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Evaluate the Impact of the Alcoholic Extract of *Eruca sativa* Seeds on the Physiological and Productive Responses of Broiler Chickens Exposed to Lead Acetate-Induced Oxidative Stress

Sulwan J. Hanna* and Khalid C. K. Al-Salhie
Animal Production Department, College of Agriculture, University of Basrah, Iraq

**Corresponding author: E-mail*: sulwan575@gmail.com

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ABSTRACT

The study aimed to assess the effects of adding an alcoholic extract of *Eruca sativa* seeds and lead acetate to drinking water on the productive and physiological performance of broiler chickens. A total of 144 one-day-old Ross 308 broiler chicks were used, divided into four treatments of 36 birds each, with three replicates per treatment. The control treatment received no additives, while the second treatment received 350 mg/L of lead acetate. The third treatment received 250 mg/L of *Eruca sativa* seeds extract, and the fourth treatment received a combination of both 350 mg/L lead acetate and 250 mg/L *Eruca sativa* seeds extract. Results showed that the third treatment had the best results, including higher body weight, final weight gain, cumulative feed consumption, and better feed conversion efficiency. This treatment also had significant ($P \leq 0.05$) increases in packed cell volume (PCV), total red blood cell (RBC), and hemoglobin concentration (Hb) compared to other treatments. Additionally, the third treatment had lower heterophil percentages and a more positive lymphocyte percentage and heterophil to lymphocyte (H/L) ratio. The antioxidant enzyme activities, superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), and catalase (CAT) were significantly ($P \leq 0.05$) higher in the third treatment, while malondialdehyde (MDA) levels were lower. The study concludes that the alcoholic extract of *Eruca sativa* seeds effectively reduces lead acetate-induced oxidative stress and enhances both and productive and physiological performance of broiler chickens.

تقييم تأثير المستخلص الكحولي لبذور الجرجير في الاستجابة الفسيولوجية والإنتاجية لفروج اللحم المعرض للإجهاد التأكسدي الناجم عن خلات الرصاص

سلوان جوزيف حنا وخالد جلاب كريدي الصالحي
قسم الإنتاج الحيواني، كلية الزراعة، جامعة البصرة، العراق

الخلاصة

هدفت الدراسة إلى تقييم تأثير إضافة المستخلص الكحولي لبذور الجرجير وخلات الرصاص إلى مياه الشرب في الأداء الإنتاجي والفسيولوجي لفروج اللحم. أُستخدم 144 فرخاً من سلالة روس 308 بعمر يوم واحد، وُزعت على أربع معاملات كل منها 36 طائرًا، بثلاث مكررات لكل معاملة. لم تعطى معاملة السيطرة أي إضافات، بينما أُعطيت المعاملة الثانية 350 ملغم / لتر من خلات الرصاص. أُعطيت المعاملة الثالثة 250 ملغم / لتر من مستخلص بذور الجرجير، وأُعطيت المعاملة الرابعة مزيجًا من كل من خلات الرصاص 350 ملغم / لتر و 250 ملغم / لتر من مستخلص بذور الجرجير. أظهرت النتائج أن المعاملة الثالثة حققت أفضل النتائج، بما في ذلك وزن الجسم النهائي والزيادة الوزنية النهائية واستهلاك العلف التراكمي وافضل كفاءة تحويل غذائي. كما أظهرت هذه المعاملة زيادة معنوية ($P \leq 0.05$) في حجم خلايا الدم المرصوصة وعدد خلايا الدم الحمراء وتركيز الهيموغلوبين مقارنة بالمعاملات الأخرى. بالإضافة إلى ذلك، سجلت المعاملة الثالثة نسب منخفضة من الخلايا المتغايرة وزيادة في نسبة الخلايا الليمفاوية واقل نسبة للخلايا المتغايرة إلى الخلايا الليمفاوية. كانت أنشطة إنزيمات مضادات الأكسدة، سوبر أكسيد ديسميوتاز وغلوتاثيون بيروكسيديز وكاتاليز أعلى معنوياً ($P \leq 0.05$) في المعاملة الثالثة، بينما كانت مستويات المالونديالدهيد أقل. وخلصت الدراسة إلى أن المستخلص الكحولي لبذور جرجير يقلل بشكل فعال من الإجهاد التأكسدي الناجم عن خلات الرصاص ويعزز كل من معايير الدم وأداء النمو في فروج اللحم.

