



Effects of *Sarcocephalus latifolius* Afzel. Ex R.Br. Leaf Powder on the Kidney Function of Alloxan-Induced Diabetes Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study assessed the effects of *Sarcocephalus latifolius* Afzel. Ex R.Br. leaf powder on the kidney function of alloxan-induced diabetes rats.

Forty-five healthy female albino rats were used in the experiment and assigned into 9 different groups. Diabetes was induced intravenously with 150 mg/kg body weight alloxan. Normal and diabetic rats were administered orally with 300, 600, 750mg/kg/ b.w of *S. latifolius*. After 28 days, the animals were sacrificed and blood with the kidney were harvested for biochemical and histological studies.

In our result, significant ($p < 0.05$) increase was observed in creatinine concentration of diabetic rats, which was significantly ($p < 0.05$) decreased upon administration of 300 and 750 mg/kg body weight

of *Sarcocephalus latifolius* leaf powder. No significant ($p>0.05$) difference was observed in the urea concentration of all the groups. Significant ($p<0.05$) difference in sodium concentration was only observed between the diabetic untreated and metformin treated groups whereas, potassium concentration varied significantly ($p<0.05$) across the groups. Certain degenerative changes in the kidney of normal and diabetic rats treated and untreated with *S. latifolius* leaf powder were observed but at a lower degree in the group treated with the 300 mg/kg/bw of the leaf powder. The result of this study showed the possible renal toxicity potential of the plant at high dose.

Keywords: *Sarcocephalus latifolius*; diabetes; renal toxicity; alloxan; rats.

1. INTRODUCTION

Chronic kidney disease (CKD) is far more prevalent worldwide than was previously assumed. It affects about 10 - 15% of the adult population in the western countries, many of whom require costly treatments or renal replacement therapy. Moreover, it has been recognized that CKD is a major risk factor for increased cardiovascular disease and death. The increasing prevalence of diseases that predispose individuals to CKD, such as hypertension, diabetes, and obesity has rendered the prevention and early detection of CKD a health-care priority in both developed and developing countries [1]. It is now known that diabetic nephropathy is the most common cause of chronic kidney disease (CKD) in both type 1 and II diabetes (Bojestig et al., 2004). Increase in glomerular capillary growth, because of glomerular hypertrophy, is one of the primary responses to hyperglycemia caused by diabetes [2]. Hyperglycemia in diabetes causes the kidney to suffer impaired function and structural changes, which consequently tends to excretion of protein [3]. The aetiology of renal disease during diabetes is linked to chronic hyperglycaemia, which causes protein glycation and formation of advanced glycation products (AGE), thus contributing to both microvascular and macrovascular complications [4,5]. In addition, hyperglycaemia can instigate reactive oxygen species (ROS) formation and simultaneously cause depletion of the antioxidant defence mechanisms [6,7,8].

The kidney perform several functions including control of the volume of body fluids, fluid osmolality, acid-base balance, various electrolyte concentrations, and removal of toxins. In addition, other processes such as conversion of vitamin D to its active form, calcitriol and synthesis of erythropoietin and renin hormones are carried out by the kidney [1]. Therefore, the kidney is a major organ for survival and development of remedy against chronic kidney damage is imperative.

Sarcocephalus latifolius is one of the commonest names in the list of medicinal plants of the world and identified with various names. In West Africa, it has acquired different names among nations and tribes. In Nigeria, the Igbo people call it ubulu-inu, the Hausas call it Doundake, Tafashiya or tashiyaigia, while the Yoruba tribe identifies it as opepe. The various English names are pincushion tree, African peach, Guinea peach, or Sierra Leone peach. *Sarcocephalus latifolius* has been identified with so many pharmacological activities that have made it attractive in herbal market. Potentially it could be a source of lead for development of a number of effective and essential modern drugs (Enemor and Okaka, 2013).

In this study, we assessed the renal protective effects of *S. latifolius* leaf powder in alloxan induced diabetic rats.

2. METHODS

2.1 Experimental Animals

Healthy female wistar rats with average weight of 230 g, which have not been subjected to previous experimental activities, were used. Their weights were determined prior to feeding. The rats were acclimatized for 2 weeks. The experimental animals were housed in standard plastic cages and provided access to food and water ad-libitum.

2.2 Collection and Preparation of Plant

The plant *Sacrocephalus latifolius* was identified by a Plant Taxonomist at the Department of Pure and Applied Biology, Ladoke Akintola University of Technology (LAUTECH) Ogbomoso, Oyo State. The fresh leaves of *Sarcocephalus latifolius* were air dried for three weeks. It was pounded and later blended to powdered form. The leaves were sieved to get a complete powdery form. The plant powder was preserved in a desiccator until it was ready to use.

2.3 Animal Grouping

A total number of 45 female Wistar rats were randomly selected and divided into nine (9) different treatments groups, each groups comprises of 5 healthy animals.

