Journal of Advances in Medical and Pharmaceutical Sciences
5(2): 1-9, 2016, Article no.JAMPS.22121
ISSN: 2394-1111

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Effects of Administration of Methanol Root Bark Extract of *Cussonia arborea* on Serum Biochemical Markers of Kidney Damage and Renal Histomorphology of Alloxan-induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among both authors. Author PEA carried out the work while author IUA conceptualized and supervised the study. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2016/22121
Editor(s):
(1) Faiyaz Shakeel, Department of Pharmaceutics, King Saud University, Riyadh, Saudi Arabia.
Reviewers:
(1) Arun Kumar, Hindu Post Graduate College, Zamania, India.
(2) Daniela Hanganu, Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca, Romania.
Complete Peer review History: http://sciencedomain.org/review-history/11986

Original Research Article

Received 19th September 2015
Accepted 6th October 2015
Published 26th October 2015

ABSTRACT

Aim: This study evaluated the effects of oral administration of varied doses of methanol root bark extract of *Cussonia arborea* on some serum biochemical markers of kidney damage and renal histomorphology of alloxan-induced diabetic rats.
Methodology: A total of Seventy two (72) male albino wistar rats weighing between 100-105 g were assigned into six groups of 12 rats per group. Groups 1- 5 rats were made diabetic by single intraperitoneal injection of alloxan monohydrate at the dose of 160 mg/kg and treated with 62.5, 125, 250 mg/kg bw of the extracts, 2 mg/kg bw Glibenclamide and 10 ml/kg DW respectively while the non diabetic group 6 rats received 10 ml/kg DW as serve as normal control rats. The treatment was daily through the oral route for 84 days. Serum urea and Creatinine were measured on days 42, 56 and 84 post treatment. At the end of the experiment, the rats were humanly sacrificed and

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their kidney harvested for histomorphometric assessment.
Results: Administration of 125 mg/kg bw of the extract, significantly ($p<0.05$) reduced the serum levels of urea and creatinine and equally ameliorated histopathologic lesions in the kidney when compared to the diabetic untreated group (negative control).
Conclusion: It was concluded that the methanol extract of *C. arborea,* at the dose of 125 mg/kg was strongly protective against kidney damages occasioned by diabetes

Keywords: Cussonia arborea; kidney damage markers; histopathology.

1. INTRODUCTION

Diabetes mellitus is a group of metabolic disorder resulting from defects in insulin secretion or reduced sensitivity of the tissues to insulin action or both [1]. It is a disease characterized by inability to regulate blood glucose as a result of relative or absolute deficiency in insulin. This results in hyperglycemia, often accompanied by glycosuria, polydipsia and polyuria [2]. Diabetic nephropathy is a chronic complication of both type-1 DM and type-2 DM. Globally as of 2010, an estimated 285 million people had diabetes with type 2 making up about 90% of the cases. In 2013 according to international Diabetes Federation, an estimated 381 million people had diabetes. Its prevalence is increasing rapidly and by 2030, this number is estimated to double [3]. Diabetic nephropathy is a common cause of high rates of dialysis and death [4,5]. Once people begin kidney dialysis, the diabetic process affects the kidneys in a few ways: glomerulosclerosis, and arteriosclerosis of the entering and leaving renal arteries; arteriosclerosis of the renal artery and its internal branches; and deposits of glycogen, fat and glycopolysaccharides around the tubules. Early on, nephropathy has no symptoms, but as it advances we see edema (swelling, particularly around the eye), nausea, fatigue, headache, and generalized itching [6]. Plants have been used since time immemorial in the management of diabetes mellitus; however their effects are usually exaggerated, unscientific or unstandardized. *Cussonia arborea* is a tropical plant belonging to the family of Araliacea with about twenty species [7]. Folklorically, the plant is used in the treatment of malaria [8] fungi diseases and chronic diarrhea [9] and diabetes [10]. The objective of the study was to evaluate the effects of varied oral doses of the methanol root bark extract of *C. arborea* on some biochemical markers of kidney damage and renal histomorphology of alloxan-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Experimental animals

