

A Pilot Study to Evaluate the Effectiveness of *Carica Papaya* Leaf Extract in Increasing the Platelet Count in Cases of Dengue with Thrombocytopenia

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Abstract

The pilot study was conducted to investigate the platelet increasing property of *Carica papaya* leaf extract (CPLE) in patients with dengue fever (DF). An open labeled randomized controlled trial was carried out at two centres of Bangalore metropolis on 30 subjects in patients with thrombocytopenia associated with dengue.

The subjects were randomized into two groups, as control and intervention group. Both the groups were managed by the standard management guidelines for dengue except steroid administration. In addition to this the intervention group received CPLE tablet three times daily for five days. All of them were followed daily with platelet monitoring. The results showed that CPLE had significant increase in the platelet count ($p < 0.003$) over the therapy duration, in dengue fever patients, reiterating that it accelerates the increase in platelet count compared to the control group. There were few adverse events related to GI disturbance like nausea and vomiting which were similar in both groups. Thus this study concluded that *Carica papaya* leaf extract (CPLE) does significantly increase the platelet count in patients with thrombocytopenia associated

with dengue with fewer side effects and good tolerability.

Keywords

carica papaya leaf extract, thrombocytopenia, increased platelets, dengue

Introduction

Dengue:

Dengue is an acute viral infection with potential fatal complications. Dengue viruses (DV) belong to family Flaviviridae and there are four serotypes of the virus referred to as DV-1, DV-2, DV-3 and DV-4. DV is a positive-stranded encapsulated RNA virus and is composed of three structural protein genes, which encode the nucleocapsid or core (C) protein, a membrane-associated (M) protein, an enveloped (E) glycoprotein and seven non-structural (NS) proteins. It is transmitted mainly by *Aedes aegypti* mosquito and also by *Ae. Albopictus*¹.

All four serotypes can cause full spectrum of disease from a subclinical infection to a mild self limiting disease, the dengue fever (DF) and a severe disease that may be

fatal, the dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS). The 1997 WHO classification divided dengue into undifferentiated fever, dengue fever (DF), and dengue haemorrhagic fever (DHF)¹. Four main characteristic manifestations of dengue illness are (i) continuous high fever lasting 2-7 days; (ii) haemorrhagic tendency as shown by a positive tourniquet test, petechiae or epistaxis; (iii) thrombocytopenia (platelet count $<100 \times 10^9/l$); and (iv) evidence of plasma leakage manifested by haemoconcentration (an increase in haematocrit 20% above average for age, sex and population), pleural effusion and ascites, etc².

An incubation period varying from 3 to 14 days is followed by a febrile illness consisting of sudden-onset fever, headache, myalgia, arthralgia and rash. Thrombocytopenia is a common feature of the illness⁶. The patient develops hemorrhagic manifestations such as petechiae and bleeding through the nose, gastrointestinal tract and gums. After the incubation period, the illness begins abruptly and, in patients with moderate to severe disease, is followed by three phases—febrile, critical and recovery (Fig. 1). Due to its dynamic nature, the severity of the disease will usually only be apparent around defervescence i.e during the transition of the febrile to the afebrile phase, which often coincides with the onset of the critical phase.

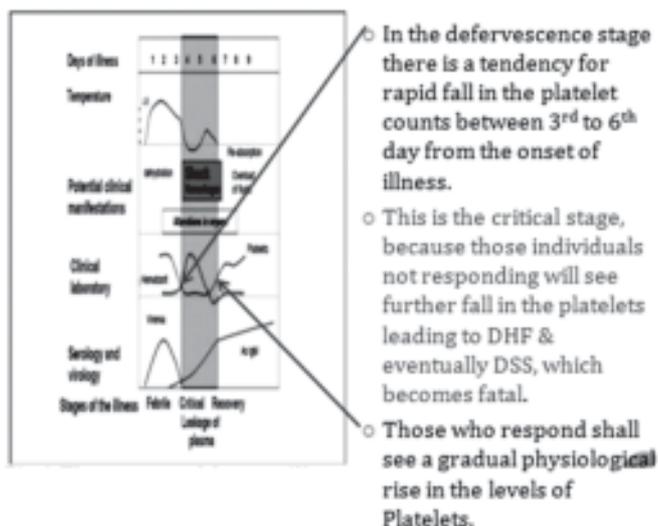


Fig. 1

The course of dengue illness

IgM = immunoglobulin M, IgG = immunoglobulin G,

Temperature in degree celcius (°C)

Source : Adapted from. 1980(2)authors.

