Zagazig Veterinary Journal, ©Faculty of Veterinary Medicine,
Zagazig University, 44511, Egypt.
Volume 49, Number 1, p. 102-113, March 2021
DOI: 10.21608/zvjz.2021.55792.1132

**RESEARCH ARTICLE**

# The Ameliorative Role of Cranberry Extract Use on Hematological Changes Induced by Lead acetate in Rats

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***Article History: Received: 12/03/2021 Received in revised form: 28/03/2021 Accepted: 31/03 /2021***

## Abstract

Lead poisonousness is a widely recognized type of heavy metal poisoning in humans and animals. So, this study aimed to assess the ameliorative role of cranberry extract use on hematological changes induced by lead acetate in rats. A total number of 40 adult male albino rats weighing approximately 200 ± 20 g were randomly assigned into four groups; Normal control group, group 2; Positive control, lead acetate at a dose of (50 PPM) for 45 days, group 3; Lead acetate at a dose of (50 PPM) then Cranberry extract (75 mg/kg) for 45 days also group 4; Lead acetate (50 PPM) then Cranberry extract (150 mg/kg) for 45 days. Blood samples were collected in EDTA tubes for hematological examinations. Oral administration of lead acetate (50 PPM) significantly decreased total erythrocyte count, hemoglobin, packed cell volume and mean cell volume levels in comparison with the normal control group *(P< 0.0001)*. Addition of cranberry extract at a dose of 75 and 150 mg/kg significantly increased the total erythrocyte count, hemoglobin, packed cell volume and mean cell volume levels in comparison with the positive control group *(P< 0.0001)*. Oral administration of lead acetate (50 PPM) significantly increased total leukocytes count, lymphocyte, neutrophils, eosinophil and monocytes count in comparison with the normal control group *(P< 0.0001)*. Addition of cranberry extract at a dose of 75 and 150 mg/kg significantly decreased the total leukocytes count, lymphocyte, neutrophils, eosinophil and monocytes count in comparison with the positive control group *(P< 0.0001)*. Our results clearly indicate that cranberry extract ameliorates hematological changes in lead acetate-treated rats.

**Keywords**: Lead-induced toxicity, Oxidative stress, Cranberry extract, Hematological investigations.

## Introduction

Environmental pollution, especially by heavy metals, represents a grave danger to the environment and a cause of extraordinary concern [1, 2]. Numerous specialists have worked extensively in the exploration of these minerals because of their critical dangerous effect on human health and nature [3]. One of the most widespread and heavy metal toxicity is the lead, which is plentiful in aquatic environments and soil near industrial areas [4].

Lead metal is distinguished by its popular silver, a little bluish color, and its brightness in dry weather. Drinking water, food, cigarettes, domestic sources and manufacturing activities such as petrol, home gilding, plumbing tubes, buckshot slugs, batteries, toys of kids and water taps are among the main sources of exposure to lead in our daily life [5]. Emission of lead into the atmosphere occurs through industrial processes and from vehicle exhausts as well, and from there to soil and water bodies, and then absorbed by plants, and then humans are exposed to lead from drinking water as well as food [6]. Lead accumulates in

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mammalian tissues such as the liver, kidneys and bones as well and these organs are usually analyzed in toxicological studies of wildlife [7].

Lead applies poisonous impacts through various mechanisms on many different organ systems. The hematologic system and the developing nervous system are the systems most especially sensitive to lead poisonousness [8, 9]. In the hematologic system, lead causes oxidative stress and early cell death by damaging the red cell membrane [9]. It inhibits the synthesis of hemoglobin by restricting the necessary enzymes needed in the pathway of heme synthesis. In addition, it increases the fragility of cell membranes, which leads to a decrease in the life span of red blood cells. These factors ultimately lead to anemia [10]. There are two types of anemia of lead poisoning. The first is hemolytic anemia due to unexpected exposure to lead at a high level. And frank anemia, which occurs when the level of lead in the blood rises dramatically for an extended period of time [11].

