



AMELIORATION OF THROMBOCYTOPENIA AND COAGULATIVE PARAMETERS BY *CARICA PAPAYA* FRUIT WITH HEMATOLOGICAL AND HISTOPATHOLOGICAL EVIDENCE – AN IN-VIVO STUDY

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Abstract

Introduction: Recent research on analyses of the platelet-boosting abilities of polyphenolic compounds and nutritional supplements, and their efficacy to impact health parameters has been established. *C. papaya* is renowned for its excellent digestive, antioxidant, and nutraceutical properties derived from bioactive substances such as papain, latex, carpaines, benzyl glucosinolates, choline, zeaxanthin, and more.

Material and Methods: Twenty-four rats were randomly divided into four groups, each consisting of six: NC & CPF₁ (normal control & negative control), CPF₂, and CPF₃ (300 & 600 mg/kg dried papaya fruit). The Wistar albino rats were exposed to thrombocytopenia by cyclophosphamide (s.c.). Blood samples were collected on days 0, 10, 20, and 30 to analyze the serum platelet count and coagulation profile. Following 30 days, the kidney and liver were dissected for histopathological evaluation.

Results: The Platelet count was significantly elevated ($p \geq 0.05$) compared to normal and negative control in experimental groups. The platelet count within the CPF₃ group (a dose of 600 mg CPF), showed more promising results as compared to the CPF₂ group (a dose of 300 mg CPF) while observed at 0, 10, 20, and 30th days. The histopathology analysis on the kidney and liver revealed a reversal toward normal parenchymal structure on an administered dose of 600 mg of *C. papaya* L. fruit.

Conclusion: *C. papaya* fruit provides promise for its potential application in treating and controlling thrombocytopenia and associated bleeding disorders. This effect may be attributed to carpaines, ascorbic acid, and carotenoids in *C. papaya* L.

Keywords: Coagulation profile, Thrombocytopenia, Cyclophosphamide, Papaya (*C. papaya* L.), Histology

INTRODUCTION

Certain diseases like dengue, aplastic anemia, idiopathic thrombocytopenic purpura, chikungunya, hypersplenism, and drug-induced thrombocytopenia are linked to a drop in thrombocyte count in the blood (Anjum, Arora, et al. 2017). The leading causes of thrombocytopenia in intensive care units (ICUs) are severe illness, organ failure, sepsis, shock, and renal failure. Thrombocytopenic anemia patients have a higher probability of bleeding, commonly requiring transfusions of platelets, plasma, and red blood cells. They experience prolonged hospital stays and a significantly increased mortality risk in the intensive care unit (ICU) (Santoshi, Patel, et al. 2022).

Dengue fever, an arboviral illness impacting an estimated global population of 390 million individuals, significantly alters thrombocyte suppression and platelet function, serving as vital signs of infection. Activated platelets can release granular components, along with mitochondrial failure and subsequent death, contributing to thrombocytopenia (Singh, Bisht et al. 2020). Furthermore, dengue virus (DENV) infection may lead to dengue hemorrhagic fever with a lower platelet count, causing abnormalities in blood clotting (Lien, Chan, et al. 2021). Indeed, several viruses can directly infect bone marrow hematopoietic stem cells, causing alterations in hematopoiesis through altered cytokine production or reduced numbers of progenitor cells. Specific viruses that infect bone marrow hematopoietic stem cells might impede thrombopoietin synthesis, potentially resulting in liver tissue injury. The damage caused by the viruses could delay megakaryocyte maturation, limiting platelet output (Franchini, Veneri, et al. 2017).

Thrombocytopenia commonly arises without underlying health issues and leads to a drop in blood platelet count to between 150 and 400 x 10⁹/L, which is the normal range. Patients with platelet counts above 50 x 10⁹/L are often asymptomatic. Although spontaneous bleeding is rare in thrombocytopenic settings, the risk of bleeding markedly increases when platelet counts drop below 20 x 10⁹/L. (Ashworth, Thielemans et al. 2022). Platelet counts at peak symptomatic illness have been discovered as predictors of disease severity in specific viral infections, or they can act as an initial indicator for detecting persistent viral infections. The link between platelet counts and disease severity in specific viral infections is typically attributed to the impact of the viruses on platelet function rather than changes in platelet quantity. Platelets, small anucleate cells, circulate in the bloodstream for roughly 7 to 10 days following their production. Their primary function is hemostasis, which involves producing blood coagulation to uphold vascular integrity. Platelets come from megakaryocytes, gigantic polyploid cells dwelling in the bone marrow, which arose from hematopoietic stem cells. Proplatelets, produced by megakaryocytes, release many platelets into the bloodstream as part of their endoplasmic maturation process (Raadsen, Du Toit, et al. 2021). Thrombocytopenia can come from various pathophysiological factors, including decreased platelet formation, increased platelet death by immunological or non-immune mechanisms, and increased platelet sequestration in the reticuloendothelial system (Santoshi, Patel, et al. 2022). Viral infection of megakaryocytes can impair platelet production through various processes, including cell death, delayed maturation, and decreased receptor expression. (Rasizadeh, Ebrahimi et al. 2024).

Foods and nutrients that impact platelet activity can yield surprising outcomes in laboratory examinations (McEwen 2014). Recently, researchers have been interested in exploring phytochemicals, nutraceuticals, and herbal medicines as they offer potential solutions to the limitations associated with conventional drugs, such as drug resistance, side effects, and chronic toxicities from long-term use. (Sarker, Khan et al. 2021). In patients with refractory immune thrombocytopenia (ITP), several therapeutic drugs have been released, including high doses of corticosteroids, intravenous immunoglobulin (IVIG), TPO-Ras, rituximab, azathioprine, cyclosporine, mycophenolate mofetil, and others. However, despite these treatments, only a minority of patients, approximately 10-15%, experience disease stability in moderate- and short-term follow-up trials. Patients and their families often struggle with the financial burden and side effects associated with repeated use of these treatments. Natural medicines may offer a promising

avenue for effectively enhancing platelet count, decreasing bleeding, and promoting general well-being in patients suffering from immune thrombocytopenia (Tran, Pham, et al. 2023).