Group A: the control group,

Group B: untreated diabetes mellitus group,

Group C: served as diabetes mellitus treated with 300 mg/kg of *Sacrocephalus latifolius* powder,

Group D: served as non- diabetic rats treated with 300 mg/kg of *Sacrocephalus latifolius* powder,

Group E: served as diabetes mellitus treated with 600 mg/kg of the *Sacrocephalus latifolius* powder,

Group F: served as non- diabetic rats treated with 600 mg/kg of the *Sacrocephalus latifolius* powder,

Group G: served as diabetes mellitus treated with 750 mg/kg of *Sacrocephalus latifolius* powder,

Group H: served as non- diabetic rats treated with 750 mg/kg of the *Sacrocephalus latifolius* powder,

Group I: served as diabetes mellitus treated with Metformin.

NB: Diabetes was induced by i.p administration of 150 mg/kg of alloxan to animals in cages B, C, E, G and I.

2.4 Experimental Design

At the end of the two weeks acclimatization, the animals were fasted overnight and groups B, C, E, G, and I were administered 150 mg/kg of alloxan intra-peritoneally. The fasting blood sugar (FBS) was determined 48 hours after induction, and animals with FBS \geq 200 mg/dl were considered diabetic. Upon diabetic induction, the animals were treated with *Sacrocephalus latifolius* leaf powder for a period of 28 days. Nadia et al. [9] had earlier reported the LD₅₀ of the aqueous extract to be greater than 5000 mg/kg/b.w., therefore doses lower than the LD₅₀ were selected in this study. Regular monitoring of weight was ensured throughout the experiment and the weights were recorded in grams. In addition, the FBS was determined prior animal sacrifice.

2.5 Blood Collections

At the end of the 28 days treatment, the animals were fasted overnight and sacrificed by cervical dislocation. The blood samples were collected through retro-orbital process into sterile EDTA bottles. Samples were centrifuge at 4000 rpm for 5 minutes to obtain the plasma. The liver was excised and preserved in formalin buffer for histological examination.

2.6 Biochemical Analysis

2.6.1 Determination of plasma concentration of sodium and potassium electrolytes

Plasma concentrations of Sodium and Potassium were determined using the Corning 410 Clinical flame photometer. The equipment was operated according to its operation manual. Briefly, 0.1 mL of Lithium heparinized plasma sample, control and standard was mixed each with 4.9 mL of distilled water and mixed thoroughly. The absorbance was read at 589 nm for Sodium and 768 nm for Potassium.

2.6.2 Determination of plasma concentration of urea and creatinine

Plasma concentrations of Urea and Creatinine were estimated using the modified Berthelot method [10] and the method of Taursky [11], respectively. Briefly, 10 μ L of the sample was mixed with 100 μ L Sodium nitroprusside and urease and incubated for 10 min at 37°C. 2.5 mL each of Phenol and Sodium hypochlorite were then added and incubated for 15 min at 37°C. This procedure was done for the control also. The Blank contained 10 μ L of distilled water while the Standard contained 10 μ L of Standard solution and were treated as done for the test sample.

$$\text{Urea concentration} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{standard concentration}$$

For determination of Creatinine concentration, 1000 μ L of working reagent was mixed with 100 μ L of the heparinized plasma sample (Test) and 100 μ L of standard solution (Standard). First (T₁) and second (T₂) absorbance values were taken 60 seconds apart.

$$\text{Creatinine concentration } (\mu\text{mol/L}) =$$

$$\frac{T_2 - T_1 \text{ of sample}}{T_2 - T_1 \text{ of standard}} \times 177 \text{ (concentration of standard)}$$

2.7 Histological Examination of the Kidney

The histological procedure was carried out by the method described by Biswas et al. (2010) with some modifications. The kidney from both the treated and control groups was processed with automatic tissue processor (STP 120) by tissue processing method as described by Galen and Gambino (1975). Histology of tissues 4 μ m sections was prepared with the help of Microtome (Leica, RM 2145). These sections then de-paraffinated in xylene, dehydrated through a graded ethanol series, and stained with haematoxylin and cleared in xylene I and xylene II and these organs were preserved for microscopic examination. The slides prepared by this process were observed under microscope (Model Nikon Labophot. 223425 Japan) and photographed through Nikon labophot Advanced Research Microscope, Model 223425 Japan, with Sony Digital 12.1 MEGA PIXELS.

2.8 Data Analysis

Data obtained are expressed as Mean \pm Standard error of mean. The data was subjected to one-way analysis of variance (ANOVA) and differences between means were determined using the Graph Pad Prism 5 (Graph Pad Software Inc., San Diego, CA). The level of significance was set at $p < 0.05$.

