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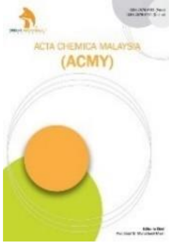

RESEARCH ARTICLE

NEPHROPROTECTIVE POTENTIAL OF *ARTOCARPUS HETEROPHYLLUS* FRUIT PULP AGAINST MERCURIC CHLORIDE–INDUCED RENAL INJURY IN WISTAR RATS: PHYTOCHEMICAL, BIOCHEMICAL, AND HISTOLOGICAL PERSPECTIVES

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ABSTRACT

Objective: *Artocarpus heterophyllus* fruit pulp extract were tested for protective effects against Mercuric Chloride– induced nephrotoxicity experimentally induced in Wistar rats, using phytochemical screening, biochemical parameters and histological studies. Methods: A total of twenty five adult male Wistar rats were randomized into five groups each containing five rats; Group A(control), group B which received Mercuric Chloride(3mg/kg), group C received *A. heterophyllus* (1000 mg/kg), extract+Mercuric Chloride group D received vitamin E (200mg/kg) plus Mercuric Chloride and group E which received *A. heterophyllus* only. All groups received their respective treatments for four weeks. Phytochemical screening and quantitative phytochemical analysis of *Artocarpus heterophyllus* fruit pulp were done. Body weight gain, Kidney weight, kidney index, serum urea, serum creatinine and serum electrolytes were carried out. Histological study of kidney tissues using HE stain were performed. Graphpad Prism version 9 was used for data analysis and level of significance was set at $p<0.05$. Results: Phytochemical screening of the extract showed the presence of high amounts of phenols (29 ± 0.48 mg/100 g) and saponins (19 ± 1.1%), while flavonoids, tannins, and alkaloids were present in moderate quantities. Injection of Mercuric Chloride significantly reduced body weight gain, caused nephromegaly, increased serum urea and creatinine levels, and resulted in marked histopathological damage in kidneys characterized by tubular necrosis and inflammatory cell infiltration. Treatment with aqueous extract of *A. heterophyllus* fruit pulp and vitamin E effectively reversed body weight loss, restored biochemical parameters, and ameliorated histopathological architecture of kidneys induced by Mercuric Chloride (ANOVA, $p < 0.05$). Oral administration of *A. heterophyllus* extract alone did not alter body weight gain, renal functions and caused no significant histopathological changes in kidneys. Conclusions: Pretreatment with aqueous extract of fruit pulp of *A. heterophyllus* offered significant nephroprotection against Mercuric Chloride–induced nephrotoxicity which can be attributed to the synergistic antioxidant effect of its phytoconstituents.

KEYWORDS

Artocarpus heterophyllus, jackfruit pulp, nephroprotection, Mercuric Chloride, oxidative stress, phytochemicals

1. INTRODUCTION

Heavy metals are elements that occur naturally in the environment, distinguished by their relatively high atomic weights and densities greater than water. At low concentration, heavy metal even though they occur in the earth crust natural, can be harmful to organisms (Clarkson *et al*., 2003). Human activities like mining, industrial operations, and agricultural practices are major contributors that drives most of the heavy metal contamination. (Farina *et al*., 2011).

Due to its status as a pervasive global pollutant, Mercury stands out as one of the most documented heavy metal. It ability to accumulate in tissue and cause oxidative stress is one of it harmful tendencies leading to a number damaging effects (Bernhoft, 2012). The primary target for mercury exposure is the central nervous system, however, prolong exposure at high level will lead to impairment of the kidneys and gastrointestinal tract and disrupt immune functions (Zarups, 2000).

Mercuric chloride which is the inorganic form of mercury is particularly harmful to a number of organs including the kidneys, liver, and digestive organs (Bridges and Zalups, 2005). Mercuric chloride toxicity involves a host of connected mechanisms like oxidative stress, depletion of cellular thiol groups, and a strong affinity for binding thiol-containing molecules in the body (Zahir *et al*., 2005). After absorption, mercury can distribute widely, and acute exposures may produce severe systemic toxicity that can progress to multi-organ failure (Rooney, 2007). Reports by Valko *et al.* (2006) suggest that in mild cases, there is a possibility of improvemet after exposure if the source of exposure is removed, however, damage may not be reversed if the exposure is severe or prolonged, especially in the renal and nervous tissue. This is due to oxidative stress been mercury's central mechanism of action. This has led to researchers exploring possible

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antioxidants as potential protective agents (Halliwell and Gutteridge, 2015).

