

**EFFECT OF IRAQI SWEET ALMOND (*Prunus amygdalus*) ON
SOME PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS
IN ROCK DOVE PIGEONS (*Columba livia gaddi*)
TREATED WITH LEAD ACETATE**

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ABSTRACT

The current study aims to evaluate the effect of Iraqi sweet almond (*Prunus amygdalus*) on the toxicity of lead acetate in wild pigeons Rock Doves (*Columba livia gaddi*) on body weight and certain hematological and biochemical parameters. Forty healthy adults of both sexes Rock Dove pigeons were randomly selected and categorized into four groups, A,B,C, and D. Group A (control group), were fed on a balanced ration, group B, were administered lead acetate 72 mg/kg body weight orally, group C orally administered with an aqueous extract of Iraqi sweet almond (*Prunus amygdalus*) 300 mg/kg body weight, and group D were orally administered with 72 mg/kg body weight of lead acetate and 300 mg/kg body weight of the aqueous extract of Iraqi sweet almond (*Prunus amygdalus*). These materials were administered daily and for the whole study period which extended to 28 days. Results showed that lead acetate alone caused significant decrease ($P \leq 0.05$) in the body weight, hemoglobin concentration, total erythrocyte count, PCV% and triglycerides, while there were a significant increase ($P \leq 0.05$) in total leukocyte count, heterophils, monocytes, lymphocytes, glucose, uric acid, and AST and ALT activities. On the other hand, combined treatment with Iraqi sweet almond and lead acetate to members of group (D) caused significant increase ($P \leq 0.05$) in hemoglobin, total erythrocyte count, PCV% and lymphocytes, and significant decrease ($P \leq 0.05$) in cholesterol and uric acid, while no significant differences in triglyceride and AST and ALT activities. The present finding concludes that adding of Iraqi sweet almond has significant

effects on certain hematological and biochemical properties of Rock Dove pigeons (*Columba livia gaddi*) affected by administering lead.

INTRODUCTION

Lead (Pb) is one of the abundant environmental pollutants that make a wide range of physiological and biochemical problems in animals (1). Commonly, lead produces acute and chronic poisoning and induces an expansive variety of dysfunctions in animals, certainly many enzymes, biochemical processes, and macromolecules have been shown to be affected by lead (2).

Animals are exposed to lead by many sources, also from the general environment, but the sources of lead poisoning are soil, industrial pollutant, agricultural technology, food processing, and ingested lead (3). Gathered lead is toxic in most of its chemical forms, whether it is inhaled or ingested by food or water (4). However, lead toxicity is variable depending on the condition of the animal, nutritional composition (both type and amounts), exposure duration and chemical structure of lead derivatives (many lead compounds, except lead acetate are difficulty solve in water) (5). The most obvious manifestation of lead poisoning is anemia due to inhibition of heme synthesizing enzymes. Lead is also diminished stability of erythrocyte membrane (6), and finally, lead blood level measurement to consider the most important indicator to lead poisoning (7). There are many indications show that lead is a toxic agent with multiple target organs such as hematopoietic system, nervous system, immune system, and kidneys (8).

Substances derived from medicinal plants endure the basis for a large part of viable medications used in the treatment of various disorders (9). Sweet almond is a fruit whose phytochemical ingredients consider a powerful remedy; it offers a wide-ranging collection of healthful benefits (10). It is contain a high levels of vitamin E, fibers, magnesium, arginine, polyphenolic compounds, and monounsaturated fatty acids (11), since vitamin E is the main antioxidant which contests free radicals produced in the body, and the efficient antioxidant ingredient of plants origin play significant roles in improving tissue defense against oxidative stress toxicity in both human and animals (12).

There are no available data published about the toxic effect of lead acetate, hematological, biochemical parameters, and role of Iraqi sweet almond for Rock Dove (*Columba livia gaddi*) model, therefore the goal from the present work was to discover that's data.

MATERIALS AND METHODS

Experimental animals: The birds used in the present study were apparently healthy adults of both sexes of wild pigeons Rock Dove (*Columba livia gaddi*) (14,15) were commonly obtained from a local market in Mosul, Iraq. The birds were individually kept in captivity cages (80 × 60 × 70 cm) of about 25 C° with water and poultry feed available *ad libitum* for 7-14 days before experiments. All the experiments were complied with institutional regulations addressing animal use, and proper attention and care were given to the birds used in this study.

The euthanasia of the birds was conducted on the (28) day of the experiment in accordance with the laws and regulations applied to Laboratory animals, especially those related to the prevention of torture (Use of less lethal killing technique) (16).

Lead acetate used in this experiment was Lead Acetate $Pb(C_2H_3O_2)_2 \cdot 3H_2O$, Sp.gr.:2.50, m.p.:200C. Basfe company Ltd. (Email: Basfe.material@yahoo.com) at a dose of 72 mg/kg body weight orally, which documented by (17) .

