

Evaluation of the Therapeutic potential of Ethanol Leaf Extract of *Anacardium occidentale* in the management of experimentally induced kidney damage in Wistar rat

Abstract

The aim of this study was to evaluate the renoprotective potentials of ethanolic leaf extract of *A. occidentale* in wistar rats. Freshly harvested leaf of *A. occidentale* was shade dried and ground to fine powder. 500 g of powdered plant sample was macerated in ethanol for 72 hrs. Twenty five adult male wistar rats were divided into five groups of five rats each. **Group 1** was the normal control and was fed normal rat feed and water only, **Group II** was the negative control and was induced renal damage without treatment, **Group III-V** were induced with renal damage and treated with 100, 300, 500 mg/kg b.w of extract orally respectively. Animals were sacrificed, blood and tissue samples were harvested and analysed using standard procedures. Induction of renal damage in rats caused a significant ($P<0.05$) decrease in serum urea and creatinine levels as well as severe weight loss. However, administration of 100, 300 and 500 mg/kg bw of extract of *A. occidentale* caused a dose dependent reversal of the serum creatinine and urea to values that were not significantly different ($P<0.05$) from the those recorded for the control. Similar observation was made on their weight. In conclusion, it was deduced from this study that leaf of *A. occidentale* could be a repository of active therapeutic ingredient for the treatment of renal dysfunction.

Keywords: Kidney, Creatinine, Urea, *Anacardium. occidentale*,

Introduction

The kidney is a pair of bean shaped organ each being about 10-15 cm long, positioned on the either sides of the spine deep in the abdomen. Each kidney weighs about 160 g and discharges between one and one-and-a-half litres of urine per day. The two kidneys together filter 200 litres of fluid every 24 hours. They are saddled mainly with the task of eliminating toxins from the blood. The inability of the kidney to function properly is caused by the accumulation of harmful toxins and excess fluid in the body and consequently kidney failure with characteristic symptoms

such as high blood pressure, extreme tiredness or lethargy, persistent headaches, swelling in the face and ankles, fluid retention and or lower back pain [1].

Although dialysis and kidney transplantation are the most frequently employed treatment measures for advanced renal failure, unfortunately, they are associated with a number of pitfalls. Such as immunological rejection of kidney graft, in addition to the adverse effects that characterize drugs used to prevent graft rejection such as suppress immune defense mechanism, thereby paving way for the emergence of some unusual and difficult-to-treat infections among numerous other challenges that come with tackling kidney dysfunction [1].

It has been estimated that 80% of the world's population relies on traditional medicine to treat their diseases [2]. Medicinal plants have a long history of use and are globally safer than the synthetic drugs [3]. They are regarded as cheap, easily available, and safe sources of active compounds for pharmaceuticals [4].

. *Anacardium occidentale* L. commonly called cashew and a member of *Anacardiaceae* family is a small-sized tree. Its leaf is leathery and obovate with a rounded apex and has been used in the treatment of dysentery, diarrhea, diabetes and piles etc [5].

Although research has revealed the importance of the various parts of this plant in the treatment of disease, no research effort had revealed its potential in the treatment of kidney disease despite the fact that the therapeutic effects of several medicinal plants on kidney disorders have been variously studied [6].

METHODOLOGY

Collection and processing of plant material

Mature green leaves of *Anacardium occidentale* was harvested from a local farm in Uli in Ihiala Local Government Area of Anambra State. The leaves were washed with clean tap water after which they were dried at room temperature. The dried leaves were subsequently ground and sieved to fine powder.

Animals

Adult male wistar rats weighing 140-220 g were held in plastic cages in the Animal House of the Department of Human Physiology, College of Health Sciences Anambra State University and were fed rat chow and water *ad libitum*. They were acclimatized for three weeks prior to experiment.

Extraction of Plant Material

Exactly 500 g of the powdered plant sample was soaked in 2 litres of 70% ethanol for about 72 hours and stirred intermittently. The extract was filtered and the filtrates concentrated [7].

Median lethal dose 50% (LD50)

The determination of the acute toxicity test on extract involved three groups of three wistar rats each. The various groups were separately administered with 10, 100 and 1000 mg/kg of extract orally. The rats were observed for 24 hrs for effects of toxicity. Being that mortality was not observed in any of the groups, another three groups of one rat each was each administered with 1600, 2900 and 5000 mg/kg of extract separately. The animals were observed for 48 hrs for signs of toxicity Lorke [8].

Induction of renal toxicity study

Twenty five adult male wistar rats were divided into five groups of five rats per group. Group I was the normal control fed with rat feed and water, Meanwhile, Groups II-V were administered with 1 mL/kg body weight CCl₄ (Sigma Aldrich, USA) intraperitoneally in 30% v/v olive oil for induction of renal toxicity [9].

