Aspartame, Chronic kidney insufficiency and *Ocimumgratissimum* extract.

ABSTRACT

Objective:The paired human kidneys are retroperitoneal organs responsible for the maintenance of the internal meliu of the human body. Chronic kidney disease is major cause of morbidity and mortality globally. Nutrition and life style are two main factors in the aetiopathogenesis of chronic kidney disease. Aspartame is a non-nutritive sweetener commonly consumed worldwide. *Ocimumgratissimum* is a naturally growing plant with some medicinal benefits. This study investigated if oral administration of aspartame could induce renal insufficiency in rat and the plausible ameliorative role of *O. gratissimum* extract.

Methodology: Fifty animals allotted to four groups were used for the study. The control group (CN) had normal feed. The Aspartame group (Asp) had 250 mg/kg/day of aspartame for 28 days.The Aspartame low dose *O.gratissimum* extract group (ALO) had 250 mg/kg/day of aspartame for 28 days followed by once daily dose of the extract at100 mg/kg for 28 days.For the Aspartame high dose *O.gratissimum* extract group (AHO); aspartame was administered at 100 mg/kg once daily for 28 days followed by another 28-day daily administration of the plant extract at 200mg/kg. Both aspartame and plant extract were administered orally. Periodically, blood samples were collected for biochemical analyses and kidneys harvested for histopathological examination.

Results: The marked biochemical indicators of renal insufficiency were significantly pronounced in the Asp group but not in the AHO. Oxidative stress was pronounced in the Asp and ALO but not in group AHO at day 57. Histopathological examination of the kidney obtained from the Aspartame group revealed destruction of glomeruli.

Chronic administration of aspartame in rat caused sustained renal insufficiency which was ameliorated by prolonged administration of high dose of*Ocimumgratissimum* extract. Thus *O. gratissimum* extract is beneficial to aspartame induced renal toxicity in a dose dependent manner.

Key words: Aspartame, chronic kidney insufficiency, *Ocimumgratissimum.*

1.INTRODUCTION

The human kidneys are paired retroperitoneal organs responsible for the maintenance of the body fluid, electrolytes and acid-base balance ie homeostasis amongst other functions. The total daily blood flow through the adult human kidneys is about 1,700 litres from which about 1 litre of concentrated urine is produced [1]. The kidney can be affected by a myriad of pathologies raging from mild infection to end stage renal disease (ESRD). Chronic kidney disease (CKD) is a progressive pathology with a global prevalence rate that is more than 10 % of world population.This translates to about 843.6 million people as at 2017 and according to the Global Burden of Disease Study of 2019 [2]. In the 21st century, CKD has emerged as a major cause of morbidity and mortality.By way of definition, CKD refers to persistence of renal dysfunction for more than 3 months [3-5].The major risk factors of CKD are obesity, diabetes mellitus and hypertension [2,3].The aetiology of chronic kidney disease include genetic predisposition, adverse drug reaction, drug overdose, injurious herbal preparations, acute renal insult,protracted hypovolemia, dyslipidemia, alcoholic beverages and dietary indiscretion.

Aspartame is a white crystalline powder normally used as an artificial sugar-free sweetener and commercially marketed under different brand names [6]. It is metabolized in the body to aspartic acid, phenylalanine and methanol[7]. The methanol is further degraded to formic

acid which is toxic to tissue [8,9].Aspartame is said to cause renal injury via oxidative stress [10]. *Ocimumgratissimum* is a perennial herbal plant with some attributable medicinal relevance and also of culinary importance [11,12]. It may thus be able to prevent or reduce the renal injury that may be occasioned by dietary consumption of aspartame. This thus formed the nidus of this study.

2. MATERIALS and METHODS

2.1 Plant Materials

2.1.1 Plant collection and authentication

Fresh leaves of *Ocimumgratissimum* were harvested from the parent plant within the University of Ibadan Campus. Confirmatory identification and authentication were at the Herbarium division of the Botany Department, University of Ibadan. A sample was deposited at the herbarium for future reference.

2.1.2 Extract preparation

The leaves of*Ocimumgratissimum*were initially washed under potable running water,subsequently left to dry at ambient room temperature and thereafter blended till fine-textured. Seven hundred and sixty grammes of the powder was used to prepare the ethanolic extract and a 7.05% yield was obtained and refrigerated till use.