Deaths due to dengue are usually a consequence of patients developing complications like dengue hemorrhagic fever and dengue shock syndrome⁸. Dengue hemorrhagic fever, if untreated, has a mortality rate of 10-20%. It occurs due to progression of thrombocytopenia and development of increased vascular permeability and plasma leakage. It progresses to dengue shock syndrome, which is again associated with high mortality⁶.

Primary infection is thought to induce lifelong protective immunity to the infecting serotype⁹. Individuals suffering an infection are protected from clinical illness with a different serotype within 2-3 months of the primary infection but with no long-term cross-protective immunity.

Current knowledge about the physiopathology of DHF suggests amplification of the immune response due to the presence of heterotypic antibodies against a serotype of the dengue virus at the time of new infection^{10, 11} as an explanation for the higher frequency of DHF in repeat dengue infections^{12, 13}.

Individual risk factors determine the severity of disease and include secondary infection, age, ethnicity and possibly chronic diseases (bronchial asthma, sickle cell anaemia and diabetes mellitus). Young children in particular may be less able than adults to compensate for capillary leakage and are consequently at greater risk of dengue shock².

Treatment for dengue is usually symptomatic. Some cases require platelet transfusions and fluid management⁵. One of the most disturbing aspects of the problem of dengue is that there are no effective antiviral agents available to treat dengue complications. Though symptomatic treatment works in most mild cases, some cases progress to complications very fast and this often make it difficult to save the life of the patient.

Problem statement:

The incidence of dengue has grown dramatically around the world in recent decades. Over 2.5 billion people – over 40% of the world's population – are now at risk from dengue. WHO currently estimates there may be 50–100 million dengue infections and half a million DHF worldwide every year, with an average case fatality rate of around 5 %².

Today endemic in over 125 countries and various studies estimated that it infects nearly 50-270 million people every

year, resulting in a sizable number of deaths³. In fact, dengue appears to be overtaking malaria in terms of morbidity and economic impact of the disease.[4] Unfortunately, due to lack of adequate surveillance systems in the underdeveloped and developing countries, the exact extent of the problem is not known. Travelers from non-endemic areas to the dengue-affected areas are also exposed to the possibility of infection⁵. This makes it an international public health concern, affecting individuals from countries even where the disease is not prevalent.

The epidemiology of dengue fevers in the Indian subcontinent has been very complex and has substantially changed over almost past six decades in terms of prevalent strains, affected geographical locations and severity of disease. The average total economic burden was estimated to be US\$27.4 million (US\$25.7—29.1 million)^{1,23}.

Dengue and Thrombocytopenia:

The most common virus responsible for dengue is DV-2 (dengue virus-2). DV-2 inhibits *in vitro* megakaryopoiesis and induces apoptotic cell death in a sub-population of early megakaryocytic progenitors which may contribute to thrombocytopenia in dengue disease¹⁴.

In another study it was shown that DV-2 may directly interact with and activate platelets and thus may be responsible for thrombocytopenia. Significant ultra structural changes in DV infected cells especially endo-membrane re-organization and formation of auto-phagosomes have been shown using whole mount transmission electron microscopy. These changes, taken together with a later study, that showed marked elongation of endothelial cell processes after transfection with the DV-E protein, provided early insights that the replication biology of the virus is coupled closely with the host cell physiology¹⁵.

Such molecular mimicry occurs between platelets/ endothelial cells and dengue virus antigens. Platelets and endothelial cells are bound by the cross-reactive anti-dengue virus antibodies such as anti-NS1 or anti-prM antibodies²².

Postulated Mechanisms of Thrombocytopenia in Dengue:

Two mechanisms have been suggested that could be responsible for dengue-induced thrombocytopenia- impaired thrombopoiesis and peripheral platelet destruction. In

support of the theory of impaired thrombopoiesis studies have suggested reduced megakaryopoiesis at the onset of infection, which is normal at the time of clinical recovery¹⁶. This effect could be due to a direct effect of the virus on the megakaryocytes, or an effect on the stromal cells which are responsible for the release of cytokines and control of megakaryopoiesis. Studies have also indicated altered proliferative capacity, inhibition of differentiation and megakaryocytic progenitor apoptosis as possible mechanisms of thrombocytopenia.

