Original Article

Exploring the Nephroprotective Efficacy of *Carica papaya* Leaf Extract on Serum Creatinine and Kidney Histopathology in Cyclophosphamide-Induced Thrombocytopenic Long-Evans Rats

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Abstract

Background: Cyclophosphamide, a widely used chemotherapeutic agent, is known to cause nephrotoxicity and thrombocytopenia, leading to renal dysfunction marked by increased serum creatinine and oxidative stress-induced kidney damage. Despite limited treatment options, *Carica papaya* leaf extract has shown promising antioxidant properties due to its rich content of flavonoids, tannins, and vitamin E. **Objective:** To investigate the nephroprotective potential of *C. papaya* leaf extract by examining its modulatory effects on serum creatinine levels and renal histopathological alterations in Long-Evans rats subjected to cyclophosphamide-induced thrombocytopenia. **Methods:** A total of 30 healthy rats (8–10 weeks, 150–250 g) were acclimatized for 14 days and divided into Control (Group A) and Experimental (Group B) groups. Group A was further subdivided into A1 (Baseline control) and A2 (Cyclophosphamide-induced thrombocytopenic control), with 10 rats in each subgroup. All rats received a basal diet for 23 days, while Group A2 and Group B were administered cyclophosphamide (70 mg/kg/day, subcutaneously) on days 1, 3, and 5 to induce stable thrombocytopenia. Group B additionally received *C. papaya* leaf extract (400 mg/kg/day) from day 10 to day 23. Blood samples were collected on day 1 and day 10 via tail vein, and on day 24 through cardiac puncture for serum creatinine evaluation, along with histopathological examinations of kidney tissues. Statistical analysis was performed using SPSS version 22, with significance set at $p \leq 0.05$. **Result:** Group A2 had significantly elevated serum creatinine levels ($p<0.05$) compared to Group A1. In contrast, Group B exhibited markedly reduced creatinine levels ($p<0.05$) relative to Group A2, with values comparable to Group A1, demonstrating robust nephroprotective effects. Histopathological analysis revealed kidney abnormalities in 40% of Group A2 and 10% of Group B, with no abnormalities in Group A1. **Conclusion:** *C. papaya* leaf extract significantly reduces serum creatinine levels and histopathological damage in cyclophosphamide-induced thrombocytopenic Long-Evans rats, indicating its potential as an effective adjunctive therapy for renal complications, likely due to its antioxidant properties.

Keywords: Thrombocytopenia, Cyclophosphamide, *C. papaya* leaf, Long Evans male rats.

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Introduction

Thrombocytopenia, defined as a platelet count of less than 150,000 per microliter, poses significant clinical challenges, particularly in patients undergoing chemotherapy. It is a common hematological complication associated with various malignancies and treatments, including cyclophosphamide, a widely utilized alkylating agent in cancer therapy [1]. Cyclophosphamide, while effective in treating a range of cancers, is notorious for inducing myelosuppression and nephrotoxicity, resulting in elevated serum creatinine levels and structural damage to renal tissues [2]. The kidneys are especially susceptible to oxidative stress generated by cyclophosphamide metabolism, leading to the production of reactive oxygen species (ROS), which can initiate lipid peroxidation, protein damage, and eventual cellular apoptosis [3].

Current therapeutic interventions to manage cyclophosphamide-induced nephrotoxicity are limited and often associated with significant adverse effects, necessitating the exploration of safer and more effective adjunctive therapies. Natural products, particularly plant extracts, have emerged as promising candidates for protecting against oxidative stress and enhancing renal function [4]. Among these, C. papaya leaf extract has gained attention due to its rich profile of bioactive compounds, including flavonoids, tannins, saponins, and vitamin E, which are known for their potent antioxidant and anti-inflammatory activities [5].

Numerous studies have highlighted the protective effects of C. papaya leaf extract on various organ systems. For instance, Nandini et al. [6] demonstrated its ability to improve platelet counts in cyclophosphamide-treated rats, suggesting its role in mitigating chemotherapy-induced hematological side effects. Furthermore, Alzahrani et al. [7] and El-Sayed et al. [8] reported that the extract effectively reduced oxidative stress markers and inflammatory cytokines in experimental models of nephrotoxicity. Despite these promising findings, the specific nephroprotective effects of C. papaya leaf extract in the context of cyclophosphamide-induced thrombocytopenia have yet to be thoroughly investigated.

