HISTOLOGY

Histologic Evaluation of Adult Wistar Rat Kidneys Exposed to Generator Fumes, Petrol, Herbicides, Electrocution and Starvation for Infarction

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Abstract

Introduction: The increasing human population has led to the use of agrochemicals for weeding which improve food production, likewise the use of fuel and other amenity which has exposure human to various stressors is on the increase. **Aim:** This study histologically assessed the effect of these various stressors such as generator fumes, petrol, paraquat herbicides, electrocution and starvation in Adult Wistar rats' kidneys. **Methodology:** It was centered on 42 rats, randomly divided into six groups (1-6): control (normal room air), exposed to generator fumes, paraquat herbicides, electrocution, starvation and petrol respectively. The weight of each rat was recorded and analysed using one-way analysis of variance (ANOVA) for significant difference between each stressor group against control. At interval, the rats were sacrificed, kidneys were harvested, tissue processed, photomicrographs were reported. **Result:** Findings demonstrate significant weight loss in starvation, and no much changes among other groups except the petrol-exposed groups. The histologic architecture of the rat's kidney placed on starvation showed acute renal injury, while kidney of generator fumes had peritubular spaces and a darker glomerulus when stained with Masson trichrome probably due to carbon monoxide. An irregular vascular wall pointing to an acute kidney and glomerular injury showing signs of infarction when observed in the electrocution group. **Conclusion:** There was no significant weight loss in rats exposed to petrol, paraquat, generator fumes, and electrocution, but starvation led to a marked reduction in body weight and death. Histological assessment showed signs of acute kidney injury such as tubular damage, dilated capillaries, and glomerular changes especially in the generator fume, electrocution, and starvation groups. In contrast, the kidneys of rats in the petrol, paraquat, and control groups appeared normal. Both Hematoxylin and Eosin and Masson's Trichrome stains showed similar results, with no signs of fibrosis, supporting the accuracy and consistency of the observed changes.

Keywords: Stressors; kidney; histology; starvation; electrocution

1 Introduction

The need to feed the world's increasing human population has prompted the use of agrochemicals for increasing food production and ensure the continuation of the human race (Mesnage et al., 2015). Such agrochemicals include pesticides, several formulations of inorganic fertilizers, and herbicides marketed as harmless, with little or no side effects by the manufacturers. However, these agrochemicals have been viewed by some researchers as chemicals whose environmental fate is poorly understood, despite the rapidly increasing use (Dill et al., 2010).

Gasoline is one of the refined liquid products of crude oil and contains more than 2000 hydrocarbons with some additives and blending agents (Isa et al., 2013). These hydrocarbon contents are highly

volatile if left exposed and the vapor released into the atmosphere may be considered as gasoline fumes. Gasoline which is mainly used as vehicle fuel, it is also used to power electricity/generator at homes and industries (Mitri et al., 2015). The rising daily consumption of gasoline has led to humans being exposed to its fumes more often. An assessment from the California Office of the Environmental Health Hazard (2014) noted that gasoline and its fumes contain some toxic substances including benzene, toluene, ethylbenzene and xylene (BTEX), which are capable of causing various health hazards. And in accordance with the Centre of Management Development, 40% of households in Nigeria own and use generators to meet electricity requirements as reported in 2022 (The Nigeria Punch Newspaper, 2022), between 2015 and 2019, Nigeria spent approximately $500m on the importation of generators, which is the largest among nations of the world (Adesina, 2012). The estimated carbon dioxide (CO2) emitted from the world's electrical power industry is a 6% increase from 2020 pushed emissions to 36.3 gigatonnes (GT) (Centre for Global Development [CGD], 2021). With continuous growth in industrialization and urbanization, one can only expect a commensurate increase in the rate of generator importation and usage in Nigeria, thereby leading to a simultaneous increase in the country's contribution to the global CO2 burden. Besides its CO2 release, these generators have also been reported to generate a broad range of gaseous, particulate environmental pollutants, and sound etc of which CO is a notable constituent (Geiss et al., 2010).

Starvation is a serious deficiency of energy intake for a long period (Cahill, 2016). During the food restriction, the glucose level declines between 24 and 72 hours, resulting in release of glucagon. In these conditions, the body switches from carbohydrate to fat and protein usage to produce glucose and energy (Fuentebella & Kerner, 2009). Therefore, glucose is replaced with fatty acids and ketone bodies as the main energy source (Crook et al., 2011). Generally, Food is any substance consumed by living organisms to provide essential nutrients that support growth, energy production, and maintenance of vital bodily functions, which has proven that starvation adversely affect various functions in the body (Seriki et al., 2015). Earlier studies have determined that individuals with insufficient nutrition during development often experience poorer later-life health and fitness (Paris et al., 2006; Prado & Dewey, 2014).

