Original Article

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Effect of *Carica papaya* Linn. Unripe and Ripe Fruit Pulp Juice in Augmenting Platelet Count in Rats: An Experimental Study

Animal Research Section

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ABSTRACT

Introduction: Thrombocytopenia is often associated with diseases like dengue, Idiopathic Thrombocytopenic Purpura (ITP), malignancy, aplastic anaemia, drug induced thrombocytopenic purpura and haemolytic uremic syndrome. There are no effective methods to treat thrombocytopenia. *Carica papaya (C. papaya)* fruit pulp juice contains many constituents that can act on the bone marrow and enhance its ability to produce platelets.

Aim: To evaluate the effects of fresh *Carica papaya* unripe and ripe fruit pulp juice on platelet count in cyclophosphamide induced thrombocytopenic rat model.

Materials and Methods: A total of 24 male Albino Wistar rats were grouped into 'Control' (A), 'Drug control' (B), 'Papaya unripe fruit pulp' (C) and 'Papaya ripe fruit pulp' (D) groups. Thrombocytopenia was induced by three doses of cyclophosphamide (50 mg/kg body weight) given subcutaneously to Groups B, C and D from day 1 to 3. Groups C and D received *Carica papaya* unripe and ripe fruit pulp juice orally from day 11 to 14 respectively. Platelet count, Packed Cell Volume (PCV), Red Blood Cell (RBC) count, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and serum creatinine levels was measured on day 1, 10, 14 and 21. For comparison between groups, 'unpaired t-test' and within the group, paired t-test were used. A p-value ≤0.05 was considered significant.

Results: There was significant increase in platelet count in Group C (6.27×10^5/μL) and D (20.5×10^5/μL) compared to drug control B (3.94×10^5/μL) on day 14. Within the group, there was significant increase in platelet count in Group C and D on day 14 compared to day 10. Between the groups, Group D showed higher platelet increasing effect on thrombocytopenic rats.

Conclusion: *Carica papaya* ripe and unripe fruit pulp juice has platelet enhancing effect. Further preclinical studies are required to identify those chemicals of *C. papaya* fruit responsible for boosting thrombopoiesis.

Keywords: Cyclophosphamide, Dengue, Thrombocytopenic model

INTRODUCTION

Thrombocytopenia is a condition associated with the lower production of platelets than the normal numbers in the bone marrow and is often multifactorial. Thrombocytopenia is seen in diseases such as dengue, ITP, malignancy, aplastic anaemia, drug induced thrombocytopenic purpura and haemolytic uremic syndrome [1]. The dengue infection is the most rapidly spreading mosquito-borne viral disease in the world and an estimated 50 million dengue infections are reported annually [2]. Thrombocytopenia is a constant manifestation in dengue fever, which often leads to life threatening Dengue Haemorrhagic Fever (DHF) and the Dengue Shock Syndrome (DSS). Both haemorrhagic diathesis and circulatory collapse are the fatal complications of the dengue infection [3]. The standard treatment protocol for management of dengue includes symptomatic treatment with fluid management; with platelet transfusion when platelet count lowers to levels less than 20000/μL.

Carica papaya leaves and fruit pulp juice have been successfully employed in folk medicine for the treatment of dengue infections with haemorrhagic manifestations. *C. papaya Linn*. belongs to the plant family Caricaceae. A lot of work has been carried out on plant parts like fruits, seeds and roots, indicating the presence of biologically active compounds [4]. *C. papaya* contains alkaloids, glycosides; polyphenols and hydroxymethyl anthraquinones in the seed, leaf and pulp extracts while saponins and flavonoids are absent in the seed and pulp extracts, respectively [5]. Chemical analysis showed the presence of considerable amount of carpaine, malic acid, quinic acid, manghaslin and clitorin, minor quantities of various malic acid derivatives, nicotiflorin, rutin and unidentified constituent [6]. These constituents can act on the bone marrow, prevent its destruction and enhance its ability to produce platelets [7]. Study by Ikpeme EV et al., on Albino Wistar rats using ethanolic extracts of leaf, unripe fruit pulp and seeds of *C. papaya* showed, pulp extract boosted the thrombocytes number more than leaf and seed extract [5]. *C. papaya* fruit pulp juice could be a cost effective alternative for treatment of thrombocytopenia if proven by scientific research.

Cyclophosphamide, an antineoplastic and immunosuppressive agent, was used as toxicant in the current study because of its capacity to induce stable thrombocytopenia [8]. Subcutaneous injection of cyclophosphamide 50 mg/kg/day for three consecutive days is found to be one simple, feasible and rat thrombocytopenia model [9].

In the current study, the possible effects of fresh *C. papaya* unripe and ripe fruit pulp juice in increasing the platelet count in cyclophosphamide induced thrombocytopenic rat model was determined.