Researches have proven that natural antioxidants help the body defend itself against damage from free radicals (Flora *et al.*, 2008). One of such natural antioxidant is Vitamin E which is a fat-soluble antioxidant and plays a central role in preserving cellular health and limiting oxidative stress (Flora *et al.*, 2008). It is concentrated in membrane-rich regions of cells, where it helps prevent lipid peroxidation and modulate oxidative reactions (Valko *et al.*, 2007; Ekor, 2014). Aside from been a free radical scavenger, vitamin E also posseses anti-inflammatory effects and supports immune function (Morton, 1965). Because the main functions of the kidneys are filtration of blood to remove waste and balance electrolyte, they are constantly exposed to circulating toxins which makes them at risk to toxic injury (Raihandhany *et al.*, 2018). For these reasons, investigating whether vitamin E can protect the kidneys from Mercuric Chloride–induced damage is an important and timely area of biomedical research.

Fruits are rich sources of antioxidants, vitamins, and minerals, and jackfruit (*Artocarpus heterophyllus*) is a good example. Widely cultivated across Asia, Africa, and South America, this large tropical fruit supplies carbohydrates, protein, and vitamins such as A and C, along with minerals like potassium, calcium, and magnesium (Baliga *et al.*, 2011; Khan *et al.*, 2021). Some of the Phytochemicals bioactive compounds identified in jackfruit, includes carotenoids and polyphenols which have been studies to possess antioxidant, anti-inflammatory, and anti-diabetic properties (Mandhare *et al.*, 2020; Banc *et al.*, 2022). These properties make it plausible that jackfruit could help protect organs from toxic insults, including kidney injury caused by Mercuric Chloride.

Against this background, the present study was undertaken to assess whether vitamin E and jackfruit (*Artocarpus heterophyllus*) pulp extract can guard against Mercuric Chloride–induced renal toxicity in Wistar rats, with evaluation focused on biochemical, histological, and antioxidant endpoints.

2. Materials And Methods

2.1 Chemicals and Plant Material

Mercuric Chloride ($HgCl_2$) was manufactured in the UK for ANOSANTEC Laboratory and purchased from Emmytex Biomedical Store, Benin City, Nigeria. Jackfruit (*Artocarpus heterophyllus*) was obtained from the Scripture Union Camp, Oluku Road, Benin City.

2.2 Experimental Animals

Twenty-five adult Wistar rats (133–182 g) were housed in standard plastic cages with wire-gauze tops and acclimatized for two weeks. Animals had ad libitum access to Top Feed Ltd. growers' mash and water. Cages were cleaned daily and disinfected at intervals. All procedures conformed to institutional ethical guidelines for laboratory animal care.

2.3 *Experimental Design*

Rats were randomly divided into five groups (n = 5) and treated for 28 days:

- Group A (Control): 1 mL distilled water daily.
- Group B ($HgCl_2$): 3 mg/kg $HgCl_2$ daily (Ali *et al.*, 2020).
- Group C (Jackfruit): 1000 mg/kg *A. heterophyllus* extract daily (Efendi *et al.*, 2020).
- Group D (Jackfruit + $HgCl_2$): 1000 mg/kg extract, 30 mins before 3 mg/kg $HgCl_2$.
- Group E (Vitamin E + $HgCl_2$): 200 mg/kg vitamin E, 30 mins (Obayuwana and Nweke, 2024) before 3 mg/kg $HgCl_2$.

The oral LD_{50} of *A. heterophyllus* is >5000 mg/kg (Prakash *et al.*, 2015). Substances were administered by oral gavage using a modified method (Alflen *et al.*, 2017

2.4 Plant Extraction

Jackfruit extract was prepared using freeze-drying (Liapis and Bruttini, 2020; Waseem et al., 2023).

3. Sample Collection

On day 29, rats were weighed and sacrificed under chloroform anesthesia. Kidneys were harvested, blotted dry, and weighed. Organ weight ratio (%) was calculated as:

$$\text{Organ index (\%)} = \left(\frac{\text{Organ weight (g)}}{\text{Body weight (g)}}\right) x100$$

Kidneys were fixed in 10% buffered formalin for histology; blood was collected into heparinized tubes for renal function assays. Tissue processing was done at the University of Benin (Histopreparatory Lab), and biochemical assays at UBTH Hematology Lab.

3.1 Histological Analysis

Tissues were dehydrated, cleared in xylene, and embedded in paraffin. Sections (5 µm) were stained with hematoxylin and eosin and examined under a Leica DM750 microscope with a digital camera (Leica ICC50), at ×100 magnification.