There are three main enzymes that are essential for heme synthesis, and the lead influences greatly the synthesis path heme with dose-dependent manner by reducing the regulation of these enzymes. The first enzyme is a cellular enzyme called delta-aminolevulinic acid dehydratase (ALAD) which works to regulate porphobilinogen from δ-aminolevulinic acid (ALA). The second type is a mitochondrial enzyme called aminolevulinic acid synthetase (ALAS), which stimulates and synthesizes aminolevulinic acid (ALA). The third type is the mitochondrial enzyme ferrochelatase, which works greatly to stimulate the manufacture of heme by binding iron to protoporphyrin [12].

A potential biomarker for detection of lead toxicity observed at the onset of the effects of blood diseases is the basophilic stippling's of red blood cells. This aggregates as products of RNA degradation [13]. Other detectable blood changes related to lead poisoning include; Anemia characterized by a lack of leukocytes and red blood cell lipid peroxidation, which leads to increased fragility of red blood cells [14, 15] low thrombocytopenia and leukocytopenia [16, 17].

Antioxidants have a great and effective role in reducing oxidative stress in human body. During stress, different types of plants have biologically active compounds, including flavonoids, glycosides, rutins, tannins, terpenoids and alkaloids play as antioxidants as well as important protective roles against tumors, inflammation and genetic mutation [18]. Cranberry is one of these plants, and it has been valued for its healing capabilities for centuries and was preferred by Native Americans, who used it to treat diseases of the bladder and kidneys.

Cranberry is distinguished by its natural antioxidant compounds. Flavonoids are among these compounds, which belong to three groups: anthocyanins, flavonoids and proanthocyanidins [19]. Cranberries are also an especially affluent source of phenolic phytochemicals, which include phenolic acids (benzoic, hydroxy-cinnamic, and ellagic acids) and flavonoids (anthocyanins, flavonols and flavans-3-ols) [20- 22]. Studies using different measures of antioxidant activity have shown that cranberries and their products have among the highest antioxidant capacity of fruits and fruit juices [22- 24].

The latest evidence for cranberry-health connection shows the following key points:

Cranberries are thought to provide health benefits due to their flavonoid and phytonutrient content [25, 26]. These naturally occurring compounds have antioxidant and antimicrobial benefits that are evident in the oral cavity, gastrointestinal (GI) tract and urinary tract [25].

A specific type of flavonoid, proanthocyanidins (PAC), in cranberries provide urinary tract benefits by interfering with the ability of pathogenic P-fimbriated *Escherichia coli* (*E. coli*) to cause infections in the urinary tract [25, 27- 29].

The majority of studies have focused on the cranberry's role in urinary tract health, but the benefits extend beyond the urinary tract. Other key areas include the berry's antimicrobial

activities, cardiovascular and Type 2 diabetes, and anti-cancer properties.

Cranberries provide numerous cardiovascular benefits. They have been shown to reduce low-density lipoprotein (LDL)-oxidation, maintain or improve high-density lipoprotein (HDL) levels, reducing platelet aggregation and improve vascular function [19, 20].

The principal objective of this study highlighted the evaluation of the hematological changes of cranberry extract in rats that induced during lead acetate administration.

## Materials and Methods

Cranberry water extract (CBE) was purchased from Virgin Extracts (TM), China. Also, Lead acetate was purchased from Sigma, USA. Prior to administration, it was dissolved in distilled water. All reagents were analytical grade.

