Sahel Journal of Life Sciences FUDMA (SAJOLS)
December 2024 Vol. 2(4): 15-21
ISSN: 3027-0456 (Print)
ISSN: 1595-5915(Online)
DOI: *https://doi.org/10.33003/sajols-2024-0204-03*

Research Article

Evaluation of the Hepato and Reno protective Effects of Methanol Seed Extract of Unripe *Carica papaya* (pawpaw) Seed in Wistar Rats Exposed to Carbon tetrachloride (CCl_4)

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ABSTRACT

Rapid urbanization leads to high rates of organ failure due to toxic industrial emissions. This necessitates the need to develop solutions from natural materials, with the perceived potential to protect vital organs against the damaging effects of these emissions that can be easily transformed into beverages. This study evaluated the hepato and reno-protective effects of methanol seed extract of unripe *Carica papaya* (pawpaw). Twenty-five adult male Wistar rats were divided into five groups of five rats each. Group I served as the normal control and received 2 ml of distilled water. Group II was the negative control (administered CCl_4 only). Groups III-V were administered 200, 400, and 600 mg/kg of methanol seed extract of unripe fruit before induction of renal and hepatic damage with carbon tetrachloride (CCl4). The extract was administered to animals for 28 days, after which the animals were euthanized, and samples were collected in appropriately labeled containers. Evaluation of serum markers of hepatic and renal injury was performed using standard procedures. Both serum hepatic and renal markers alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), creatinine, and urea) were elevated in the negative control group beyond the levels reported for the normal control. However, a contrary observation was made in urea, creatinine, Na+, K+, Cl-, HCO_3, and ALT, AST, and ALP, respectively. In conclusion, it can be inferred from the results of this study that methanol extract of unripe pawpaw seed has the potential to protect against hepatic and renal damage.

Keywords: *Carica papaya*; Alkaline phosphatase; Hepatic; Renal; Damage

Citation: Nweke, E.O., Ogbonnaya, E., Mohammed, K., Miracle, N.T., Collins, O.K, Dimgba, U.C. & Fidelis, O.O. (2024). Evaluation of the Hepato and Reno protective Effects of Methanol Seed Extract of Unripe *Carica papaya* (pawpaw) Seed in Wistar Rats Exposed to Carbon tetrachloride (CCl_4). *Sahel Journal of Life Sciences FUDMA*, 2(4): 15-21. DOI: *https://doi.org/10.33003/sajols-2024-0204-03*

INTRODUCTION

The liver is fundamentally recognized for its roles in detoxification of xenobiotics, protein synthesis, and production of digestive enzymes, among others. It sits below the diaphragm in the upper quadrant of the body. Additional functions of the liver, which are of immense significance to life, include red blood cell regulation, synthesis, and storage of glucose, etc. (Iluz-Freudlich *et al*., 2020).

The kidney is a bean-shaped organ that is saddled with the metabolic responsibilities of regulating the osmolarity of plasma, modulation of the blood water, solutes, and electrolytes content, among others. It ensures an enduring maintenance of the acid-base balance and contributes to the production of red blood cells through its ability to produce erythropoietin. Its role in the regulation of blood pressure, a task it performs due to its ability to produce renin, is widely acknowledged and has also been implicated in the conversion of vitamin D to its active form (Johannes *et al*., 2022). With all the critical functions for which the liver and the kidney are known, it is not out of place to infer that damage to the organs would translate to outright loss of life.

Efforts to meet the rising human material needs are evident by the birthing of rapid industrialization, which, although forms the pivot upon which the economy rests, thwarts environmental and public health integrity being a viable source of hazardous emissions. Ambient air pollution has been identified as a major cause of global diseases (Brook *et al*., 2017). Several research outcomes have established a link between exposure to ambient air pollution and liver and kidney diseases (Go *et al*., 2004) to which humans unavoidably fall victims, thus necessitating the need for an innovative approach towards curbing the menace, which primarily should begin with investigations to unveil food-based materials with the potential to protect against the damaging effects of ambient air pollution.

