Effect of Resveratrol on Liver Histopathology of Lead-induced Toxicity in Wistar Rats

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Authors’ contributions

This work was carried out in collaboration between all authors. Author SMH designed the study, performed the histopathological analysis, wrote the protocol and wrote the first draft of the manuscript. Author MA managed the analyses of the study. Author BYM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the effect of resveratrol on liver histopathology of lead-induced toxicity in wistar rats.

Study Design: Experimental Study.

Place and Duration of Study: Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria (11° 10’ N, 07° 38’ E), at the elevation of 650 m above sea level, located in the Northern Guinea Savannah zone of Nigeria and August-September, 2014.

Methodology: The study employed wistar rats (150 - 250 g) which were administered carboxymethylcellulose 10 g/l (control), lead acetate solution (120 mg/kg), lead acetate solution (120 mg/kg) and succimer (10 mg/kg BW); lead acetate solution (120 mg/kg) and resveratrol (200 mg/kg); lead acetate solution (120 mg/kg) and resveratrol (400 mg/kg); and resveratrol alone (400 mg/kg) then administered lead acetate solution (120 mg/kg) daily for 2 weeks and considered as

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prophylactic group. All treatments were through the oral route for different days. After the animals were euthanized, liver was removed from the rats and fixed in 10% formalin for at least 48 h. Livers were then processed routinely, and the tissues were embedded in paraffin wax. Histological sections was cut at 5 – 6 µm and stained with routine haematoxylin and eosin (H and E). A detailed microscopic examination was carried out by a consultant histopathologist. Photomicrograph of the liver was taken at magnification (x 250).

Results: In the liver, necrotic cell (hepatocyte), vacuolated hepatocyte, fatty changes and hydropic degeneration were observed in positive control group. In addition, there was an interrupted liver parenchyma with evidence of hyperemia in the liver sinusoids, complete congested central vein.

Conclusion: We concluded that lead poisoning in wistar rats causes toxicopathological changes in the liver of the wistar rats. Furthermore, the use of resveratrol as a protective agent can reduce the toxic effect of lead poisoning and improve the histopathological lesions observed in wistar rats at doses tested.

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

1. INTRODUCTION

Inorganic lead is one of the oldest occupational toxins and evidence of lead poisoning can be traced to Roman times. Lead production started at least 5000 years ago and outbreaks of lead poisoning occurred from this time [1]. Lead which is a soft, grey-blue heavy metal is a common cause of poisoning in domestic animals throughout the world [2]. Lead is a poisonous metal, which exist in both organic (Tetraethyl lead) and inorganic (lead acetate, lead chloride) forms in the environment [3]. It has been used in medicines, paintings, pipes, ammunition and in more recent times in alloys for welding storage materials for chemical reagents [4].

Liver is the major organ of drug metabolism and is highly exposed to both indigenous and exogenous chemical substances. Studies have shown that the liver is one of the primary target in lead associated toxicity. There is also some report from some quarters on lead induced liver damage which was mitigated by some chemical substances. In this section we critically examine literature on the mitigating effects of some chemical agents on lead associated liver damage.

Researchers have shown that some extracts of plant and substances of animal origin have ameliorated lead impaired liver damage in experimental animal studies. Some synthetic chemical substances with known antioxidant properties were also reported to mitigate hepatotoxicity associated with lead.

The hepatotoxicity of lead and the roles of mitigating chemical substances can be seen from the study performed by [5]. He and colleagues administered 0.5 mg/g concentration of lead acetate to rats in diet for 60 days and observed significant increase in lipid peroxidation and transaminases while SOD, GPx and other biochemical parameters were decreased. The impaired biochemical parameters were normalized when 8 mg/100 g of rat body weight of methanol extract of C. Sempervirens, 0.3 mg/100 g of quercetin and 0.1 mg/100 g of rat body weight of rutin were administered prior to lead acetate administration. Similar observation was reported by [6], when he injected rats (i.p) with subacute dose (100 mg/kg body weight/day) of lead acetate and documented significant increase in serum glutamate oxaloacetate, transaminases, serum glutamate pyruvate transaminases and lactate dehydrogenase level. Pretreatment with grape seed extract (Vitisvinifera) (100 mg/kg body weight/day) normalized these biochemical parameters. Liver enzymes were elevated while antioxidant enzymes were decreased and histopathological changes in the liver were noted when rats were exposed to lead acetate (0.2%) in drinking water for 4 weeks. Pretreatment with 1.5 ml/kg of natural honey orally for 4 weeks alleviated these lead induced changes [7]. [8] also reported the protective effect of 1:50 diluted latex/kg body weight of ficus latex against 500 mg/L of lead acetate induced impairments of biomarkers of liver function and alterations in liver architecture of rats.