### *Gas Chromatography/mass spectrometry analysis (GC–MS) of CBE*

The chemical composition of cranberry extract was performed using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) at the Regional Center for Mycology and Biotechnology, Al-Azhar University Campus, Nasr city, Cairo, Egypt. The GC–MS system was equipped with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 50 ℃ and then increased by 5°C /min to 230°C hold for 2 min. increased to the final temperature 290°C by 30°C /min and hold for 2 min. The injector and MS transfer line temperatures were kept at 250, 260°C respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min and diluted samples of 1 µl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200°C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

### *Experimental animals*

The experimental investigation of this study used a total of 40 adult male albino rats weighing approximately 200 ± 20 g, obtained from the Central Animal House, Faculty of Veterinary Medicine, Zagazig University. They were allowed to acclimatize for 2 weeks. They were held in stainless steel cages in controlled environment-conditioned space with a temperature of 22 ± 2 ℃ and a humidity of 60% for a 12-hour light-dark period. Each animal was raised on an *ad libitum* diet (Dyets Inc., Bethlehem, PA) with free access to water during the adaptation period. The institutional Animal Care and Use Committee of Zagazig University approved all experimental procedures with approval number (ZU-IACUC/2/F/169/2019).

### *Experimental design*

After acclimatization, rats were divided into four groups (10 rats per group).

Group 1: (Normal control) received distilled water (1 mL) every day for 45 days.

Group 2: Positive control, in which each rat was given 50 PPM of lead acetate orally for 45 days [30] . Group 3: Each rat was given 50 PPM of lead acetate and 75 mg/kg BW of cranberry extract suspended in distilled water for 45 days [31].

Group 4: Each rat was given 50 PPM of lead acetate and 150 mg/kg BW of cranberry extract suspended in distilled water for 45 days [31].

The exercise protocol was initiated daily at 10.00 am and continued for 45 days.

### *Sampling*

Each group of rats was fasted overnight, weighed, and euthanized by cervical dislocation at the end of the experiment. EDTA test tubes were used to collect blood samples for haematological examinations.

### *Haematological examinations*

Using a Hema Screen 18-Automated Haematology Analyser (Hospitex Diagnostics, Sesto Fiorentino, Italy), blood samples in

EDTA tubes were used for haematological investigation of total erythrocyte count (TEC), hemoglobin (Hb), packed cell volume (PCV), mean cell volume (MCV), mean corpuscular haemoglobin concentration (MCHC), and total leukocyte count, lymphocyte, neutrophil, Eosinophil and monocyte counts [32].

### *Analytical statistics*

SPSS version 25 was used to analyze all of the data (Armonk, NY: IBM Corp). The results have been shown as a mean ± SE. For certain parameters, a one-way ANOVA was used to see if there were any differences between groups. For count data, the analyses were done on log10 transformed data. The post-hock test was done using Duncan's multiple range test. It was statistically significant if $P < 0.05$.

## Results

The GC/MS results of oil samples of the cranberry extract, displayed in Table (1), which revealed qualitative and quantitative variation in the chemical composition. In cranberry extract compounds were identified which accounted for 29.33% of stearic acid, 10.44% Palmitic acid, 3% Oleic acid, 7.59% Myristic acid, 6.94% furfural, 4.84% marphinan, 5.05% tridemorph, 3.81% oxirane, 1.68% docosane, 1.7% lycopene, 19.97% Methyl furfural, 2.56% D-glucital, 1.5% glycan, 2.5% Dimethyl propanol and 1.22% siloxane.

Our findings revealed that there was highly significant difference on Erythrogram including: (RBCs, Hb, PCV and MCV levels) according to type of treatment applied ($P< 0.0001$) except MCHC level which has non-significant difference in all treated groups (Table 2). When compared to the normal control group, oral administration of lead acetate (50 PPM) resulted in a significant decrease in RBCs, Hb, PCV and MCV levels. In comparison to the group that received lead acetate, supplementation with cranberry extract at 75 and 150 mg/kg resulted in a significant increase in RBCs, Hb, PCV and MCV levels (Figure 1).

In addition, our results showed that there was highly significant difference on Leukogram levels according to type of treatment applied ($P< 0.0001$) (Table 3). Oral use of lead acetate (50 PPM) resulted in significant increases compared to normal control group in the overall leukocyte count, lymphocytes, neutrophils, eosinophils, and monocyte count. Addition of 75 and 150 mg/kg BW cranberry extract led to a significant decrease in total leukocytes count, lymphocyte, neutrophils, eosinophil and monocytes counts compared to the group that received lead acetate (Figure 2).