Electrocution is referred to injury or death caused by the passage of electric current through the body. Electricity itself is the flow of electric charge usually carried by electrons through a conductor because the body is made up mostly of water and electrolytes, it can easily conduct electricity (Fish & Geddes, 2009). When exposed, the current can pass through tissues and vital organs, causing damages to the vital organs. The impact of electric shock on the body depends on several factors, such as the voltage, the strength of the current, how long the exposure lasts, and the path the current takes (Zemaitis et al., 2025). It can lead to burns, irregular heart rhythms, breathing difficulties, nerve and muscle damage, or even failure of internal organs (Fish & Geddes, 2009). In severe cases especially with high-voltage exposure it can cause extensive tissue destruction and affect the heart, brain, and kidneys (Teodoreanu et al., 2014). The kidneys are especially vulnerable due to their rich blood supply which make electric current disrupt blood flow, injure the delicate renal tubules, and set off inflammation and cell death, potentially leading to areas of tissue infarction (Basile et al., 2012).

A kidney or a kidney segment may suddenly stop receiving blood, leading to ischemia and ultimately necrosis of the infarcted area. This condition is known as a Renal infarction (Saeed, 2012). An infarction is the death or necrosis of tissue as a result of insufficient blood flow to the affected area. It could be brought on by vasoconstriction, mechanical compression, arterial rupture, or obstruction (Namgyal, 2020). A rare disorder called renal infarction is brought on by an abrupt interruption of blood flow in the renal artery. Because of its rarity and usually ambiguous clinical appearance, renal infarction is

frequently misdiagnosed and this increases the risk of subsequent embolic events affecting other organs or causes irreparable damage to the renal parenchyma (Bourgault et al., 2013). Atherosclerosis of the aorta or renal artery, aortic aneurysms, smoking, trauma, vasculitis, connective tissue illnesses, valvar endocarditis (natural or artificial valve) and vasculitis are additional etiological promoters of renal infarction (Mulayamkuzhiyil Saju & Leslie, 2023). As renal reperfusion therapy is ineffective in a significantly delayed presentation, an undiagnosed condition can cause irreversible loss of renal function (Seetho et al., 2009).

A general or local in situ mechanism may be to blame for a renal infarction. The primary causes of general renal infarction are traditionally categorized as "thrombophilic" from a variety of hypercoagulable conditions or "embolic" from atrial fibrillation, valvar or aortic wall disease. This condition of hypercoagulability appears to cause both arterial and venous thrombosis (Piazza & Morrow, 2020). Patients with COVID-19 may, like those with other viral pneumonias, be at risk for acute pulmonary embolism, however, the frequency of this link has not yet been shown. The first to document an acute arterial thrombosis associated with COVID-19 was Burke et al. (2020). Renal artery lesion in the parietal lobe makes up the local mechanism. Every mechanism is influenced by different causes. Finally, renal infarction is categorized as "idiopathic" when no cause is identified despite a thorough investigation (Bourgault et al., 2013). Furthermore, without systematic thorough explorations of adjacent arterial sites, nor without embolic and thrombophilic investigations, arterial blockage or isolated dissection might occasionally make the etiologic diagnosis challenging (Faucon et al., 2018). Atrial fibrillation may first manifest as renal infarction. Additionally, many atrial fibrillation patients who get renal infarction are discovered to be taking anticoagulants below the recommended dosage (Caravaca-Fontán et al., 2016). Since Renal infarction can mimic many other pathologic conditions, such as pyelonephritis, renal colic, acute abdomen, pulmonary embolism, rupture of an aortic aneurysm, and more, the diagnosis of renal infarction is frequently difficult to make (Antopolsky et al., 2012). When renal infarction is discovered and recognized early in the disease process, endovascular intervention and subsequent revascularization are advantageous, however, early detection is unusual due to the non-specific presentation (Ou et al., 2020). This study investigates and understands the effects of starvation, herbicides, electrocution, generator fumes, including petrol on the kidneys of Adult Wistar rats. As exposure to all these stressors increases, raising concern about their potential toxic effect on human health and their impact on the environment is not well understood, this study aims to investigate the potential effects of those stressors on the renal health of adult Wistar rats.

2 Materials and methods

2.1 Materials

Apparatus for this study were as follows: Cages, Rat Chow, Dissecting Kits, specimen bottles, Syringes, Oral Cannula, glass slide, light and primo star microscope, a laptop, Pasteur pipettes, water bath, refrigerator for storage of collected samples, digital weighing balance to weigh the Wistar rats, glass slides and cover slip on tissues are placed and passed through staining and covered, graduated cylinder, Microtome, absorbent paper, generator.