Seventy two (72) male albino rats weighing between 100 to 105 g were obtained from the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka laboratory animal house for the study. The rats were randomly assigned into six groups (groups 1-6) of 12 each. The rats were acclimatized for two weeks. The environmental temperature where the animals were housed varied between 28- 32°C. The animals were kept in stainless wire mesh cages and provided with good clean water *ad libitum*. They were fed with standard commercial feed (Guinea^{R} growers). The experimental protocol used in this study was approved by the ethics committee of the University of Nigeria, Nsukka and conforms with the guide to the care and use of animals in research and teaching of University of Nigeria, Enugu state, Nigeria.

2.1.2 Plant material

The root bark of the plant material (*Cussonia arborea*) used in this study was collected from Orukpa Local Government Area of Benue state and identified by a plant taxonomist, at the International Centre for Ethnomedicine and Drug Development, Echara, Aku Road, Nsukka, Enugu State, Nigeria.

2.2 Methods

2.2.1 Preparation of the plant extract

Cold maceration method of extraction was employed. The root bark of *C. arborea* was air dried at a very low intensity of sunlight to avoid denaturation of the active ingredient. It was pulverized and stored in an air tight container pending its usage. About 2 kg of the powdered

stem bark was soaked in 10 liters of 80% methanol (SIGMA ALDRICH, UK) with intermittent shaking every 2 h for 48 h. The mixture was filtered using Whatmann No 1 filter paper. The filtrate was concentrated using rotary evaporator and the extract stored as *Cussonia arborea* extract (CAE) at 4℃

2.2.2 Induction of experimental diabetes mellitus

Diabetes was induced in rats in groups 1-5 using the method described by Venugopal [11]. The rats were injected with alloxan monohydrate (SIGMA ALDRICH, UK) dissolved in distilled water at dose of 160 mg/kg body weight intraperitoneally, after overnight fasting (18 h). Before the injection with alloxan monohydrate, the blood sugar (FBS) levels of the rats were taken using Accu-Check glucometer. This was done by tail snip of the rats and allowing blood to drop on the Glucometer strip. The value was read off on the screen of the glucometer. After injecting alloxan, the rats were kept in clean stainless cages and fed with commercial feed and were also given clean water for about 2 days before they came down with diabetes. On the 2nd day, diabetes was confirmed. The rats were fasted overnight before the assessment of their glucose blood status on the 2nd day. Fasting blood glucose values above 7 mMol/L (126 mg/dl) were considered diabetic.

2.3 Treatments

The rats were treated as follows:

- Group 1: Diabetic rats treated with 62.5 mg/kg CAE
- Group 2: Diabetic rats treated with 125 mg/kg CAE
- Group 3: Diabetic rats treated with 250 mg/kg CAE
- Group 4: Diabetic rats treated with 2 mg/kg Glibenclamide
- Group 5: Diabetic rats treated with 10 ml/kg Distilled water (DW)
- Group 6: Non diabetic rats treated with 10 ml/kg DW

The rats were treated daily for eighty four (84) days. Serum urea and Creatinine were determined on days 28, 56 and 84. At the end of the 84 days experimental period, the rats were humanly sacrificed and the kidneys collected into a bottle containing formol saline for histopathology processing.

Serum urea and creatinine tests were done following standard procedures, using test kits manufactured by Randox, UK. Serum urea was determined by the modified Berthelot-Searcy method [12] for the *in-vitro* determination of urea in serum or plasma using urea test kit while the serum creatinine was determined using the modified Jaffe method [13] for the *in-vitro* determination of creatinine in serum, plasma or urine using creatinine test kit

The histological examination of the tissues of the kidneys was done using the method of Drury et al. [14].

2.4 Statistical Analysis

Data generated from the study was subjected to One-way Analysis of Variance (ANOVA) and variant means were compared by post hoc using the Duncans Multiple Range Test. Probability values less than 0.05 was accepted as significant. The statistical analyses were done using the SPSS software, version 20.