**Table 1: Chemical composition of cranberry extract : Virgin Extracts (TM), China**

| RT | Compound Name | % |
|---|---|---|
| **5.28** | Stearic acid | 29.33 |
| **6.41** | Palmitic acid | 10.44 |
| **6.74** | Furfural | 6.94 |
| **10.73** | Morphinan | 4.84 |
| **13.92** | Tridemorph | 5.05 |
| **15.75** | 0xirane | 3.81 |
| **15.94** | Docosane | 1.68 |
| **18.02** | Lycopene | 1.7 |
| **19.30** | Methyl furfural | 19.97 |
| **21.71** | D-glucital | 2.56 |
| **23.45** | Glycan | 1.5 |
| **27.17** | Myristic acid | 7.59 |
| **35.23** | Dimethyl propanol | 2.5 |
| **39.46** | Oleic acid | 3 |
| **57** | Siloxane | 1.22 |

**Table 2: Effects of orally administered lead acetate and cranberry extract on the Erythrogram of normal and experimental groups of rats**

| | Negative control group | Positive control (lead acetate 50 PPM) | Lead acetate (50 PPM) + Cranberry extract (75 mg/kg) | Lead acetate (50 PPM) + Cranberry extract (150 mg/kg) |
|---|---|---|---|---|
| **RBCs ($x10^6$/µl)*** | 6.92 ± 0.01[a] | 6.53 ± 0.01[d] | 6.73 ± 0.006[c] | 6.82 ± 0.01[b] |
| **Hb (g/dl)** | 16.3800 ± 0.13565[a] | 9.4800 ± 0.23537[d] | 12.7400 ± 0.17776[c] | 14.5600 ± 0.31875[b] |
| **PCV (%)** | 54.8000 ± 0.86023[a] | 33.2000 ± 1.06771[d] | 43.2000 ± 1.06771[c] | 49.0000 ± 0.70711[b] |
| **MCV (fl)** | 79.19 ± 1.56759[a] | 50.84 ± 3.97160[d] | 64.19 ± 1.15397[c] | 71.85 ± 3.22822[b] |
| **MCHC (%)** | 29.9060 ± 0.35122[a] | 28.55 ± 0.66969[a] | 29.49 ± 0.90777[a] | 29.71 ± 1.06667[a] |

[a,b,c,d] Means with different superscript within same row are statistically different at level P<0.05 according to Duncan's multiple range test.
***** Denotes result of log transformed data.

**Table 3: Effects of orally administered lead acetate and cranberry extract on the Leukogram of normal and experimental groups of rats**

| | Negative control group | Positive control (lead acetate 50 PPM) | Lead acetate (50 PPM) + Cranberry extract (75 mg/kg) | Lead acetate (50 PPM) + Cranberry extract (150 mg/kg) |
|---|---|---|---|---|
| **WBCs ($x10^3$/µl)*** | 12.27 ± 0.01[d] | 13.79 ± 0.02[a] | 13.26 ± 0.007[b] | 12.86 ± 0.007[c] |
| **Lymphocyte ($x10^3$/µl)*** | 3.52 ± 0.02[d] | 3.82 ± 0.03[a] | 3.71 ± 0.01[b] | 3.65 ± 0.004[c] |
| **Neutrophil ($x10^3$/µl)*** | 3.30 ± 0.009[d] | 3.73 ± 0.003[a] | 3.59 ± 0.01[b] | 3.51 ± 0.01[c] |
| **Eosinophil ($x10^3$/µl)*** | 2.56 ± 0.02[d] | 2.96 ± 0.009[a] | 2.87 ± 0.009[b] | 2.69 ± 0.02[c] |
| **Monocyte ($x10^3$/µl)*** | 2.89 ± 0.009[d] | 3.28 ± 0.005[a] | 3.09 ± 0.009[b] | 3.01 ± 0.01[c] |

[a,b,c,d] Means with different superscript within same row are statistically different at level P<0.05 according to Duncan's multiple range test.