Papaya (*C. papaya*) fruit and leaf extracts have been reported to aid in treating dengue fever and raise blood platelet counts. Various components of *C. papaya* have documented therapeutic activities, including anti-hypertensive, antibacterial, diuretic, antifertility, antifungal, and anticancer properties. Additionally, *C. papaya* plants have significantly affected wound healing, immunomodulation, and illnesses connected with hypolipidemia and hypoglycemia. (Sharma, Bachheti et al. 2020). A prior study showed that *C. papaya* fruit aqueous extracts may reduce lipid peroxidation, boost glutathione levels, promote the activity of catalase and superoxide dismutase, and improve immunological status. Rats subjected to acrylamide poisoning showed a rise in IgG and IgM levels, indicating the impact of acrylamide poisoning on the immune system. (Od-Ek, Deenin et al. 2020). The enormous spectrum of phytoconstituents in *C. papaya* has tremendous promise for evidence-based medical treatments. These phytoconstituents offer feasible remedies for different illnesses, emphasizing the versatility and promise of *C. papaya* as a therapeutic agent (Amin, Bughdadi, et al. 2019).

This research proposes to explore the effects of *C. papaya* fruit on numerous health indicators, including platelet count, prothrombin time, and clotting time, utilizing a rat model with cyclophosphamide-induced thrombocytopenia. Additionally, the study assesses its potential effectiveness in alleviating thrombocytopenia and examining cyclophosphamide's potentially harmful effects on the liver or kidneys.

2. MATERIALS AND METHODS

Chemicals and material

Materials

A freeze dryer (Labconco, USA), and an electric grinder (Panasonic) were used for sample preparation. Sysmex 21 Hematology analyzer was used for hematological evaluation. EDTA vials (BD) were purchased locally.

Sample preparation

Fresh *C. papaya* fruit was procured from a farm near Lahore, ensuring equal maturity in shape, size, color, and ripeness stage for consistency. The ripe fruits had yellow skin with yellow to dark orange pulp, meeting the maturity index standards according to criteria (Santamaría Basulto, Sauri Duch et al. 2009) which consider factors such as sugar content, firmness, and color for an accurate assessment. Before processing, the fruits underwent surface cleaning to remove contaminants, using a dry cotton cloth and water at 48°C for 10 minutes to ensure thorough sanitation. Only complete and insect-free fruits were selected for the study to ensure consistent quality and eliminate potential contaminants that could affect the results. Following selection, the fruits were carefully peeled and deseeded, and the pulp was meticulously chopped into small cubes measuring 2 x 2 cm, using a sterilized stainless-steel knife for hygiene. For freeze-drying, cubes were freeze-dried for 24h (Labconco, USA) followed by grinding into a fine powder using a grinder (Panasonic) and storage at 4°C.

Composition of diet

The calculated doses of CPF/rat/day/kg body wt. were combined with an iso-caloric and iso-nitrogenous diet, as specified in Table 1, to attain the required weight and made into diet balls for administration to Wistar rats twice daily, at 8 am and 6 pm, respectively. According to AIN-93 (Reeves, Nielsen, et al. 1993), all essential vitamins and minerals were provided throughout this research period, providing unrestricted access to both food and water sources during this study period.

Table 1.: Composition of diet used in the study

Ingredients (g/1000 g)	NC	CPF ₁	CPF ₂	CPF ₃
Soybean meal	420	420	420	420
Maltodextrin	100	100	100	100
Sucrose	100	100	100	100
Corn starch	230	230	215	200
Maize Bran	50	50	50	50
Papaya fruit (dried)	-	-	15	30
Soybean oil	50	50	50	50
DL – methionine	3	3	3	3
Choline Bitartrate	2	2	2	2
AIN-93-VX Vitamin mix	10	10	10	10
AIN-93G-MX mineral mix	35	35	35	35
Total Energy, kcal	3822	3822	3822	3822

The dose was calculated based on average food consumption per rat/day which is 20g approx. Papaya dry fruit was substituted with corn starch based on calories (kcal).

Experimental Design

24 Wistar albino rats aged 6-7 weeks, weighing 150±10g from the Department of Zoology, University of Punjab, Lahore. These rats were housed in metabolic cages to enable the collection of their feces (for digestibility studies) in a controlled laboratory environment that included humidity levels between 45-55%, temperature between 22-25°C, and an alternate lighting schedule with 12 hours of light/12 hours dark (Nandini, Madhunapantula, et al. 2021). All rats were provided with standard rat pellets and unlimited access to water and each group was identified using permanent ink markers of different colors on their tail. All procedures were performed according to the given guidelines (Council, Earth, et al. 2010), (Council, Nutrition, et al. 1995) and approved by the Animal Ethical Committee of Government College University, Faisalabad, Pakistan. The Wistar rats were divided into a completely randomized design of 4 equal groups (n=6) as discussed in Table 2.

Table 2. Experimental design

Group No.	Group Name	Cyclophosphamide-induced thrombocytopenia (1-10 days)	Experimental Supplement (11-30 days)
1	NC	-	Standard rat pellets (normal control)
2	CPF ₁	Induction of thrombocytopenia (s.c. injection of 100 mg/kg body wt. cyclophosphamide on day 1, and subsequent 70 mg/kg body wt. for next two days), Induction period for the next 07 days (Erhirhie, Ekene, et al. 2014) and (Kamali, Khazaei, et al. 2018)	Rat pellet without <i>C. papaya</i> Disease induced animals (negative control)
3	CPF ₂		Rat pellet with <i>C. papaya</i> dried fruit (300 mg/kg body wt./ day)
4	CPF ₃		Rat pellet with <i>C. papaya</i> dried fruit (600 mg/kg body wt./ day)

p.o.; per oral; s.c.: subcutaneous

Cyclophosphamide-injected animals were left for 07 days to develop stable thrombocytopenia after 03 days of induction, confirmed by decreased platelet count and increased prothrombin time (PT) (Nandini, Madhunapantula, et al. 2021).