3. RESULTS

The serum urea levels of all other rat groups were significantly ($p<0.05$) lower than that of group 5 rats on day 28 of the experiment and those of groups 1, 2 and 4 were comparable ($p>0.05$) to that of group 6 rats (Table 1). On day 56, the serum urea levels of group 5 rats were significantly ($p<0.05$) higher than those of all other groups and among all other rat groups. Group 3 rats had a significantly ($p<0.05$) higher serum urea than others and none of the groups was comparable to the group 6 rats. On day 84, the serum urea levels of the group 6 rats were also significantly ($p<0.05$) higher than those of all other groups, but those of groups 1, 2 and 4 were comparable ($p>0.05$) to that of group 6 rats.

The serum creatinine levels of the rats in group 5 were significantly ($p<0.05$) elevated compared to that of the other groups while that of the groups 1, 2, 3 and 4 were statistically comparable ($p>0.05$) on day 28 post treatment. Serum creatinine levels of rats in group 6 were however significantly ($p<0.05$) lower than that of the other groups. On day 56, the serum creatinine levels of rats in groups 2, 4 and 6 were comparable ($p>0.05$) but significantly ($p<0.05$) lower than that of the rats in groups 3 and 5. Serum creatinine levels of those rats in groups 1 and 3 were statistically comparable ($p>0.05$) but were

Table 1. Effects of administration of *Cussonia arborea* root bark extract on serum urea (mg/dl) of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	13.60±0.36^{ab}	13.29±0.35^{b}	13.57±0.36^{a}
2	12.44±0.44^{a}	14.52±0.29^{c}	14.95±0.79^{a}
3	14.71±0.48^{b}	16.85±0.13^{d}	18.33±0.38^{b}
4	12.15±0.65^{a}	13.56±0.62^{bc}	13.77±0.64^{a}
5	16.55±0.90^{c}	20.45±0.25^{e}	27.24±2.17^{c}
6	12.14±0.29^{a}	12.15±0.14^{a}	12.95±0.24^{a}

Table 2. Effects of administration of *Cussonia arborea* root bark extract on serum creatinine (mg/dl) of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	0.70±0.00^{b}	0.80±0.00^{bc}	0.82±0.01^{b}
2	0.72±0.01^{b}	0.73± 0.00^{ab}	0.69± 0.00^{a}
3	0.77± 0.02^{bc}	0.83±0.02^{c}	0.80±0.00^{b}
4	0.72±0.03^{b}	0.71±0.01^{a}	0.71±0.01^{a}
5	0.82±0.02^{d}	0.98±0.04^{d}	1.12±0.04^{c}
6	0.62±0.02^{a}	0.66±0.01^{a}	0.67±0.000^{a}

Different superscripts along the same column indicate significant difference at p<0.05

significantly (p<0.05) lower than that of the group 5 rats. The serum creatinine levels of rats in groups 2,4 and 6 on day 84 post treatment remained statistically comparable (p>0.05) and significantly (p<0.05) lower than those of groups 1, 3 and 5 rats. Those of the group 5 rats were significantly (p<0.05) higher than that of the other groups (see above Table 2).

There was no obvious histopathology observed. The glomerulus and the renal tubules appeared normal (Fig. 1).

The renal epithelial cells appeared to have undergone degeneration and necrosis and subsequent erosion of the cells. The tubules equally appeared dialated compared to that of the normal control rats (Fig. 2).

The epithelial cells were mildly eroded and glomerulus infiltrated with mononuclear cells compared to that of the diabetic untreated group (Fig. 3).

The arrows (black and white) show normal epithelial cells which compared favorably with that of the normal control rats (Fig. 4).

There was a mild localized necrosis of the renal tubular cells comparable to that of the rats treated with 62.5 mg/kg of the extract (Fig. 5).

There were no visible lesions. The kidney section compared very well with that of the normal control rats and those treated with 125 mg/kg of the extract (Fig. 6).