Comment [LCA1]: appointment

2.2 Animals

Fifty adult Wistar rats weighing 135 to 190 g were sourced from the Central Animal house of the College of Medicine, University of Ibadan. They were acclimatized for three weeks in a well ventilated and illuminated environment with optimal ambient temperature (26±2°C, 12 hours light / dark cycle) that was conducive for the study. The animals were fed liberally with locally sourced but standard pelletized rat feed and had free access towater

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Comment [LCA2]: References used.

2.3 Design of the Experiment

Comment [LCA3]: appointments

The creation of the experimental groups was premised on aspartame and dosage of O.*gratissimum*ethanolic extract administered. Consequently, four groups were created and the animals were randomly allotted.

The details of the groups were;

(1) Control (CN)-had normal rat feed and water.

(2) Aspartame (Asp)- had only aspartame.

(3) Aspartame with low dose O.*gratissimum* extract (ALO)- Sequential administration of aspartame and low dose ethanolic extract of O.*gratissimum*

(4) Aspartame with high dose O.*gratissimum* extract (AHO)- Sequential administration of aspartame and high dose ethanolic extract of O.*gratissimum*.

2.5 Conduct of the Experiments

Comment [LCA4]: appointments

The control group had only normal rat feed and water while the Aspartame group had 250 mg/kg/day of aspartame orally for 28 days. The Aspartame low dose *O.gratissimum* extract group (ALO) had 250 mg/kg/day of aspartame orally for 28 days. Thereafter, had the extract orally at a daily single dose of 100 mg/kg for 28 days. For The Aspartame high dose *O.gratissimum* extract group (AHO); aspartame was administered at 100 mg/kg once daily for 28 days followed by another 28-day daily administration of the plant extract at 200mg/kg. Venous blood samples were collected through intraocular puncture from five animals in each group at day 36,43,50 and 57(ALO & AHO groups) and on day 29 (CN &Asp groups) for

biochemical analyses.The biochemical parameters evaluated were the serum electrolytes (Potassium-K^+, bicarbonate- HCO_3^-,Cl^- , sodium-Na^+), ureaand creatinine. The activities of Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and Catalase; and the degree of lipid peroxidation as measured by Malondialdehyde (MDA) levels were used to assess the extent of oxidative stress occasioned by the lead toxicity.
Also, on day 36,43,50 and 57, each group had five animals (whose blood were collected) sacrificed by cervical dislocation with prior light sedation for the purpose of organ harvesting. The groups CN and Asp animals were sacrificed on day 29.
The harvested liver specimens were initially washed in buffered saline and thereafter stored in 10 % formaldehyde solution for subsequent light microscopy.

2.6Data Analysis and Processing
The numerical aspects of the results were analyzed with Statistical Package for the Social Sciences (SPSS) version 24 and expressed as percentages, means plus standard deviation of means (SD). The student t- test was used for inter group comparison and level of significance was set at $p<0.05$.

RESULTS
All the groups recorded weight gain with the highest being the Control and the least being the Asp.
Comment [LCA5]:
Both the mean weight and the relative weight of the kidney were similar across the groups.
Renal function test
The plasma potassium (K^+) level was markedly elevated in the Asp group and in the ALO group on day 36,43 and 50. The rise in the plasma level of K^+ was least in the AHO group and by day 50, its value was similar to that of the control.
The plasma bicarbonate (HCO^{3-}) level was significantlydepressed in the Asp, ALO (day 36,43&50) and AHO on day 50.
Significant hyponatremia (low plasma sodium-Na^+ level) was observed in groups Asp and ALO (day 36). While the levels of Na^+ of groups ALO (day 57) and AHO (day 43 & 57) were markedly higher than that of Asp.
Significant elevated chloride (Cl^-) level was observed in groups Asp and ALO (day 43&57).
The creatinine levelsof Asp and ALO groups were significantly higher than normal (Table 1)
Oxidative stress assessment
The activities of catalase and sodium dismutase were significantly depressed in the Asp, ALO and AHO groups except on day 57 for AHO. There was no remarkable difference in the activities of glutathione peroxidase between the groups. Groups Asp, ALO and AHO had markedly depressed activities of sodium dismutase. The extent of lipid peroxidation was most severe in the Asp group and only at day 28 in the AHO as evidenced by the significantly elevated levels of malondialdehyde (Table2).
Histopathology
In the Asp group, some glomeruli with reduced capillary content were observed also within the same group, the capillary tufts were noted to be fragmented. Besides these observations, the glomeruli, renal tubules, interstitium and vessels appeared normal in all the other groups (Plate1).

