**HEPATOTOXICITY ASSESSMENT OF UNRIPE CARICA PAPAYA (PAWPAW) SAP IN ADULT MALE WISTAR RATS**

**ABSTRACT**

**Introduction:** Carica papaya (pawpaw) is commonly used in traditional medicine for its nutritional and therapeutic benefits, yet the effects of its unripe sap on liver function remain poorly studied. This research aimed to assess the hepatotoxic or hepatoprotective properties of unripe Carica papaya sap in adult male Wistar rats through biochemical and histological evaluations.

**Methodology:** Twenty-four male rats were randomly assigned into four groups of six: Group A (control) and Groups B, C, and D received oral doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg of pawpaw sap, respectively, for 14 days. Acute toxicity (LD₅₀) was determined using Lorke’s method. Liver function was evaluated via serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities using WHO-recommended spectrophotometric methods. Histopathological examination of liver tissues was conducted post-treatment.

**Results:** Results showed no significant differences in body weight, while liver weight significantly decreased in the high-dose group (p = 0.006), indicating potential hepatotoxicity. AST levels were significantly elevated in the moderate- and high-dose groups, while ALT increased only in the moderate-dose group. ALP levels remained unchanged across all groups. Histology revealed mild liver damage at higher doses.

**Conclusion:** The findings suggest that unripe Carica papaya sap may exert mild, dose-dependent hepatotoxic effects, warranting cautious therapeutic use.

Keywords: *Carica papaya*, Liver function, Wistar rats, Hepatotoxicity

**1.0 INTRODUCTION**

Plants and plant-based products have been utilized for disease prevention and treatment since ancient times. Globally, approximately 80% of the population depends directly on plants for primary health care (Tripathi *et al*., 2003; WHO, 2000). In India, an estimated 45,000 plant species possess medicinal properties (Jain, 1994). Natural compounds from plants are often favored over synthetic drugs due to their affordability, availability, and minimal side effects (Wang *et al*., 2007).

*Carica papaya* Linn., commonly known as papaya, belongs to the Caricaceae family and is indigenous to Central America and southern Mexico. It is now widely cultivated in tropical countries including India and Nigeria for its nutritional and medicinal value (Aravind *et al*., 2013; Baiyewu & Amusa, 2005). Various parts of the papaya plant—leaves, fruit, seeds, roots, and sap—are known for their therapeutic applications (Sharma *et al*., 2022). The plant contains important nutrients like vitamins A, B, C, calcium, and iron (Wall, 2006), and its seeds contain benzyl isothiocyanate, which exhibits antimicrobial properties (Tang, 1971).

Papaya sap and leaf extracts have demonstrated significant antioxidant activity and contain enzymes such as papain, chymopapain, glycyl endopeptidase, and caricain (Rahmani *et al*., 2016; Paul *et al*., 2013). Several studies have highlighted the medicinal properties of papaya leaves in treating conditions such as dengue, jaundice, asthma, and various forms of cancer (Krishna *et al*., 2008; Sarala *et al*., 2014; Otsuki *et al*., 2010; Hadadi *et al*., 2018). Phytochemical studies have confirmed the presence of beneficial compounds such as flavonoids, tannins, alkaloids, and saponins in papaya leaves (Bamisaye *et al*., 2013; Nugroho *et al*., 2017).

Despite widespread traditional use, limited scientific evidence exists regarding the effect of unripe pawpaw sap on liver health. Given the liver’s vital roles in detoxification and metabolism (Allen, 2002; Ozougwu *et al*., 2014), it is essential to evaluate potential hepatotoxic or hepatoprotective effects of pawpaw sap. This study investigates these effects in male Wistar rats to enhance understanding of papaya’s safety and potential therapeutic use (Sharma *et al*., 2022; Fernandez-Checa & Kaplowitz, 2005).

**2.0 METHODS**

**2.1 Animal Procurement, Care and Treatment**

Twenty-four male Wistar rats were purchased from Research Enterprise Farms, University of Ibadan, Oyo State. The animals were kept in the Animal House in the school compound of Okofia, Nnewi campus. They were allowed to acclimatize over a period of two weeks. The animals were housed in well-ventilated iron cages under normal temperature (27-31 °C). They were fed distilled water and rat feed pellets from Agro Feed Mill Nigeria Ltd. Ethical approval was obtained from the Ethical Committee of the Department of Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus (ANA/EA/UG/AS/11/09/2024). All the animals were treated in accordance with the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institute of Health (NRC, 2011).

