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The Preliminary Study on the Histopathological Effect of Aqueous Extract of Zingiber Officinale on Lead Acetate Induced Toxicity of Adult Sprague-Dawley Rats.

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Abstract

Lead Acetate is one of the environmental pollutants that can threaten the life of living creatures in many ways. The use of plant derived herbal compounds in herbal preparations as alternative sources of medication has continued to play major roles in the general wellness of people all over the world Farombi 2003; Rajesh et al. [1], Ekor [2]. However the treatment with the extract of Zingiber officinale modulates the toxicity caused by lead acetate administration. The aim of this study is to investigate the ameliorative properties of ginger on lead acetate induced on kidney induced toxicity. A total of forty adult rats of weight 150 ± 20 g were used for this research.

The animals were grouped into four (A, B, C and D) of 10 animals each. Group A-C was the treatment groups while group D served as the control. Group A received 0.4 g of Lead Acetate, Group B received 0.4 g of Lead Acetate and then 0.1 g of Ginger, Group C received 0.1 g of Ginger and Group D received 1 ml of distilled water. The mode of administration was oral and the study was for 25 days after a 2 week acclimatization period. At the end of the experiment, the animals were sacrificed using ketamine and an anterior abdominal incision was performed and the kidney was excised. The organs were fixed in 10% formal saline and processed for histology. The histological findings revealed cytoarchitectural distortion in the podocytes, dilation in the capsular space and cells with pyknotic nuclei in the distal and proximal convoluted tubules in the Group A and to lesser extent in Group B compared to normal cytoarchitectural photomicrographs obtained in the Group C and D. These findings thus underline the protective effect of Zingiber officinale in Lead acetate induced toxicity as it affects ultrafiltration as well as tubular fluid reabsorption properties of the kidney.

Keywords: Zingiber officinale; Lead Acetate; Sprague Dawley; Histology

Introduction

Man has persistently increased the toxicity suffered from heavy metal exposures due to increased environmental pollution. Generally, heavy metals produce their toxicity by forming complexes or ligands with organic compounds thereby affecting the function of biological molecules, inactivate some biochemical enzymes and affect protein structures. Pirkle et al. [3]. Lead (Pb) is a dangerous heavy metal and harmful even in small amounts. Nevertheless, humans get exposed to lead through their environment and diet Gidlow [4]. Lead has no known function in the body as it has diverse and deleterious effects on man and animal health Suradkar et al. [5].

The effects of Lead toxicity are systemic and multiple organs are involved. It damages the cellular material and alters cellular genetics and produces oxidative damage leading to increased production of free radical and decrease availability of antioxidant reserves to respond to the resultant damage Sujatha and Srilatha [6]. It also affects the hematopoietic system where it causes anemia Sohler et al. [7]; Papionnou et al [8]; Anetor [9], in cardiovascular system causes hypertension Landis and Flegal [10]; Schwartz [11], in nervous system it causes neurotoxicity Shannon [12] including cognitive deficits Weisskopf et al. [13], in the gastrointestinal system it causes gastric ulcer Olayeye et al. [14] and in renal system causes renal damage Kimber et al. [15], in the hepatic system it causes hepatitis and hepatic failure Beattie et al. [16]. Lead induces wide range of physiological, biochemical and behavioral dysfunction Suradkar et al. [5].

Lead poisoning is, and for centuries has been, one of the most significant preventable causes of neurological morbidity
from an environmental toxin. The manifestation of its poisoning in humans is nonspecific as it can include weight loss, anemia, Waldron [17]; Khalil-Manesh et al. [18] memory loss, Hopkins [19] nephropathy, infertility, liver, testis and heart damages Patocka et al [20]; Gurur-Orhan et al [21]. Sources of lead exposure may include air, water supplies, food, soil and industry such as lead-based paint, leaded gasoline, battery manufacture and reclamation, pottery/ceramics and eye cosmetics Mage. The toxic effects of lead are treated by chelating therapy which also depletes the body store of essential cations Ruff et al [22], therefore there is need to look for an alternative solution to lead poisoning.

