Original Research Article

ASSESSMENT OF THE PROTECTIVE EFFECT OF DACRYODES EDULIS METHANOL SEED EXTRACT ON GLYCEROL-INDUCED ACUTE KIDNEY INJURY

ABSTRACT

Aim: The present study was conducted to evaluate the effect of methanol seed extract of *Dacryodes edulis* (African pear) on the kidney of Albino rats.

Study Design: Using a preclinical experimental model, twelve (12) albino rats were grouped from A to D.

Study Location: This study was conducted at the Department of Medical Laboratory Sciences University of Nigeria Enugu Campus.

Methodology: Group A and B served as baseline control group and negative control group respectively and received distilled water and rat pellets for 7days respectively. Groups C-D received *Dacryodes edulis* extract in the following concentration 30mg/kg and 60mg/kg respectively for 7days. All administration was done once daily via oral gavage. At the end of the experiment, blood samples were collected for serum analysis for levels of Sodium, Potassium, Chloride and Bicarbonate using QCA (Quinica Clinical applicator assay kits). All the rats were sacrificed on the eighth day, the kidney tissues were dissected, weighed, processed using paraffin wax embedding technique and further stained using Hematoxylin and Eosin technique for histological studies.

Results: The results showed an increase in the body weight after the administration of the *Dacryodes edulis* extracts when compared to the body weights of the rats before the experiment. A statistically significant increase in the Bicarbonate level was observed in the rats that received 30mg/kg *Dacryodes edulis* extracts [18.86±1.03] when compared with the negative control [10.73±0.19]. Histological findings on the kidney include cellular infiltration of inflammatory cells and necrotic cells.

Conclusion: Ultimately, there was no significant protective effect recorded in the treated groups.

Keywords: Alternative medicine, *Dacryodes edulis*, nephrotoxicity, plant extracts, glycerol-induced kidney injury, traditional herbal medicine.

INTRODUCTION

1.1 Background of Study

Due to the financial limitations among developing Countries, sociocultural beliefs and recent research findings, people from various walk of life have engaged in the use of herbal and traditional medicine as an alternative, or as a compliment to orthodox medicines [1,2,3]. Nevertheless, while it provides a solution to the limitation posed by the socioeconomic variable in assessing of health as one of the sustainable development goals of the United Nation Organisation, questions have been asked of its efficacy as well as its effects in the body [4,5]. This is particularly important for low and middle income populations which research has shown as those that make use of these extracts the most [5]. Various plant products including *Dacryodes edulis* have been shown to have not just nutritional benefits but also potent medicinal effects. In Africa and China, much of these medicinal properties have been passed down through oral tradition in the form of folklore with varying degree of scientific evidence to back it up [6,7]. It is therefore pertinent to establish the pharmacological as well as the toxicological effects of these traditional medicines through various means [8]. This will not only help improve health coverage for low and middle-income populations but also provide substantial breakthroughs in biotechnology, biomedicine and sustainable public health or global health coverage [6].

The anatomy and physiology of albino rats' kidneys are comparable to those of other mammals including human beings. In addition to this, their genetic makeup maintain a striking resemblance with that of human beings making it an ideal experimental model in analyzing human diseases and processes in-vitro [9]. The kidney, a part of the urinary system, plays a primary role in filtering blood and urine and removing waste from the body [10]. Beyond that, it helps in promoting the development of red blood cells, maintaining the volume of all body fluids, controlling the concentration of dissolved substances like sodium ions, potassium ions, and calcium, and regulating the concentration of hydrogen ions in the blood among other vital functions it performs in the human body [10,11]. Particularly, its role as an excretory organ and in the pharmacokinetics of various medicinal agents makes it a primary target of toxic assaults from these foreign agents [12]. Understanding the effect of these plant extract and in fact any other medicinal agent on kidney toxicity, is therefore of great scientific and public health importance [6,8].