The objective of this study is to evaluate the nephroprotective efficacy of C. papaya leaf extract by assessing its impact on serum creatinine levels and renal histopathological changes in Long-Evans male rats subjected to cyclophosphamide-induced thrombocytopenia. By elucidating the mechanisms underlying the protective effects of this natural extract, the study aims to provide valuable insights into potential therapeutic strategies for preventing renal complications associated with cyclophosphamide treatment. The findings may contribute to the development of adjunctive therapies that enhance patient outcomes while minimizing the risks associated with conventional chemotherapy.

Methodology

Study Settings and Population: This experimental study was conducted at the Department of Physiology, Sir Salimullah Medical College (SSMC), Dhaka, Bangladesh. The study involved 30 healthy male Long-Evans rats, aged 8 to 10 weeks, with body weights ranging from 150 to 250 grams. These animals were sourced from the Animal Resource Facility at the International Center for Diarrheal Disease Research, Bangladesh (ICDDR,B) in Dhaka. Only rats exhibiting normal health were included; those showing signs of illness or disease were excluded to ensure the integrity of the experimental outcomes.

Grouping of the Rats: Following a 14-day acclimatization period, the 30 Long-Evans rats were randomly allocated into two primary groups. Group A (control) was further subdivided into

Group A1, which comprised 10 rats receiving only a basal diet for 23 days, and Group A2, consisting of 10 rats that received a basal diet along with subcutaneous injections of cyclophosphamide (70 mg/kg) on days 1, 3, and 5. Group B (experimental group) also included 10 rats that were administered the same diet and cyclophosphamide injections as Group A2, but additionally received an oral dose of C. papaya leaf aqueous extract (400 mg/kg/day) from days 10 to 23.

Intervention: Group A1 served as the baseline control, receiving a basal diet throughout the 23-day study period. Group A2 acted as the cyclophosphamide-induced thrombocytopenic control group, being fed a basal diet and receiving subcutaneous cyclophosphamide injections (70 mg/kg body weight) on days 1, 3, and 5. Group B, the experimental group, underwent similar treatment as Group A2 but was also treated with C. papaya leaf aqueous extract (400 mg/kg body weight) delivered orally via gastric gavage from day 10 to day 23. All interventions, including dietary provisions and cyclophosphamide administration, were carried out daily between 9:00 AM and 10:00 AM throughout the study.

Study Procedure: The research protocol received ethical approval from the Institutional Ethics Committee (IEC) at Sir Salimullah Medical College (SSMC) in Dhaka, Bangladesh. The experiment was performed at the animal facility of the Institute of Nutrition and Food Science, Dhaka University, under controlled conditions (temperature: 27–28°C; 12-hour light/dark cycle). The 30 male Long-Evans rats underwent a 14-day acclimatization phase with unrestricted access to food and water. The experimental phase lasted 23 days, during which body weights were measured on day 1 and day 23.

On day 1, baseline assessments of platelet count, bleeding time (BT), and clotting time (CT) were conducted for all rats. Thrombocytopenia was induced in Groups A2 (cyclophosphamide-induced thrombocytopenic control) and B (experimental) through subcutaneous injections of cyclophosphamide (70 mg/kg) on days 1, 3, and 5. Group A1 received only a basal diet. By day 10, platelet counts, BT, and CT were reassessed to confirm thrombocytopenia development in Groups A2 and B, evidenced by prolonged BT and CT, while Group A1 maintained normal values.

From day 10 to day 23, Group B was administered C. papaya leaf aqueous extract (400 mg/kg/day) via gavage, while Group A2 continued with a basal diet. Blood samples were collected again on day 10 to reassess platelet counts, BT, and CT. On day 24, all rats were subjected to anesthesia using 30% chloroform and then euthanized. Serum creatinine levels were quantified using established laboratory methods, specifically the Kinetic Alkaline Picrate Method (Jaffe reaction) utilizing the Atelica CI Analyzer (Siemens Healthineers).

