Original Research Article

Effect of Ethanolic Leaf Extract of *Piper Guineense* (Uziza) on the Kidneys of Paraquat-Induced Adult Male Wistar Rats.

Abstract

Piperguineense (Uziza in Igbo) is a medicinal plant with leaves commonly used as spices. The effects of the ethanolic leaf extracts of *P*. guineense on the histology of the kidneys, and kidney biomarker enzymes of paraquat-induced Wistar rats were determined in this study. Thirty (30) adult male Wistar rats weighing between 160g – 200g were randomly assigned to six Groups A, B, C, D, E, and F of 5 rats each. The control Group A was not induced with paraquat, while Groups B, D, E, and F were induced with 20mg/kg of paraquat. Group C received 200mg/kg of the extract only. After the fourteenth day, groups E and F were withdrawn and treated with 80mg/kg and 100mg/kg of the extract, respectively, for another fourteen days; while Group D was withdrawn without treatment. The results showed that animals in all Groups ($P<0.05$) except B (-21.67 ± 22.55) and D (-21.21 ± 15.67) had decreased body weight; severely degenerated intra lobular inflammatory cells, with a severe aggregate of inflammation cell,?? intra renal hemorrhage, congestion of the cast nephropathy, necrotic debris around the hemorrhagic area and severe degeneration inflammatory infiltrate in kidneys when compared with the control. The effects were ameliorated in Groups E and F which received variable doses of the ethanolic leaf extracts of *P. guineense*. It was therefore concluded that ethanolic leaf extracts of *P. guineense* have ameliorative effects on paraquat-induced kidney damage.

Keywords: *Piper Guineense*, Paraquat, Kidneys, Histology

INTRODUCTION

The knowledge and use of plants as herbs and spices are plant-derived seasonings used for culinary purposes. The terms ‘herbs’ and ‘spices’ are often used interchangeably, but they have specific definitions in botany (Okwute, 1992). Herbs store flavor components in their leaves, whereas spices store theirs in seeds, bark, and root (Opara and Chohan, 2014). A spice may be

theflower bud (clove), bark (cinnamon), ~~root~~rhizome(ginger), aromatic seed (cumin), and flower stigma (saffron) of a plant. In addition to ~~making food taste good,~~increasing palatability of food,culinary spices have been used as food preservatives ~~and~~as well as for their health-enhancing properties for centuries (Kaefer and Milner, 2011). Moreover, for people of the world, spices stimulate appetite and create visual appeals to food (Opara and Chohan, 2014).

Herbs and spices ~~elaborate~~possess secondary metabolites that form part of the plant's chemical defense. They make food taste good but may not be delicious themselves, and many of them possess marked pharmacological and medicinal properties (Newman and Cragg, 2012).

In traditional herbal medicine, the seeds of Uziza (*Piper guineense*) are put to a variety of uses; for instance, in some parts of Nigeria, the seeds are consumed by women after childbirth (Mbah et al., 2022) to enhance uterine contraction for the expulsion of the placenta and other remains from the womb (Mbongue et al., 2005);as an adjuvant in the treatment of rheumatic pains and as an antiasthmatic (Negbenebor et al., 1999) and alsoused for the control of weight. Uziza leaves contain Protein, Dietary fiber, Alkaloids, Glycosides, Flavonoids, Essential oils, Tannins, Saponins, and Phenols. The chemical piperine, which gives the leaves their flavoring taste, is found in 5–8% of the leaves.?? They also contain a lot of beta-caryophyllene, an anti-inflammatory compound. ~~It also includes~~They also include significant levels of myristicin, elemicin, safrole, and dillapiol, as well as a small quantity of apiole, all of which are health-promoting compounds (Mbah et al., 2022).

Paraquat, a widely used herbicidal chemical, plays a pivotal role in modern agriculture due to its potent weed-control capabilities. It is a quaternary nitrogen herbicide and brown syrupy liquid ~~being~~belongingto the Bipyridinium compounds and its chemical name is 1, 1'-dimethyl 4, 4'-bipyridinium (Dasta, 1978; Bismuth et al., 1982, 1990; Raghu et al., 2013). Paraquat is widely used for weed control in fruit orchards and plantation crops, including coffee, cocoa, coconut, oil palms, rubber, bananas, vines, olives and tea, ornamental trees and shrubs, and in forestry (Hood et al., 1963; Hood, 1965). Paraquat is classified by the WHO as a moderately hazardous herbicide and class II poison for acute toxicity (WHO, 2010). In paraquat poisoning, no antidote is available and the potential delay in the onset of clinical signs by several days differentiates it from organophosphates (Ellenhorn et al., 1997).