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

INTRODUCTION:

Poultry is considered one of the most important food sources due to its high nutritional value, low cost, and ease of preparation, in addition to the high-quality protein it provides (Barbut and Leishman, 2022). Heavy metal pollution has attracted the attention of researchers who want to reduce the toxicity or accumulation of these pollutants in living organisms (Alengebawy *et al.*, 2021). Researchers have linked lead (Pb), one of the main environmental pollutants, to more accidental poisoning deaths in pets and birds than any other substance (Roegner *et al.*, 2013).

Lead pollution is widespread in cities and surrounding areas and is likely to be significant near smelters, other industrial facilities, or major highways where vehicle exhaust fumes contaminate the surrounding area (Levin *et al.*, 2021). However, the animal's health and the availability of nutrients determine how much lead the intestines absorb (Assi *et al.*, 2016). Lead affects proteins and enzymes, thus interfering with amino acids and impacting protein synthesis, reducing the total protein amount, and associating with cell membranes (Sanders *et al.*, 2009). Lead alters cell membrane permeability by reducing the effectiveness of the metabolic

glutathione compound, which works to eliminate free radicals that cause cell wall damage and breakdown, allowing enzymes to pass into the bloodstream (Lobo *et al.*, 2010). Given this understanding, the search for solutions and treatments has become a vital issue.

Researchers have used medicinal plants and their extracts to reduce oxidative stress and improve the productive and physiological performance of poultry (Al-Salhie and Al-Waeli, 2019; Al-Ashoor and Al-Salhie, 2020; Al-Mosawy and Al-Salhie, 2021). *Eruca sativa*, known as arugula, is a medicinal plant whose seeds contain several components that significantly contribute to biological activities, including antioxidant activities, as they are rich in vitamins E and C and beta-carotene (Sarwar Alam *et al.*, 2007). These compounds are of great importance to human health as they are antifungal, antibacterial, anticancer, and antioxidants (Sharifi-Rad *et al.*, 2015). *Eruca sativa* seeds contain flavonoids and phenolic compounds, which are effective antioxidants both *in vivo* and *in vitro* because they suppress free radicals and have antibacterial and antiviral activity (Sarwar Alam *et al.*, 2007). Scientific studies on the effects of *Eruca sativa* seeds extract on poultry are relatively uncommon, despite the plant's and its seeds' enormous significance and the variety of applications and effects they have in human nutrition and medicine. Thus, the purpose of the current study was to evaluate the impact of the alcoholic extract of *Eruca sativa* seeds on the physiological and productive responses of broiler chickens exposed to lead acetate-induced oxidative stress.

MATERIALS AND METHODS:

The current study was conducted in the poultry hall of the Animal Field at the College of Agriculture, University of Basrah, from October 9, 2023, to November 12, 2023, lasting 35 days.

Preparation of Alcoholic Extract of *Eruca sativa* Seeds:

The alcoholic extract was prepared according to the method described by WHO (2018). Fifty grams of *Eruca sativa* seeds powder were placed in a 500-ml glass beaker, and 250 ml of 70% ethyl alcohol was added. The mixture was left for 24 hours in a water bath at 37°C. It was then stirred with an electric mixer for one hour. The solution was filtered using a medical gauze cloth. The filtrate was distributed into test tubes for centrifugation at 3000 rpm for 15 minutes. The supernatant was collected, and the sediment was discarded. The supernatant was placed in glass petri dishes inside a drying incubator at 37°C. After complete drying, the extract was scraped off. The resulting extract weighed 5 grams from the original 50 grams of *Eruca sativa* seeds powder. It was then dissolved in 100 ml of distilled water and stored in tightly sealed glass bottles in the refrigerator until use.

Birds Management:

The broiler chicks were reared for 35 days under similar conditions in a closed hall. The birds were distributed into single-tier iron cages with dimensions of 120 cm (length) × 80 cm (width) × 70 cm (height). Each cage housed twelve chicks. In the first week, the temperature was set at 33.5°C, 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 8 to 35 days. The birds were fed a starter diet containing 22.34% crude protein and 3074 kcal/kg of metabolizable energy from 1 to 21 days. From 22 to 35 days, they were fed a grower diet containing 20.21% crude protein and 3170.5 kcal/kg of metabolizable energy. Table 1 shows the composition and chemical analysis of the diets based on NRC (1994).