3.2 Statistical Analysis

Data are expressed as mean ± SEM. One-way ANOVA followed by Tukey's HSD post hoc test was used. Statistical significance was set at $P < 0.05$. Analysis was performed using GraphPad Prism 9.

4.RESULTS

The phytochemical evaluation of *Artocarpus heterophyllus* fruit demonstrated the presence of several biologically active constituents (Table 1). Qualitative screening revealed positive results for compounds such as saponins, phenols, eugenols, terpenoids, alkaloids, flavonoids, and tannins. However, glycosides, steroids, and reducing sugars were not detected in the sample analyzed.

Further quantitative assessment showed that phenolic compounds were present in the highest concentration (29.0 ± 0.48 mg/g). This was followed by saponins (19.0 ± 1.10 mg/g), flavonoids (5.4 ± 1.20 mg/g), tannins (4.7 ± 0.41 mg/g), and a relatively smaller amount of alkaloids (0.70 ± 0.09 mg/g). Overall, these findings indicate that *A. heterophyllus* fruit contains appreciable levels of phenolic compounds and saponins, which may play an important role in the biological and therapeutic activities associated with the fruit.

Table 1: Phytochemical composition of *Artocarpus heterophyllus* fruit (mean ± SEM).

S/N	Phytochemical	Test Method	Qualitative Inference	Quantitative Content (Mean ± SEM, mg/100 g DW)
1	Glycosides	General Test	–	0.45 ± 0.08
2	Saponins	Frothing Test	+	19.0 ± 1.1
3	Phenolics	Ethanol/Ferric Chloride	+	29.0 ± 0.48
4	Eugenols	Ethanol/Ferric Chloride	+	1.2 ± 0.05
5	Terpenoids	Salkowski Test	+	3.8 ± 0.22
6	Steroids	KOH Test	–	ND (not detected)
7	Alkaloids	Picric Acid	+	0.70 ± 0.09
8	Flavonoids	Lead Acetate	+	5.4 ± 1.2
9	Tannins	Ferric Chloride	+	4.7 ± 0.41
10	Reducing Sugar	Fehling's A & B	–	ND (not detected)

Key: + = Present; – = Absent; ND = Not Detected; DW = Dry Weight

Table 2 presents the effects of *Artocarpus heterophyllus* extract and vitamin E on body weight and kidney-related parameters in rats exposed to Mercuric Chloride. At the beginning of the experiment, the body weights of the animals were generally similar across all groups (p = 0.0016). By the end of the study period, however, rats treated with Mercuric Chloride showed a noticeable decline in body weight when compared with both their initial measurements and the control group.

In contrast, animals that received either *A. heterophyllus* extract or vitamin E did not experience this reduction in body weight. Instead, their body weights remained comparable to those of the control animals, and they demonstrated significant improvements when compared with the Mercuric Chloride–only group (p = 0.0005). Despite these changes in body weight, there were no significant differences observed in kidney weight or in the kidney–body weight index among the experimental groups (p > 0.05).

Table 2: Effect of *Artocarpus heterophyllus* and Vitamin E on Body Weight, Kidneys Weight, and Kidney-Body Weight Index in Mercuric Chloride-Treated Rats

Groups/Test	Control	3mg/kg of Mercuric Chloride	1000mg/kg of *Artocarpus heterophyllus*	1000mg/kg of *Artocarpus heterophyllus* + 3mg/kg of Mercuric Chloride	Vitamin E	p-value
Initial weight(g)	150.70±1.50	152.00±6.90	155.70±8.40	151.00±3.10	157.00±7.20	0.0016
Final Weight (g)	176.30±3.80*	147.00±4.40	171.70±10.30*	170.70±0.70*	171.00±7.80*	
Weight Change (g)	25.60±4.10	-5.00±5.40 $^{\alpha}$	16.00±10.90	19.70±2.80 $^{\gamma}$	14.00±8.20 $^{\gamma}$	0.0005
Kidneys Weight (g)	0.90±0.12	1.03±0.19	0.80±0.17	0.97±0.12	0.77±0.07	0.6437
Kidneys- Body Weight index (%)	0.56±0.08	0.70±0.12	0.48±0.13	0.57±0.07	0.45±0.04	0.4500

Note: Values are mean ± SEM (n = 5). One-way ANOVA followed by Tukey's post-hoc test was used for analysis.