The function of kidneys as a vital role in the excretion of waste products and toxins such as urea, creatinine, and uric acid, regulation of extracellular fluid volume, serum osmolality and electrolyte concentrations, as well as the production of hormones like erythropoietin and 1, 25-dihydroxyvitamin D and renin. The functional unit of the kidney is the nephron, which consists of the glomerulus, proximal and distal tubules, and collecting duct. Assessment of renal function is important in managing patients with kidney disease or pathologies affecting renal function.

Tests of renal function have utility in identifying the presence of renal disease, monitoring the response of kidneys to treatment, and determining the progression of renal disease. According to the National Institutes of Health, the overall prevalence of chronic kidney disease (CKD) is approximately 14%. (citereference , last accessed) Worldwide, the most common causes of CKD are hypertension and diabetes.

Materials and Methods

Thirty (30) male adult albino Wistar rats weighing 160g – 200g were obtained from Amaka Animal Farm in David Umahi University of Health Sciences, Uburu, Ebonyi State. The rats were housed in Wire gauze cages and allowed to acclimatize in the animal house of the Department of Anatomy, David Umahi University of Health Sciences, Uburu, Ebonyi State for one week before exposure. The rats were fed with rat chow and were provided with water ad libitum throughout the experiment. They were randomly assigned to six Groups (A-F) (n- 5). The control Group A was not induced with paraquat, while Groups B, D, E, and F were induced with 20mg/kg of paraquat. Group C received 200mg/kg of the extract only. After the fourteenth day, groups E and F were withdrawn and treated with 80mg/kg and 100mg/kg of the extract, respectively, for another fourteen days; while Group D was withdrawn without treatment and the animals were sacrificed at the end of the experiment of day 28. Blood was obtained from the animals via ocular puncture, and it was used for hormonal assay of the kidneys, immediately the animals were sacrificed and the liver was measured immediately the animals were sacrificed and fixed in a 10% normal saline which is used for the histology. Raw data such as body weights, relative organ weights, and hormonal results were analyzed using the SPSS (version 21) software package. All the results obtained were expressed as Mean value ± SEM (Standard error of the mean) in each group. All the tested parameters were subjected to statistical analysis using the Paired T-test and the one-way analysis of Variance (ANOVA). Differences between means were regarded as significant at $P<0.05$

Results and Discussion

The effect of Ethanolic Extract of Uziza (*Piper guineense)* and Paraquat on Body Weight of adult male Wistar rats.