Growth performance

The feed given to each group was weighed and recorded daily. Split and leftover feed were also measured to get the exact estimation of daily feed intake. The body weight (using Weighing Balance) of each rat was monitored daily (Manjula and Krishna 2016). The gain in body weight (BWG) was measured by using a formula.

$$\text{Body weight gain} = \text{Final weight (g)} - \text{Initial weight (g)}$$

Hematological analysis

Blood samples were obtained from rats after an overnight fast. The target area was wiped with an antiseptic solution containing 70% alcohol. The rat was restrained and blood was collected using a 22-gauge needle from the lateral tail vein. The collected blood was divided into two aliquots, one aliquot was transferred into BD Vacutainer which contains EDTA as an anticoagulant. The vacutainer was rotated at 2,100 x g in a refrigerated centrifuge (4°C) for 10 min within 10 min of collection. The plasma was eluted to avoid disturbing the red and white blood cell layers. The complete blood count including platelet count was observed. All EDTA-anticoagulated blood samples were analyzed using a Sysmex 21 automated hematology analyzer (Sysmex Co., Kobe, Japan). The second aliquot was transferred into Vacutainer (without anticoagulant) at room temperature for up to 30 min to enable clotting. The tubes were rotated in a refrigerated centrifuge (4°C) at 2,000 x g and the serum was eluted (Lee and Goosens 2015).

Tissue preparation and histological analysis of liver and kidney

Liver and kidney tissues were first washed in phosphate-buffered saline before drying, weighting, and being preserved in 10% formalin. Next, desiccation with increasing ethanol concentrations occurred before being cleaned with xylene before embedding in paraffin wax for sectioning into 5µm slices. Finally, after deparaffinization and dehydration with decreasing grades of ethanol concentrations, the sections were stained using Hematoxylin/Eosin stain, according to (Khazaei, Ghanbari, et al. 2020). Horizontal sections of stained tissues were examined under a microscope at magnifications between 10x and 40x to detect histopathological alterations, with Dr. Ghulam Mustafa, Lecturer in Pathology at the University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan, conducting histopathological assessments for both liver and kidney pathologies.

Statistical analysis

IBM SPSS 27 (SPSS Inc.) was used for statistical analysis. Before conducting any analyses, data were assessed for normality and homogeneity using Shapiro-Wilk and Levene tests. Feed Intake (FI), Final Weight Gain (FWG), Platelet count, Clotting Time (CT), and Prothrombin Time (PT) were put through a general linear model including univariate and post hoc multiple comparisons for observed means.

Results

Growth performance

The growth performance of the rats in terms of weight gain, and feed intake before and after induction of thrombocytopenia and administration of CPF is presented in Fig.1. Cyclophosphamide induced thrombocytopenic rats that were fed CPF₁ revealed decreased ($p \geq 0.05$) FWG at wk 2,3,4,5,6, whereas CPF₂ and CPF₃ containing *C. papaya* dry fruit exhibited increased ($p \geq 0.05$) FWG from 2 to 6 wk (Fig. 1). An interaction between CPF₂ and CPF₃ were also observed on FWG ($p \geq 0.05$) as compared to NC but it was observed that CPF₂ had decreased FWG as CPF₃.

The feed intake of rats for all groups before and after thrombocytopenia induction and CPF administration is presented in Fig.2. In the first week, the feed intake of all groups was not significant ($p \geq 0.05$). The disease-induced rats' CPF₁ showed decreased ($p \geq 0.05$) feed intake (18.6) in the wk 2 which tends to decrease until week 6 (17.2). The study groups that were given CPF₂ and CPF₃ had a higher intake in week 6, 21.5, and 22.5 respectively ($p \geq 0.05$) compared to CPF₁ had a decreased intake (17.2) but were non-significant when compared to NC having intake 22.05.

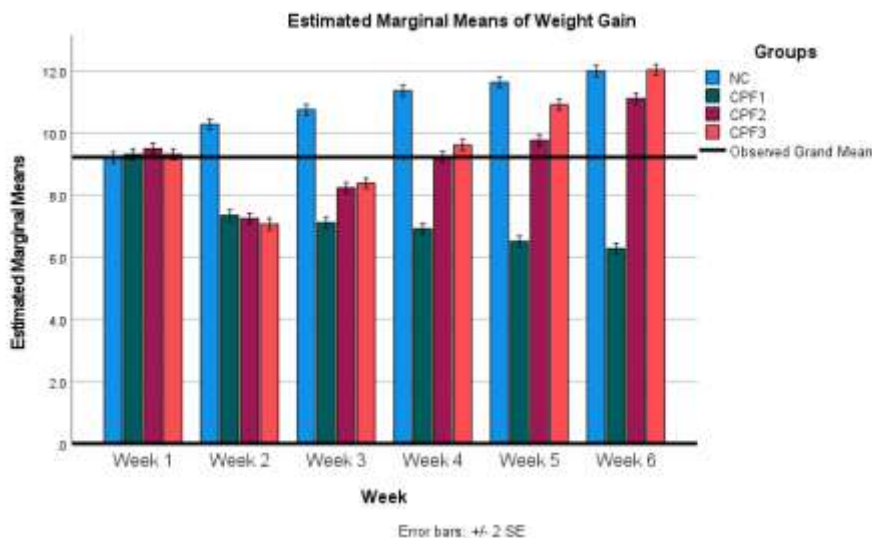


Fig.1: Final weight gain (FWG) of control and study groups

NC Control group, CPF₁ Negative control group, CPF₂ experimental group given 300 mg *C. papaya* dried fruit, CPF₃ experimental group given 600 mg *C. papaya* dried fruit.