3. RESULTS

Fig. 1 showed the effect of *Sarcocephalus latifolius* leaf powder on urea concentration in diabetic female wistar rats. The result revealed insignificant ($p > 0.05$) increase ($P < 0.05$) in the urea concentration of the DM group when compared to the other groups. From the result, there was no significant ($p > 0.05$) difference in the urea concentration across all the groups when compared with the diabetic and normal control groups.

The effect of *Sarcocephalus latifolius* leaf powder on plasma sodium concentration of diabetic and normal rats is illustrated in Fig. 2. Significant ($p < 0.05$) difference was only observed between the diabetic untreated and metformin treated groups. The sodium concentration increased significantly ($p < 0.05$) in the metformin treated group relative to the diabetic untreated group. No significant ($p > 0.05$) difference was observed

between the control groups and the normal and diabetic rats administered *Sarcocephalus latifolius* leaf powder.

Fig. 3 below illustrates the plasma potassium concentration of diabetic and normal rats administered *Sarcocephalus latifolius* leaf powder. Potassium concentration increased significantly ($p < 0.05$) in diabetic rats administered 600 and 750 mg/kg body weight and normal rats administered 300, and 750 mg/kg body weight of *Sarcocephalus latifolius* leaf powder when compared with the normal control group. The concentration in normal rats administered 300 mg/kg body weight of *Sarcocephalus latifolius* leaf powder increased significantly ($p < 0.05$) when compared with the normal and diabetic control groups. No significant ($p > 0.05$) difference was observed between the normal control and the group treated with metformin whereas the concentration decreases when compared with the diabetic control group.

Creatinine concentration in the plasma of normal and diabetic rats administered *Sarcocephalus latifolius* leaf powder is illustrated in Fig. 4. The result showed variation in the concentration of creatinine across the groups. In the untreated diabetic group, creatinine concentration increased significantly ($p < 0.05$) compared to the normal control and diabetic groups treated with 300 and 750 mg/kg body weight of *Sarcocephalus latifolius* leaf powder, whereas, no significant difference ($p > 0.05$) was observed when compared with the diabetic group administered 600 mg/kg body weight and normal rats administered 750 mg/kg body weight of *Sarcocephalus latifolius* leaf powder. No significant difference ($p > 0.05$) was observed between the normal and diabetic rats administered 300 mg/kg body weight of *Sarcocephalus latifolius* leaf powder compared to the normal control group.

3.1 Effect of *Sarcocephalus latifolius* Leaf Powder on Kidney Histomorphology of Alloxan-induced Diabetic Rats

Fig. 5a-l is the representative photomicrograph of kidney section of diabetic and normal rats treated with metformin and leaf powder of *Sarcocephalus latifolius*. Different morphological changes observed in are indicated with arrows and reported in the legend.

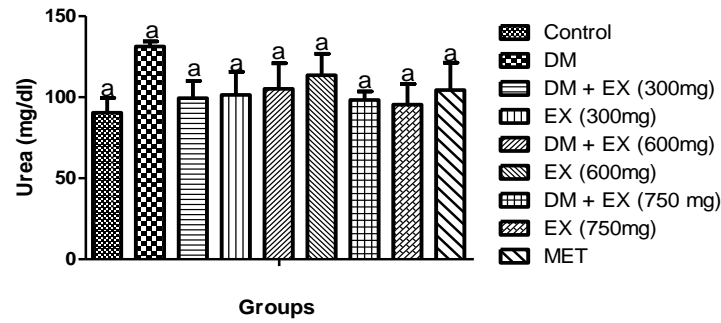


Fig. 1. Effect of *Sarcocephalus latifolius* leaf powder on Urea concentration in alloxan- induced diabetic female wistar rats

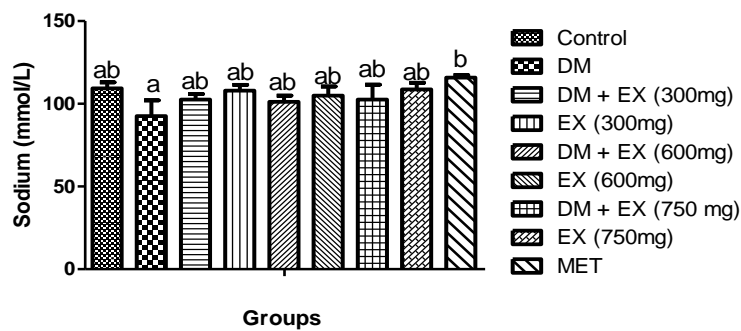


Fig. 2. Effect of *Sarcocephalus latifolius* leaf powder on Sodium concentration in alloxan- induced diabetic female wistar rats