Reagents for this study were Paraquat dichloride, normal saline, paraffin wax, Papanicolaou stain, Bouin (75% saturated picric acid, 25% formaldehyde, 5% glacial acetic acid), 70% ethanol, 90% alcohol, 95% alcohol, Absolute alcohol, xylene, petrol lastly, haematoxylin and eosin stain then special stain (Masson Trichrome) for collagen.

2.2 Experimental design

The Ethics Committee of Delta State University, College of Health Sciences, Faculty of Basic Medical Science, Department of Human Anatomy and Cell Biology provided ethical permission for this work. Forty-eight healthy adult Wistar rats, weighing between 85 and 95 grammes, were taken from Delta State University's (DELSU) animal house's breeding section in Delta State. Given two weeks to acclimatize, the animals were housed in a well-ventilated cage in the departmental animal house. After that, the animals were divided into six groups: with eight rats in each group.

Groups 2, 3, 4, 5 and 6 were the exposure groups, while Group 1 was the control group, meaning it was not subjected to any stressors.

While the stressor groups were exposed to generator fumes (group 2), Starvation (group 3), electrocution (group 4), herbicides (group 5) and petrol (group 6) for varying amounts of time, Petrol and Paraforce (Herbicide) were obtained from nearby fuel station and pest control center in Abraka, Delta state, Nigeria.

The weight change was observed periodically for all groups (control inclusive). Briefly, the body weight was measured weekly with a digital balance throughout the experimental period before subjecting each group to their stressors.

The kidney, which is the experiment's vital organ, was removed for histological analysis, and the rats were killed by cervical dislocation 24 hours after the final exposure and were fixed in 10% formalin which was followed by enclosure in paraffin wax. The tissues were sectioned and stained using hematoxylin and eosin and special stain, Masson Trichrome. The prepared slides were then observed with the aid of a light microscope at a magnification of x100 and was captured using Primo Star Light Microscopy.

3 Results

There was statistically significant difference in the weight of the Wistar rats across all groups. The weekly body weight gain following the 3 weeks' duration of exposure to the various stressors is presented in Table 1. The control group showed no significant increase or decrease in body weight. The starvation group showed a significant decrease in weight due to deprivation of feed. The weight of rats exposed to generator fumes increased. The weight of rats treated with paraquat increased due to decreased activities and increased feeding mode. The electrocution group's weight remained consistent with the control group. The highest weight was observed in the Petrol group, and the lowest in the starvation group.

Table 1. Comparison of mean body weight (g) of the various groups across the weeks of the experiment.

Week	Control	Starvation	Generator fumes	Herbicides	Electrocution	Petrol
Week 1	144.7	123.2	162.5	118.8	151.4	199.7
Week 2	149.8	103.2	159.4	120.7	158.2	175.8
Week 3	143.8	47.4	164.2	121.1	171.4	201.8
Significance	–	0.01*	0.01*	0.01*	0.01*	0.01*

Note: Key: * = 0.1, ** = 1.0 (as given by the authors).

Photomicrographs of the kidneys of control and stressor-exposed rats are shown in Figures 1–5.