4. DISCUSSION

The increases in serum urea and Creatinine levels observed in group 5 rats (diabetic untreated) compared to the normal control rats may be attributed to the effect of diabetes on the kidney (diabetic nephropathy). Diabetes has been associated with impairment of kidney function. The kidney is actively involved in the development, maintenance and resolution of hyperglycemia through gluconeogenesis and glucose excretion [15]. The kidney is involved in the regulation of glucose via gluconeogenesis, taking up glucose from the glomerular filtrate. Glucose utilization by the kidney after overnight fast accounts approximately to 10% of glucose utilized by the body [16]. The cellular responses of kidney to noxious incursions vary from minor biochemical abnormalities to cell death. The effects usually reported following toxic injury to the kidney reflect decreased elimination of wastes such as urea or increased creatinine levels [17]. The urea and creatinine are considered important biomarkers of kidney dysfunction [18]. The urea and creatinine levels of 125 mg/kg extract-treated rats and glibenclamide-treated rats were significantly (p<0.05) reduced compared to the negative control groups across the duration of the experiment. This may probably be as a result of mitigation of diabetes by the agents (extract and

glibenclamide) resulting to a corresponding amelioration of the complication (diabetic nephropathy). The result is in agreement with the findings of [19] who reported decreases in the creatinine and urea values of *Allium sativum*-treated diabetic rats. This biochemical finding of most severe kidney damage (degeneration and necrosis of the tubular epithelial cells and hypersegmentation of the glomerulus) in diabetic rats and ameliorated lesions in the treated groups correlates positively with the most severe kidney histopathology in the diabetic untreated and the less severe lesions in the treated groups (Figs. 1-6).

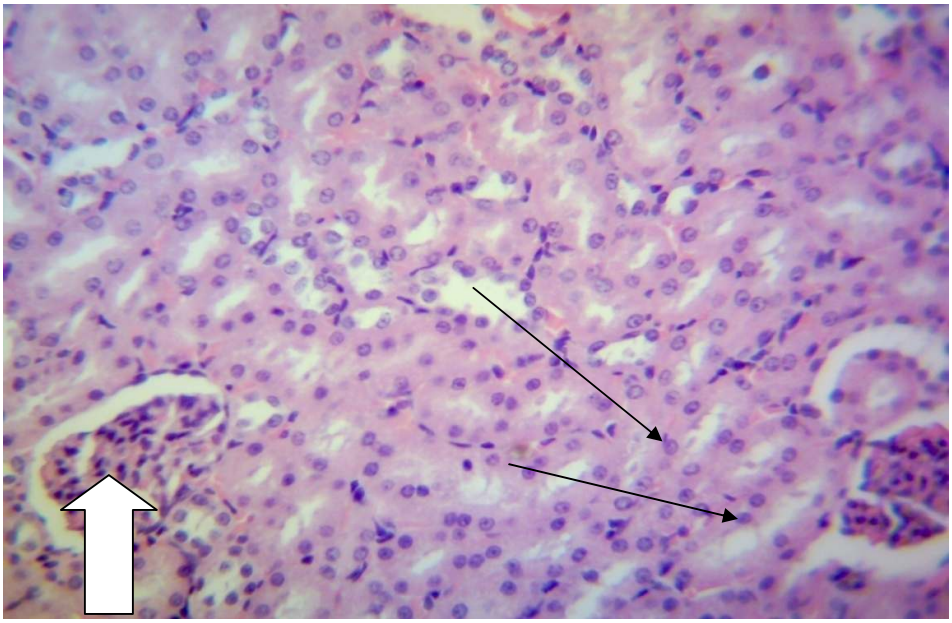

Fig. 1. Kidney of normal control rats (group 6) showing glomerulus (white arrow) and normal renal tubular epithelial cells (black arrows) (H&E X400)