Today, it is estimated that about 80% of the world population rely on medicinal plants as they play important roles in pharmacology and medicine for many years to meet health needs Ogbera et al [23]. Ginger (Zingiber officinale) which belongs to the family Zingiberaceae is an example of plants that is rapidly gaining popularity among modern physicians and its underground rhizomes are medicinally useful part Mascolo et al. [24]. Zingiber officinale is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals. The pharmacological actions of ginger and compounds isolated include immuno-modulatory, antitumorogenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic actions Amir and Arash [25]. Among the pharmacological effects demonstrated are anti-platelet, anti-oxidant, anti-tumour, anti-rhinoviral, anti-hepatotoxicity and anti-arthritic effect Fisher et al; Sharma et al; Kamthouing et al [26]. Zingiber officinale was found to have hypcholesterolaemic effect and cause decrease in body weight, glucose in blood, serum total cholesterol and serum alkaline phosphatase in adult male rats Bhandari et al. [27].

Ginger extracts have been extensively studied for a broad range of biological activities, especially antioxidant activities Miller et al ; Ahmed et al. [28] which showed that Ginger significantly lowered lipid per-oxidation by maintaining the activities of the antioxidant enzymes such as super oxide dismutase, catalase and glutathione peroxides in rats. For decades, the cellular effects of Lead Acetate and Ginger have been discussed extensively but the effect on the cyto-architecture of the kidney has not studied when administered simultaneously. Lead acetate has a deleterious effect on the organs and systems in the body but the level of its damage when administered with ginger on the kidney still need to be further investigated, hence the birth of this study.

Preparation of Extract

The aqueous extract was prepared using cold extraction techniques. Forty grams of ginger powder were placed in 160 ml of sterile distilled water and left at room temperature for 24 hours with continuous mixing using magnetic stirrer. Then mixture was filtered and after filtration it was dried using incubator at 40°C. The liquid has evaporated, and the precipitated extract was left at the base of the baker. Five mls of distilled water was added to 1 g of this extract powder to produce 20% (w/v) as standard stock solution.

Experimental Animals

Animals

Forty healthy Sprague-Dawley rats between 150 ± 20 g were used for this study. They were housed in well standard ventilated wire mesh plastic cages in the animal house of the Department of Anatomy, Faculty of Basic Medical Sciences of Ladoke Akintola University of Technology under standard room temperature ranging between 26oC-28oC; relative humidity 50-55% and were exposed to twelve hours light and twelve hours dark cycle. They were allowed unrestricted access to water and commercial rat chow ad libitum. They were left to acclimatize for a period of two weeks before the commencement of the experiment. The animals were identified by different ear tags. All experimental procedures and techniques were approved by the departmental committee on the use and care of animals and tissue collection. The weights of the animals were taken every week.

Experimental Design

a) Group A: received oral dose of 0.4 g of Lead Acetate daily
b) Group B: received oral dose of 0.4 g of Lead Acetate + 0.1 g of Zingiber officinale daily
c) Group C: received oral dose of 0.1 g of Zingiber officinale daily
d) Group D: received distilled water only, served as control

At the end of the experiment of 25 days, the animals were sacrificed using ketamine and an anterior abdominal incision was performed and the kidneys were excised for histology.

Histological Procedures

The excised organs were fixed in 10% formal saline for 72 hours. The tissues were processed for microscopic examination using a standard protocol and 5 μm thick paraffin sections were made. Slides were stained with routine haematoxylin and eosin stains and photomicrographs were made at a magnification of 100 and 400 using Olympus and Leica microscopes.

Statistical Analysis

The data obtained were analysed statistically by students’ T-test and one-way Analysis of Variance (ANOVA) test. The level of significance was at P<0.05. The data were expressed as mean ± SEM.
Results

No mortality was observed during the duration of the experimentation. All animals in the groups appeared healthy and showed normal behavior throughout the study.

Effect On Body Weight

At the end of the study, there was decrease in body weight of the treatment groups (A and B) when compared to the Control, there was increase in the group C that was administered with extract alone (Table 1).