Carica papaya, commonly known as pawpaw, is a succulent fruit widely known for its nutritional and medicinal values (Afolabi *et al*., 2011). It is a member of the *Caricaceae* family (Jeon *et al*., 2022). It is native to southern Mexico and Central America and originates in South Mexico and Costa Rica. Its commercial production is practiced in Florida, Hawaii, South Africa, Sri Lanka, India, Canary Islands, Malaysia, and Australia (Milind and Gurditta, 2011). The seeds are numerous, small, black, round, and covered with gelatinous aril (Afolabi *et al*., 2011), and those from unripe pawpaw fruit have been used locally in the treatment of jaundice in neonates, a condition that manifests following the liver's inability to process red blood cells. Thus, the imperativeness of probing the viability of unripe pawpaw fruit seed as a therapeutic option against hepatic and renal damage is defined.

MATERIALS AND METHODS

Collection of Plant Material

Unripe *Carica papaya* fruits harvested from a home garden were subsequently conveyed in a black polythene bag to the herbarium unit of the Department of Forestry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Processing and Extraction of Plant Material

Unripe pawpaw fruits were sliced into two halves, and the seeds were obtained and spread on a clean flat surface to dry at room temperature for 12 days. The dried seeds were then ground into a fine powder. One hundred grams (100 g) of the powdered plant material were soaked in 2000 mL of methanol for 72 hours, with shaking three times a day. The resulting mixture was filtered using Whatman No. 1 filter paper. The filtrate was then extracted using a Soxhlet apparatus and concentrated under pressure to dryness in a rotary evaporator at 30°C (Phrompittayarat *et al*., 2008).

Animal

A total of thirty-four (34) adult male Wistar rats weighing 120-140 g were purchased from the Animal House of the Department of Science Laboratory Technology and subsequently housed in transparent plastic cages for 14 days for acclimatization. The rats were provided with rat chow and water *ad libitum*.

Lethal Dose 50%

This was a dual-phase experiment in which three groups of three rats per group were each administered 10 mg, 100 mg, and 1000 mg/kg of extract orally in the initial phase. This was followed by strict observation of the rats for 24 hours. Following the absence of mortality in the observed animals, the second phase of the experiment involved three groups of one rat per group that were administered 1600 mg, 2900 mg, and 5000 mg/kg of extract, respectively, and subsequently observed for 48 hours (Lorke, 1983).

Experimental Design

Twenty-five adult male Wistar rats were divided into five groups of five rats per group.

Group I (Normal control) – 2 mL/kg distilled water (orally)

Group II (Negative control) - induced hepatic and renal damage without treatment

Group III – Pretreated with 200 mg/kg methanol seed extract of *Carica papaya* orally

Group IV – Pretreated with 400 mg/kg methanol extract of *Carica papaya* orally

Group V – Pretreated with 600 mg/kg methanol extract of *Carica papaya* orally

Treatment with the extract lasted for 28 days, after which 1 mL/kg of CCl_4 dissolved in corn oil was administered intraperitoneally 3 hours post-extract treatment. Two hours after the injection, the rats were anesthetized with isoflurane. Subsequently, the rats were sacrificed, and blood was collected and centrifuged at 1000 × g for 10 minutes at 4 °C.

The liver enzymes (alkaline phosphatase, alanine transaminase, and aspartate aminotransferase) using

commercial enzyme kits produced by Biorexfars company with the aid of an automated analyzer.

Determination of Serum Electrolytes

Chloride levels were assessed using the mercury chloride colorimetric method. Sodium and potassium levels were determined using the flame photometric method (Flame Photometer - CL 26D - ELICO, UK), and bicarbonate levels were measured using the Roche Cobas method (c501 module).

Urea and Creatinine Determination

A 5 ml venous blood sample was taken to determine serum urea and creatinine levels. Creatinine was measured using the modified Jaffe's method (Bower, 1980), and urea was determined using the Urease-Berthelot's method (Richterich and Kuffer, 1973) using the Cobas Integra (Roche) completely automated analyzer.

Histopathology

Liver and kidney tissues harvested from the animals were kept in 10% formalin for preservation purposes. Tissues were sectioned with the aid of a microtome. The tissue was dehydrated using graded alcohol before embedment with paraffin section. Tissues were stained with haematoxylin and eosin (H&E) and then studied under a light microscope (Akharaiyi *et al*., 2015).

