Research Article

BASJVETRES

BASRAH JOURNAL OF VETERINARY RESEARCH, 2024, 23(3):13-23
https://bjvr.uobasrah.edu.iq/

The Effect of The Alcoholic Extract of *Eruca sativa* Seeds on Some Blood Biochemical Indicators and Histological Characteristics of Liver in Broiler Chickens Exposed to Lead Acetate Poisoning

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DOI: https://doi.org/10.23975/bjvr.2024.150390.1092

Received: 10 June 2024 Accepted: 24 June 2024.

Abstract

The current study investigated the impact of adding an alcoholic extract of *Eruca sativa* seeds and lead acetate to drinking water on some biochemical blood parameters and liver histological changes in broiler chickens. A total of 144 one-day-old Ross 308 broiler chicks, with an initial weight of 40 grams, were randomly distributed into four groups (each group included 36 birds) with three replicates for each (12 birds per replicate). The first group was the control group (without any addition); the second group added 350 mg of lead acetate per liter of drinking water; the third group added 250 mg of the alcoholic extract of *Eruca sativa* seeds per liter of drinking water, and the fourth group added 350 mg of lead acetate and 250 mg of the alcoholic extract of *Eruca sativa* seeds per liter of drinking water. The results indicated a significant increase ($p \leq 0.05$) in the third group's total protein and globulin concentration. In contrast, a significant decrease ($p \leq 0.05$) in the total protein and globulin concentration was observed in the second group compared to the other groups. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly ($p \leq 0.05$) increased in the second group compared to other groups. A significantly higher ($p \leq 0.05$) amount of lead concentration was found in the blood serum, breast muscle, liver, and feces of second group. On the other hand, these parameters were significantly ($p \leq 0.05$) decreased in the third group compared to the others. The findings showed no significant difference between the control and fourth groups in lead concentration, total protein, and globulin. The results showed no significant differences in the groups' albumin, cholesterol, and triglyceride concentrations. The findings showed that treating broiler chickens with lead acetate adversely affected liver tissue. It can be concluded that adding the alcoholic extract of *Eruca sativa* seeds with lead acetate to drinking water for broiler chickens reduced oxidative stress caused by lead acetate and improved some biochemical blood parameters.

Keywords: Broiler chickens, *Eruca sativa* seeds, Lead acetate. Oxidative stress.

Introduction

Oxidative stress is one of the significant challenges poultry faces, impacting not only the welfare and health of the birds but also hindering production performance and the quality of meat and eggs produced (1). More than any other substance, lead (Pb) is a major environmental pollutant that has caused accidental poisoning deaths in pets and birds (2). The primary sources of lead compound contamination in agricultural ecosystems are emissions from industrial establishments, quarries, mines, thermal power plants, fuel combustion products, battery production, and the use of metal-containing pesticides (3). Lead contamination is widespread in cities and surrounding areas, with significant pollution likely occurring near factories, other industrial facilities, or major highways where vehicle exhaust fumes contaminate the environment (4). However, the amount of lead absorbed from the intestines varies depending on the healthy state of the animal (5). To mitigate the effects of oxidative stress, medicinal plants and their extracts have been used to improve the productive and physiological performance of poultry (6-8). Medicinal plants have gained prominence in global agricultural production due to their natural chemical compounds, which have significant physiological and therapeutic effects on humans and animals. (9).

The rocket plant (*Eruca sativa*) is one of the most important medicinal plants known for its antioxidant and beneficial properties. The seeds' bioactive compounds include beta-carotene, vitamins E and C, and other nutrients (10). *Eruca sativa* is also a rich source of minerals such as calcium, manganese, potassium, sodium, iron, copper, and zinc, as well as glycosides and other active compounds (11). Flavonoids, which are significant bioactive substances found in *Eruca sativa* seeds, are known for their ability to suppress free radicals and chelate heavy metals (12).

Eruca sativa seed extract, despite its significant importance and diverse uses and benefits in humans and animals, has limited studies in poultry. Therefore, the goal of the current study was to assess the effectiveness of the alcoholic extract of *Eruca sativa* seeds on broiler chickens exposed to lead acetate-induced oxidative stress, as well as its effects on certain biochemical blood parameters and liver histological changes. (more fluency)

Materials and Methods

The current study took place over the course of 35 days, from October 9, 2023, to November 12, 2023, in the poultry hall of the animal field at the College of Agriculture, University of Basrah.