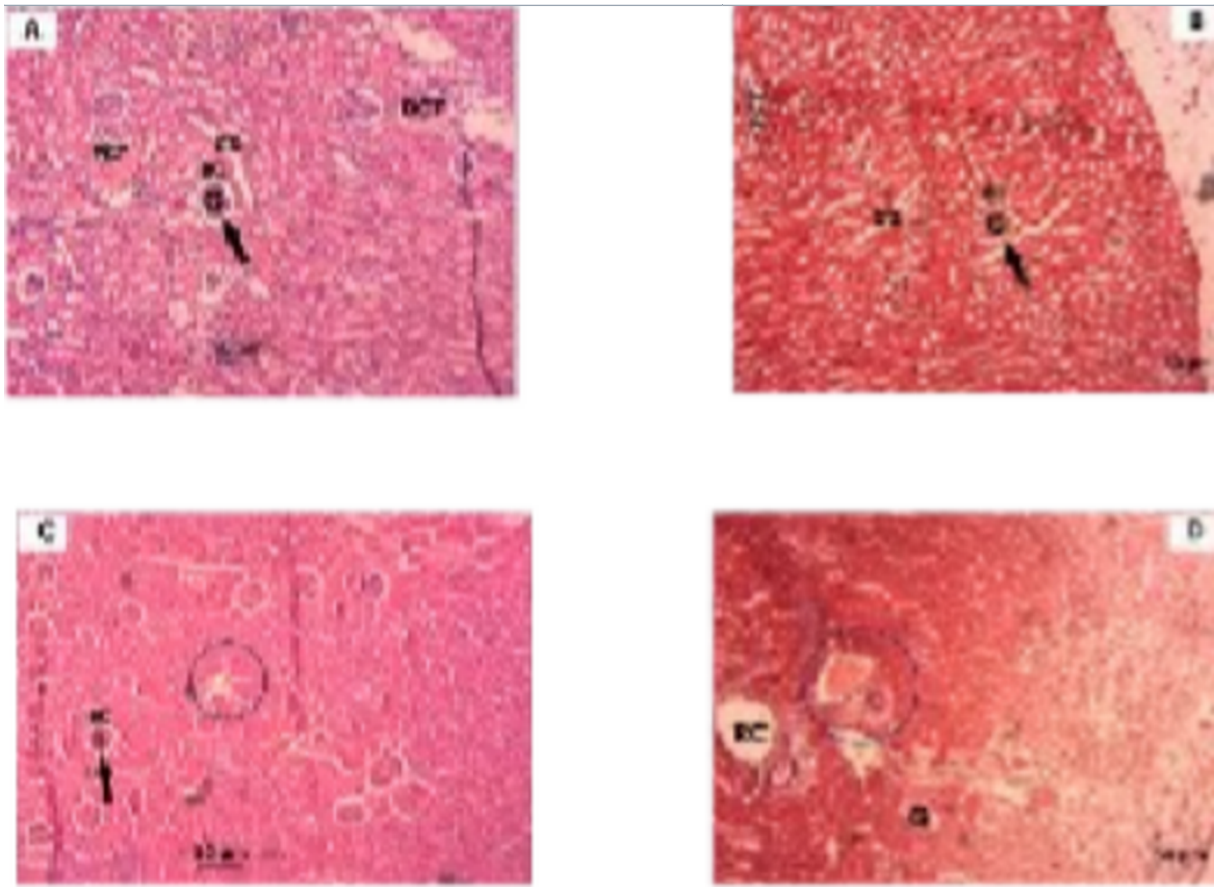


Figure 1. Photomicrographs of the kidney: control and generator-fume groups.

(A) Control group stained with H&E; (B) Control group stained with Masson trichrome. (C) Generator fumes stained with Masson Trichrome, showing sign of peritubular space and renal cortex. (D) Generator fume stained with H&E showing sign of peritubular space and renal cortex.

Key: G – Glomerulus. The black arrow is pointing to the BC-bowman capsule space. CD – indicates the collecting ducts. PCT – Proximal convoluted tubule and DCT indicates the distal convoluted tubules.