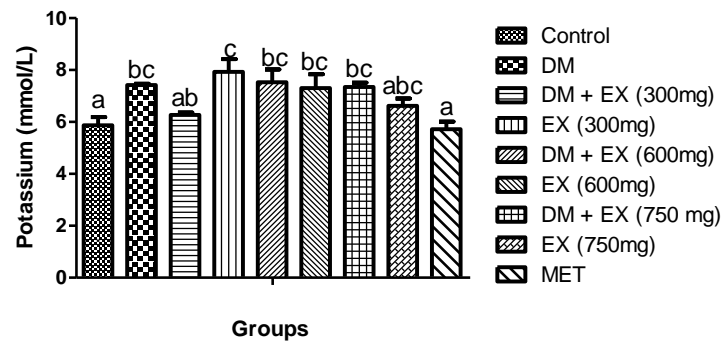


Fig. 3. Effect of *Sarcocephalus latifolius* leaf powder on Potassium concentration in alloxan- induced diabetic female wistar rats

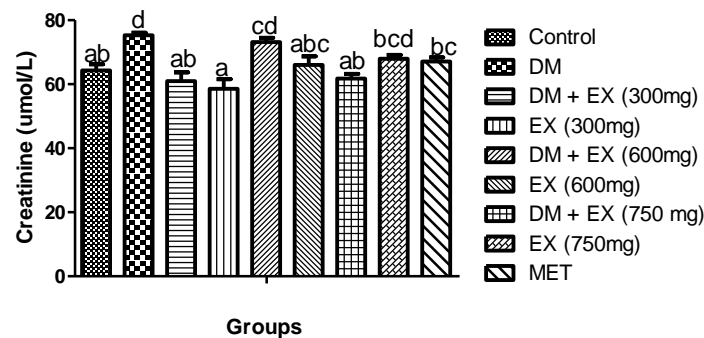


Fig. 4. Effect of *Sarcocephalus latifolius* leaf powder on Creatinine concentration in alloxan- induced diabetic female wistar rats

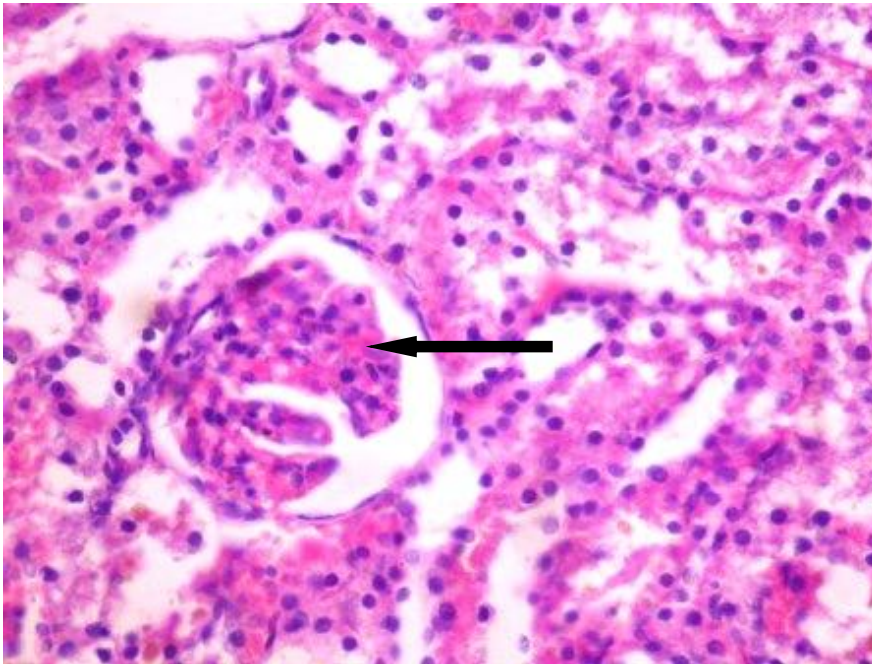


Fig. 5a. Control Group (Mag x400)

A section of the kidney showing the glomerulus with compact apparatus and wide capsular space (black arrow), the renal tubules appear normal, the interstitial spaces appear normal.

