Renal Morpho-Functional Changes in Wistar rats administered Citrullus *lanatus* (watermelon)

Abstract:

Introduction

The kidneys are paired organs that function as eliminators of harmful metabolites from the blood. Considering the nutritional profile, *Citrullus lanatus* is reportedly a vital source of anti-oxidants that may affect renal functions.

Aim

Using the adult male wistar rats, this study examined the effect(s) of *Citrullus lanatus* fruit seeds on serum electrolytes and histology of the kidney.

Methodology

Twenty rats (95-200 g) were purchased and grouped into four (n=20) of five (5) rats per group. Group 1 (control group) received growers mash and water only, groups 2, 3 and 4 were fed with 100, 200 and, 400 mg/kg of *C. lanatus* fruit seeds extract respectively (for 21 days). Following period of extract administration, animals were fasted overnight and carefully restrained. Equal volume of blood samples were collected from their retro-orbital plexus for biochemical analysis. Abdominal cavities were cut opened and kidney excised for histo-architectural study.

Result

With serum Na^+, Cl^-, Ca^{2+}, HCO_3^- , levels returning insignificant results for all doses (100mg/kg, 200mg/kg and 400mg/kg) of watermelon fruit seed extracts treated rats upon comparison with control; there was however a significant increase ($p < .05$) in serum K^+ and Urea levels of extract treated rats when compared with control. Study also observed no histological alteration in the kidney of extract treated rats when compared to control.

Conclusion

Thus, administration of *C. lanatus* seed extract did not affect serum Na^+, Cl^-, HCO_3^- and creatinine levels of wistar rats; even though there were significance increase in K^+ and urea levels.

Keywords: Electrolytes, Renal, Histo-architeture, *Citrullus lanatus*

Introduction

With plant kingdom becoming a treasure house for a plethora of potential drugs, in recent times, increasing awareness about the importance of medicinal plants [1] has attracted the research community. According to the world health organization (WHO), medicinal plants have great potential of becoming a great source to obtaining a variety of drugs; however, such plants should be investigated to better understand their properties, safety and efficiency [2]. From time immemorial**,** plant products have been part of phytomedicines, and are obtained from such plant parts as the barks, leaves, flowers, roots, fruits, seeds, etc [3].

Knowledge of the phyto-chemistry of plants is therefore desirable as such information will help in the synthesis of complex chemical substances, including drugs [4, 5]. Watermelon (*Citrullus lanatus*) is one of numerous fruits that are of great medicinal and

economic importance. It is enjoyed by several people across the globe as a fresh fruit. It is reportedly low in calories, and is highly nutritious with thirst-quenching potential [6]. Though several of its traditional medical uses have been documented, most of these claims are yet to be fully validated by scientific protocols.

C. lanatus is an excellent source of vitamin A, B and C; which are necessary for energy production. Pink watermelon is also said to be a great source of argentine ($C_{23}H_{26}N_4O_3$), carotenoids, lypcopene, carbohydrate, sodium, magnesium, potassium and water [7]. It is thought to have originated from southern Africa, where it is found growing wild, and reaches its peak. It is considered to be indigenous to tropical Africa [8], and contains about 93% water, earning it the name "water melon". *C. lanatus* belongs to the Eucurbitacaceae family. It possesses long, weak, trailing or climbing stems that are five-angled and up to 3m (10ft) long. Their leaves are stemmed and alternate, with large pinnately-lobed, stiff and rough when old. Their flowers grow singly in the leaf axils and the corolla is white or yellow inside. They have unisexual flowers, with males and females occurring on the inside (monoecious). Their fruit in the wild form is subglobose, indehiscent and up to 200mm in diameter [9, 10].

Often time, *C. lanatus* seeds are discarded by its eaters, while their fruits are eaten without bark.