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	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 Treatments:

The current study included four treatments. A total of 144 one-day-old broiler chicks weighing 40 grams were used. The birds were divided into four treatment groups (36 birds per treatment), with three replicates per treatment (12 birds per replicate). The first treatment served as the control group without any additional substances. In contrast, the second treatment was added 350 mg of lead acetate per litre of drinking water. The third treatment was added 250 mg of alcoholic extract of *Eruca sativa* seeds per litre of drinking water, while the fourth treatment combined 350 mg of lead acetate with 250 mg of alcoholic extract of *Eruca sativa* seeds per litre of drinking water.

Studied Traits:

Productive Traits: At the end of the experiment, overall weight, final weight gain, cumulative feed intake, and feed conversion efficiency were calculated based on Kyakma *et al.*, (2022).

Haematological Traits: Blood samples were randomly collected from the birds at 32 days of age from the leg vein for one male per replicate. One milliliter of blood was put into tubes that had the anticoagulant EDTA in them to find out the packed cell volume (PCV), total red blood cell count (RBC), haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), heterophil percentage (H), lymphocyte percentage (L), and the heterophil to lymphocyte ratio (H/L). These parameters above were estimated according to Campbell, (1995).

Antioxidant Parameters: Based on Beers and Sizer (1952), Flohe and Gunzler (1984), and Yagi (1998); the study assessed antioxidant status by measuring glutathione peroxidase (GSH-PX), superoxide dismutase (SOD), catalase (CAT) activities, and malondialdehyde (MDA) concentration in serum.

Statistical Analysis:

The data were analyzed using the statistical software program SPSS (2018) according to a completely randomized design (CRD). Duncan's test (1955) was used to test the differences between treatments 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: Treatment effect. ei: Experimental error effect.

RESULTS AND DISCUSSION:

Table 2 presents the effect of adding an alcoholic extract of *Eruca sativa* seeds and lead acetate to the drinking water on the productive performance of broiler chickens. Highest overall body weight, final weight gain, and cumulative feed intake, with improved feed conversion efficiency were recorded in the third treatment compared to other treatments. The second treatment group showed a significant reduction ($P \leq 0.05$) in overall body weight, final weight gain, cumulative feed intake, with worse feed conversion efficiency compared to the other treatments. The fourth treatment group did not differ significantly the control treatment. The presence of bioactive compounds in the alcoholic extract of *Eruca sativa* seeds, particularly flavonoids, glycosides, and phenols, which are known antioxidants. By serving as hydrogen or electron donors, these compounds inhibit or delay the oxidation process. They then interact with free radicals and the oxidizing agent to form compounds empty of free radicals, which reduce oxidative stress. Therefore, it stimulates the secretion of digestive enzymes and improve digestion (Villatiro-Pulido *et al.*, 2012; Abdel-Moneim *et al.*, 2020; Omar *et al.*, 2020; Favela-González *et al.*, 2020).

Table (2): Effect of *Eruca sativa* seed extract and Lead acetate on productive performance (Mean± SE)

Treatments Parameters	T_1	T_2	T_3	T_4
Initial body Weight (g)	40 [a] ± 0.00	40 [a] ±0.00	40 [a] ±0.00	40 [a] ±0.00
Overall Body Weight gain (g)	2138.33 [bc] ± 56.27	2005.13 [c] ±52.51	2468.27 [a] ±8.66	2214.66 [b] ±28.41
Final Weight gain (g)	2098.33 [bc] ±56.27	1965.13 [c] ±52.51	2428.27 [a] ±8.66	2174.66 [b] ±28.41
Cumulative Feed Intake (g)	2961.42 [b] ±30.14	2872.09 [b] ±19.65	3136.11 [a] ±27.90	2927.12 [b] ±38.26
Feed Conversion Ratio (g.g)	1.41[ab] ±0.02	1.46[a] ±0.03	1.29[c] ±0.08	1.34[bc] ±0.08

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

By enhancing digestion, absorption, and metabolism, phenols improve nutrient utilization and protect cells from oxidative stress. These compounds prevent or delay the oxidation process by acting as supporters of hydrogen or electrons. Following their interaction with the oxidizing agent and free radicals, they produce compounds empty of free radicals that decrease oxidative stress (Hu *et al.*, 2019; Abdel-Moneim *et al.*, 2020; Mahfuz *et al.*, 2021). Lead acetate causes oxidative stress, which raises the production of free radicals and lipid peroxidation and damage and death of cells (Matović *et al.*, 2015; Ebrahimi *et al.*, 2023). These findings are consistent with Borekar *et al.*, (2021), who reported a significant decrease in body weight in broiler chickens fed a diet with 200 mg/kg lead acetate for 28 days compared to the control group. Similarly, Al-Shammari and Batkowska, (2021) found that adding 200 ppm lead acetate to the drinking water of broiler chickens for 42 days led to poor feed conversion efficiency compared to the control.