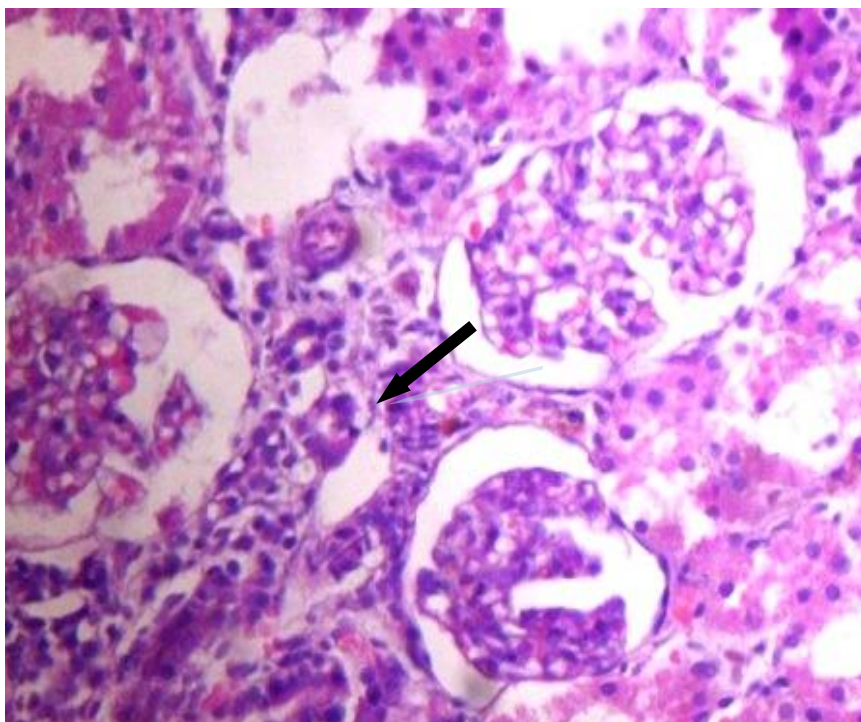


Fig. 5b. Untreated Diabetes mellitus (Mag x400)

A section of the kidney from this group shows a moderate architecture but the interstitial spaces show focal area of inflammation with infiltration of inflammatory cells and degeneration (Black arrow) when compared with the control group

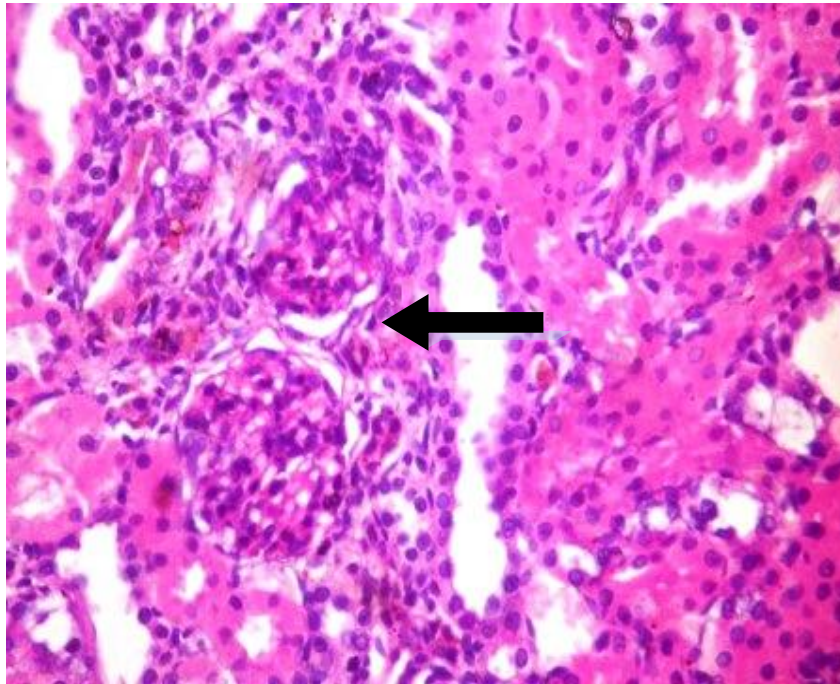


Fig. 5c. Diabetes mellitus treated with extract 300mg/kg (Mag x400)

A section of the kidney (black arrow) shows a poor architecture, the renal cortex show some glomeruli with mesangial cells hyperplasia, mid renal tubules degeneration and interstitial spaces with focal inflammation when compared with the control group.

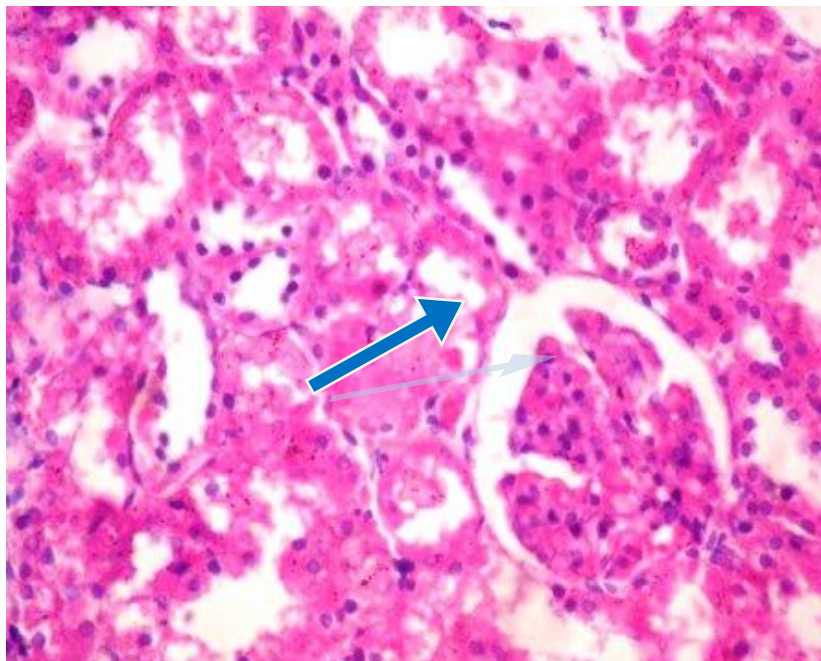


Fig. 5d. Extract only 300mg/kg (Mag x400)