Aim of Study

This study aimed at determining if intake (with experimental rats) of a given dose of *C. lanatus* seed will have minimal or no effect on serum electrolyte (Na^+, Cl^-, Ca^{2+} and HCO_3) levels and the histology of the kidney. Specifically, Study attempted to:

i. Determine the effects of *C. lanatus* seed intake on serum sodium, potassium, urea, creatinine , chloride and calcium levels
ii. Examine the histo-architectural changes in the kidney of wistar rats fed with varying doses of *C. lanatus* seed extract
iii. Ascertain the nutritional values of *C. lanatus* seed intake

Materials and Methods

Study Design

Study used a total number of twenty (20) male wistar rats and randomly divided into a group of four (4) rats for treatment within three weeks (21 days) of experimentation as follows;

GROUP 1: control group, no extract was administered (n=5).
GROUP 2: was administered with 100mg/kg of *C. lanatus* seed extract (n=5).
GROUP 3: was administered with 200mg/kg of C. *lanatus* seed extract (n=5).
GROUP 4: was administered with 400mg/kg of *C. lanatus* seed extract (n=5).

Preparation of Plant's extract

With the assistance of a renowned taxonomist from the department of botany, Delta State University, Abraka, *C. lanatus* fruit was identified and procured from local market in Abraka, Delta State, Nigeria. The *C. lanatus* seeds were then harvested from the fruit, washed, air-dried (for 48 hours), and milled into fine powder. Using a weighing balance, powder extract was weighed and placed in a beaker, soaked in 1400ml of absolute methanol and 400ml of distilled water for 48hours. The solution was then filtered using handkerchief and concentrated with water bath at a temperature of 15^{o}C.

Animal Preparation

Obtained animals were house in a suitable condition with a 24 hours light/dark cycle and nutritionally balanced pellet and water ad-libitum to acclimatize for a period of one week before the commencement of the experiment. This was carried out in line with recommendations from the declaration of Helsinki (1996) as reported by Mojab *et al* (2003)[10] in his Guide on the use and care of laboratory animals.

Samples Collection

Blood and Kidney collection

Following the twenty one (21) days of extract administration, animals were fasted overnight, re-weighed and humanely killed by cervical dislocation. Blood samples were collected from their retro-orbital plexus, and cautiously emptied into a lithium heparinized tube. Next, obtained blood samples were mixed with anticoagulants to prevent them from clothing. Samples were then centrifuged at 3500 rpm for 10 minutes, with plasma were aspirated from centrifuged samples into another set of labelled tubes for biochemical assay. Also for any case, abdominal cavities of sacrificed rats were cut open, and their kidneys were excised and fixed in 10% formal saline for histological studies.

Determination of Serum Electrolytes

Chloride

Method:

This was based on the principle that chloride ions form a soluble, non-ionized compound, with mercuric ions and will displace thiocyanate ions from non-ionized mercuric thiocyanate. The released thiocyanate ions react with ferric ions to form a colour complex that absorbs light at 480nm. The intensity of the produced colour is directly proportional to the chloride concentration. To approach this, 1.5ml of chloride was pipetted into a test tube labelled blank. About 10▪ 1 of water and blood sample were placed into the tube and mixed. They were then incubated for at least 5minutes at 37^0C and calculated using;

Concentration of chloride sample (mEq/L) = $\frac{Abs.of sample}{Abs.of standard}$

Determination of sodium

Method:

This worked on the principle that sodium is precipitated as the triple salt, sodium magnesium uranyl acetate, with an excess of uranium [12] than being reacted with ferrocyanide; producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test specimen. In lieu of this, about 1.0ml of sodium filtrate reagent was pipetted into a blankly labelled test tube. 50ul of water and blood sample were then added to the tube. The mixture was then shaken vigorously and mixed continuously for 3 minutes. Tubes were centrifuge at high speed (1500G) for 10 minutes, and the supernatant were tested and calculated using;

Sodium Conc. in sample = $\frac{Abs.of blank - Abs.of sample}{Abs.of blank - Abs.of standard}$ x Conc. of standard (mEq/L).