Sweet almond seeds were commonly obtained from a local market in Mosul, Iraq, the whole almond seeds sliced and dried in an oven in about temperature 25°C until the almonds become free of moisture and the extraction was carried out by Soxhlet apparatus using watery extract that considered as a very effective in extracting the active ingredients of the almond (18).

Experimental design:

Rock Dove were randomly divided into four groups (10 birds per group) and fed for 28 days the following diets:

1. Group A: (control group): Diet contained essential nutrients optimal to the requirement of chickens (19).
2. Group B: lead acetate 72 mg/kg body weight orally by steel gavage needle.
3. Group C: Iraqi sweet almond 300mg/kg body weight orally by steel gavage needle.

4. Group D: lead acetate 72 mg/kg body weight and sweet almond 300mg/kg body weight orally by steel gavage needle.

Sampling and analysis:

At the end of experiment (28) days, the control and treated birds were euthanised by decapitation and blood samples were collected using heparin zed test tubes. The hematological studies were performed within two hours of blood collection. Total erythrocyte count (TEC), White blood cells count (WBCs) were obtained with haemocytometer. The differential WBC counts were determined on the same blood smears, by counting 200 WBC from a representative part of the smears.

Hemoglobin (g/dl) concentration and packed cell volume (PVC %) were determined by sahli haemometer and microhaematocrit methods, respectively immediately before serum separation by blood centrifugation (Shanghai instruments factory 80-2 centrifuge, China) at 3000 cir/min for 15 minute.

Serum biochemical analyses: Serums were separated and immediately analyzed for total cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities, glucose, uric acid and serum triglyceride concentrations were measured on an analyzer (Reflotron plus 2011, Roche, Germany) the instrument is work on the principle of reflectance photometry (21).

Statistical analysis: Analysis of data was performed using analysis of variance ANOVA test by SPSS program (ver.12) to determine mean \pm S.E. Duncan's Multiple range test were used to determine significant difference between the groups at $p \leq 0.05$ (22).

RESULTS AND DISCUSSION

In the current study, table 1 revealed that the effect of lead acetate at 72 mg/kg BW orally reduced growth in terms of BW at the end of the study, body weight gain was statically lower in the lead-treated group than that in the control and Iraqi sweet almond treated group ($P \leq 0.05$.), the results obtained coincide with the finding of (23), who stated that growth inhibition was reported as a sing of lead toxicity in birds that may be due to the lead decrease appetite and low energy (24). The effect of sweet almond on weight gain was not statically different compared to control, these results agree with (25), they stated that using of sweet almond in the diet had no adverse effect on quail growth performance. There is no report regarding the effect of sweet almond on the toxicity of lead on body weight in pigeons. However the decrease in

body weight gain in supplementation of both lead acetate and sweet almond which may be due to the harmful effect of lead acetate in high dose on body weight gain (26). Growth depression may be due to metabolic disorders associated with lead, such as inhibition of enzymes involved in heme synthesis and the oxidase system. Lead also has a strong affinity to mitochondria and many of its pathological effects may be caused by ultra-structural and functional changes in these organelles (27). It has been demonstrated that lead has a potential for inducing oxidative stress, which may, in turn, result in loss of cellular functions and tissue damage, possibly leading to growth depression and impairment of health (28).

Table 1 : Effect of Lead Acetate and Iraqi sweet almond on body weight gain (g) in Rock Dove (*Columba livia gaddi*).

Groups of birds *			
(control)	Lead acetate 72mg/kg BW orally	Iraqi sweet almond 300mg/kg BW orally	Lead acetate 72mg/kg BW and Iraqi sweet almond 300mg/kg BW orally
377.47g±1.62 A	279.81g±1.09 b	379.73g±0.63 a	281.51g±3.12 B

* Mean ± S.E. for 10 birds in each group.

Different letters mean significant differences ($p \leq 0.05$).

The results of hematological parameters are summarized in table (2), showed that lead acetate has a significant dose dependent decreasing effect on Hb concentration, erythrocytes and PCV% in treated groups when compared with control and sweet almond groups, this may be due to the deleterious effect of lead on blood causing anemia by two basic detects : a shortened erythrocyte lifespan and impairment of heme synthesis, however, lead cause increased concentration of protoporphyrin by inhibiting heme synthetase, the enzyme which combined protoporphyrin and iron to form heme (29). The negative effects of lead on

erythrocytes may be due to lead attaches and damages the red blood cells as a whole or it is membrane (30). The combination of sweet almond with lead acetate group causes significant increases ($p \leq 0.05$) in (PCV%) and (Hb) concentration and this may be due to sweet almonds containing a good proportions of iron, manganese, potassium, calcium and magnesium, as well as a group of vitamins that collectively play an important role in the increase of (PCV%) and (Hb) concentration, this result is agree with (31) and (32) who mentioned that sweet almonds play an important role in the treatment of severe anemia because of its important role in the production of red blood cells by activating stem cells in the bone marrow as well as the production of immune cells that have a role in immune activation and the sweet almonds have a very effective role in the treatment of anemia because it contains a percentage of copper is estimated (1.15mg/100gm almond), so sweet almonds is one of the important sources involved in the formation of hemoglobin in the body.

