Assessing The Safety Profile Of Carica Papaya Leaves And Seeds On Hepatic And Renal Function In Healthy Rabbits

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Abstract:
Objective: To assess the impact of Carica papaya leaves and seeds on hepatic and renal function in a controlled rabbit model**.**
Methodology: In a 45-day experimental study at Baqai Medical University, Karachi, 18 healthy adult rabbits were allocated into five groups. All groups received varying doses of papaya seed or leaf extracts. Liver and renal function assessments were performed at 15-day intervals, providing insights into the impact of Carica papaya extracts on these vital physiological functions.
Results: The administration of papaya extracts demonstrated a generally favorable safety profile, with no disruption to liver enzymes and renal biochemistries. However, the group receiving papaya seed extract at 500mg exhibited a noteworthy increase in albumin levels. While this may suggest a potential risk of dehydration, it did not impact liver synthetic function. Notably, renal function remained within clinically acceptable ranges across all groups, emphasizing the overall safety of the papaya extracts.
Conclusion: The findings suggest that Carica papaya extract, particularly from leaves, holds therapeutic potential for promoting liver and kidney health. Importantly, the observed effects were without significant adverse consequences. These results provide valuable insights into the safety and potential benefits of Carica papaya extracts, laying the foundation for further research and exploration of their applications in supporting hepatic and renal functions.

Keywords: Papaya Seeds, Papaya Leaves, Hepatic function, Renal function, Safety profile, Rabbit model.

INTRODUCTION

Carica papaya, commonly identified as papaya, is a tropical fruit utilized for centuries for its medicinal properties (1). The various parts of the papaya plant, including leaves and seeds, have been traditionally employed in folk medicine for their potential health benefits (2). The plant is rich in phytochemicals, including papain, carpaine, alkaloids, and flavonoids, which contribute to its therapeutic properties (3).

Studies have suggested that Carica papaya possesses anti-inflammatory, antioxidant, and immunomodulatory effects, making it a subject of interest in the field of natural medicine and pharmacology (4). Persistent interest has surrounded the utilization of C. papaya leaf as an adjunctive treatment, in conjunction with standard care, to augment platelet counts. This interest has been particularly notable in conditions such as dengue fever (5). Moreover, this interest has recently expanded to explore the potential role of C. papaya in cancer treatments (6).

In addition to demonstrating efficacy, the assurance of safety for a medicinal herb and its formulation holds significant importance. According to the 2019 Global Report on Traditional and Complementary Medicine by the World Health Organization (WHO), the safety evaluation of herbal medicines is typically mandated to undergo thorough assessment processes similar to those applied to conventional medicine in many countries (7).

The safety of Carica papaya leaves, commonly incorporated into diets and traditional medicine, underwent assessment through acute and subacute toxicity studies in rats. The researchers discovered that oral administration of Carica papaya aqueous leaf extract was tolerated, even at a single high dose, with an LD50 surpassing 5000 mg/kg. Additionally, the frequent administration of test doses had no adverse effects on hematological parameters or organ/body weight profiles assessed. In the subacute study, there were no indications of hematological mortality or organ alterations. However, significant variations were noted in biochemical parameters. (8, 9)

Papaya leaves and seeds holds potential therapeutic benefits across various physiological systems, including the hepatic and renal systems. (10) However, the existing scientific literature does not provide a thorough examination of the safety profile linked to Carica papaya, especially in the assessment of hepatic and renal function.
Rabbits, owing to their anatomical and physiological similarities to humans, are often utilized as experimental models for situdying the effects of natural products on organ systems (11).
The hepatic and renal systems play crucial roles in maintaining homeostasis within the body, and any disturbance in their functions could have significant health implications (12). The impact of Carica papaya on these vital organs is essential for establishing its safety profile and providing evidence-based recommendations for its use in traditional and complementary medicine (10).
This study aims to bridge the existing gap in knowledge by evaluating the impact of Carica papaya leaves and seeds on hepatic and renal function in a controlled rabbit model.

METHODOLOGY

Study Design: This interventional and experimental study was conducted at Baqai Medical University, Karachi, over a period of 45 days.

Animal Selection: Eighteen healthy adult rabbits of either sex, with a mean weight of 2.8 kg, were selected for the study. The rabbits were housed in the university's animal facility, maintained under standard conditions, including a well-aerated room, with access to fresh hay and water.