A member of the *Burseracea* family, *Dacryodes edulis* is a plant found in the tropical region of the world. It goes by several names, including African pear, Bush fruit tree, Bush butter tree, Safou (French), Atanga (Gabon), Ube (Igbo), Elemi (Yoruba), and Orumu (Benin) [13,14].The fruit of *Dacryodes edulis* can be roasted, eaten raw, or boiled in salt water in Nigeria, especially in the South East. We can also eat the roasted or boiled fruit with roasted or boiled corn [14]. Beyond its biological, pharmacological and overall medicinal implications, *Dacryodes edulis* have been implicated for its cosmetic uses, primarily derived from its exudates (resins) and oil extracted from the fruit and seeds [15]. In addition, *Dacryodes edulis* has been used in traditional medicine to treat ringworm, wounds, scabies, skin conditions, and inflammation due to the presence of various bioactive components in the plant [13,15].

In recent years, research evidence points to the fact that these plants display a wide range of biological and pharmacological actions, including anti-inflammatory, diuretic, laxative, antihypertensive, and antibacterial activities [16]. This has been accredited to their various nutritional components including, lipids, protein, vitamins, and minerals. However, beyond these basic nutritional component, these plant contain potent phytochemical components including saponins, tanins, glycosides, gallium, carotenoids, polyphenols, terpenoids, alkaloids, and glucosinolates among others [13,15,16] Specifically, numerous bioactive substances, including alkaloids, terpenes, flavonoids, tannins, thiamine, and saponins, are found in *Dacryodes edulis* [13]*.* Perhaps this is why evidence shows that a lot of ailments can be treated permanently with *Dacryodes edulis.* The plant has a long history of usage in traditional medicine for the treatment of a variety of illnesses, including fever, dysentery, wounds, and skin conditions [14]. Also, its extracts has been shown to have biological effects against bacteria, oxidative stress, and sickle cell anemia [17]

Glycerol-induced kidney injury can lead to acute or chronic renal failure. Acute renal failure is characterized by an abrupt loss in the renal system's ability to remove wastes, concentrate urine, reserve electrolytes, and maintain fluid balance. Also characterized by an increase in

serum creatinine and blood urea nitrogen (BUN) levels on an hourly, daily, or weekly basis [18].It is important to understand the composition, characteristics, and applications of glycerol, and how it affects the kidney. Glycerol otherwise referred to as glycerin has been used in various industry for commercial purposes as well as for personal use [19]. These uses include in cosmetic companies as a humectant, in the food industry as a sweetener, as a solvent in pharmaceutical industries, as a lubricant, explosives in various industries [18,20]. This suggests its wide range of applicability and how easily it can make way into the body of human beings. Also, research has provided evidence of its nephrotoxic effect, providing the need not just to create sustainable solutions or alternative to this, but also in providing sustainable medicinal therapy [6]. As a traditional medication, methanol seed extract of *Dacryodes edulis* has the advantage of containing antioxidant, anti-inflammatory and anti-bacterial components, which may make it a great regimen for treating various kidney conditions[13,14]. Consequently, the study will contribute to demonstrating the effectiveness of methanol seed extract of *Dacryodes edulis* in the management of not just glycerol-induced kidney injury but other various nephrotoxic conditions. The study was conducted to assess the nephroprotective properties of methanol seed extract of *Dacryodes edulis* on glycerol-induced Albino rats.

MATERIALS AND METHODS

3.1 Collection of Plant Material

Fruits of *Dacryodes edulis* (African pear) were bought at the Ogbete market in Enugu and were identified by a Taxonomist at the University of Nigeria Herbarium (UNH) domiciled in the Department of Plant Science and Biotechnology, Faculty of Biological Sciences, University of Nigeria, Nsukka and assigned a voucher number UNH/05/0315C

3.2 Ethical Consideration

The ethical approval for this study was obtained from the Animal welfare and ethics committee, Department of Animal science, University of Nigeria, Nsukka.

3.3 Experimental Animals

Twelve (12) male albino rats obtained from Nsukka; Enugu State were used for this study. They were kept at the Animal house of the Human Anatomy department, University of Nigeria, Enugu campus (UNEC). They were allowed for a period of two weeks for acclimatization before the commencement of the study. Acclimatization of animals and treatment were carried out in a well-ventilated room. They were placed in clean metallic cages and kept under ambient temperature (27±2° C) and 12-hour light/ dark periodicity. They were allowed free access to water and rat pellets.

