British Journal of Pharmaceutical Research
9(4): 1-7, 2016, Article no.BJPR.22341
ISSN: 2231-2919, NLM ID: 101631759

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Effect of Resveratrol on Some Haematological Parameters of Lead-intoxicated Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author SMH performed the experiments, designed the study, managed the analyses of the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author DNM supervised the study and managed the literature searches. Author MA supervised the study and did all correction mentioned by the reviewers. Author BYM supervised and did some corrections on manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2016/22341
Editor(s):
(1) R. Deveswaran, M.S. Ramaiah College of Pharmacy, Bangalore, India.
(2) Alyautdin Renad N, Chair of the Department of Pharmacology (Pharmaceutical Faculty), I. M. Sechenov MSMU, Moscow, Russia.
Reviewers:
(1) Carolina Baraldi Araujo Restini, University of Ribeirão Preto, SP, Brazil.
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(3) Juan Carlos Troiano, University of Buenos Aires, Argentina.
(4) Anonymous, Cairo University, Egypt.
Complete Peer review History: http://sciencedomain.org/review-history/12360

Original Research Article

Received 29th September 2015
Accepted 9th November 2015
Published 20th November 2015

ABSTRACT

The aim of this experiment was to investigate the effect of resveratrol on haematological parameters of lead-induced toxicity in male wistar rats. The study employed 36 male wistar rats (150 - 250 g) divided equally into six (6) groups. The first group (negative control) was administered carboxymethylcellulose (CMC) 10 g/L body weight (BW) daily for 19 days. The second group (positive control) was administered lead acetate solution (120 mg/kg BW) daily for 2 weeks. The third group was administered lead acetate solution (120 mg/kg BW) daily for 2 weeks then treated with succimer (10 mg/kg BW) daily for 5 days. The fourth group was administered lead acetate

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solution (120 mg/kg BW) daily for 2 weeks then treated with resveratrol (200 mg/kg BW) daily for 5 days. The fifth group was administered lead acetate solution (120 mg/kg BW) daily for 2 weeks then treated with resveratrol (400 mg/kg BW) daily for 5 days. The sixth group was pretreated with resveratrol (400 mg/kg BW) daily for 5 days then administered lead acetate solution (120 mg/kg BW) daily for 2 weeks and considered as prophylactic group. All treatments were administered orally by *gavage*. The animals were euthanized and blood sample were taken for heamatological analysis. In the result, there was significant ($P < 0.05$) increase in platelet counts in group 5 compared to both negative and positive control groups. No significant ($P > 0.05$) change was recorded for the other heamatological parameters, when the resveratrol-treated groups were compared to negative and positive control groups. No significant ($P > 0.05$) change was recorded for the other heamatological parameters, when the resveratrol-treated groups were compared to negative and positive control groups. Thus, the effect of resveratrol in platelet level is dose dependent. This study also observed that all the groups had normal levels of packed cell volume (absence of anemia).

Keywords: Resveratrol; lead acetate; succimer; male rats.

1. INTRODUCTION

Lead is a heavy, low melting, bluish-gray metal that occurs naturally in the Earth's crust. However, it is rarely found singly as a metal. It is usually found combined with two or more other elements to form lead compounds. For these reasons, lead has been used by humans for millennia and widespread today in products as diverse as pipes, storage batteries, pigments and paints, glazes, vinyl products, weights, and ammunition, cable covers, and radiation shielding. Tetra-ethyl lead was used extensively from the 1930s to the 1970s as a petrol additive to improve engine performance [1,2]. Tetra-ethyl lead has been eliminated from petrol in majority of countries, but is still used in about nine countries [3].

Lead inhibits the body's ability to make hemoglobin by interfering with several enzymatic steps in the heme pathway. Specifically, lead decreases heme biosynthesis by inhibiting *d*-aminolevulinic acid dehydratase (ALAD) and ferrochelatase activities. Ferrochelatase, which catalyzes the insertion of iron into protoporphyrin IX, is quite sensitive to lead. A decrease in the activity of this enzyme results in an increase of the substrate, erythrocyte protoporphyrin (EP), in the red blood cells (also found in the form of ZPP-bound to zinc rather than to iron). Also associated with lead exposure is an increase in blood and plasma d-aminolevulinic acid (ALA) and free erythrocyte protoporphyrins (FEP) [4,5].

Environmental Protection Agency (EPA) estimated the threshold BLL for a decrease in hemoglobin to be 50 µg/dL for occupationally lead exposed adults and approximately 40 µg/dL for children, although other studies have indicated a lower threshold (25 µg/dL) for children [4,5].

Recent data indicate that the erythrocyte protoporphyrin (EP) level, which has been used in the past to screen for lead toxicity, is not sufficiently sensitive at lower levels of blood lead and is therefore not as useful a screening test as previously thought.

Lead can induce two types of anemia, often accompanied by basophilic stippling of the erythrocytes [5]. Acute high-level lead exposure has been associated with hemolytic anemia. Frank anemia is not an early manifestation of lead exposure and is evident only when the BLL is significantly elevated for prolonged periods. In chronic lead exposure, lead induces anemia by both interfering with heme biosynthesis and by diminishing red blood cell survival. The anemia of lead intoxication is hypochromic and normo- or microcytic with associated reticulocytosis.