Data Analysis

Data generated were expressed as Mean ± Standard Deviation using SPSS (Ver. 23). Data were analyzed using one-way Analysis of Variance (ANOVA). Differences in mean were compared using Tukey's Test. p-values less than 0.05 were considered statistically significant.

RESULTS

The activities of liver enzymes in rats with experimentally induced hepatic damage are displayed in Figure 1, showing that the activities of alanine transaminase (ALT) and aspartate aminotransferase (AST) were significantly ($p < 0.05$) higher than those reported for the normal control. However, these were significantly ($p < 0.05$) reduced following oral administration of methanol seed extract of unripe *Carica papaya* (pawpaw seed) to levels that were not significantly ($p > 0.05$) different from those reported for the normal control group. A similar observation was made on alkaline phosphatase, except that the enzyme activity observed following oral administration of 200 mg/kg of the methanol extract of unripe *Carica papaya* (pawpaw) seed was not significantly ($p < 0.05$) different from that recorded for the negative control group.

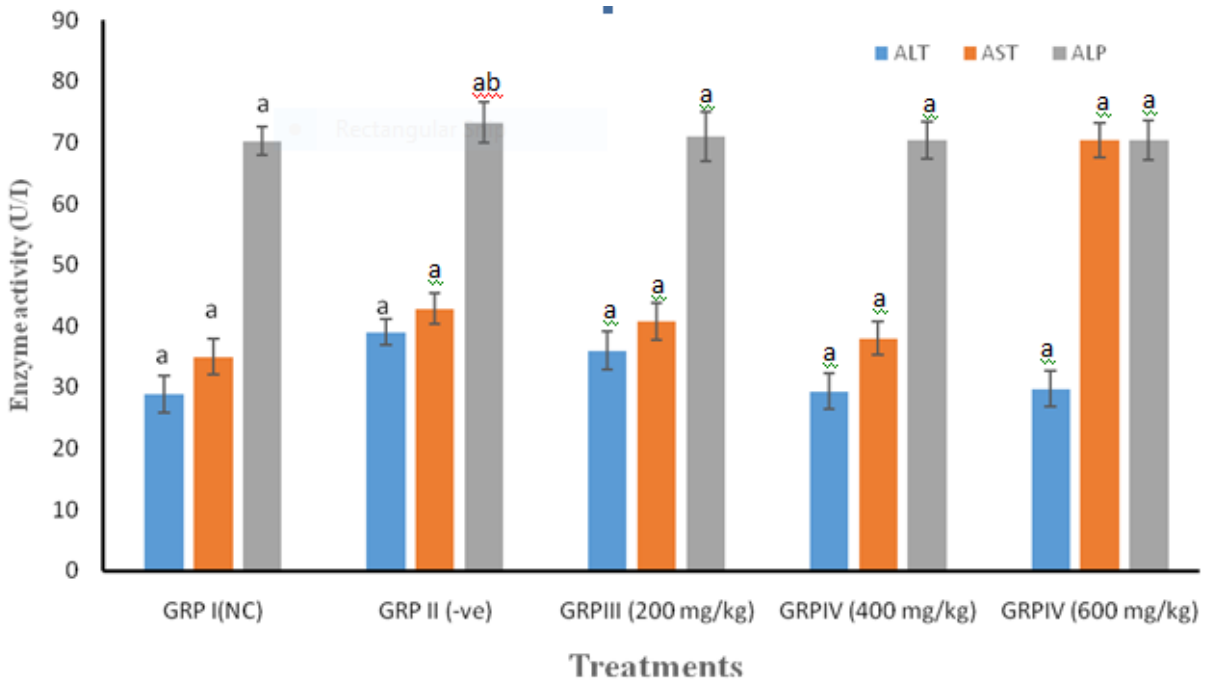

Figure 1: Liver Enzyme Activity in rats administered methanol extract of unripe *Carica papaya* seed
Grp [Group], **-ve** [Negative control]

The renal function markers in rats with experimentally induced renal damage treated with methanol extract of unripe pawpaw seeds are shown in Table 1. The concentrations of urea, creatinine, and electrolytes

(Na+, K+, Cl-, HCO_3) in the negative control group (induced renal damage without treatment) were significantly higher ($p < 0.05$) than those in the normal control group. However, the concentrations of these renal markers were significantly reduced ($p < 0.05$) in pretreated groups, reaching levels that were not significantly different ($p > 0.05$) from those reported for the normal control group.