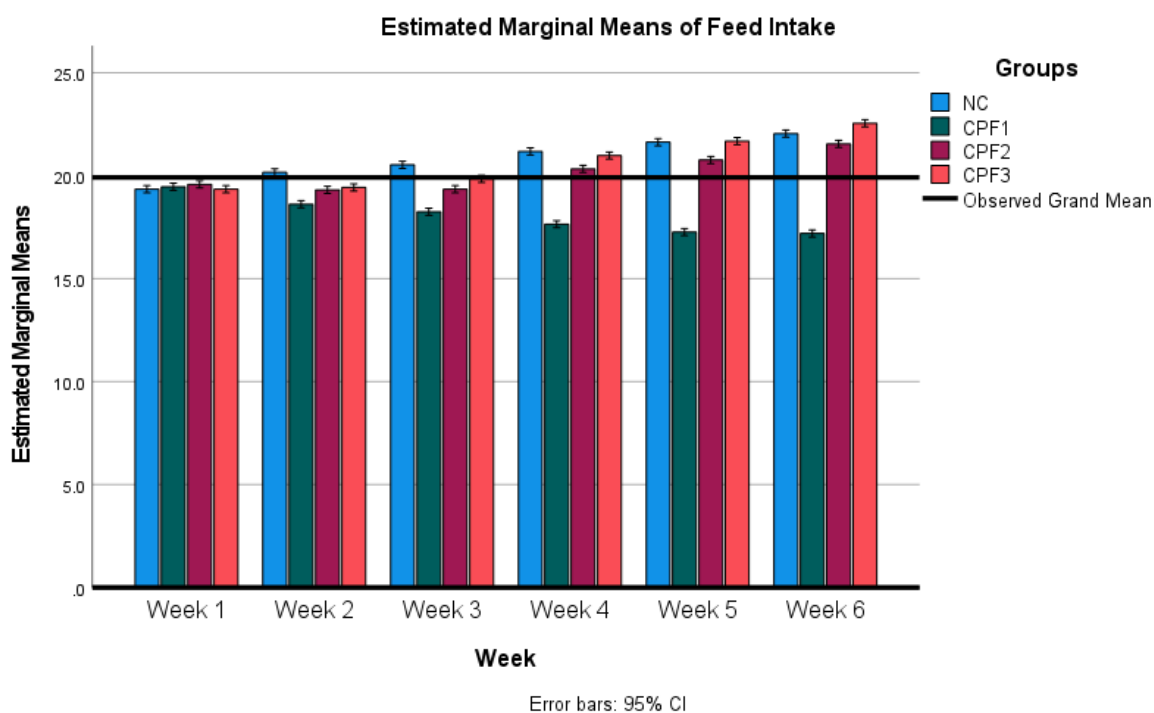


Fig.2: Feed intake (FI) of control and study groups

NC Control group, CPF₁ Negative control group, CPF₂ experimental group given 300 mg *C. papaya* dried fruit, CPF₃ experimental group given 600 mg *C. papaya* dried fruit.

Table 4. Administration of CPF orally increased platelet count in cyclophosphamide-induced thrombocytopenia

CPF increased the number of platelets in cyclophosphamide-induced thrombocytopenic rats. Thrombocytopenia is categorized by a markedly decreased platelet count. There was a time-dependent rise in the platelet count in response to CPF ratios. All the values are expressed as Mean ± SEM.

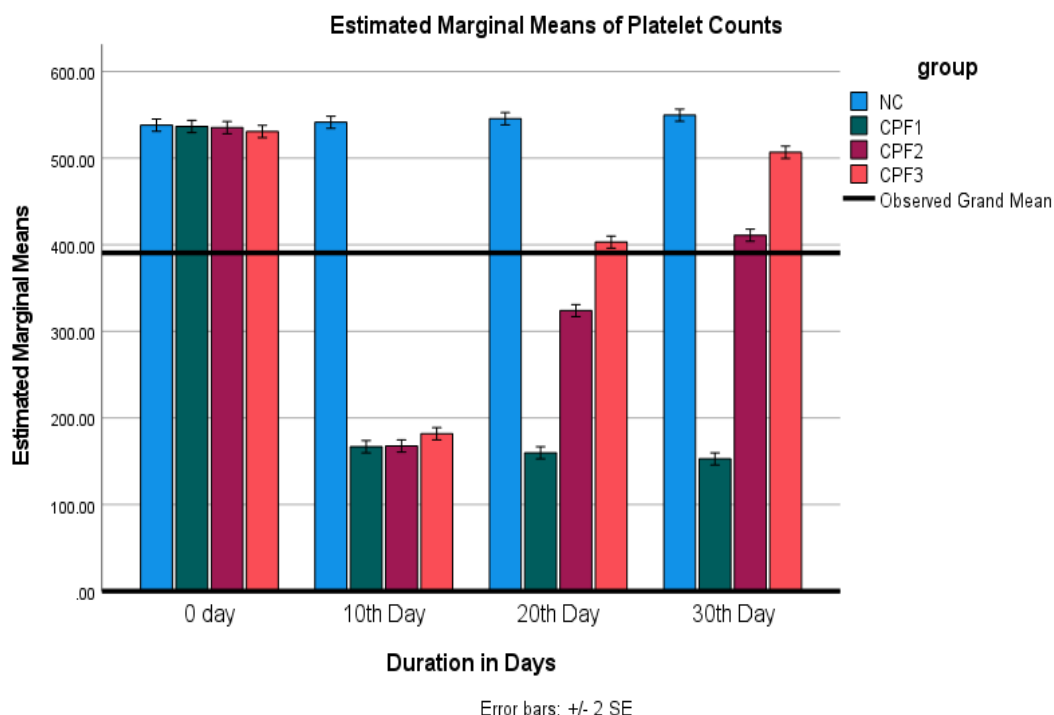


Fig.3. Platelet count of control and study groups

NC Control group, CPF₁ Negative control group, CPF₂ experimental group given 300 mg *C. papaya* dried fruit, CPF₃ experimental group given 600 mg *C. papaya* dried fruit.

To analyze the general linear model univariate was used for data analysis, and the LSD and Tukey post-hoc test was used for any multiple comparisons. A significance level of $p < 0.05$ indicates how different a treatment day's results are from those of the normal group.

The average serum platelet counts of the experiment and control groups were 535.25×10^3 cells/ μ L. Observed data for the platelet count showed that the administration of cyclophosphamide resulted in a significant decrease ($p \geq 0.05$) by 68.91% in CPF₁ (negative controlled group), 68.68% in CPF₂ (experiment group with low dose), 65.84% in CPF₃ (experiment group with high dose) in the platelet count on the 10th day when compared with the normal control group. Administration with CPF resulted in a significant increase ($p \geq 0.05$) in the platelet count by 93.24% in CPF₂ and 122.65% in CPF₃ on the 20th day when compared to the decrease of 4.2% in CPF₁ to further increase till the 30th day by 21.17% in CPF₂ and 20.45% in CPF₃. The serum platelets increased significantly ($p \geq 0.05$) in CPF₂ and CPF₃ as compared to CPF₁. The levels of serum platelet in all treatment groups had shown non-significant ($p \geq 0.05$) results compared to NC.

