COMPARATIVE ASSESSMENT OF HISTOPATHOLOGICAL AND BIOCHEMICAL INDICES OF RENAL FUNCTION IN ALLOXAN-INDUCED MALE AND FEMALE DIABETIC RATS

ABSTRACTS

It has been observed that alloxan-induced diabetes causes free radical production, which ultimately damages the pancreatic β-cells and impact several organs especially the kidney. This study was a comparative assessment of histopathological and biochemical indices of renal function in alloxan-induced male and female diabetic rats. A total of twenty-four (24) male and female albino wistar rats were divided into five groups (6 rats per group): normal control female (group A), normal control male (group B), diabetic female (group C) and diabetic male (group D). Diabetes mellitus was induced in the rats via intraperitoneal injection of alloxan at a dose of 120 mg/kg bwt. The rats were observed for 48 hours and were allowed access to water and food as much as they wanted and were then sacrificed 48 hours post alloxan-induction.Serum urea, creatinine and electrolyte profile (sodium, potassium, chloride and bicarbonate) were determined using standard laboratory methods while the histology of the kidney determined by H and E technique. The histopathological section of the group C rats showed thickened glomeruli which are closely adherent to the bowman's capsular space. There was interstitial fibrosis, hyaline changes, epithelial cellular vacuolar degeneration and arteriopathy. The group D rats showed milder histological alterations of glomeruli and arterioles. There was significantly increased mean serum Urea, sodium, chloride and bicarbonate levels in the female diabeticrats than in female control ($p=0.000$; 0.020; 0.009; 0.027) respectively while potassium was significantly increased in male diabetic rats than in male control ($p=0.018$). Also, the mean serum urea and creatinine level was significantly increased in female diabetic rats compared to male diabetic rats ($p=0.017$; 0.010) respectively. This study has revealed the variable alterations in kidney histology and functions due to alloxan-induced diabetes mellitus.The kidney functions of male and female wistar rats with alloxan-induced diabetes was found to exhibit varying degrees of significant histological and biochemical changes.

Key Words: diabetic kidney disease, diabetic nephropathy, hyperglycemia, , kidney function, Renal excretion, alloxan toxicity

INTRODUCTION

Alloxan and streptozotocin (STZ) are the most commonly utilized diabetogenic agents in investigations (Ighodaro *et al*., 2017; Ezeugwunne*et al*., 2018), however other known agents include dithizone, monosodium glutamate, gold thioglucose, high fructose load, high glucose load, and anti-insulin serum. Nevertheless, some authors have documented the variability and inconsistency of alloxan as a chemical that causes diabetes both within and between animal species (Soni *et al*., 2019; Osibemhe*et al*., 2023). Alloxan induces diabetes through a mechanism that essentially involves the beta cells of the pancreatic islets partially degrading and then producing less insulin overall, both in terms of quality and quantity (Ighodaro *et al*., 2017). It has been observed that alloxan-induced diabetes causes free radical production, which ultimately damages the pancreatic β-cells (Martin *et al*., 2023). The use of it as a diabetogenic drug in test animals was initially reported by Dunn and McKetchie (Dunn and McKetchie, 1943).

Diabetes mellitus (DM) is a major public health concern, especially in developing countries like Nigeria. The International Diabetes Federation (IDF) estimates that 642 million people worldwide will have diabetes mellitus by 2040, accounting for 8.8% of the global population (Garhwal *et al*., 2020). In actuality, diabetes mellitus is becoming more and more common over time (Garhwal *et al*., 2020). Diabetes mellitusis a chronic metabolic disorder with impaired insulin production and increased insulin resistance as its key pathophysiological characteristics (Ogbodo *et al*., 2019).It is a metabolic disease that is characterized by hyperglycemia associated with alterations in carbohydrate, protein, and fat metabolism (Dilworth *et al*., 2021) when the body cannot cope with blood glucose levels (Frolova *et al*., 2022). Diabetic individuals are unable to metabolize glucose efficiently and cannot synthesize fatty acids and triglycerides from carbohydrates or amino acids, due to the failure of insulin secretion or action and since the cells cannot detect and absorb glucose in the blood, enzymes in the glycolytic, lipogenic, and pentose phosphate pathways are suppressed, while gluconeogenic, glycogenolytic, and lipolytic activities are elevated, reversing the metabolic pathway in nondiabetic individuals (Dilworth *et al*., 2021). The increased risk of morbidity and mortality from vascular complications in diabetic persons is linked to genetic factors such as family history, elevated glucose levels, hypertension, obesity,

oxidative stress,blood lipid disorders, and smoking (Xu *et al*., 2005; Alharithy*et al*., 2018; Ogbodo *et al*., 2019; Okwara *et al*., 2021). The long-term consequences of diabetes are caused by hyperpolarization, which results from persistent oxidative stress in diabetic cells (Zheng *et al*., 2010).