Determination of Potassium

Method:

The amount of potassium is determined by using sodium tetrephylboron in a specifically prepared mixture to produce a colloidal suspension. The turbidity of which is proportional to potassium concentration when measured spectrophotometrically. Here, about 1.0ml of potassium reagent was pipetted into a blankly labelled test tube. Next, 10ul of

sampled blood and distilled water were added to the test tube and mixed; allowing it to sit at room temperature for 3 minutes. The spectrophotometer was zeroed with reagent blank and absorbencies of all tubes were measured at a wavelength of 500nm using;

Potassium Conc. (mEq/L) = $\frac{Abs.of\,sample}{Abs.of\,standard}$ x concentration of standard (mEq/L)**.**

Calcium determination in serum:

Method:

Calcium is known to react with cresolphthalein complexone in 8-hydroxyquinoline to form a complex colour (purple colour) that absorbs at 570 nm (550 -580 nm). The intensity of the formed colour is proportional to the calcium concentration. Here, the working reagent (1000ul) was transferred into a blank labelled test tube. 20µl of samples and standard reagents were added to the test tube and mixed. The mixtures were incubated for at least 1 minute at room temperatures. Absorbance of samples was then read against reagent blank at 570 nm. Calcium level (mg/dl) in sample was calculated using the formula below;

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard.}$$

Determination of creatinine

Method:

Under alkaline conditions, Creatinine is known to react with picric acid to form a complex colour, which absorbs light at 510nm. The rate of formation of the colour is proportional to creatinine concentration in the sample. In the endpoint, the difference in absorbance is measured after colour formation value and corrected for interfering substances.

Determination of Serum Biocarbonate

Method:

This is based on the principle that Phosphoenol pyruvate carboxylase (PEPC) catalyses the reaction between phosphoenol pyruvate and carbon dioxide (bicarbonate) to form oxaloacetate and phosphate ion. Formed Oxaloacetate is then reduced to malate with simultaneous oxidation of an equimolar amount of reduced Nicotiamide Adenine Dinucleotide (NADH) to NAD; the reaction is catalysed by malate dehydrogenase (MDH). This results in a decrease in absorbance at 340 nm that is directly proportional to CO_2 concentration in the sample. To this point, 1.0ml of CO_2 Reagent was pipetted into test tubes labelled blank. All tubes were incubated for 3 minutes at 37^0c and 5 µl of water and blood

sample were added and mixed. The absorbance values of all samples were then measured and recorded at 340 nm wavelength.

Ethical Considerations

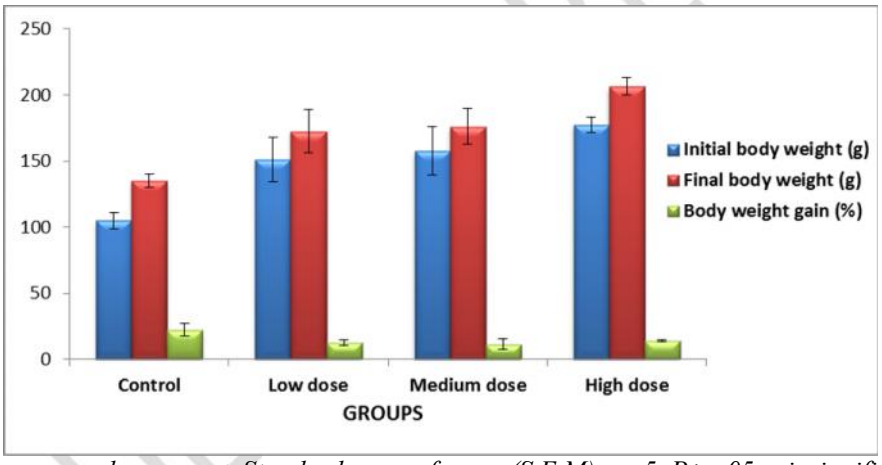
Ethical approval was sought and granted from the Research and Ethics committee of the College of Health Sciences, Delta state University, Abraka, Delta state.

Statistical Analysis

Results were expressed as Mean ± SEM (Standard Error of Mean). Statistical comparisons were performed by One Way Analysis of Variance ANOVA) followed by Fisher's Least Significant Difference (LSD) test. A P-value less than .05 ($p < .05$) was considered as significant.