EXPERIMENTAL DESIGN

Group I: was fed with rat chow and water *ad libitum*.

Group II: was induced without treatment.

Group III: was administered with 100 mg/kg of *A. occidentale* leaf extract

Group IV: was administered with 300 mg/kg of *A. occidentale* leaf extract.

Group V: was administered with 500mg/kg of *A. occidentale* leaf extract.

Administration of treatment was performed at interval of one day for 30 days. After the last treatment, the animals were euthanized following the administration of 2 µg/kg medetomidine after which blood sample was collected by cardiac puncture. The blood samples were centrifuged at 4 °C, 500×g for 15 min to obtain serum. The kidney of each animal was excised, washed with ice-cold water to get rid of debris prior to being stored in 10% formalin (Sigma Aldrich, USA) for histopathological evaluation [10].

Determination of serum creatinine and urea

Analysis of serum creatinine and urea was determined with the aid of a diagnostic kit (AMP Krenngasse 12, 1810 Graz, Australia) with strict adherence to manufacturer's guidelines and instruction.

Histopathological study

The animals were euthanized and the kidney fixed process. This was followed by dehydration in alcohol (90%). Kidney tissue was further processed in accordance to the method of Burki et al. (2020). Finally, the thin slice of kidney tissue (3-4 μm) was subjected to the removal of wax and then stained with hematoxyline-eosin (Sigma Aldrich, USA).

Statistical Analysis

Data generated were expressed as Mean \pm Standard Deviation using SPSS (Ver. 23). Data were analysed using one way Analysis of Variance (ANOVA). Differences in mean were compared using Turkey Test. *p-values* less than 0.05 was considered statistically significant.

Table 1: Urea and Creatinine Levels of Wistar Rats induced with Renal Damage treated with extract of *A. occidentale*

GROUPS	TREATMENT	UREA (mg/dL)	CREATININE(mg/dL)
Group I	Rat feed + DH ₂ O	51.00±0.00 ^a	0.25±0.96 ^a
Group II	CCl ₄	62.11±0.22 ^d	0.46±0.00 ^d
Group III	100 mg/kg	58.98±1.35 ^c	0.37±0.00 ^c
Group IV	300 mg/kg	56.15±0.05 ^b	0.27±0.00 ^b
Group V	500 mg/kg	54.29±0.90 ^{ab}	0.26±0.100 ^{ab}

Results are mean ± standard deviation from three determinations. Values with same superscripts in a column are significantly different at (P<0.05).

Table 2 : Changes in Body Weight of Rats over the Period treatment

GROUPS	TREATMENT	Mean initial wt (g)	Final mean wt (g)
Group I	Rat feed + DH ₂ O	141.42±8.57	141.42±8.56
Group II	CCl ₄	155.71±12.80	55.71±7.82
Group III	100 mg/kg	59.58±10.56	66.42±7.45
Group IV	300 mg/kg	85.71±19.25	81.42±3.40
Group V	500 mg/kg	121.42±18.44	145.71±4.28