Histopathological Findings on the Liver

Findings from the histopathology of the liver show healthy hepatocytes (Plate Ia), sinusoidal congestion (Plate Ib), minimal Kuffer cell hyperplasia (Plate Ic), slight sinusoidal congestion and Kuffer cell hyperplasia (Plate Id) and also healthy hepatocytes (Plate Ie).

Histopathological Findings on the Kidney

Findings from the histopathology of the kidney reveals features of a healthy kidney (Plate IIa), slight hyperplasia of inflammatory cells (Plate IIb), slight tubular distortions (Plate IIc), tubular necrosis (Plate IId) and characteristics of a normal kidney (Plate e).

Table 1: Renal Function Markers in Rats with experimentally induced Renal Damage treated with Pawpaw Seed Extract

Groups	Urea (mg/dl)	Creatinine(mg/dl)	Na (mg/dl)	K^+ (mg/dl)	Cl^- (mg/dl)	HCO_3 (mg/dl)
Group I	16.93±0.85^a	0.70±0.12^a	128.21±1.26^a	4.54±0.07^a	86.50±1.62^a	19.40±0.20^a
Group II	19.32±0.45^b	0.98±0.35^b	144.45±1.56^b	7.03±0.05^b	99.09±2.21^b	23.08±0.31^b
Group III	17.56±0.49^a	0.72±0.05^a	131.43±1.18^a	4.51±0.03^a	97.31±1.52^a	19.10±0.11^a
Group IV	17.40±0.20^a	0.74±0.01^a	132.03±1.41^a	4.42±0.12^a	96.36±1.21^a	19.40±0.21^a
Group IV	17.02±0.96^a	0.72±0.06^a	133.02±2.12^a	4.50±0.12^a	97.02±1.30^a	19.50±0.63^a

Results are expressed as mean ± standard deviation from three determinations. Values with different superscripts in a row are significantly different at ($p < 0.05$).