Table1.Morphological and biochemical parameters

Parameter↓ Group→	CN(n=5)	Asp (n=5)	ALO(n=20)	AHO (n=20)
Body weight gain (%)	45.63±3.69	22.70±1.45	35.03±1.01	35.68±1.21
Kidney weight (g)	0.70±0.09	0.59±0.08	0.64±0.13	0.63±0.06
Relative Kidney weight (g/100g body weight)	0.34±0.01	0.31±0.00	0.27±0.00	0.26±00
Renal Function Test				
i) K^+(mmol/l)				
Day 29	4.17± 0.37	10.9± 0.31$^{\alpha}$	-------	------
Day36			7.66± 0.36$^{\alpha}$	7.53± 0.80
Day 43			8.17± 0.59$^{\alpha}$	6.02± 0.36
Day 50			7.77± 0.27$^{\alpha}$	4.26± 1.04
Day 57			6.03±0.90	4.10± 0.30$^{\beta}$
ii) HCO^-_3 (mmol/l)				
Day 29	25.83± 1.88	16.26± 0.99$^{\alpha}$	-------	--------
Day 36			17.6± 1.32$^{\alpha}$	17.53± 2.19
Day 43			16.08± 0.15$^{\alpha}$	19.8± 1.47
Day 50			18.8± 0.10$^{\alpha}$	16.15± 1.37$^{\alpha}$
Day 57			20.75± 1.52	20.15± 1.14
iii) Na^+ (mmol/l)				
Day29	60.83± 2.08	43.48± 1.11$^{\alpha}$	--------	--------
Day36			42.61± 1.00$^{\alpha}$	43.97± 1.99
Day 43			44.74± 1.55	50.17± 1.87$^{\beta}$
Day 50			48.79± 1.92	47.86± 1.31
Day 57			51.43± 1.24$^{\beta}$	55.9± 1.69$^{\beta}$
iv)Cl^-(mmol/l)				
Day 29	56.63±2.65	67.5± 1.78$^{\alpha}$	-------	--------
Day 36			60.7± 3.69	62.68± 9.73
Day 43			68.5± 1.91$^{\alpha}$	66.4± 1.38
Day 50			61.2± 3.00	65.02±0.19
Day 57			66.21±1.84$^{\alpha}$	50.37±6.11
v) Urea (mmol/l)				
Day 29	4.46± 0.51	7.97± 0.56$^{\alpha}$	-------	---------
Day 36			7.43± 0.28$^{\alpha}$	6.63± 0.53$^{\alpha}$
Day 43			6.77± 0.66	5.09± 0.25$^{\beta}$
Day 50			6.35± 1.21	4.33± 0.14$^{\beta}$
Day 57			5.50± 0.35$^{\beta}$	4.09± 0.26$^{\beta}$
vi) Creatinine (µmol/l)				
Day 29	41.39±1.17	68.47±2.57$^{\alpha}$	------	------
Day 36			69.18±2.06$^{\alpha}$	63.25±2.65
Day 43			63.21±1.64$^{\alpha}$	65.82±3.12
Day 50			65.77±1.65$^{\alpha}$	61.61±1.98$^{\beta}$
Day 57			62.27±4.90	55.26±2.93$^{\beta}$