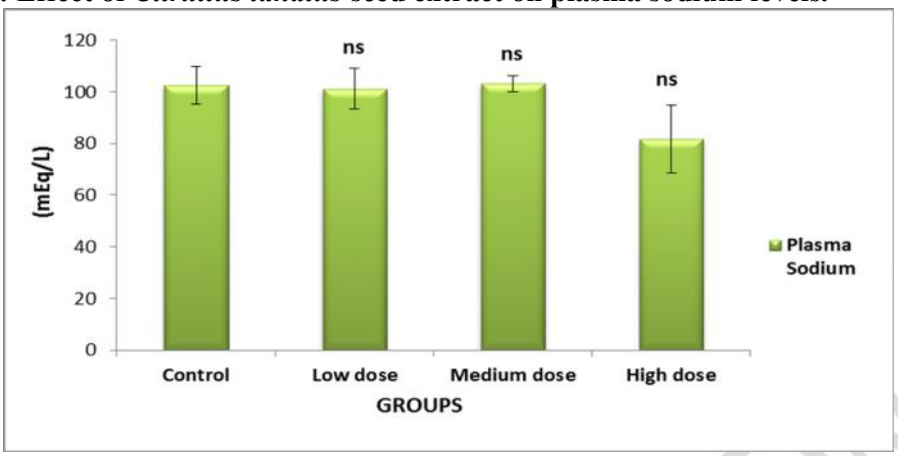
Results

Figure **I:** Effects of *Citrullus lanatus* seed extract on body weight of male Wistar rats.



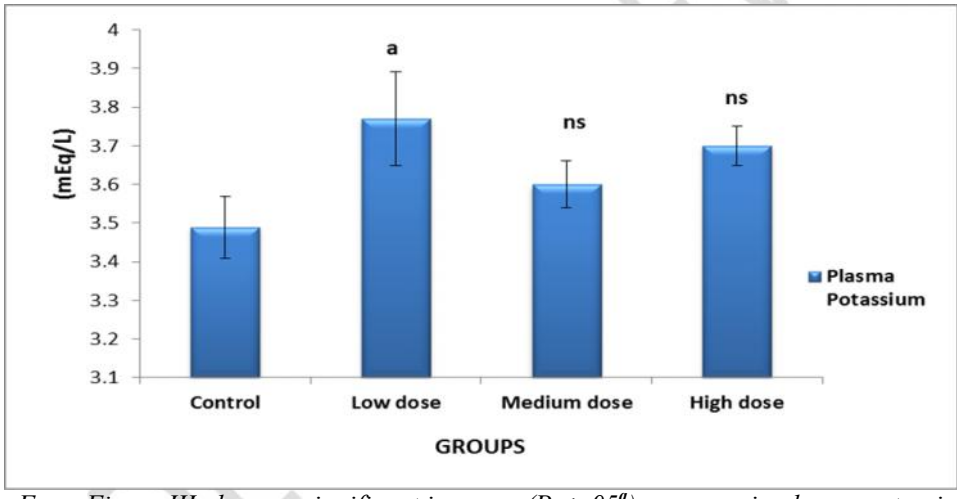
Values are expressed as mean ± Standard error of mean (S.E.M), n=5. P > .05 = insignificant. From Figure I above, there was a decrease in body weight gain of animals administered with Citrullus lanatus seed extract when compared with control rats. This decrease was seen to be lowest in medium dose group, followed by low dose and high dose rats.

Figure II: Effect of *Citrullus lanatus* seed extract on plasma sodium levels.



