

## ORIGINAL ARTICLE

# Effect of *Carica papaya* Leaf Extract Capsule on Platelet Count in Patients of Dengue Fever with Thrombocytopenia

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## Abstract

**Objective:** Thrombocytopenia in dengue fever is a common and serious complication. However, no specific treatment is available for dengue fever induced thrombocytopenia. In few countries (Pakistan, Malaysia, Sri Lanka and other Asian countries) the leaf extract of *Carica papaya* has been effectively used for thrombocytopenia. So, the study is planned to access effect of *Carica papaya* leaf extract on platelet count in dengue fever patients.

**Methods:** All participants were randomised into two groups, study group and control group; the study group was given papaya leaf extract capsule of 500 mg once daily and routine supportive treatment for consecutive five days. The controls were given only routine supportive treatment. Daily complete blood counts, platelet counts and haematocrit level, liver function test, renal function test of both groups were observed.

**Result:** On the first day platelet count of study group and control group was (59.82±18.63, 61.06±20.03 thousands, p value 0.36). On the 2nd day platelet count of both study and control groups was not significantly different (61.67±19.46 and 59.93±19.52 thousands, p value 0.20) but on 3rd day platelet count of study group was significantly higher than control group (82.96±16.72, 66.45±17.36 thousands, p value < 0.01). On 4th and 5th day platelet count of study group (122.43±19.36 and 112.47±17.49 thousands respectively) was also significantly higher than the control group (88.75±21.65 and 102.59±19.35 thousands) (p value < 0.01). On 7th day platelet count of study group and control group were not significantly different (124.47±12.35 and 122.46±19.76 thousands respectively, p value 0.08). Average hospitalization period of study group v/s control group was 3.65±0.97 v/s 5.42±0.98 days (p value < 0.01). Average platelet transfusion requirement in study group was significantly less than control group (0.685 units per patient v/s 1.19 units per patient) (p value < 0.01)

**Conclusion:** It is concluded that *Carica papaya* leaf extract increases the platelet count in dengue fever without any side effect and prevents the complication of thrombocytopenia. So, it can be used in dengue fever with thrombocytopenia patients.

## Editorial Viewpoint

- Many active components of *Carica papaya* extract inhibit immune mediated platelet destruction, bone marrow suppression and stabilizes the membrane of infected cells in dengue.
- Papaya leaf extract capsule raised the platelet count by 3rd day with decreased requirement of platelet transfusion.
- The drug did not produce any major side effects.

## Introduction

Dengue is the most common arthropod-borne viral (arboviral) illness in humans. It is transmitted by mosquitoes of the genus *Aedes*. In the last 50 years; incidence has increased 30-fold. An estimated 2.5 billion people accounting to 40% of world population live in over 100 endemic countries and areas where dengue viruses can be transmitted. Up to 50 million infections occur annually with 500 000 cases of dengue haemorrhagic fever and 22,000 deaths mainly among children. Prior to 1970, only nine countries had experienced cases of dengue haemorrhagic fever (DHF); since then the number has increased more than 4-fold and now widely distributed in subtropical and tropical areas of the world.<sup>1</sup> Based

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on the data of National Vector Borne Disease Control Programme (NVBDCP), the number of cases reported in India in 2013 was 74,454 for dengue with 167 deaths.<sup>2</sup>

Four main characteristic manifestations of dengue illness are; continuous high fever lasting 2-7 days; haemorrhagic tendency as shown by a positive tourniquet test, petechiae or epistaxis; thrombocytopenia (platelet count  $<100 \times 10^9/L$ );<sup>3</sup> and evidence of plasma leakage manifested by haemoconcentration (an increase in haematocrit 20% above average for age, sex and population), pleural effusion and ascites.<sup>4,5</sup>

The management of dengue virus infection is essentially supportive and symptomatic. No specific treatment is available.<sup>6,7</sup> Until now, no licensed dengue vaccine is available, several vaccine candidates are currently being evaluated in clinical studies.<sup>8</sup> Therefore, there is an urgent need of development of alternative therapy for dengue. It has been reported that several plant species prevent complication of thrombocytopenia but do not cure dengue. *Carica papaya* (CP) otherwise known as the papaya pear is found in most tropical and subtropical countries of the world. The fruits of papaya are much sought after by human as valuable foodstuff and have anti-hypertensive activity.<sup>9</sup> The leaves of papaya have been shown to contain many active components such as papain, chymopapain, cystatine, toopherol, ascorbic acid, flavonoids, cynogenic glucosides and glucocynolates. These components are related with anti-inflammatory activity. *Carica papaya* leaves extract is also found to have antitumor and immunomodulator activity.<sup>10,11</sup> It has been also hypothesized that certain genes have been shown to influence platelet production and platelet aggregation, namely, the arachidonate 12-lipoxygenase (ALOX 12) also known as the platelet-type lipoxygenase as well

