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Original Research Article

Evaluation of nephrotoxicity effects of the methanol leaf extract of *A. angustifolia* in Wistar rats

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ABSTRACT

Background: *Agave angustifolia* is a common traditional remedy in localities for disease treatment. This study focussed on evaluating the nephrotoxicity activity of the methanol extract of *A. angustifolia* leaf.
Methods: Twenty-five Wistar rats of both sex were randomly shared into five groups. Group 1 received 10 mL/kg distilled water, group 2 gentamicin 80mg/kg, extract groups (3, 4 and 5) were placed on 100 mg/kg, 200 mg/kg and 400 mg/kg of *A. angustifolia* leaf extract administered orally through an orogastric tube for 14 days. The rats were sacrificed using chloroform, and their kidneys were harvested, weighed and immediately fixed in 10% buffered formalin for histological analysis and blood samples were collected by cardiac puncture for biochemical and haematological analysis.
Results: Results showed there was no marked difference in the levels of the packed cell volume (PCV) in all the concentrations of the extract as well as the controls. The gentamicin group showed a remarkable rise in the serum urea and creatinine level when compared to both the control and extract groups. Similar effects were observed in the 100 mg/kg and 200 mg/kg of the extract. However, severe kidney injury was observed in the group treated with 400 mg/kg of the extract.
Conclusions: Despite the beneficial potential of *A. angustifolia*, it also exhibited toxic effects on the kidney thereby causing significant damage to the kidney morphology at higher doses. The damages inflicted in these tissues are dose-dependent. Therefore *A. angustifolia* should be taken in low doses within shortest period of time.

Keywords: *A. angustifolia*, Leaf extract, Nephrotoxicity, Rats

INTRODUCTION

The use of medicinal plants to treat various pathologies dates back to the time. They are an important part of our daily lives. The reasons for the usage of plants are natural, traditional, cultural, economic and other reasons. According to the World Health Organization (WHO), up to 80% of the world's population uses medicinal plants for their health care.[1] The effectiveness, availability, cost and minimization of side effects would contribute to this situation. Medicinal plants can play an important role in drug discovery and their studies are logical search

strategies in the discovery of new drugs.[2] Several studies have reported the efficacy of medicinal plants in treating various diseases.[3] However, in most circumstances the safety or otherwise of these products have often been neglected and even in cases of reported toxicity, such reports have largely been ignored and left undocumented as a result of lack of scientific data to back up such reports

Agave angustifolia, is one such plants which have been used traditionally as medicine to treat various ailments in man. It is also referred to as 'centaury plant', 'American aloe', and 'Caribbean *Agave* in English language', originated from Mexico but located in several regions of the world especially North and South America. The genus *Agave* belongs to the Agavaceae family of which 200 species have been documented; 150 (75%) are distributed in Mexico and 116 (58%) are endemic.[4,5] These agaves were a source of carbohydrates (before the corn crop was established) for western Mexico and the southwestern United States of America.[6] Furthermore, the agaves have had and continue to have great social, cultural, ecological, environmental, and medicinal importance. *A. angustifolia* is an example of this family, which has been used in various ways such as the production of alcoholic beverages such as the "bacanora" of Sonora and the "mezcal" in southern Mexico. Its leaves are used to cook roasted lamb and flowers in various dishes. In some parts, the flowers and leaves are used as fodder. Likewise, its fibers are extracted to make ropes, baskets, clothes, sandals, brushes, etc. In traditional Mexican medicine, it has been widely used by the indigenous people to heal different ailments such as dehydration, scurvy, blows, wounds, indigestion, "reumas," toothache, stings of poisonous animals, and to heal livestock diseases. In addition, it is used as a remedy for the sprains of bones of people and animals.[7] A large range of secondary metabolites have been reported from different Agave species.[7-9] Evidence of pharmacological activity has been reported for this genus, where immunomodulatory, anti-inflammatory, cytotoxic, and antiparasitic activity are some of the most important.[8-11] There is a general perception that natural products of plant origin are non-toxic and without side effects which in some cases has proven to be false.[12] The overwhelming importance attached to this plant resulting from its use in traditional medicine and as source of tea has necessitated this research into its toxicity on the kidney which has not been reported in any study previously.

