



The Nephro -Toxicity of Aqueous Leaf Extract of *Costus Afer* Ker Gawl on the Kidney of Adult Wistar Rat

Hyacinth Tochukwu EZE¹, Ezejindu Damian NNABUIHE², Joshua Izuchukwu ABUGU^{2*}, Idorenyin UMOH³, Kingsley Akaninyene OKON³

¹Department Of Internal Medicine, Nnamdi Azikiwe University, College Of Medicine Nnewi Campus, Anambra State, Nigeria.

²Department of Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria

³Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Akwa Ibom State, Nigeria

*Corresponding Author

Joshua Izuchukwu
ABUGU

Department of Anatomy,
Faculty of Basic Medical
Sciences, Nnamdi Azikiwe
University, Nnewi Campus,
Anambra State, Nigeria.

Article History

Received: 18.03.2024

Accepted: 28.04.2024

Published: 04.05.2024

Abstract: The consumption of medicinal herbs in Nigeria has seen a notable surge in recent years due to their widespread availability, affordability, and the prevailing belief in the natural and non-toxic nature of herbal treatments. The objective of this research was to examine the potential toxicity of the dehydrated leaf extract derived from *Costus afer* Ker Gawl on the hepatic and renal systems of male albino Wistar rats. The research used a cohort of 20 male albino Wistar rats weighing between 100 and 170 grams. These rats were placed into four groups, each consisting of five individuals. Group A was provided with unrestricted access to conventional feed and water, and it was designated as the control group. Participants in Group B, C, and D were administered doses of 400, 700, and 1000mg/kg of an aqueous leaf extract derived from *Costus afer*, respectively. The medication was administered orally on a daily basis for a duration of twenty-one days. The animals were sedated with chloroform vapor and subsequently dissected twenty-four hours following the previous dosing. The kidneys were collected, measured, and preserved in a 10°C formal saline solution for the purpose of histological examination. Serum preparation included the collection of blood by eye puncture in order to conduct histochemical investigations. The assessment of marker enzymes was conducted using the randox kit methodology. An investigation was conducted to examine the impact of *C. afer* on the renal biomarkers urea and creatinine. Group D had substantially elevated serum levels of creatinine and urea ($P \leq 0.05$) compared to the control group A, as well as groups B and C. In the histological examination, it was shown that Group D of the kidney, which received a dosage of 1000mg/kg of an aqueous leaf extract, exhibited significant renal tissue damage characterized by necrosis, renal bleeding, loss of renal tissue, and deteriorated glomeruli. *Costus afer* was found to possess alkaloids, saponins, flavonoids, phenolic compounds, and tannins, as indicated by the conclusion. High dosages of *Costus afer* may be renally toxic. The use of medicinal herbs in Nigeria has seen a notable surge in recent years due to their widespread availability, affordability, and the prevailing belief in the natural and non-toxic nature of herbal treatments. The objective of this research was to examine the potential toxicity of the dehydrated leaf extract derived from *Costus afer* Ker Gawl on the hepatic and renal systems of male albino Wistar rats. The research used a cohort of 20 male albino Wistar rats weighing between 100 and 170 grams. These rats were placed into four groups, each consisting of five individuals. Group A was provided with unrestricted access to conventional feed and water, and it was designated as the control group. Participants in Group B, C, and D were administered doses of 400, 700, and 1000mg/kg of an aqueous leaf extract derived from *Costus afer*, respectively. The medication was administered orally on a daily basis for a duration of twenty-one days. The animals were anesthetized with chloroform vapor and subsequently dissected twenty-four hours following the previous dosing. The kidneys were collected, measured, and preserved in a 10°C formal saline solution for the purpose of histological examination. Serum preparation included the collection of blood by eye puncture in order to conduct histochemical investigations. The assessment of marker enzymes was conducted using the randox kit methodology. An investigation was conducted to examine the impact of *C. afer* on the renal biomarkers urea and creatinine. Group D had substantially elevated serum levels of creatinine and urea ($P \leq 0.05$) compared to the control group A, as well as groups B and C. In the histological examination, it was shown that Group D of the kidney, which received a dosage of 1000mg/kg of an aqueous leaf extract, exhibited significant renal tissue damage characterized by necrosis, renal bleeding, loss of renal tissue, and deteriorated glomeruli. *Costus afer* was found to possess alkaloids, saponins, flavonoids, phenolic compounds, and tannins, as indicated by the conclusion. High dosages of *Costus afer* may be renally toxic.

Keywords: kidney, *Costus afer*, Nephro -Toxicity, Histoarchitecture, medicinal plant.

Cite this article:

EZE, H. T., NNABUIHE, E. D., ABUGU, J. I., UMOH, I., OKON, K. A., (2024). The Nephro -Toxicity of Aqueous Leaf Extract of *Costus Afer* Ker Gawl on the Kidney of Adult Wistar Rat. *ISAR Journal of Multidisciplinary Research and Studies*, 2(5), 1-14.

Introduction

The genus *Costus*, belonging to the Costaceae family, has a diverse group of 150 species of robust, perennial, and rhizomatous plants (Edeoga et al., 2000). The woodland belt of Senegal, South Africa, Guinea, Niger, Sierra Leone, Ghana, Cameroon, and Nigeria is where it is located. The botanical specimen is well recognized as ginger lily or bush cane. In Igboland, it is referred to as "Okpete" or "Okpoto," while in Hausa it is known as "Kakizawa," in Yoruba as "tete-egun," and in Efik as "Mbriem" (Edeoga et al., 2000). It is referred to as "Monkey sugar cane" in Anglophone Cameroon. It has blooms of both white and yellow hues. The wild plant is gathered for its stem, seeds, leaves, and rhizomes, which are known to contain many bioactive compounds (Oliver et al., 1960). This botanical specimen is used as a therapeutic intervention for several ailments such as cough, inflammation, arthritis, laxative, aperient, purgative, diuretic, rheumatism, and the management of numerous other medical conditions. The using of plant extract as fodder for the treatment of goats with retained placenta has been documented in Nigeria (Awouters et al., 1978). The infusion derived from the stem or powdered fruits is used as a remedy for coughing. The whole cooked root is used for the treatment of wounds and sores. A formulation designed to alleviate rheumatic pains is made using boiling leaves, as described by Awouters et al. (1978). Previous research conducted by Awouters et al. (1978) has shown the ability of this substance to mitigate carageenan-induced edema in the rat paw, as well as alleviate diarrhea generated by arachidonic acid and castor oil. According to Iwu et al. (1993), the administration of a dosage ranging from 100-300 mg/kg per day in people effectively alleviated all symptoms related to adjuvant-induced polyarthritis in rats. The decoction of the plant is offered to diabetic patients in Ohafia, Abia State, Nigeria, as well as in some regions of Anglophone Cameroon, with the aim of alleviating the clinical symptoms (Iwu et al., 1993). Tachycardia is treated by administering an infusion of the inflorescence (Dioka et al., 2002). Stomach issues may be treated by administering either a rhizome infusion or the same infusion. To cure cough, respiratory issues, and a sore throat, one may consume a stem decoction, which consists of mashed stem or pounded fruit, sometimes combined with sugar cane juice.

According to Iwu et al. (1993), the debarked stem is masticated in Nigeria for the purpose of alleviating nausea and reducing thirst. The stem is subjected to a cold water extract for the treatment of minor epileptic episodes. Rhizome pulp is used for the purpose of healing abscesses and ulcers, treating toothache by applying it to teeth, and treating diarrhea and amoebic dysentery by mixing it with water. Leprosy and venereal infections may be treated by consuming either a rhizome decoction or the raw rhizome. Leaf sap is used as ocular and nasal drops for the treatment of headaches accompanied by vertigo. For the treatment of edema and fever, it is also used. Malaria is treated by consuming leaf sap or a decoction made from rhizomes. Stem sap is used for the treatment of urethral secretions, venereal ailments, jaundice, and as a preventive measure against miscarriage. Stem sap has acidic and rubefacient properties, making it a potential burn on exposed wounds. However, it also exhibits analgesic and healing properties, making it suitable for many skin conditions. The use of the stem decoction is prevalent in the treatment of rheumatoid arthritis. Hypertension is treated by administering an infusion of the desiccated aerial portions. In the treatment of worms and hemorrhoids, the

powdered stems are used as an enema. The pulped stem, when soaked in water, has potent diuretic properties. *Costus lucanusianus*, a species of the genus, is used in the Ivory Coast for the purpose of treating imminent abortion, as documented by Iwu et al. in 1993. Additionally, it has uterine relaxing properties. In the management of diabetes mellitus, *Costus schlechteri* and *Costus afer* are used as therapeutic interventions. *Costus* plants have been shown to have anti-inflammatory, purgative, antiarthritic, and antifungal properties, as reported by Bandara et al.

In 1988, Kirtikar and Basu conducted a study. The hepatoprotective and antifertility action of the rhizomes of the *costus* plant in rats has been shown in prior studies. The study conducted by Bhuyan and Zaman (2008) and Verma and Khosa (2009) revealed the hepatoprotective effect of the ethanolic extract derived from the rhizomes of the *Costus* plant in rats treated with carbon tetrachloride. Tewari et al. (1973) demonstrated the antifertility properties of saponins derived from the rhizomes of the *costus* plant in rats.