as the platelet-activating factor receptor (PTAFR). An increase in activity of these genes is required for platelet production and activation. The ALOX 12 gene is strongly expressed in megakaryocytes and has been known to be responsible for the 12-hydroxyeicosatetraenoic acid (12-HETE) production of platelets. The PTAFR gene has been found to be expressed in megakaryocytes indicating that it could be a precursor for platelet production in addition to its well known role in platelet aggregation.<sup>13</sup>

In a few countries the leaf extract of *Carica papaya* has been effectively used in dengue fever disease.<sup>12,13</sup> The clinical use of *Carica papaya* leaves extract in dengue fever patients should be evaluated in large comparative trial. Therefore, this study was conducted in our hospital to see the effects of *Carica papaya* leaf extract on platelet count, to compare the duration of hospitalization and the requirement of platelet transfusion between study and control groups.

## Material and Methods

The present study was conducted in Department of Medicine, S.P. Medical College & Associated Group of P.B.M. Hospitals, Bikaner from September 2013 to January 2014. Total 400 cases, (275 male and 125 female) of dengue fever with thrombocytopenia were selected for the study with the inclusion criteria of, age >16 yrs, a proven case of dengue fever by positive NS1 or IgM antibody and platelet count less than  $1,50,000/mm^3$ . Patients who were known case of primary thrombocytopenia, known case of blood malignancy, known case of idiopathic thrombocytopenic purpura, known case of chronic liver disease and chronic kidney disease which affect platelet count and other cases of fever with thrombocytopenia like malaria, brucellosis, leptospira, and enteric fever were excluded from the study.

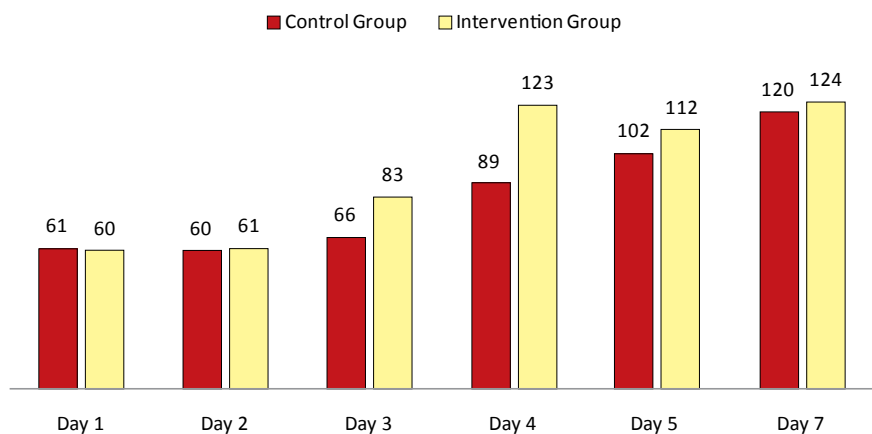
Other causes of thrombocytopenia were ruled out by detailed history, clinical examination and appropriate investigation including complete blood count, peripheral blood film, liver function test, malaria antigen rapid card test, brucella serology, blood culture, serial widal tests and Ultrasonography of abdomen.

Clearance for the study was taken from the ethical committee. A written informed consent from subjects >18 years of age and for subjects <18 years of age written informed consent was taken from their parents.

All participants were randomized into two groups, study group and control group by simple randomization (odd - even method). The study group was given papaya leaf extract capsule (500 mg) once daily and routine supportive treatment (antipyretic Paracetamol, intravenous 0.9% normal saline, antiemetic) for consecutive five days. The control group was given only routine supportive treatment and no placebo drug was given. Complete blood counts, platelet counts, haematocrit level, liver function test and renal function test for both groups were screened daily. The patients were followed from the day of admission till their discharge from hospital. The platelet counts, average hospital stay duration and requirement of platelet transfusion in both study and control groups were compared statistically by student t-test.

### Method of Papaya Leaf Extraction

Air dried papaya leaves of 250 grams were crushed and charged for extraction in a round bottom flask of glass. It was extracted at 80°C thrice with triple volume of demineralised water. All three washings were collected and distilled under vacuum up to 20-30 TDS. Resultant syrup mass was dried in vacuum oven. Crude papaya leaf extract of approx 45 gms was obtained. Of this extract 90 capsules were formed, each



**Fig. 1: Average platelet count in thousands**

containing 500 mg out of crude extract formed from 2777 mg of dried leaves.