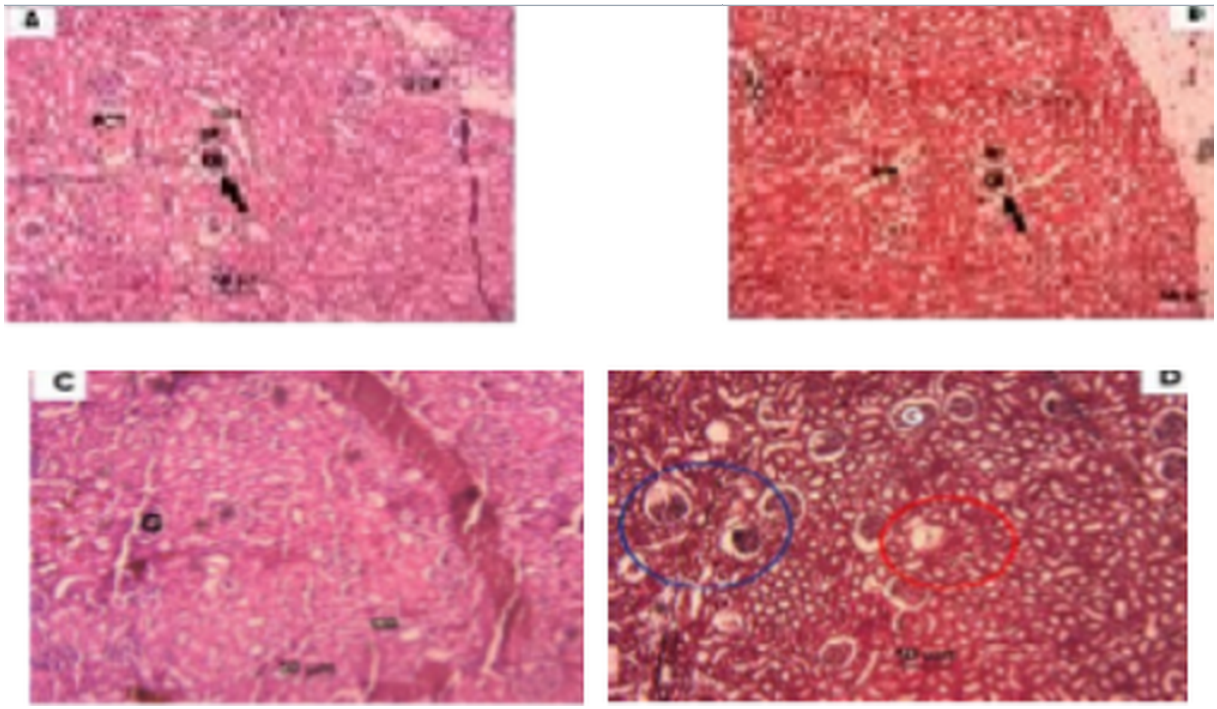


Figure 2. Photomicrographs of the kidney: control and petrol groups.

(A) Control group stained with H&E; (B) Control group stained with Masson Trichrome. (C) Petrol group stained with H&E shows normal glomerulus and Bowman's capsule. (D) Petrol group stained with Masson Trichrome shows normal glomerulus and Bowman's capsule.

Key: G – Glomerulus. The black arrow is pointing to the BC- bowman capsule space. CD – indicates the collecting ducts. PCT – Proximal convoluted tubule and DCT indicates the distal convoluted tubules.

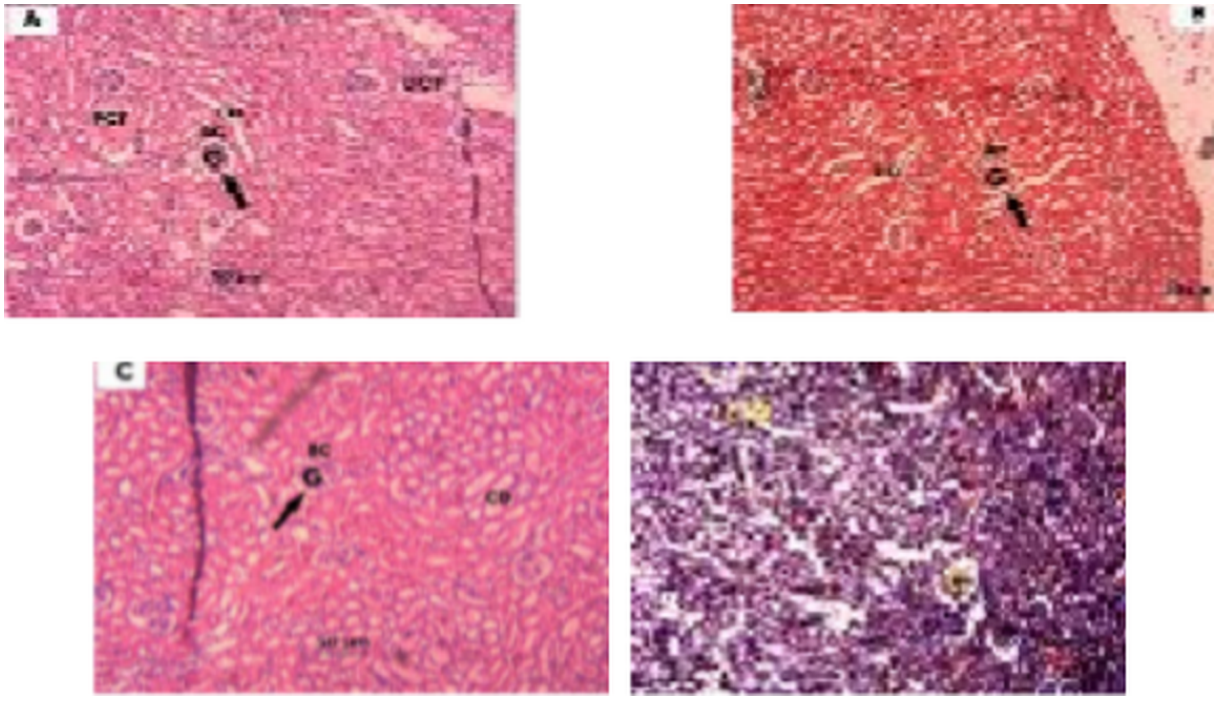


Figure 3. Photomicrographs of the kidney: control and herbicide groups.

(A) Control group stained with H&E; (B) Control group stained with Masson Trichrome, (C) Herbicides group stained with Masson Trichrome showed no changes in the histoarchitecture of the kidney after inhalation. (D) Herbicides group stained with H &E showed no changes in the histoarchitecture of the kidney after inhalation

Key: G – Glomerulus. The black arrow is pointing to the BC- bowman capsule space. CD – indicates the collecting ducts. PCT – Proximal convoluted tubule and DCT indicates the distal convoluted tubules.