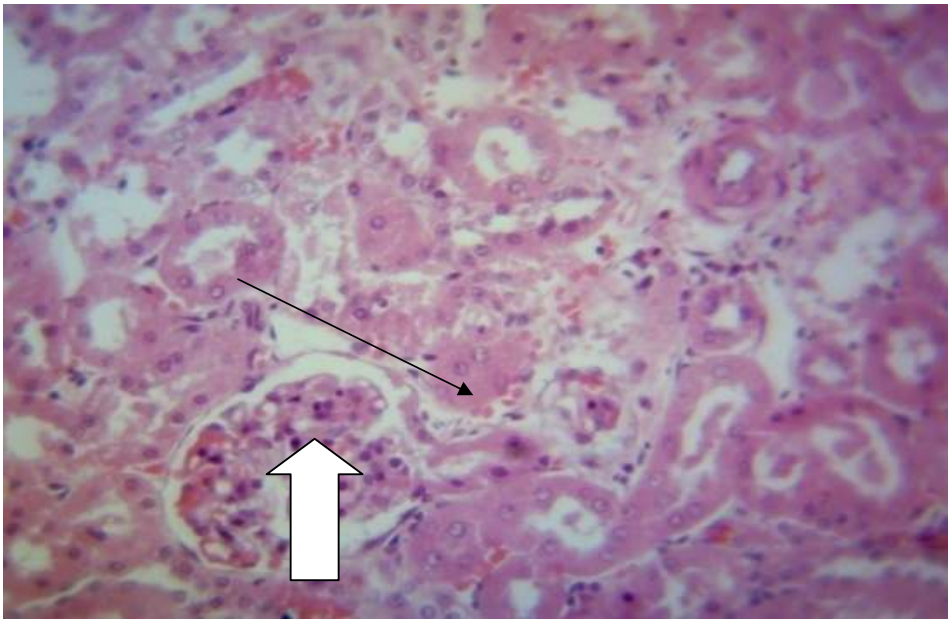

Fig. 2. Kidney of rats in group 5 showing erosion of epithelial cells of the renal tubules (black arrows) and hypersegmented glomerulus (white arrows) (H&E X400)

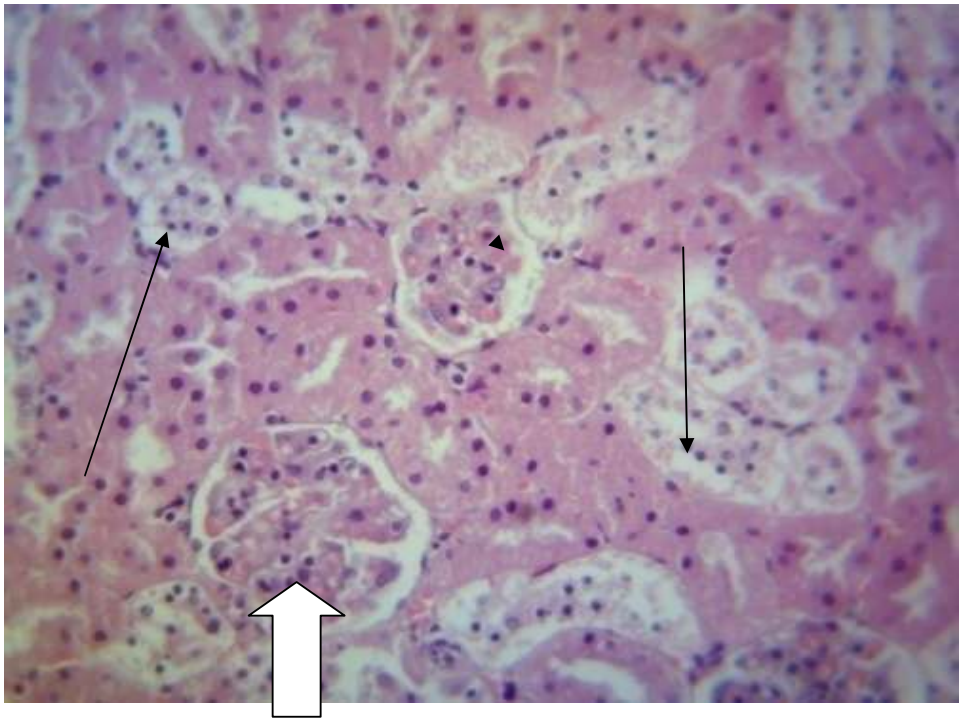

Fig. 3. Kidney of group 1 rats (diabetic rats treated with 62.5 mg/kg of extract) showing infiltrations with mononuclear cells in the glomerulus (white arrow) and necrosis of the tubular epithelial cells (black arrows) (H&E X400)