The heme synthesis pathway, on which lead has an effect, is involved in many other processes in the body including neural, renal, endocrine and hepatic pathways. There is a concern about the meaning of and possible sequelae of these biochemical and enzyme changes at lower levels of lead.

Resveratrol (3, 5, 4'-trihydroxystilbene) is a polyphenol that occurs naturally in foods and drinks, available in grapes and peanuts, and also in a number of herbal remedies, both alone and as part of plant extracts [6]. Resveratrol attracted little interest until 1992, when its use was postulated to explain some of its cardioprotective

properties and was thought to account in part for the so-called 'French Paradox', that is, the finding that the rate of coronary heart disease mortality in France is lower than that observed in other industrialized countries with a similar risk factor profile [7]. Since then, reports have shown that resveratrol prevents or slows the progression of a wide variety of illnesses, including cancer, cardiovascular disease [8] and ischaemic injuries [9]. Resveratrol enhances stress resistance and extends the lifespan of various organisms from yeast to vertebrates [10]; it reduces the incidence of breast cancer [11-14], cardiovascular diseases [15,16], and possesses antioxidant property [17]. Resveratrol's antioxidant property has been demonstrated to ameliorate adverse effects of heat stress-induced toxicity [18-20]. Information on the ameliorative effect of resveratrol on heavy metals induced organ toxicity is however scanty. The present study was undertaken to assess the ameliorative effect of resveratrol on lead induced organ toxicity in rats.

Succimer is an analogue of dimercaprol (2, 3-dimercapto-1-propanol, British anti-lewisite, BAL), and has replaced dimercaprol as one of the main antidotes used in the management of poisoning by lead and other heavy metals. The advantages of succimer are that it is effective by oral administration because it is soluble in water; it is well-tolerated, has relatively low toxicity and can be given at the same time as iron supplements to treat iron deficiency anaemia. It does not cause significant increase in urinary excretion of essential minerals unlike the other widely used lead chelating agent, sodium calcium EDTA.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals

Trans-resveratrol (60g) of analytical grade was purchased from Candlewood Stars Incorporated, Danbury, USA (Batch Number: MR 110218). Lead acetate (product No; 10142, BDH Laboratory chemicals limited Poole, England), Carboxymethylcellulose CMC (10 g) (Product No: 27929, BDH Laboratory chemicals limited Poole, England) were obtained from the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. *Trans*-Resveratrol, due to its low solubility in water, was suspended in 10 g/L CMC [21].

2.1.2 Equipment

Lead Care II User's Guide, Lead Care II blood analyser, Automated Haematology Analyzer (Sysmex model 2X-12N, USA). Automated Biochemistry Analyzer (Selectra XL, Vital Scientific, Netherlands) Dissecting sets, syringes, and needles, spatula, regent bottles, digital weighing balance, Sensors (2 containers of 24 each), Treatment Reagent tubes, Capillaries/plungers, Transfer droppers, Calibration button, Alcohol wipes, Gauze pads, Power free Gloves, High and low control

2.1.3 Experimental animals

Thirty six (36) male wistar rats (weighing 150 - 250 g) were used for this study. The animals were housed in the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. They were given access to pelletized growers marsh and water *ad libitum*. The rats were acclimatized for two weeks in the home cages and environment before commencement of the experiment. All experimental protocols were in accordance with the Ahmadu Bello University research policy and regulations governing the care and use of experimental animals (NIH publication number 85-23, revised 1996). The experiments were conducted in a quiet environment between the hours of 900 and 1600.

2.2.4 Experimental Site

The experiments were carried out during the hot-humid (rainy) season (August- September, 2014) at the Department Pharmacology and Therapeutics, Ahmadu Bello University, Zaria (11^{o} 10' N, 07^{o} 38' E), at the elevation of 650 m above sea level, located in the Northern Guinea Savannah zone of Nigeria [22].

2.2 Experimental Procedure

2.2.1 Resveratrol preparation and administration

Trans-resveratrol (Batch Number: MR 110218), due to its low solubility in water, was suspended in 10 g/L Carboxymethylcellulose (CMC), and administered orally once daily for 14 days [23].

2.2.2 Lead acetate induction and resveratrol pretreatment

Male wistar rats were divided into six groups, of six (6) animals each. The first group servered as negative control and animals were given

carboxymethylcellulose (CMC) (10 g/L body weight) orally. The second, third, fourth and fifth groups were given lead acetate (120 mg/kg) body weight orally for 14 days and the sixth group was pretreated with resveratrol (400 mg/kg body weight) [24,25] orally for 5 days serving as prophylaxis.

2.2.3 Treatments with succimer and resveratrol

After the lead acetate induction for 14 days and resveratrol pretreatment for 5 days, treatment with succimer and resveratrol commenced on the 15th day and lead acetate induction on the 5th day, where the second group was serving as the positive control (lead poisoned), the third group was treated with succimer (10 mg/kg body weight) [26,27], the fourth group was treated with Resveratrol (200 mg/kg body weight) [24,25], the fifth group was treated with resveratrol (400 mg/kg body weight) [24,25] orally for five (5) days and the sixth group was induced with lead acetate (120 mg/kg body weight) orally for 14 days serving as prophylactic group [24].