In the past, the discovery that certain tissues and cells are resistant to the long-term harm and problems caused by hyperglycemia raised awareness of the variations in how various cell types deal with extracellular glucose overload. kidney cells, including immune system cells, endothelial cells, and podocytes, mesangial cells, and tubular cells. Furthermore, glucose enters the neuron system by enhanced diffusion via the insulin-independent glucose transporter 1 in cells such neurons and glia (Heilig *et al*., 1995; Forbes and Cooper, 2013; Daza-Arnedo *et al*., 2023). Because of this, hyperglycemia in these cells might result in intracellular excess glucose, which forces the cell to metabolize it and get rid of metabolic intermediates, which has negative biochemical effects. This highlights why the kidney, neurological system, cardiovascular system, and eye are the primary target organs of diabetic macular degeneration. Nephropathy, or damage to the kidneys that leads to renal failure, is one of the main microvascular complications of diabetes mellitus (Ihim*et al*., 2021; Abu *et al*., 2022).The primary cause of the onset and advancement of diabetic nephropathy in diabetic kidney disease is hyperglycemia-induced metabolic damage (Natesan and Kim, 2021).

Diagnosing renal dysfunction or impairment is commonly achieved using serum urea, creatinine and electrolytes profile tests, otherwise kidney function tests measurements. The kidneys play 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 dihydroxy vitamin D and renin (Gounden *et al*., 2024). Renal impairment or muscular injury are associated with elevated blood urea and creatinine levels. Traditional markers of renal function include blood urea and creatinine. Protein breakdown results in the byproduct urea. Renal excretion accounts for around 90% of urea generated (Walmsley *et al*., 2010). The kidneys alone are responsible for excreting creatinine, a waste product of muscle catabolism (Treasure, 2003). Due to decreased kidney function, urea and creatinine build up in the bloodstream as a result of renal injury (Abu *et al*., 2023). Electrolyte imbalance is indicated by specific ion levels, such as sodium (Na^+), potassium (K^+), chloride (Cl^-), and bicarbonate (HCO_3^-). Through the production of impulses, fluid

absorption, and blood volume maintenance, electrolytes support fluid balance. Electrolyte imbalance in pathological situations is characterized by low potassium and elevated amounts of sodium and chloride (Abu *et al*., 2023).

Although several studies have reported significant alterations in renal functions of alloxan-induced diabetic rats previously (Pourghasem*et al*., 2014; Frolov *et al*., 2022), there is paucity of published data on the comparative assessment of histopathological and biochemical indices of renal function in alloxan-induced male and female diabetic rats. Regardless of the fact that diabetes affects both sexes in individuals and in certain genetic animal models, most examinations of diabetic complications have focused on streptozotocin-induced diabetes in male mice only. NationalInstitutes of Health now mandates that researchers include both sexes in their research (Negara *et al*., 2020). Hence, the need for this study.

MATERIALS AND METHODS

Experimental Animals

A total of twenty-four males and female Wistar rats (*Rattus norvegicus*) weighing between 145g and 160g was purchased from the Research Aid Animal House, Federal University of Technology Owerri, Nigeria. The animals were kept in metallic cages and housed in a room with temperature of about 25°C. All experimental procedures were carried out in compliance with Guidelines for the Care and Use of Laboratory Animals.

Experimental design and animal treatment

The rats were acclimatized for two weeks before the experiment commenced and divided into four groups of six animals each. Diabetes was induced by a single intraperitonial (i.p) injection of alloxan (120mg/kg). The animals had free access to feed and water throughout the period of experiment and they were divided into four groups (group I-4) as follows:

Group A (normal control): This group was the nondiabetic female rats designated as"**NF**". They were fed with rat diet and water all through the period of experiment.

Group B (normal control): This group was the nondiabetic male rats designated as"**NM**". They were fed with rat diet and water all through the period of experiment.

Group C (diabetic female): These were the diabetic female rats. Diabetes was induced by a single intraperitonial(i.p) injection of alloxan(120mg/kg). They were designated as" **DF**".