Preparation of the Alcoholic Extract of *Eruca sativa* seeds

The alcoholic extract of *Eruca sativa* seed was prepared according to the method described by (13). The study involved combining *Eruca sativa* seed powder with 70% ethyl alcohol in a 500-ml glass beaker, allowing it to sit in a 37°C water bath for 24 hours, and then stirring it with an electric mixer for an hour. The solution was filtered using medical gauze. The filtrate was

distributed into test tubes for centrifugation at 3000 rpm for 15 minutes. The supernatant was collected, and the residue was discarded. The supernatant was placed in glass petri dishes inside a drying incubator at 37°C. After drying, the extract was scraped off and weighed, yielding 5 grams from the original 50 grams of *Eruca sativa* seed powder. This was then dissolved in 100 ml of distilled water and stored in tightly sealed glass bottles in the refrigerator until use.

Birds Management

A total of 144 one-day-old broiler chicks, with an initial weight of 38-42 grams, were raised for 35 days. The chicks were raised under similar conditions in a closed hall. The birds were raised in metal cages measuring 120 cm in length, 80 cm in width, and 70 cm in height. Each cage contained twelve chicks. In the first week, the temperature was set at 33.5°C, and then reduced by 2°C each week until the end of the fifth week. A lighting program of 23 hours of light and 1 hour of darkness was used from day 8 to day 35. At 7 days old, the birds were vaccinated with the Newcastle vaccine via drinking water. During the experiment, there was no mortality. The birds were fed a starter diet containing 22.34% crude protein and 3074 kcal/kg of metabolizable energy from days 1 to 21. From days 22 to 35, the birds were fed a grower diet containing 20.21% crude protein and 3170.5 kcal/kg of metabolizable energy. Table 1 presents the composition and chemical analysis of the diets basing on (14).

Table (1): Diets nutritional and chemical compositions.

Ingredient %	Starter diet (1-21 days) (%)	Grower diet (22-35 days) (%)
Yellow corn	50	55
Wheat	12	12
Soybean meal (48%)	29	25.5
Protein concentrate (40%)	5	3
Plant oil	2	3
Limestone	1	0.5
NaCl	0.2	0.2
Premix (29%)	0.5	0.5
L – Lysine	0.2	0.2
Methionine	0.1	0.1
Total	100	100
Calculated chemical composition		
Metabolizable energy (Kcal. Kg^{-1})	3074	3170.5
Crude protein (%)	22.34	20.21
Calorie: Protein ratio	137.60	156.87
Ether extract (%)	5.02	5.94
Crude fibre (%)	3.45	3.26
Calcium (%)	0.71	0.42
Available Phosphorus (%)	0.30	0.24
Lysine (%)	1.25	1.11
Methionine + Cysteine (%)	0.83	0.75

Study design

The birds were divided into four groups (36 birds per treatment), with three replicates per group (12 birds per replicate). The groups were as follows: The first group served as the control group, with no additions made to the drinking water. Second group: add 350 mg of lead acetate per liter of drinking water. Third group: adding 250 mg of the alcoholic extract of *Eruca sativa* seeds per liter of drinking water. Fourth group: adding 350 mg of lead acetate and 250 mg of *Eruca sativa* seeds' alcoholic extract per liter of drinking water.

Studied Tests

Biochemical Blood Parameters: At 34 days old, blood samples (5 ml) were collected from two males per replicate in tubes without anticoagulants. Serum AST and ALT activities were measured according to (15). Total protein, albumin, globulin, cholesterol, and triglyceride concentrations were measured using commercial kits.

Lead Concentration: Lead concentration in the blood serum, breast muscle, liver, and feces were measured according to (16;17) and (18).

Histological Study: Liver samples were taken from males at 35-days-old and placed in 10% formalin until histological processing was performed. The histological sections were prepared according to (19).