METHODS

This was prospective cohort study which was conducted between December 2022 and February 2023. The study was carried out in Pharmacology Department Laboratory of University of Calabar, Calabar, Nigeria.

Collection, identification of A. angustifolia leaf

Fresh leaves of *A. angustifolia* were collected from the locality of Ikot Offiong Ambai, Akpabuyo Local Government Area of Cross River state, Nigeria. The plant was identified and authenticated by a taxonomist Mr Effa A. Effa of the Department of Botany, University of Calabar, Nigeria, where the leaf specimen was deposited in the herbarium with a voucher number [Bot/Herb/UCC/156].

Preparation of methanol extract of A. angustifolia

The fresh leaves (25kg) *A. angustifolia* were plucked from their stalk and was thoroughly washed using tap water, in order to remove sand, debris and dirty. The leaves *A. angustifolia* collected were air dried under room temperature for 2 weeks. It was grinded to fine powder with a lab manual milling machine. 400g of the ground sample were weighed with our lab manual weighing balance (Camry model: EK3132, Max: 5kg/11 1b,). The grinded fine plant material was mixed and was de-fatted with 1500ml of ethanol at 2:30pm. This solution was properly mixed by using a starring rode to stir it and was allowed to 48 hours, after which it was filtered using Whitman No. 1 filter paper to harvest the filtrate on a tray pan. Our filtrated got dried within 24 hours weighed about 5.6g. We reweighed the dry residue and it weighed about 350g before re-sucked into a 99% methanol for 72 hours a round bottom flask, after which it was filtered using a Whitman No: 1 filtered paper to harvest filtrate. The filtrate was poured on a tray pan, and was allowed to evaporate to dryness for about 48 hours, dried methanol extract of *A. angustifolia* weighed about 25g. The AAE extract was stored in transparent specimen bottle.

Animals for the study

This prospective cohort study which was conducted between December 2022, and February, 2023, involved the use of adult Wistar rats of both male and female with body weight range of 200 g obtained from the animal breeding unit, Department of Pharmacology, Faculty of Basic Medicals Sciences, University of Calabar, Calabar. The rats were kept in a plastic cage for acclimatization in the department for seven days. The animals were fed with standard diet and have free access to water at libitum. The rats were maintained under standard conditions of humidity, temperature and 12hrs light/12hrs darkness cycles. The rats were marked for identification using markers, denoted with five different marker colours on their tails. A standard protocol was drawn up in accordance with the Good Laboratory Practice (GLP) of University of Calabar. The rats were used in accordance with the National Institute of Health (NIH) guide for the care and use of laboratory animals (National Research Council, 1985).[13] An ethical approval was obtained from the ethical committee from the faculty of basic medical science, university of Calabaar, Calabar with an issued number: 213PHA1023.

Acute toxicity testing

The acute toxicity (LD_{50}) study was determined in rats, on the methanol extract of *A. angustifolia*, using Locke's

method with slightly adjustments.[14] A total of 9 Wistar rats of either sex were fasted overnight prior to the study. In phase one, 9 Wistar rats were randomized into 3 groups of 3 per cage. Groups 1, 2, and 3 rats received 10 mg/kg, 100 mg/kg, 1,000 mg/kg of the extract administered orally. The animals were observed for signs of toxicity which includes; paw licking, weakness, feeling sleepy, respiratory distress, hyperactivity, coma and death for the first 4 hours, and subsequently 24 hours. Since no signs of toxicity were observed, the second phase was initiated. In this phase, 4 rats were also grouped into 4 with one rat per cage. Doses was selected based on the results of the phase 1 since no death occurred, higher doses were selected and administered; 1600 mg/kg, 2900 mg/kg, 5000 mg/kg and 10 ml/kg distilled water of the extract. The animals were observed for signs of toxicity and mortality for 48 hours and thereafter 72 hours for late toxicity.

Experimental design

The nephrotoxicity study was done by the experimental protocols described by OECD Guideline for animal toxicity studies. Test No. 423 with slight modifications.[15] Briefly, 25 rats were grouped into 5 group of 5 rats each. And extract administration dose was calculated as: Dosage = Average weight in each group (g)/1000 x Standard Dose.