Table 3 shows the effect of adding an alcoholic extract of *Eruca sativa* seeds and lead acetate to the drinking water of broiler chickens on some haematological parameters. The third treatment group did significantly ($P \leq 0.05$) better than the other treatments in the PCV, RBC, and Hb. The third treatment group did not differ significantly from the control and fourth groups. This might be because of the bioactive compounds in the *Eruca sativa* seed extract, mostly phenols and flavonoids. These compounds work as antioxidants to protect body cells from free radical damage (Kaurinovic and Vastag, 2019). This improvement is evident in the enhanced live weights, weight gain, and feed conversion efficiency (Table 2). The significant increase in the PCV and Hb is generally associated with the total red blood cell count and stable health conditions of the birds (Campbell, 1995). The results indicated a significant decrease ($P \leq 0.05$) in the PCV, RBC, and Hb in the second treatment group compared to third treatment. These values did not differ significantly between the control and fourth treatments. The significant ($P \leq 0.05$) increase in the H/L ratio was recorded in the second treatment compared to other treatments. These findings may be because lead acetate-induced oxidative stress. Exposure to lead causes a reduction in red blood cell lifespan, leading to anaemia,

and inhibits haemoglobin synthesis (Jain, 1986). The results showed no significant differences in the MCV, MCH, and MCHC among the treatments.

Table (3): Effect of *Eruca sativa* seed extract and Lead acetate on some blood parameters (Mean± SE)

Treatments Parameters	T_1	T_2	T_3	T_4
RBC (x 10^6 .mm^3)	2.83 ^{ab} ±0.03	2.64 ^{b} ±0.07	3.06 ^{a} ±0.10	2.77 ^{ab} ±0.18
Hb (g.dL)	8.50 ^{ab} ±0.09	8.00 ^{b} ±0.19	9.11 ^{a} ±0.29	8.33 ^{ab} ±0.50
PCV (%)	25.50 ^{ab} ±0.28	24.00 ^{b} ±0.57	27.33 ^{a} ±0.88	25.00 ^{ab} ±1.52
MCV (fl)	90.10 ±0.18	90.90 ±0.29	89.31 ±0.29	90.25 ± 0.71
MCH (pg)	30.03 0.06±	30.30 0.09±	29.77 0.09±	30.07 0.23±
MCHC (g.L^1)	33.33 ±0.008	33.33 ±0.008	33.33 ±0.008	33.32 ±0.008
Heterophils (%)	22.33 ^{b} 1.45±	35.00 ^{a} 1.15±	17.33 ^{c} 0.88±	26.00^{b} 1.73±
Lymphocytes (%)	63.66 ^{b} 1.45±	56.00 ^{c} 1.15±	77.66^{a} 0.88±	62.00 ^{b} 1.73±
H/L ratio	0.35 ^{b} 0.02±	0.62 ^{a} 0.03±	0.22 ^{c} 0.01±	0.41 ^{b} 0.04±

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

The present results are consistent with the findings of Borikar *et al.*, (2024), who reported the addition of lead acetate at a concentration of 200 mg/kg to broiler chickens resulted in a significant decrease in packed cell volume, red blood cell count and haemoglobin concentration in the birds' blood compared to the control treatment. The results indicate that the second treatment group recorded the highest rate of heterophil percentage compared to the other study treatments. In contrast, the third treatment group recorded the lowest average compared to the other study treatments. The fourth treatment group did not significantly differ from the control group. This may be attributed to the oxidative stress experienced by the birds in the second treatment group, leading to increased secretion of corticosterone from the adrenal cortex, which increases heterophil cells and reduces lymphocyte cells (Siegal, 1980). The significant decrease in heterophil percentage in the third treatment group can be attributed to the role of the alcoholic extract of *Eruca sativa* seeds, which contains phenols acting as antioxidants and playing a crucial role in protecting cells from oxidative stress (Kaurinovic and Vastag, 2019). The third treatment group recorded the highest lymphocyte percentage compared to other treatments. In contrast, the second treatment group recorded the lowest average compared to the other study treatments. A low heterophil-to-lymphocyte (H/L) ratio is a good indicator of bird health and stress levels. An elevated ratio indicates severe stress. The decreasing in the lymphocytes can be attributed to increased secretion of corticosterone from the adrenal cortex, which reduces lymphocyte and increases heterophil in poultry (Tetel and Fraley, 2022)