According to Agbaje et al. (2009), nephrotoxicity is a significant problem that involves the morphological destruction of intracellular organelles and cellular necrosis. This is followed by functional changes such as the depletion of the antioxidant defense system and mitochondrial damage. It is widely believed that oxidative damage plays a significant role in the development of almost all chronic renal diseases (Joy et al., 2008).

Materials and Methods

Ethical Consideration And Approval

Ethical approval for this research was sort and obtained from the ethical committees of the faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University Nnewi Campus

Location and Duration of the Study

This research was carried out at the Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi, Nigeria.

The duration of the study were three weeks for administration of the *Costus afer* Ker Gawl leaves and two weeks for acclimatization of the animals

Procurement And Preparation of Extract

The study collected fresh *Costus afer* Ker Gawl leaves from a field located in Okofia, Otolu, Nnewi South Local Government Area of Anambra State, Nigeria. Dr. Mrs. B.O. Aziagba from the Department of Botany at Nnamdi Azikiwe University Awka, Anambra State, identified and verified the leaves. The leaves underwent a drying process at room temperature for a duration of three weeks. Soon after, it was pulverized. The coarse leaf extract was immersed in distilled water for a duration of 24 hours, consisting of an overnight session. Next, it is sifted using a linen made of porcelain. Subsequently, it underwent further filtration using a filter paper. Next, it was placed in the refrigerator for use. The aqueous leaf extract of *Costus afer* Ker Gawl is prepared at regular intervals of three days to prevent spoiling.

Experimental Animals

The study was conducted using a sample of twenty male albino Wistar rats, with a weight range of 100-170g. This A total of twenty male albino rats, weighing between 100 and 170 grams, were

procured from a privately-owned animal farm located in Enugu, Enugu State, Nigeria.

The animals were let to thrive inside the Animal House facility at Nnamdi Azikiwe University, College of Health Sciences Nnewi. They were accommodated in conventional cages with ambient temperature ranging from 27 to 2°C, relative humidity ranging from 50 to 15%, and a typical photo cycle of 12 hours of darkness followed by 12 hours of light. An analytical weighing scale was used to quantify the weight of each rat. Wood shavings were acquired from a grain mill in close proximity and used as bedding materials. During the acclimation stage, the beddings were replaced a minimum of two times per week, during which they were provided with unrestricted access to water and growers feed. Eke Amobi Nnewi, Anambra State, Nigeria, was the source of the feed purchase. To assess the growth of the rats, their weight was measured. The study adhered to the guidelines outlined in the Guide for the Care and Use of Laboratory Animals (2011).

Design of the Experiment:

The present study was conducted in accordance with the guidelines outlined in Helsinki's Declaration, which provides a set of standards for the ethical treatment and use of animals. Following acclimatization and toxicity assessments, the animals were weighed and then categorized into four distinct groups, denoted as A-D. Each group consisted of five male albino rats, as specified by the OECD in 2008. The control group was designated as Group A, while the test groups were designated as Groups B, C, and D. Group A was provided with unrestricted access to normal feed and water, while Group B, C, and D were administered aqueous extracts of *Costus afer* leaves at doses of 400, 700, and 1000mg/kg, respectively. The test samples were administered to the test animals for a duration of three weeks. The groups of four animals were housed in standard cages that provided sufficient room for their free mobility and enough ventilation throughout the duration of the experiment. During nighttime, kerosene lamps were positioned in close proximity to their cages to provide warmth for the animals. The cages' bedding, consisting of sawdust, was

replaced on a daily basis. Additionally, the cages were subjected to weekly washing and disinfection to ensure the animals' comfort and overall welfare. They were provided with feed (Top Feed Nig. LTD) and water.

Group A(control group): received only water *ad libitum* and feed for 21 days

Group B: received 400mg/kg of the extract for 21 days.

Group C: received 700mg/kg of the extract for 21 days.

Group D: received 1000mg/kg of the extract for 21 days.

QUALITATIVE PHYTOCHEMICAL ANALYSIS:

Phytochemical screening was performed on the freshly prepared sample of *C.afer KerGawl* in the Department of Anatomy, Nnamdi Azikiwe University, Nigeria using standard procedures to identify chemical constituents as described by Harborne (1973) and Sofowora (1993).

Alkaloids: The aqueous extracts were evaporated to dryness and the residue (0.5g) was stirred in 5 ml of 1% HCl on a steam bath and filtered while hot. Distilled water was added to the residue and 1 ml of the filtrate was treated with a few drops of Wagner's reagent. A reddish brown precipitate indicates the presence of alkaloids.

Flavonoid: Two milliliters of dilute sodium hydroxide was added to 2 ml of the extract. The appearance of a yellow colour indicates the presence of flavonoids.

Phenols: Equal volumes (1 ml) of extracts and iron(III)chloride were mixed. A deep bluish green solution gave an indication of the presence of phenols.

Tannins: A portion of the extract was dissolved in water, after which the solution was clarified by filtration 10% ferric chloride solution was then added to the resulting filtrate. The appearance of a bluish black.

Table.1 Qualitative Phytochemical Composition (Mg/100g) Of Aqueous Extract Of *Costus Afer*.

PHYTOCHEMICAL	AQUEOUS EXTRACT
Alkaloids	+
Flavonoids	++
Saponins	++
Tannins	+
Phenols	++

Acute Toxicity (Ld50):

The median lethal dose (LD50) of aqueous leaf extract of *costus afer ker Gawl* was carried out in the department of Physiology, Faculty of Basic Medical Sciences, Nnamdi Azikiwe university, Nnewi Campus. This was determined using the modified method of Dietrich Lorke (1983). The median Lethal dose was found to be above 2000mg/kg.

Organ Collection

On the 14th night, the rats were fasted and on the 15th day they were sacrificed under ether anesthesia and the kidneys were harvested. The organ weights were taken. Whole blood was obtained from ocular puncture from each of the rats, collected into heparinized bottles and the blood samples (plasma) were analyzed for the activity of the biochemical parameters of the kidney (renal function tests) and liver (liver function tests).

The liver and kidney were immediately fixed in 10% formal saline. Other tissue processing techniques were carried afterwards to prepare a tissue section.

Tissue Processing: After weighing the organs, a small part of the Amygdala tissues were cut out and immediately fixed in 10% formal saline in order to preserve the various constituents of the cells in their normal micro anatomical position and to prevent autolysis and putrefaction. After fixation the tissues were dehydrated to remove water and other substances. This was carried out in different percentages of alcohol 50%, 70% and 95% absolute. In each grade of alcohol, tissues were changed twice for two (2) hours, one (1) hour for each change. After dehydration, tissues were cleared in xylene for two (2) hours after which infiltration was done in molten paraffin wax at a temperature of 60oc for two (2) hours, each in two changes. When the paraffin wax cools, it sets as a hard block which allows for easy sectioning of the tissues. The tissue sections were produced by normal histochemical methods of dehydration, clearing, impregnation, embedding, sectioning and staining (with H&E). The micrographs of the relevant stained sections were subsequently taken with the aid of a light microscope.

Statistical Analysis: The data were analyzed using SPSS version 23. Values were represented as MEAN and SEM, Relative Organ weight (Brain) were analyzed using One way ANOVA, followed by Post Hoc LSD multiple comparison. Body weight was analyzed using Student dependent T-test. Values were considered significant at $P < 0.05$.

Results

Physical and Behavioural Observations

During the period of acclimatization, all the rats were healthy, with smoothly laid hairs on their skin, pinkish eyes, normal skin colour and they increased in size. After commencement of the experiment, group A showed no clinical signs while the remaining groups had staggering, decreased food intake and No death was recorded in all groups

Effect of Aqueous Leaf Extract Of *Costus Afer Ker Gawl* (Bush Cane) On Body Weight Changes

TABLE 2: The effect aqueous leaf extract of *costus afer ker gawl* on Body weight.

		MEAN±SEM	P-VALUE	T-VALUE
Group A	Initial	100.00±0.00		
	Final	170.00±12.90	0.012*	-5.422
Group B	Initial	160.00±15.00		
	Final	145.00±13.14	0.007*	-6.789
Group C	Initial	165.00±8.16		
	Final	155.00±14.14	0.041*	-3.464
Group D	Initial	200.00±0.00		
	Final	182.00±11.81	0.013*	-5.290

Data was analyzed using Student dependent T-test, and values were considered significant at $P < 0.05$. * $P < 0.05$ means significant and $P > 0.05$ means not significant.