Group-1 received 10 mL/kg Normal Saline. Group-2 received 080mg/kg Gentamicin (Refers to standard or positive control). Group-3 received 100 mg/kg of methanol extract of *A. angustifolia*. Group-4 received 200 mg/kg of methanol extract of *A. angustifolia*. Group-5 received 400 mg/kg of methanol extract of *A. angustifolia*.

The extract administrations lasted from day 1-14 days for rats in group 1 which received normal saline and rats in groups 3-5 which received *A. angustifolia* extract. While rats in group 2 received gentamicin (80 mg/kg) to induce nephrotoxicity from day 7-14 day of the study. The rats were closely observed for general and behavioural signs & symptom of toxicity, body weight changes and mortality during the entire period of the experiment. Thereafter, all animals were sacrificed under ether anaesthesia and blood collected through cardiac puncture.

Collection of blood, tissue and preparing of serum

The night after last day of *A. angustifolia* leaf extract administration, rats from each group were weighed and fasted overnight, and their body weight was measured and recorded. The rats were sacrificed through cervical dislocation under ether anaesthesia and blood samples were collected for both biochemical and haematological analysis.

Determination of body weight changes and relative organ weight

The rats weighed before the start of the experiment and also weighed every three days. The recorded changes were used as parameters to determine the changes in body weighed resulting from the use of the extract. The rats were also weighed on the last day of the experiment just before the sacrifice and their weighed recorded, after sacrifice the kidneys of the rats were harvested and weighed. The weight of the kidneys were used to calculate the relative organ weight of the kidney thus:

Relative organ weight = (weight of kidneys/weight of the entire rat) x 100

Determination of biochemical and haematological parameters

The blood was collected into two different sample bottles for each group of rats; one of the sample bottles contains EDTA and was used to the determination of haematological parameters like white blood cell count (WBC) and its differentials as well as haemoglobin levels. The other sample bottle had no EDTA and was used for the determination of biochemical parameters like the urea and creatinine levels as well as other kidney function parameters.

Histopathology studies

The kidneys were harvested, weighed and fixed in 10% formalin solution. The kidney morphology was examined using Haematoxylin and Eosin staining techniques. Thereafter, microscopic slides were photographed and interpreted.

Statistical analysis

The results were presented as a mean ± standard error mean (SEM) comparison of means, caring out by one-way analysis of variance ANOVA. The level of acceptable error will be set at a P value of <0.05 and value less than this will be considered as statistical significance. The (SPSS) version 16 statistical software was used for all statistical analysis.

RESULTS

Acute toxicity tests

The results of the acute toxicity test for the methanol extract leaf extract of *A. angustifolia* showed that the extract has an LD_{50} value of 1,264.91mg/kg/body weight in rats.

The results of the effects of the methanol leaf extract of A. angustifolia on the weight of rats

The result of the change of weight is the percentage of the difference in weight recorded between the days of the experiment (Table). From day one to day 14 there were variable changes in the weight of the rats. These changes were recorded every 3 days until the last day of the experiment. The control group was seen to have gained

weight up till the seventh day and continued on days 12 and 14. Also, the positive control group treated with gentamicin was observed to gain weight up till day 7 and then continued on days 12 and 14. The group treated with 100 mg/kg of extract witnessed weight loss for the first 7 days. The loss was significant when compared to control groups (p<0.05). The 200 mg/kg and 400 mg/kg of extract groups also recorded weight loss however, the loss was not significant when compared to the control groups (p<0.05).

Table 1: The effect of methanol leaf extract of *A. angustifolia* on the body weight of rats.