Only a few studies have been done on toxicity and safety profile of *Carica papaya* leaf extract. However, a dose up to 0.25 g to 0.5 g/Kg body weight is considered safe. Common side effects are rash, itching, pain abdomen, nausea and vomiting, but no signs and symptoms of toxicity and overdose has been reported.<sup>12-14</sup> Rescue plan for management of adverse effects was withdrawal of drug and symptomatic management.

## Results

The present study was carried out on 400 subjects out of which 200 (male 131 and female 69) were given papaya leaf extract capsule considered as study group and other 200 (male 144 and female 56) constituting the control group, showed that there was a faster and higher increase in platelet count in study group as compared to controls. No any side effect of papaya leaf extract capsule was observed. On first day, platelet count of study group was 59.82±18.63 thousands, 95% CI ± 2.58 and platelet count of control group was 61.06±20.03 thousands, 95% CI ± 2.78 (p value 0.36). On 2nd day, platelet count of both study group and control group was 61.67±19.46 thousands, 95% CI ± 2.7 and 59.93±19.52 thousands, 95% CI

± 2.71 respectively, difference was not significant (p value 0.20) but on 3rd day platelet count of study group 82.96±16.72 thousands, 95% CI ± 2.32 was significantly higher than control group 66.45±17.36 thousands, 95% CI ± 2.41 (p value <0.01). On 4th and 5th day, platelet count of study group (122.43±19.36 thousands, 95% CI ± 2.68 and 112.47±17.49 thousands, 95% CI ± 2.42 respectively) was also significantly higher than the control group (88.75±21.65 thousands, 95% CI ± 3 and 102.59±19.35 thousands, 95% CI ± 2.68) (p value < 0.01). On 7th day, platelet count of study group and control group was not significantly different (124.47±12.35 thousands, 95% CI ± 1.71 and 122.46±19.76 thousands, 95% CI ± 2.74 respectively, p value 0.08). This indicates that *Carica papaya* leaves extract tends to fasten the natural course of rate of platelet rise in dengue fever (Figure 1).

The average hospitalization period in control group was 5.42 ± 0.98 days, 95% CI ± 0.14 while, in the study group it was 3.65 ± 0.97 days, 95% CI ± 0.13. Statistical analysis showed significant difference (p value <0.01) (Table 1).

We followed platelet transfusion criteria of; platelet count <20000/mm<sup>3</sup>, bleeding tendency and platelet count <30000/mm<sup>3</sup> with decreasing trend. Total number of patients required platelet transfusion was 148. Out of total 200 study patients only 55 (27.5%)

**Table 1: Average hospitalization period (days) of both groups**

Admission day	Study group (n)	Control group (n)
1	0	0
2	18	2
3	71	3
4	82	23
5	70	53
6	8	80
7	2	21
After 7 days	2	18

**Mean hospitalization days in both groups**

Study group	Control group	P value
3.65±0.97	5.42±0.98	<0.01

**Table 2: Platelet transfusion in both groups**

Units of platelets	Study group	Control group
0	145	107
1	11	15
2	21	38
3	13	26
4	6	7
5	3	3
6	1	4

**Average platelet transfusion (units/patient) in both groups**

Study group	Control group	P value
0.685	1.19	<0.01

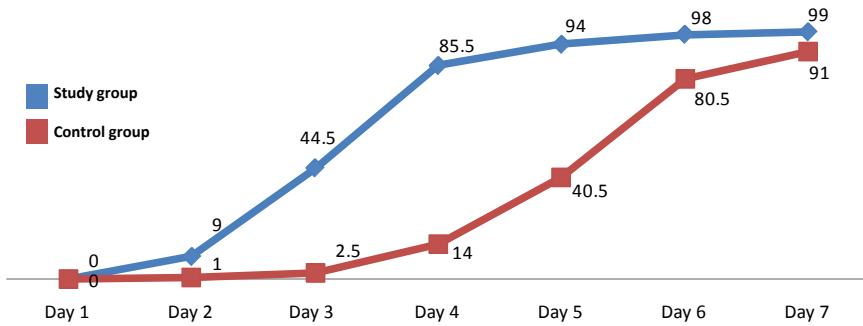
required platelet transfusion while in control group 93 patients (46.5%) required platelet transfusion. It was seen that average platelet transfusion requirement of study group (0.685 units/patient) was significantly less compared to control group (1.19 units/patient) (p<0.01) (Table 2).