Clotting Time:

After the induction of thrombocytopenia on the 10th day, clotting time exhibited a substantial increase of 28.57% in the negative control group compared to the normal control group by 1.39% while it was observed as non-significant as compared to CPF₂ by 29.6% and CPF₃ by 30.2%. Treatment with CPF, however, decreased the clotting time on the 20th day by 9.09% CPF₂ and by 11.57% in CPF₃ compared with the negative control group by 5.25%. However, the values were significantly higher than that observed for the normal control by 0.77%. On the 30th day, the clotting time was significantly pronounced ($p \geq 0.05$) in the CPF₂ by 9.57% and CPF₃ groups by 17.32% compared to CPF₁ by 3.73%.

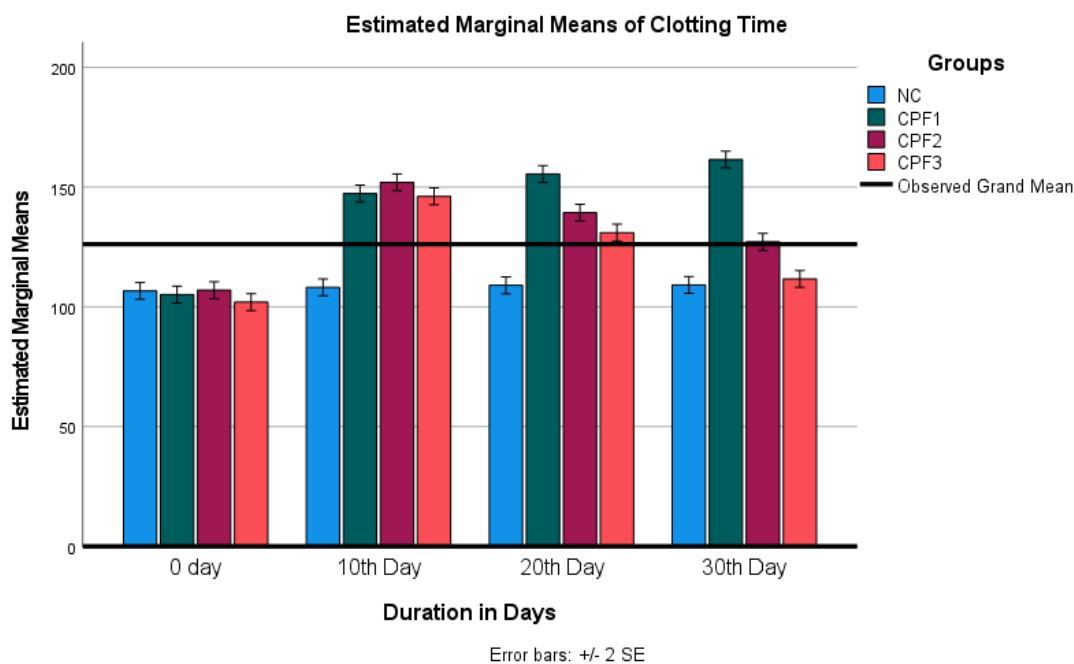


Fig.4. Clotting Time

NC Control group, CPF₁ Negative control group, CPF₂ experimental group given 300 mg *C. papaya* dried fruit, CPF₃ experimental group given 600 mg *C. papaya* dried fruit.

Prothrombin Time:

Prothrombin time (PT) measurements in rats were established to range between 13.6 and 16.6 seconds (Wohlauer, Moore et al. 2011). After the administration of cyclophosphamide, significant ($p \geq 0.05$) prolongation of prothrombin time was observed on the 10th day in CPF₁ by 50.27%, CPF₂ by 48.26%, and 51.93% in CPF₃ when compared with the normal control group by 3.76%. After the application of the experimental supplement CPF, a significant decrease ($p \geq 0.05$) in prothrombin time was observed in CPF₂ by 34.36% and 51.29% in CPF₃ groups, when compared with the NC by -2.25% and CPF₁ 5.42%, respectively on 20th day. A similar trend was seen on the 30th day with a significant decrease ($p \geq 0.05$) in prothrombin time by 29.27% in CPF₂ and 26% in CPF₃ compared with the NC by 2.24% and 4.14% in CPF₁ respectively.

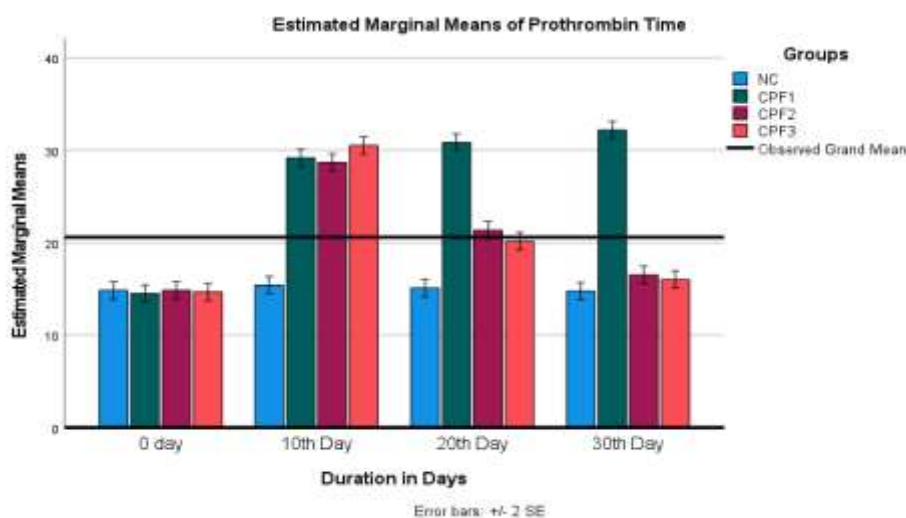


Fig.5. Prothrombin Time

NC Control group, CPF₁ Negative control group, CPF₂ experimental group given 300 mg *C. papaya* dried fruit, CPF₃ experimental group given 600 mg *C. papaya* dried fruit.