Table 1: mean values of Body Weights obtained at the end of the experiment

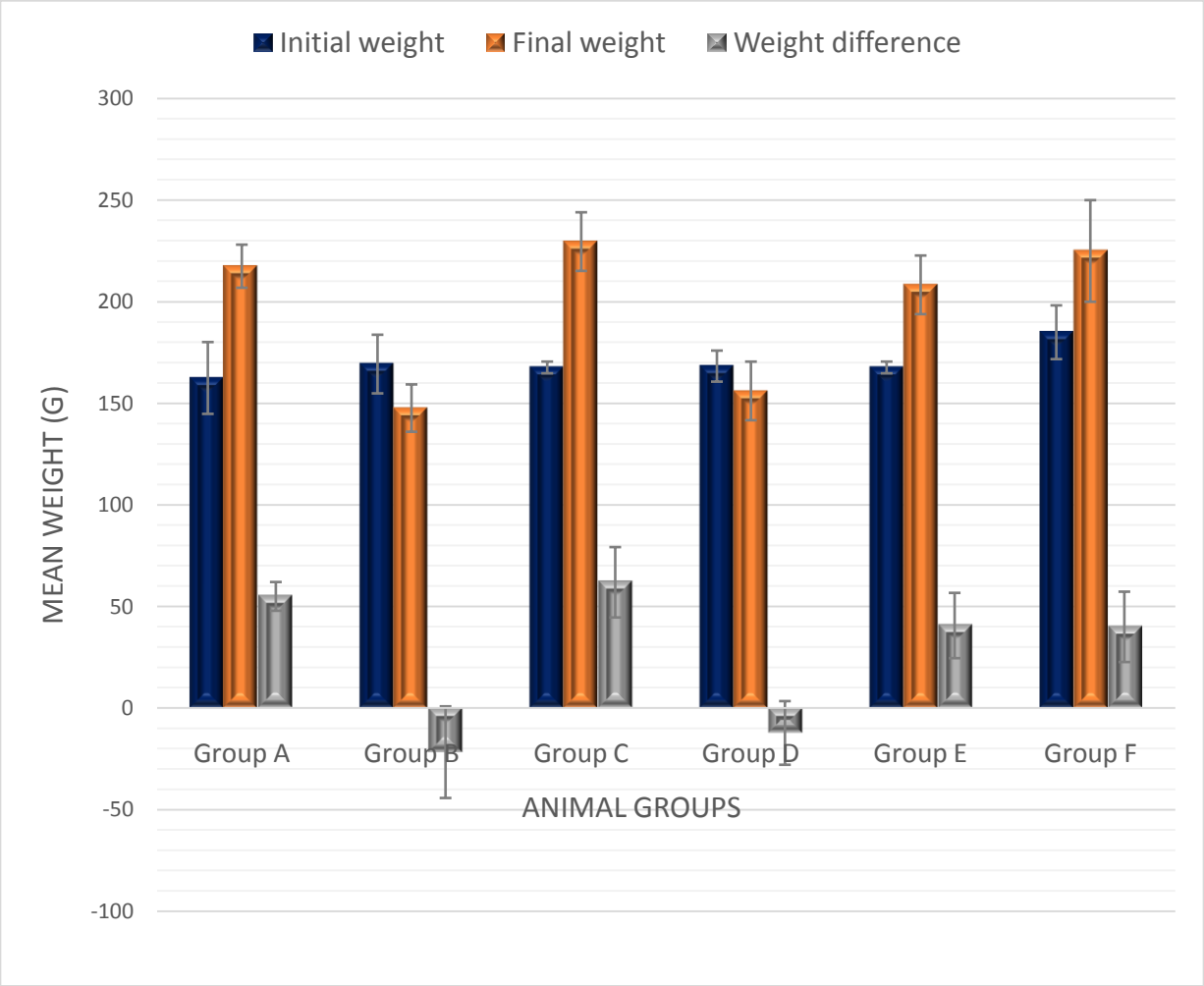
	Initial Body Weight (g)	**Final Body Weight (g)**		**T-test**
Group	**Mean ± SEM**	**Mean ± SEM**	**p-value**	
Group A	161.50 ±16.68	216.50 ± 09.61	0.057	-12.00
Group B	168.34 ± 13.43	146.67 ± 10.65	0.127	0.664
Group C	166.67± **1**.89	228.61 ± 13.43	0.056	-3.000
Group D	167.33 ± **6**.64	155.12 ± 14.13	0.043*	3.619
Group E	166.67 ± 1.89	207.33 ± 13.73	0.075	-2.413
Group F	184.00 ± 12.23	224.00 ± 24.00	0.056	-3.000

*p < 0.05 when compared with group A (control)

Table 1: shows the analysis of the body weight ~~showed~~indicatingthat there was a significant decrease in weight difference in group B and group D compared with group A. There was however insignificant increase in other groups when compared with group A. Dates were analyzed using a Paired T-test and the one-way analysis of Variance (ANOVA), followed by post-HOC Bonferroni's multiple Comparison Values were expressed as mean ± SEM (Standard error of mean) and were considered significant at *p < 0.05. Group A served as control and received only distilled water, and was not induced with paraquat, while Groups B, D, E, and F

were induced with 20mg/kg of paraquat. Group C received 200mg/kg of the extract only. After the fourteenth day, groups E and F were withdrawn and treated with 80mg/kg and 100mg/kg of the extract, respectively, for another fourteen days; while Group D was withdrawn without treatment

Figure 1. Composite Bar chart showing the Initial and Final Mean Body weight change for the various Experimental Groups studied as shown in Tables 1



Effect of Ethanolic extract of *Uziza (Piper guineense)* and paraquat on relative kidney weight of adult male Wistar rats.

