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The Effect of *Aloe Vera* on the Kidney Function of Male Wistar Rat

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Abstract

Acute kidney injury is a sudden loss of kidney function which manifests within a few hours to few days and remains associated with high morbidity and motality in the society, despite the advances in the field of medicine. Aloe vera is taken by many orally as it is medically important in the areas of skin treatments, digestive health, improvement of blood sugar and many more. The present study shows the effect of oral intake of different doses of Aloe vera on the kidney. Twenty (20) male albino Rats were randomly grouped in to five (5: Groups A-D and received the following treatments for two (2) weeks; Rats in group A (control group) no Aloe vera gel was administered, group B received high dose of Aloe vera gel (6ml/kg), group C received a medium dose (4.5ml/kg) and group D received a low dose (3ml/kg).Renal injury was assessed by measuring Serum creatinine (Jaffe-slot method), BUN (Diacetyl Monoxime method) Na^+ and K^+ (Perlong Medical PL1000A Electrolyte Analyser). Comparing the result with the normal control BUN (19.42± 1.21mg/dl), Creatinine (0.76 ± 0.32 mg/dl), K^+ (5.24 ± 0.28 mg/dl) and Na^+ (138.12 ± 1.09 mg/dl). Oral administration of high dose of Aloe vera showed significantly increase in concentration of BUN (22.25± 1.07 mg/dl), Creatinine (1.01± 0.13 mg/dl), K^+ (7.83 ± 0.84 mg/dl) and reduction in Na^+ (132.07 ± 0.36 mg/dl), from the low dose group the biochemical parameters tends to occur at the normal range with the Control group BUN (20.12 ± 1.72 mg/dl), Creatinine (1.51 ± 0.08 mg/dl), K^+ (6.94 ± 0.05 mg/dl) and a slight increase in concentration of Na^+ (133.49 ± 0.41 mg/dl). Histopathological studies also support the biochemical observations as the the kidney sections of Groups A and D showed normal Glomeruli and tubules, while that in Groups B and C showed eroded Glomeruli and sloughed off epithelia and eosinophilic casts in the tubules. This study reveals that Aloe vera possess a dose-dependent nephrotoxicity.

Keywords: Aloe Vera, Phytochemicals, Nephrotoxicity, Kidney Function Test

Introduction

Aloe Vera (*Aloe barbadensis miller*) is derived from the Arabic word Alloeh, meaning shining bitter substance, and a Latin word Vera which means True. Aloe Vera belongs to the kingdom Plantae, Family of Asphodelaceae (Liliaceae), it grows mainly in dry regions with a characteristic shrubby, perennial and Xerophytic nature [1].

The plant has triangular fleshy leaves with serrated edges, yellow tubular flowers and fruits that contain numerous seeds. Each leaf is composed of three layers, the first layer which is an inner clear gel that contains 99% water, and the remaining 1% constitutes of glucomannans, amino acids, lipids, steroid and vitamins, the second layer is the middle layer made up of Latex, which is a bitter yellow sap, and constitutes of anthraquinones and glycosides, the third layer is the outer thick part of 15-20 cells, this has a protective function and synthesizes carbohydrates and proteins, there are also vascular bundles in this region which are responsible for the transportation of substances such as water (xylem) and starch (phloem) [1].

Studies have shown that Aloe Vera has medicinal properties and is one of the most powerful and well known medicinal plants [2]. It potentially consist of 75 active constituents [3] which includes; **Sugars** (glucose, fructose, glucomannans/polymannose, all are derived from the mucilage layer of the plant known as Mucopolysaccharide, the most prominent monosaccharide is the mannose-6-phospate and the prominent polysaccharide is the glucomannans [beta (1,4)-acetylated mannan]), **Anthroquinones** which are phenolic compounds known traditionally as laxatives, **Fatty acids** like cholesterol, campesterol, *B*-sisosterol and Lupeol, these exhibits anti-inflammatory, antiseptic and analgesic properties. There are also **Hormones** which includes auxins and gibberellins which helps in healing wound, Aloe Vera also provides 20 out of the 22 required amino acids, 7 of the 8 essential amino acids, salicylic acid and Ligin, **Vitamins** (Vit A, C and E which are antioxidants and Vit B12, folic acids and Choline neutralizes free radicals), **Enzymes** (Aliiase, amylase, bradykinase, Carboxypeptidase, which aids in reduction of excessive inflammation when applied to the skin and equally breaks down sugar and fats when taken orally),**Minerals** (calcium, chromium, selenium, magnesium, manganese, sodium and zinc which are essential for the proper functioning of various enzyme system in different metabolic pathways).