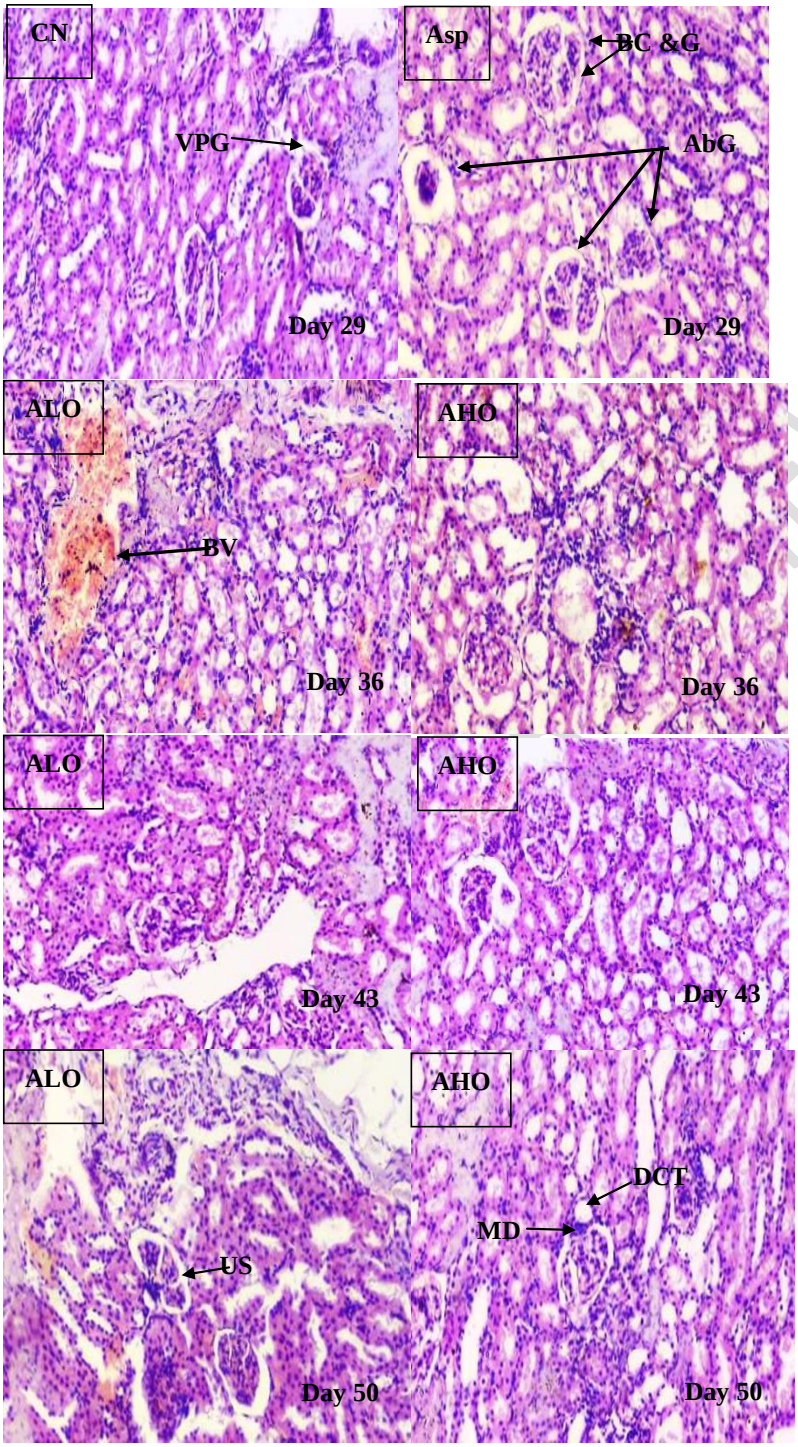
Legend: CN- Control group, Asp-Aspartame group, ALO- Aspartame low dose extract, AHO- Aspartame high dose extract group.Cl^-- Plasma level of chloride ,HCO^-_3- Plasma level of bicarbonate, K^+- Plasma level of potassiumNa^+- Plasma level of sodium.

Table2.Oxidative stress parameter

Parameter ↓ Group →	**CN** (n=5)	**Asp**(n=5)	**ALO** (n=20)	**AHO**(n=20**)**
Catalase (µM/mg)				
Day 29	44.20±2.77	19.77±1.67$^{\alpha}$	-------	------
Day 36			21.71±2.61$^{\alpha}$	25.39±2.85$^{\alpha}$
Day 43			25.00±0.96$^{\alpha}$	29.86±3.66$^{\alpha\beta}$
Day 50			29.49±3.35$^{\alpha\,\beta}$	29.38±4.00$^{\alpha}$
Day 57			28.23±1.61$^{\alpha}$	36.15±2.01$^{\beta}$
Glutathione Peroxidase (µmol/mg)				
Day 29	9.73±0.28	6.49±2.11	-------	--------
Day 36			8.68±1.13	8.90±0.23
Day 43			8.37±0.79	9.81±0.67
Day 50			10.12±0.65	7.74±1.26
Day 57			9.68±0.54	10.26±0.68$^{\beta}$
Sodium dismutase (µmol/mg)				
Day 29	14.96±0.95	6.83±1.43$^{\alpha}$	------	-----
Day 36			7.77±1.73$^{\alpha}$	7.43±1.63$^{\alpha}$
Day 43			7.48±1.33$^{\alpha}$	7.96±1.56$^{\alpha}$
Day 50			8.66±1.51$^{\alpha}$	8.09±0.52$^{\alpha}$
Day 57			8.22±1.28$^{\alpha}$	14.02±0.51$^{\beta}$
Malondialdehyde (mmol/l)				
Day 29	10.2± 2.03	23.01± 2.40$^{\alpha}$	------	-----
Day 36			17.51±3.12	19.54±2.77$^{\alpha}$
Day 43			15.63±3.77	13.85±2.72
Day 50			16.63±3.49	9.35±1.31$^{\beta}$
Day 57			10.23±5.17	7.07±1.68$^{\beta}$