*P < 0.05 compared with initial weight of the same group; $^{\alpha}$P < 0.05 compared with control; γP < 0.05 compared with Mercuric Chloride group.

The results of the one-way ANOVA showed that there were significant differences among the experimental groups in chloride ion concentration (p < 0.0001), urea levels (p < 0.0001), and creatinine levels (p = 0.0027). In contrast, the concentrations of sodium, potassium, and bicarbonate did not show any statistically significant variation across the groups (p > 0.05).

Further post hoc analysis indicated that exposure to Mercuric Chloride caused a significant decline in chloride ion concentration and urea levels when compared with the control group (p < 0.05). However, administration of *Artocarpus heterophyllus* pulp, either given alone or alongside Mercuric Chloride, led to a significant improvement in chloride levels compared with the group that received Mercuric Chloride alone (p < 0.05).

Similarly, urea concentrations in rats treated with the extract returned toward normal values, showing significant differences when compared with both the control and the Mercuric Chloride–treated groups. Mercuric Chloride exposure also resulted in an increase in serum creatinine relative to the control group (p < 0.05). Interestingly, rats treated with *Artocarpus heterophyllus* pulp alone exhibited a marked reduction in creatinine levels when compared with both the control and Mercuric Chloride groups (p < 0.05).

Table 3: Serum Electrolytes and Renal Indices in Mercuric Chloride-Treated Rats Following *Artocarpus heterophyllus* Fruit Pulp Administration

Groups/Test	Control	3mg/kg of Mercuric Chloride	1000mg/kg of *Artocarpus heterophyllus*	1000mg/kg of *Artocarpus heterophyllus* + 3mg/kg of Mercuric Chloride	Vitamin E	p-value
Sodium ion (mEq/L)	142.00±0.58	140.70±1.20	143.30±1.20	142.00±1.53	141.30±1.45	0.6377
Potassium ion (mEq/L)	5.23±0.19	5.00±0.20	4.67±0.18	4.97±0.12	5.00±0.21	1.248
Bicarbonate ion (mEq/L)	20.67±1.20	22.67±0.33	23.67±0.88	23.00±1.73	23.00±1.73	0.7800
Chloride ion (mEq/L)	103.70±0.33	98.33±0.33 $^{\alpha}$	105.30±0.67	106.30±0.67 $^{\gamma}$	105.30±0.67 $^{\gamma}$	<0.0001
Urea (mmg/dL)	46.33±0.88	37.33±0.67 $^{\alpha}$	40.33±0.88 $^{\alpha}$	38.33±0.67	44.67±0.88 $^{\gamma}$	<0.0001
Creatinine (mg/dL0	0.97±0.03	1.17±0.09 $^{\alpha}$	0.70±0.06 $^{\alpha}$	0.80±0.06 $^{\gamma}$	1.00±0.06	0.0027

Note: Values are mean ± SEM (n = 5). One-way ANOVA followed by Tukey's post-hoc test was used for analysis.
*P < 0.05 compared with initial weight of the same group; $^{\alpha}$P < 0.05 compared with control; γP < 0.05 compared with Mercuric Chloride group.

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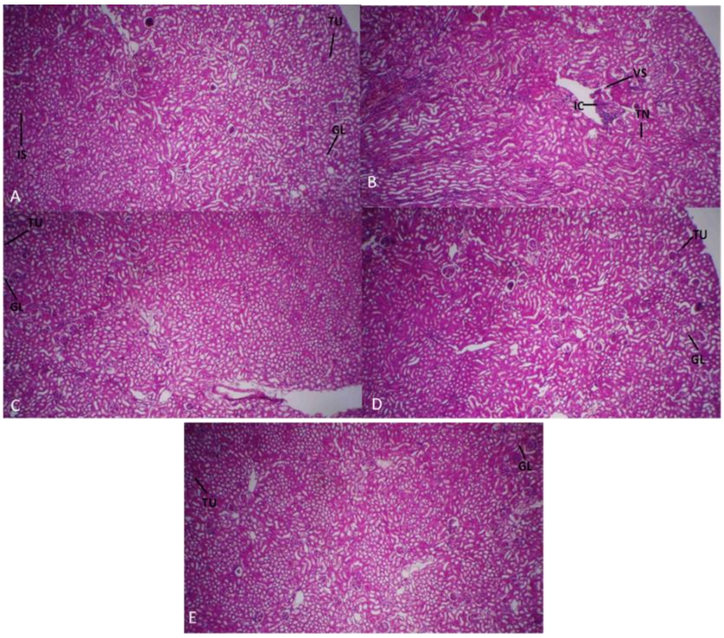