3.4 Preparation of the Extract

The fruits were de-fleshed to separate the seed from the pulp. The seeds were washed and dried under the sun and ground using Gasoline powdered grinding machine. Methanol was the extraction solvent. The methanol was diluted to 80%. Two hundred (200) gram of the powdered seed was soaked into 1000ml of methanol in a beaker and the set up was allowed to stand for 48 hours in a fridge. The suspension was stirred intermittently. On the second day, it was filtered using Whatman filter paper. The filtrate was evaporated to dryness using a rotary evaporator; the concentrate was reconstituted with 500ml of water and then stored in an airtight container and stored in the refrigerator for administration to the experimental rat models.

3.5 Experimental Design

Twelve (12) male albino rats of about 3-4months old weighing between 110-145g were used for this study. The rats were divided into four (4) groups of 3 rats each according to their

weights. The groups were labeled A-D. Rats in group A served as the baseline control while rats in group B served as the negative control. Rats in group C and D served as the test groups. Rats in group A received water and food only. Rats in group B served as the negative control and received water and food without extract but kidney injury was induced with glycerol in this group. Rats in group C received low dose of the extract, 30mg/kg of methanol extract *Dacryodes edulis* and rats in group D received high dose of the extract, 60mg/kg methanol extract *Dacryodes edulis* via oral gavage respectively for seven (7) days. All animals were handled in accordance to Institutional approved guidelines for the care and use of animals for scientific research.

Table 1: Animal grouping and treatment

Groups	Number of Rats	Concentration of extract administered
A	3	Nil
B	3	Nil
C	3	30mg/kg of methanol extract of *Dacryodes edulis*
D	3	60mg/kg of methanol extract of *Dacryodes edulis*

3.6 Induction of Kidney Damage Using Glycerol

On the eighth day, upon completion of administration of the methanol extract of *Dacryodes edulis*, in the test groups, all the rats were fasted overnight. Upon completion of the fasting period, rats in groups A and B received 10mg/kg of normal saline while rats in groups C and D received 30mg/kg and 60mg/kg of methanol extract of *Dacryodes edulis* orally respectively. After 45minutes of the extract administration, kidney damage was induced in rats in groups B, C and D using 10mg/kg of 80% glycerol given to the rats intramuscularly. These rats were observed for one hour before the collection of blood specimens from the rats.

3.7 Biochemical Analysis

After an hour of kidney damage induction, blood samples were collected by retrobital puncture from medial canthus of the rats. The serum was separated from each blood specimen and the electrolyte (sodium, potassium, chloride and bicarbonate) levels were analysed.

3.8 Tissue Histology

The excised kidney tissues were observed grossly and the images of the lesions on the kidney tissues were captured with Samsung digital camera, the kidney tissues were cut and fixed in 10% formal saline. They were processed according to paraffin wax embedding method using an Automatic Tissue Processor. Sections of 5um thickness were obtained using the Rotary microtome. Hematoxylin and Eosin staining technique was used to stain the tissue sections.

3.9 Microscopy and Photomicrography

The sections were examined under the Olympus light microscope and then the images of areas of interest in the sections were captured using Samsung NX 1000 digital camera attached to the microscope.

3.10 Statistical Analysis

Data analysis was done using statistical package for social sciences software program (SPSS, Chicago, IL, version 23.0). The results were expressed where appropriate as mean ± standard error of mean (SEM). Differences between mean values were determined with one way analysis of variance (ANOVA) followed by Tukey's post hoc test. The results were considered statistically significant with values of $P < 0.05$.

RESULTS

4.1 Weight of Rats

Figure 1 shows a significant increase in the weights of rats after the administration of extract when compared to the weight before the administration of extract.