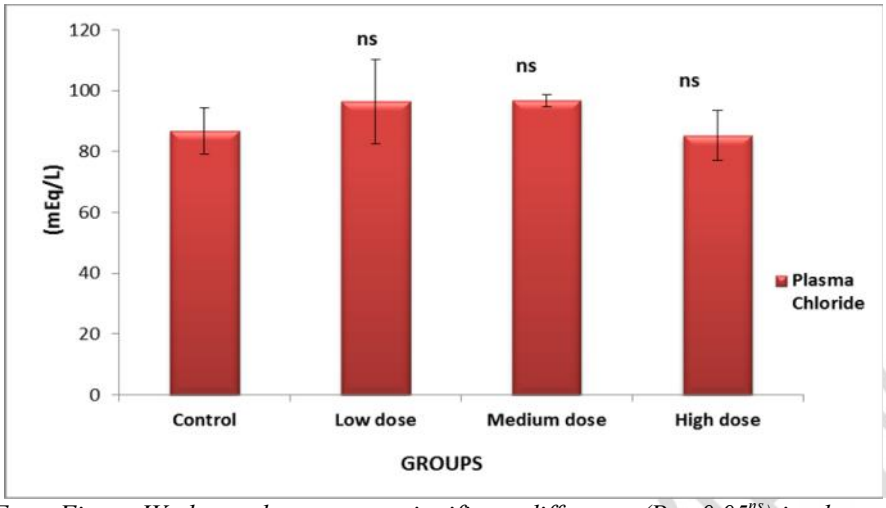
From figure II above, there was an insignificant difference (P > .05) in plasma sodium levels of animals administered with Citrullus lanatus seed extract across all doses when compared with control group.

Figure **III: Effect of *Citrullus lanatus* seed extract on plasma potassium levels.**



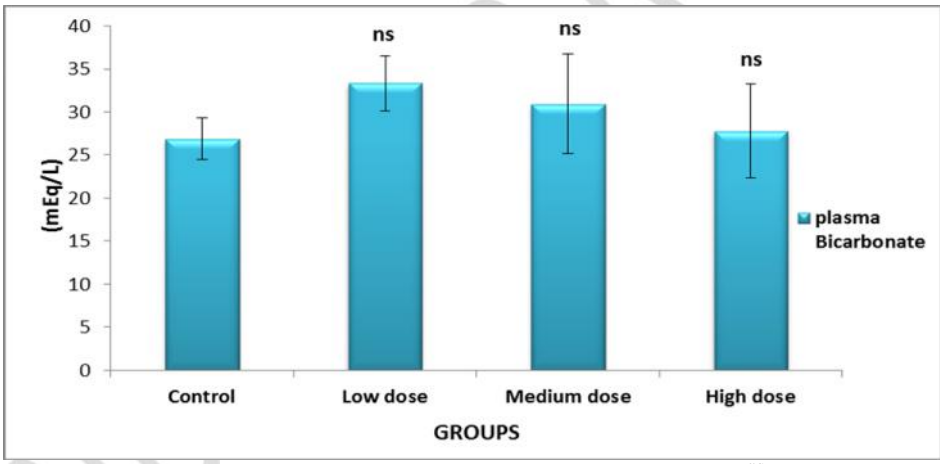
From Figure III above, a significant increase (P < .05[a]) was seen in plasma potassium level of animals administered with low dose of Citrullus lanatus seed extract when compared with control group. However, an insignificant difference was observed in experimental groups administered with medium and high doses of Citrullus lanatus seed extract when compared with control group. Here = significant, ns = not significant

Figure IV: Effect of *Citrullus lanatus* seed extract on plasma chloride level



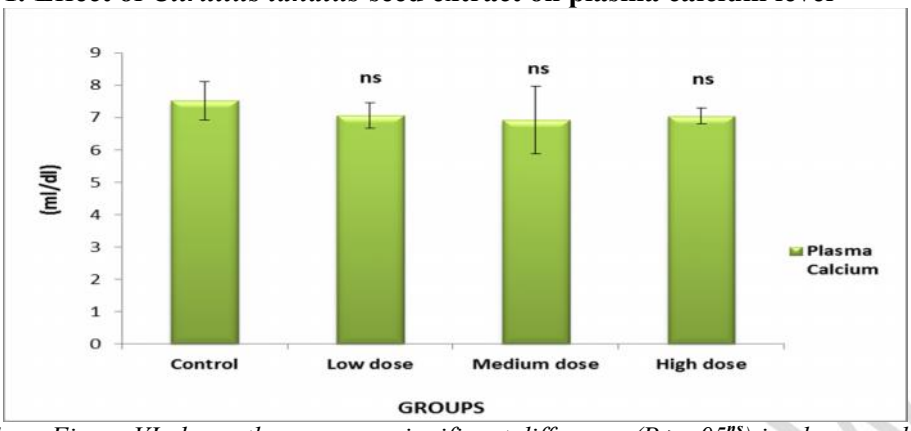
From Figure IV above, there was no significant difference ($P > 0.05^{ns}$) in plasma chloride level of animals administered with Citrullus lanatus seed extract across all doses when compared with control group. Here = significant, ns = not significant