Table 1: Effect on the average body weight.

| Duration (Weeks) | Treatment Groups |
|------------------|------------------|
|                  | A                | B                | C                | D                |
| 1                | 212.50±9.32      | 182.00±3.96      | 190.80±8.70      | 208.3±19.00      |
| 2                | 147.50±6.92      | 152.50±12.05     | 191.00±8.57      | 212.20±6.04      |
| 3                | 140.0±4.41       | 140.60±5.23      | 196.00±6.86      | 205.00±5.48      |
| 4                | 142.90±3.06*     | 157.50±10.44*    | 204.00±7.48      | 228.00±10.44     |
| 5                | 142.50±3.23*     | 177.80±18.19*    | 203.20±9.68      | 229.00±12.39     |

Values are mean ± standard error of mean; n=10,*p<0.05 compared to control (student’s test)

Effect On Kidney Histology

The section from Group A treated rat showed an increased luminal diameter of the renal tubules particularly the proximal convoluted tubule. The morphological features appeared to be distorted with dilation in the capsular space and cytoplasmic vacuolated cells with pyknotic nuclei were found. The glomeruli also showed adhesive glomerulitis, with damage varying from single adhesions to complete obliteration of the capsular space (plate B). The section from group B demonstrates marked reduction of the histological features of renal injury, consisting of more focal and mild tubular necrosis. Also there is attenuation of the histological lesions observed in this section compared with A(plate C). Photomicrograph of Group C showing normal morphology of the renal corpuscle, proximal tubules (P), the capsular space (CS) and the glomeruli (G) were seen to be prominent and morphologically normal (plate D). The photomicrograph from control (Group D) rat showed a normal renal corpuscle. The Bowman capsule and the glomeruli appeared to be prominent and normal. The podocytes and luminal diameter were found to be normal, with no distortion and there were no cytoplasmic vacuolations (Figure 1).

Section of Group A showing an increased luminal diameter of the renal tubules particularly the proximal convoluted, dilation in the capsular space and vacuolation with pyknotic nuclei were found (H&E, X 400) (Figure 2).

Figure 1: Effect of lead Acetate and Ginger on weight.

Figure 2: Showing an increased luminal diameter of the renal tubules.

Figure 3: Showing a less severe damage to the renal corpuscle with normal renal tubules (H&E X 400).

Photomicrograph of the longitudinal section of the kidney in the treatment Group B showing a less severe damage to the renal corpuscle with normal renal tubules (H&E X 400) (Figure 3).

Figure 4: Photomicrograph of the longitudinal section of the kidney rat.
Photomicrograph of the longitudinal section of the kidney rat in treatment Group C presenting the normal morphology of the renal corpuscle. Proximal tubules (P), the capsular space (CS) and the glomeruli (G) were seen to be prominent and morphologically normal (H&E X 400) (Figure 4).

Section of Group D group presenting the normal morphology of the renal corpuscle. Proximal tubules (P), the capsular space (CS) and the glomeruli (G) were seen to be prominent and morphologically normal (H&E, X 400) (Figure 5).

Discussion

The study demonstrates the adverse effect of Lead Acetate on the kidney of the Sprague-Dawley rats. The morphometric result showed a significant decrease in the body weight of the rats in the treatment Group A receiving lead acetate which agrees with Harvey et al. [29] that the significant decrease in weight is as a result of loss of appetite and gastrointestinal disturbances via lead effects on the satiety set up studies Pentenusci, [30]; Hamilton [31]; Djeblil et al. [32]. Also, there was decrease in the body weight of treatment Group B that received Lead Acetate and the extract of Zingiber officinale, although, it was quite better than the group A, which can be as a result of the extract when compared with Group C that had increase in body weight.

Histological examination of the kidney of the control group revealed entirely normal structures of the renal cortex which comprised renal corpuscles, proximal and distal convoluted tubule, which quite similar to section of Group C that was administered with the extract of Zingiber officinale alone. However, the Group A that was administered with Lead Acetate alone showed a complete deviation from the control, the section showed dilation in the capsular space and cytoplasmic vacuolated cells with pyknotic nuclei. Also there was an increased lumen diameter of the renal tubules particularly the proximal convoluted tubule as compared with renal tubules in group D. The glomeruli also showed adhesive glomerulitis, with damage varying from single adhesions to complete obliteration of the capsular space. The less severe damage of the renal corpuscle with normal renal tubules observed in the photomicrograph of the section of Group B that received both Lead Acetate and the extract of Zingiber officinale show an improvement when compared to Group A. This shows the ameliorative property of the extract on the Lead Acetate toxicity which agrees with the antioxidant effects as documented by Jagetia et al. [33]; Haksar et al. [34,35].

Conclusion

In conclusion, the present body showed that the extract of Zingiber officinale possess protective potentials on Lead Acetate induced renal toxicity. This study therefore suggests that the extract of Zingiber officinale may be a useful preventive agent against the adverse effect of Lead Acetate and other heavy metals that possess threat to the kidneys.

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