Group D (diabetic male): These were the diabetic male rats. Diabetes was induced by a single intraperitonial (i.p) injection of alloxan (120mg/kg). They were designated as"**DM**".

The research animals were sacrificed after forty-eight (48 hours) of the alloxan-induction.

Collection of Blood Sample

At the end of the experimental feeding period, the rats were fasted overnight and sacrificed. Five milliliters (5ml) of whole blood was collected by ocular puncture into plain tubes, allowed to clot and the serum separated by centrifugation at 3500 rpm for five minutes. Samples were transported to De-life Family Diagnostic center, Owerri, for the analysis of biochemical parameters.

Collection of Organs: The kidneys were collected at sacrifice and kept in ten percent buffered formalin and taken to the Histopathological unit of the Federal Medical Center Owerri for tissue processing.

Determination of Biochemical Parameters

Ion Selective Electrode (ISE) method was used to determine serum electrolytes (Na^+, K^+, Cl^-, HCO_3^-). While creatinine was determined using the Jaffe-Slot alkaline picric acid method, as described by Ochei and Kolhatkar (2007), serum urea was determined using the Berthlot method, as described by Taussky (1956).

Histological Examination

The kidney tissues were fixed in 10% neutral buffered formalin, embedded in paraffin,and then, following normal protocols, 5 µm thick slices were cut and stained with eosin and haematoxylin. A light microscope (Leica DM 1000 binocular microscope) was used to examine the slides, and 400× photomicrographs were taken.

Statistical analysis

Data collected on all biochemical parameters was analyzed with statistics provided by the software statistical package for social sciences (SPSS) version 27. Difference in mean values between groups was assessed using student t-test and the relationship was determined using the Pearson correlation (r). Tests with a probability value of $p<0.05$ was considered statistically significant.

RESULTS

Fig 1shows the Histological section of kidney of control rat. There was no alteration in the morphology of the kidney in the control rat.

Fig 2 shows the histopathological section of the kidney of the female alloxan induced diabetic rats showing thickened glomeruli which are closely adherent to the bowman's capsular space. There was interstitial fibrosis, hyaline changes, epithelial cellular vacuolar degeneration and arteriopathy.

Fig 3 shows Histopathological section of the kidney of alloxan induced male diabetic rats which showed thickened glomeruli which was closely adherent to the bowman's capsule. Milder histological alterations of glomeruli and arterioles were observed.

The mean values of serum urea, sodium, chloride and bicarobonate were significantly increased ($p=0.000$, $p=0.020$, $p=0.009$ and $p=0.027$) in female diabetics when compared to healthy female albino rats (Control) respectively. There was no significant difference ($p=0.071$ and $p=0.446$) in

the mean value of serum creatinine and potassium in female diabetics when compared to healthy female albino rats (Control). See table 1.

The mean serum concentration of potassium was significantly increased in male diabetics when compared to male control albino rats (4.93±0.38 Vs 4.32±0.38; p=0.018). However,

There was no significant difference (p>0.05) in the mean serum levels of urea, creatinine, sodium and chloride and bicarbonate in the male diabetics when compared to healthy male albino rats (Control) respectively. See table 2.

The mean serum levels of urea and creatinine was significantly increased (p=0.017, p=0.010) in female diabetics when compared to male diabetic albino rats respectively but there was no significant difference (p>0.05) in the mean value of sodium, potassium, chloride and bicarbonate in female diabetics when compared to male diabetic albino rats. See table 3.

There was no significant correlation observed between levels of glucose, urea, creatinine, sodium, potassium, chloride and bicarbonate in the female diabetic albino rats (p>0.05) respectively. See table 4.

Furthermore, there was no significant correlation observed between levels of glucose, urea, creatinine, sodium, potassium, chloride and bicarbonate in the male diabetic albino rats (p>0.05) respectively. See table 5.