***** Denotes result of log transformed data.

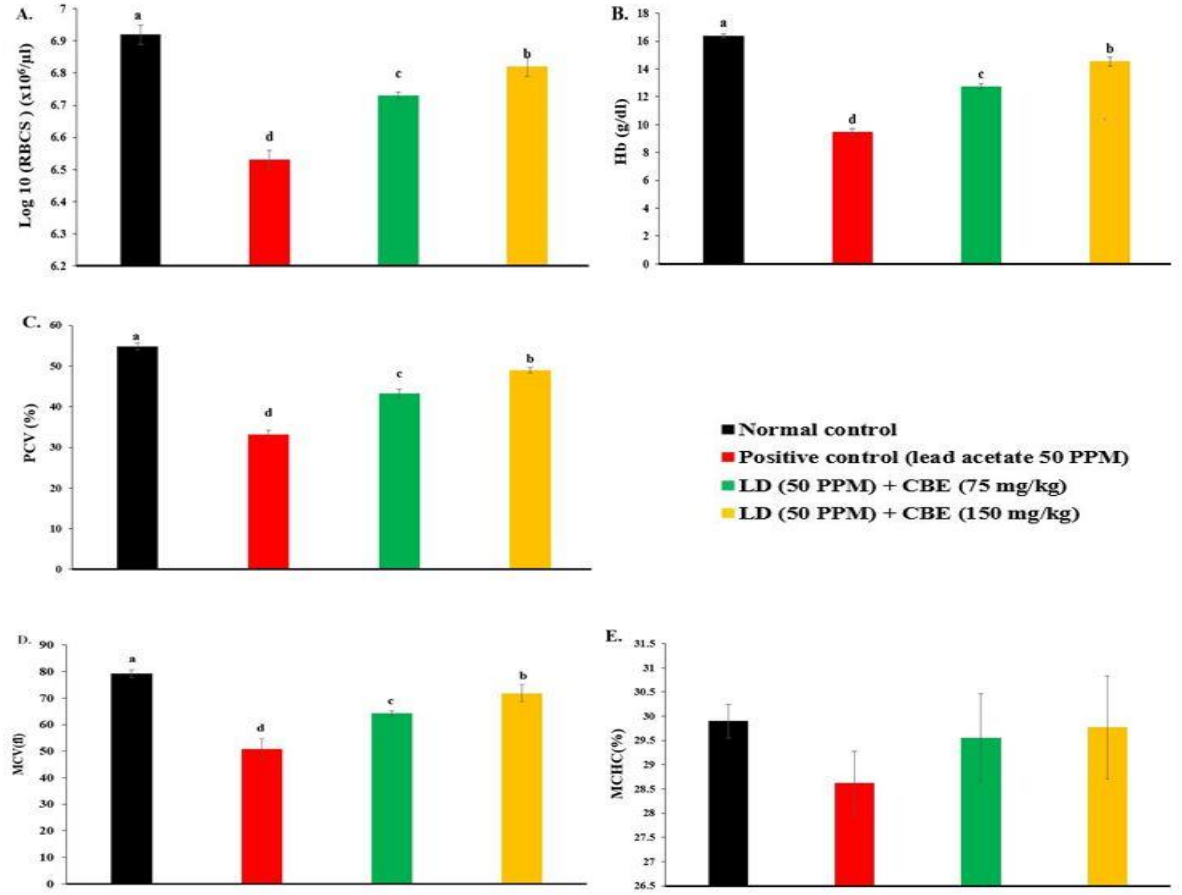


**Figure 1: Effects of orally administered lead acetate and cranberry extract on the Erythrogram of normal and experimental groups of rats (A-E).**