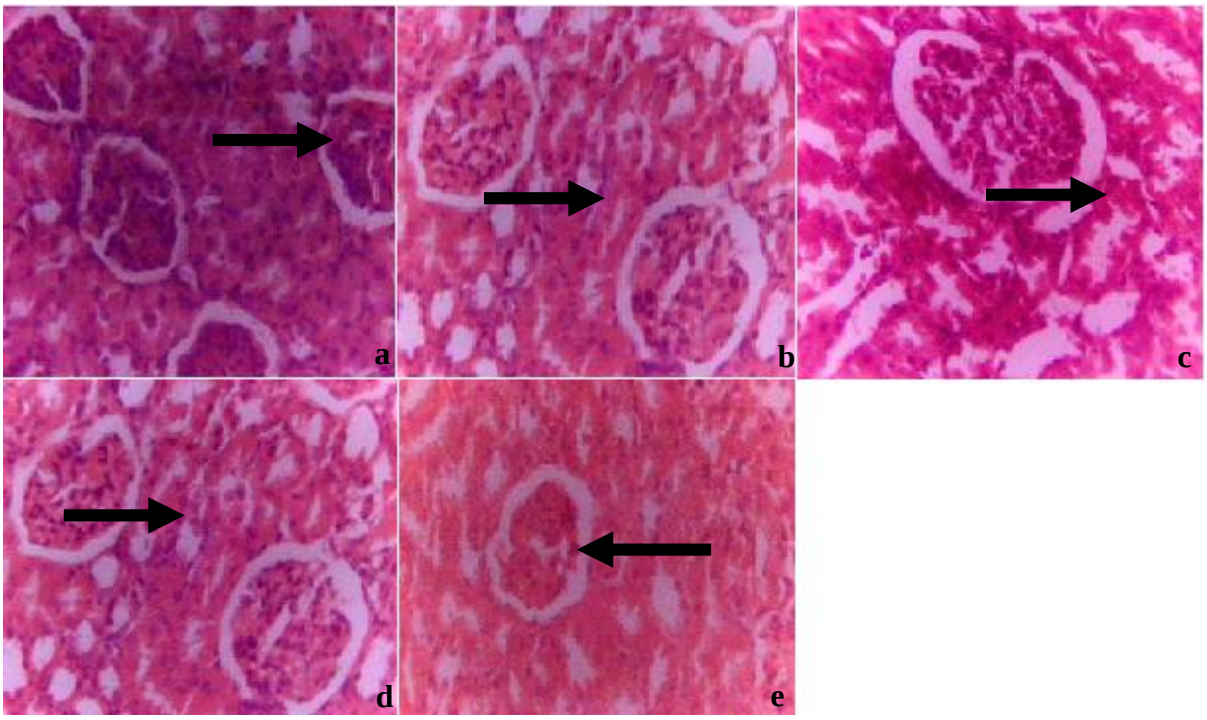

Plate I (a-e): Show the photomicrographs of the the kidney of rats Rats with experimentally induced Renal damage treated with pawpaw seed extract