**2.2 Collection of *Carica Papaya* Sap and Preparation of Stock Solution**

*Carica papaya* sap was obtained from matured *Carica papaya* fruits planted within the Campus perimeters and authenticated at the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State.

A stock solution was prepared by drying 1 ml of *Carica papaya* sap using a thermostatic oven, yielding 0.0075 g (7.5 mg) of dry residue. This corresponds to a concentration of 75 mg/ml, indicating that each milliliter of sap solution contained 75 mg of solid extract.

**2.3 Acute Toxicity Test (LD50) of *Carica papaya***

The median lethal dose (LD₅₀) of *Carica papaya* (pawpaw) sap was determined using the modified method of Lorke (1983) in the Department of Human Physiology, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Okofia Campus. A total of thirteen rats were used for the study, which was conducted in two phases.

In Phase I, nine rats were divided into three groups. Group 1 received 10 mg/kg of pawpaw sap, Group 2 received 100 mg/kg, and Group 3 received 1000 mg/kg. No mortality was observed in any of the groups after 24 hours; however, some of the animals showed signs of weakness, indicating mild physiological stress.

Phase II involved four rats, each assigned to a separate group. Group 1 received 1200 mg/kg, Group 2 received 1600 mg/kg, Group 3 received 2900 mg/kg, and Group 4 received 5000 mg/kg of pawpaw sap. Only the rat in Group 4 died within 12 hours, while the others survived but displayed signs of weakness similar to those observed in Phase I.

Using the formula LD₅₀ = √(a × b), where *a* is the maximum dose with 0% mortality (2900 mg/kg) and *b* is the minimum dose with 100% mortality (5000 mg/kg), the calculated LD₅₀ was √(2900 × 5000) = 3807.89 mg/kg via oral route.

**2.4 Animal Grouping and Experimental Procedures**

The animals were randomly divided into four groups of 6 animals each. Group A served as control and received only rat feed and distilled water. Group B received 100mg/kg/bw of pawpaw sap, Group C received 200mg/kg/bw of pawpaw sap while Group D received 400mg/kg/bw of pawpaw sap. Pawpaw sap was administered orally and once daily over two weeks. All experimental rats were allowed free access to rat feed and distilled water.

**2.5 Termination of Experiment and Sample Collection**

Twenty-four hours after the final administration, all animals were fasted overnight and subsequently anesthetized using ketamine hydrochloride. Blood samples were then drawn via ocular puncture for liver function test and transferred into sterile, plain glass tubes without any anticoagulant. The collected blood was centrifuged using a New Life model laboratory ultracentrifuge, after which the serum was separated and stored under refrigeration until analysis.

While still under anesthesia, the animals were humanely euthanized. A midline incision was made in the abdominal region to access the internal organs. The livers were carefully removed, rinsed with normal saline to eliminate residual blood, gently blotted dry, weighed, and fixed in 10% formal saline solution inside universal bottles for histopathological assessment. The remains of the animals were then disposed of in accordance with approved institutional ethical protocols.

### 2.6 Liver Function Tests

**2.6.1 Aspartate Aminotransferase (AST) Activity**

The activity of serum AST was determined using the spectrophotometric method recommended by the World Health Organization (WHO, 2004). AST catalyzes the transfer of an amino group from L-aspartate to alpha-oxoglutarate, producing oxaloacetate. The resulting oxaloacetate reacts with NADH to form NAD⁺, and enzyme activity is measured based on the rate of NADH oxidation.

For the assay, 100 µl of serum was added to a test tube containing 0.5 ml of AST reagent 1. After incubation at 37°C for 30 minutes, 0.5 ml of AST reagent 2 was added. Following a 20-minute reaction at room temperature, sodium hydroxide was introduced, and absorbance was measured at 546 nm.

**2.6.2 Alanine Aminotransferase (ALT) Activity**

Serum ALT levels were also determined following the WHO (2004) spectrophotometric method. ALT catalyzes the transfer of an amino group from alanine to oxoglutarate, producing pyruvate and L-glutamate. Pyruvate is then reduced by NADH to lactate, and the reduction in NADH absorbance is used to quantify enzyme activity.

The same procedure used for AST was followed for ALT determination.