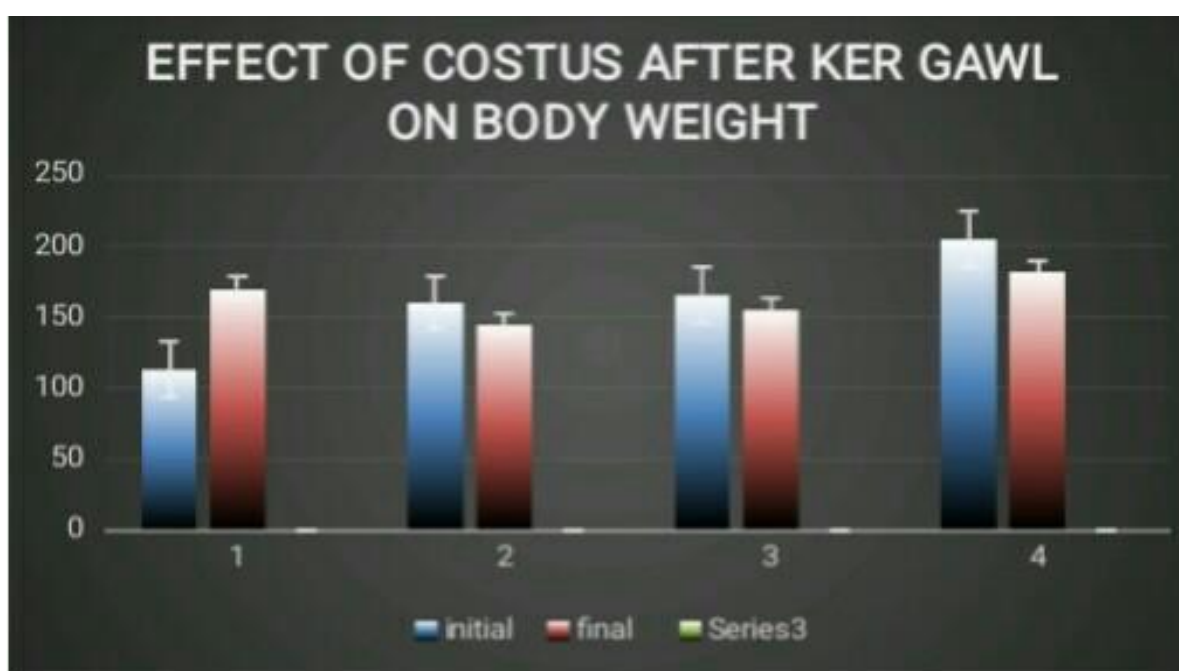


Table 2: shows that, there was a significant increase in the body weight in group 1 (control), when comparing the Initial weight (100.00 ± 0.00) to the Final weight (170.00 ± 12.90). For group 2, there was a significant decrease in the body weight when comparing the Initial weight (160.00 ± 15.00) to the Final weight (145.00 ± 13.14). For group 3, there was a significant decrease in the body weight when comparing the Initial weight (165.00 ± 8.16) to the Final body weight (155.00 ± 14.14). For group 4, there was a significant decrease in the body weight when comparing the Initial weight (200.00 ± 0.00) to the Final weight (182.00 ± 11.81).

Fig 1: Bar chart showing the Effect of aqueous extract of *costus afer ker gawl* on Body weight.

The chart above represents the result as shown in the table 4.1 it shows that in all the experimental groups (B,C, D) there was significant decrease in the body weight when initial body weight was compared to final body weight.

Effect Of Aqueous Extract Of *Costus Afer Ker Gawl* (Bush Cane) On Relative Kidney Weight.

Table 3: Effect Of Aqueous Leaf Extract Of *Costus Afer Ker Gawl* (Bush Cane) On Relative Kidney Weight

		MEAN	$\pm$ SEM	P value	F value
Relative KIDNEY weight (g)	Group A	0.38	± 0.01		
	Group B	0.23	± 0.00	0.055	
	Group C	0.22	± 0.03	0.078	2.153
	Group D	0.20	± 0.00	0.282	

Data were analyzed using One way ANOVA followed by Post HOC Fisher's LSD multiple comparism, and data were considered significant at $P < 0.05$. * $P < 0.05$ means significant and $P > 0.05$ means not significant.

There is an insignificant decrease in group B (0.23 ± 0.00), group C (0.22 ± 0.03) and group D (0.20 ± 0.00) when compared to group A (0.38 ± 0.01).

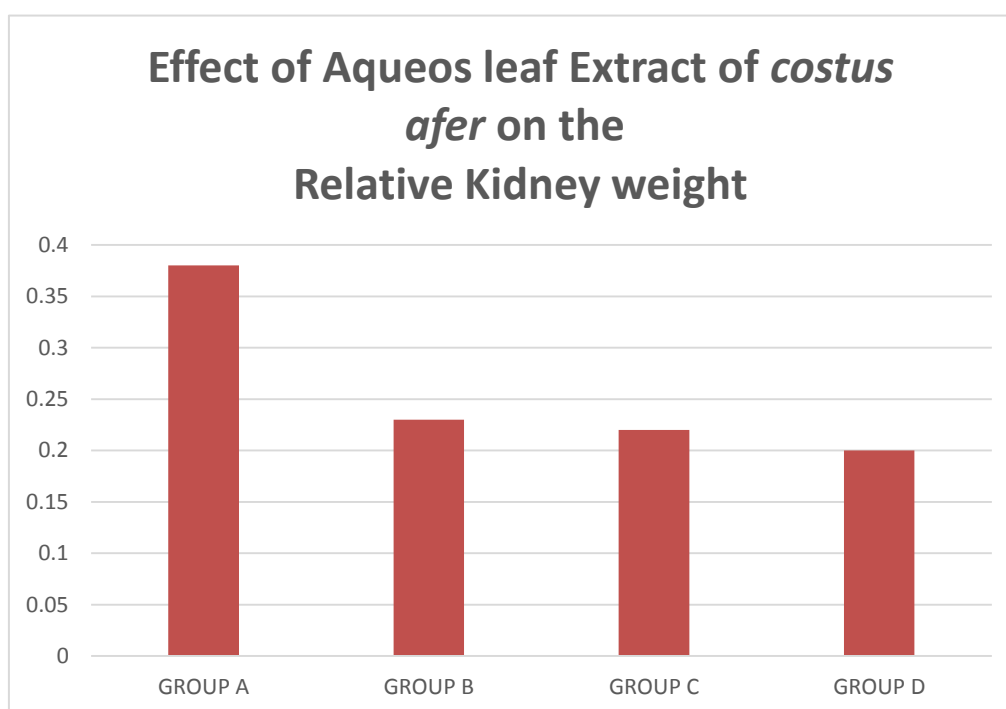


Fig 2: Bar chart showing the Effect of of *costus afer ker gawl* on Relative Kidney weight.

The chart above shows the relative kidney weight in all the groups, group C that received 700mg/kg of *costus afer ker gawl* followed by group D that received 1000mg/kg of *costus afer ker gawl* had the lowest kidney weight and group B that received 400mg/kg of *costus afer ker gawl* had insignificant decrease when compared to group A while group A had the highest relative kidney weight.

Effect Of Aqueous Leaf Extract Of *Costus Aferker Gwal* On The Urea And Creatinine Of Male Albino Wistar Rats

Table 4: ACTIVITIES OF SERUM LEVELS OF UREA

GROUP	UREA	p	F
Group A	23.26±3.40		
Group B	24.30±2.66	0.071	6.235
Group C	25.22±1.75	0.054	
Group D	30.24±1.22	0.048	

The data above was analysed using the SPSS Anova and Post Hoc LSD test and data was flagged significant at $P \leq 0.05$ and $P > 0.05$ means not significant.

The serum urea level of the controls group was 23.26±3.40, that of group B was 24.30±2.66. group C had urea level of 25.22±1.75 while that of group D was 30.24±1.22.

A test of Anova and Post Hoc LSD test revealed that compared with control group, group B had insignificantly higher urea level ($p = 0.071$); group C was rather significantly higher than the control ($p=0.054$) and group D was also significantly higher, ($p=0.048$) when compared with the control.

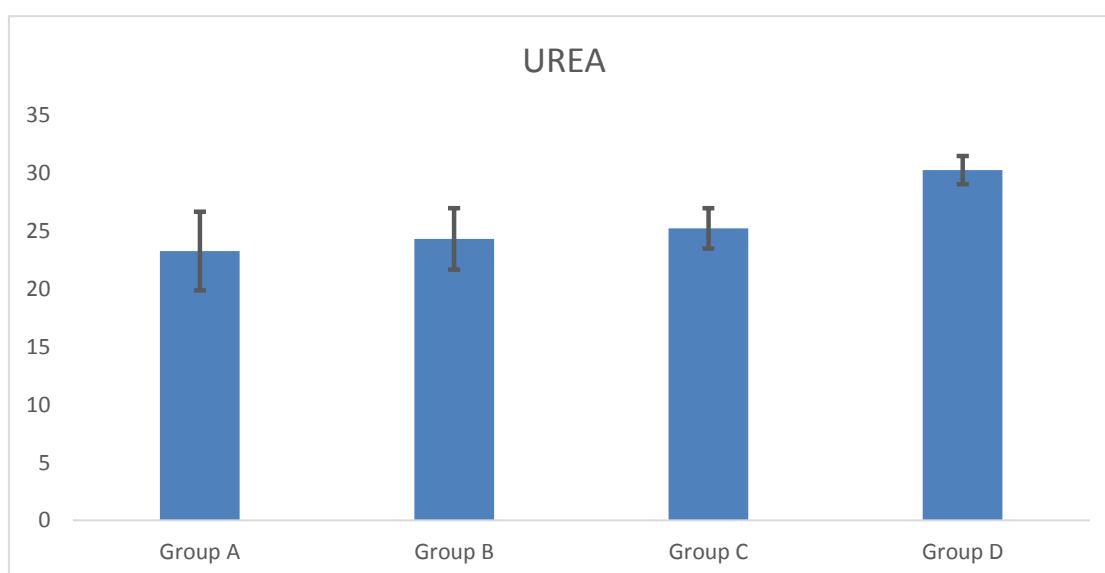


Fig 3: A chart showing serum Urea levels of the various groups in mean and standard deviation. The above chart was prepared using Microsoft Excel 2016.