Figure V: Effect of *Citrullus lanatus* seed extract on plasma bicarbonate level



From Figure V above, there was no significant difference ($P > 0.05^{ns}$) in plasma bicarbonate level of animals administered with watermelon seed extract across all doses when compared with control group. Here = significant, ns = not significant

Figure VI: Effect of *Citrullus lanatus* seed extract on plasma calcium level



From Figure VI above, there was no significant difference ($P > .05^{ns}$) in plasma calcium level of animals administered with watermelon seed extract across all doses when compared with control group. Here = significant, ns = not significant.

Figure VII: Effect of *Citrullus lanatus* seed extract on the histology of the kidney

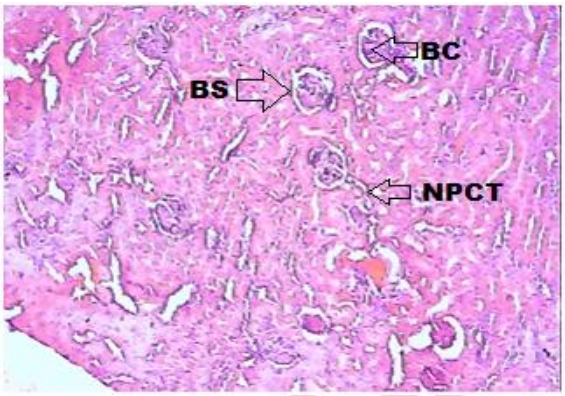


Plate A (control): Kidney. H and E (x 100)

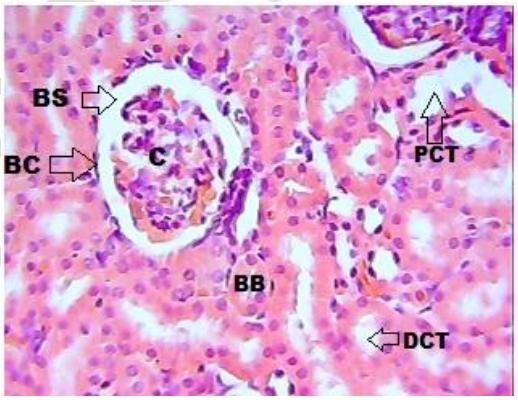


Plate B: Kidney administration with low dose of citrullus lanatus seed extract. H and E (x 400)

Micrographs shows sections of the Bowman's capsule (BC), Bowmn's space (BS), Capillaries (C), Proximal convoluted tubule (PCT), Distal convoluted tubule (DCT) and Brush border (BB).

Discussion

With special reference to lycopene, ascorbic acid and citruline, Watermelon is a valued source of natural antioxidant. These functional ingredients act as protection against chronic health problems like cancer insurgence and cardiovascular disorders [15]. Lycopene is characterized by its distinctive red colour in fruits and vegetable [11].

Watermelon is an excellent source of vitamin A, B and C necessary for energy production. Pink watermelon is also a source of the arginine, carotenoids, lycopene, carbohydrate, sodium, magnesium, potassium and water [6]. Considering the nutritional profile, consumption of 100 g watermelon provides 30 kcal. It contains almost 92 % water and 7.55 % of carbohydrates out of which 6.2 % are sugars and 0.4 % dietary fiber. It is enriched with carotenoid, vitamin *C, citrulline*, carotenoids and flavonoids and fat and cholesterol free, thus considered as low caloric fruit [8]. Additionally, watermelon is rich source of β-carotene acts as an antioxidant and precursor of vitamin A. Besides the presence of lycopene, it is a source of B vitamins, especially B1 and B6, as well as minerals such as potassium and magnesium [9].