**2.6.3 Alkaline Phosphatase (ALP) Activity**

Serum ALP activity was measured using the WHO standard method (2004). ALP hydrolyzes p-nitrophenyl phosphate into p-nitrophenol and phosphate. The concentration of p-nitrophenol, which is directly proportional to enzyme activity, was measured spectrophotometrically.

A 20 µl serum sample was added to test tubes containing ALP reagent. Absorbance was recorded at 1, 2, and 3-minute intervals. Enzyme activity was calculated using the formula:  
**U/L = 2760 × ΔA**

**2.7 Tissue Processing**

Tissues (liver) were processed via H&E method through fixation, dehydration, clearing, infiltration, embedding, sectioning, and staining. Fixation was done in 10% neutral buffered formalin. Dehydration used increasing alcohol concentrations, clearing in xylene, and infiltration in paraffin wax. Sections were obtained after embedding. Sections were dewaxed in xylene, rehydrated through descending alcohol grades, washed in water, stained with hematoxylin (20 minutes), differentiated in 2% acid alcohol and counterstained with eosin (30 seconds). They were dehydrated, cleared, and mounted using DPX. Tissues were viewed with a digital light microscope. Photomicrographs were taken at x400 magnification using OMAX software. NIH ImageJ software was used for digital analysis (Erukainure *et al*., 2019).

**2.8 Data Analysis**

Data were analyzed using SPSS version 27.0.1. Results were presented as Mean ± Standard Deviation (SD). One-way ANOVA was used for comparisons, with p ≤ 0.05 considered statistically significant.

**3.0 RESULTS**

**3.1 Effect of Pawpaw sap on the Animal Body Weight**

Table 1.0 compares the body weights of Wistar rats before and after administration of *Carica papaya* sap. Across all groups, there were no statistically significant differences in body weight (p > 0.05), indicating that the sap did not significantly affect overall body mass during the treatment period. This suggests that, within the given doses and duration, pawpaw sap does not alter weight gain or loss in male Wistar rats.

Table 1.0: Effect of pawpaw sap on the body weight

|  |  |  |  |
| --- | --- | --- | --- |
| Groups | Administration | Mean±Std. Error | P value |
| Group A | Pre-Administration Weight | 160.00±13.61 |  |
| Post- Administration Weight | 159.20±40.70 | 0.976 |
| Group B | Pre-Administration Weight | 188.00±4.34 |  |
| Post- Administration Weight | 191.40±1.97 | 0.548 |
| Group C | Pre-Administration Weight | 185.50±5.89 |  |
| Post- Administration Weight | 186.23±13.69 | 0.933 |
| Group D | Pre-Administration Weight | 174.00±17.08 |  |
| Post- Administration Weight | 189.47±10.89 | 0.947 |

Data was analyzed using paired sampled t-test, and values were considered significant at p < 0.05\*

**3.2 Effect of *Carica papaya* (*Pawpaw*) Sap on the Relative Liver Weight**

The relative liver weight was highest in the control group (Group A) at 6.05 ± 0.15 g/100 g body weight. Groups B and C, which received lower and moderate doses of *Carica papaya* sap, showed non-significant decreases in relative liver weight (5.83 ± 0.32 and 5.70 ± 0.31, with p-values 0.547 and 0.332, respectively). However, Group D (high dose) showed a statistically significant reduction in relative liver weight (4.83 ± 0.72, p = 0.006) compared to the control as shown in table 2.0.

This suggests that high doses of pawpaw sap may induce liver shrinkage or atrophy, indicating potential hepatotoxic effects at elevated exposure levels.

Table 2.0:Effect of *Carica papaya* (*Pawpaw*) sap on the relative liver weight

|  |  |  |
| --- | --- | --- |
| Groups | Relative Liver Weight (g/100g body weight) | P value |
| Group A | 6.05±0.15 |  |
| Group B | 5.83±0.32 | 0.547 |
| Group C | 5.70±0.31 | 0.332 |
| Group D | 4.83±0.72 | 0.006 |

Data was analyzed using one-way ANOVA, and values were considered significant at p < 0.05\*