The serum Urea level of the controls group was 27.48±8.35, that of group B was 28.19±9.43. group C had Urea level of 28.70±9.39 while that of group D was 31.36±7.20.

From the chart, it can be inferred that in order of increasing magnitude, there is the control, the group B, group C and group D. Group D animals clearly showed higher serum Urea concentration level.

Table 5: Activities Of Serum Levels Of Creatinine

GROUP	CREATININ	p	F
Group A	2.80±0.60		
Group B	2.84±0.42	0.1	6.235
Group C	2.46±1.26	0.05	
Group D	2.95±0.23	0.05	

The data above was analysed using the SPSS Anova and Post Hoc LSD test and data was flagged significant at $P \leq 0.05$ and $P > 0.05$ means not significant.

The serum creatinine level of the controls group was 2.80 ± 0.60 , that of group B was 2.84 ± 0.42 . group C had creatinine level of 2.46 ± 1.26 while that of group D was 2.95 ± 0.23 .

A test of Anova and Post Hoc LSD test revealed that compared with control group, group B had insignificantly higher creatinine level ($p = 0.1$); group C was significantly lower in creatinine level than the control ($p=0.05$). Finally, Group D was also significantly higher in creatinine level, ($p=0.05$), when compared with the control.

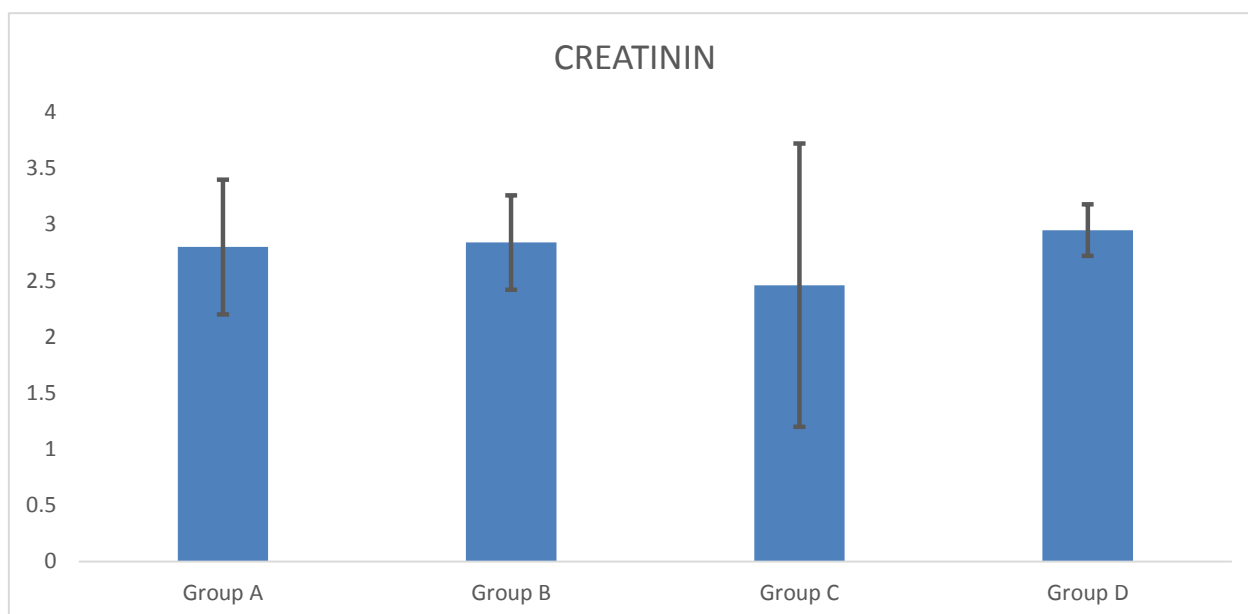


Fig 4: A chart showing serum creatinine levels of the various groups in mean and standard deviation. The above chart was prepared using Microsoft Excel 2016.

The serum creatinine level of the controls group was 2.8 ± 0.60 , that of group B was 2.84 ± 0.42 . group C had creatinine level of 2.46 ± 1.26 while that of group D was 2.95 ± 0.23 . From the chart, it can be inferred that in order of increasing magnitude, there is the group C, the control group, the group B, and group D. Group D animals clearly showed higher serum Creatinine concentration level. Most conspicuous quality of the chart is the large standard deviation bar of the group C animals

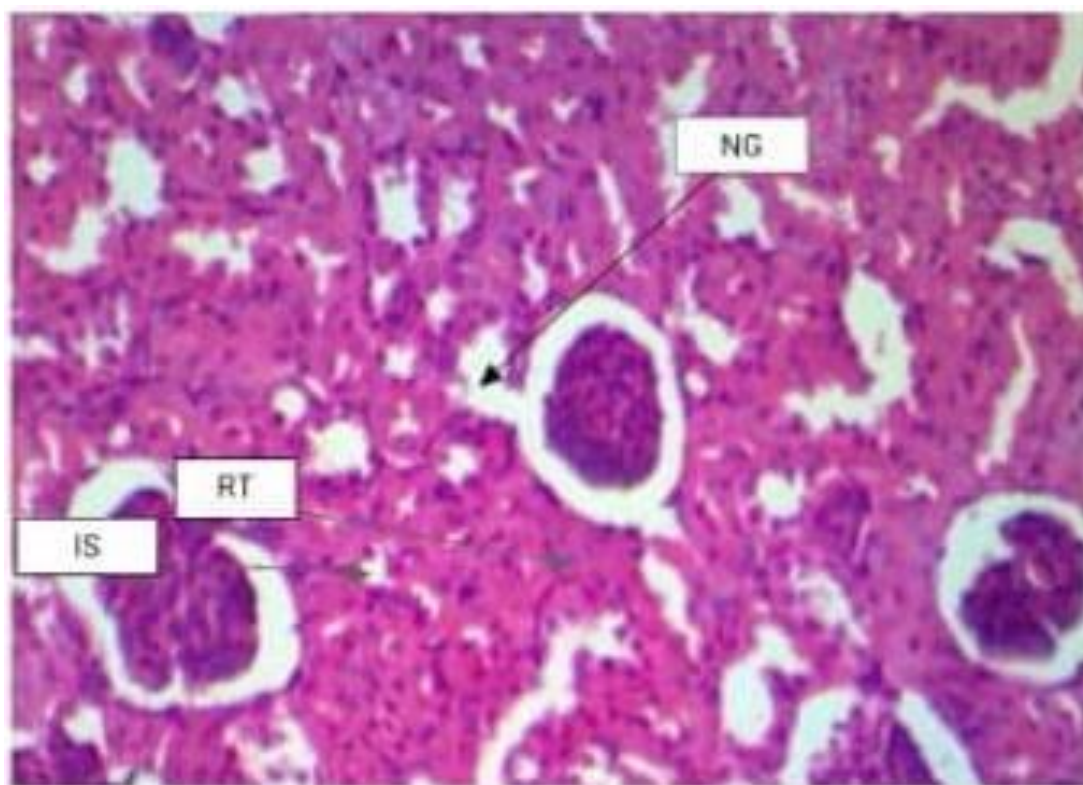


Fig 5, Photomicrograph of group A kidney section ($\times 400$) (H/E) shows normal histology with numerous glomeruli, renal tubules and blood vessels. The interstitial space appears unremarkably.