Similar observation was reported by [9] on the hepatoprotective effect of Ficuscarica L in animal studies. The protective effect of the methanolic extract of Pongamia Pinnata flowers was studies in rats with lead acetate induced hepatotoxicity. Administration of 160 mg/kg body weight/day of lead acetate for 90 days to male albino rats
resulted in significant elevation of transaminases and lipid peroxidation. These changes were ameliorated by 150 mg/kg body weight/day of methanolic extract of pongamia pinnata flowers administered for 90 days [10]. Ginger extract has been shown to mitigate lead-induced hepatotoxicity. This was well documented by [11] who reported that treatment of rats with 100 mg/kg of ginger for 10 weeks prevented lead impairments of antioxidant functions and lipid peroxidation.

Similar observations using ginger to mitigate lead induced toxicity were also reported [12]. Studies have shown that *Tinospora cordifolia* stem and leave extracts protective property against lead nitrate induced toxicity in albino male mice. Oral treatment with 400 mg/kg body weight of aqueous stem extract and 400 mg/kg weight of aqueous leaves extract for 30 days restored functions of transaminases, antioxidants system and architecture of the liver of lead exposed rats [13]. Coadministration of various doses of lead and cadmium to rats synergistically and significantly impaired liver enzymes and cellular integrity of liver. Treatment of rats with various doses of calcium and magnesium restored the function of liver enzymes and cellular integrity of the liver [14]. Green tea extract (GTE) is observed to have hepatoprotective property on lead induced toxicity in Sprague Dawley rats. Exposure of rats to 0.4% lead acetate in distilled water for 2 weeks was found to impair antioxidant function in the liver of treated rats, which was however mitigated when supplemented with (1.5%, W/V) of green tea extract. Histopathological study of liver showed that supplementation with green tea extract resulted in mild degeneration and decongestion of the blood vessels and enhanced regenerative capacity [15].

Resveratrol (3, 5, 4′-trihydroxystilbene) is a polyphenol that occurs naturally in foods and drinks made from grapes and peanuts, and also in a number of herbal remedies, both alone and as part of plant extracts. Resveratrol attracted little interest until 1992, when it 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 [16]. Since then, reports have shown that resveratrol prevents or slows the progression of a wide variety of illnesses, including cancer, cardiovascular disease [17] and ischaemic injuries [18]. Resveratrol enhances stress resistance and extends the lifespan of various organisms from yeast to vertebrates [19]; it reduces the incidence of breast cancer [20,21,22,23], cardiovascular diseases [24,25], and possesses antioxidant properties [26]. Resveratrol is a potent antioxidant, demonstrated to ameliorate adverse effects of heat stress-induced toxicity [27,28,29]. Information on the effect of resveratrol on heavy metals induced organ toxicities is scanty. The present study was undertaken to assess the effect of resveratrol on liver histopathology in lead-induced toxicity in wistar rats.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals

Trans-resveratrol (60 g) 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 [30].

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 controls.

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 of 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.

2.1.4 Experimental site

The experiment carried out in (August - September, 2014) at the Department Pharmacology and Therapeutics, Ahmada Bello University, Zaria (11° 10’ N, 07° 8’ E), at the elevation of 650 m above sea level, located in the Northern Guinea Savanna zone of Nigeria [31].

2.1.5 Experimental procedures

2.1.5.1 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 [32].

2.1.5.2 Lead acetate induction and resveratrol pretreatment

Male wistar rats were divided into six groups, of six (6) animals each. The first group served 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) [37,34] orally for five (5) days and the fifth group was treated with resveratrol (400 mg/kg body weight) [38,34] 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 [38].

2.2 Methods

2.2.1 Induction of lead toxicity and measurement of blood lead level (BLL)

In six groups animals were assessed for clear signs of lead toxicity viz., weakness or aggressiveness, food refusal, loss of weight, diarrhea, discharge from eyes and ears, noisy breathing and mortality.