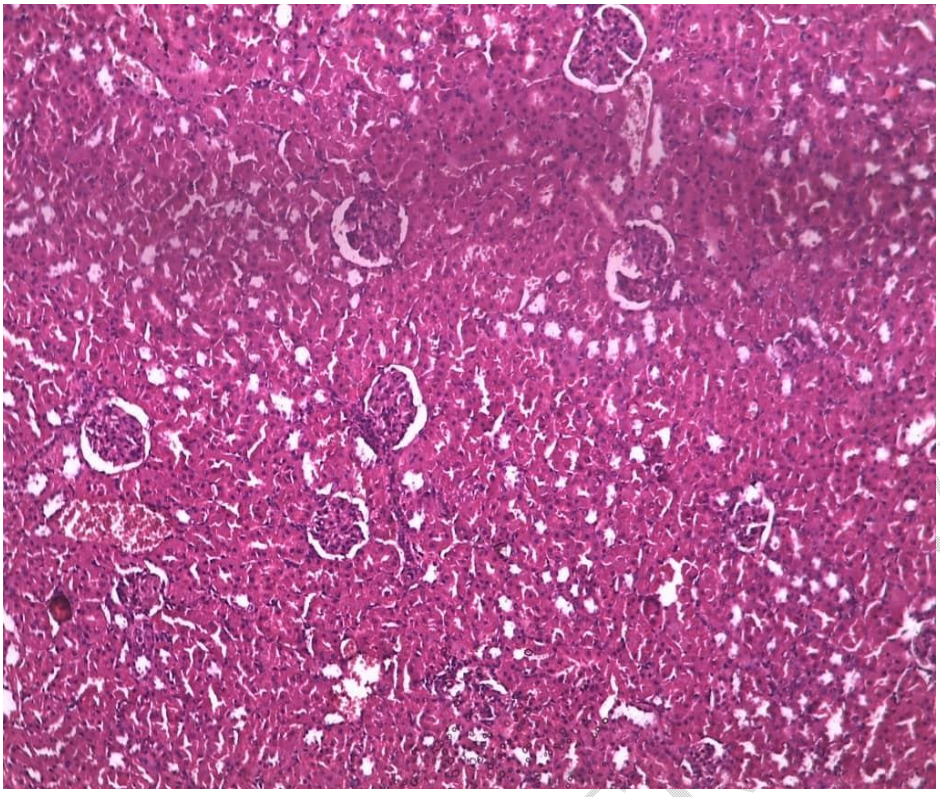

Fig 1: Histological section of kidney of control rat.

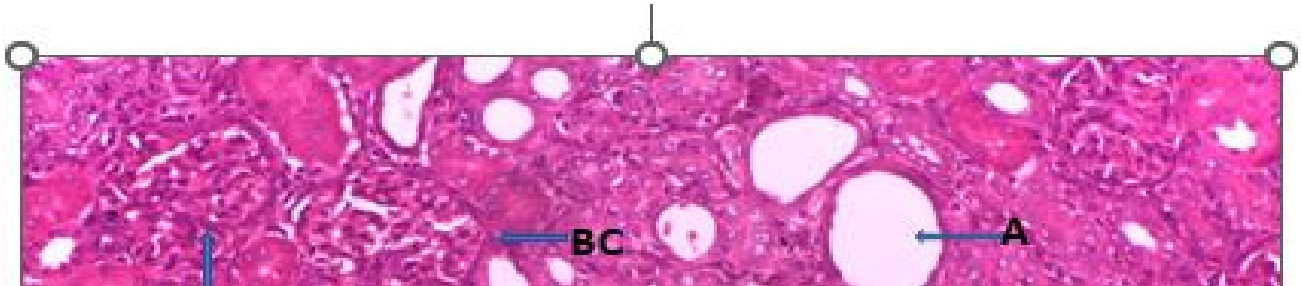

Fig 2: Histopathological section of kidney of female diabetic mice

Key:

A- Arteriopathy

B- BC- Bowman's capsule

EP- Epithelial cellular vascular degeneration

G- Glomeruli

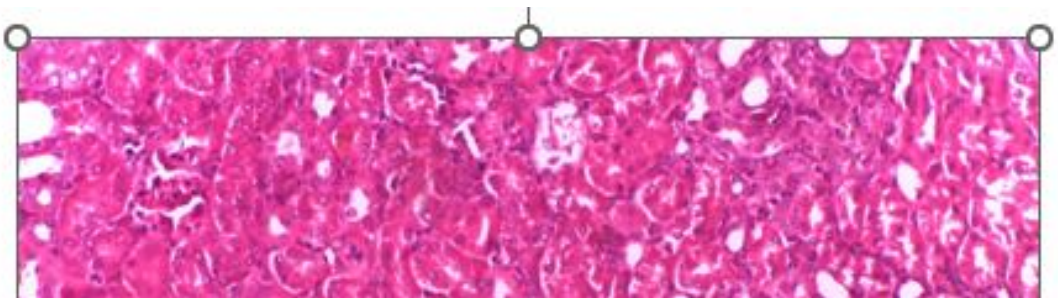

Fig 3: Histopathological section of kidney of male diabetic mice

Key:

A-Arteriopathy

B-BC- Bowman's capsule

EP- Epithelial cellular vascular degeneration

G- Glomeruli

Table 1: Mean Serum Levels of Urea, Creatinine, Sodium, Potassium, Chloride and Bicarbonate in Female Diabetics Vs Female Control

Parameters	Female (Diabetics)	Female (Control)	t-value	p-value
Urea (mg/dl)	135.11±33.89	54.89±9.23	5.48	0.000*

Creatinine (mg/dl)	1.38±0.06	0.99±0.42	1.88	0.071
Sodium (mmol/l)	150.60±1.67	142.50±6.16	2.25	0.020*
Potassium (mmol/l)	5.12±0.13	4.35±0.50	3.56	0.446
Chloride (mmol/l)	105.00±3.24	100.33±1.21	3.69	0.009*
Bicarbonate (mmol/l)	31.40±1.14	29.50±1.22	2.41	0.027*

***Statistically Significant at P<0.05.**

Table 2: Mean Value of Urea, Creatinine, Sodium, Potassium, Chloride and Bicarbonate in Male Diabetics Vs Male Control

Parameters	Male (Diabetics)	Male (Control)	t-value	p-value
Urea (mg/dl)	82.98±25.11	61.57±22.61	1.55	0.152
Creatinine (mg/dl)	1.12±0.17	1.19±0.15	0.81	0.436
Sodium (mmol/l)	146.50±4.68	141.83±4.53	1.75	0.110
Potassium (mmol/l)	4.93±0.38	4.32±0.38	2.83	0.018*
Chloride (mmol/l)	103.00±3.89	103.50±5.01	0.19	0.851
Bicarbonate (mmol/l)	29.83±1.47	30.33±1.75	0.54	0.604

***Statistically Significant at P<0.05.**

Table 3: Mean Value of Urea, Creatinine, Sodium, Potassium, Chloride and Bicarbonate in Female Diabetics Vs Male Diabetics

Parameters	Female (Diabetics)	Male (Diabetics)	t-value	p-value

Urea (mg/dl)	135.11±33.89	82.98±25.11	2.93	0.017*
Creatinine (mg/dl)	1.38±0.06	1.12±0.17	3.27	0.010*
Sodium (mmol/l)	150.60±1.67	146.50±4.68	1.85	0.098
Potassium (mmol/l)	5.12±0.13	4.93±0.38	1.05	0.323
Chloride (mmol/l)	105.00±3.24	103.00±3.89	0.91	0.385
Bicarbonate (mmol/l)	31.40±1.14	29.83±1.47	1.94	0.085

***Statistically Significant at P<0.05.**

Table 4: Correlation of Glucose Concentration with Renal Profile in Female Diabetics

Dependent Variable	N	R	p-value
Urea	6	-0.69	0.131
Creatinine	6	0.78	0.070
Sodium	6	-0.78	0.070
Potassium	6	-0.40	0.429
Chloride	6	0.10	0.846
Bicarbonate	6	-0.63	0.177

***Statistically Significant at P<0.05.**

Table 5: Correlation of Glucose Concentration with Renal Profile in Male Diabetics

Dependent Variable	N	R	p-value
Urea	6	0.32	0.531
Creatinine	6	0.24	0.644
Sodium	6	0.12	0.816
Potassium	6	-0.38	0.464
Chloride	6	0.29	0.570
Bicarbonate	6	0.75	0.086

***Statistically Significant at P<0.05.**

DISCUSSION

Alloxan is reported to cause acute tubulointerstitial nephritis, consequently resulting in nephrotoxicity. Renal changes, varying from swelling to necrosis, occur predominantly in the epithelia of the proximal convoluted tubules (Zhang *et al.*, 2016). The histopathological section of the kidney of the female alloxan induced diabetic rats showed thickened glomeruli which are closely adherent to the bowman's capsular space. There was interstitial fibrosis, hyaline changes, epithelial cellular vacuolar degeneration and arteriopathy. The males showed milder histological alterations of glomeruli and arterioles. This is in agreement with the work of Teramaya*et al*., (2016) who observed that two days after alloxan treatment, tubular degeneration and regeneration was apparent in the thick ascending limbs of Henle with severe degeneration and necrosis were in distal convoluted tubules, and proximal convoluted tubules exhibiting vacuolar degeneration and necrosis with severe dilation of lumen. Similar to this work, Gande *at al*., (2023) observed alterations in the kidney histology, which was described as glomerular shrinkage. Other similar study also did agree with the current results (Frolova *et al*., 2022).