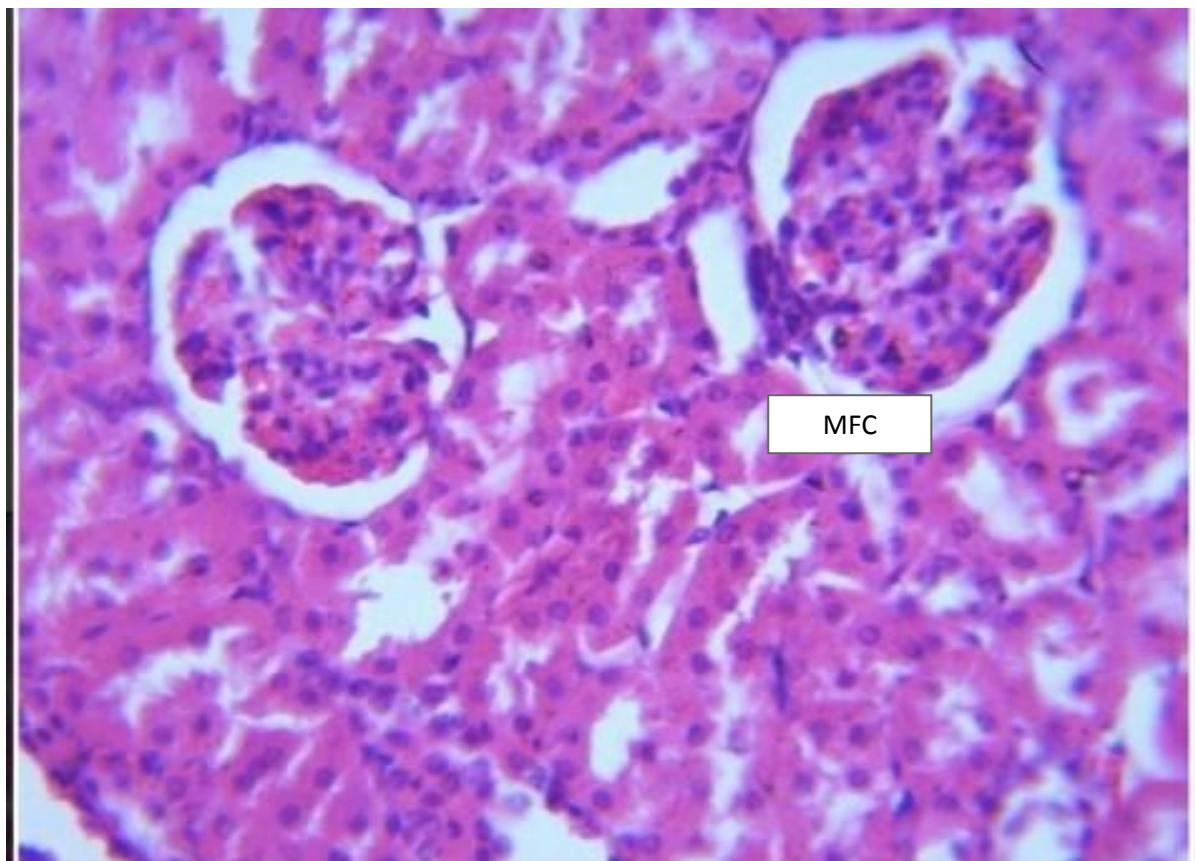


Fig 6: Photomicrograph of group B kidney section treated with 400mg/kg of Aqueous leaf extract of *costus afer* ($\times 400$) (H/E) shows normal renal architecture.

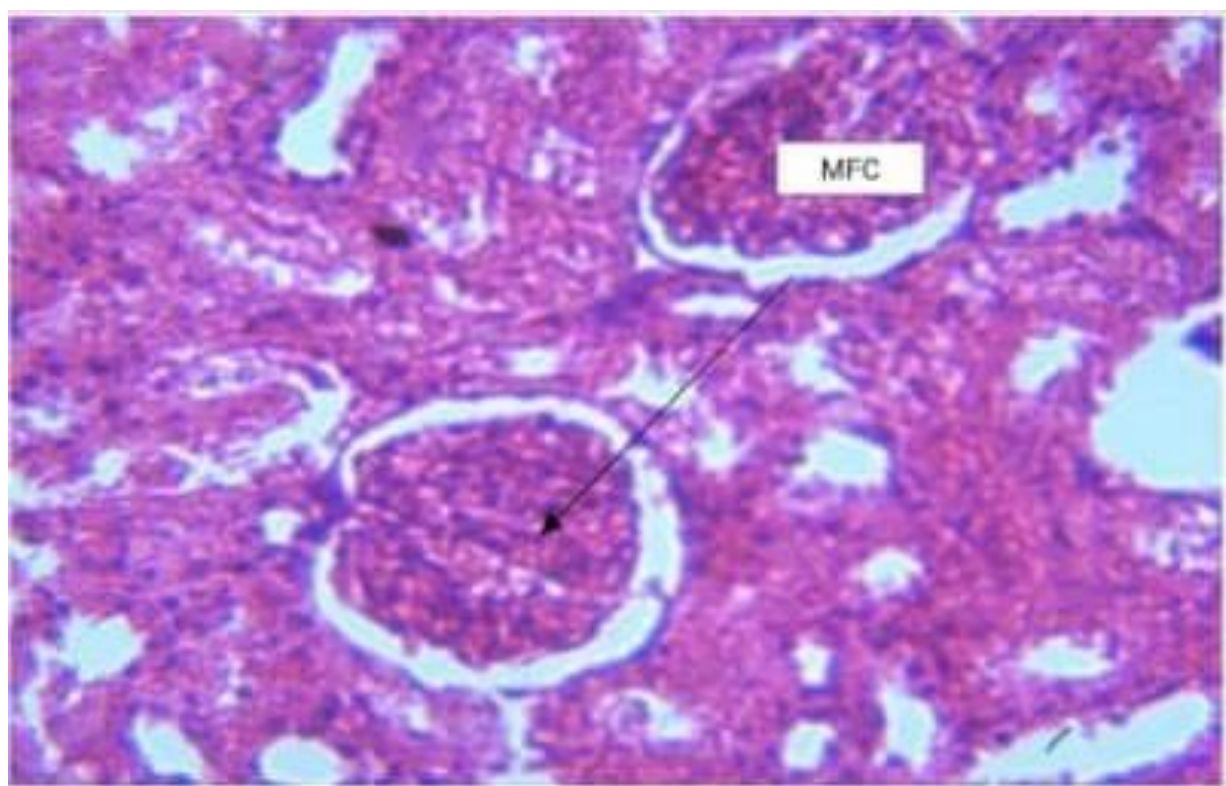


Fig 7: Photomicrograph of group C kidney section treated with 700mg/kg of aqueous leaf extract of *costus afe* $\times 400$) (H/E) shows renal tissue with mild fatty change otherwise normal.

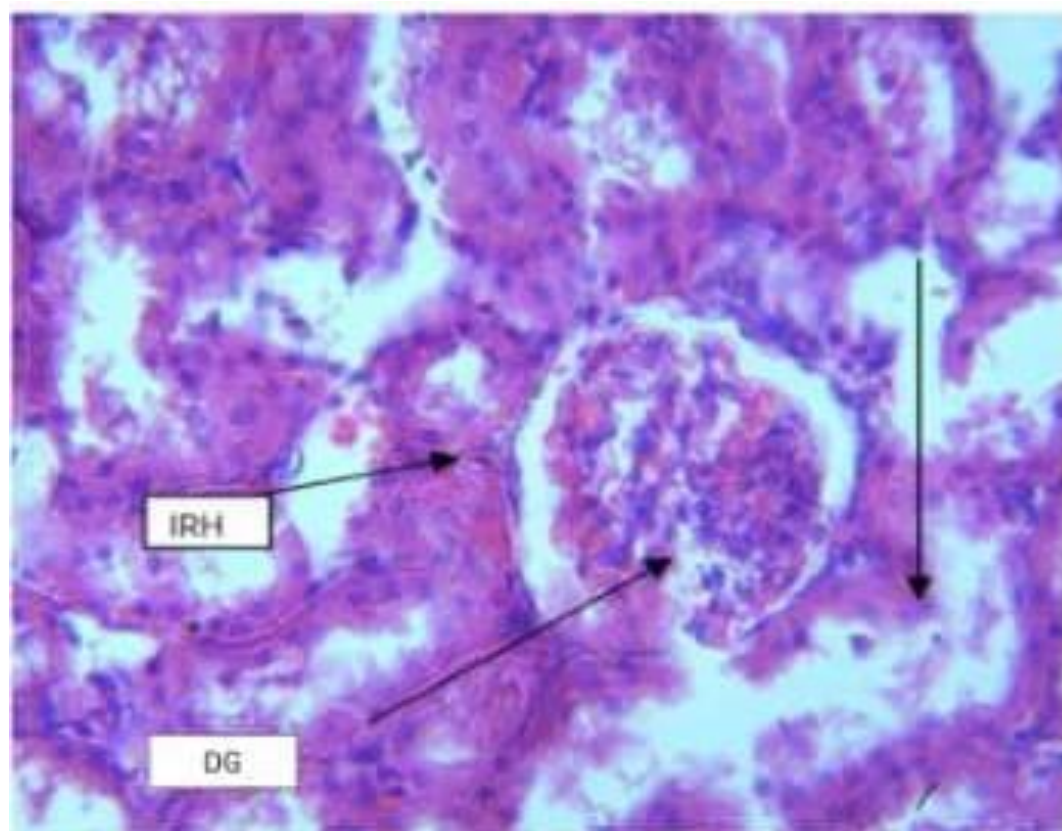


Fig 8: Photomicrograph of group D kidney section treated with 1000mg/kg of aqueous leaf extract of *costus afer* ($\times 400$) (H/E) shows severe damage on the renal tissue with necrosis, renal hemorrhage, Loss of renal tissue and degenerated glomeruli.