Table 2: mean values on the relative kidney weight obtained at the end of the experiment

		Mean±STD	**p-value**	**F-value**
Relative kidney weight (%)	Group A	0.95±0.04		
	Group B	1.58±0.17	0.00	10.35
	Group C	0.99±0.16	0.74	
	Group D	1.09±0.09	0.24	
	Group E	0.91±0.06	0.76	
	Group F	0.95±0.09	0.96	

*$p < 0.05$ when compared with group A (control)

Table 2: shows a significantly higher relative kidney weight in group B (1.58±0.17) compared with the control (0.95±0.04). There was also a significantly lower relative organ weight in group D compared with the control. Group C (0.99±0.16), group D (1.09±0.09), group E (0.91±0.06) and group F (0.95±0.09) did not vary significantly from the control

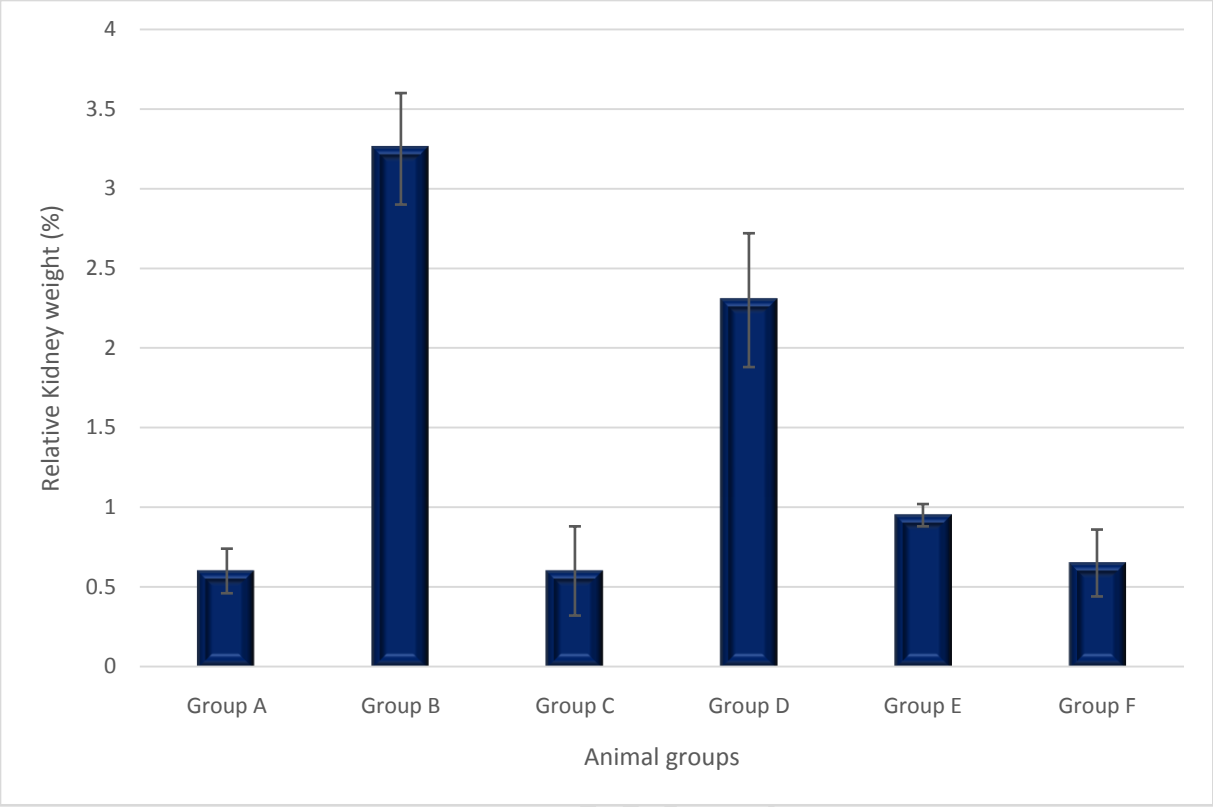


Figure 2: Barchart showing the relative kidney weight of animals in the experimental group. The bar chart represents Table 2

Effect of Ethanolic extract of *Uziza (Piper guineense)* and paraquat on Serum Urea level of adult male wistar rats.

