gain and adipose tissues, enhancing metabolic processes, and preventing the induction of inflammatory mediators, OLE may help prevent obesity and the immune inflammation that goes along with it (De Cicco *et al.*, 2020). Similarly, OLE has been linked to lowering the risk of coronary heart disease to promote cardiovascular health because it lowers dangerous cholesterol, keeps blood pressure stable, and improves atherosclerosis (Olmez *et al.*, 2015; Efentakis *et al*., 2015). Additionally, by lowering hepatic damage and fibrosis and enhancing liver tissues, OLE protects against the hepatotoxicity and DNA damage brought on by tetrachloride carbon (Taamalli *et al.*, 2020).

Thus, the purpose of this study was to determine the protective role of olive leaf extract (*Olea europea* L.) (OLE) against the effects of lead acetate (PbAc) on the activity of liver enzymes in adult male rabbits (AST, ALT and LDH).

Materials and methods

Chemical compounds

Lead acetate (PbAc): According to Greenwood and Earnshaw (2012), lead acetate was prepared in the chemistry laboratory at the Faculty of Science, Derna University, at a concentration of 40

http://www.doi.org/10.62341/habd2579

mg/kg. By mixing 10 grams of lead oxide with acetic acid in a heat-resistant glass flask and diluting with 20 ml of distilled water. Heat the mixture while stirring continuously until the lead oxide is completely dissolved and reacts with the acid. After complete dissolution, allow the solution to cool to form lead acetate crystals by the following equation:

$$PbO + 2\ CH_3COOH \rightarrow Pb(CH_3COO)_2 + H_2O$$

The aqueous extract of olive leaves: The aqueous extract of olive leaves, Olea europaea L., was prepared following the methodology outlined by Zari and Al-Attar (2011). The green leaves of Olea europaea L. were collected from olive trees in the Ain Mara region of Libya during the months of November and December 2024.

After collecting the leaves, it was washed well to remove dust, and 10 gm of green leaves were taken and placed between two layers of filter paper to absorb the washing water. Next, they were placed in an electric mixer with one liter of distilled water and blended for 15 minutes. The extract was filtered through several layers of medical gauze to ensure the removal of insoluble material and then through Whatman filter paper to remove plant fibers, thus obtaining a crude extract. The filtrate was placed in an opaque flask and stored in the refrigerator for oral supplementation administration using a 10 ml syringe equipped with a measuring device in the form of a thin metal tube.

Experimental animals' groups

In this study, sixteen adult male rabbits, aged between 5 and 7 months and with an average weight of 1500 ± 200 g, were obtained from a local market in the Al-Qubbah region of eastern Libya. The rabbits were randomly distributed and placed in clean, sterilized individual metal cages at room temperature (22–25°C) with a 14-hour daily light cycle. They were acclimated for one week prior to the start of the experiment to ensure that they were free of any diseases. The animals were provided with a balanced diet and water as needed.

Rabbits were divided into four equal groups (n = 4), and the doses of OLE and PbAc were prepared as follows: The rabbits receive lead acetate at a dose of 40 mg/kg of body weight daily and olive leaf extract at a dose of 200 mg/kg of body weight daily. Doses given

http://www.doi.org/10.62341/habd2579

orally by gavages approximately at the same time each morning for 30 consecutive days.

1. Group (1): Control (untreated rabbits).
2. Group (2): Was orally administered with PbAc (40 mg/kg BW/day).
3. Group (3): Was orally administered with OLE (200 mg/kg BW/day).
4. Group (4): Rabbits were orally administered with PbAc (40 mg/kg BW/day) and then with OLE (200 mg/kg BW/day).

Collection of blood samples:

At the end of the experiment and twenty-four hours after the last dosing, blood was drawn from the bare ear vein. The blood was kept in glass tubes without an anticoagulant (non-heparinized) for 30 minutes. The tubes were then placed in a centrifuge for 15 minutes at a speed of 3000 rpm to obtain blood serum for biochemical analysis. After that, the rabbits were slaughtered and dissected, and the liver and kidneys were removed after removing the fatty tissue and the surrounding connective tissue.

Statistical analysis:

The results were expressed as means ± SE, and data were statistically analyzed using SPSS software (v.17) for all experiment groups with one-way analysis of variance (ANOVA). A post-hoc test was performed to compare mean values among all groups using the $LSD_{0.05}$ (Howell, 1995).

Results and discussion

The results shown in Table (1) and figures (1and 2) illustrate the effect of administering lead acetate (PbAc) and an aqueous extract of olive leaves (OLE) to adult male rabbits on the activity of various liver enzymes. Statistical analysis reveals the negative effect of lead acetate, as its addition to the rabbits' daily diet resulted in a significant increase ($P<0.05$) in the activity of various liver enzymes. Aspartate aminotransferase AST (U/L) enzyme concentration reached its highest value in the experimental group treated with lead acetate (421.3 ± 133.704) compared to the control group, which was recorded with the lowest concentration (163.2 ± 19.8). On the other hand, the use of the aqueous extract of olive

http://www.doi.org/10.62341/habd2579

leaves when used alone did not record any significant differences when compared to the control, as the concentration recorded was 165.13 ± 36.13. Additionally, the positive and protective effect of using the aqueous extract of olive leaves on male rabbits that were treated with lead acetate was evident, as the results showed that male rabbits doused with the aqueous extract of olive leaves and doused with lead acetate had a significant decrease ($P<0.05$) in enzyme activity (197.86 ± 49.08) when compared to the lead acetate group (421.3 ± 133.704).