Research also indicates that Aloe Vera is applied in the treatment of constipation, regulates cancer cell apoptosis and helps in the treatment of malignant cancer cells [4]. It has impacted so much in life as it is widely used in cosmetics to treat burns, wounds, sun burns and in cell aging, In medication it is used to reinforce immune system, cleans the heart, improve blood circulation, reduces high blood pressure, reduces weight, increases white blood cell count and improves erythropoiesis. On prolonged usage at high dosage it might lead to Diarrhea, Hepatotoxicity (the impairment of the liver function due to exposure to xenobiotics such as drugs, chemicals and food substances) and Nephrotoxicity (the rapid deterioration in the Kidney function due to toxic effects of some chemicals, additives and some medicinal plants. The likely effect of high intake of Aloe Vera gel on kidney function cannot be over emphasized as kidney is a vital organ and plays an important role in the body.

The kidneys are bean shaped organs on either side of the spine, about 4-5 inches long, they are responsible in filtering the blood, removal of waste products and drugs from the body and releases hormones that regulate blood pressure. When there's decreased blood flow to the kidney, inflammation or excessive exposure to toxins an acute kidney injury (AKI) develops [5] these harmful substances include some drugs such as antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs) which weakens the kidney function, and excessive intake of some medicinal plants as some herbs possesses nephroprotective properties such as *Vitex negundo* Linn*, Oroxylum indicum vint*, Ginger and *Barringtonia acutanggula* Linn [6]. Aloe Vera as a medicinal plant might exert injury to the kidney there by affecting the kidney function if taken at high dose for a long period of time.

The high consumption of Aloe Vera as a beverage and as local medication recently is on the increase, and is not supported by clinical studies as some medicinal plants possesses nephrotoxicity properties which leads to acute kidney injury, a potential factor in morbidity and mortality in the world today.

Although Aloe Vera is a medicinal plant recent research has shown that it has a nephrotoxicity property which might affect the kidney, therefore we propose that the intake of high dose of Aloe Vera gel might affect the kidney functions. The aim of this study is to investigate the effects of Aloe Vera gel on kidney function of male Wistar Rat.

Materials and Methods

Plant Materials

The fresh Aloe vera (*Aloe barbadensis* miller) used for the study was obtained from Ogbete market, a local market in Enugu, Enugu state, Nigeria. The plant material was authenticated by a consultant taxonomist at the herbarium section of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Reagents

Reagent(s): Perlong medical Analyser PL1000A electrolyte Analyser, Diacetyl monoxime, strong H_2SO_4, Sodium tungstate, Picric acid, all the reagents were purchased at a standard Science Store located at Ogbete main market Enugu state.

Aloe vera juice extraction

Fresh *Aloe vera* plants were washed, with the aid of a sharp knife, the bottom scarred end and thorny sides were cut off to ensure that the gel is carefully separated from the leave. About 20g of the gel is homogenized in a blender (Qasa blender made in Nigeria). It is blended, until it is frothy and liquefied.The resultant Aloe vera juice (AVJ) is stored at 4^{o}C in the refrigerator.

Animals

A total of twenty (20) adult albino rats, weighing 120-180g were obtained from the animal house of the College of Veterinary Medicine, University of Nigeria. The animals were housed in metallic under standard conditions of temperature (22 ± 3^{o}C) and a 12 h light, 12 h dark cycle. The animals were kept under observation for about 14 days before the onset of the experiment for acclimatization. Experimental protocol and handling was according to Institutional guidelines describing the use of rats and in accordance with the American Physiological Society guiding principles for research involving animals and human beings.

Phytochemical analysis of the *Aloe vera*

Preliminary phytochemical screening of the *Aloe vera* Leaf for the presence of glycosides, flavonoids, saponins,

steroids, tannins, carbohydrates, proteins and terpenoids was carried out at Department of Pharmacognosy, Faculty of Pharmaceutical Science, University of Nigeria Nsukka. Methods according to Trease and Evans were used for the analyses [23].

Experimental Design

The twenty (20) albino rats were grouped into grouped into (A-D) of five rats each and they received the following treatments which lasted for 21 days:

Group A: (Normal Control): No treatment was administered to this group.

Group B: received oral administration of High dose of AVJ (6 ml/kg)

Group C: received oral administration of medium dose of AVJ (4.5 ml/kg)

Group D: received oral administration of low dose of AVJ (3 ml/kg).