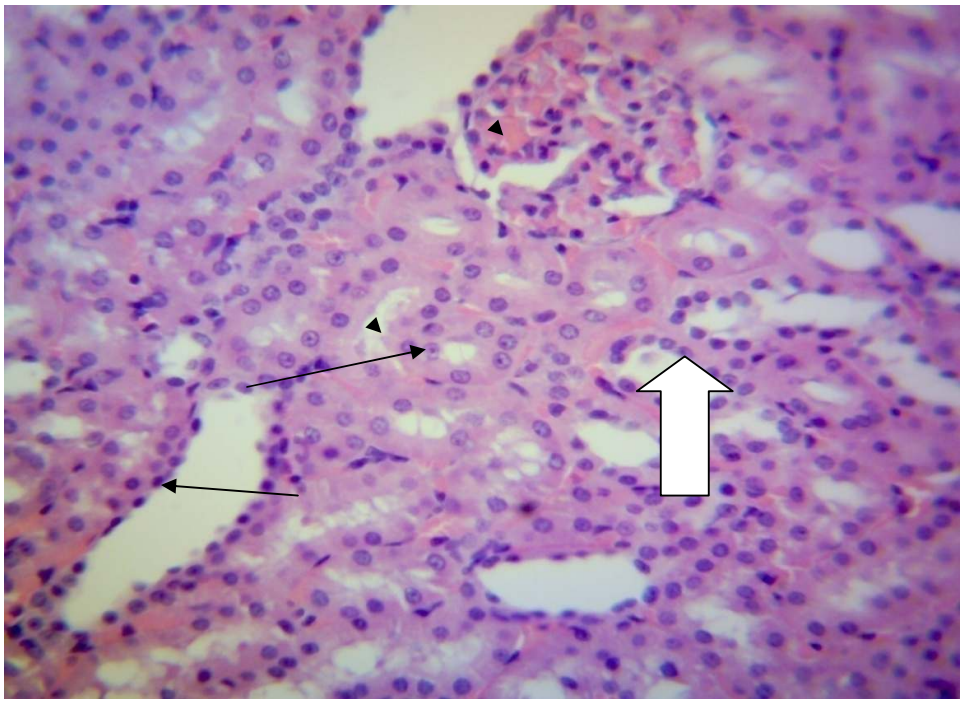

Fig. 4. Kidney of group 2 rats (diabetic rats treated with 125 mg/kg extract) showing normal tubular epithelial cells (arrows) (H&E X400)