Sample Preparation - Plant Extract: Leaves of the selected plant were thoroughly washed and air-dried for two weeks at room temperature. Subsequently, the dried leaves were coarsely powdered with the use of an electric grinder (Anex) and filtered through a sieve. The resulting powdered sample was weighed using an Electric Balance (Snowrex Ej-120), placed into compact plastic envelopes, and stored at room temperature. Fresh preparations were created as required by blending the measured leaf powder with 10 cc of ordinary tap water, and this mixture was then directly administered into the oral cavity using a syringe. (13)

Sample Preparation - Seeds Extract: Ripe papayas were acquired from the nearby vegetable and fruit market in Karachi. The fruits were sliced into pieces, and their seeds were carefully extracted, washed thoroughly, and left to air-dry for a duration of two weeks. The air-dried seeds were finely ground into powder using a household grinder. The resulting fine powder was evaluated using an Electric Balance (Snowrex Ej-120), then packed into small plastic envelopes and stored at room temperature. For the research, a specific amount (ranging from 250 to 500 mg) of papaya powder was administered. The viability of the powder was considered to be 7 days. The measured seeds powder was mixed with 10 cc of clear tap water and administered directly into the oral cavity using a syringe. (13)

Animal Model: Healthy adult rabbits, regardless of gender, ranging in age from 16 to 24 months and weighing approximately 2.5 to 3.0 kg, were selected for the study. These rabbits were housed under normal conditions in a well-ventilated room maintained at a temperature of 22°C, with a 12-hour light/dark cycle. They had access to fresh hay and water throughout the experiment. A total of eighteen rabbits were randomly allocated into five groups, each comprising six animals, as detailed below:

Table 1: Experimental Groups and Treatment Regimens

Group Label	Type	Dose	Frequency/Duration
Group-A	Control	Standard diet (Placebo)	-
Group-B	Test	Seed-fed	Primary-dose-250mg
Group-C	Test	Seed-fed	Escalated-dose-500mg
Group-D	Test	Leaves-fed	Primary-dose-250mg
Group-E	Test	Leaves-fed	Escalated-dose-500mg

Note: Animals in Group-A (Control) received a standard diet serving as a placebo, while Groups B-E received seed or leaves-fed extracts at specified doses.

1. Liver Function Assessment:

- **Bilirubin**: Serum total and direct bilirubin levels were determined using the Jendrassik-Grof method, involving the formation of azo dyes and photometric measurement.Reagents included sulfanilic acid, sodium nitrite, caffeine, sodium benzonate, and Fehling solution II.
- **Serum Glutamate Pyruvate Transaminase (SGPT) Assessment:** SGPT levels were quantified through a reaction involving 2-oxyglutarate, L-alanine, and NADH, providing insight into liver health. Reagents included Tris buffer, L-alanine, LDH, NADH, and oxyglutarate.

- **Alkaline Phosphatase (ALP) Assessment:** ALP levels were determined using an optimized standard method based on the p-nitrophenylphosphate reaction, indicating liver and bone health. Reagents included diethanolamine, magnesium chloride, and p-nitrophenylphosphate.

2. Renal Function Assessment:

- Serum urea levels were measured using the modified urease-Berthehot method, forming a green complex with salicylate and hypochlorite.
- Serum creatinine levels were assessed through a kinetic colorimetric assay, involving the formation of a creatinine-picric acid complex.

Statistical Analysis:

The data input and analysis were conducted using the SPSS (Statistical Packages for the Social Sciences) version 19.0 computer package. Results present the mean ± standard deviation (S.D.) of continuous variables for liver and renal function tests on days 0, 15, 30, and 45, as well as cumulative values. The percentage change from day 0 to the end of treatment (day 45) will be calculated. Statistical comparisons will employ ANOVA (analysis of variance) and the student t-test for quantitative variables within groups. Significance will be evaluated based on a p-value < 0.05 in all statistical analyses.

RESULTS

Oral administration of papaya plant leaves and seeds extract at doses of 250mg and 500mg occurred over 45 days in healthy rabbits. In Group C, receiving a seed-fed extract at 500mg, a substantial rise in albumin levels compared to the control was observed (33.52 ± 2.34 g/L vs. 27.10 ± 1.90 g/L), while other treatment groups showed no significant difference in albumin levels.

Across all groups, the levels of total bilirubin, direct bilirubin, and indirect bilirubin remained insignificant. Serum Glutamate Pyruvate Transaminase (SGPT) and alkaline phosphatase levels exhibited variations, with no significant differences observed among the treatment groups.

Regarding liver enzymes, including Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), and Alanine Aminotransferase (ALT), no significant differences were noted among the treatment groups.

Table 1: Comparison of Hepatic Parameters (Cumulative Mean ± S.D) of Test Groups B, C, D & E from Control Group A