Table 2: Individual comparison of the mean values of certain hematological parameters corresponding to the treated groups with the mean of control group.

Groups of birds *				
Parameters	(control)	Lead acetate 72mg/kg BW orally	Iraqi sweet almond 300mg/kg BW orally	Lead acetate 72mg/kg BW and Iraqi sweet almond 300mg/kg BW Orally
TEC ($10^{12}/L$)	3.89±0.09 a	2.67±0.33 b	3.93±1.41 a	3.62±0.78 a
Hb (g/dl)	14.76±0.11 a	12.71±1.29 b	15.92±9.91 a	14.39±1.32 a
PCV (%)	47.75±0.32 a	35.30±0.92 b	49.11±0.01 a	46.76±1.61 a

* Mean ± S.E. for 10 birds in each group.

Different letters mean significant differences ($p \leq 0.05$).

Table (3) demonstrated that administration of lead acetate alone, total leukocyte count had increased mainly due to an increase in heterophil and monocyte count. There was also an increase in lymphocyte count, which was statistically significant ($p \leq 0.05$). In some reports, leukocytosis has been attributed to the lead-induced inflammation (33). A three-fold increase in heterophil and monocyte count along with severe leukocytosis in the laboratory animals that were exposed to lead has also been reported (34). Controversies exist about monocytes; since in some studies lead-induced monocytopenia (35,36) and in others significant increases in monocyte count have been reported (37,38). The reason for such difference is probably due to the extent of lead-induced inflammation. Consistent with other reports severe eosinopenia were observed in this study (36).

The results showed that the Iraqi sweet almond when added with lead acetate had a regulating role of the immune system through Its role in the reduction of inflammatory cells and increase the lymphocytes that have the largest role in inhibiting inflammation by reducing the effectiveness of the foreign body (39), and that the vitamin E in the composition of sweet almond helps reduce the stress factors caused by heavy metals and raise the immune response by stimulating centers white blood cells by the bone marrow (40,41).

Table 3 : Individual comparison of the mean values of certain leukocytes parameters corresponding to the treated groups with the mean of control group .

Groups of birds *				
Parameters	(control)	Lead acetate 72mg/kg BW orally	Iraqi sweet almond 300mg/kg BW orally	Lead acetate 72mg/kg BW and Iraqi sweet almond 300mg/kg BW Orally
TLC($10^9/L$)	27.93±2.71 a	40.56±7.22 b	26.67±0.43 a	24.09±1.88 a
Heterophil(%)	39.73±0.33 a	50.81±0.78 b	38.81±0.20 a	39.98±0.65 a
Eosinophil(%)	2.86±0.10 a	1.94±3.76 c	2.97±1.67 a	2.64±2.90 a
Basophil(%)	2.17±0.12 a	2.18±1.02 a	2.10±2.09 a	2.09±0.54 a
Lymphocyte(%)	51.31±0.92 a	67.19±0.55 b	52.60±0.56 a	65.22±1.09 b
Monocyte(%)	2.19±0.22 a	1.13±3.45 b	2.39±1.98 a	2.15±1.56 a
H/L ratio	0.77±0.36 a	0.78±1.42 a	0.74±0.36 a	0.65±0.60 b

* Mean ± S.E. for 10 birds in each group.

Different letters mean significant differences ($p \leq 0.05$).

From table (4) it is noted that there is a significant increase in the activity of AST and ALT in birds exposed to lead acetate compared with control and Iraqi sweet almond groups. The increasing activity of these enzymes plays an important role in the use of amino acids in the oxidation process or glycogenesis, it can be considered a useful clinical index to detect damages to the liver (42). In addition, heart failure, muscular dystrophy, bile duct obstruction, hemolytic and anemia increases the level of these enzymes in plasma (43). In the present study, the concentration of glucose in birds treated with lead significantly increased. However, environmental stress and tension could result in a significant increase in plasma glucose levels (44,45).

The increase in glucose may be partly influenced by changes in the endocrine glands and an increase in cortisol, which consequently increases the metabolism of glucose (glycolysis) (46,47). The uric acid rise may be due to the adverse effect of lead on kidney function. Based on the results of this study, there was no significant change in cholesterol. However, triglyceride concentration was significantly dropped

compared with the control group, triglyceride drop in the treated birds may result from damage to the small intestine villi and impaired absorption of fatty acids. Moreover, damage to the liver may reduce the synthesis of triglyceride (46).