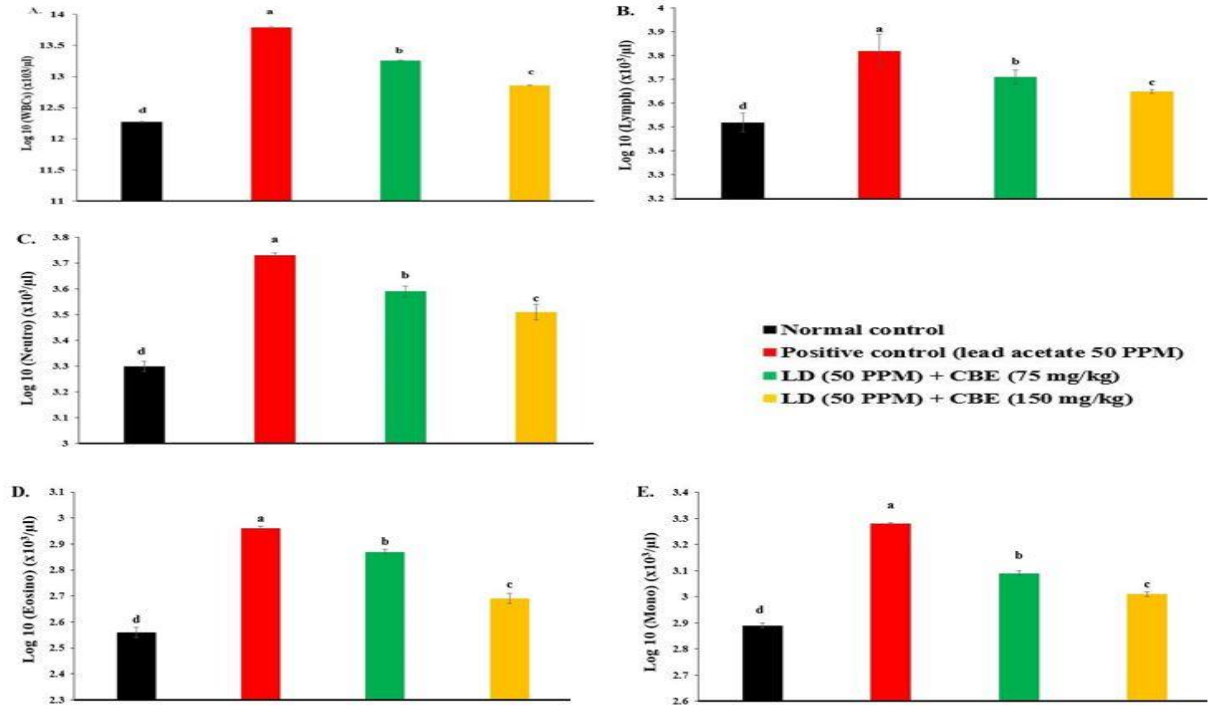


**Figure 2: Effects of orally administered lead acetate and cranberry extract on the Leukogram of normal and experimental groups of rats (A-E).**

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Volume 49, Number 1, p. 102-113, March 2021
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## Discussion

In this study, we hypothesized that cranberry extract would improve the blood picture in rats suffering from oxidative stress caused by lead acetate. Lead is one of the most permanent poisonous pollutants in the environment, and it is a major public health trouble [33]. Lead-induced toxicity can lead to serious and irreversible health effects such as cardiovascular, kidney, liver, nervous and blood diseases, causing molecular, cellular and intracellular changes in living organisms through apoptosis, ionic mechanism and increased oxidative stress generation.

Oxidative stress is caused by an unbalance between the production of free radicals and the biological system's ability to quickly purify the reaction mediator or repair the damage [34]. Blood disorders such as leukemia, anemia, lymphomas, hematopoietic stem cell transplantation, graft-versus-host disease, and others have been linked to oxidative stress.