Groups	Day 1	Day 4	Day 7	Day 10	Day 12	Day 14
1 (normal saline)	179.8±10.1	203.2±6.74	200.27±8.82	195.1±12.6	201.17±5.5	207.98±7.24
2 (80mg/kg gentamicin)	98.08±18.1	111.68±8.2	111.18±9.6	117.88±6.11	149.08±8.10	165.18±2.03
3 (500mg/kg extract)	197.68±3.3	153.48±11.89**†	156.44±12.8*†	157.88±4.8	149.8±2.7*†	165.18±13.0**
4 (200mg/kg extract)	133.95±7.4	172.75±2.0*	166.45±10.1*	173.25±18.2*	158.38±3.8*	179.75±17.1*
5 (100mg/kg extract)	230.9±6.33	230.45±7.0*†	228.35±15.1*†	227.65±2.3†	223.5±2.4*†	225.25±6.4*†

The values represent mean SEM., n=5; *Significantly different from normal control p<0.05; **Significantly different from normal control p<0.01; †Significantly different from positive control

The results of the effects of methanol leaf extract of A. angustifolia on relative organ weight

The results of the relative organ weight of the kidney (Figure 1) shows that the extract did not produce a significant change in the kidney in relation to the weight of the body (p<0.05).

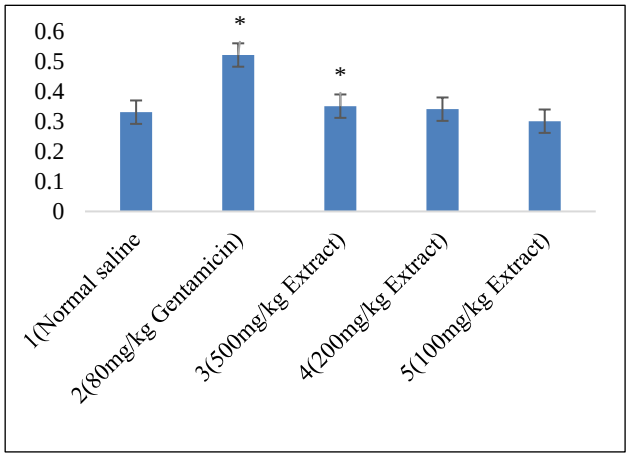

Figure 1: The effect of methanol extract of *A. angustifolia* on the relative kidney weight of rats.
The values represent mean SEM., n=5; *Significantly different from normal control p<0.05; **Significantly different from normal control p<0.01

Although, the result not significant when compared to the normal control group, there was a steady increase in the values of the relative organ weight as the dose increased. However, the positive control group treated with gentamicin showed significant increase in the value of the relative organ weight of the rats when compared to both normal control group and the groups treated with different concentration of the extract.

The results of the effect of the methanol leaf extract of A. angustifolia on the haematological indices

The effect on haematological parameters of methanol leaf extract of *A. angustifolia* was carried out to determine the activity of the extract on blood indices. Parameters tested for includes; packed cell volume (PCV), total white blood cell count (WBC), neutrophils, lymphocytes, monocytes and eosinophils count. The results (Table 3) show that the group treated with gentamicin had the lowest amount of PCV but values were not significant when compared to the normal control group (p<0.05. The positive control group also recorded a significant reduction in the levels of WBC and neutrophils (p<0.05). Furthermore, the highest concentration of the extract (400 mg/kg) recorded a significantly elevated levels of PCV and neutrophil when compared to the control groups (p<0.05). However, there was no other observed significant changes in the haematological parameters under investigated (Table 2).

Table 2: The effects of methanol extract of *A. angustifolia* on the haematological parameters of Wistar rats.

Groups	PCV (%)	WBC (10/L)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosophil (%)	Basophils (%)
1 (normal saline)	42±2.0	8.3±1.2	37±4.5	59±7.3	2±0	2±1	0±0
2 (80mg/kg gentamicin)	38±3.8	3.6±0.5*	35±6.2*	62±2.9	1±0	2±0	0±0
3 (500mg/kg extract)	47±1.6*	4.5±0.9	63±3.9*	37±3.5	0.5±0.5	0.2±0.3	0±0
4 (200mg/kg extract)	39±2.2	8.0±2.1	37±1.8	59±6.1	2±1	2±1	0±0
5 (100mg/kg extract)	43±2.7	4.7±1.3	52±9.1	46±3.2	1±0.5	1±1	0±0

The values represent mean SEM., n=5; *Significantly different from normal control p<0.05; **Significantly different from normal control p<0.01