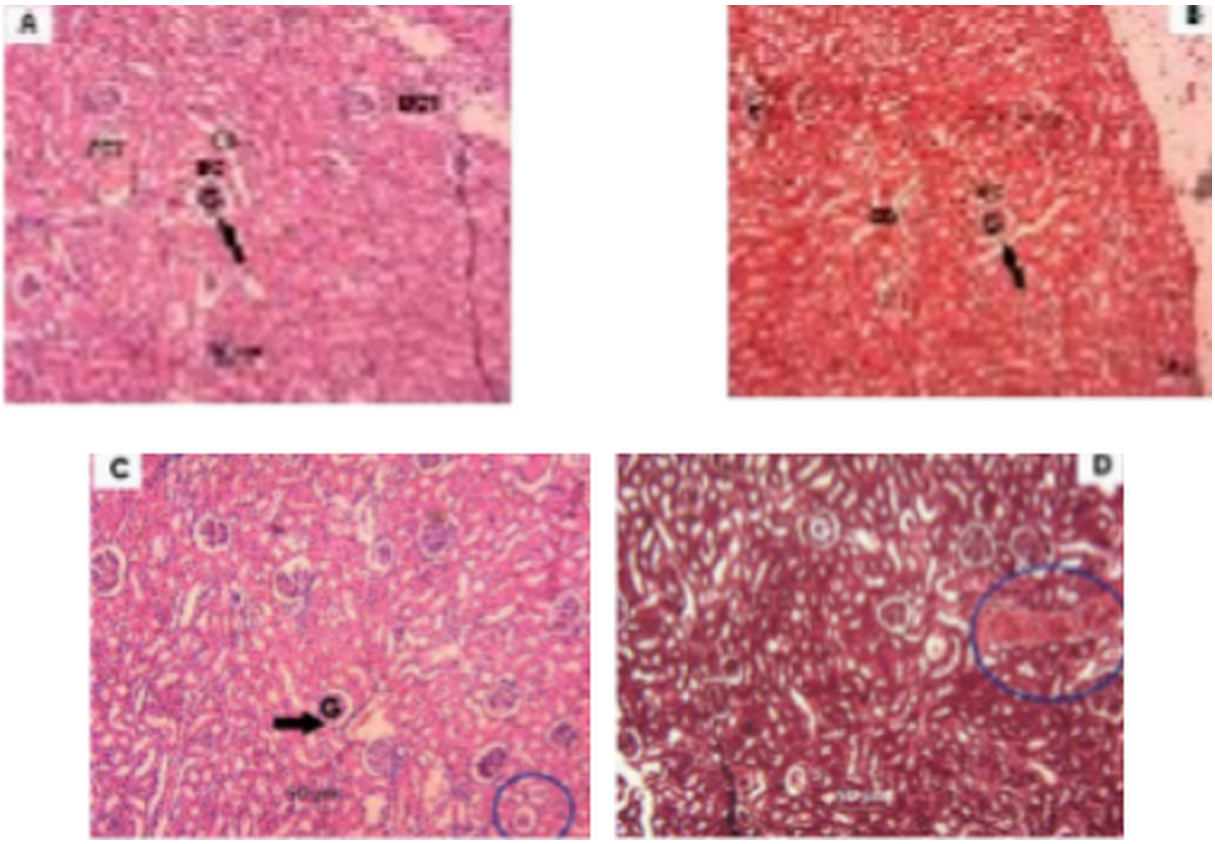


Figure 4. Photomicrographs of the kidney: control and electrocution groups.

(A) Control group stained with H&E; (B) Control group stained with Masson Trichrome, (C) Electrocution stained with Masson Trichrome showed an acute kidney injury (D) Electrocution stained with H & E stain showed an acute kidney injury.

Key: BC- Bowman's capsule, G – Glomerulus. The black arrow is pointing to the bowman capsule space. CD – indicates the collecting ducts. PCT – Proximal convoluted tubule and DCT indicates the distal convoluted tubules.