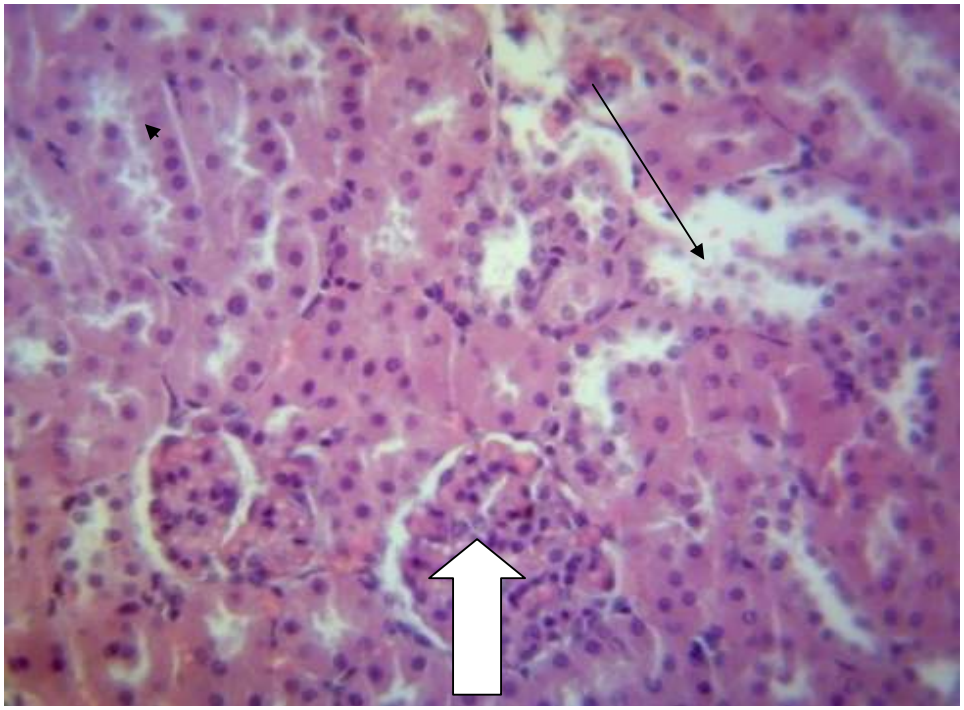

Fig. 5. Kidney of group 3 rats (diabetic rats treated with 250 mg/kg of extract) showing glomerulus (white arrow) and mild necrosis of renal tubular epithelial cells (black arrow) (H&E X400)

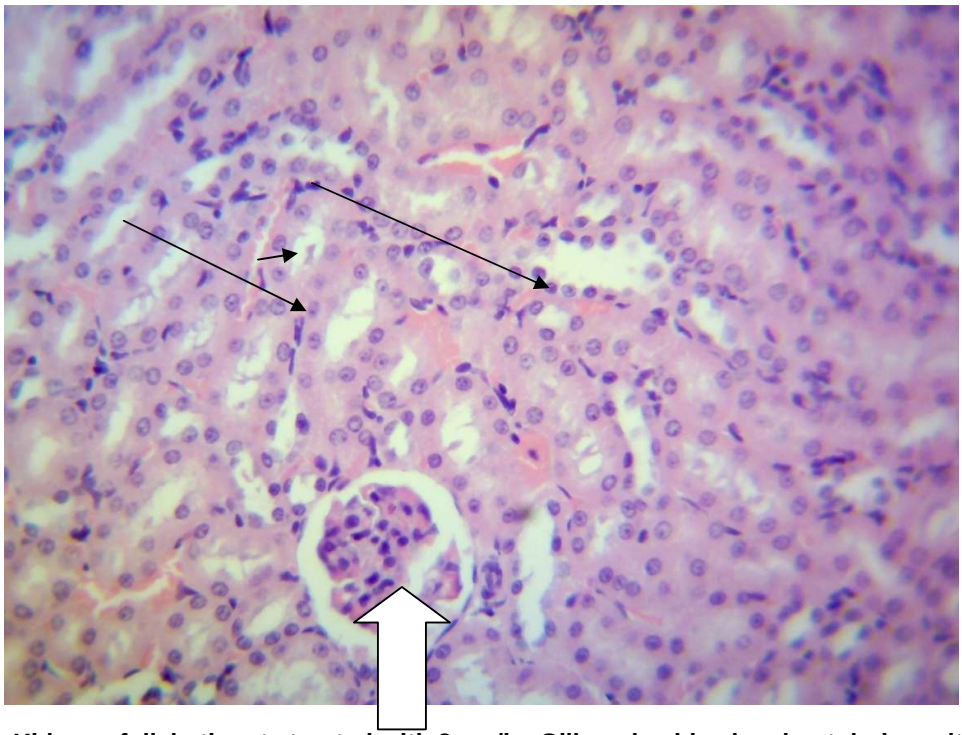

Fig. 6. Kidney of diabetic rats treated with 2 mg/kg Glibenclamide showing tubular epithelial cells (black arrows) and glomerulus (white arrow) with no observable pathologic lesions (arrows) (H&E X400)

5. CONCLUSION

In conclusion therefore, treatment of alloxan-induced diabetic rats with methanol extract of *Cussonia arborea* especially at the dose of 125 mg/kg demonstrated the highest curative ability by ameliorating histopathological lesions occasioned by diabetes mellitus in the kidney as evidenced by the findings of kidney damage markers.

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. All experiments have been examined and approved by the appropriate ethics committee.

ACKNOWLEDGEMENTS

We wish to sincerely appreciate the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka for giving us access to equipment

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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