Results are expressed as mean ± standard deviation from three determinations

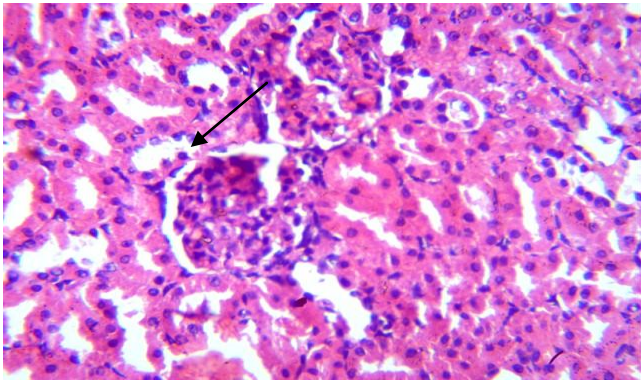


Plate 1: Normal control

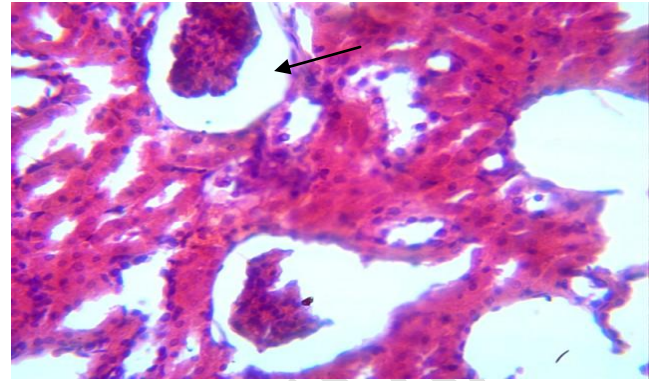


Plate 2: Rats induced without treatment

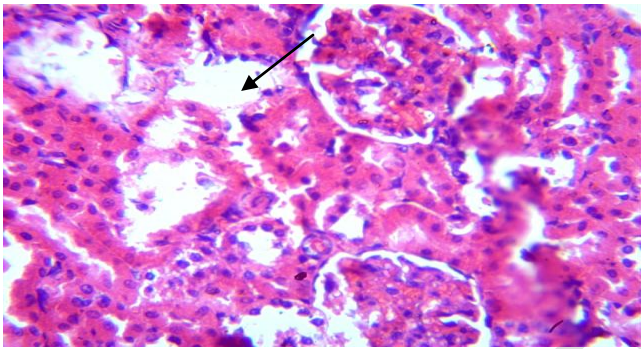


Plate 3: Rats with renal damage treated with 100 mg/kg extract

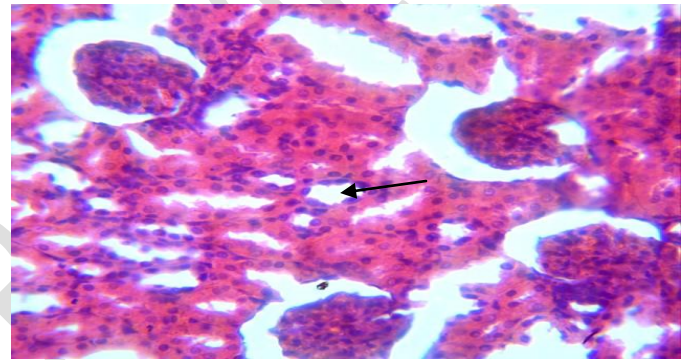


Plate 4: Rats with renal damage treated with 300 mg/kg extract

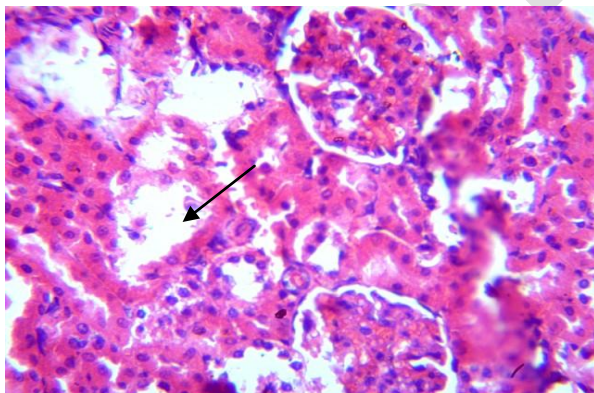


Plate 5: Rats with renal damage treated with 500 mg/kg of extract

Plate 1: shows a well perfused normal renal architecture with Glomeruli(G) and Convuluted Tubles (T), Bowman's Space (BS) and Tubular Cells (Tc).

Plate 2: Shows a severely damaged renal tissue with Necrossed Glomeruli (NG) and Tubular Atrophy (TA), Intra Renal Inflammation and Intra Renal Hmorrahage (IRH).

Plate 3: Shows moderate regeneration with mild Intra Renal Inflammation (IRI) Clumping of Glomeruli (CG), and focal Loss of Renal Tissue.

Plate 4: Shows moderate regeneration of damaged renal tissue with mild Intra Renal Inflammation (IRI), Clumping of Glomeruli and Dilated Tubules (DT).

Plate 5: shows an advanced stage of damaged renal tissue regeneration

RESULT AND DISCUSSION

The kidney is saddled mainly with the task of eliminating toxins from the blood transforming the resulting waste to urine. Table 1 shows the urea and creatinine levels of serum obtained from wistar rats induced with renal damage and treated with different doses of ethanolic extract of *A. occidentale*. The result indicates that serum urea (62.11 ± 0.22 mg/dL) and creatinine (0.46 ± 0.00 mg/dL) of rats induced without treatment (**Group I**) was significantly higher compared to the normal control (51.00 ± 0.00 mg/dL) and (0.25 ± 0.96 mg/dL) for urea and creatinine respectively. However, administration of the ethanolic extract of *A. occidentale* at doses of 100, 300 and 500 mg/kg b.w brought about a dose dependent decrease in the levels of urea and creatinine. The inability of the kidney to function properly is characterized by the accumulation of harmful toxins and excess fluid in the body and consequently kidney failure results with characteristic symptoms one of which is loss of appetite and hence weight loss. **Table 2** shows changes in body weight of wistar rats induced with renal damage and treated with ethanolic extract of *A. occidentale*. Induction of renal damage caused a significant weight reduction in all groups. However, a reversal of the condition was observed as treatment progressed. The renoprotective potential of the said extract could be attributed to the presence the presence of certain therapeutic phytochemicals. This result is consistent with the work of Leonard et al [12] which established that n-hexane leaf extract of *Anacardium occidentale* has the potential to diabetes-induced functional and histological alterations in the kidneys.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of

knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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