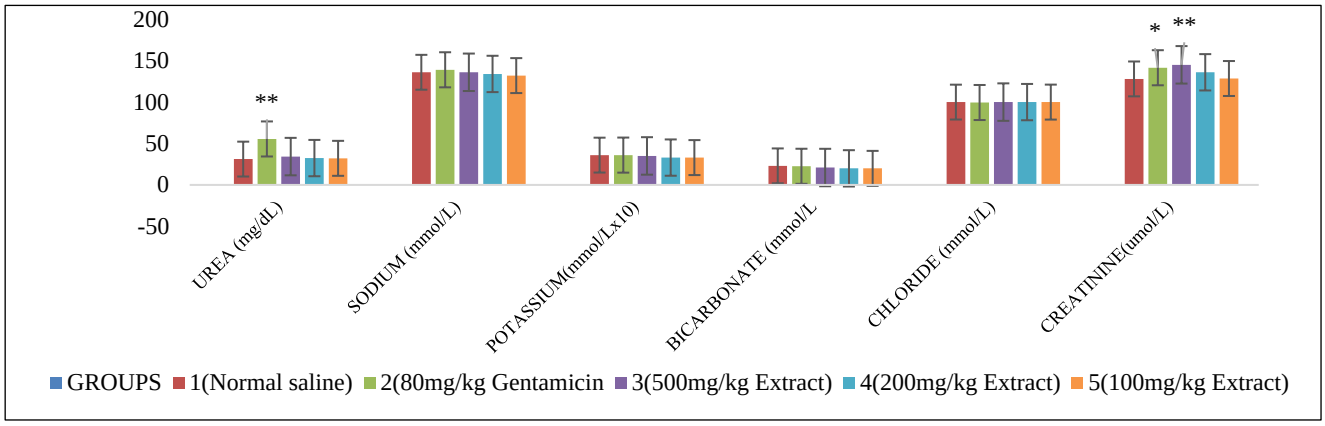

Figure 2: Effects of methanol leaf extract of *A. angustifolia* on kidney function parameters of Wistar rats.
The values represent mean SEM., n=5; *Significantly different from normal control $p<0.05$;**Significantly different from normal control $p<0.01$

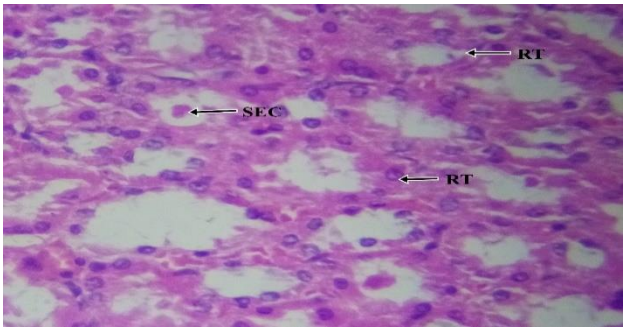

Figure 3: Kidney function of rats on normal control.
Group 1 (Normal saline) Kidney, Medullary Area X400- Section of the kidney shows prominent glomeruli and renal tubules of various sizes and shape. The glomeruli appear swollen with a cellular mesangial matrix and congested arterioles. The bowman spaces are distinct and narrowed and the renal tubules are lined by cuboidal epithelial cells with an empty luminal cavity. The interstium is compact consisting of congested blood vessel. No features suggestive of nephrotoxicity.

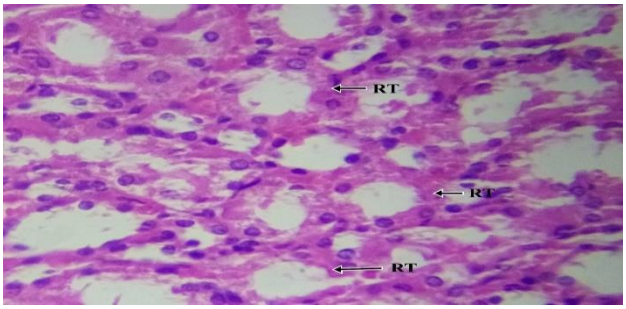

Figure 4: Mild cellular injury of rats induced with gentamycin.
Group 2 (80 mg/kg of Gentamicin) Kidney, Medullary Area X400- Section of the kidney shows prominent glomeruli and renal tubules of various sizes and shape. The glomeruli appear mildly swollen with a hypercellular mesangial matrix and mildly congested arterioles. The bowman spaces are distinct and narrowed and the renal tubules are lined by cuboidal epithelial cells with an empty luminal cavity. The interstium is scanty and consists of stroma cells and thickened wall blood vessels. Features suggestive of mild cellular injury.