***C. papaya* fruit (CPF) restored cyclophosphamide-induced thrombocytopenia associated histological changes in the liver and kidney:**

Histological examination of liver and kidney sections revealed signs of pathological changes ranging from mild focal cell necrosis to sinusoidal damage and hemorrhage in cyclophosphamide-induced thrombocytopenic rat groups. Cyclophosphamide is considered a potent inducer of thrombocytopenia. As evidenced by several studies, the administration of cyclophosphamide may lead to hepatotoxicity and nephrotoxicity in rats (Khazaei, Ghanbari et al. 2020) To determine the possible protective benefits of *C. papaya* fruit against cyclophosphamide-induced toxicity in the liver and kidney, rat organs were collected and examined through hematoxylin and eosin staining for their morphology and architecture. Administration of *C. papaya* fruit in CPF₂ with 300 mg/kg body weight showed limited recovery, whereas in CPF₃ 600 mg/kg body weight was capable of restoring normal morphology in the liver sinusoids. Likewise, administration of CPF₂ and CPF₃ exhibited reduced cyclophosphamide-induced nephrotoxicity. Treatment groups showed recovery in renal cells as opposed to thrombocytopenic ones, though CPF₂ exhibited a slight hemorrhagic tissue. The histopathology analysis of the liver and kidney in the negative control group presented evidence of atrophy and a reduction in the pore size of the sinusoids.

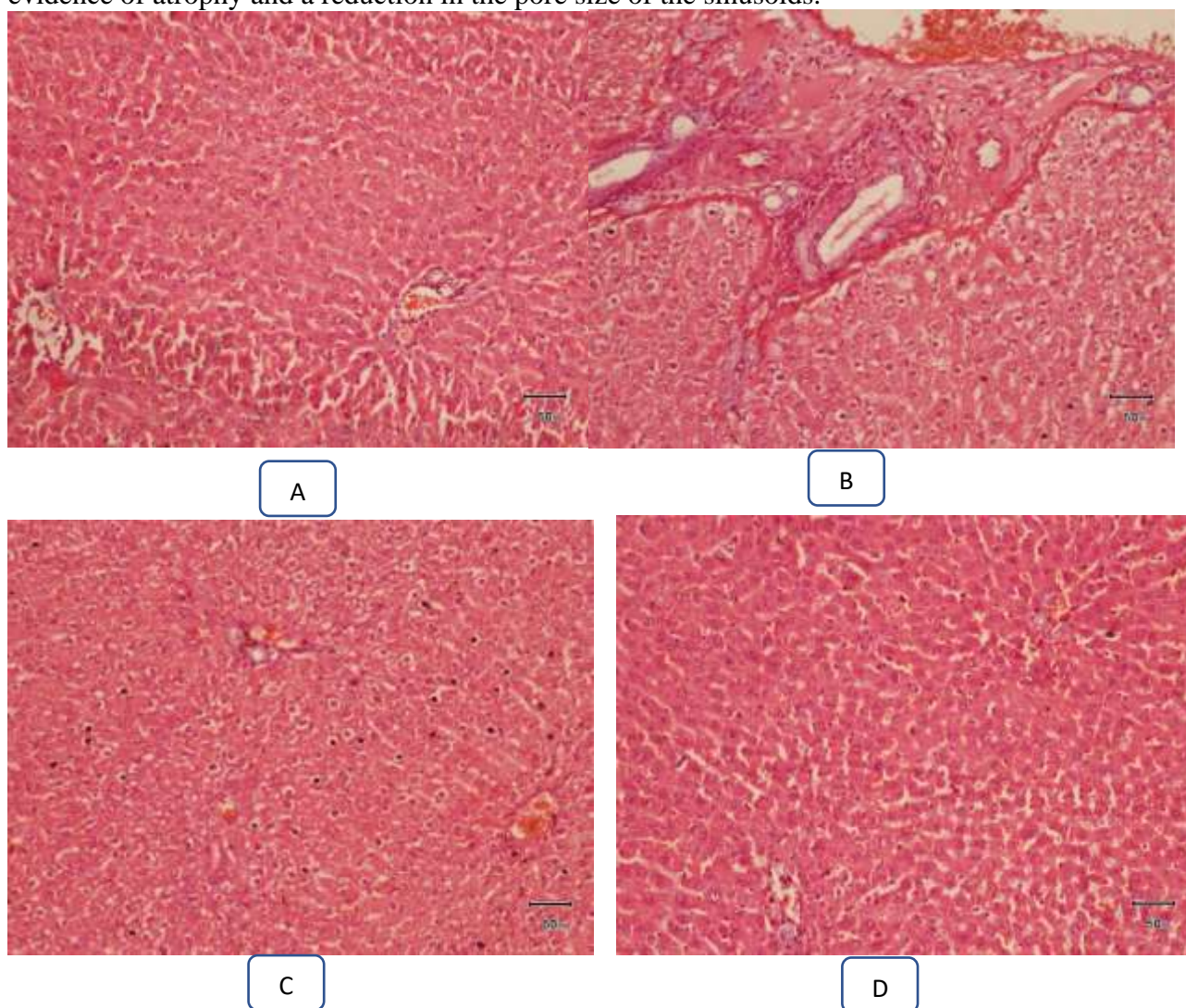


Fig. 6. Histopathological observation of rat liver tissue. Histological examination of the section of the liver (10 x 10) showed sinusoidal damage and hemorrhage in the rats receiving

cyclophosphamide. Hepatocytes had almost normal histology and sinusoids (A). Marked hydropic degeneration in the hepatocytes of the periportal area is seen. Mild infiltration of inflammatory cells is seen in the portal area (B). Mild cellular swelling is seen in the hepatocytes of the centrilobular, midzonal, and periportal areas (C). Mild congestion is seen in the sinusoidal capillaries (D).