Discussion

In this work, the cytotoxic effects of an aqueous leaf extract derived from *Costus afer* were examined in male albino Wistar rats. The modification in the comparative body weight ratio serves as an indicator of dysfunction in the regular operation of the organs. The use of organ weights is extensively acknowledged in the assessment of toxicities linked with test articles.

The findings of this study indicate a notable reduction in the average body weight of rats that were administered aqueous leaf extract of *C. afer* ker Gawl at moderate and high doses, in comparison to the control group. However, the precise mechanism underlying this decrease in body weight remains unclear, potentially due to the presence of one or more phytochemicals within the extract. This finding is consistent with a prior investigation conducted by Ezejiofor and Orisakwe (2013).

The findings also indicated a negligible reduction in the weight of the kidney. However, the exact mechanism of action remains uncertain, but it is possible that the presence of phytochemicals in the extract may be responsible. This aligns with the research conducted by Ezejiofor et al. (2013), which found no significant difference in the average weight of the liver and kidney when administered an aqueous leaf extract of *Costus afer*.

The extract had an impact on the levels of creatinine and urea in group D, as seen in the current investigation. Nevertheless, a marginal but noteworthy elevation in the concentrations of urea and creatinine was seen in comparison to the control group. This finding contradicts the earlier study conducted by Ezejiofor et al.

(2013), which reported no significant disparity in creatinine and urea levels.

The treated animals exhibited a reduction in the relative organ weight of the kidney, along with notable alterations in other biochemical markers, across all tested dosages. This suggests the potential for renal toxicity when administering large doses. According to the Environmental Health and Safety Toxicology and Exposure Guidelines (2000), the findings are consistent with a prior research.

The histological examination of the kidney revealed that Group D had histopathological alterations in the kidney. The potential toxicity of the aqueous leaf extract of *Costus afer* on renal function may be attributed to the presence of one or many phytochemical compounds within the extract. Additionally, it might be attributed to their synergistic impact.

Based on the findings derived from the current investigation, there is evidence suggesting that *Costus afer* may possess potential. The renal tissue in group D exhibits renotoxicity, characterized by significant damage, necrosis, renal bleeding, loss of renal tissue, and degeneration of glomeruli.

To summarize The findings of this study have shown that the administration of larger dosages of *Costus afer* may result in renal toxicity. It is advisable to refrain from regular ingestion of the aqueous leaf extract of *Costus afer* ker Gawl.

RECOMMENDATION

I recommend the need for relevant health/nutritional agencies in Nigeria should create awareness on the effect of *Costus afer* ker

Gawl on the Liver and Kidney for public health importance toward the effect of consumption of *Costus afer* in the Liver and Kidney.

I also recommend that further research should be carried out on the effect of leaf extract of *Costus afer* on other vital organ like Ovary, Testis etc.

REFERENCES

1. Abdel-Misih, S. R., & Bloomston, M. (2010). Liver anatomy. *Surgical Clinics*, 90(4), 643-653.
2. Agbaje EO, Adeneye AA, Daramola AO, (2009). Biochemical and toxicological studies of aqueous extract of *Syzgium aromaticum* (L) Merr. and Perry (Myrtaceae) in rodents. *African Journal of Traditional Complement and Alternative Medicine*.;6:241–254.
3. Akendengue, B., & Louis, A. M. (1994). Medicinal plants used by the Masango people in Gabon. *Journal of ethnopharmacology*, 41(3), 193-200.
4. Akpan, M.M., Odeomena, C.S., Nwachukwu, C.N and Danladi, B, (2012). Antimicrobial assessment of ethanolic extract of *Costus afer* leaves. *Asian Journal of Plant Science and Research*, 2(3); 335-341.
5. Anaga, A. O., Njoku, C. J., Ekejiuba, E. S., Esiaka, M. N., & Asuzu, I. U. (2004). Investigations of the methanolic leaf extract of *Costus afer*. Ker for pharmacological activities in vitro and in vivo. *Phytomedicine*, 11(2-3), 242-248.
6. Anyasor, G. N., Ogunwenmo, O., Oyelana, O. A., & Akpofunure, B. E. (2010). Phytochemical constituents and antioxidant activities of aqueous and methanol stem extracts of *Costus afer* Ker Gawl.(Costaceae). *African Journal of Biotechnology*, 9(31), 4880-4884.
7. Anyasor, G. N., Funmilayo, O., Odutola, O., Olugbenga, A., & Oboutor, E. M. (2014). Chemical constituents in n-butanol fractions of *Castus afer* ker Gawl leaf and stem. *Journal of Intercultural Ethnopharmacology*, 3(2), 78.
8. Aweke, G., (1997). Costaceae. In: Edwards, S., Mesfin Tadesse, Demissew Sebsebe & Hedberg, I. (Editors). Flora of Ethiopia and Eritrea. Volume 6. Hydrocharitaceae to Arecaceae. The National Herbarium, Addis Ababa University, Addis Ababa, Ethiopia and Department of Systematic Botany, Uppsala University, Uppsala, Sweden. pp. 330–332.
9. Awouters, F., Niemegeers, C. J. E., Lenaerts, F. M., & Janssen, P. A. J. (1978). Delay of castor oil diarrhoea in rats: a new way to evaluate inhibitors of prostaglandin biosynthesis. *Journal of Pharmacy and Pharmacology*, 30(1), 41-45.
10. Banerjee, T., Crews, D. C., Wesson, D. E., Tilea, A., Saran, R., Rios Burrows, N., ... & Centers for Disease Control and Prevention Chronic Kidney Disease Surveillance Team. (2014). Dietary acid load and chronic kidney disease among adults in the United States. *BMC nephrology*, 15, 1-12.
11. Berg, T., DeLanghe, S., Al Alam, D., Utley, S., Estrada, J., & Wang, K. S. (2010). β -catenin regulates mesenchymal progenitor cell differentiation during hepatogenesis. *Journal of Surgical Research*, 164(2), 276-285.
12. Berg T, DeLanghe S, Al Alam D, Utley S, Estrada J, Wang KS (2010). " β -catenin regulates mesenchymal progenitor cell differentiation during hepatogenesis" . *Journal of Surgical Restoration* . 164 (2): 276–85.
13. Borchers, A. T., Leibushor, N., Naguwa, S. M., Cheema, G. S., Shoenfeld, Y., & Gershwin, M. E. (2012). Lupus nephritis: a critical review. *Autoimmunity reviews*, 12(2), 174-194.
14. Brenner, B. M.; Meyer, T. W.; Hostetter, T. H. (1982). "Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease". *The New England Journal of Medicine*. 307 (11): 652–659.
15. Burkill, H.M., (2000). The useful plants of West Tropical Africa. 2nd Edition. Volume 5, Families S–Z, Addenda. Royal Botanic Gardens, Kew, Richmond, United Kingdom. 686 pp.
16. Chen, W., & Abramowitz, M. K. (2014). Metabolic acidosis and the progression of chronic kidney disease. *BMC nephrology*, 15, 1-8. doi: 10.1186/1471-2369-15-55.
17. Chhabra, S.C., Mahunnah, R.L.A. & Mshiu, E.N., (1993). Plants used in traditional medicine in eastern Tanzania. 6. Angiosperms (Sapotaceae to Zingiberaceae). *Journal of Ethnopharmacology* 39: 83–103.
18. Chu, J., & Sadler, K. C. (2009). New school in liver development: lessons from zebrafish. *Hepatology*, 50(5), 1656-1663.
19. Clemente, C. D. (1997). Anatomy: a regional atlas of the human body. (No Title).
20. Hansen, K. L., Nielsen, M. B., & Ewertsen, C. (2015). Ultrasonography of the kidney: a pictorial review. *Diagnostics*, 6(1), 2.