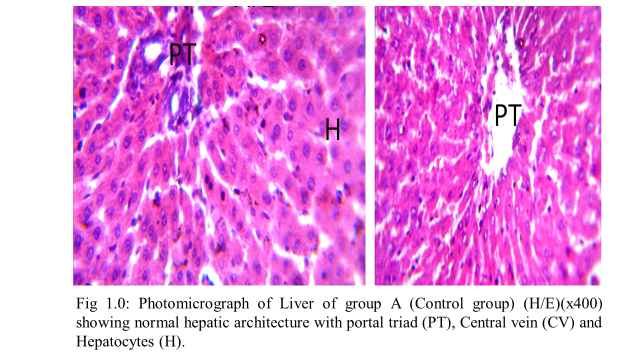
**3.3 Effect of *Carica papaya* (*Pawpaw*) sap on liver biochemical markers**

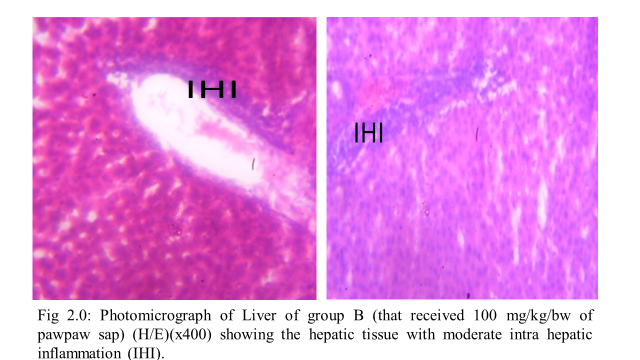
This table shows the effects of *Carica papaya* sap on liver enzyme activities (AST, ALT, and ALP) in Wistar rats. A significant increase in AST was observed in Groups C and D (p = 0.001 and 0.002), indicating potential dose-dependent hepatocellular injury, while ALT showed a significant rise only in Group B (p = 0.016), suggesting mild liver stress at lower doses. ALP activity showed no significant differences across all groups (p > 0.05), implying no biliary or cholestatic dysfunction as shown in table 3.0.

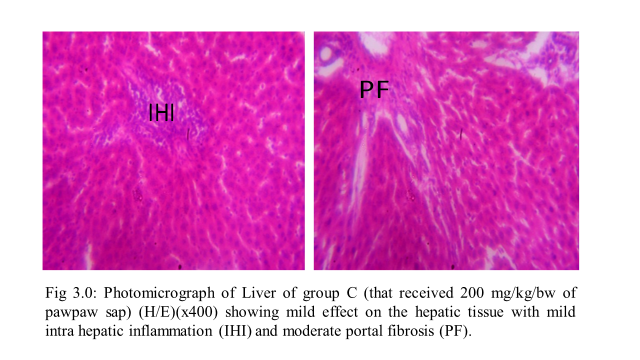
Table 3.0: Effect of *Carica papaya* (*Pawpaw*) sap on liver biochemical markers

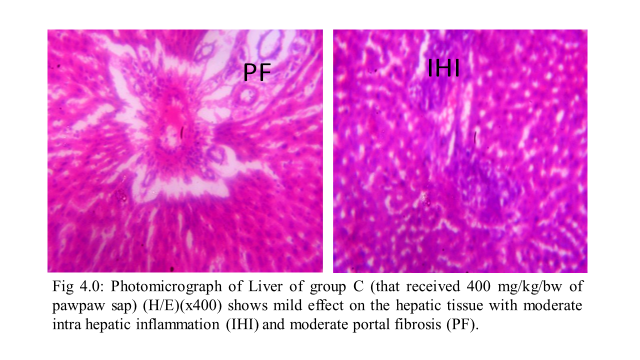
|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | Group | Value (u/l) | P value |
| Serum Aspartate Aminotransferase (AST) Activities (u/l) | Control (Group A) | 31.00±0.00 |  |
| Group B | 32.67±1.20 | 0.238 |
| Group C | 34.33±0.33 | 0.001 |
| Group D | 35.00±0.58 | 0.002 |
| Serum Alanine Aminotransferase (ALT) Activities (u/l) | Control (Group A) | 13.00±0.00 |  |
| Group B | 14.33±0.33 | 0.016 |
| Group C | 12.67±0.33 | 0.374 |
| Group D | 12.67±0.33 | 0.374 |
| Serum Alkaline Phosphatase (ALP) Activities (u/l) | Control (Group A) | 78.68±0.45 |  |
| Group B | 79.60±0.39 | 0.122 |
| Group C | 80.07±0.58 | 0.092 |
| Group D | 80.48±1.02 | 0.162 |

**3.4 Histological Findings**



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**4.0 DISCUSSION**

This study investigated the effects of *Carica papaya* (pawpaw) sap on liver function in adult male Wistar rats by assessing body and organ weights, liver enzyme activity, and histopathological changes. The goal was to evaluate the safety profile and potential hepatotoxic effects of pawpaw sap, thereby contributing to its pharmacological understanding for both traditional and modern medicinal applications.