Statistical Analysis

The statistical software program (20) analyzed the data using a completely randomized design (CRD). (21) was used to test the differences between groups at a significance level of ($P \leq 0.05$) according to the following mathematical model: $Yij=\mu+Ti+ei$Where: Yij: Observation value. μ: General mean of the studied trait. Ti: Group effect. ei: Experimental error effect.

Results and discussion

Table 2 shows the effect of adding the alcoholic extract of *Eruca sativa* seeds and lead acetate to the drinking water of broiler chickens on some biochemical blood parameters. Table 2 reveal a significant increase ($p\leq0.05$) in total protein and globulin concentration in the third group compared to other study groups. This could have been caused by the flavonoids and other active compounds in the *Eruca sativa* seed alcoholic extract. Flavonoids protect cells from oxidative stress by increasing the activity of antioxidant enzymes (22, 23). Alkaloids in the *Eruca sativa* seed alcoholic extract, which enhanced the immune system and reduced inflammation, may have been responsible for the increase in the globulin concentration (24, 25). The results also showed a significant ($p\leq 0.05$) decrease in the concentration of total protein and globulin in the second group compared to the first, third and fourth groups. These findings could have been caused by the adrenal cortex releasing more corticosterone hormone when the birds were exposed to oxidative stress due to lead acetate. This hormone breakdown proteins to produce energy (26). No significant differences were found in the albumin, cholesterol, and triglyceride concentrations among the groups. Also (27), found that giving 200 ppm led acetate to broiler chickens for 42

days had no significant effect on their cholesterol and albumin levels. Similarly, (28) found no significant impact on the albumin concentration in broilers exposed to 400 ppm lead acetate in their drinking water at 42 days of age. The results showed that the activity of AST and ALT was significantly higher ($p \leq 0.05$) in the second group compared to the other groups. These findings were similar because lead acetate was toxic and raised the basal metabolic rate, which damaged cells (29). (30) also suggested that lead may cause increased cell membrane permeability or liver cell membrane damage. These results agreed with those of (31), who found that when broilers were given 2500 ppm lead acetate in their drinking water for 35 days, the AST and ALT activities significantly increased compared to the control group. Additionally, research (32) revealed a significant increase in AST and ALT activities in broilers fed 200 mg/kg lead acetate in their diet at 21 and 42 days of age. The table also showed no significant differences in total protein, globulin concentration and AST and ALT enzyme activities between the fourth group and the control group. These findings indicated that the *Eruca sativa* seed extract had a positive role in mitigating lead acetate toxicity.

Table (2): Effect of *Eruca sativa* seed extract and lead acetate on some blood biochemical parameters of broiler chickens (Mean± SD)

Groups Parameters	group1	group2	group3	group4
Total protein	2.46 [ab]	1.80 [b]	2.71 [a]	2.34[ab]
(g/100ml)	±0.10	±0.30	±0.21	0.17±
Albumin	1.43	1.20	1.33	1.30
(g/100ml)	±0.08	±0.15	±0.03	±0.05
Globulin	1.03[ab]	0.60 [b]	1.38 [a]	1.04 [ab]
(g/100ml)	±0.11	±0.17	±0.22	±0.12
Cholesterol	139.96	136.94	138.72	142.78
(mg/100ml)	±10.94	±21.63	±1.19	±2.73
Triglycerides	96.11	75.28	91.81	91.71
(mg/100ml)	±18.17	±8.02	±6.17	±5.64
AST	36.00[b]	44.33 [a]	33.66 [b]	38.33 [b]
(Ul/L)	0.57±	3.48±	0.88±	0.33±
ALT	10.33 [b]	15.66 [a]	9.33 [b]	11.00 [b]
(UI/L	0.88±	0.88±	1.85±	1.73±

Different Letters in the same row mean there are significant different at ($p \leq 0.05$)