21. Coresh, J., Selvin, E., Stevens, L. A., Manzi, J., Kusek, J. W., Eggers, P., ... & Levey, A. S. (2007). Prevalence of chronic kidney disease in the United States. *Jama*, 298(17), 2038-2047.
22. Cotran, R., Stanley, L., Abbas, K., & Nelson, F. (2005). *Robins and Cotran pathologic basic of disease*, St Louis, MO.
23. Dancygier, H., & Dancygier, H. (2010). Primary Biliary Cirrhosis. *Clinical Hepatology: Principles and Practice of Hepatobiliary Diseases*, 895-907.
24. Dekkers, A. J., Rao, A. N., & Goh, C. J. (1991). In vitro storage of multiple shoot cultures of gingers at ambient temperatures of 24–29 °C. *Scientia Horticulturae*, 47(1-2), 157-167.
25. Deny, P. (2006). Hepatitis delta virus genetic variability: from genotypes I, II, III to eight major clades?. *Hepatitis delta virus*, 151-171.
26. Deriemaeker, P., Aerenhouts, D., Hebbelinck, M., & Clarys, P. (2010). Nutrient based estimation of acid-base balance in vegetarians and non-vegetarians. *Plant foods for human nutrition*, 65, 77-82.
27. Dioka, C., Orisakwe, O. E., Afonne, O. J., Agbasi, P. U., Akumka, D. D., Okonkwo, C. J., & Ilondu, N. (2002). Investigation into the haematologic and hepatotoxic effects of rinbacin in rats. *Journal of Health Science*, 48(5), 393-398.
28. Ebenso, I. E., & Okafor, N. M. (2002). Alternative diets for growing Archachatina marginata snails in south-eastern Nigeria.
29. Edeoga, H. O., & Okoli, B. E. (2000). Chromosome numbers of Costus lucanusianus (Costaceae) in Nigeria. *Folia Geobotanica*, 35, 315-318.
30. Edeoga, H. O., & Okoli, B. E. (1996). Apomictic behaviour in Costus afer–C. lucanusianus (Costaceae) complex in Nigeria. *Feddes Repertorium*, 107(1-2), 75-82.
31. Elias, H., & Bengelsdorf, H. (1952). The structure of the liver of vertebrates. *Cells Tissues Organs*, 14(4), 297-337.
32. Ezeonwumelu, J. O. C., Julius, A. K., Muhoho, C. N., Ajayi, A. M., Oyewale, A. A., Tanayen, J. K., ... & Reddy, P. M. K. (2011). Biochemical and histological studies of aqueous extract of Bidens pilosa leaves from Ugandan Rift Valley in rats. *British Journal of Pharmacology and Toxicology*, 2(6), 302-309.
33. Jain, N. C., & Zinkl, J. G. (Eds.). (2000). *Schalm's veterinary hematology*. Lippincott Williams & Wilkins.
34. Ghent, Cam N (2009). "Who should be performing liver biopsies?" *.Canadian Journal of Gastroenterology*. 23(6):437–438.
35. Ghent, Cam N (2009). "Who should be performing liver biopsies?".*Canadian Journal of Gastroenterology*. 23 (6): 437–438. PMC 2721812.
36. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015" *.Lancet*. 388 (153): 1545–1602. 2016.
37. Goraya, Nimrit; Simoni, Jan; Jo, Chan-Hee; Wesson, Donald E. (2013). "A comparison of treating metabolic acidosis in CKD stage 4 hypertensive kidney disease with fruits and vegetables or sodium bicarbonate". *Clinical Journal of the American Society of Nephrology*. 8 (3): 371–381.
38. Goraya, Nimrit; Wesson, Donald E. (2014). "Is dietary Acid a modifiable risk factor for nephropathy progression?".*American Journal of Nephrology*. 39 (2): 142–144.
39. Greaves, P. (2011). *Histopathology of preclinical toxicity studies: interpretation and relevance in drug safety evaluation*. Academic Press.
40. Guo, B., Zhang, S., Wang, S., & Liang, Y. (2009). Expression, mitogenic activity and regulation by growth hormone of growth hormone/insulin-like growth factor in Branchiostoma belcheri. *Cell and tissue research*, 338(1), 67-77.
41. Heporaitis, L; Freeny, PC (1998). "Hepatic and portal surface veins: A new anatomic variant revealed during abdominal CT". *AJR. American journal of roentgenology*.
42. Emmanuel, E. C., & Delesi, K. H. Evaluation of antimalarial effect of methanol root extract of Costus Lucanusianus in the treatment of malaria-infested albino mice.
43. Hirschfield, GM; Gershwin, ME (2013). "The immunobiology and pathophysiology of primary biliary cirrhosis". *Annual Review of Pathology*. 8:303–330.
44. Ichimura T, Hung CC, Yang SA, Stevens JL, Bonventre JV, (2004). Kidney injury molecule-1: A tissue and urinary biomarker for nephrotoxicant-induced renal injury. *American Journal of Renal Physiology*.286:552–563.

45. Idee, J. M., Boehm, J., Prigent, P., Ballet, S., & Corot, C. (2006). Role of apoptosis in the pathogenesis of contrast media-induced nephropathy and hints for its possible prevention by drug treatment. *Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Inflammatory and Anti-Allergy Agents)*, 5(2), 139-146.
46. Iwu, M. M. (1993). African medicinal plants. *CRC Press, Maryland. Jamkhande, PG, Ajgunde, BR, & Jadge, DR (2017). Annona cherimola Mill.(Custard apple): A review on its plant profile, nutritional values, traditional claims and ethnomedicinal properties. Oriental Pharmacy and Experimental Medicine, 17(3), 189-201.*
47. Iwu, M. M., & Anyanwu, B. N. (1982). Phytotherapeutic profile of Nigerian herbs I: anti-inflammatory and anti-arthritic agents. *Journal of ethnopharmacology*, 6(3), 263-274.
48. Jelkmann, Wolfgang (2001). "The role of the liver in the production of thrombopoietin compared with erythropoietin". *European Journal of Gastroenterology & Hepatology*. 13(7): 791–801.
49. Jelkmann, Wolfgang (2001). "The role of the liver in the production of thrombopoietin compared with erythropoietin". *European Journal of Gastroenterology & Hepatology*. 13 (7): 791–801.
50. Joy J, Nair CK, (2008). Amelioration of cisplatin induced nephrotoxicity in Swiss albino mice by *Rubia cordifolia* extract. *Journal of Cancer Restoration Therapy*. 4:111–5.
51. Juárez-Rojop, I. E., Díaz-Zagoya, J. C., Ble-Castillo, J. L., Miranda-Osorio, P. H., Castell-Rodríguez, A. E., Tovilla-Zárate, C. A., ... & Bermúdez-Ocaña, D. Y. (2012). Hypoglycemic effect of *Carica papaya* leaves in streptozotocin-induced diabetic rats. *BMC complementary and alternative medicine*, 12, 1-11.
52. Kambu, K., Tona, L., Luki, N., Cimaga, K., & Makuba, W. (1989). Evaluation de l'activité antimicrobienne de quelques préparations traditionnelles antidiarrhéiques utilisées dans la ville de Kinshasa-Zaïre. *Bulletin de Médecine Traditionnelle et Pharmacopées*, 3(1), 15-24.
53. Kampf, Caroline; Mardinoglu, Adil; Fagerberg, Linn; Hallström, Björn M.; Edlund, Karolina; Lundberg, Emma; Pontén, Fredrik; Nielsen, Jens; Uhlen, Mathias (2014). "The human liver-specific proteome defined by transcriptomics and antibody-based profiling". *The FASEB Journal*. 28(7): 2901–2914.
54. Kanda, E., Ai, M., Kuriyama, R., Yoshida, M., & Shiigai, T. (2014). Dietary acid intake and kidney disease progression in the elderly. *American Journal of Nephrology*, 39(2), 145-152.
55. Kawarada, Y; Das, BC; Taoka, H (2000). "Anatomy of the hepatic hilar area: the plate system". *Journal of Hepato-Biliary-Pancreatic Surgery*. 7(6): 580–586.
56. Kluwe WM, (1981). Renal function tests as indicators of kidney injury in subacute toxicity studies. *Toxicol Appl Pharmacol.*; 57:414–424.
57. Kmiec Z (2001). "Co-operation of liver cells in health and disease". *Advanced Anatomy and Embryology Cellular Biology*. 161: III–XIII, 1–151.
58. Krishna, Gopi ; Hillman, James (1970). *Kundalini – the evolutionary energy in man*. London: Stuart & Watkins. p. 77. ISBN 1570622809.
59. Kuntz, E., & Kuntz, H. D. (2009). *Hepatology: Textbook and atlas*. Springer Science & Business Media.
60. Lin, Julie; Fung, Teresa T.; Hu, Frank B.; Curhan, Gary C. (2011). "Association of dietary patterns with albuminuria and kidney function decline in older white women: a subgroup analysis from the Nurses' Health Study" *.American Journal of Kidney Diseases*. 57 (2): 245–254.
61. Lin, Julie; Hu, Frank B.; Curhan, Gary C. (2010). "Associations of diet with albuminuria and kidney function decline" *.Clinical Journal of the American Society of Nephrology* . 5 (5): 836–843.
62. Lin, R. C., Lacaille Dubois, M.A., Hanquet, B., Correia, M. & Chauffert, B., 1997. New diosgenin glycosides from *Costus afer*. *Journal of Natural Products* 60(11): 1165–1169.
63. Lin, R.C., Hanquet, B. & Lacaille Dubois, M.A., (1996). Aferoside A, a steroidal saponin from *Costus afer*. *Phytochemistry* 43(3): 665–668.
64. Lock, J.M., (1985). Zingiberaceae. In: Polhill, R.M. (Editor). *Flora of tropical East Africa*. A.A. Balkema, Rotterdam, Netherlands. 40 pp.
65. Maton, Anthea; Jean Hopkins; Charles William McLaughlin; Susan Johnson; Maryanna Quon Warner; David LaHart; Jill D. Wright (1993). *Human Biology and Health*. Englewood Cliffs, New Jersey, USA: Prentice Hall. ISBN 0-13-981176-1.