As per discharge criteria considering; platelet count more than 1.5 lakhs and asymptomatic, it was seen that more than 94% patients were discharged on day 4th in study group while on the same day only 40.5% patients were discharged in control group.

On 7th day 90% of control group patients were discharged as compared to 99% discharged in study group (Figure 2).

## Discussion

Thrombocytopenia is a characteristic feature of dengue fever. Dengue virus induces bone



**Fig. 2: Summation of percentage of discharged patients**

marrow suppression; it can bind to human platelets in presence of virus specific antibody and immune mediated clearance of platelets, spontaneous aggregation of platelets to vascular endothelial cells pre-infected by virus inducing aggregation, lysis and platelet destruction. Anti-platelet antibody generated after dengue virus infection causes destruction of platelets.<sup>15</sup>

Schexneider KI, et al reviewed the pathophysiology of thrombocytopenia and clinical bleeding in dengue across the spectrum of disease. The study found that platelet counts do not correlate well with clinical bleeding. Although serious bleeding is rare, patients should be monitored closely for hemorrhagic manifestations and thrombocytopenia. Symptomatic thrombocytopenia may require platelet transfusion.<sup>16</sup>

Funahara Y, et al have done an in-vitro study and showed that: 1. Dengue virus antigen are attached to human platelets without immune-mediated reaction, 2. A decrease in platelet count was more markedly demonstrated by the binding of anti-dengue virus antibody on the dengue virus antigen associated with platelets than by the binding of the antigen-antibody complex on platelets, 3. A modulation of endothelial cell by the infection of dengue virus to the cell was suggested as one of the causes of thrombocytopenia.<sup>17</sup>

*Carica papaya* fruit juice

and leaf extracts are known to have many beneficial medical properties. Recent reports have claimed possible beneficial effects of *Carica papaya* leaf juice in treating patients with dengue viral infections.

A study at University of Colombo, Sri Lanka, to evaluate the membrane stabilization potential of *Carica papaya* leaf extracts using an in-vitro haemolytic assay, showed a significant inhibition of haemolysis in vitro and could have a potential therapeutic effect on disease processes causing destabilization of biological membranes.

Ahmad, et al described the potential of *Carica papaya* leaves extract against dengue fever in 45 years old patient, before the extract administration the blood sample from patient was analyzed. Platelets count (PLT), White Blood Cells (WBC) and Neutrophils (NEUT) decreased from  $176 \times 10^3/\mu\text{L}$ ,  $8.10 \times 10^3/\mu\text{L}$ , 84.0% to  $55 \times 10^3/\mu\text{L}$ ,  $3.7 \times 10^3/\mu\text{L}$ , 46.0%. Subsequently, the blood sample was rechecked after the administration of leaves extract. It was observed that the platelet count increased from  $55 \times 10^3/\mu\text{L}$  to  $168 \times 10^3/\mu\text{L}$ , WBC from  $3.7 \times 10^3/\mu\text{L}$  to  $7.7 \times 10^3/\mu\text{L}$  and Neutrophils from 46.0% to 78.3%.<sup>18</sup>

Chandi et al have done an experimental study. Total 36 mice were used for the trial. Fresh *Carica papaya* leaf extract [0.2 mL (2 g)/mouse] was given only to the test group (18 mice) and found that fresh *Carica papaya* leaf extract significantly increased the platelet

and RBC counts in the test group as compared to control.<sup>19</sup>

S Hettige have done a pilot study on salutary effects of *Carica papaya* leaf extract in dengue fever patients; study has shown the effects of papaya leaf juice in dengue patients of elevating the total white cell counts, platelet counts and recovery without hospital admission.<sup>20</sup>

A similar study on effect of *Carica papaya* leaf extract capsule on platelet count and hematocrit level in Indonesia by Fenny et al showed that *carica papaya* leaf extract capsule had significant increase in platelet count maintain stability of hematocrit in the normal level and shorten the hospitalization period in dengue patient.<sup>21</sup>

A study for evaluation of platelet augmentation activity of *Carica papaya* leaf aqueous extract in mice with cyclophosphamide induced thrombocytopenia by Patil et al showed significant increase in platelet count and decreasing clotting time.<sup>22</sup>

Results of our study were similar to the mentioned studies. It may be hypothesised that many active components of papaya carica extract such as papain, chymopapain, cystatin, tocopherol, ascorbic acid, flavonoids, cyanogenic-glucosides and glucosinolates inhibit immune mediated platelet destruction, bone marrow suppression by virus and stabilise the membrane of infected cells in dengue fever, so fasten the natural course of recovery with increasing the platelet count and prevents the complication of thrombocytopenia without any side effects.

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