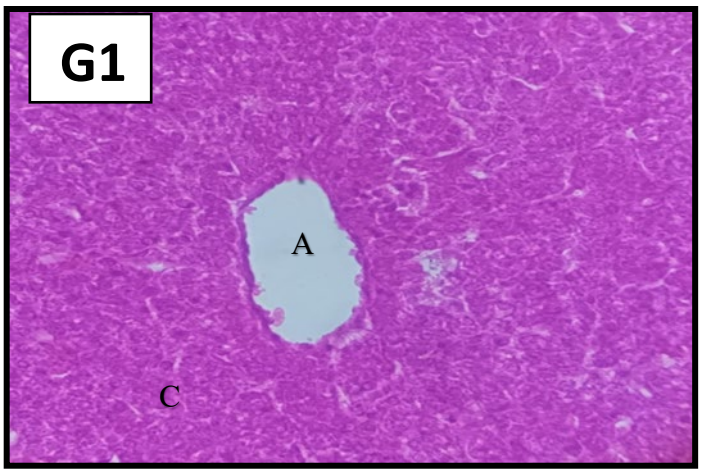
Table 3 shows the effect of adding the alcoholic extract of *Eruca sativa* seeds and lead acetate to the drinking water of broiler chickens on serum, tissues, and feces lead concentrations. The results indicated a significant increase ($p \leq 0.05$) in lead concentration in the serum, breast muscle, liver, and feces in the second group compared to other groups. These findings may have been because the birds were directly exposed to toxic lead acetate. The liver was also the primary organ for lead storage and other tissues (33). The third group had a significantly ($p \leq 0.05$) lower

level of lead in the serum, breast muscle, liver, and feces compared to the other groups. Because of *Eruca sativa* seeds contained mucilaginous compounds that worked as chelating agents to stop lead distribution in the body. Chelating agents reduced lead toxicity (34). The levels of lead in serum, breast muscle, liver, and feces were not significantly different between the fourth and control groups. This clearly demonstrated that *Eruca sativa* seeds' alcoholic extract and active compounds were responsible for lowering lead levels in broiler tissues. These findings were consistent with those of (31), who found an increase in liver lead concentration in broilers given 2500 ppm lead acetate in drinking water for 35 days. Also, (35) reported increased liver lead concentrations in broiler fed diets, as well as increased lead acetate concentrations.Figure 1 shows result of histological changes of the broiler liver. In the control group, the histological section of the bird's liver appeared to have normal central hepatic veins and hepatocytes (G1). However, in the second group, the histological section of the bird's liver showed necrosis of the hepatocyte, severe congestion, dilation, hemorrhage in the central hepatic vein, severe hemorrhage in the portal area, and infiltration of inflammatory cells (G2 A and B). Lead acetate may have caused oxidative stress, leading to excessive free radical production, increased lipid peroxidation, and oxidative stress, which could destroy or kill cells (36). Broilers that received 2500 ppm lead acetate in their drinking water for 35 days experienced liver cell death and congestion in the central vein, as reported in (31). In the third group, the liver cells of birds appeared normal (G3). On the other hand, histological sections of birds' livers in the fourth group showed a normal central hepatic vein with no congestion (G4). The hepatocytes around the hepatic vein also got better, and there was only a little hemorrhage in the portal area. It's possible that this improvement was caused by the alcoholic extract of *Eruca sativa* seeds, which had antioxidants like phenols and flavonoids that kept the body from getting hurt by free radicals (37).

Table (3): Effect of *Eruca sativa* seed extract and lead acetate on serum, tissues and feces lead concentration (Mean±SE)

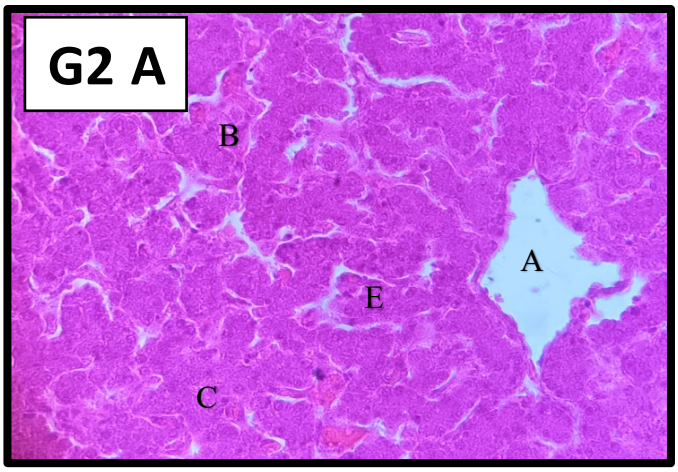
Groups Parameters	group 1	group 2	group 3	group 4
Serum lead (mg/L)	0.58 [b] ±0.043	0.72 [a] ±0.013	0.44 [c] ±0.008	0.64 [b] ±0.010
Breast lead (mg/g)	3.13 [b] ±0.33	4.17 [a] ±0.09	2.25 [c] ±0.02	3.31 [b] ±0.32
Liver lead (mg/g)	3.50 [b] ±0.09	4.87 [a] ±0.12	2.49 [c] ±0.03	3.71 [b] ±0.02
Feces lead (mg/g)	36.84 [b] ±1.77	57.84 [a] ±1.64	26.14 [c] ±0.03	38.38 [b] ±4.19