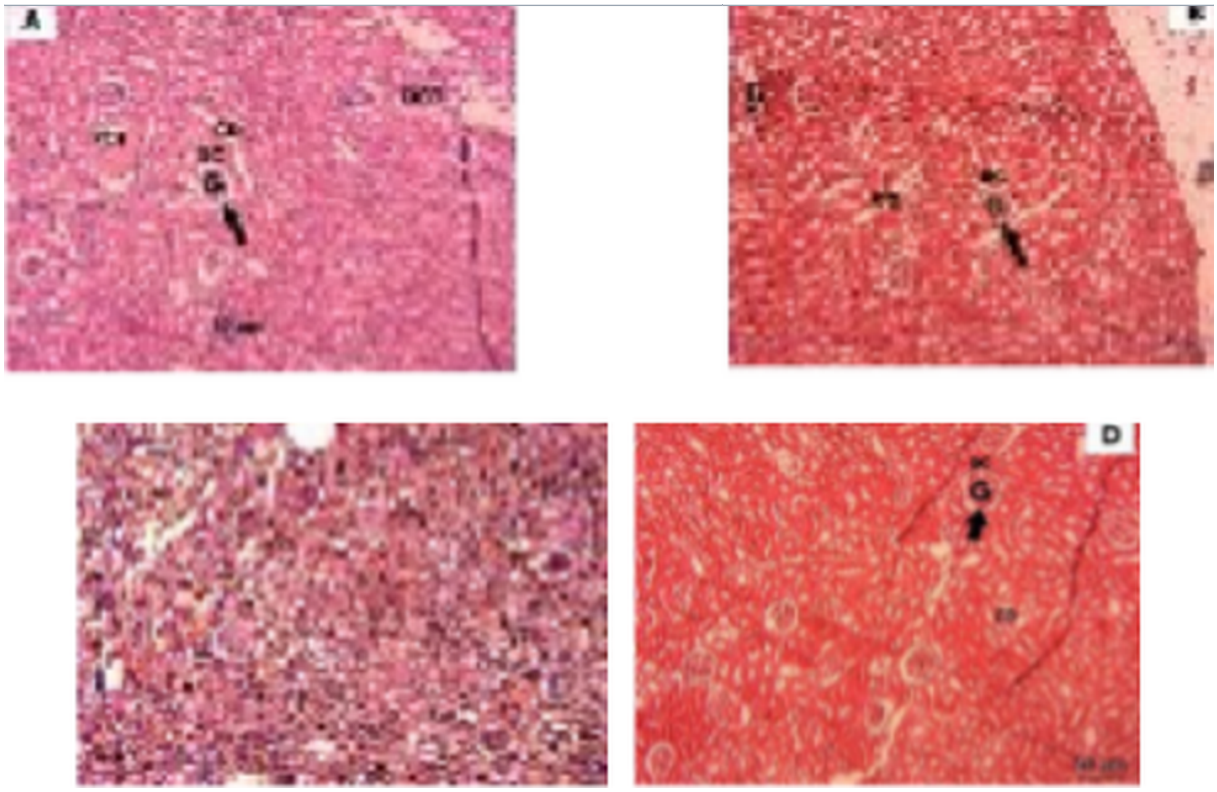


Figure 5. Photomicrographs of the kidney: control and starvation groups.

(A) Control group stained with H&E; (B) Control group stained with Masson Trichrome, (C) Starvation stain with Masson Trichrome shows irregular shape and injury to the glomerulus. (D) Starvation stain with H & E shows irregular shape and injury to the glomerulus.

Key: G – Glomerulus. The black arrow is pointing to the BC- bowman capsule space. CD – indicates the collecting ducts. PCT – Proximal convoluted tubule and DCT indicates the distal convoluted tubules.

4 Discussion

According to the study's duration, control and experimental animals were weighed before and after being subjected to stress as previously done by Ruth et al. (2015). This study showed that the control group during week 1 had a higher weight compared to the starvation group, the decrease in weight loss was still observed in the starvation group compared to the control group during the second week, as a result of deprivation of feed and water to the rats subjected to starvation which led to cannibalism amongst the Wistar rats. A rapid decrease in weight gain was seen in the third week after a chronic period of starvation and this led to slow response to activities and numbness most of the time before death. Thus, Wistar rats subjected to starvation were not euthanized but died after a rapid weight loss, showing that the Wistar rats subjected to test of starvation had a drastic loss of weight during the test which is in line with the work of Orellana et al. (1993), Eleftheriades et al. (2016) and Dietze et al. (2016) that also showed Wistar rats subjected to starvation had a significant difference in weight loss between the fed control group and the starved group. Thus, this study does not align with the finding of Ruth et al. (2015) that showed that the starved rats had increase in body weight than the fed control group.

In comparison with the Wistar rats exposed to generator fume, the rats showed little or less weight gain compared to the control group throughout the period of testing. During the first week of

exposure, the Wistar rats weighed more than the control group and during the second week, there was slight decrease in the weight gain compared to the control group, showing little or no difference to the control group during the third week of exposure.

The body weight of Wistar rats subjected to herbicides had a significant weight loss compared to the control group during the first week of testing. There was rapid weight gain during the second week of testing until the third week of exposure to herbicide (paraforce) having no decrease in weight gain rather it increased. The paraforce administered to the Wistar rats made the rats slow to external activities but more active in terms of feeding. During the third week of exposure, the Wistar rats had found the introduction of herbicides harmful and tend to stay away from areas affected with paraforce thus an increase in weight gain.