Table 3: The serum urea level obtained at the end of the experiment

		Mean±STD	p-value	F-value
	Group A	20.5±2.12		
Urea (mg/dL)	Group B	54±8.49	0.00	8.32
	Group C	20.5±3.54	1.00	
	Group D	35±7.07	0.07	
	Group E	24±8.49	0.61	
	Group F	22.5±6.36	0.77	

*$p < 0.05$ when compared with group A (control)

Table 3: shows significantly higher plasma urea levels in group B (54±8.49) compared with the control (20.5±2.12). The rise in urea level in group D (35±7.07) was not significant compared to group A. Group C (20.5±3.54), group E (20.5±3.54) and group F (22.5±6.36) did not vary significantly from the control.

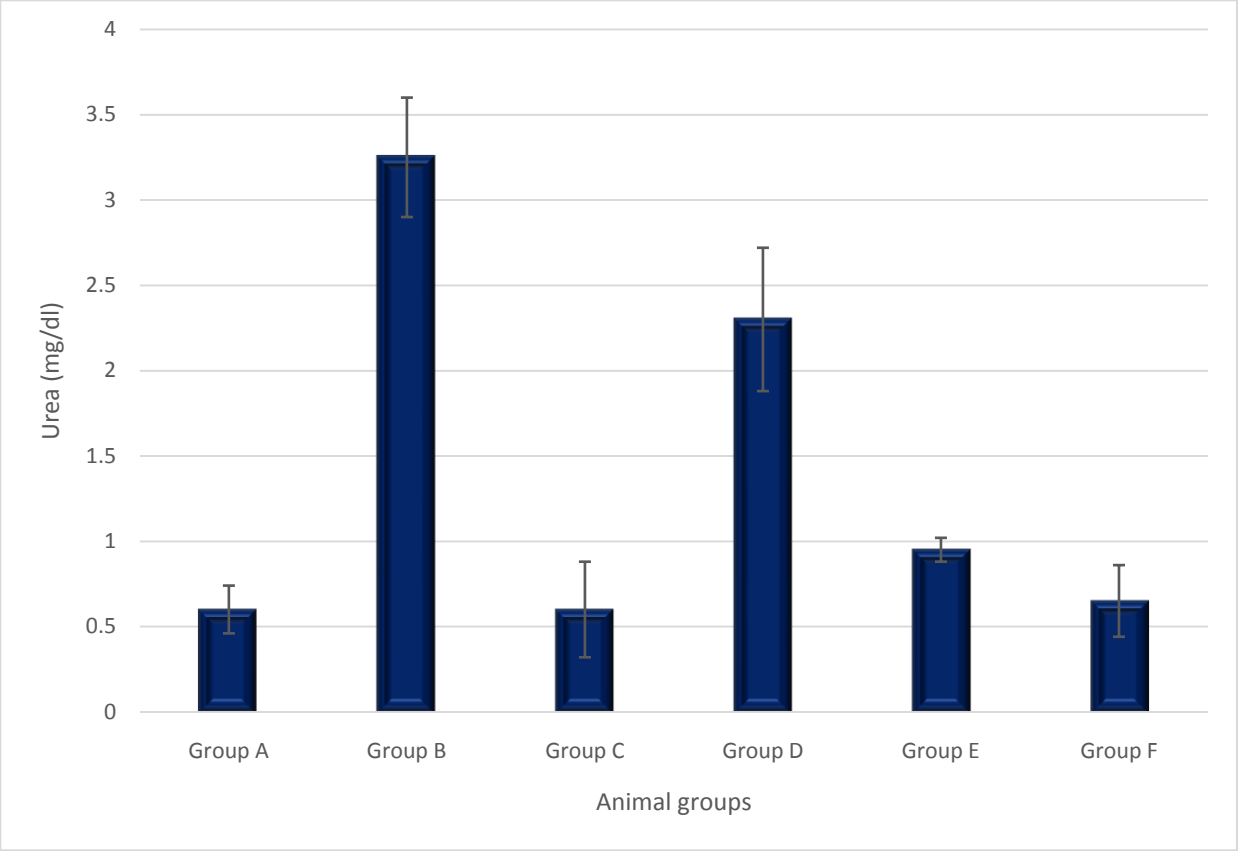


Figure 3. Bar chart showing the serum urea level of animals in various experimental groups. The bar chart represents the results in table 3

Effect of Ethanolic extract of *Uziza (Piper guineense)* and paraquat on serum creatinine level of adult male wistar rats.

