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Research Article

Histomorphology and Nephroprotective Effect of *Cajanus cajan* in Lead-acetate-Induced Kidney Damage

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Abstract

Background: Nephrotoxicity results from an overload of chemicals and drugs including Lead-acetate (Pb), an environmental pollutant. This study examined the nephroprotective potential of *Cajanus cajan* in male Wistar rats.

Material and Methods: Nephrotoxicity was induced by exposure to Lead at 20mg/kg while *Cajanus cajan* was administered at 200mg/kg, 400mg/kg and 800mg/kg for 14 days. Markers of nephrotoxicity including urea and serum creatinine were evaluated, and histoarchitecture alteration of the tissue was examined.

Results: The lead exposed group showed a marginal increase in urea and serum creatinine significantly ($p < .05$) compared to control values. The *Cajanus cajan*-treated groups showed a significant ($p < .05$) decrease respectively in urea and serum creatine. *Cajanu cajan* protected kidney tissue morphology.

Conclusion: The results indicate that *Cajanus cajan* protected Kidneys against Lead-induced toxicity

Keywords: Lead; *Cajanus cajan*; Nephrotoxicity; Urea; Serum Creatinine

Introduction

A major environmental pollutant is lead (Pb). The environmental contaminant lead (Pb) is substantial. It can enter the body by ingesting contaminated food and water, inhaling polluted dust, or inhaling air that is polluted [1]. Lead poisoning has grown to be a significant health danger for people in Nigeria due to the rising industry. Children's kidneys and other organ physiologic functions, as well as their neurodevelopment, can suffer greatly from it [2]. The kidney, which is second only to the brain in terms of susceptibility to subacute or acute lead exposure, is one of the organs most at risk [3,4]. According to other researchers, lead-induced nephrotoxicity also involves oxidative stress, an inflammatory response, and alterations in histopathology [5].

Lead-induced oxidative stress develops when the kidney's levels of reactive oxygen species (ROS) and antioxidants are out of equilibrium [6,7]. Additionally, because these free radicals are so reactive with DNA and nephron-membrane lipids, excessive lead generation might cause stress damage to the glomeruli and nephron tubules [8]. For the treatment of lead-induced kidney disease, there are numerous pharmacological antioxidant and anti-inflammatory drugs accessible, but they have unfavorable side effects [2].

In recent years, it has been demonstrated that herbal components derived from Nigerian native plants can guard against oxidative stress and inflammation. Flavonoids, which make up the majority of the bioactive components, have demonstrated promise as prospective therapies for lead-induced nephrotoxicity [8,9]. A class of polyphenolic compounds known as flavonoids has a diverse range of pharmacologic actions. These include antioxidant, anti-inflammatory, hepatoprotective, and anticarcinogenic effects [10,11].

Pigeon pea is the common name for *Cajanus cajan*. It is a plant that is used medicinally [12]. It strengthens the immune system and is used to treat infections, bedsores, malaria, and wounds [13]. It has several therapeutic qualities, including antioxidants, anthelmintics, and protection against liver damage caused by N-nitroso diethylamine [14,15]. Studies have demonstrated the ability of *C. cajan* to protect against the liver-damaging effects of acetaminophen in rats [14]. Additionally, *C. cajan's* antioxidant activity was tested against liver toxicity [15]. This present work aimed to examine the hepatoprotective activity of ethanol extract of *C. cajan* leaf against lead-induced nephrotoxicity so that in future an efficient formulation could be produced or developed which will be specific for imparting nephroprotection.

Material and Methods

Chemicals

Lead acetate was purchased from a well-known chemical store in Enugu state, southeast Nigeria. Lead acetate were dissolved in distilled water, and administrated to the experimental animals by oral gavage using a gavage tube.

Animals and Managements

Healthy Twenty (20) male albino Wistar rats (n = 4) weighing 150–180 g body weight were used for the experiment. They were purchased from the National Institute of Research, Vom, Plateau State. They were separated randomly into five groups consisting of four animals each in different aluminum cages, placed in a well-ventilated house with optimum conditions (temperature 300C photoperiod; 12 hours natural light and 12 hours dark; humidity is 40-50%). The animals were fed growers' mash manufactured by Top Feed Nigeria Limited and allowed free access to water. Rats were acclimatized for two weeks and throughout the experimental period; the animals were handled according to the guidelines for animal research in the National Institute of Health guidelines for the care and use of laboratory animals. The study was carried out per the principles of laboratory animal care and standard experimental procedures.