To investigate the histopathological impacts of the interventions, kidney tissues were harvested from all sacrificed rats. These tissue samples were processed to prepare histological slides, which were then examined under a light microscope. Photomicrographs of the kidney sections were captured to document any pathological changes. The histopathological analysis was performed at the Department of Pathology, Sir Salimullah Medical College, Dhaka. Tissue samples were evaluated for structural alterations such as necrosis, inflammation, and cellular degeneration, thereby elucidating the potential protective or damaging effects of C. papaya leaf extract on renal health following cyclophosphamide-induced toxicity.

Statistical Analysis: Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 22. Results were presented as

mean values ± standard deviation (SD). One-way ANOVA was employed to compare differences across groups, followed by post hoc Bonferroni tests for pairwise comparisons. Where appropriate, paired t-tests and Fisher's Exact tests were conducted. A p-value of ≤ 0.05 was considered statistically significant.

Ethical Approval: The study protocol received approval from the Institutional Ethics Committee (IEC) of Sir Salimullah Medical College (SSMC), Dhaka, Bangladesh.

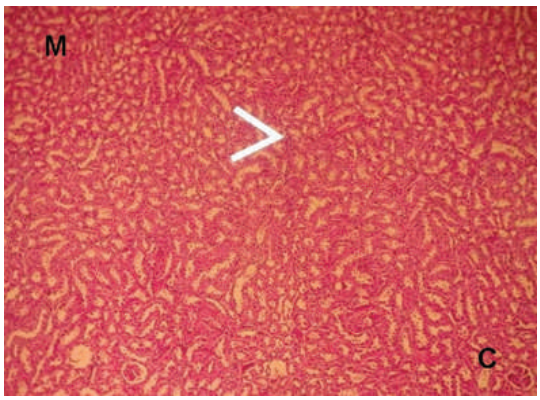
Result

The mean (± SD) serum creatinine was 0.63 ± 0.20, 0.78 ± 0.19 and 0.55 ± 0.17 mg/dl in group A1, group A2 and group B, respectively (Figure 1).

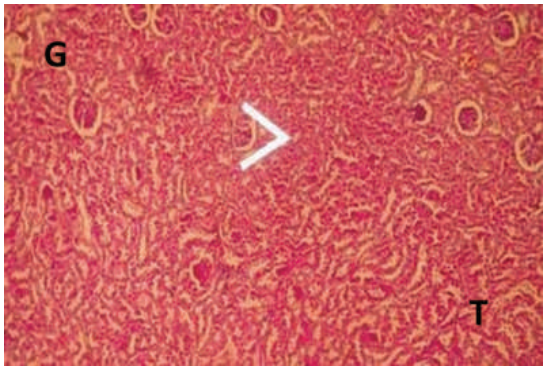
Table I. Mean serum creatinine level at day 24 in different groups of rats (N=30).

Group	*p* value
A1 vs A2 vs B	0.033*
A1 vs A2	0.212 ^{ns}
A1 vs B	1.000 ^{ns}
A2 vs B	0.034*

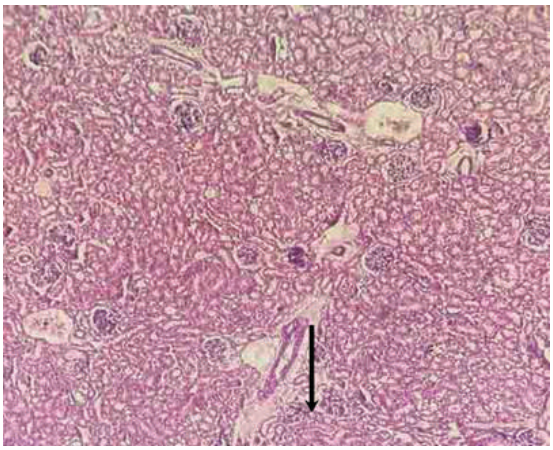
Data are presented as mean ± SD. Statistical analysis was performed using one-way ANOVA for group comparisons, followed by post hoc Bonferroni tests for pairwise group comparisons. Values in parentheses indicate the range. Group A1 served as the baseline control group, Group A2 as the cyclophosphamide-induced thrombocytopenic control group, and Group B as the experimental group, where thrombocytopenic rats were treated with C. papaya leaf extract. The total number of rats is represented by N, with n indicating the number of rats in each group. Statistical significance is denoted as follows: ns for non-significant, * for p<0.05, and *** for p<0.001.