Variables	Group A (n=6)	Group B (Seed-fed 250mg)	Group C (Seed-fed 500mg)	Group D (Leaf-fed 250mg)	Group E (Leaf-fed 500mg)
Albumin (g/L)	27.10 ± 1.90	27.90 ± 2.30	33.52 ± 2.34*	28.63 ± 1.83	28.78 ± 2.50
Bilirubin-Total (mg/dl)	0.6 ± 0.04	0.6 ± 0.07	0.6 ± 0.04	0.6 ± 0.05	0.6 ± 0.04
Bilirubin-Direct (mg/dl)	0.3 ± 0.04	0.3 ± 0.04	0.3 ± 0.04	0.3 ± 0.04	0.3 ± 0.05
Bilirubin-Indirect (mg/dl)	0.3 ± 0.04	0.3 ± 0.07	0.3 ± 0.05	0.3 ± 0.05	0.3 ± 0.06
SGPT (U/L)	111 ± 20.6	130 ± 23.65	130 ± 19.6	102 ± 27.9	98 ± 12.2
Alkaline Phosphatase (U/L)	152 ± 22.0	173 ± 19.28	173 ± 17.1	151 ± 14.6	141 ± 13.7
AST (U/L)	176.27 ± 29.20	160.73 ± 31.34	162.67 ± 32.67	148.54 ± 27.51	145.90 ± 23.51
ALT (U/L)	43.20 ± 7.62	40.25 ± 8.18	56.20 ± 9.14	57.78 ± 9.82	58.78 ± 9.45

*Significant difference compared to Control Group A (*P < 0.05). Levels were obtained at 45th day

In Table 2 Group C (Seed-fed 500mg) demonstrated the highest mean urea level at 19.5 ± 4.42 mg/dl however it was insignificant. Creatinine levels were consistently maintained, from 0.05 ± 0.05 mg/dl to 0.06 ± 0.07 mg/dl across all groups. Overall, the results imply that administering papaya plant leaves and seed extract at specified doses did not significantly affect the renal function, with observed values within clinically acceptable ranges.

Table 2: Comparison of Renal Parameters (Cumulative Mean ± S.D) of Test Groups B, C, D & E from Control Group A

Variables	Group A (n=6)	Group B (Seed-fed 250mg)	Group C (Seed-fed 500mg)	Group D (Leaf-fed 250mg)	Group E (Leaf-fed 500mg)
Urea (mg/dl)	17.6 ± 3.49	18.3 ± 5.34	19.5 ± 4.42	15.8 ± 0.65	16.1 ± 1.28
Creatinine (mg/dl)	0.05 ± 0.07	0.05 ± 0.07	0.06 ± 0.07	0.05 ± 0.05	0.05 ± 0.07

*Significant difference compared to Control Group A (*P < 0.05). Levels were obtained at 45th day

DISCUSSION

Ensuring the prolonged safety of medicinal plants is crucial, especially when utilized for general health over an extended period, requiring careful consideration of potential side effects. Researchers and healthcare practitioners must navigate this delicate balance, striving for the therapeutic benefits of medicinal plants while mitigating potential risks to ensure sustained well-being. Previous research has raised concerns about potential liver dysfunction in studies involving herbal products or plants.(14)

In our research involving rabbits, liver enzymes such as AST, ALT, and ALP did not demonstrate significant differences when compared to the control group. However, an rise in albumin levels was noted at higher doses with a seed-fed diet, reaching the maximum concentration. Siegel has suggested that an elevated albumin level could be indicative of abnormal liver function or a state of dehydration. (15) Furthermore, Kwo has emphasized that abnormalities in AST, ALT, and ALP levels are more strongly associated with indications of liver cell injury. (16)

Our investigation revealed that Carica papaya leaves and seeds exhibited a nephroprotective effect, even at high doses, without causing noticeable changes in healthy rabbits. Similarly, in a study conducted by Madinah et al. and Gheith et al observed that an aqueous extract of Carica papaya seeds demonstrated effective nephroprotective activity in albino Wistar rats. This was evidenced by a reduction in biochemical parameters and an improvement in kidney architecture among rats with kidney injury (17, 18).

The alignment between the findings of this study and existing literature suggests that the nephroprotective effects observed in medicinal plants, including Carica papaya extract, are likely attributed to the antioxidant properties characteristic in alkaloids, flavonoids, and saponins. These insights emphasize the potential therapeutic significance of these plant components, particularly in the context of liver and kidney injuries (17, 19).

Tarkang et al. led a study on both aqueous and ethanol extracts of C. papaya leaves over 28 and 90 days. The results revealed no irregularities in liver enzymes and renal biochemistries in rats after the administration of C. papaya leaf extract for these durations. However, the ethanol extract exhibited certain variations in liver and renal toxicity at a dosage of 1 g/kg BW. This disparity in findings might be attributed to the use of a different extract in their study. They utilized air-dried leaves extracted with water or ethanol, whereas our study employed juice extracted from fresh leaves (20).

CONCLUSION:

In conclusion, the evaluation of Carica papaya leaves and seeds on hepatic and renal function in a controlled rabbit model indicated an overall favorable safety profile, with no adverse effects on liver enzymes and renal biochemistries. Of significance is the seed-fed extract at 500mg, revealing a notable elevation in albumin levels. The renal function consistently remained within clinically acceptable ranges across all groups. These findings underscore the potential therapeutic significance of Carica papaya extract, particularly in addressing liver and kidney concerns

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