A section of the kidney from this group shows a poor architecture, the renal cortex and the glomeruli with the mesangial cells shows hyperplasia, the renal tubules shows moderate to severe tubular necrosis seen (blue arrow), other tubules seen are collapsed and lack luminal spaces, the interstitial spaces appear normal when compared with control

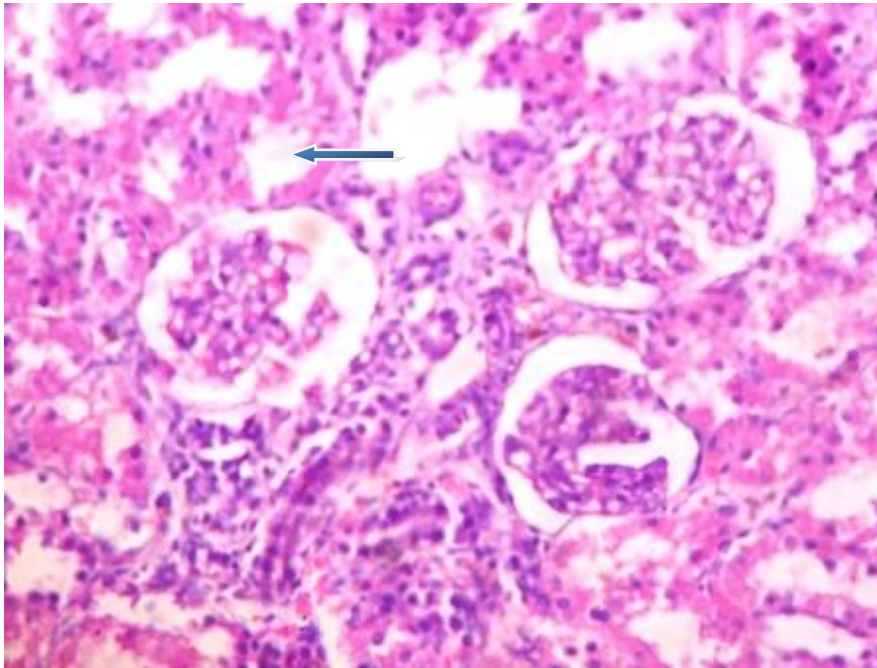


Fig. 5e. Diabetes mellitus treated with extract 600mg/kg (Mag x400)

A section of kidney from this group shows the interstitial spaces around the glomeruli are infiltrated with inflammatory cells with mild vascularization, a focal area of mild fluid accumulation was observed (blue arrow)

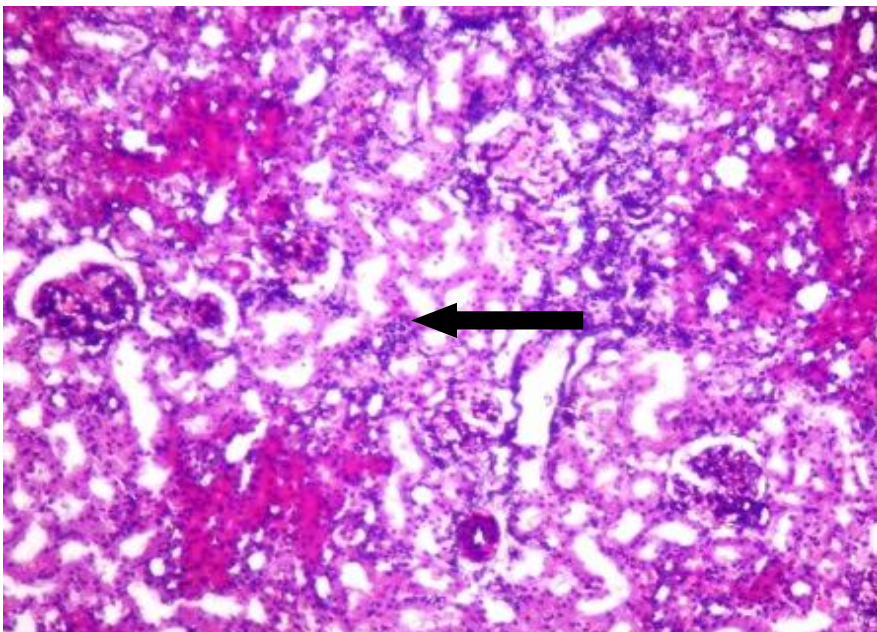


Fig. 5f. Extract only 600mg/kg (Mag x400)