Twenty (20) adult Wistar rats were divided randomly into five groups of four: Group A served as the control and received no treatment. Group B was the negative control and was given oral gavage of only Lead acetate mixed in water at 20 mg/kg. Group C, D and E were given oral intubation of *C. cajan* ethanolic leaf extract at 200, 400 and 800 mg/kg respectively for 14 days followed by oral gavage of Lead acetate mixed in water at 20 mg/kg for 7 days.

Collection and Preparation of Plant Extract

The leaves of *C. cajan* were obtained in June 2022 from a market in Calabar, Cross River State, Nigeria and properly identified and authenticated by a qualified taxonomist. They were plucked from their branches, washed and air dried at room temperature (26 ◦ C) for four weeks. The air-dried leaves of *C. cajan* were pulverized using an electric blender which yielded 300.1 g and were soaked in 99% ethanol (900 ml) for 72 h in two successive extractions. It was then sieved out using a muslin cloth. The extract was concentrated using a Rotary evaporator at 50 ◦ C and a water bath at 40 ◦ C to obtain a yield of 26g semi-solid crude substance [26].

Collection of blood and kidney tissue

Blood was collected from all groups directly in to the test tube and allowed to clot. The serum was collected for estimation of creatinine, urea and stored at 40C in a fridge.

The Kidney was quickly excised, washed in saline and was stored in 10% formalin solution at room temperature for histopathological studies.

Histological studies

Histological sections were prepared from paraffin blocks and stained with haematoxylin and eosin (H & E) to examine changes in the morphology of the tissue.

Statistical analysis

SPSS (Version 24) was used for all statistical analyses. Statistical significance was determined at $p \leq .05$. Data was expressed as mean ± standard error of mean (SEM).

Results

Effect of *Cajanus cajan* on urea and creatinine

Lead acetate induction significantly ($p \leq 0.05$) increased urea and creatinine when compared to the control group (Table 1). *C. cajan* treated groups were seen to significantly ($p \leq 0.05$) reduce urea and creatinine to near normal levels when compared to the lead-treated group. This reduction was comparable with normal control and statistically significant in 800 mg/kg and 200 mg/kg.

Enzymes Markers	ANIMAL TESTED PER GROUP (2)	GROUP A(mg/dL) (Mean ± SD)	GROUP B(mg/dL) (Mean ± SD)	GROUP C(mg/dL) (Mean ± SD)	GROUP D(mg/dL) (Mean ± SD)	GROUP E(mg/dL) (Mean ± SD)	p-value
Urea		6.31 ± 1.69	19.08 ± 4.03[a]	15.12 ± 1.24	10.46 ± 0.47	6.75 ± 2.01[b]	0.009
Creatinine		1.18 ± 0.28	7.73 ± 1.90[a]	6.38 ± 1.08	3.42 ± 1.47	2.40 ± 0.52	0.012

Table 1: Effect of *Cajanus cajan* on serum urea and creatinine activity.

Values are expressed as mean ± SD; [a] Values differ significantly from the control group; [b] Values differ significantly from lead-treated group.

Effect of *Cajanus cajan* on Histological Features of the kidney

Figure 1 shows photomicrographs of the kidney showing the general structure, renal tissues (arrow), glomeruli (G), and general parenchyma. The control group showed normal architecture of the nephrocytes (Figure 1A). Administration of Lead acetate was seen to cause non-proliferative glomerular atrophy (arrow), and tubular metaplasia with progressive parenchyma pigmentation as shown in (Figure 1B). Nephrotic damage was protected by the administration of *Cajanus cajan,* and the histological index of tubular metaplasia, non-proliferative glomerular atrophy, and parenchyma pigmentation were significantly decreased to some extent (Figure 1C–E).