Table (1) Effect of lead acetate and olive leaf extract on liver enzymes levels in adult rabbits

Treatment	AST (u/l)	ALT (u/l)	LDH (u/l)
Control	163.2 ± 19.8	185.2 ± 49.4	1128.4 ± 350.3
Lead acetate (PbAc)	421.3 ± 133.704	225.7 ± 79.78	3239.2 ±1497.63
Olive leaf extract (OLE)	165.13 ± 36.13	169.40 ± 45.96	1375.2 ± 587.75
PbAc + OLE	197.86 ± 49.08	188.14 ± 45.17	2109.1 ± 1439.94

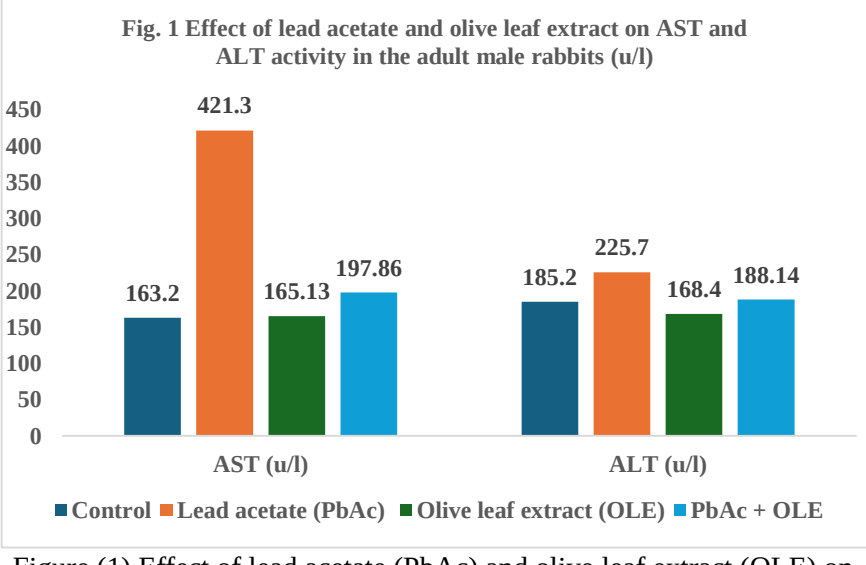

Figure (1) Effect of lead acetate (PbAc) and olive leaf extract (OLE) on AST and ALT activity in the adult male rabbits (u/l)

http://www.doi.org/10.62341/habd2579

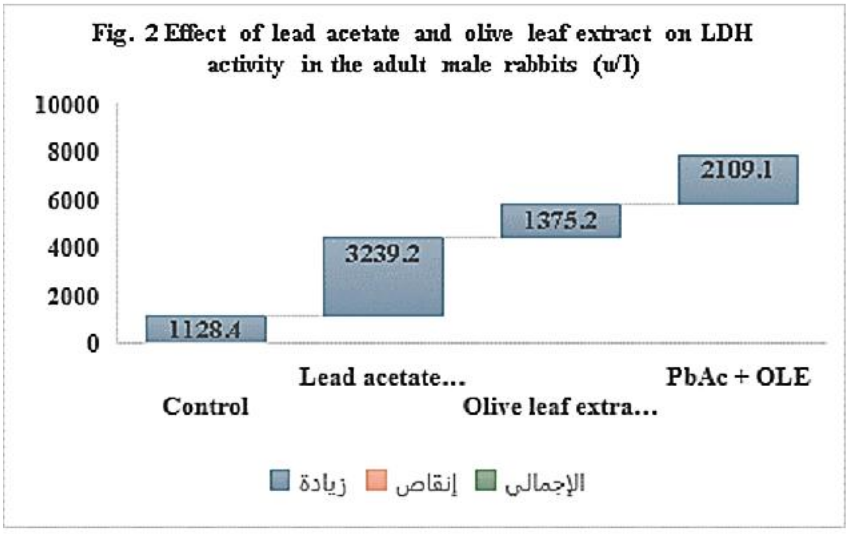

Figure (2) Effect of lead acetate (PbAc) and olive leaf extract (OLE) on LDH activity in the adult male rabbits (u/l)

Likewise, lead acetate showed a significant increase ($P<0.05$) in alanine aminotransferase (ALT) enzyme activity (U/L) in the second experiment group (G2) that was treated with it (225.7 ± 79.78) when compared with the control group (G1) (185.2 ± 49.4), while the fourth group (G4) that was doused with the aqueous extract of olive leaves and lead acetate showed a significant decrease ($P<0.05$) in ALT enzyme activity (188.14 ± 45.17) when compared with the lead acetate group (225.7 ± 79.78), which indicated the protective role of olive leaf extract against the toxicity of lead acetate in male adult rabbits. While the third group (3) that was treated with the aqueous extract of olive leaves did not show significant differences in ALT enzyme activity (169.40 ± 45.96) compared with the control group (185.2 ± 49.4).