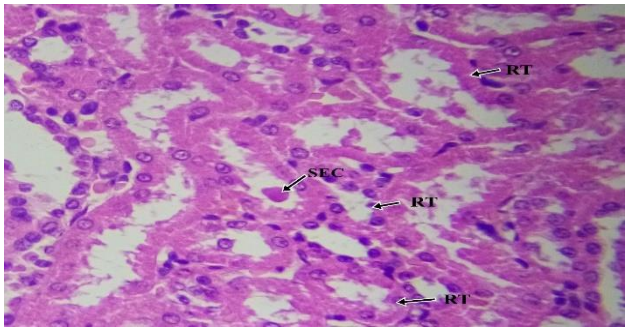

Figure 5: Mild response to cellular injury effects of *A. angustifolia* on kidney function in rats.
Group 3 (400 mg/kg of A. angustifolia extract) Kidney Medullary Area X400- Section of the kidney shows prominent glomeruli and renal tubules of various sizes and shape. The glomeruli appear enlarged with a hypercellular mesangial matrix and congested arterioles. The bowman spaces are distinct and narrowed and the renal tubules are lined by cuboidal epithelial cells with an empty luminal cavity. The intervening interstium is scanty. Features suggestive of mild response to cellular injury.

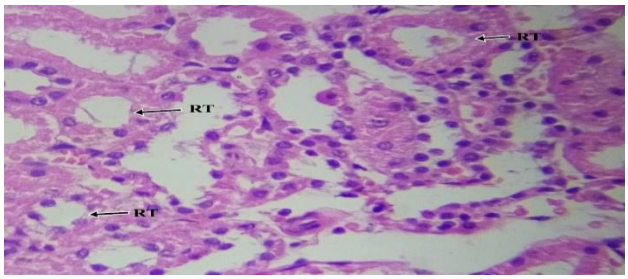

Figure 6: Mild glomerular injury effects of *A. angustifolia* on kidney function in rats.
Group 4 (200 mg/kg of *A. angustifolia* extract) Kidney Medullary Area X400- Section of the kidney shows prominent glomeruli and renal tubules of various sizes and shape. The glomeruli appears swollen with a hypercellular mesangial matrix and congested arterioles. There is mild focal sclerosis. The bowman spaces are distinct but narrowed. The renal tubules are lined by cuboidal epithelial cells with an empty luminal cavity. The intervening interstium is compact. Features suggestive of mild glomerular injury.

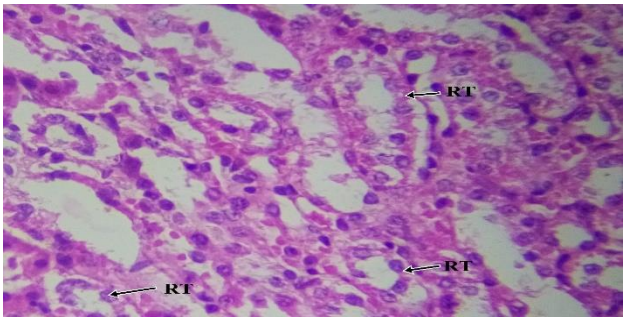

Figure 7: Glomerular injury effects of *A. angustifolia* on kidney function in rats.

Group 5 (100 mg/kg of *A. angustifolia)* Kidney Medullary Area X400- Section of the kidney shows atrophic prominent glomeruli with a dilated bowman capsules and contracted mesengial matrix. The mesangium appears cellular. The renal tubules of various sizes and shape. Some of the tubules are lined by tall columnar cells with distinct nuclei and some display poorly delinated outline. The intervening stroma is compact. Finding suggestive of glomeruli injury.

The effects of the methanol leaf extract of A. angustifolia on kidney function parameters

Biochemical analysis was carried to ascertain effect of the extract on serum electrolytes (sodium, potassium, bicarbonate and chloride), urea and creatinine. The results (Figure 2) showed that there was no significant difference in the values of serum electrolytes in all the concentrations of the extract as well as in the negative and positive controls ($p<0.05$).