Table 4: The serum creatinine level obtained at the end of the experiment.

		Mean±STD	p-value	F-value
Creatinine (mg/dL)	Group A	0.6±0.14		
	Group B	3.25±0.35	0.00	33.11
	Group C	0.6±0.28	1.00	
	Group D	2.3±0.42	0.00	
	Group E	0.95±0.07	0.25	
	Group F	0.65±0.21	0.86	

*$p < 0.05$ when compared with group A (control)

Table 4: shows the levels of creatinine. There was a significantly higher plasma creatinine level in group B (3.25±0.35) and group D (2.3±0.42) compared with the control (0.6±0.14). Group C (0.6±0.28), group E (0.95±0.07) and group F (0.65±0.21) did not vary significantly from the control.

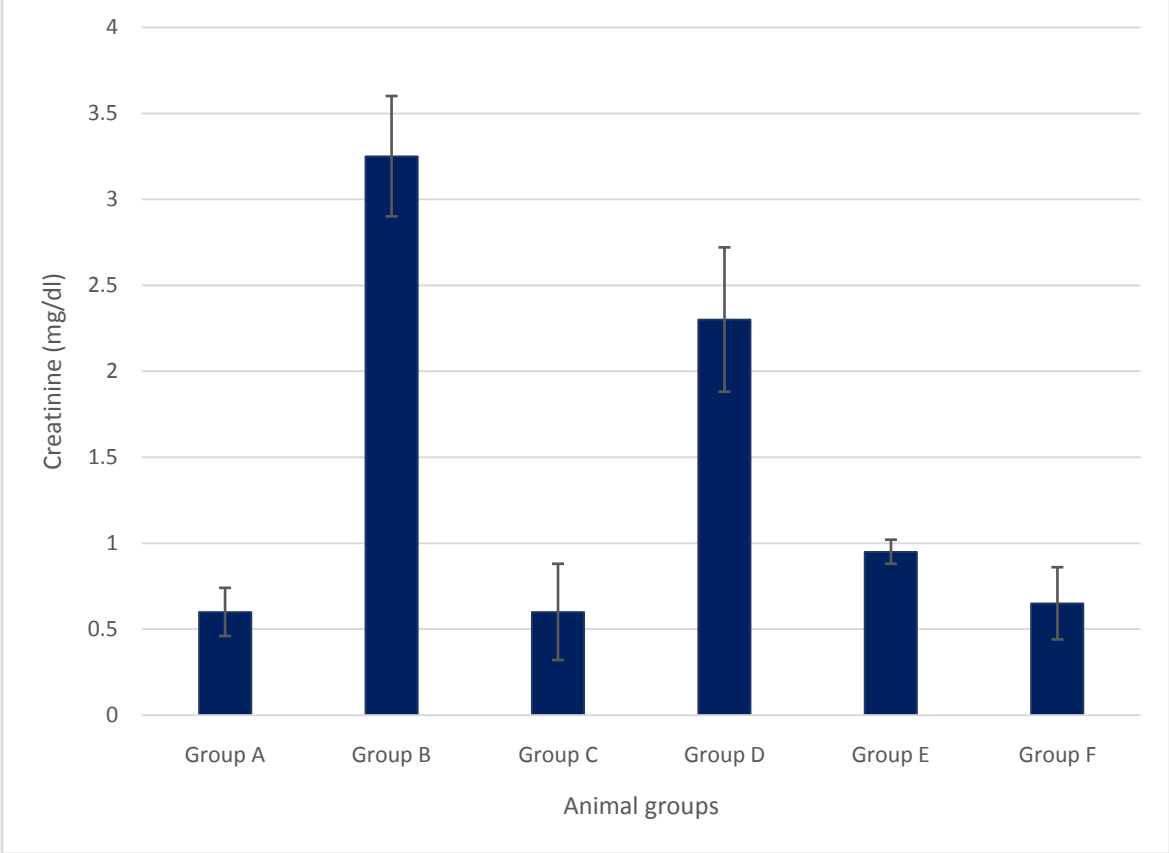


Figure 4: Bar chart showing creatinine level of animals in various experimental groups. The bar chart represents the results in table 4.