2.2.2 Effect of resveratrol on histopathological studies

After the animals were euthanized, liver was removed from the rats and fixed in 10% formalin for at least 48 h, liver was then processed routinely, and the tissues was embedded in paraffin wax. Histological sections was cut at 5 – 6 µm and stained with routine haematoxylin and eosin (H & E). A detailed microscopic examination was carried out by a consultant histopathologist. Photomicrograph of the organs was taken at magnification (x 250).

3. RESULTS

3.1 Effect Resveratrol on Liver Histopathology of Lead-induced Toxicity in Male Wistar Rats

The normal architecture of liver sinusoid with Kupffer cell; central vein, viable hepatocyte were observed in negative control group (Plate 1). Positive control group showed an interrupted liver parenchyma with evidence of hyperemia in the liver sinusoids, complete congested central vein. There is damp focal necrosis of some hepatocytes and some appear vacuolated (Plate 2). Rats that were treated with succimer (group 3) after lead acetate induction for 2 weeks showed slightly distorted liver parenchyma evidenced by dilated sinusoids and prominent kuffer cells. Some hepatocytes appear Karyolytic and pyknotic conspicuous (Plate 3). The rats treated with resveratrol (200 mg/kg) (group 4) after lead acetate induction for 2 weeks showed an improved liver parenchyma. Liver hepatocytes look viable; however, there is some evidence of
prominent kuffer cells and focal necrosis of some hepatocytes (Plate 4). Rats treated with resveratrol (400 mg/kg) (group 5) after lead acetate induction for 2 weeks showed a preserved liver parenchyma, hepatocytes appear viable. However, sinusoids appear dilated and prominent kuffer cells in sinusoids (Plate 5). Rats that were pretreated with resveratrol (400 mg/kg) for 5 days than lead acetate for 2 weeks showed preserved liver parenchyma, hepatocytes appear viable and prominent kuffer cells in sinusoids (Plate 6). Rats that were treated with distilled water for 2 weeks showed normal central vein and viable hepatocytes (Plate 7).

4. DISCUSSION

In toxicological studies, histopathological examination provides supportive evidence for biochemical and haematological observations [39]. In the present study, the levels of lead in tissues of liver were significantly higher in positive control group which results in necrosis of liver cell (hepatocyte) and vacuolated hepatocyte. Disruption of pro oxidant/antioxidant balance might result in the tissue injury. In addition to an interrupted liver parenchyma with evidence of hyperemia in the liver sinusoids, complete congested central vein, there was damp focal necrosis of some hepatocytes and some appear vacuolated. Ingestion of lead is one of the primary causes of its hepatotoxic effects.

The molecular understanding of lead effects on hepatic drug metabolizing enzymes, cholesterol metabolism, oxidative stress, and hepatic hyperplasia suggest a potential role for lead in damaging extrahepatic systems, including the cardiovascular system. While treatment with succimer showed slightly distorted liver parenchyma evidenced by dilated sinusoids and prominent kupffer cells, some hepatocytes appear karyolytic and pyknotic conspicuous. Furthermore, resveratrol (200 mg/kg and 400 mg/kg) showed an improved and preserved liver parenchyma. Liver hepatocytes looked viable; however, there was evidence of prominent kupffer cells and focal necrosis of some hepatocytes. Also, similar results were reported by [3,12,2]. A similar result was also seen in group pretreated with resveratrol (400 mg/kg) before lead acetate induction. Similar result was reported by [40] who used Vitamin E as supplement. However, these preserved and prominent liver hepatocytes which look viable and kupffer cells observed in resveratrol treated and pretreated animals is suggested to be as a result of chelating property of resveratrol. [41], found a significant higher levels of lead in liver of rats exposed to lead for 4 weeks and a significant reduction of lead levels after treatment with chelating agent, EDTA, after 5 week of treatment [42,3]. The most common and constant findings was a portal leukocytic infiltration, hydropic degeneration and loss of
normal architecture in the liver. Resveratrol reduced tissue lead burden; the oral administration of resveratrol to lead-intoxicated rats augmented the antioxidant potential by affecting the antioxidant enzyme activities besides reducing the tissue injury of liver cells [43].

5. CONCLUSION

This study concluded that lead poisoning in male wistar rats causes toxicopathological changes in the liver. Resveratrol has exhibited ameliorative effects on lead-induced liver toxicities in male wistar rats. Furthermore, the use of resveratrol as a protective agent can reduce the toxic effect of lead poisoning and improve liver histopathological lesions in male wistar rats at dose tested.

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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.
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