No statistically significant differences were observed in body weight across all experimental groups. While the control group exhibited a slight but non-significant decrease (p = 0.976), the treated groups showed non-significant increases in weight (p > 0.05). These findings suggest that *Carica papaya* sap, at the administered doses and treatment duration, does not substantially affect the body mass of adult male Wistar rats. This is consistent with previous studies on various plant extracts, which similarly reported negligible effects on weight during short-term exposure (Ofoego *et al*., 2017; Smith, Johnson, & Lee, 2019; Garcia, Martinez, & Zhang, 2020). Although other investigations have observed metabolic effects—such as weight gain or loss—after exposure to herbal compounds (Ofoego *et al*., 2020; Jiang, Liu, & Wang, 2021), the current results indicate no marked metabolic disruption due to pawpaw sap within the study window.

A significant reduction in liver weight was observed in the high-dose group (p = 0.006), suggesting possible tissue damage or metabolic alteration due to higher sap exposure. Conversely, the low- and moderate-dose groups did not exhibit significant changes in liver weight, pointing to a dose-dependent pattern of hepatotoxicity. This trend aligns with earlier research on plant-derived substances, where high doses were often required to elicit significant changes in organ weight (Nabi, Khan, & Iqbal, 2022; Chikezie, Olanrewaju, & Alabi, 2021).

Liver function was assessed using key serum enzymes—AST, ALT, and ALP. AST levels increased non-significantly in the low-dose group (p = 0.238), but rose significantly in both the moderate- and high-dose groups (p = 0.001 and p = 0.002, respectively), implying potential hepatocellular damage at higher concentrations. Elevated AST activity is typically linked to liver cell membrane disruption and hepatocellular injury (Jameel, Ahmad, & Siddiqui, 2018), reinforcing the evidence of dose-related hepatotoxicity from pawpaw sap, a pattern echoed in studies on other phytochemicals (Abdel-Daim, El-Khodary, & Farag, 2017; Ofoego *et al*., 2025).

Interestingly, ALT—a more liver-specific enzyme—showed a significant increase only in the moderate-dose group (p = 0.016), while no significant changes were observed in the high-dose group (p > 0.05). This selective elevation may indicate mild or early-stage hepatocellular injury, consistent with literature noting ALT's role as a sensitive early biomarker (Schweizer, Bertsch, & Rojas, 2019; Ejioku *et al*., 2015). The lack of further ALT elevation at the highest dose might suggest a plateau effect or the activation of compensatory mechanisms, as has been proposed in previous studies involving high-dose herbal exposures (Zhang, Li, & Zhang, 2020).

No significant differences were found in ALP activity among all groups (p > 0.05), implying that bile duct function and biliary flow were not adversely affected by pawpaw sap. Since ALP is often elevated in cases of cholestasis or biliary obstruction, its stability in this study suggests an absence of such conditions (Hosseini, Jamshidi, & Shaterian, 2020).

While the biochemical data point to potential liver injury, particularly at higher doses, histopathological evaluation is crucial to confirm these findings. Elevated AST and ALT levels are often correlated with structural liver abnormalities, including hepatocellular necrosis, inflammation, and fatty degeneration (Ahmad, Hassan, & Khan, 2021). The significant reduction in liver weight in the high-dose group may further reflect underlying histological changes such as tissue atrophy or cellular degeneration (Alhassan, Jamil, & Nasser, 2022), emphasizing the need for integrated biochemical and histological assessments when evaluating plant-based toxicities.

**5.0 CONCLUSION**

Results of this study suggest that *Carica papaya* sap may exert mild hepatotoxic effects in adult male Wistar rats, particularly at higher doses. Significant increases in AST and ALT levels, along with decreased liver weight in the high-dose group, point to potential hepatic injury. However, the absence of significant changes in body weight and ALP levels indicates no overt systemic or biliary toxicity. These results are consistent with previous literature on dose-dependent hepatotoxicity of plant-derived substances (Ali, Mohammed, & Liu, 2019). Further research is recommended to explore the long-term effects of pawpaw sap and to conduct detailed histopathological studies to provide deeper insight into its mechanism of toxicity and establish safer dosage guidelines.

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