Also, all the concentrations of the extract tested in this study showed no significant change in the serum levels of urea when compared to the negative control treated with normal saline ($p<0.05$). However, the positive control group showed a significant increase in the level of serum urea when statistically compared to normal control and the various concentrations of the extract. Also, the highest concentration of the extract (400 mg/kg) showed similar elevated values of serum creatinine with the group treated with gentamicin and these values were significant when compared to the normal control ($p<0.05$). Again, the rise in the serum creatinine levels effected by the methanol extract of *A. angustifolia* was shown to be dose-dependent amongst all the concentrations used in the experiment.

The effects of the methanol extract of A. angustifolia on the histology of the kidney

Sections of the kidney was prepared for histopathological analysis under the light microscope. The results showed that the normal control group treated with normal saline (Plate 1) showed no sign of nephrotoxicity. The positive control group treated with gentamicin (Plate 2) showed features swollen glomeruli, mildly congested arterioles and thickened wall blood vessels which were features suggestive of cellular injury. The groups treated with 100 mg/kg and 200 mg/kg (Plate 3 and 4) showed features of glomeruli injury while the highest concentration of the extract (400 mg/kg) (Plate 5) showed features similar to the group treated with gentamicin, features suggestive of mild response to cellular injury.

DISCUSSION

This project work was tailored to the evaluation of the nephrotoxicity potential of the methanol extract of *A. angustifolia* in Wistar rats. Nephrotoxicity focuses on the deterioration in the function of the kidney resulting from toxic effect of drugs and other chemicals.[16] There are different mechanisms through which these nephrotoxins exert toxicity to the kidney which ranges from thrombotic micropathy, crystal nephropathy, inflammation, glomerular damage and renal tubular toxicity.[17] It is common knowledge that herbal medicine and their formulations are generally considered safe mostly as a result of their "negligible" side effects. This has resulted in indiscriminate use of these herbal formulations especially amongst rural populace. These formulations are used over a long period of time with indiscriminate dosage without proper monitoring by experts on the toxic consequences that might arise from such usage.[18] Therefore, for proper guidance on usage and accurate dosing regimen, scientific knowledge towards oral toxicity is imperative. Novel medicinal plants like *A. angustifolia* no matter how beneficial they may prove to be are subjected to toxicity studies and one of the key organs in toxicity testing is the kidney because it is a continuing concern during drug development. Immediately an individual is diagnosed with acute kidney injury after exposure to toxic chemicals or medications, it means that rapid loss of renal function and the precursor of acute renal failure and sometimes chronic kidney disease that may require dialysis or kidney transplant. This again makes it important and critical to determine potential kidney toxicity of new drugs or medicinal plant before talking about clinical trial.

The evaluation of toxicity potentials of medicinal plant is usually a preliminary step towards the determination of its pharmacological activity. Along with several other processes in the toxicity testing, the determination of LD_{50} is usually the beginning step.[19] In line with this protocol, the LD_{50} of the methanol leaf extract of *A. angustifolia* on Wistar was investigated.

According to United States Food and Drug Administration (FDA), a number of parameters are used to assess renal function and damage in clinical safety assessment studies such as this. The first is the physical examination and behaviour which includes changes in weight and signs of dehydration. The second factor to look out for is the haematology; checking the changes in packed cell volume (PCV) as indicators of change in dehydration and for longer duration exposure, progressive non-regenerative anaemia is noted as well as changes in shape and size of red blood cell. The third factor of testing nephrotoxicity is the serum chemistries which put into consideration changes in creatinine, potassium, phosphorous, calcium and their ratios indicating glomerular injury leading to a