Figure 1: Histopathological Evaluation of Rat Kidneys (H&E ×40)

Representative photomicrographs of kidney sections showing treatment-related changes. Plate A: Control group displaying normal renal architecture with intact tubules (TU), interstitial space (IS), and glomeruli (GL). Plate B: Rats treated with 3 mg/kg $HgCl_2$ alone showing marked pathological alterations, including heavy interstitial infiltrates of inflammatory cells (IC), vascular stenosis (VS), and focal tubular necrosis (TN). Plate C: Rats treated with 1000 mg/kg *Artocarpus heterophyllus* alone exhibiting normal tubules (TU) and glomeruli (GL). Plate D: Rats co-treated with 1000 mg/kg *A. heterophyllus* and 3 mg/kg $HgCl_2$ demonstrating preserved renal histoarchitecture with intact tubules (TU) and glomeruli (GL). Plate E: Rats co-treated with Vitamin E and $HgCl_2$ also showing normal renal morphology with preserved tubules (TU) and glomeruli (GL).

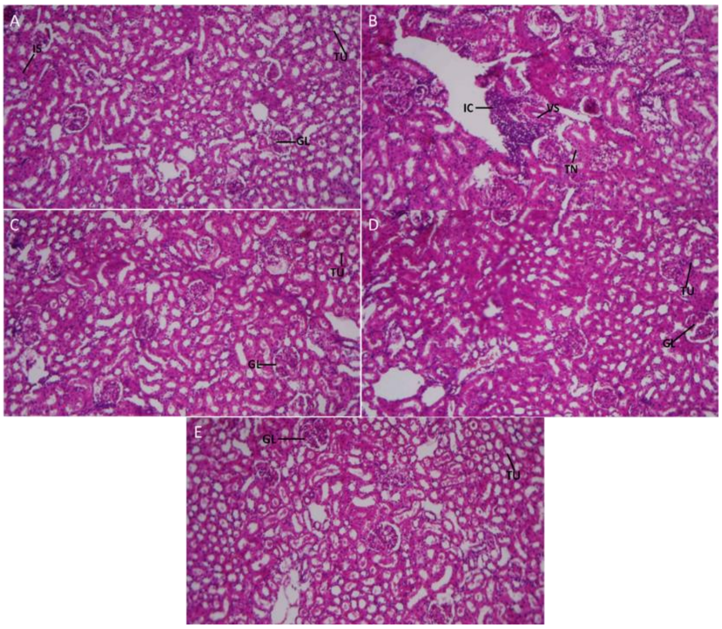

Figure 2: Representative photomicrographs of kidney sections showing treatment-related changes.

Plate A: Control group showing normal renal architecture with intact tubules (TU), interstitial space (IS), and glomeruli (GL). Plate B: Rats treated with 3 mg/kg $HgCl_2$ alone, exhibiting pronounced pathological changes including heavy interstitial infiltration by inflammatory cells (IC), vascular stenosis (VS), and focal tubular necrosis (TN). Plate C: Rats given 1000 mg/kg Artocarpus heterophyllus alone, displaying normal tubules (TU) and glomeruli (GL). Plate D: Rats co-treated with 1000 mg/kg A. heterophyllus and 3 mg/kg $HgCl_2$, showing largely preserved renal histoarchitecture with intact tubules (TU) and glomeruli (GL). Plate E: Rats co-treated with vitamin E and $HgCl_2$, also demonstrating normal renal morphology with preserved tubules (TU) and glomeruli (GL).

5. Discussion

Mercuric chloride is widely knowns as a powerful toxin that can produce both systemic and renal injury through mechanisms such as oxidative stress, mitochondrial dysfunction, and disruption of electrolyte balance. This study therefore examined whether pulp extract from *Artocarpus heterophyllus* (jackfruit) could attenuate those effects, using phytochemical profiling, biochemical assays, and histological evaluation to assess any protective benefits.

Phytochemical Analysis: Phytochemical screening of the extract showed significant amounts of phenols, saponins, flavonoids, tannins, and alkaloids, with phenols and saponins present in the highest concentrations. These classes of compounds are well known for their antioxidant activities and their ability to regulate inflammatory responses (Sreeja et al., 2021). Other studies also reported similar rich phytochemical compounds in the leaves and seeds of *Artocarpus heterophyllus*, indicating it potential across multiple parts of the plant (Druga *et al*., 2022).