A section of the kidney shows the renal cortex with some peri-glomerular infiltration of inflammatory cell (black arrow), few renal tubules appear mildly degenerated, others appear normal, the interstitial spaces appear normal when compared with the control and untreated DM

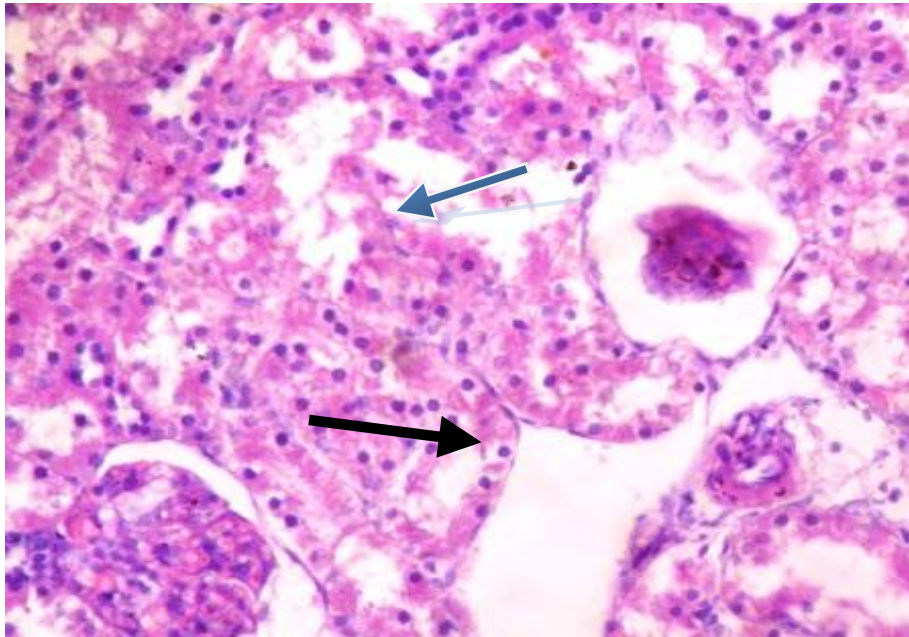


Fig. 5g. Diabetes mellitus treated with extract 750mg/kg (Mag ×400)

This section of the kidney form the group shows the renal cortex with few glomerular sclerosis (blue arrow), the renal tubules appear normal (black arrow), the interstitial spaces appear normal too when compared with the control and Untreated DM

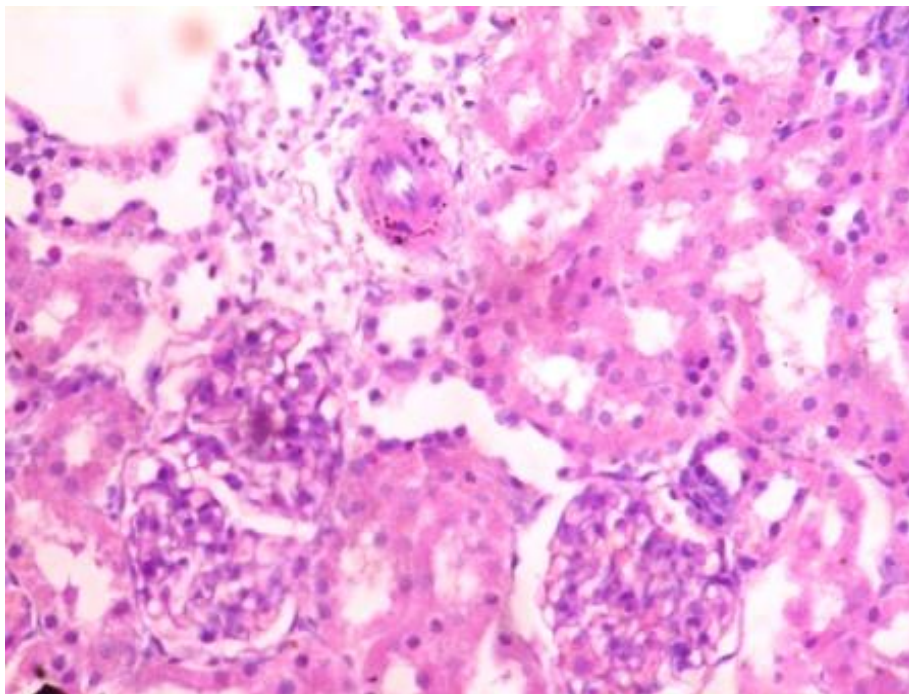


Fig. 5h. Extract only 750mg/kg (Mag ×400)

This section of the kidney shows the renal cortex with normal glomeruli with normal mesangial cells and capsular spaces, the renal tubules appear normal, the interstitial spaces appear normal when compared with control.