2.3 Methods

2.3.1 Determination of effect of resveratrol on haematological parameters in lead-induced toxicity in male wistar rats

Blood samples were collected at the end of treatment period. (3 – 4 mls) was collected into K^+ EDTA bottles for estimation of packed cell volume (PCV), platelets, white blood cell count (WBC) and differentials using Automated Haematology Analyzer (Sysmex model 2X-12N, USA).

2.3.2 Statistical analysis

Data obtained were expressed as mean ± SEM. Statistical analysis was carried out using SPSS version 20 and all the analysis were done using one way ANOVA followed by Tukey post *hoc* test for multiple comparisons. Values of $P < 0.05$ were considered significant.

3. RESULTS

3.1 Effect of Resveratrol on Haematological Parameters of Lead-induced Toxicity in Male Wistar Rats

There was a statistical significant (P < 0.05) increase in platelets count (392.33±31.81 L/L) in Lead acetate (120 mg/kg) + Resveratrol (400 mg/kg) group when compared to positive control (Lead acetate 120 mg/kg) (210.50±24.99 L/L) and negative control (carboxymethylcellulose 10 g/L) (219.50±30.50 L/L) group. There was no statistical significant (P > 0.05) difference in PCV, WBC, neutrophils, lymphocytes and monocytes in resveratrol-treated groups when compared to negative (carboxymethylcellulose 10 g/l) and positive control groups (lead acetate 120 mg/kg) (Table 3.1).

4. DISCUSSION

Lead is a ubiquitously found environmental and industrial pollutant that has been detected in nearly all phases of environment and biological system. Its persistence in human and animal tissues has quite often been associated with considerable health risks [28]. This study was to investigate the effects of resveratrol on some heamatological parameters lead induced organ toxicity in male wistar rats.

Blood platelet aggregation under physiological conditions is an important process that arrests bleeding, but excessive platelet aggregation causes thrombosis and atherosclerosis [29,30]. The haemopoietic system serves as important target for toxic chemicals and is a sensitive index of pathological conditions. In the present study, treatment with resveratrol did not cause any alteration in haematological parameters except in the platelet (PLT) level. Increase in platelets count observed in group 5 might be an indication of resveratrol non-toxic effects on the bone marrow and it is possible that prolonged consumption of resveratrol could prevent thrombocytopenia (reduced PLT level), preventing bleeding disorders, due to its ability to cause an increased platelet count at dose of 400 mg/kg. Although, this finding was actually in disagreement with the finding of [30] who demonstrated that resveratrol significantly reduced platelet count but in hyperhomocysteinaemia induced wistar rats as against the present study which used lead-induced toxicity in male wistar rats. Thus, the effect of resveratrol in PLT level is dose dependent. This study also observed that all the groups had normal levels of packed cell volume (absence of anemia). However, anemia has been reported following lead poisoning which was attributed to various inhibitory effects of Pb on heme biosynthesis [31]. Besides, excessive Pb exposure inhibits the body's ability to make hemoglobin by interfering with several enzymatic

Table 3.1 Effect of resveratrol on haematological parameters in lead-induced toxicity in male wistar rats

Treatments	Packed cell volumes (L/L)	White blood cells (L/L)	Platelets (L/L)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)
CMC(10 g/L)	0.95±3.53	12.83±5.51	219.50±30.50	25.17±7.29	70.67±6.71	4.60±1.01
LA(120 mg/kg)	0.42±0.03	14.33±4.18	210.50±24.99	22.83±3.90	76.33±3.98	3.00±0.00
LA(120mg/kg)+ S (10 mg/kg)	0.39±0.02	9.98±3.30	261.00±30.51	37.20±4.96	59.60±4.96	4.00±1.56
LA(120 mg/kg)+ R (200 mg/kg)	0.50±0.02	12.16±2.87	237.00±19.72	15.80±2.46	83.80±2.25	_
LA(120mg/kg)+ R (400 mg/kg)	0.40±0.02	19.18±5.25	392.33±31.81[a]*	38.00±6.22	49.83±5.93	3.50±0.65
R(400mg/kg)+ LA(120 mg/kg)	0.44±0.02	6.87±0.41	181.67±47.57	18.67±2.91	81.33±2.91	_

Values are represented as means ± SEM. [c] = P < 0.001 compared to CMC (10 g/l) and Lead acetate (120 mg/kg) one way ANOVA followed turkey test. LA-Lead-acetate, R-Resveratrol, CMC-Carboxymethylcellulose, S-Succimer*

steps in the heme pathway, through inhibiting aminolevulinic acid dehydratase and ferrochelatase activity, leading to anemia and erythrocytes degeneration or destruction [32].

5. CONCLUSION

In conclusion, intervention with resveratrol has significantly increased platelet counts (392.33±31.8) in the lead poisoned male wistar rats. Resveratrol's effectiveness in platelet count in this experimental group was found to be dose dependent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No.85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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