Plants like *Moringa oleifera* and *Lawsonia inermis* has shown similar antioxidant profiles which have been linked their protective effects against kidney and liver toxicity (Karthivashan *et al*., 2016; Enoghase *et al*., 2024). Similar compounds found in this study likely explains the

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protective effects *Artocarpus heterophyllus* against heavy metal toxicity through antioxidant mechanisms.

Body Weight Changes: Exposure to Mercuric Chloride significantly reduced the body weight of treated rats while increasing the weight of the kidneys. Similar findings were reported by Bridges and Zalups, (2017) and Gao *et al*. (2022), stating that heavy metal exposure may lead to interference in body mechanisms and lead to renal. Co-administration of mercuric chloride and jackfruit pulp prevented weight loss observed in the mercuric chloride only group, this result indicates a protective effect. Similar reports of other fruit-derived antioxidants such as grape seed extract and pomegranate juice in rodent models of nephrotoxicity (Zhang *et al*., 2014; Aksu *et al*., 2017) had earlier been published.

Jackfruit pulp was not able to completely protect against the increase in the kidney weight caused by mercuric chloride, however, Vitamin E showed a better protective effect against mercuric chloride-induced nephromegaly. Studies have shown that although plants have the ability to reduce heavy metal toxicity, they may not have the ability to completely protect against these toxic compounds (Partovi *et al*., 2018).

Biochemical Parameters: Mercuric Chloride exposure caused a significant rise in serum urea and creatinine, markers of impaired renal function. This result is consistent with prior studies showing that mercury and other heavy metals disrupt kidney physiology (Gao *et al*., 2022; Clarkson and Magos, 2006). Co-administration of jackfruit extract, however, reduced these markers, demonstrating a protective effect. Similar outcomes have been reported with jackfruit seed extract in neomycin-induced kidney damage (Shah *et al*., 2024) and with flavonoid-rich plant extracts against cadmium toxicity (Josiah *et al*., 2020).

Interestingly, rats given the extract alone showed slight variations in baseline urea and creatinine, suggesting that the pulp itself may exert intrinsic physiological effects. Comparable findings have been noted with polyphenol-rich products like green tea, which can influence metabolic and antioxidant processes even without toxic exposure (Mohabbulla *et al*., 2016). Collectively, these results support the idea that jackfruit pulp can reduce biochemical indicators of heavy metal–induced kidney injury.

Histological Findings: Histological examination reinforced the biochemical data. Rats exposed to Mercuric Chloride displayed clear pathological changes, including tubular necrosis, vascular narrowing, and inflammatory infiltration—hallmarks of mercury-induced renal damage (Bridges and Zalups, 2017). In contrast, rats treated with jackfruit pulp alongside Mercuric Chloride showed largely preserved kidney architecture. This protective effect mirrors findings from studies on plant extracts rich in saponins and flavonoids, which have been shown to reduce kidney damage caused by heavy metals such as lead and cadmium (Obafemi *et al*., 2019; Adebayo *et al*., 2023). Morphologically, the protection offered by jackfruit pulp resembled that of vitamin E, supporting earlier evidence that natural antioxidants can limit structural injury to renal tissues (Agarwal *et al*., 2010). Together, the histological and biochemical findings confirm that jackfruit pulp provides nephroprotective benefits by maintaining both kidney function and tissue integrity.

Environmental and Practical Implications: Given mercury's persistence and widespread presence in the environment, the protective effects observed with natural antioxidants such as jackfruit and vitamin E are particularly significant. These findings suggest that dietary sources of antioxidants may help reduce the risk of kidney damage linked to heavy metal exposure. Incorporating jackfruit into the diet could therefore represent a simple, affordable, and locally available strategy to mitigate mercury's harmful effects. Nonetheless, further research involving human subjects is essential before these results can be translated into clinical or public health recommendations.

6. Discussion

Overall, the findings of this study indicate that the fruit pulp of *Artocarpus heterophyllus* offers considerable protective effects against renal damage caused by Mercuric Chloride exposure. The rich phytochemical composition of the extract likely contributes to its antioxidant and anti-inflammatory activities, which in turn help restore body weight, improve renal biochemical markers, and maintain the normal structure of kidney tissues. In line with earlier studies on plant-derived agents with nephroprotective potential, the present results further suggest that jackfruit pulp may serve as a useful dietary supplement or phytotherapeutic option for reducing kidney damage associated with heavy-metal toxicity.

Conflict Of Interest

The authors declare no conflict of interest.

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