Because many plant products are rich in antioxidants and micronutrients, it is probable that antioxidant supplements protect against oxidative stress that is mediated by disease progression. Therefore, antioxidant supplementation is becoming a growingly common practice to support optimal body functions. In recent years, products derived from natural plants such as flavonoids, steroids, alkaloids and terpenoids have gained much attention because of their various phytochemical constituents, which include anti-oxidant and anticancer action. Antioxidants play a critical role in inhibiting and scavenging free radicals, providing significant protection against infection and degenerative diseases in humans [35]. According to Vinson *et al.* [36], the antioxidant properties of cranberries occupy a unique position among the fruits in terms of quality and amount of antioxidants because they contain a large amount of flavonoids as well as phenolic acids in abundance.

Our findings revealed that oral administration of lead acetate (50 PPM) resulted in a significant decrease in RBCs, Hb, PCV and MCV levels, when compared to the normal control group. In comparison to the group that received lead acetate, supplementation with cranberry extract at 75 and 150 mg/kg resulted in a significant increase in RBCs, Hb, PCV and MCV levels.

Lead is the most toxic of the heavy metals for both humans and animals due to its many behavioral, biochemical and pathological impacts [37, 38]. The current work has led to a substantial reduction in the erythrocyte count, hemoglobin level and PCV ratio with normal blood indices for rats' treatment, which indicates normocytic normochromic anaemia, as indicated by Andjelkovic *et al.* [33]. The hematopoietic system is suppressed by lead, which can result in anaemia, which prevents the formation of erythrocytes or it can be attributed to lead's binding to erythrocytes resulting in increased erythrocyte membrane impairment, leading to accelerated red blood cell destruction. Moreover, lead is capable of combatting divalent cations, such as iron, leading to the inhibition of ALAD or to iron metabolism dysfunction, lowering haemoglobin synthesis [38, 39, 40]. Also, Offor *et al.* [41] clarified that rats that had been given 60 mg/kg of LA orally for 28 days were intoxicated and have a significantly reduced RBCs count, Hb, and PCV ratio. Dewanjee *et al.* [42] have reported similar findings.

Our results also showed that oral use of lead acetate (50 PPM) resulted in significant increases compared to normal control group in the overall leukocyte count, lymphocytes, neutrophils, eosinophils, and monocyte count. Addition of 75 and 150 mg/kg.b.w cranberry extract led to a significant decrease in total leukocytes count, lymphocyte, neutrophils, eosinophil and monocytes counts compared to the group that received lead acetate. According to the lead form, dosage, route, exposure time, host age, and genetic sensitivity the immune response is affected [43, 44]. Lawrence [45] and Ercal *et al.* [46] observed that chronic exposure to elevated levels of lead suppresses

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immunity. In contrast, Lawrence [47] reported that the effects of the immune stimulation can be achieved by exposing the body to a low amount of lead. Consequently, bone marrow toxicity and lymphoproliferative tumors development have been indicated by leukocytosis with lymphocytosis and neutropenia [43]. In the present study, leukogram results are consistent with the studies which have shown that lead's toxicity on lymphoid organ leucopoiesis of LA-intoxicated rats led to leukocytosis and lymphocytosis, which led to an increase in production from the germinal center of the lymph organs [14]. Increased total leukocytes count has also been associated with the occurrence of lead-induced inflammation [48]. Our findings are similar to those of Karamala *et al.* [49] and Aladaileh *et al.* [50], who conducted their experiments on rabbits and rats subjected to LA disease.

In this study, the usage of cranberry extract phenolics and lead acetate seemed to improve the changed blood profile. These results agree with those reported by El-Maddawy and El-Sayed [51], in which levels of blood picture parameters induced by lead acetate toxicity were normalized by phenolic compounds.

## Conclusion

Based on the obtained results, Cranberry supplementation is very important and could be a natural product in the future to prevent lead acetate poisoning and ameliorate the hematological changes in lead acetate-treated rats. In addition, this can be a step in the discovery of safe and successful new free radical products.

## Conflict of interest

The authors declare no conflicts of interest, financial or otherwise.