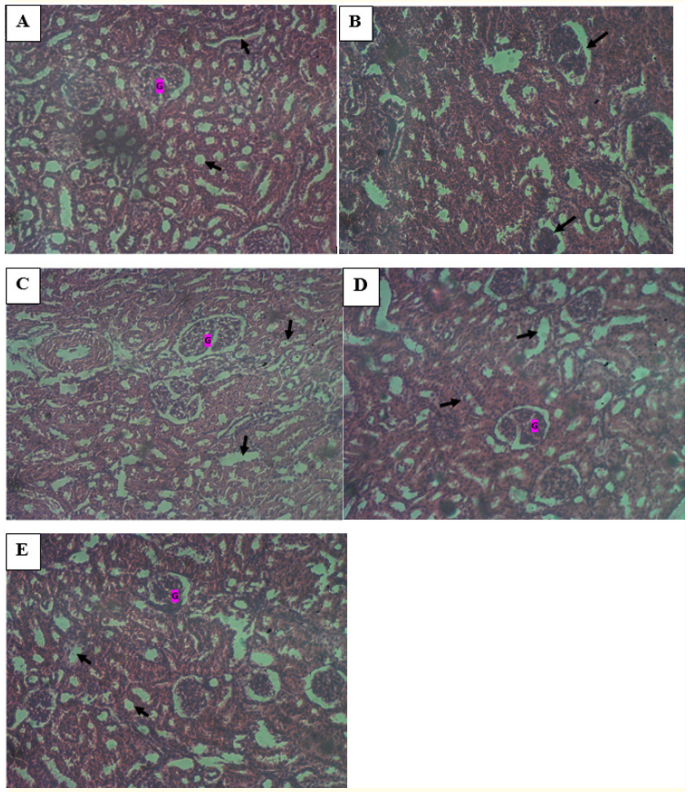

Figure 1: Photomicrograph of hematoxylin and eosin-stained section (X200) of normal Kidney. Histology showing control and experimental groups. Group (A); Served as the control animals (B); Animals receiving Lead acetate (20 mg/kg) (C); Animals receiving *Cajanus cajan* (200 mg/kg) + lead (20 mg/kg) (D); Animals receiving *Cajanus cajan* (400 mg/kg) + Lead)20 mg/kg) (E); Animals receiving *Cajanus cajan* (800 mg/kg) + Lead (20 mg/kg).

Discussion

There are numerous biochemical and physiological dysfunctions in both humans and laboratory animals as a result of the prevalent environmental toxin lead.

According to recent studies [17,18], medicinal herbs can remove lead ions from blood and organs. *C. cajan* was employed as a medicinal plant to cure toxicity hundreds of years ago. According to the current study's findings, in the blood and kidneys of Wistar rats with lead-induced nephrotoxicity, lead was significantly antagonistic to *C. cajan* flavonoid extract (Table 1). This result is in line with data from Adhikari and colleagues' investigation. Their studies have discovered that a combination of soluble Pb-flavonoids (naringin) can minimize lead toxicity in vivo and in vitro due to its chelation and antioxidant characteristics [19]. The liver and other soft tissues have the second-highest concentrations of lead in the

human body, behind the kidneys [20]. According to Gargouri., *et al*. [21], the kidney mostly removes xenobiotics through urine excretion. Additionally, we found the dose-dependent facilitation of urinary lead excretion by *C. cajan* (Table 1).

Lead-induced nephrotoxicity attracts increasing attention in developing nations. Urea and serum creatinine are measures of renal function. Urea and serum creatinine levels are typically abnormally elevated as a result of renal dysfunction [8]. According to the study's findings, lead-exposed rats had significantly higher serum levels of urea and creatinine than the control group. *C. cajan* provided excellent protection for the contents of several renal function markers (Table 1). Additionally, the main indication of renal function is glomerular filtration (GF), which is dependent on the number and effectiveness of nephrons [22].

Lead buildup in renal tissue can damage glomerular structures directly and reduce GFR (Figure 1). This finding is in line with clinical research, which found that the flavonoids quercetin protected the glomerular structure in patients with iodinated contrast-induced nephropathy, as demonstrated by a drop in serum creatinine and a rise in GFR [23]. By raising the GFR, *C. cajan* may be able to protect glomerular structures. Furthermore, Wistar rats exposed to Pb alone developed obvious kidney injury. The pathologic alterations to the kidney brought on by Pb could be greatly reduced by *C. cajan* (Figure 1). These results indicated that *C. cajan* may be a valuable renal-protective substance.

Conclusion

We demonstrated, for the first time, that *Cajanus cajan* protected the kidney against lead-induced nephrotoxicity by reducing Serum Creatinine and Urea levels in Wistar rats. The ethanolic leaf extract of *C. cajan* showed a strong protective effect in the restoration of renal tubules and glomeruli in groups that received both low and medium doses but more pronounced in the group with the high dose of the extract. Our findings suggest that *C. cajan* could be a natural antioxidant and anti-inflammatory agent for treating lead-induced nephrotoxicity.

Ethics Statement

The animal study was reviewed and approved by the Animal Ethics Committee of our university with Ethical number UC/FEC/2022/0014.

Author Contributions

All the authors contributed conception and design of the study. Mbang J.E performed the animal experiments and Nwanama E.K and Ogbo F.O carried data analysis. All the authors wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of Interest

None.

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Availability of Data and Material

All data generated or analyzed during this study are included in this published article.

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