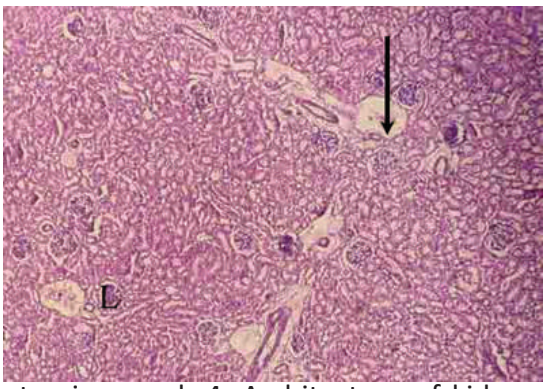
Photomicrograph 1: Architecture of kidney in baseline control group of rats (here C represents cortex and M represents medulla in ×100).



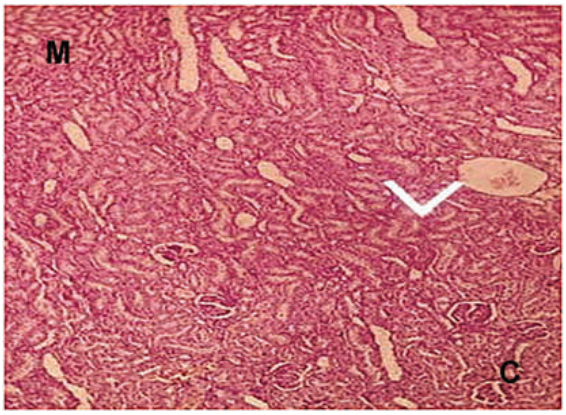
Photomicrograph 2: Architecture of kidney in baseline control group of rats (here T represents tubules and G represents glomerulus in ×400).



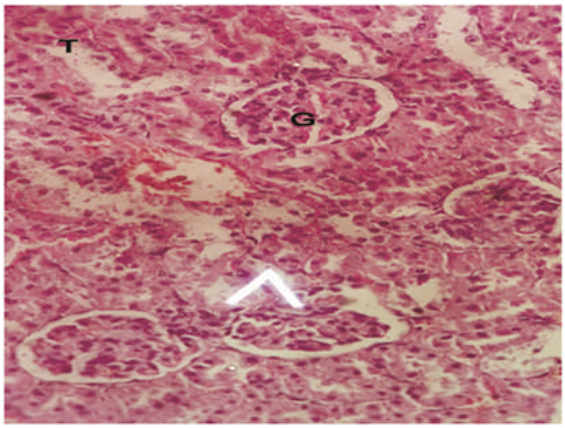
Photomicrograph 3: Architecture of kidney in cyclophosphamide induced thrombocytopenic control group of rats (here arrow represents mild tubular necrosis in x 100).



Photomicrograph 4: Architecture of kidney in cyclophosphamide induced thrombocytopenic control group of rats (here arrow represents slight tubular dilatation and L represents lymphocyte infilation X 400).



Photomicrograph 5: Restoration of normal architecture of kidney in thrombocytopenic rats with C. papaya leaf treated group of rats (here M represents medulla C represents cortex X 100).



Photomicrograph 6: Restoration of normal architecture of kidney in thrombocytopenic rats with C. papaya leaf treated group of rats (here G represent glomerulus X 400).

Table II. Multiple comparisons of mean distribution of Rats Kidney by the histopathological findings in kidney (N=30).