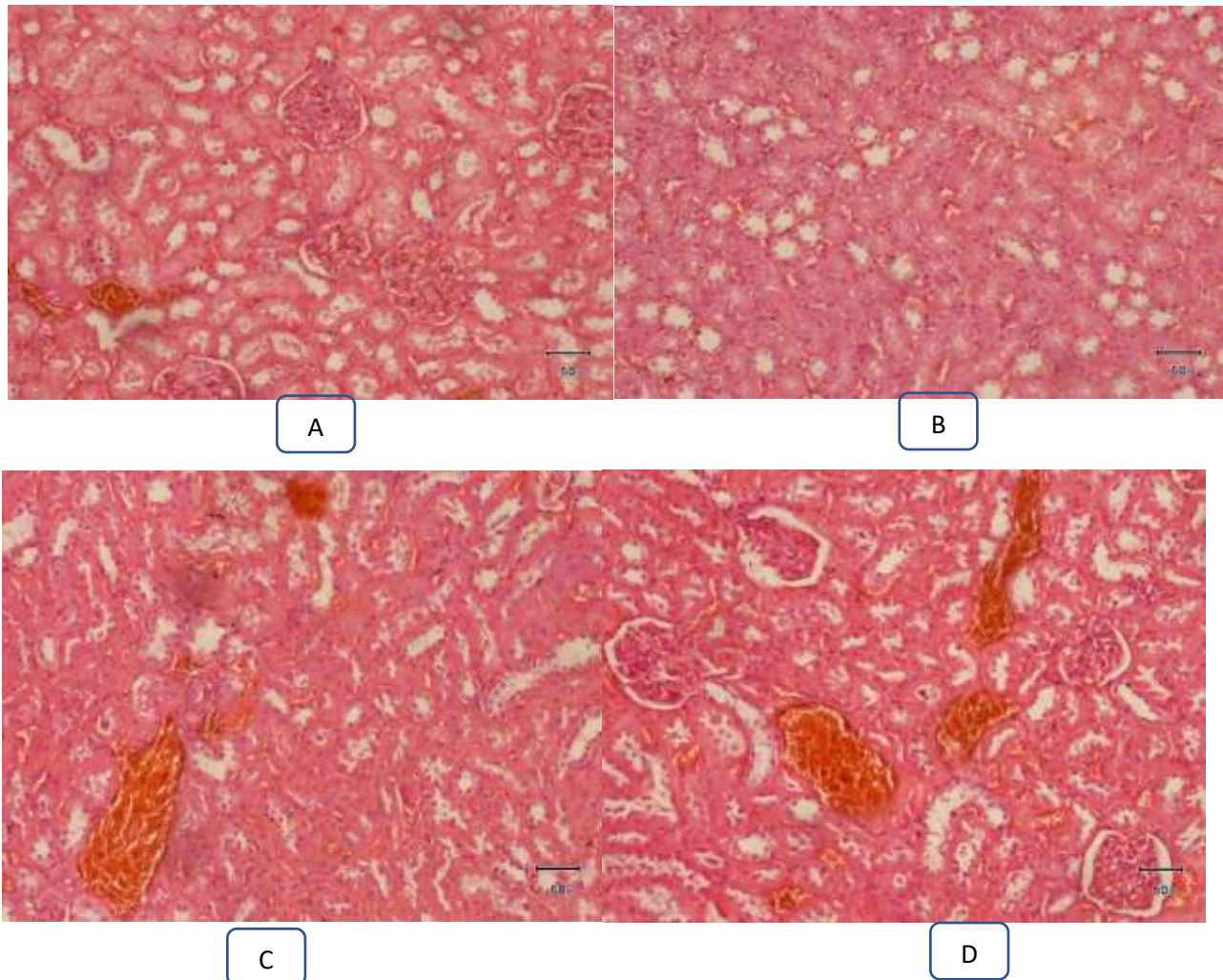


Fig. 2. Histopathological observation of rat kidney tissue. Histological examination of the section of the kidney (10 x 10) showed sinusoidal damage and hemorrhage in the rats receiving cyclophosphamide. Renal cells had almost normal histology and sinusoids (A). Marked peritubular congestion and coagulative necrosis in the tubular epithelial cells are seen (B). Moderate peritubular congestion is seen (C). Mild congestion and cellular swelling in renal tubular epithelial cells are seen (D).

Discussion:

The observed data in the current study indicated changes in body weight after administration of cyclophosphamide, which indicates a possible correlation with the drug's impact on suppressing appetite. This association suggests that the drug may cause a mild to moderate alteration in neurochemical signals in the hypothalamus that regulate appetite. The decrease in appetite may result in a reduction of feed intake, which consequently causes a decline in body weight, as highlighted in Fig.2. Nevertheless, the administration of CPF has counteracted the adverse effects of cyclophosphamide, but it also enhances the feed intake, which eventually results in restoring body weight changes, as indicated in Fig.1.

In another particular study, mice in the model group exhibited a gradual weight loss after administration of cyclophosphamide when compared to the control group. However, the data have

revealed a progressive increase in body weight gain in administering study supplements to the treatment group compared to the control group, which validates the study's findings (Zhong, Huang et al. 2022). The study revealed that cyclophosphamide induction significantly reduced body weight (164 ± 14.5 versus the initial weight of 201.9 ± 7.6). Following the administration of an experimental supplement, all groups demonstrated increased weight gain; no statistically significant difference existed between the treatment and control groups (Khazaei, Ghanbari et al. 2020). Another finding exhibited that the rats of each dose group did not show any signs of general toxicities except that the body weight and weight gain were decreased in the high-dose group. Further, no mortality was seen during the treatment. Thus, 10 mg/kg cyclophosphamide is a dose that did not produce significant stress, malnutrition, or fatalities, but ideally produced some measurable sign of general toxicity (loss of body weight), which accords with the demand of the biochemical test guidelines (Hou, Yang et al. 2007).