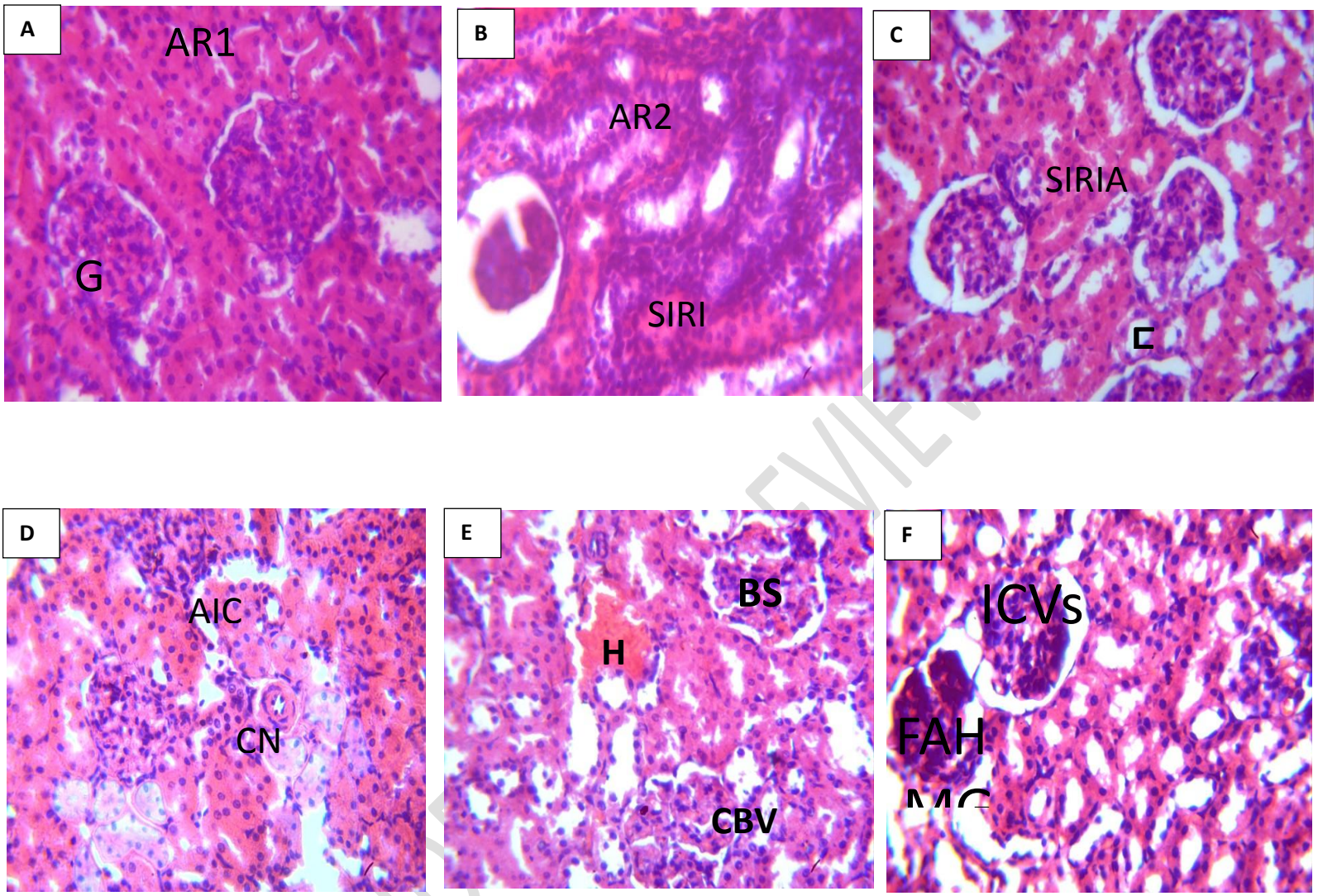


Plate 1: glomeruli (G), bowman space (BS), renal tubules (RT), and tubular cell (TC), Showing mild aggregate of moderate degeneration with moderate intra renal inflammation arrest (SIRIA), severe intra renal inflammation (SIRI), intra renal hemorrhage (H), cast nephropathy (CN) and aggregate of inflammation cell (AIC), congestion of blood vessel (CBV), important congestive vessels (ICVs), mild focal area of hemorrhage (FAH).