Sacrificing of Animals and Sample Collection

Blood samples for the determination of serum analyses of Serum creatinine, Glomerula filteration rate and Blood urea nitrogen were taken by cardiac puncture of the left ventricle of the heart under chloroform anesthesia and the Kidneys were harvested for histopathological analyses.

Biochemical analysis

The levels of serum electrolyte, urea and Creatinine were estimated.

Measurement of Serum Electroyte

Method: Perlong Medical PL1000A Electrolyte Analyser

The Electrolyte analyser applies the principle of ion-selective elecytrode [24], which gives the instrument a stable and reliable measurement. It measures the ion concentration of K^+, Na^+, Cl^-, HCO^3 and pH values in whole blood, serum and urine samples.

Procedure

90ul of sample was fed into the machine through the sample niddle.

50-60 seconds later, the results were ready and printed through a built in printer.

Measurement of Blood Urea Nitrogen

Method: Diacetyl Monoxime (DAM) method

Principle

Urea is heated with diacetyl monoxime in the presence of a strong acid and an oxidizing agent to form a yellow diazine derivative. Thiosemicarbazide is added to the mixture to enhance and stabilize the color [25].

Procedure

1. A protein-free filtrate was prepared by mixing 1.0ml of blood, 7.0ml of distilled water and was mixed. 1.0ml of sodium tungstate and 1.0ml of $^2/_3$ N H_2SO_4, were added, mixed, allowed to cool and was filtered.
2. 3 test tubes were set up
3. 5.0ml of color reagents were added to 0.5ml of the filtrate.
4. For the standard, 5.0ml of colored reagent was added to 0.5ml of urea standard
5. Then, for the blank, 5.0ml of color was added to 0.5ml of water.
6. All the test tubes were heated in a boiling water bath for 15minutes, cooled and absorbed.

Calculation

Urea concentration = $A_{sample}/A_{standard}$ X Standard conc. (mmol/l)

Calculation of Blood Urea Nitrogen (BUN):

BUN = Urea/2.14

Measurement of Serum Creatinine

Method: Jaffe-slot Method

Principe

Creatinine in an alkaline solution reacts with picric acid to form an orange coloured complex read at 490nm. The amount of the complex formed is directly proportional to the creatinine concentration [26].

Procedure

1. Two set of tubes were required
2. 2.0ml of working reagent was added to 0.2ml of sample.
3. 2.0ml of working reagent was added to 0.2ml of standard.
4. Mixed and left at room temperature for 90minutes.
5. The absorbance of the first set of tubes was read at absorbance of 490nm
6. 50ul 60% v/v acetic acid, was added to all tubes, left at room temperature for 8 minutes and absorbance read at 490nm.

Calculation

A2 - A1 = ΔA

Serum creatinine conc.µmol/l = $\Delta A_{sample}/\Delta A$ X standard conc.

Histopathological analysis

The excised kidneys were processed using the paraffin wax embedding technique, sectioned at 5 microns and stained using the Haematoxylin and Eosin [H and E] staining procedure. The histological sections were examined using an Olympus TM light microscope.

Statistical analysis

Data analysis was done using GraphPad prism version 7.0 (GraphPad, San Diego, CA, USA). The results of the biochemical assays were reported as mean±SEM(standard error of mean). The level of significance was tested using one-way analysis of variance (ANOVA), followed by the Tukey post hoc analysis. Probability levels less than 0.05 ($p<0.05$) was considered significant.

Results

Biochemical Results

The function of the kidneys was assessed by evaluating the biochemical markers at different levels in the blood, such as

creatinine, blood urea nitrogen (BUN), potassium (K^+), and sodium (Na^+) (Table 1). From the results, a statistically significant ($P<0.05$) elevated levels of blood urea nitrogen (BUN), creatinine and potassium (K^+) were seen in group B (AVG 6ml/kg, high dose) and group C (AVG 4.5ml/kg, medium dose) separately, when compared with group A (normal control). Furthermore, we observed that the *Aloe vera* gel (3ml/kg, low dose) did not pose any renotoxic effects in the animals (Table 1).

Histopathological Results

In Figure 1(group A), The glomeruli (G) and tubules appear normal. The kidney section from rat administered with *Aloe vera* gel (group B) (6ml/kg, high dose) showed that the glomeruli (G) appear eroded while the tubules showed sloughed off epithelia and eosinophilic casts (Figure 2). In *Aloe vera* gel (4.5ml/kg, medium dose) treated group (group C), it was observed that The glomeruli (G) appear mildly constricted while the tubules (arrows) appear normal with some tubules having intraluminal eosinophilic casts (Figure 3). In *Aloe vera* gel (3ml/kg, low dose) treated group (group D), it was observed that the glomeruli (G) and tubules (arrows) appear normal with some tubules having intraluminal eosinophilic casts. The histopathological findings were in tandem with the biochemical results as we observed that *Aloe vera* gel showed dose-dependent toxicity.