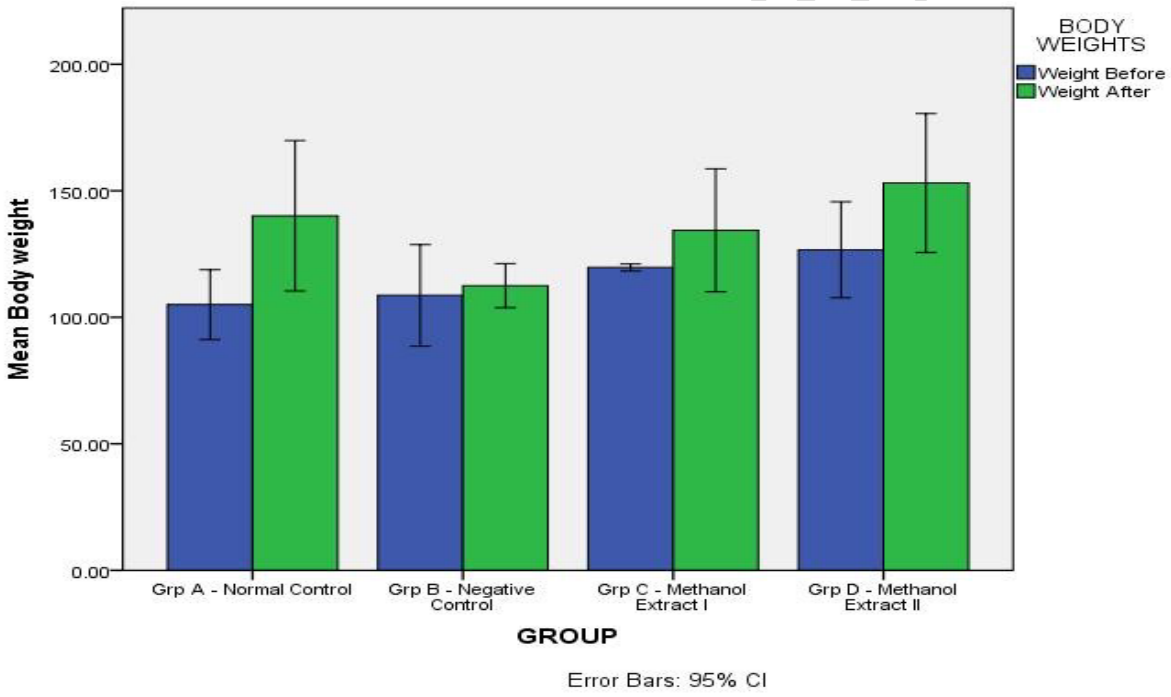

Figure 1 Bar chart showing the Mean body weight of Rats before extract administration and after extract administration.