Piperguineense, or Guinea pepper, stands in stark contrast to paraquat as a natural?? plant with diverse culinary and medicinal applications. The dried seeds and leaves of this spice plant are integral to West African cuisine, imparting a unique and robust flavor to a variety of dishes (Mbah et al., 2022). Furthermore, *Piperguineense* has garnered recognition for its medicinal properties. It has been employed traditionally to alleviate pain, harness anti-inflammatory effects, and explore its therapeutic potential for various health conditions. The significance of *Piper guineense i*s particularly pronounced in the context of traditional medicine, as it aligns with indigenous healing practices that have been passed down through generations. The spice plant's potential therapeutic benefits warrant further investigation, as they hold promise for the development of natural remedies and pharmaceutical compounds (Mbongue et al., 2005). (include in Introduction)

The result from this study of the effect of the Ethanolic extract of Uziza (*Piper guineense)* on body weight revealed that all groups showed increases in body weight except for Group D which received 20mg/kg of paraquat. Group D showed a significant decrease when compared with Group A which served as the control. The reason for the increase in group A may be physiological due to the high fiber and carbohydrate content in the rat feed given to the control group. The reason for the increase in Groups B, C, E, and F may be physiological too due to the high fiber and carbohydrate content in the rat feed. This is in agreement with the findings of Ezejindu et al., (2014) and Mbah et al., (2022). In their research, there was body weight gain in the experimental groups that received Uziza (*Piperguineense*) when compared to the control group. The reason for the weight loss in group D might be due to the effect of paraquat being able to cause cell death by free radicals induced by Nicotinamide Adenine Dinucleotide Phosphate (NADPH) depletion (Dinis-Olivera et al., 2009).

The relative kidney weight of paraquat-induced rats in group D was significantly higher than that of the control. This suggests that the kidneys were functioning suboptimally leading to fluid accumulation in the kidneys. This is further supported by the significantly higher urea and creatinine levels in group D compared to the control. Similarly, Sharifi-Rigi and Heidarian, (2018) reported a significant rise in urea and creatinine levels in rats induced with oral paraquat. Histopathological evaluation of the kidneys of group D animals showed renal degeneration,

hemorrhage, and infiltration by leucocytes. Many other studies have also observed a similar pattern of renal damage after oral administration of paraquat in rats (Michaelis, 2018; Peng et al., 2007; Pronczuk et al., 2004). As suggested by Peng et al., (2007), ROS produced by paraquat can damage proteins and alter their structures, and decrease protein enzymes. However, the animals that received Uziza extract only showed minimal changes from the normal. This protective effect observed may be due to the presence of antioxidant flavonoids and phenols in Uziza extracts (Michaelis, 2018).

This raises the concern that exposure to paraquat might also increase the risk of development of cancer. Histopathology revealed that in the future, therapies for kidney disease could be based on cell- derived factors rather than on the cells themselves, thereby circumventing the risks associated with cell administration, such as tumor formation. The kidneys of uziza-treated rats showed signs of healing of the tumor; suggesting that uziza extract has an ameliorative effect on paraquat-induced kidney toxicity. This is likely due to the presence of antioxidants like Niacin in Uziza extracts.

Conclusions

From this study, the researcher concluded that:

- Ethanolic extract of uziza leaf offered some protection against paraquat-induced kidney damage.
- Ethanolic extract of uziza leaf offered some amelioration on paraquat-damaged kidney

Recommendations

This study recommends that farmers, agriculturists, and instrustral workers, avoid accidental ingestion of herbicides such as paraquat by their children or family members because it shares a resemblance with (Hibiscus ~~Sobdariffa~~)sabdariffaZobo juice which has proven to be deleterious to health. They should also include Uziza in their diet for protection

Ethical statement

This research was sought and obtained from the Chairman Committee of the Faculty of Basic Medical Sciences, David Umahi University of Health Sciences, Uburu, Ebonyi State, Nigeria.

The experimental procedures of this Study complied with ARRIVE guidelines, National Institutes of Health (NIH) guidelines, and National Health Research Ethics Laboratory Animals

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