Table 4 shows the impact of adding the alcoholic extract of *Eruca sativa* seeds and lead acetate to the drinking water on the antioxidant status of broiler chickens. The results showed a significant increase ($P \leq 0.05$) in the SOD activity in the serum of the third treatment group compared to the other treatments. While the second treatment group recorded a significant decrease ($P \leq 0.05$) in the SOD activity compared to the other treatments. The results also indicated that the third treatment group showed a significant increase ($P \leq 0.05$) in the GSH-PX activity compared to the other study treatments. In contrast, the second treatment group showed a significant decrease ($P \leq 0.05$) in this enzyme activity compared to the other treatments. The results also showed that the third treatment group recorded a significant increase ($P \leq 0.05$) in the CAT activity compared to the other study treatments. On the other hand, the second treatment group showed a significant decrease ($P \leq 0.05$) in the CAT activity compared to the other treatments. The results indicated a significant increase ($P \leq 0.05$) in the MDA concentration in the serum of the second treatment group compared to the other study treatment. The third treatment group showed a significant decrease ($P \leq 0.05$) in the MDA concentration compared to the other treatments. The control group did not significantly differ from the fourth treatment group in these parameters. The reduced antioxidant enzyme activity and increased MDA level in the serum of the second treatment group can be attributed to oxidative stress induced by lead acetate. Lead acetate increases free radical generation and reduces antioxidant enzyme activity.

The elevated MDA concentration in the serum of the second treatment group may be due to lead's effect on the hepatic cell membrane and its impact on unsaturated fatty acids such as arachidonic acid (Lawton and Donaldson, 1991). Lead causes oxidative stress, DNA damage and cell death (Xu *et al.,* 2008). The increased antioxidant enzyme activity and decreased MDA level in the serum of the third treatment group can be attributed to the role of the alcoholic extract of *Eruca sativa* seeds. *Eruca sativa* seeds contain the phenols and flavonoids. These compounds act as antioxidants, suppressing free radicals, thereby reducing oxidative stress or preventing it altogether and consequently reducing cell damage (Kaurinovic and Vastag, 2019). The results clearly show the positive role of the alcoholic extract of *Eruca sativa* seeds in mitigating the negative effects of lead acetate on the antioxidant system and maintaining blood cell integrity. This is evident from the lack of significant differences in antioxidant enzyme activity, MDA concentration, and haematological parameters between the control group and the fourth treatment group. Al-Shammari and Batkowska, (2021), reported a significant decrease in antioxidant enzyme activity (SOD, CAT, and GPX) and a significant increase in MDA level in the serum of broiler chickens when 200 ppm lead acetate was added to drinking water for 42 days. These findings also agree with Soliman *et al.,* (2021), who reported a decrease in SOD enzyme activity and a significant increase in MDA level in the serum of broiler chickens at 35 days old when 500 mg/L lead acetate was added to drinking water.

Table (4): Effect of *Eruca sativa* seed extract and lead acetate on serum antioxidant status (Mean±SE)

Treatments Parameters	T_1	T_2	T_3	T_4
SOD (U/MI)	27.32[b] ±0.008	25.60[c] ±0.335	30.23[a] ±0.603	27.84[b] ±0.350
GSH-PX (U I)	1804.66[b] ±2.32	1567.52[c] ±4.64	1856.91[a] ±4.64	1816.91[b] ±5.29
CAT (U/MI)	2.39[b] ±0.014	1.93[c] ±0.088	2.95[a] ±0.005	2.42[b] ±0.028
MDA (UM/L)	1.21[b] ±0.066	1.65[a] ±0.038	0.25[c] ±0.063	1.28[b] ±0.069

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

CONCLUSIONS:

From the data presented above, we might conclude that the active component in the alcoholic extract of *Eruca sativa* seeds plays a beneficial role in decrease the effects of lead acetate-induced oxidative stress. When 250 mg/L of alcoholic extract from *Eruca sativa* seeds was added, both physiological blood parameters and the growth performance of broiler chickens subjected to lead acetate-induced oxidative stress improved.

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