MATERIALS AND METHODS

The experiments were carried out taking appropriate measures in accordance with the guidelines laid down by Committee for the Purpose of Control and Supervision of Experiments on Animals, India (CPCSEA) with regard to the care and use of animals for experimental procedures, and with approval from the Institutional Animal Ethics Committee. The study was conducted during the period of October 2016 to February 2017.

Experimental animals: A total of 24 inbred male, Albino Wistar rats weighing 150-200 gm were used. Animals were kept in metallic cages (six rats in each cage) in the Small Animal Research facility (SARF) of the Jubilee Mission Medical College and Research Institute, Thrissur, Kerala. The animals were housed under standard conditions of temperature, humidity and light and maintained on a standard pellet diet and water ad libitum.

Establishment of thrombocytopenia in the rat model: Induction of thrombocytopenia was performed by the subcutaneous administration of freshly prepared cyclophosphamide. Each rat was given 50 mg/kg body weight dose of cyclophosphamide dissolved with normal saline and was administered by subcutaneously once a day for three consecutive days from day 1 to 3. Then it successfully initiated thrombocytopenia by day 10 [8,9].

Preparation of *C. papaya* unripe and ripe fruit pulp juice: Fresh *C. papaya* unripe and ripe fruit pulps were harvested, after removal of skin and seeds, weighed and blended separately. It was filtered and stored at 4°C and administered orally at 2 gm/kg body weight along with normal saline.

The fruit was washed with distilled water, peeled, deseeded and the pulp was then blended, and the resulting pulp was squeezed through a mesh cloth to produce the pulp juice.

Experimental procedure: An amount of 24 rats were divided into four groups each having six rats. The animals had free access to pellet food and water ad libitum. The same conditions were maintained throughout the experiment. Groups were labelled as Group A, Group B, Group C and Group D.

Group A: Rats received normal saline (10 mL/kg orally) from day 1 to day 3 and thereafter from day 11 to day 13.

Group B: Rats received cyclophosphamide 50 mg/kg subcutaneously (s/c) from day 1 to day 3 and thereafter normal saline from day 11 to day 13.

Group C: Rats received cyclophosphamide 50 mg/kg s/c from day 1 to day 3 and thereafter *C. papaya* unripe fruit juice at the dose 2 gm/kg body weight/day with normal saline orally from day 11 to day 13.

Group D: Rats received cyclophosphamide 50 mg/kg s/c from day 1 to day 3 and thereafter *C. papaya* ripe fruit juice at the dose 2 gm/kg body weight/day with normal saline orally from day 11 to day 13.

Blood samples were collected on 1st, 10th, 14th and 21st day of the experiment from the retro orbital venous plexus of the rats after giving halothane inhalation (open drop method). Platelet count, PCV, RBC count, White Blood Cell (WBC) count, ALT, AST and serum creatinine levels were measured. The scheme of the experiment is given in [Table/Fig-1].

STATISTICAL ANALYSIS

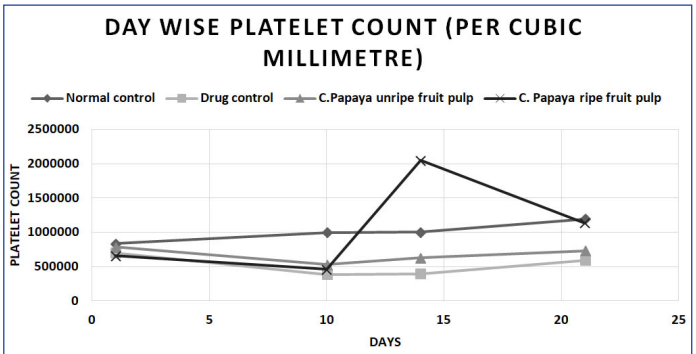
Data were represented as mean±SD. For comparison between groups, one-way ANOVA (Analysis Of Variance) followed by Tukey's post-hoc test and 'unpaired t-test' was done. For comparison within the group, paired t-test was used. A p-value ≤0.05 was considered significant for all tests. Statistical analysis were performed using commercially available software (SPSS version 22.0).

RESULTS

The *C. papaya* unripe fruit pulp and ripe fruit pulp juice were found to significantly increase the platelet count in cyclophosphamide induced rat model. The mean platelet count of the normal control (A), drug control (B), *C. papaya* unripe fruit pulp (C) and *C. papaya* ripe fruit pulp (D) were 8.33×10^5/µL, 7.02×10^5/µL, 7.88×10^5/µL and 6.61×10^5/µL respectively before the experiment. The mean platelet count was not significantly different between the groups on day 1 (One-way ANOVA: F=1.263, df=3, p=0.314). The day wise platelet count in all four groups on day 1, 10, 14 and 21 are shown in [Table/Fig-2].