Table 1: Statistical Comparison of biochemical concentrations of the kidneys of treated groups with negative controls Groups after *Aloe vera* gel administration

Group	BUN (mg/dl)	Creatinine (mg/dl)	K^+ (mmol/l)	Na^+ (mmol/l)
A: Normal Control	19.42± 1.21	0.76 ± 0.32	5.24 ± 0.28	138.12 ± 1.09
B: AVG (6ml/kg)	22.25± 1.07*	1.01± 0.13*	7.83 ± 0.84*	132.07 ± 0.36*
C: AVG (4.5ml/kg)	21.46 ± 3.53*	0.91± 0.29*	6.05 ± 1.87	137.28 ± 3.14
D: AVG (3ml/kg)	20.12 ± 1.72	1.51 ± 0.08	6.94 ± 0.05	133.49 ± 0.41

Values given as Mean ± SEM. **p<0.01 or *p<0.05 is significant when normal control is compared with all other groups (*Aloe vera* gel- administered groups).

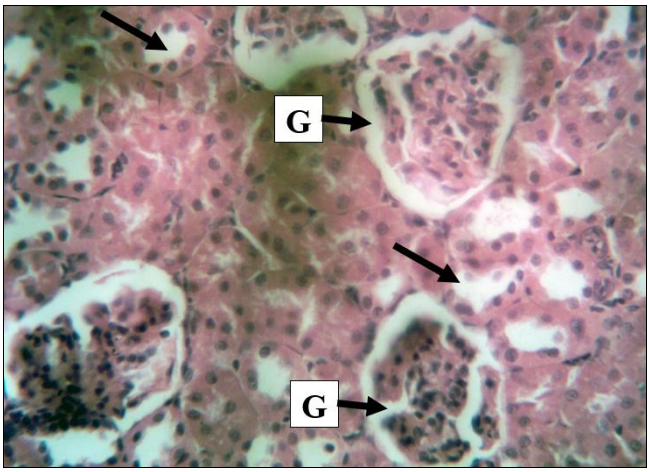

Fig 1: Representative micrograph of kidney of animals in group A. The glomeruli (G) and tubules (arrows) appear normal. **Stain**: Haematoxylin and Eosin. **Magnification**: X400.

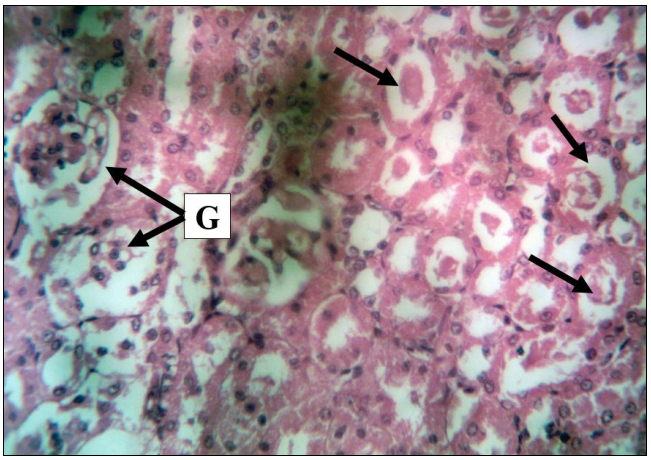

Fig 2: Representative micrograph of kidney of animals in group B. The glomeruli (G) appear eroded while the tubules (arrows) showed sloughed off epithelia and eosinophilic casts. **Stain**: Haematoxylin and Eosin. **Magnification**: X400.

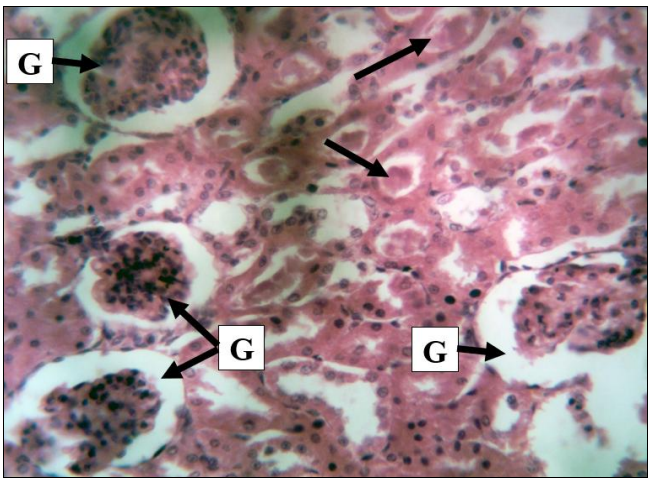

Fig 3: Representative micrograph of kidney of animals in group C. The glomeruli (G) appear mildly constricted while the tubules (arrows) appear normal with some tubules having intraluminal eosinophilic casts. **Stain**: Haematoxylin and Eosin. **Magnification**: X400.