Specific biochemical components found in *C. papaya* fruit may be associated with increased feed intake. This association may be responsible for the release of digestive secretions like cholecystokinin (CCK), glucagon-like peptide 1, and peptide YY, which will result in decreasing gastric emptying time, slowing down gastric motility, and decreasing intestinal transit time. These effects will eventually improve the absorption of food. Moreover, the fruit possesses a significant amount of dietary fiber, linked to enhanced nutrient absorption, especially trace elements, and water retention. This process makes it easier for food to pass through the gastrointestinal system. The study revealed that including *C. papaya* in the diet resulted in weight reduction, attributed to its weight-reducing properties. The nutrient-enriched *C. papaya* fruit has health benefits and can also be beneficial in managing metabolic syndrome in rats (Matsuane, Kiage, et al. 2023). Another finding revealed a significant reduction in the final body weight and weight gains by 7.73% in the low-dose and 12.49% in the high-dose group relative to the high-fat group. Notably, the experimental groups had no statistically significant variance (Od-Ek, Deenin et al. 2020). potent antioxidant effect of aqueous fruit extract of *C. papaya* might be attributed to its rich antioxidant contents such as vitamin C, β -carotene, and other flavonoid compounds. (Athesh, Karthiga et al. 2012)

The study data has revealed a significant reduction in platelet count in the CPF₁ (disease-induced) compared with the NC (normal control group) Fig.3. According to certain studies, cyclophosphamide can induce the oxidation of membranes of cellular blood components, making them more vulnerable to mechanical abrasion and eventual destruction. It suggests a possible correlation between hemolytic phenomenon - defined by decreased sinusoidal pores in hepatocytes and renal cells - and blood cell breakdown. CPF (*C. papaya* fruit) appears to possess anti-sickling and hematopoietic properties attributed to its traditional medicinal use, helping restore and preserve both liver and renal structure and function after administration. This phenomenon could be attributable to various bioactive compounds in CPF, such as potassium, copper, zinc, magnesium, and vitamins A, B, ascorbic acid, folate, and lycopene, associated with improved hematological parameters.

A previous study suggests that *C. papaya* ripe fruit pulp demonstrates a more substantial effect on raising platelet count in thrombocytopenic rats than the immature fruit pulp. Additionally, a separate experiment evaluating the nutritional composition of *C. papaya* fruit at different ripening stages indicated a significant amount of vitamin C in mature papaya. The impact of vitamin C on platelet activities includes the lowering of reactive oxygen species, the change of pro-inflammatory CD40 ligand expression, the suppression of thromboxane B2 synthesis, and the stimulation of prostaglandin E1 generation. These findings likely explain the increased platelet count in the ripe papaya group compared to the unripe papaya group in the current experiment. (Jose, Sasmi et al. 2018).

Prothrombin time (PT) measures how long plasma blood takes to clot when triggered by a tissue factor, typically through the extrinsic pathway. Combined with the international normalized ratio (INR), this test provides a standardized assessment for various thromboembolic and hemorrhagic

disorders. (Saha, Bajpai et al. 2021). The elevated prothrombin time observed in the negative control group reveals a potential impediment in the tissue factor pathway, necessitating factor VII or due to lack of coagulation factors V and X. Conversely, the reduction in prothrombin time after the administration of CPF may be associated with the impact of fruit fractions in enhancing fibrinogen synthesis. The observed modulation in platelet count and coagulation assay by CPF suggests a potential utility of these fruit fractions in managing coagulation disorders. This impact may be associated with the presence of flavonoid components present in the fruit. The thromboembolic effect of the fruit is further substantiated by the reduction in prothrombin time and clotting time observed in the treatment groups compared to the negative control group.

The induction of thrombocytopenia through cyclophosphamide administration resulted in sinusoidal injury and hemorrhaging within hepatocytes and renal cells. Thrombopoietin (TPO) synthesis exhibits constitutive characteristics, and the circulating levels are determined primarily by the circulating platelet mass. Notably, hepatocytes are critical contributors to TPO synthesis, and their expression decreases due to liver cell injury. This process reduces thrombopoiesis within the bone marrow, subsequently manifesting as thrombocytopenia. Histological examination of hepatocytes and renal cells demonstrated that the administration of CPF restores the normal parenchymal structure that contributes to adequate thrombopoietin production, leading to prompt restoration of platelet count. It signifies the potential of CPF in optimizing crucial pathways involved in platelet biogenesis and regulation.

Histological examination of hepatocytes and renal cells revealed that the administration of CPF restores the normal parenchymal structure. This restoration of tissue architecture contributes to adequate thrombopoietin production, facilitating a prompt increase in platelet concentration. This examination depicts the potential efficacy of CPF in restoring hepatocytes and renal cells that facilitate thrombopoietin production.

It has been reported that extracts from different parts of *C. papaya*, such as the leaves, seeds, or fruit, exhibited significant efficacy in treating liver toxicity by lowering hepatic damage and improving antioxidant enzyme activity such as superoxide dismutase (SOD), glutathione (GSH) and catalase. Additionally, reports indicated reductions in the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) enzymes. Similar results, such as decreased kidney damage and improved renal function, were documented in nephrotoxicity caused by CCl₄ in rats treated with *C. papaya* seed water extract, with outcomes dependent on the dose and duration of therapy. These outcomes included significant reductions in biochemical indicators such as blood levels of uric acid, urea, and creatinine, along with confirmed kidney protection demonstrated through histological testing post-recovery from renal lesions. (Santana, Inada et al. 2019).

Conclusion

This research signifies the impact of ripe *C. papaya* fruit on cyclophosphamide-induced thrombocytopenia, particularly its efficacy in augmenting platelet count and treating coagulative disorders. The observed data reveal a significant elevation in platelet counts and a concurrent reduction in prothrombin and clotting times in cyclophosphamide-induced thrombocytopenic rats after administration of CPF for 18 days. The research outcomes demonstrate the potential therapeutic effects of dried *C. papaya* fruit in ameliorating thrombocytopenia due to flavonoids and other phenolic compounds likely to possess membrane-stabilizing properties and protect blood cells against stress-induced destruction. This property may be helpful in patients with dengue infection where the CPF could prevent platelet lysis. Considering its modest efficacy and safety, CPF may be a feasible option in treating thrombocytopenia induced by dengue fever and chemotherapy. However, further studies are required to validate its beneficial effects. Considering its modest efficacy and safety, *C. papaya* fruit fraction may be feasible in treating CIT. However, further studies are required to validate its beneficial effects.

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