Group	Day 1-3	Day 4-10	Day 11-13
A	Normal saline (10 mL/kg p.o)	No intervention	Normal saline (10 mL/kg p.o)
B	Cyclophosphamide (50 mg/kg s/c)	No intervention	Normal saline (10 mL/kg p.o)
C	Cyclophosphamide (50 mg/kg s/c)	No intervention	Papaya unripe fruit pulp juice (2 gm/kg body weight/ day) with normal saline
D	Cyclophosphamide (50 mg/kg s/c)	No intervention	Papaya ripe fruit pulp juice (2 gm/kg body weight/ day) with normal saline

[Table/Fig-1]: Scheme of the experiment.
p.o: per orally; s/c: subcutaneous
Group A: Control; B: Drug control; C: Papaya unripe fruit pulp juice; D: Papaya ripe fruit pulp juice



[Table/Fig-2]: Line graph for day wise platelet count (per cubic millimetre).

Group	Day 1	Day 10
A	833830±230040	995830±260850 [a]
B	702170±185040	389000±211070 [b]
C	788170±156340	535500±92170 [b]
D	661670±100442	461330±132470 [b]

[Table/Fig-3]: Platelet count of Group A, B, C, D on day 1 and day 10.
Data expressed as Mean±SD; platelet count in cells per cubic millimetre; Paired t-test done in groups A, B, C, D between day 1 and 10; p-values: a-0.39; b-<0.05
Group A: Control; B: Drug control; C: Papaya unripe fruit pulp juice; D: Papaya ripe fruit pulp juice

Day 14	Group B	Group C	Group D	p-value
Platelet count	394030±96332	627500±105950	--	0.003
	394030±96332	--	2050833±368370	0.001
		627500±105950	2050833±368370	0.001

[Table/Fig-4]: Platelet count of Group B, C, D on day 14.
Data expressed as Mean±SD; platelet count in cells per cubic millimetre; Unpaired student's t-test done between B and C; B and D; C and D

Groups	Day 10	Day 14	p-value
A	995830±260850	1004800±261520	0.950
B	389000±211070	394030±96332	0.940
C	535500±92170	627500±105950	0.001
D	461330±132470	2050833±368370	0.001

[Table/Fig-5]: Platelet count of Group A, B, C, D on day 10 and day 14.
Data expressed as Mean±SD; platelet count in cells per cubic millimetre; Paired t-test done in groups A, B, C, D between day 10 and 14
Group A: Control; B: Drug control; C: Papaya unripe fruit pulp juice; D: Papaya ripe fruit pulp juice

The platelet count reduced significantly in all the three groups (B, C, D) by day 10 compared to day 1 [Table/Fig-3]. There was no significant difference in mean platelet count between Group B (3.89×10^5/µL) and Group C (5.35×10^5/µL, p-0.150) and Group D (4.61×10^5/µL, p-0.493) on day 10.

From day 11, *C. papaya* unripe fruit pulp and ripe fruit pulp was given to the test groups (C and D) for three days. There was significant increase in platelet count in Group C (6.27×10^5/µL) and D (20.5×10^5/µL) compared to drug control B (3.94×10^5/µL) on day 14 [Table/Fig-4]. Within the groups, there was significant increase

Parameters	Day 1			Day 10			Day 14		
	B	C	D	B	C	D	B	C	D
RBC ($\times10^6$/mm^3)	7.74±0.41	6.22±0.42	5.64±0.31	5.81±0.88	4.41±0.53	5.09±0.41	5.44±0.86	4.34±0.58	4.95±0.22
WBC ($\times10^3$/mm^3)	14.33±4.90	12.36±2.54	14.45±3.15	8.35±5.66	12.25±6.28	7.30±3.09	24.06±14.35	15.31±9.50	23.53±11.17
PCV (%)	42.81±1.42	33.56±1.23	32.73±1.09	32.20±4.68	25.43±1.87	29.28±2.15	31.95±3.46	25.68±1.83	31.43±1.75
AST (U/L)	117.58±49.00	148.76±0.78	134.37±40.84	115.74±29.44	177.93±51.22	134.94±36.36	156.44±55.66	143.20±47.91	177.12±49.89
ALT (U/L)	100.45±44.28	105.60±39.13	135.94±84.21	78.68±56.15	68.09±19.04	147.47±85.45	74.63±31.50	78.39±17.76	105.77±28.84
S. Creatinine (mg/dL)	0.88±0.69	0.57±0.05	0.69±0.06	0.63±0.08	0.72±0.12	0.72±0.07	0.74±0.09	0.72±0.13	0.70±0.03

[Table/Fig-6]: Haematological and biochemical parameters in the groups.
Data expressed as Mean±SD
RBC: Red blood cell; WBC: White blood cell; PCV: Packed cell volume; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase

in platelet count in Group C and D on day 14 compared to day 10 [Table/Fig-5]. Between the groups, Group D showed higher platelet increasing effect on thrombocytopenic rats on day 14. The increase in mean platelet count was maintained till day 21 compared to day 10 in Groups C and D. The mean RBC, WBC counts and PCV were within normal limits. The Serum creatinine, ALT, AST values were not significantly altered in the test rats (Group C, Group D) compared to drug control (Group B) rats on day 14 [Table/Fig-6].