Different Letters in the same row mean there are significant different at (p≤ 0.05)

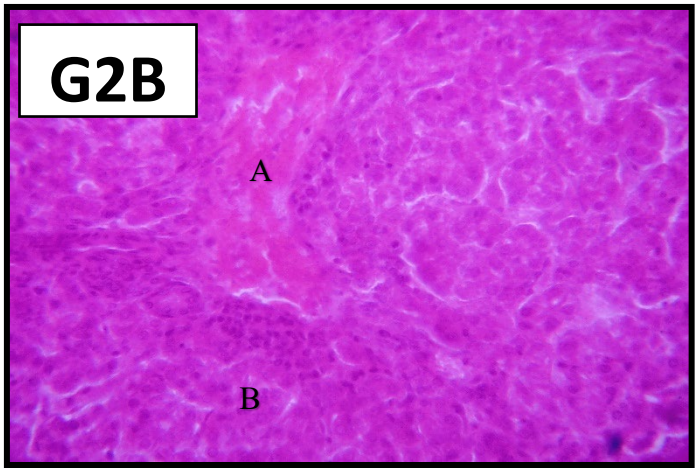


G1: A= normal hepatic vein

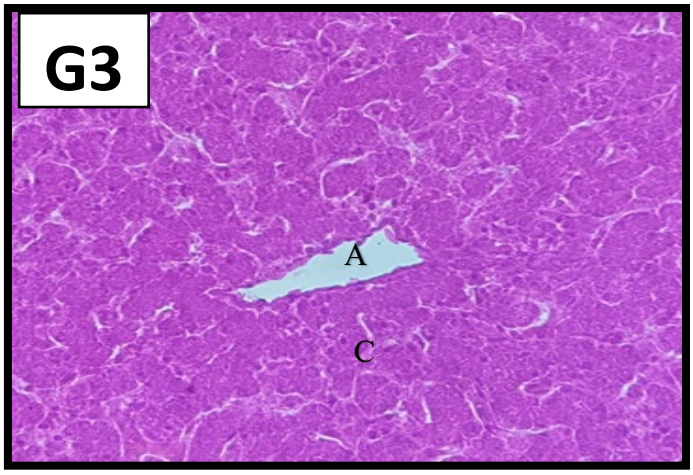
C= normal hepatic cell



G2A: A = abnormal hepatic vein with dilation, B = sever hemorrhage in hepatic tissue, C = abnormal hepatic cell (necrotic hepatic cell), E= infiltration of inflammatory cells.



G2B: A = sever hemorrhage with necrotic hepatocytes B= infiltration of inflammatory cells.



G3: A= normal hepatic vein C= normal hepatic cells

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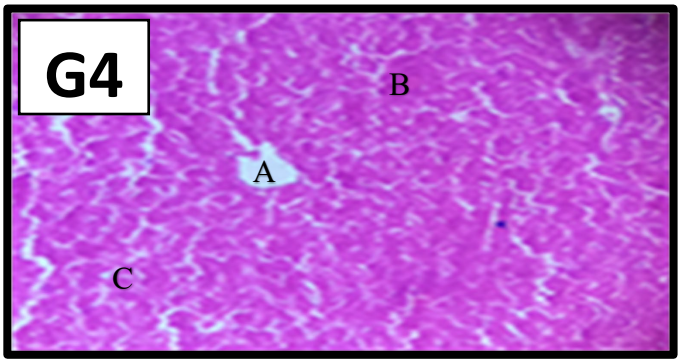