In the group of lead acetate and Iraqi sweet almond group, there were no significant differences in serum triglyceride, glucose, AST and ALT levels, but the blood cholesterol and uric acid level were significantly lower in the treated group. The findings presented are in agreement with previous reports which showed the application of almond nut in human diet can reduce blood total cholesterol, these effects of almonds are mediated by components in the oil fraction of this nut (48) or probably in part because of the non-fat (protein and fiber) and monounsaturated fatty acid components of the nut (49).

From the present study, severe changes in blood indices by the lead that were found in the present investigation and other studies indicate the necessity of even more concerns about the bio-environment pollution of lead. Designation and provision of the health programs to limit causal exposure to this toxic element is highly important for health. It is concluded from this study positive role of the Iraqi sweet almonds in improving the blood and biochemical image of the Rock dove model exposed to lead acetate poisoning.

Table 4 : Individual comparison of the mean values of certain serum biochemical parameters corresponding to the treated groups with the mean of control group .

Groups of birds *				
Parameters	(control)	Lead acetate 72mg/kg BW orally	Iraqi sweet almond 300mg/kg BW orally	Lead acetate 72mg/kg BW and Iraqi sweet almond 300mg/kg BW Orally
Total cholesterol (mg/dl)	147.98±2.12a	150.43±0.02 a	133.11±0.45 b	135.03±0.66 b
Triglyceride, (mg/dl)	104.20±0.59a	84.75±1.65 b	107.04±0.32 a	107.13±1.34 a
AST, (IU/l)	47.56±1.67a	69.80±0.55 c	48.99±1.76 a	46.15±2.08 a
ALT, (IU/l)	17.48±0.99 a	29.22±1.09 c	19.04±0.22 a	16.68±0.45 a
Glucose (mg/dl)	183.31±1.34a	195.02±1.11 c	185.02±3.77 a	184.71±0.54 a
Uric acid (mg/dl)	8.49±1.09 a	9.89±0.87 c	8.99±0.16 a	6.89±1.79 c

* Mean ± S.E. for 10 birds in each group.

Different letters mean significant differences (p≤ 0.05).

تأثير اللوز الحلو العراقي على الصفات الفسلجية في الحمام الجبلي المعامل بخلات الرصاص

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الخلاصة

هدفت الدراسة الحالية إلى تقييم تأثير اللوز الحلو العراقي على التأثير السمي لخلات الرصاص في الحمام البري في كل من وزن الجسم ومعايير الدم والكيميوحيوية في هذه الطيور. تم اختيار أربعين طائر من البالغين الأصحاء من كلا الجنسين عشوائياً وقسمت إلى أربع مجموعات متساوية (ن = 10) هي أ، ب، ج و د. اعتبرت المجموعة (أ) مجموعة السيطرة تناولت عليقة متوازنة، المجموعة (ب) جرعت خلات الرصاص بالفم بجرعة ٧٢ ملغم / كغم من وزن الجسم، المجموعة (ج) جرعت المستخلص المائي من اللوز الحلو العراقي بجرعة ٣٠٠ ملغم / كغم من وزن الجسم وجرعت المجموعة (د) بكل من خلات الرصاص ٧٢ ملغم / كغم من وزن الجسم والمستخلص المائي من اللوز الحلو العراقي ٣٠٠ ملغم / كغم من وزن الجسم على التوالي خلال ٢٨

يوم من الفترة التجريبية. اظهرت النتائج ان خلايا الرصاص وحدها تسببت بانخفاض معنوي في وزن الجسم وتركيز خضاب الدم وعدد الكريات الحمراء الكلي وحجم الخلايا المرصوصة% والدهون الثلاثية، في حين كانت هناك زيادة معنوية في مجموع عدد الكريات البيض والعدلات والوحيدة النواة واللمفية وجلوكوز الدم وحمض اليوريك وفعالية انزيم ناقل الامين الاسبارتيت والالنين (ALT) (AST). من ناحية أخرى، أدى إضافة اللوز الحلو العراقي في المجموعة المعاملة بخلايا الرصاص إلى زيادة معنوية ($P \leq 0.05$) في خضاب الدم ومجموع عدد كريات الدم الحمراء وحجم الخلايا المرصوصة والخلايا الليمفاوية، بينما كان هناك انخفاض معنوي في الكوليسترول وحمض اليوريك ولم يكن هناك فروق ذات دلالة إحصائية في أنشطة الدهون الثلاثية و(ALT) و (AST). وتشير النتائج الحالية إلى أن مكملات اللوز الحلو العراقي لها تأثيرات ايجابية في معايير الدم والمعايير البيوكيميائية في نموذج الحمام البري المعامل بخلايا الرصاص.

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