Group	*p* value
A1 vs A2 vs B	0.044*
A1 vs A2	0.093^{ns}
A1 vs B	1.000^{ns}
A2 vs B	0.300^{ns}

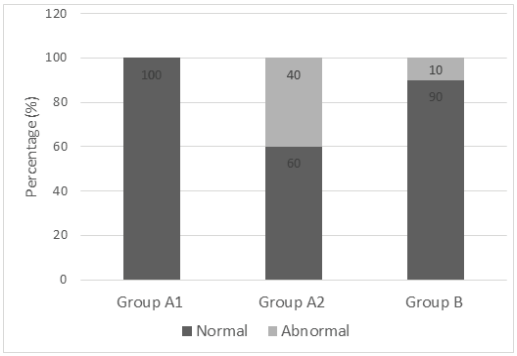


Figure 2: Mean distribution of rats by histopathological findings in kidney (N=30); Group A1: Baseline control group; Group A2: Cyclophosphamide induced thrombocytopenic control group; Group B: Thrombocytopenic rats with C. papaya leaf treated group; N= Total number of rats.

Discussion

The current study aimed to assess the anti-thrombocytopenic effects of C. papaya leaf extract in cyclophosphamide-induced thrombocytopenic rats. To evaluate the potential nephrotoxic effects, serum creatinine levels were measured, and histological examinations of renal tissues were conducted to observe microscopic changes in the kidneys.

Serum creatinine level: In this study, serum creatinine levels were significantly elevated ($p < 0.05$) in the cyclophosphamide-induced thrombocytopenic control group compared to the thrombocytopenic rats treated with C. papaya leaf extract. These results align with earlier findings reported by Bordoloi et al. [9] and Deli et al [10]. Furthermore, serum creati-

nine levels in the C. papaya leaf-treated group were comparable to those in the baseline control group, with no statistically significant difference observed. Similar observations have been documented by Anjum et al [11], Dharmarathna et al [12], and Hampilos et al [13].

Histological changes in kidney: In the present study, mild histological alterations, including tubular necrosis, lymphocyte infiltration, and slight tubular dilatation, were observed in some rats from the cyclophosphamide-induced thrombocytopenic control group. These findings are consistent with previous research by Nandini et al [6] and Nwangwa et al [14]. Conversely, the majority of rats treated with C. papaya leaf extract exhibited normal kidney histological architecture, corroborating results from Francis et al [15].

The cyclophosphamide-induced thrombocytopenic control group and the C. papaya leaf-treated group both displayed evidence of thrombocytopenia, as indicated by slightly elevated serum creatinine levels and mild architectural changes in the kidneys observed during histopathological examination [16, 17]. These histopathological changes may be attributed to increased free radical production, which initiates lipid peroxidation and subsequent cellular damage; however, the current study did not measure free radical concentrations to elucidate this mechanism.

C. papaya leaf extract effectively reduced elevated serum creatinine levels in thrombocytopenic rats, suggesting its non-toxic nature and protective role against renal complications associated with thrombocytopenia [6, 11, 18]. The nephroprotective effects of the extract are attributed to its content of tannins, flavonoids, saponins, triterpenoids, and vitamin E, which exhibit antioxidant properties by significantly inhibiting malondialdehyde (MDA) levels, a marker of oxidative stress [19-21]. The extract reduces lipid peroxidation, mitigates the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and scavenges free radicals, thereby preventing oxidative stress-induced kidney damage [22-23].

Furthermore, lower serum levels of creatinine in this group suggest that C. papaya leaf extract does not exert toxic effects on the liver or kidneys. Mild histopathological changes in the liver and kidneys of thrombocytopenic rats treated with C. papaya leaf may be due to its free radical scavenging activity, although the precise mechanisms underlying these effects cannot be determined from this study.

Conclusion

This study demonstrates that C. papaya leaf extract significantly improves cyclophosphamide-induced thrombocytopenia and reduces renal toxicity in Long Evans rats by lowering serum creatinine levels and preventing kidney damage. Future research should explore varying doses of the extract, identify the key bioactive compounds, and assess antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) to better understand its protective mechanisms. These findings highlight C. papaya leaf extract's potential as a natural adjunct for managing thrombocytopenia and renal complications.

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