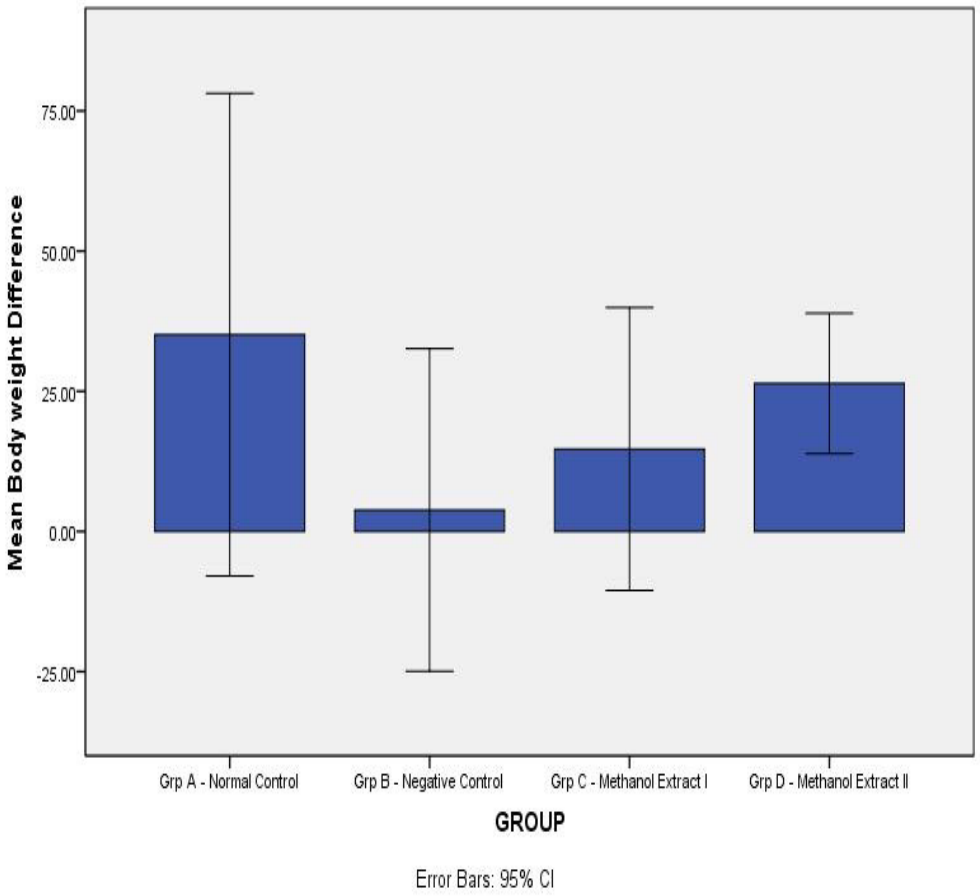

Figure 2 Bar chart showing the Mean body weight difference

4.2 Biochemical Analysis

Table 1 shows the analysis of the biochemical test. The Sodium, Potassium and Chloride levels of the Test groups (Groups C-D) against the Control groups (Group A-B) showed no significant difference while that of Bicarbonate levels of Group C that received low dose methanol extract of *Dacryodes edulis* showed an significant increase when compared with the negative control (Group B). The result is statistically significant following $p<0.05$.

Table 2: Comparison of the Electrolyte Level Between the Test and Control Groups

Groups	Number of Rats	Sodium	Potassium	Chloride	Bicarbonate
		Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
A (Baseline control)	3	141.90±1.29	4.68±0.19	94.50±0.64	18.90±2.77
B (Negative control)	3	143.43±0.17	4.95±0.00	98.60±0.20	10.73±0.19
C (Methanol low dose)	3	142.17±3.77	3.94±0.28	96.50±2.01	18.86±1.03*
D (Methanol high dose)	3	141.47±5.78	4.33±0.19	98.30±4.26	17.01±0.99