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**الملخص العربي**

**الدور التحسيني لمستخلص التوت البري على التغيرات الدموية التي يسببها أسيتات الرصاص في الفئران**

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تم استخدام مجموعة مكونة من 40 من ذكور الفئران البيضاء البالغين؛ لدراسة وتقييم الآثار التحسينية المحتملة لمستخلص التوت البري على التغيرات الدموية التي يسببها أسيتات الرصاص (LA) في الفئران. تم تقسيم الفئران إلى أربع مجموعات متساوية كل منها تتكون من 10 فئران، مجموعة 1 تسمي المجموعة الضابطة حيث تلقى كل فأرعن طريق الفم ١ ملي ماء مقطرا، ومجموعة ٢ مجموعة ضابطة إيجابية تم إعطائها أسيتات الرصاص حيث تلقى كل فأر50 مجم / كجم عن طريق الفم، ومجموعة 3 تم إعطاء كل فأر50 ملغم / كجم من أسيتات الرصاص عن طريق الفم قبل ساعة واحدة من إعطائه 75 ملجم / كجم عن طريق الفم من الخلاصة المائية للتوت البري، ومجموعة 4 تم إعطاء كل فأر50 ملغم / كجم من أسيتات الرصاص عن طريق الفم قبل ساعة واحدة من إعطائه 150 ملجم / كجم عن طريق الفم من الخلاصة المائية للتوت البري. واستمرت التجربة لمدة 45 يوم. تم استخدام عينات الدم في أنابيب EDTA لعمل صورة دم كاملة (CBC). وقد أظهرت النتائج وجود فروق ذات دلالة إحصائية عالية على مخطط الدم الاريثروجرام و يشمل: مستويات كرات الدم الحمراء (RBCs)، والهيموجلوبين (Hb)، و حجم كريات الدم الحمراء المكدسة (PCV) ، ومتوسط حجم كريات الدم الحمراء (MCV) باستثناء مستوى متوسط تركيز الهيموغلوبين في الدم (MCHC) الذي لا يوجد به فرق معنوي في جميع المجموعات المعالجة. أدى تناول أسيتات الرصاص عن طريق الفم (50 مجم / كجم) إلى انخفاض كبير في مستويات كرات الدم الحمراء والهيموجلوبين وحجم كريات الدم الحمراء المكدسة (PCV) ، ومتوسط حجم كريات الدم الحمراء (MCV) مقارنةً بمجموعة التحكم الضابطة (*P <0.0001*). أدى تناول الخلاصة المائية للتوت البري عن طريق الفم بتركيز (75 مجم / كجم) إلى تحسن كبير وبتركيز (150 مجم / كجم) إلى تحسن أكبر فقد أدى تناوله إلى زيادة ملحوظة في مستويات RBCs، Hb، PCV، MCV مقارنةً بالمجموعة الضابطة الإيجابية (*P <0.0001*). كذلك أظهرت النتائج وجود فروق ذات دلالة إحصائية عالية على مخطط الدم الليوكوجرام. أدى تناول أسيتات الرصاص عن طريق الفم (50 مجم / كجم) إلى زيادة كبيرة في عدد كرات الدم البيضاء (WBCs)، والخلايا اللمفاوية (lymphocyte)، والنيتروفيل (neutrophils)، والخلايا الحمضية (eosinophil)، والمونوسايت (monocytes) مقارنةً بمجموعة التحكم الضابطة (*P <0.0001*). أدى تناول الخلاصة المائية للتوت البري عن طريق الفم بتركيز (75 مجم / كجم) إلى تحسن كبير وبتركيز (150 مجم / كجم) إلى تحسن أكبر فقد أدى تناوله إلى انخفاض ملحوظ في عدد WBCs، lymphocyte، neutrophils، eosinophil، monocytes مقارنةً بالمجموعة الضابطة الإيجابية (*P <0.0001*). في الختام يمكن القول إن التوت البري له تأثير قوي ضد الإجهاد التأكسدي ويمكن أن تكون منتجًا طبيعيًا في المستقبل لمنع التسمم الناجم عن أسيتات الرصاص وتخفيف التغيرات الدموية في الفئران لما يحتويه على نسبة عالية من مضادات الأكسدة.