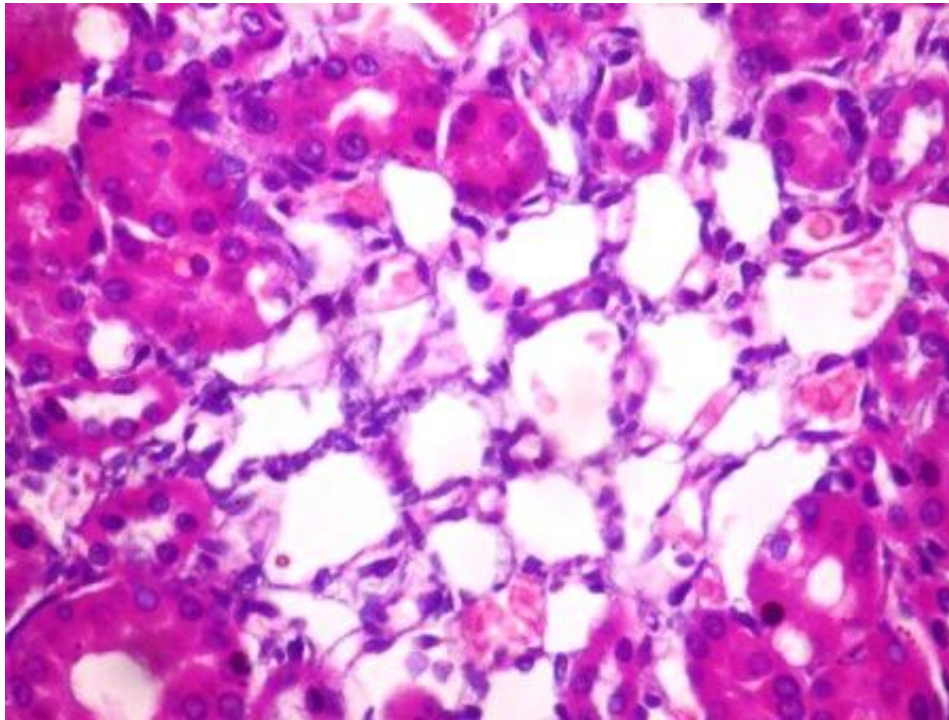


Fig. 5i. Diabetes mellitus treated with metformin (Mag x400)

This section shows a degenerated glomerulus, the renal tubules appear normal, the interstitial spaces appear normal too. The medullary ray show some collecting tubules with somewhat fat degeneration

4. DISCUSSION

The study aimed at examining the effect of *Sarcocephalus latifolius* leaf powder on kidney dysfunction instigated by alloxan-induced diabetes in rats. The current investigation revealed that induction of diabetes resulted in the elevation of urea and creatinine concentrations. Measurement of serum urea and creatinine concentrations is considered significant in the diagnosis of renal dysfunction [12,13,14]. Metabolism of creatine in the muscle gives rise to the metabolite; creatinine, whose amount in blood is constantly regulated. Elevated levels are indication of diminished renal function only, since it is easily excreted by the kidneys [15]. Serum electrolytes such as Na⁺, and K⁺ could also be employed to indicate renal dysfunction at both the tubular and glomerular levels. Enhanced serum potassium ion for instance implies dysfunction at the tubular levels [16]. In a previous study, decrease in serum sodium concentration in diabetic rats was attributed to dehydration and loss of cations to buffering of metabolic acidosis [17]. All the observation on electrolytes, urea and creatinine concentration in the blood of diabetic rats reported in this study is in consonance with the literature and *S. latifolius* leaf powder only

provided protection against diabetic induced renal damage only at low dose whereas it might be toxic to normal rats at high dose.

The hepatotoxic effect of *Sarcocephalus latifolius* extracts has previously been reported and our observation in this study indicates possible renal toxicity of the plant at high dose [18].

The histological observations further substantiate the result of the biochemical study. Observations ranging from moderate to severe inflammation, infiltration by inflammatory cells, glomeruli with the mesangial cells showing hyperplasia, few glomerular scleroses were observed across the groups. The severity was more pronounced in the diabetic untreated group and increases in severity as the dosage of *S. latifolius* increases. Our observation also supports the findings of Kolawole et al. [19]. Summarily, the observed features in our study demonstrate the extent of renal damage as a result of the toxicity level of the extract.

5. CONCLUSION

The result of this study showed that high dose of *S. latifolius* provided some protection against renal damage in diabetic rats, whereas high dose

of the plant leave might be toxic to the kidney at high dose.

DISCLAIMER

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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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