Legend:α= significantly different from the Control (CN). β= significantly different from the Aspartame group (Asp)

CN- Control group**, Asp**-Aspartame group, **ALO**- Aspartame low dose extract, **AHO**-Aspartame high dose extract group.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Day 50
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DCT
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Day 50

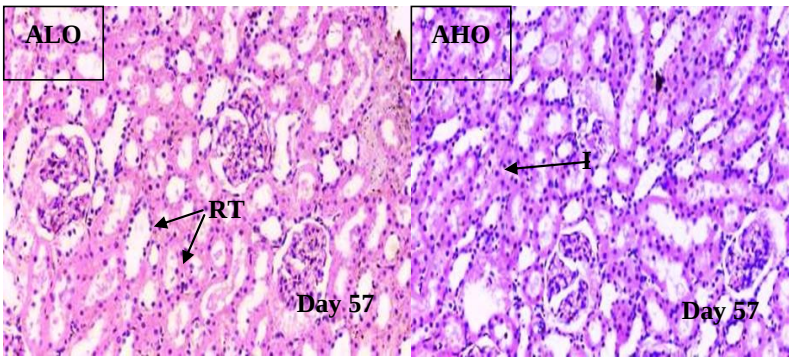

Plate1: Photomicrographs of the kidney at different periods of the study(H&E x400)
Legend-:**AbG**-Abnormal glomeruli,**BC**- Bowman's capsule,**DCT**-Distal convoluted tubule,**G**- Glomerulus,**I**-Interstitium,**MD**- Macula densa, **RT**-Renal tubules,**US**-Urinary spaceand **VPG**- Vascular pole of the glomerulus.
Control group, **Asp**-Aspartame group, **ALO**- Aspartame low dose extract, **AHO**- Aspartame high dose extract group.

DISCUSSION

All the groups gained weight in the course of the study with the Control group having the highest percentage weight gain. Body weight dynamics in kidney diseases vary from gain to loss depending on the type of pathology and its phase ie acute or chronic. This might explain the trend in body weight obtained in this study. Apart from polycystic disease of the kidney and tumours of the kidneys, other pathologies of the kidney are usually characterized by shrinkage of the kidney due to reduced parenchyma. There was no much difference between weights of the kidney of the experimental groups from those of the control group. Aside from acute renal insults such as hypovolemic shock and adverse drug reactions; chronic kidney diseases are generally of insidious origin and slowly progressing. This could explain the organ weights and the relative organ weights obtained in this study.

With reference to the control group, two of the experimental groups (Asp, and ALO) had significant hyperkalemia as evidenced by elevated plasma potassium (K^+). This was most severe in group Asp. By day 57, the K^+ of the ALO group had returned to normal.

Significant metabolic acidosis was recorded in groups Asp and ALO as evidenced by significant reduction of their bicarbonate (HCO^-_3) levels.

Azotemia which is elevated blood urea nitrogen and creatinine levels was observed in both Asp and ALO groups. The laboratory (clinical) diagnosis of chronic kidney disease (CKD) is premised largely on presence of hyperkalemia, metabolic acidosis and azotemia[1]. Since these abnormal laboratory parameters were not observed in the AHO group, the adducible inference is that aspartame caused renal insufficiency which was reversed by high dose of *Ocimumgratissimum* extract. Azotemia is largely due to reduced glomerular filtration rate thus it may be reasonably deduced that aspartame causes CKD by damaging the glomerulus this assertion is strengthened by observation of some glomeruli with fragmented or contracted capillary tufts in the micrographs obtained from kidney specimens of the Asp group. Normally, sodium (Na^+), chloride (Cl^-) and bicarbonate (HCO^-_3)ions are reabsorbed in the renal tubules while K^+ ion is secreted into the tubules. Thus it is obvious from the results of this study that besides the glomerular dysfunction, aspartame also caused kidney disease by altering renal tubular reabsorption and secretion of ions.

The administration of aspartame in this study resulted in significant depression of the activities of both catalase and sodium dismutase in all the three experimental groups ie Asp, ALO and AHO. It also caused significant lipid peroxidation in the Asp and AHO group (at day 36) by elevating the malondialdehyde levels. As documented in previous studies [7-10], one of the metabolites of aspartame is methanol that is stepwisely oxidized to formic acid which induces tissue oxidation and subsequent cellular injury. Part of the mechanisms through which aspartame causes renal injury include oxidative stress [13].