G4: A = normal hepatic vein, there is no congestion, B= mild hemorrhage in portal area, C= appearance of normal hepatic cells surrounded central vein

Figure 1: Histological sectioning of liver broiler chickens with lead acetate and an alcoholic extract of *Eruca sativa* seeds Hematoxylin and Eosin .40x.Z

Conclusion

It is concluded that adding lead acetate to the drinking water of broiler chickens causes oxidative stress and liver tissue damage. The results clearly demonstrate that the alcoholic extract of *Eruca sativa* seeds plays a positive role in reducing the adverse effects of lead acetate by improving some biochemical blood parameters and liver tissue structure.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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تأثير المستخلص الكحولي لبذور الجرجير في بعض معايير الدم الكيميو حيوية والصفات النسيجية لكبد فروج اللحم المعرضة لسمية خلات الرصاص

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قسم الإنتاج الحيواني، كلية الزراعة، جامعة البصرة، العراق

الخلاصة

هدفت الدراسة الحالية الى دراسة تأثير إضافة المستخلص الكحولي لبذور الجرجير وخلات الرصاص الى ماء الشرب في بعض معايير الدم الكيميو حيوية والتغييرات النسيجية لكبد فروج اللحم. استخدم 144 فرخا من افراخ فروج اللحم (Ross 308) بعمر يوم واحد بوزن ابتدائي 40 غم. وزعت الطيور عشوائيا على أربع معاملات (كل معاملة 36 طائراً)، بثلاث مكررات لكل منها (12 طائرا لكل مكرر). المعاملة الأولى كانت معاملة سيطرة (من دون إضافة)، المعاملة الثانية اضافة 350 ملغم من خلات الرصاص لكل لتر من ماء الشرب، المعاملة الثالثة اضافة 250 ملغم من المستخلص الكحولي لبذور الجرجير لكل لتر من ماء الشرب، المعاملة الرابعة اضافة 350 ملغم من خلات الرصاص و250 ملغم من المستخلص الكحولي لبذور الجرجير لكل لتر من ماء الشرب. اشارت النتائج الى وجود ارتفاع معنوي ($p\leq 0.05$) في تركيز البروتين الكلي والكلوبيولين في المعاملة الثالثة، بينما وجد انخفاض معنوي ($p\leq 0.05$) في تركيز البروتين الكلي والكلوبيولين في المعاملة الثانية مقارنة بمعاملات الدراسة الاخرى. اشارت النتائج الى وجود ارتفاع معنوي ($p\leq 0.05$) في فاعلية انزيمي Aspartate Aminotransferase (AST) و Alanine (ALT) Aminotransferase وفي تركيز الرصاص في مصل الدم وعضلة الصدر والكبد والبراز في المعاملة الثانية، بينما وجد انخفاض معنوي ($p\leq 0.05$) في تلك المعايير في المعاملة الثالثة مقارنة بمعاملات الدراسة الاخرى. اشارت النتائج الى عدم اختلاف المعاملتين الرابعة والسيطرة معنويا في تركيز البروتين الكلي والكلوبيولين وفاعلية انزيمي AST وALT وفي تركيز الرصاص في مصل الدم وعضلة الصدر والكبد والبراز. اشارت النتائج الى عدم وجود فروق معنوية في تركيز الالبومين والكوليسترول والكليسيريدات الثلاثية بين معاملات الدراسة المختلفة. اشارت النتائج الى تأثر نسيج الكبد سلبا بعد معاملة فروج اللحم بخلات الرصاص. نستنتج ان إضافة المستخلص الكحولي لبذور الجرجير مع خلات الرصاص الى ماء الشرب لفروج اللحم أدت الى الحد من الاجهاد التأكسدي الناجم عن خلات الرصاص وتحسين بعض معايير الدم الكيميوحيوية.

الكلمات المفتاحية: الاجهاد التأكسدي، خلات الرصاص، بذور الجرجير، فروج اللحم.