The present study showed that the serum urea concentration of the female diabetic rats was significantly increased than that of the female control group. Given that it implies that the functional integrity of the kidneys in the female diabetic rats is compromised, this may be related to the oxidative stress that diabetes mellitus places on the kidneys. Oxidative stress, or an unbalanced concentration of free radicals and antioxidants in the body, can cause damage to cells and tissues. Oxidative stress has a substantial effect on a number of renal disorders, such as ischemia-reperfusion injury, tubule-interstitial fibrosis, acute and progressive renal failure, and glomerulosclerosis (Gyurászová*et al*., 2020; Chaudhary *et al*., 2023; Jin *et al*., 2023).This is consistent with the findings of Nwogueze*et al*. (2023), which demonstrated that adult female rats subjected to constraint and invader stressors had a considerably higher urea concentration. As the last metabolite of protein nitrogen equilibrium, urea is mainly eliminated from the blood by the kidney by glomerular filtration. Exceeding the usual physiological range of serum urea concentration indicates abnormal tubular glomerular filtration (Raju *et al*., 2015). While the measurement of serum plasma concentration of urea is still used in clinical examinations to assess the functional integrity of kidneys, it is typically done in conjunction with the measurement of serum creatinine levels. The first acute renal measure is the urea concentration,

according to Borges *et al*. (Borges *et al*., 2008). When increased it indicates dysfunction and injury to the kidney.

The most reliable renal marker is an increase in plasma creatinine level, which happens when most renal function is lost. creatinine is an excretion result of muscular activity that circulates in the blood and can only be eliminated by the kidney (Belhadj *et al*., 2018). It was observed that the mean blood creatinine level did not exhibit a significant difference between the female control group and the diabetic group. This suggests that the short-term alloxan development of diabetes mellitus in the study female rats did not significantly alter the serum creatinine concentrations of the wistar rats. This agrees with the report of Nwogueze*et al*. (2023).

The present study showed significantly increased mean serum levels of sodium, chloride and bicarbonate with no significant alteration in the mean serum potassium level in the female diabetic rats than in their control counterpart. This finding partly corroborates the report of Egboh*et al*. (2022) which found that the sodium, potassium and bicarbonate electrolytes obtained in people with diabetes mellitus are significantly elevated compared with an age and gender balanced group of people without diabetes. Other previous studies have shown varying levels of electrolytes imbalance in diabetic individuals (Khan *et al*., 2019; Uppara *et al*., 2020; Eshetu *et al*., 2023). Diabetic nephropathy, which is characterized by reduced renal function can cause changes in electrolyte absorption and reabsorption as a result of damage to the nephrons caused by prevailing high blood glucose level. Electrolytes are essential for blood coagulation, nerve transmission, muscle contraction, acid-base balance, membrane potential, and bodily fluid regulation (Shrimanker and Bhattarai, 2023).

However, the current study found significantly increased mean serum level of potassium with no significant alterations in the mean serum levels of sodium, chloride and bicarbonate in the male diabetic rats compared to their male control counterpart. Our result is in consonance with several other similar studies (Rajagambeeram*et al*., 2020; Eshetu *et al*., 2023).On the other hand, Oyesola*et al*. (2022) and Martin *et al*. (2023) found no significant mean difference in plasma potassium level in alloxan-induced male diabetic rats than in male control group which is invariance with the current report.

Furthermore, the comparative evaluation of kidney function parameters in male and female alloxan-induced diabetic rats in this study showed that themean value of urea and creatinine was statistically significantly higher in female diabetics when compared to male diabetic albino rats.

This may imply that females are more susceptible to the impact of diabetes mellitus on the renal function than their corresponding male counterpart. There was no significant difference in the mean value of sodium, potassium, chloride and bicarbonate in female diabetics when compared to male diabetic rats.

Finally, there was no significant correlation observed in this study.

CONCLUSION

The kidney functions of male and female wistar rats with alloxan-induced diabetes have been found to exhibit varying degrees of significant histological and biochemical changes. These changes could put diabetics at risk for developing complications related to their condition.

Ethical Approval

Animal Ethic committee approval has been collected and preserved by the author(s)

Contribution to Knowledge

This study contributes significantly to the scientific community by addressing the differential impacts of alloxan-induced diabetes on renal function between male and female rats. It meticulously investigated both histopathological changes and biochemical indices, providing crucial insights into how diabetes affects kidney health differently based on gender.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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