From the results of this study, high dose of *O. gratissimum* extract was able to reverse the oxidative stress induced renal injury after long term duration. This assertion arose from the fact that the catalase and sodium dismutase activities of the AHO group were similar to that of the control at day 57 of the study.

Aspartame, an artificial sweetener is a food additive that provides sweet taste without adding to food calorie [10,14]. The United States Food and Drugs Administration has recommended a daily dietary intake of 50 mg /kg and that of the European Union is 40 mg/kg [10,15,16]. Although this amount is considered safe, the main problem is strict adherence to the recommended safe daily consumption limit since this is personal. In view of the reported long term health hazards of consumption of artificial sweeteners such as obesity, metabolic

syndrome and alteration in gut microbiota [10], it has become imperative to find natural products that may either counter or ameliorate these untoward effects of the artificial sweeteners.

Some studies relating to experimental toxicity of aspartame will be briefly discussed.

In a study in which aspartame heated up to 40^0C was orally administered to pregnant rats at 14g/kg; distortion was observed in the kidneys of the fetuses [17]. The finding of this referenced study may not be applicable to human since we do not consume aspartame directly but rather as a food additive. In another animal study in which aspartame was administered orally at 50 mg/kg for 15,30 and 60 days significant lipid peroxidation and marked reduction in the activities of antioxidants of hepatic and renal tissues were observed thus concluding that aspartame induced oxidative stress [18]. Again this may not be applicable to humans as aspartame is consumed only via food additive.

According to the United States Food and Drug Administration, aspartame is two hundred times sweeter than table sugar. It requires daily consumption of seventy five sachets of aspartame in order to achieve the acceptable daily intake of 50 mg/kg body weight [19]. Also it had been propounded that adults need at least ten cans of a drink fully sweetened with aspartame in order to reach the acceptable daily intake of 40 mg/kg which is the European standard [20]. In a large human population study involving 285,079 men and 188,905 women aged 50-71 years, no correlation was found between consumption of aspartame and incidences of hematopoietic and brain cancers [21]. This finding had been corroborated by more recent studies [22-24].

Aspartame is not heat stable and loses its sweetness when heated thus if used in baking it will not serve its primary purpose. It is an established fact that baked foods are consumed in very large quantity on daily basis globally, this thus reduces significantly the amount of aspartame that is consumed by humans thus reducing its probable health hazard.

The main feature of chronic kidney disease is a gradual decline in renal function culminating in end stage kidney disease that is invariably irreversible. Thus the most effective strategy of reducing CKD related mortalities lies in the prevention and early detection of onset of CKD. This will involve screening for and appropriate management of diseases that could trigger off CKD such as hypertension, diabetes mellitus, obesity, dyslipidemia and hypercholesterolemia. Such steps include life style modification a major component of which is dietary discretion. Thus if the major finding of this research is translatable to human, extract of *Ocimumgratissimum* may be beneficial as a nutritional supplement in the prevention of aspartame induced renal insufficiency.Although the beneficial effects of *Ocimumgratissimum* extract in some human ailments using animal models have been documented in chemical liver injury [25], diabetes mellitus [26], bacterial infections[27] andhuman cancer cell lines [28-30] but not in relation to aspartame and chronic kidney disease. The outcome of this study is the first documented evidence of the beneficent of *O. gratissimum* in aspartame induced kidney insufficiency.

CONCLUSION

This study has provided both quantitative and qualitative evidences that oral administration of aspartame in rats is capable of causing parenchymal and biochemical alterations in the kidney. However, sequential administration of *Ocimumgratissimum* extract in high dose ameliorated or reversed these untoward changes observed in the kidney of rat. Considering the fact that we consume aspartame as a nutritional additive and other properties of aspartame, the findings in animal (rat) model may not be translatable to human.

CONSENT
Not relevant to this study.

ETHICAL APPROVAL
The animals used in this study were handled in accordance to the guidelines asprescribed by the ethical conduct of animalresearch of the University of Ibadan.
Prior approval was sought and obtained from the University of Ibadan Animal Care and Use Research Ethics Committee.
Also, theprinciples of laboratory animal care as containedin the 8th edition (2011) of the Guide for the Careand Use of Laboratory Animals by the NationalResearch Council (US) Committee for theUpdate of the Guide for the Care and Use ofLaboratory Animals were observed [31].

COMPETING INTERESTS
We declare the nonexistence of any competing interest.

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