DISCUSSION

The present study clearly showed that platelet count increased after oral feeding with a short course of *C. papaya* unripe and ripe fruit pulp juice in cyclophosphamide induced thrombocytopenic rat model. These animals remained healthy and haematological parameters were within normal limits during the experiment.

Various extracts from different parts of *C. papaya* have been used in alternative medicine. *C. papaya* leaves, seeds, roots and unripe pulp, have been studied for their medicinal value such as, to treat dengue fever and ulcer as antidiabetic and antioxidant, antitumor and immunomodulatory wound healing and antimicrobial agents [6,10,11-15]. Controlled studies using *C. papaya* leaf juice have shown significant increase in platelet count in thrombocytopenic rat models [6,16-18]. Study by Hettige S et al., showed that *C. papaya* leaves contain complex substances that may support the release and production of platelets by the bone marrow and helps to normalise clotting and repair liver damage caused by dengue [19]. Tham SC et al., tested antioxidant and haemopoietic properties of leaves of *C. papaya* against the effect of lead acetate in experimental rats and concluded that *C. papaya* was effective against the oxidative damage caused by lead acetate in the bone marrow and had a stimulatory effect on haemopoiesis [20]. Study by Gammulle A et al., showed freshly prepared mature leaf concentrate of *C. papaya* effectively increases platelet, WBC and RBC counts in rats with no acute toxicity, and possesses potent anti-inflammatory activity [18]. The present study showed no signs of acute toxicity as the serum creatinine, ALT, AST values were not significantly altered in the test groups compared to drug control group on day 14. There was no significant difference in the mean RBC, WBC counts in the test groups compared to drug control.

In the present study, we used fresh *C. papaya* unripe and ripe fruit pulp without adding any solvent or chemical. Unripe *C. papaya* is rich in proteases such as papain [21]. Papain is one of the major cystein endopeptidases, which also include chymopapain, caricain and glycyl endopeptidase [21]. These four endopeptidases are found in latex, which can be found in differing amounts in the fruit, leaves and roots. Purified papain was reported to induce thrombocytic cytokines such as IL-6 secretion in dose dependent manner in modified mixed human lymphocyte culture and also stimulates Thrombopoietin secretion in the liver [22,23]. Study by Aziz J et al., showed that unripe papaya fruit extract induced thrombopoietic cytokines secretion by cells of diverse tissue origin (both by human peripheral blood leukocytes and stem cells from human exfoliated deciduous teeth) [24]. Subenthiran S et al., conducted a study to investigate the platelet increasing property of *C. papaya* leaves juice in patients with dengue fever and showed that there was a 15-fold increase in the *ALOX 12* gene activity among the patients in the experimental group compared to control group [10]. *ALOX 12* gene is known to be associated with increased megakaryocyte production as well as its conversion to platelets through 12-HETE mediated pathway which in turn leads to increased platelet production [10]. The present study showed *C. papaya* ripe fruit pulp has higher platelet increasing effect on thrombocytopenic rats than unripe fruit pulp. Study by Chukwuka KS et al., on nutritive value of *C. papaya* fruit at different ripening stage showed, vitamin C content of ripe papaya was found to be very high [25]. Studies have shown that vitamin C plays several roles in platelet functions, including reduction of reactive oxygen species, inhibition of expression of the pro-inflammatory CD 40 ligand, inhibition of thromboxane B2 formation and stimulation of prostaglandin E1 production [26-29]. This probably could explain the higher platelet increasing shown in ripe papaya group compared to unripe papaya group in the present study.

LIMITATION

The present study was a preliminary study and more work is needed to isolate and identify the biologically active ingredients of *C. papaya* fruit pulp to create a suitable formula responsible for the release and/or production of the platelets.

CONCLUSION

The *C. papaya* ripe and unripe fruit pulp juice has platelet increasing effect. Higher platelet increasing effect was seen with ripe papaya. The *C. papaya* fruit pulp juice may be a potential candidate for further research leading to the development of an herbal therapeutic agent for thrombocytopenia manifested in diseases such as dengue. However, this is a preliminary study and more work in future are needed to isolate and identify the biologically active ingredients of *C. papaya* fruit pulp.

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