nephrotic stage. These parameters were measured in this study in the determination of the toxic potential of the methanol leaf extract of *A. angustifolia*. First, the rate of change in body weight of rats was recorded throughout the duration of the experiment. The weights of the rats were recorded at 3-day interval (days 1, 4, 7, 10, 12 and 14). The group treated with normal saline recorded steady growth in the 14-day experiment. For the positive control, gentamicin was the drug of choice to compare the potential damage of the extract to that of gentamicin as the nephrotoxicity effect of gentamicin has since been established.[20] Surprisingly, the gentamicin treated group also witnessed increase in body weight throughout the duration of the experiment, this may not be unconnected with the weight increase observed in the organs. In the highest concentration of the extract (400 mg/kg) there was a reduction in the weight of rats in all the time interval. In comparison with the negative control (Normal saline), while the rats had a weight increase of 11.48% on the fourth day of the experiment in the control, there was a weight reduction of 0.19% in the highest dose of the extract. Furthermore, on the fourteenth day of the experiment, the group treated with normal saline recorded 13.52% increase in weight while the group treated with 500mg/kg of extract showed a 2.51 decrease in rat body weight. However, the highest reduction in weight of the rat was recorded in the group treated with 100mg/kg of extract which had a weight reduction range of 19.68% to 31.98% in all the intervals of the experiment. To buttress the implication of this weight loss, Wang et al, highlighted body weight changes as a major indicator of the deleterious effects of substances with toxic potentials and the baseline was kept at 10%.[21] This therefore suggests the extract of exhibited a level of toxicity based on the body weight changes.

The second parameter in accordance with the FDA recommendations is the haematology. The haematological indices of the animal were investigated which included the white blood cell count (WBC) and further differentials of percentage neutrophils, lymphocytes, monocytes, eosinophils and basophils while blood was also analysed for packed cell volume which represents the percentage of red blood volume of the whole volume of blood in animal and it is clinically used to signal known or suspected anaemia.[22] Unusual reduction in the PCV levels is an indication of anaemia and it is a resultant effects of damages to the myeloid tissues by toxic substances.[23] However, in this research work, there was no marked difference in the levels of the packed cell volume in all the concentrations of the extract as well as the controls.

The third parameter is the blood chemistries and in this study, serum levels of urea, creatinine and the electrolytes (sodium, potassium, bicarbonate, and chloride) were analysed using specific blood analyser. The group two (gentamicin) showed a remarkable rise in the serum urea and creatinine level when compared to both the control and extract groups. This is a further proof that gentamicin nephrotoxicity is characterized by the increase in serum level of urea and creatinine and reduction in glomerular filtration rate as reported by Afshin et al.[24] However, there was no significant change in the levels of sodium, potassium, bicarbonate and chloride.

Finally, the histopathological analysis which is referred to as gold standard was carried out the result showed sign of nephrotoxicity as the interstium was seen to be compact and consisting of congested blood vessel in the group treated with normal saline. Here again, the nephrotoxicity activity of gentamicin was confirmed as its effect on the glomeruli was well elucidated. It caused the swelling of the glomerulus with a hyper-cellular mesangial matrix and mildly congested arterioles. Similar effects were observed in the 100mg/kg and 200mg/kg of the extract. However, severe kidney injury was observed in the group treated with the highest concentration of the extract (400 mg/kg). The reason for the nephrotoxicity of the extract is not exactly known but studies have suggested that plants with high concentration of triterpenoid, despite their useful medicinal potential may exhibit a certain level of nephrotoxicity.[25] This finding was later confirmed by Olubodun et al, who working on the toxicity of a plant rich in triterpenoids (*Scoparia dulcis)* reported its nephrotoxicity marked by the elevation of serum levels of urea, creatinine and uric acid as well as histological interpretations.[26]

CONCLUSION

The study on the evaluation of the nephrotoxicity potential of the methanol leaf extract of *A. angustifolia* was done to among other things investigate the toxicity of the extract which is commonly used in the locality for its efficacy in the treatment of many disease conditions including bacterial infections. To compare this toxicity to already established nephrotoxins, 80mg/kg of gentamicin was used as the positive control. The result of the extract on the weight of the rats, the percentage organ weight as well as kidney function parameters all pointed to the toxicity effects of the extract. This toxicity effect was further confirmed by the evidence of the histological analysis which clearly shows that the extract exhibited toxic effects on the kidney. Therefore, it can be said that the methanol leaf extract of *A. angustifolia* despite its benefits exhibited toxic effects on the kidney and care must be taken in the administration of the extract especially for medicinal purposes.

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