Lead acetate exposure caused a significant ($P < 0.05$) elevation of LDH activity in the liver, where it reached the maximum (3239.2 ± 1497.63), with significant differences as compared to the control (G1), which recorded the lowest activity of LDH (1128.4 ± 350.3), as well as with group 3, which was treated with olive leaf extract, for which LDH recorded (1375.2 ± 587.75). In addition, the results show that dosing male rabbits with olive leaf extract reduced the negative effect of lead acetate on increasing LDH enzyme activity

http://www.doi.org/10.62341/habd2579

in the liver, confirming the protective effect of using the aqueous extract of olive leaves against lead acetate toxicity in rabbits, as enzyme activity was recorded when using olive leaf extract with lead acetate LDH (2109.1 ± 1439.94), but when dosing lead acetate alone, LDH enzyme activity was recorded (3239.2 ± 1497.63).

These results are consistent with what Baghshani and Shahsavani (2013) demonstrated that the use of lead acetate led to increased activity of AST, ALT, and LDH in fish liver. This is attributed to the fact that lead acetate (PbAc) interaction with biological systems, which may affect the activity of metabolic enzymes in some tissues. Another study by Mohamed et al. (2020) reported that lead acetate induced marked changes in hemato-biochemical parameters. Lead acetate significantly elevated all enzymes' ALT and AST activities, which were higher than those of the control group (untreated). Durgut *et al.* (2008) also demonstrated that lead administration to rabbits significantly increased the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), and alkaline phosphatase (ALP) enzymes in blood serum. These results are also consistent with El-belbasy et al., 2021 finding that lead acetate use led to an increase in the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) enzymes in rats blood serum.

Additionally, Kadhum noted a substantial rise in rat serum ALT and AST enzyme levels in a 2019 investigation. Lead acetate poisoning, which harms liver cells and allows these enzymes to seep into the bloodstream, is blamed for this outcome. Elevated bilirubin and cholesterol levels were also caused by liver cell death. Increased necrosis and hepatocyte enlargement, together with bleeding and congestion in the liver section, were found in histopathological investigations of the liver. Lead acetate damages or kills liver cells, which results in these effects. The findings by Mazreku *et al*., 2017 demonstrated that lead acetate therapy significantly increased AST and ALT activity in the liver, pancreas, kidney, and brain groups. lead effects via boosting the activity of enzymes (CAT, ALT, and AST) and interacting with several molecular systems.

http://www.doi.org/10.62341/habd2579

A study by Abd El-Azim in 2014 showed that AST, ALT, and LDH activities were reduced with olive leaf extract administration, so the study indicates that olive leaf extract may be an agent against lead acetate. Administration of OLE caused a significant decrease in serum AST, ALT enzyme, and LDH. Also, amelioration of oxidant–antioxidant status with olive leaf extract was observed in addition to a significant decrease in MDA and a significant increase in TAC in liver tissue with a significant increase in glutathione reductase (GR) and SOD in serum. The chemical pathological changes were in step with histopathological observation, suggesting a marked hepatoprotective result of olive leaf extract. It could be concluded that olive leaf extract (OLE) treatment may be effective in decreasing hepatic injury and oxidative stress in male albino rats (Taha *et al*., 2020).

Fki *et al*. (2020) found that olive leaf extract reduced the elevated liver enzymes (AST, ALT, LDH), enhanced the antioxidant status, and attenuated the liver inflammation and apoptosis. Oleuropein and hydroxytyrosol, as major compounds of olive leaves, have been reported to exert numerous pharmacological properties, including anticancer, antidiabetic, and anti-inflammatory activities. In addition, the oral administration of the oleuropein- and hydroxytyrosol-rich olive leaf extracts possessed hypolipidemic and hepatoprotective effects against the HFD-induced metabolic disorders by enhancing the antioxidative defense system and blocking the expression of the proteins involved in inflammation and liver damage.

Conclusion

This study aimed to evaluate the protective effect of olive leaf extract against lead acetate-induced toxicity in adult male rabbits. Four groups of rabbits were used (control, treated by PbAc, treated by OLE, and treated by a mixture of PbAc + OLE). The results showed that lead acetate increased the activity of the liver enzymes AST, ALT, and LDH. Conversely, the use of the aqueous extract of olive leaves resulted in a significant improvement and reduction of the negative effects of lead acetate. This confirms its effectiveness in mitigating the damage caused by lead poisoning. In conclusion,

http://www.doi.org/10.62341/habd2579

the study suggests the potential use of the aqueous extract of olive leaves as a natural protective agent against environmental toxins.

Recommendations

The results of the study suggest using the aqueous olive leaf extract as a protective agent to reduce the toxic effects of lead accumulation on liver enzymes activity in rabbits.

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