4.3 Photomicrographs

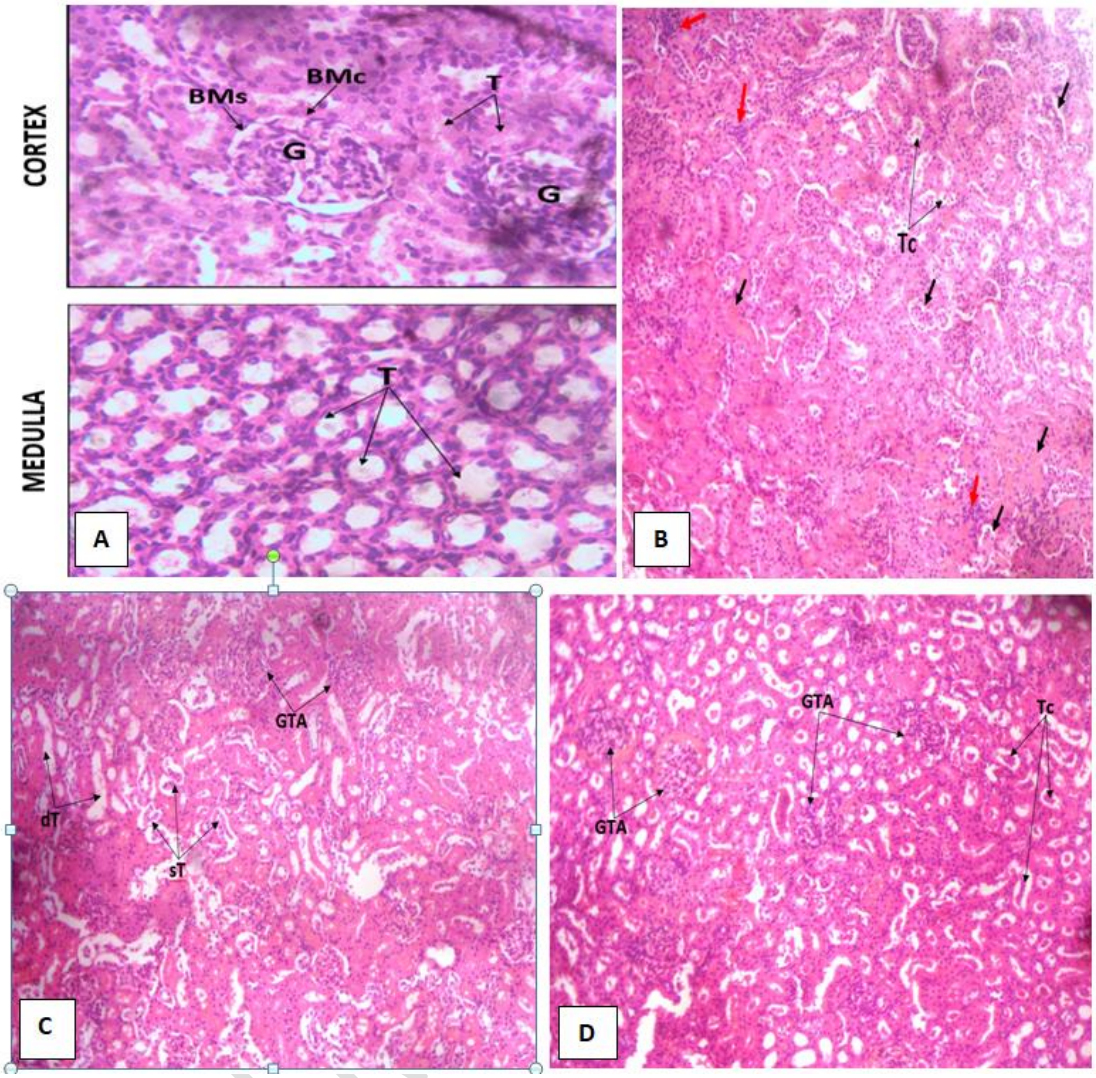

Figure 3 : A; Kidney section photomicrograph from normal control rat showing normal histoarchitecture of the renal tissue. The glomeruli (G), tubules (T), Bowman's capsule (BMc) and space (BMs) appear normal. B; Kidney section photomicrograph from rat treated with 10mg/kg glycerol only (Negative control) showing obvious structural tissue abnormalities. There is evidence of inflammatory cellular infiltration (red arrows), presence of tubular casts (Tc), degeneration and necrosis of tubules (black arrows). C; Kidney section photomicrograph from rat treated with low dose (30mg/kg) of *D. edulis* methanol seed extract prior to renal injury induction with 10mg/kg b.wt. of glycerol showing no obvious improvement in the renal histoarchitecture in comparison with negative control. Glomerular tuft adhesion (GTA) is observed while most of the degenerating tubules appear to be sloughing into the lumen (sT) or dilated (dT). D; Kidney section photomicrograph from rat treated with high dose (60mg/kg) of *D. edulis* methanol seed extract prior to renal injury induction with 10mg/kg b.wt. of glycerol showing marked glomerular tuft adhesion (GTA) and presence of tubular casts (Tc). There is no improvement on the renal histomorphology compared to negative control (Stain: H&E; Mag: 100)

DISCUSSION

The present study was designed to ascertain the potential protective effects of *Dacryodes edulis* methanol seed extract on glycerol-induced injury in albino rats. The findings of this study will contribute to the understanding of how traditional herbal remedies can be utilized in managing kidney-related disorders, particularly in the context of nephrotoxicity.