Using Wistar rats as experimental model, this study evaluated the effects of *Citrullu lanatus* seed extract on serum electrolytes levels. Result from Figure I shows a statistically significant decrease in body weight of animals given *C. lanatus* seed extract. This finding is inconsistent with the reports of Libby *et al.*[17] and Erhirhie and Ekene [18]; who reported *C. lanatus* to increase body weights due to its nutritional composition; especially its energy content (6% sugar 92% water by weight; water 91.5g, energy 32kcal, protein 0.6g, fat 0.4g, carbohydrate 7.2g, Calcium 8ml, Phosphorous 9ml, iron 0.17ml, thiamine 0.08ml, riboflavin 0.02ml, niacin 0.2ml, folate 2ml and ascorbic acid 9.6ml). For Figure II, it is observed that there is no significant difference in serum sodium levels of animals administered with *C. lanatus* seed extract when compared with control group. This may be due to the fact that *C. lanatus* has low sodium (Na^+) content (about 1ml (0%)), which may have contributed to its low serum levels as seen in experimental rats. This finding is in agreement with those of Seifter [19]; who reported that *C. lanatus* has no effect on serum sodium level and thus, causes no known renal damage from its administration as it does not affect sodium homeostasis in the body.

From Figure III, a statistically significant increase in serum potassium level was observed in animals administered with *C. lanatus* seed extract (low dose) when compared with the control group. This may be due to the fact that *C. lanatus* contains higher amounts of potassium than sodium. This is in agreement with Seifter's finding of 2011; who observed that, a half cup of *C. lanatus* contains high amount of potassium; accounting for about 112ml (2%) of the total minerals contained in *C. lanatus* Seifter. This implies that increased consumption of *C. lanatus* can lead to build up of potassium in the body, which can lead to hyperkalaemia, temporary paralysis, abnormal heart rhythm (arrhythmia) and cardiac arrest. Potassium is an essential intracellular mineral and important in maintaining fluid and

electrolyte balance in the bodies of humans and animals, its depletion results in various neurological dysfunctions. Abnormal regulation of potassium leads to hypokalaemia and hyperkalaemia.

Again from this study, an insignificant difference was observed in serum chloride, bicarbonate and calcium levels of experimental animals administered with ethanol seed extract of *C. lanatus* when compared with control (Figure IV). This may be attributed to the fact that, *C. lanatus* contains essential minerals which is however not sufficient to cause an alteration in the plasma levels of these substances despite been a good source of these minerals [20].

Result from this study also observed no major histological alteration in the kidney of experimental rats administered with *C. lanatu*s as compared with control rats (Figure VII), hence histology of the kidney in experimental group were normal (Plates A and B). This finding is in agreement with the study conducted by Oyewo and Onyije [21] who also observed no histological changes in experimental animals administered with aqueous extract of *C. lanatus*. In another study by Erhirhie and Ekene [18] on "the effects of *C. lanatus* on kidney histology", Erhirhie and Ekene revealed that the kidney histology of all experimental animals that received aqueous extract of *C. lanatus* were normal when compared with the control group. This could be due to the fact that the sugar content from watermelon had a great diuretic effect and helped to remit inflammation of the kidney due to its unique nutrients vitamins, minerals and organic compounds. These components of watermelon contribute to its major impact on health.

Conclusion

Administration of *Citrullus lanatus* fruit extract did not affect serum sodium, chloride, bicarbonate, calcium and creatinine levels of Wistar rats. However, a significant increase was seen in serum potassium levels, with an accompanied decrease in body weights. The evaluation of the possible effects of *C. lanatus* seed extract on the kidney did not record any meaningful histological distortion in kidney, using H and E stain.

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