66. McMinn, R. M. H. (2003). "Liver and Biliary Tract" . Last's Anatomy: Regional and Applied . Elsevier. pp. 342–351.
67. Moody, J.O. & Okwagbe, K.E., (2003). Topical anti-inflammatory activity of *Costus afer*. *Nigerian Journal of Natural Products and Medicine* 7: 46–48.
68. Moundipa, P.F., Melanie Flore, K.G., Bilong Bilong, C.F. & Bruchhaus, I., (2005). In vitro amoebicidal activity of some medicinal plants of the Bamun region (Cameroon). *African Journal of Traditional, Complementary and Alternative Medicines* 2(2): 113–121.
69. Okoli, I.C., Ebere, C.S., Uchegbu, M.C., Udah, A. & Ibeawuchi, I.I., (2003). A survey of the diversity of plants utilized for small ruminant feeding in south-eastern Nigeria. *Agriculture, Ecosystems & Environment* 96(13): 147–154.
70. Oliver B, (1960). Ibadan: Nigerian College of Arts, Sci and Tech, University Press Nigeria;. Medicinal Plants in Nigeria; pp. 1–33.
71. Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S, (2008). The current state of serum biomarkers of hepatotoxicity. *Toxicology*.;245:194–205. [PubMed]
72. Patel RB, Raval JD, Gandhi TP, (1988). Chakravarty BK. Hepatoprotective effect of Indian medicinal plants Part I. *Indian Drugs*.;25:224–229.
73. Pocock, Gillian (2006). *Human Physiology* (Third ed.). Oxford University Press. p. 404.
74. Portilla D, Safar AM, Shannon ML, Penson RT, (2013). Cisplatin nephrotoxicity. In: UpToDate, Palevsky PM (Ed), UpToDate, Waltham, MA, <http://www.uptodate.com/contents/cisplatin-nephrotoxicity>.
75. Rajani R, Melin T, Björnsson E, Broomé U, Sangfelt P, Danielsson A, Gustavsson A, Grip O, Svensson H, Löf L, Wallerstedt S, Almer SH (2009). "Budd-Chiari syndrome in Sweden: epidemiology, clinical characteristics and survival – an 18-year experience". *Liver International*. 29(2): 253–259.
76. Robinson, Emily S.; Khankin, Eliyahu V.; Karumanchi, S. Ananth; Humphreys, Benjamin D. (2010). "Hypertension Induced by Vascular Endothelial Growth Factor Signaling Pathway Inhibition: Mechanisms and Potential Use as a Biomarker" . *Seminars in Nephrology*. 30 (6): 591–601.
77. Romer, Alfred Sherwood; Parsons, Thomas S. (1977). *The Vertebrate Body*. Philadelphia, PA: Holt-Saunders International. pp. 354–5. ISBN 0-03-910284-X .
78. Sacher RA, Mepherson RA, (1991). 3rd ed. U.S.A., Pennsylvania: FA Davis Company;. *Widmann's Clinical Interpretation of Laboratory Test*; pp. 416–443.
79. Sagar K, Vidyasagar GM, (2010). Evaluation of acute toxicities of leaf extract of *Caesalpinia bonducella* (L.) Flem. *International Journal of Pharmacology and Biosciences*.;6:1–15.
80. Saxena, Romil; Theise, Neil (2004). "Canals of Hering: Recent Insights and Current Knowledge". *Seminars in Liver Disease*. 24 (1): 43–8. doi : 10.1055/s-2004-823100 .
81. Schwabe, Calvin W. (1979). *Unmentionable Cuisine*. University of Virginia Press. pp. 313–. ISBN 978-0-8139-1162-5 .
82. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ (2015). "Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1995 and 2015". 386 (103): 1546–1555.
83. Scialla, Julia J.; Appel, Lawrence J.; Astor, Brad C.; Miller, Edgar R.; Beddhu, Srinivasan; Woodward, Mark; Parekh, Rulan S.; Anderson, Cheryl A. M. (2011). "Estimated net endogenous acid production and serum bicarbonate in African Americans with chronic kidney disease". *Clinical Journal of the American Society of Nephrology*. 6 (7): 1526–1532.
84. Sebastian, Anthony; Frassetto, Lynda A.; Sellmeyer, Deborah E.; Merriam, Renée L.; Morris, R. Curtis (2002). "Estimation of the net acid load of the diet of ancestral preagricultural *Homo sapiens* and their hominid ancestors". *The American Journal of Clinical Nutrition*. 76 (6): 1308–1312.
85. Sheporaitis, L; Freeny, PC (1998). "Hepatic and portal surface veins: A new anatomic variant revealed during abdominal CT". *American journal of roentgenology* .171 (6): 1559–1564.
86. Shneider, Benjamin L.; Sherman, Philip M. (2008). *Pediatric Gastrointestinal Disease* .Connecticut: PMPH-USA. p. 751.
87. Simmons JE, Yang RS, Berman E, (1995). Evaluation of the nephrotoxicity of complex mixtures containing organics and metals: Advantages and disadvantages of the use of real-world complex mixtures. *Environ Health Perspect*.;103(Suppl 1):67–71.

88. Singh, Inderbir (2008). "The Liver Pancreas and Spleen" . Textbook of Anatomy with Colour Atlas. Jaypee Brothers. pp. 592–606. ISBN 978-81-8061-833-8.
89. Skandalakis, Lee J.; Skandalakis, John E.; Skandalakis, Panajiotis N. (2009). "Liver". Surgical Anatomy and Technique: A Pocket Manua. pp. 497–531.
90. Specht, C.D. & Stevenson, D.W., (2006). A new phylogeny-based generic classification of Costaceae (Zingiberales). *Taxon* 55(1): 153–163.
91. Strunk, H.; Stuckmann, G.; Textor, J.; Willinek, W. (2003). "Limitations and pitfalls of Couinaud's segmentation of the liver in transaxial Imaging". *European Radiology* . 13(11): 2472–2482.
92. Surgical Anatomy and Technique: A Pocket Manual . pp. 497–531.
93. Surgical Anatomy of the Liver . In Busuttil, Ronald W.; Klintmalm, Göran B. Transplantation of the Liver. Elsevier. pp. 23–39. ISBN 978-1-4557-5383-3.
94. Suzuki K, Tanaka M, Watanabe N, Saito S, Nonaka H, Miyajima A (2008). " Neurotrophin receptor is a marker for precursors of stellate cells and portal fibroblasts in mouse fetal liver". *Gastroenterology* . 135 (1): 270–281.
95. Taiwo, A.O. & Bolanle, A., (2003). The leaf essential oil of *Costus afer* Ker-Gawl from Nigeria. *Flavour and Fragrance Journal* 18(4): 309–311.
96. Terashima, H. & Ichikawa, M., (2003). A comparative ethnobotany of the Mbuti and Efe hunter-gatherers in the Ituri forest, Democratic Republic of Congo. *African Study Monographs* 24(12): 160–168.
97. Tietz NW, Prude EL, Sirgard-Anderson O, (1994). Tietz Textbook of Clinical Chemistry. In: Burtis CA, Ashwood ER, editors. London: W.B. Saunders Company;. pp. 1354–1374.
98. Tietz PS, Larusso NF (2006). "Cholangiocyte biology". *Current Opinion in Gastroenterology* . 22 (3): 279–287.
99. Tortora, Gerard J.; Derrickson, Bryan H. (2008). Principles of Anatomy and Physiology (12th ed.). John Wiley & Sons. p. 945.
100. Uhlén, Mathias; Fagerberg, Linn; Hallström, Björn M.; Lindskog, Cecilia; Oksvold, Per; Mardinoglu, Adil; Sivertsson, Åsa; Kampf, Caroline; Sjöstedt, Evelina (2015). "Tissue-based map of the human proteome" .*Science*. 347 (62): 126-149.
101. Van den Berg, Else; Hospers, Frédérique A. P.; Navis, Gerjan; Engberink, Marielle F.; Brink, Elizabeth J.; Geleijnse, Johanna M.; van Baak, Marleen A.; Gans, Rijk O. B.; Bakker, Stephan J. L. (2011). "Dietary acid load and rapid progression to end-stage renal disease of diabetic nephropathy in Westernized South Asian people". *Journal of Nephrology*. 24 (1): 11–17.
102. Varadarasou M, Mounnissamy S, Kavimani G, Gnanapragasam S, Sabarimuthu D, Kuppuswamy S, (2010). Evaluation of acute and sub-acute toxicity of ethanol extracts of *Cansjera rheedii* J Gmelin (Opiliaceae);;1:11–14.
103. W. T. Councilman (1913). "Two". Disease and Its Causes. New York Henry Holt and Company London Williams and Norgate the University Press, Cambridge, U.S.A.
104. Wojcikowski K, Stevenson L, Leach D, Wohlmuth H, Gobe G, (2007). Antioxidant capacity of 55 medicinal herbs traditionally used to treat the urinary system: A comparison using a sequential three-solvent extraction process. *Journal of Alternative Complement Medicine*.;13:103–9.
105. Yakubu MT, Bilbis LS, Lawal M, Akanji MA, (2003). Effect of repeated administration of sildenafil citrate on selected enzyme activities of liver and kidney of male albino rat. *Nigerian Journal of Pure and Applied Science*.;18:1395–4000.
106. Zakim, David; Boyer, Thomas D. (2002).Hepatology: A Textbook of Liver Disease (4th ed.). ISBN 9780721690513.