The Wistar rats electrocuted had body weight similar to that of the control group, there was neither rapid increase in weight gain nor decrease. This shows that this work is consistent with the work of Al-Sarraf and Mouihate (2022), which showed that the group subjected to physical resistance had similar body weight gain with that of the control group.

Further assessment in this study in the case of Wistar rats subjected to petrol test showed an increase in weight gain during the first week of exposure compared to the control group. The introduction of fuel during the second week brought about increase in activity of Wistar rats in the petrol group and the rats fed faster and consumed the most than the Wistar rats in the control group. It was also observed that the rats neither stayed away from the areas where petrol was introduced during the third week of continuous test and an increase in weight was seen during this third week of exposure. This observation of body weight is in line with that of Obanya et al. (2018) as the weight of the Wistar rats exposed to generator fume had higher difference compared to that of the control group.

At the end of the experimental study, slides obtained from both hematoxylin and eosin stain as well as special stain masson trichrome were viewed under a light microscope, and the micrographs are displayed in Figures 1–5.

The tissues of the glomeruli, which showed degenerative modifications as well as partial vacuolization, which is in agreement with Gurgen et al. (2013). It was observed that signs of infarction occurred in the blood vessel walls which showed severe effects on the histology of the kidney showing irregular shape and injury to the glomerulus after period of starvation.

The histological findings from the kidney of Wistar rats subjected to generator fumes showed great signs of carbon monoxide defects on the peritubular spaces of the kidney and renal cortex congestion (Okoh et al., 2023). In relation to the study conducted by Obanya et al. (2018), group C and D indicated regions with some perivascular swelling. Nwankwo et al. (2019) carried out a study following the Histological evaluation from Figure D of both hematoxylin and eosin and Masson Trichrome stains for herbicides exposure showed no changes in the histoarchitecture of the kidney after inhalation. This histological result is also in line with the research conducted by Onur et al. (2022) as the histological architecture were also normal to the control group.

Electricity tension can cause significant necrosis which can lead to renal failure, analysis following the histological evaluation of the slides obtained from both H&E stain and masson trichrome showed an acute kidney injury which corresponds to the report of Teramoto et al. (2020) and Alumsawi et al. (2023). The slides evaluated showed shrunken glomeruli as well as the widening of the gap between Bowman's capsule and the glomerulus which was also reported by Abo-Neima et al. (2016). Uhegbu

et al. (2015) in his study titled Effect of Exposure of Male Albino Rats to Kerosene, Diesel and Petrol on Kidney Function, analyzed the histological sections of the kidney exposed to petrol showing kidney tissue with normal glomeruli and Bowman's capsule which is in line with findings in this study. The histological analysis from Inhalation of petrol showed that the peritubular spaces were also congested due to carbon monoxide in this current study.

5 Conclusion

In this study, exposure to various stressors (petrol, generator fumes, paraquat herbicide, electrocution, and starvation) produced varying physiological and histological responses in adult Wistar rats. While no significant weight loss was observed in most groups, the starvation group exhibited a statistically significant reduction in body weight. Histological analysis revealed evidence of acute renal injury and infarction particularly in the generator fume, starvation, and electrocution groups, whereas the petrol and paraquat herbicide groups showed no observable renal effects, similar to the control group.

Importantly, both Hematoxylin and Eosin (H&E) staining and Masson's Trichrome staining demonstrated consistent findings, with no notable differences in tissue interpretation between the two methods. This indicates that while Masson's Trichrome is often used to highlight fibrotic changes and connective tissue, in this case, it did not reveal any additional pathological details beyond what was observed with H&E. Therefore, the use of both stains confirms the reliability of the observed renal injuries and supports the absence of fibrosis or chronic damage in the unaffected groups.

6 Contributions to knowledge

The study examines the impact of environmental stressors on adult Wistar rat kidneys, focusing on the kidneys exposed to generator fumes, petrol exposure, herbicides, electrocution, and starvation. The research identifies and characterize morphological alterations in renal tissues, including glomerular structure, tubular integrity, which help us to understand the relationship between environmental stressors and renal infarction, potentially impacting occupational safety, public health, environmental regulations and guide the development of preventive measures.

7 Limitations

The study's limitations include its exclusive focus on adult Wistar rats, its geographic location at Delta State University, and the complex interplay of stressors. It also lacks long-term effects and chronic conditions. The study's short-term nature suggests caution in interpretation and calls for further research on broader populations, diverse environments, and prolonged exposure durations.

8 Recommendations

This study recommends interdisciplinary collaboration, the use of advanced histological techniques like immunohistochemistry and electron microscopy, and the promotion of research findings through publication in scientific journals and conferences to advance knowledge in this area.

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