Glycerol is one of the most commonly used substances in the food industry. It is widely used in medical products and medicines. Usually as an additive, it is employed in the manufacturing of tooth paste and cosmetics such as hair care, skin care for softness and its moisturizing properties [20]. Similarly, it is widely used as a laxative with established harmful effects on kidneys. Therefore, glycerol can lead to acute inflammation of the kidneys from its consistent use especially, due to its prevalence in different food, agricultural, medicinal and industrial products consumed by humans [19].

The result from this study indicates that there was an appreciable increase in the weights of the rats after the administration of the extract. This observation is in consonance with the reports from a previous study by [21] which documented a similar increase in the body weight of rats that received *Dacryodes edulis* albeit an ethanolic extract. This strongly suggests that D*. edulis* has a potential weight gaining effect, although studies have shown that this is more of an indirect effect especially when consumed excessively amidst other dietary condition [17]. This particular study which focused on its anti-glycaemic and anti-cholesterol effects suggests that while the hexane extraxct of the seed of D. edulis possesses potential antidiabetic effect, the effect on weight loss is not clear. On the other hand, previous study by [24] has shown the phytochemical constituent of crude methanol extract of D edulis to be rich in terpenoids as opposed to the study by [17] which showed high flavonoid content. Although, it is not directly clear how they influence the weight gain since studies have shown dual effect by both phytochemical on body weight, depending on the sub-type, extract, and source [25; 26]. Perhaps this is why the study conducted by [14] recorded a dose-independent weight loss on their aqueous extract. However, the research did not attribute this weight loss to D. *edulis,* while suggesting that it may be as a result of the adaption of these animals to their environment. Similarly, it is important to add that while this study made use of methanolic extract, the study by [14] adopted an aqueous extract methodology suggesting possible differences in phytochemical content and ultimately, effect on weight. Also, the dosage is different with [14] making use of high dosages of 200,400 and 800mg'kg.

On the other hand, there were no significance difference observed in the activities of Sodium, Potassium and Chloride in animals treated with 0.3ml and 0.6ml/kg of methanol seed extract of *Dacryodes edulis* when compared with the control group but there was an appreciable increase in the Bicarbonate levels of rats in group C that received low dose (0.3ml) of methanol seed extract of *Dacryodes edulis* when compared with Group B (negative control). Nevertheless, the sodium, potassium, and chloride concentration remains unchanged when compared to the control group. While studies have shown that the hypolipidemic effect of D. *edulis* can impact on the bicarbonate levels negatively, the exact means of action remains largely unclear [17]. Also, this study worked on the hexane extract suggesting different phytochemical quantity. Generally, its effect on the electrolyte levels are insignificant with research outcomes by [22] agreeing to this, showing no significant effect in the plasma concentration of the electrolytes ($p<0.05$).
Histological examination revealed cellular infiltration and necrosis in the kidney tissues of the negative control group, consistent with the expected outcomes of glycerol-induced nephrotoxicity. However, the kidney tissues from the groups treated with *Dacryodes edulis* extract showed no significant improvement in histoarchitecture compared to the negative control group. This lack of marked improvement may indicate that while the extract has some protective properties, it may not be sufficient to reverse the cellular damage caused by glycerol.

The presence of glomerular tuft adhesion and tubular casts in the high-dose group further emphasizes that nephrotoxicity had occurred despite treatment. In contrast, the study by [23] carefully showed that this seed extract has a potential nephropotective effect through biochemical and histological analysis. However, this was done with carbon tetrachloride induced acute kidney toxicity and with aqueous extracts rather than methanol extracts. Also, this was done at significantly higher concentrations of 100-400 mg/kg as opposed to the 30 and 60 mg/kg adopted in this research. It suggests that at higher doses, this extract may provide a nephropotective effect against acute kidney diseases.

5.1 Conclusion

The use of glycerol at 10mg/kg in the present study resulted in severe inflammation of the kidney tissues and there was no significant nephroprotective effect observed in the kidney tissues of the rats in the treated groups that received the methanol seed extract of *Dacryodes edulis* compared to the negative control.

Disclaimer (Artificial intelligence)

Option 1:

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 the writing or editing of this manuscript.

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