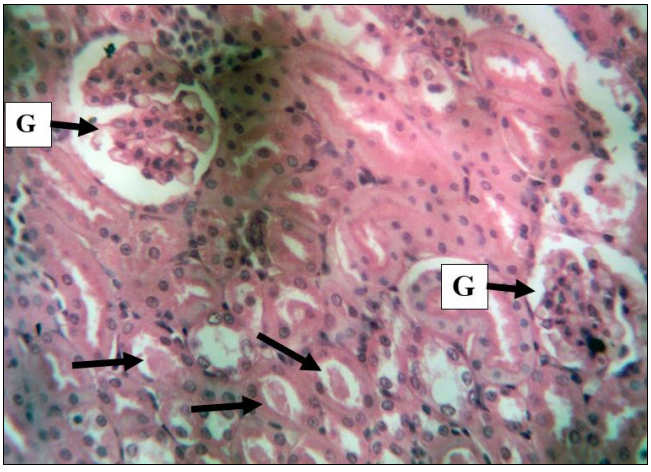

Fig 4: Representative micrograph of kidney of animals in group D. The glomeruli (G) and tubules (arrows) appear normal with some tubules having intraluminal eosinophilic casts. **Stain**: Haematoxylin and Eosin. **Magnification**: X400.

Discussion

Acute kidney injury (AKI) is a sudden loss of the kidney function which manifests within a few days [27] as a result of damage to kidney tissues, occasioned by numerous conditions such as decreased blood flow to the kidney (kidney ischemia), getting exposed to substances that are harmful to the kidney or when inflammatory process takes place in the kidney.

Despite the major advances made in the field of medicine and medical care, acute kidney failure has remained associated with a high mortality and morbidity in the society [5]. Among the substances which can cause Acute Kidney Injury are the local herbs in which Aloe vera is considered as one of them. The sole aim of this study is to evaluate the effect of Aloe vera gel on renal biochemical and histological parameters on Rats. The model for studying the effect of Aloe vera on kidney is obtained by oral administration of Aloe vera gel into Rats for two weeks on different doses.

The Rats were grouped into four, i.e groups A – D. The result of the biochemical parameters which includes Blood urea nitrogen (BUN), Serum creatinine, Potassium (K^+) and Sodium (Na^+),increases with corresponding increase in the given doses, while the low dose did not pose any renotoxic effects in the animals.

It was further observed that the extract had a dose-dependent effect on the kidney as earlier stated by [28]. Serum creatinine which is influenced by gender, age and the amount of muscle mass were kept constant as the Rats were of same age, same sex, same weight range (12 0-180g) and all had restricted movement. Therefore, the increase observed in serum creatinine, potassium and Blood urea nitrogen was due to renal toxicity caused by high intake of Aloe vera gel which agrees with the work of Ibrahim, M. A in 2019. However, these biochemical parameters were seen to be normal in the low dose group and control group when compared to the high and moderate dose group, this could be due to antioxidant and anti-inflammatory effect of aloe vera gel [29].

In histological examination as shown by the photomicrograph of all the groups, it was observed that the kidney of the control group (Group A), appeared to be structurally and functionally normal, the glomeruli and tubule showed a well conserved morphology, same goes with the low dose group. For the Rats in group B (high dose) and group C (medium dose), the glomeruli (G) appear eroded while the tubules (arrows) showed sloughed off epithelia and eosinophilic casts.

This shows that the oral intake of Aloe vera gel wouldn't pose any harm to the kidney when taken in low dose, this supports the previous review which states that prolonged intake of Aloe vera at high dosage might cause Nephrotoxicity (the rapid deterioration in the Kidney function due to toxic effects of some chemicals, additives and some medicinal plants).

Conclusion

The present study shows that oral intake of Aloe vera gel as a medicinal plant at high dose for a long period of time could impose an adverse effect on the kidney while low dose would not. Thus, this data suggests that Aloe vera should be taken at low dosage.

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