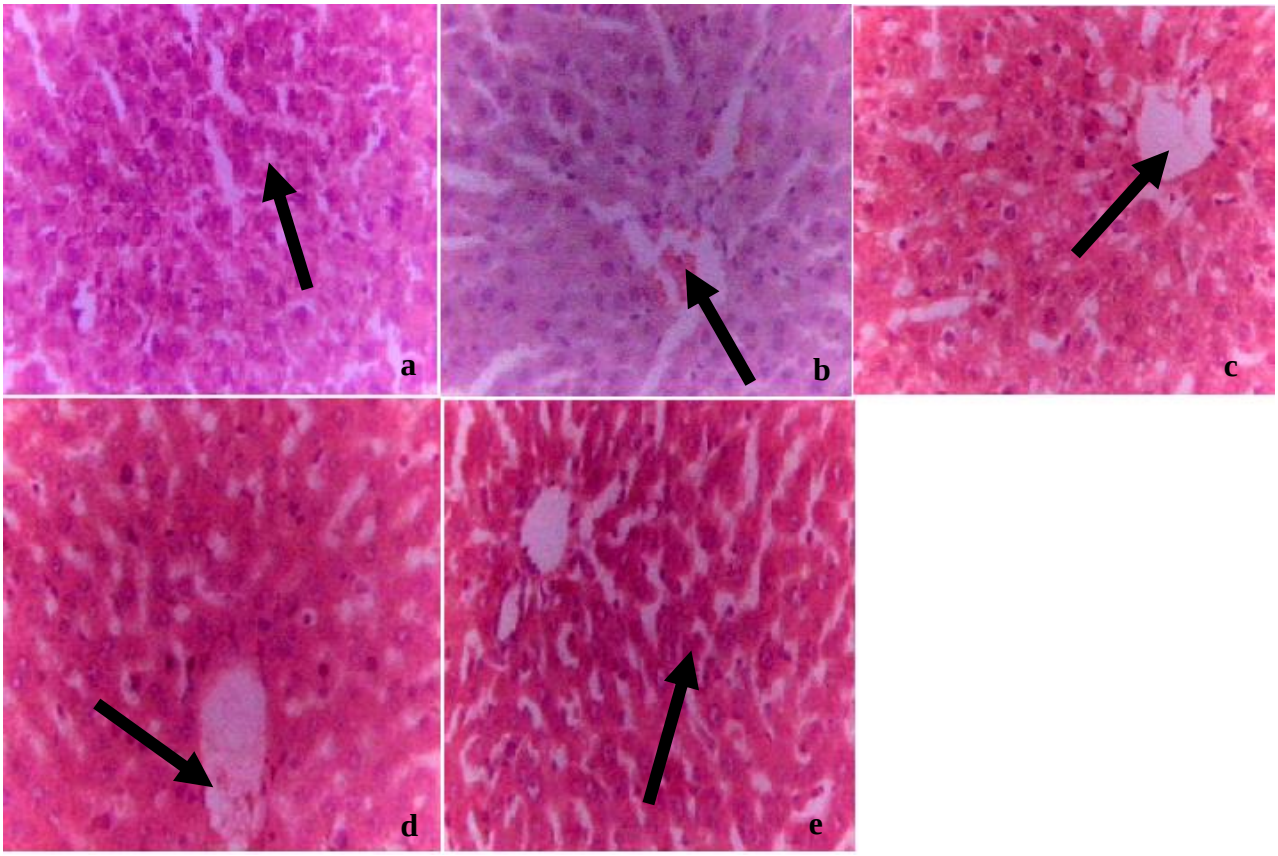

Plate II (a-e): Show the photomicrographs of the liver of rats with experimentally induced renal damage treated with pawpaw seed extract.

DISCUSSIONS

The liver and kidneys are vulnerable to the harmful effects of metabolic reactions caused by toxins. Alanine aminotransferase, alkaline phosphatase, and aspartate aminotransferase are enzymes found in liver cells. While these enzymes can be present in the bloodstream due to various physiological factors unrelated to liver damage, they are generally considered specific markers of liver damage when other factors are ruled out (Putta *et al*., 2020).

The varying doses of methanol seed extract from unripe pawpaw fruit seeds have shown the ability to reduce the activities of serum markers of hepatocellular injury, indicating the potential for regeneration of damaged hepatocytes. This effect may be attributed to the presence of phytochemicals such as phenols, flavonoids, saponins, alkaloids, and tannins (Ejeh *et al*., 2022). This finding is consistent with a study by Ogbonna *et al*. (2020), which demonstrated that methanol extract of unripe *Carica papaya* pulp significantly decreased the levels of alanine transaminase, aspartate aminotransferase, and alkaline phosphatase in a dose-dependent manner.

The kidney is responsible for eliminating metabolic wastes such as urea and creatinine, ensuring the body's optimal chemical composition. When the kidney's ability to remove these wastes is impaired due to renal damage, they accumulate in the bloodstream. Blood urea and creatinine levels are crucial diagnostic indicators for assessing kidney function and managing renal conditions (Alaasim *et al*., 2020). Serum electrolytes play a vital role in cellular functions as co-factors in enzyme-catalyzed reactions, and changes in their concentrations can indicate certain diseases (Guo *et al*., 2021). In clinical practice, electrolytes such as sodium, potassium, chloride, and bicarbonate ions are essential indicators of renal function (Ahmad *et al*. 2013). The elevated amounts of sodium, potassium, chloride, and bicarbonate ions,urea and creatinine in group II, which did not receive the extract before being exposed to CCl_4, could be attributed to the kidney's decreased ability to expel electrolytes from the tubular fluid as a result of CCl_4-induced renal injury. However, groups pretreated with unripe *Carica papaya* seed extract before CCl_4 exposure had significantly lower levels of assessed electrolytes ($p < 0.05$). This finding indicates that the kidneys were healthy and capable of excreting sufficient electrolytes from the tubular fluid as

a result of the protective roles exerted by the defensive roles of phytochemicals. The results of our study are consistent with the findings of Madinah *et al*. (2015), which confirmed that *Carica papaya* contains nephroprotective phytochemicals. This is also supported by the research of Asadulla *et al*. (2024), which demonstrated that *Carica papaya* extract, particularly from the fruit, has therapeutic potential for promoting liver and kidney health without significant adverse effects. However, our study contradicts the findings of Igbinovia *et al*. (2015), who reported that *Carica papaya* seed had a negative impact on renal function in Wistar rats. This discrepancy suggests the need to further investigate the potential sources of variation in nephro-based studies involving *Carica papaya* seed, such as environmental factors, maturation, processing, and handling, as these factors could have contributed to the differences between our results and those of Igbinovia *et al*. (2015).

CONCLUSION

The ability of seed extract of *Carica papaya* seed to preserve renal and hepatic health was evaluated in this study. Normal levels of the respective biomarkers kidney and liver functions were observed in all pretreated groups to affirm our claim on the prophylactic potential of unripe pawpaw fruit against renal and hepatic damage.

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