The other main mechanism proposed for thrombocytopenia is the increased peripheral platelet destruction by the DENV. This could be due to an autoimmune reaction, where antibodies produced by the host against the DENV bring about activation and destruction of platelets¹⁷. Platelets may also show an increased reaction with leucocytes and endothelial cells, leading to their destruction^{18,19}. Platelet dysfunction due to abnormal activation and inhibition of platelet aggregation in dengue patients may also be responsible for the destruction^{20,21}. Recent studies indicate a direct infection of the platelets by the DENV. Increased levels of mediators like tumor necrosis factor- α and interleukin-1 β were associated with thrombocytopenia⁷.

Currently available treatment modalities:

Treatment is guided by etiology and disease severity. The standard treatment protocol for management of Dengue includes symptomatic treatment with fluid management. The thrombocytopenia is not addressed till it gets lowered down to levels less than 20000 / μ l, where platelet transfusion is advocated.

Corticosteroid is advised by some which is supposed to halt further platelet destruction; however, not all prefer. The immunosuppressant effect of corticosteroids might mask the severity of the underlying condition and increase the viremic load by virtue of its immunosuppressive property.

TPO agonists and mimetics like Eltrombopag and Romiplostim are available for increasing the platelet counts however cost and accessibility factors would hamper larger proportion of people from availing them and also they are associated with adverse effects.

There is no specific or anti viral treatment available at this point of time. The dengue vaccine is being developed

and several clinical trials are ongoing, which gives a ray of hope. Alternatively other options need to be explored to tackle the nemesis by dengue.

Therefore in the current lieu, considerations for alternate therapies to combat the low platelet count, which is relatively free from the toxic side effects of the drugs, should be given^{24,25}.

The evolution of *Carica Papaya* Leaf Extract (Caripill) in the management of thrombocytopenia associated with dengue is significant as it would be-

- Better & viable option in fever associated with thrombocytopenia.
- Palatable and appropriately formulated.
- Fewer side effects.
- Decreases the cost of hospitalization.
- Cost effective.
- More affordable and accessible.
- Averting the mortalities.

Literature search has found several human and animal studies been conducted where extract of carica papaya leaf was used for treating thrombocytopenia associated with dengue. The results of these studies have been encouraging with platelets showing significant rising trend.

To evaluate whether Papaya leaf extract can be used to treat thrombocytopenia associated with Dengue: a pilot study was conducted to assess this as A Novel Therapeutic Option?

Materials and Methods

A multi-centric open labeled, randomized, comparative pilot study was conducted at two centres of Bengaluru metropolis.

The carica papaya leaf extract was formulated in appropriate dosage form of tablet in the strength of 1100mg.

A total of 30 subjects (n=30) diagnosed as dengue cases by NS1 antigen test were enrolled and randomized in this study. Exclusion and Inclusion criteria were followed and those meeting all the inclusion criteria's were allowed to participate.

Of the total subjects 14 were randomized to study group where in addition to the routine standard supportive management of dengue the investigational drug *Carica papaya* leaf extract 1100mg tid for 5 days was administered. The remaining 16 in control group received the routine standard supportive management of dengue, only. All the subjects were followed up every day for five days and their platelet counts were monitored daily.

Inclusion Criteria:

- a) Is the subject aged between 18 and 60 years?
- b) Is the patient's diagnosis confirmed as DF or DHF Grade I ,Grade 2
- c) Patients platelet count is below 100,000 and above 30,000 per μ L
- d) Is ALT/SGPT level less than 165 U/L
- e) Has the subject willingly given written informed consent?

Exclusion Criteria:

- a) Patient diagnosed with DHF Grade 3 or 4
- b) Platelet levels are less than 30,000 per μ L
- c) Patient is either pregnant or lactating
- d) Has received blood products or blood transfusion during the current hospital stay Or during last one month
- e) Is the patient diagnosed with ITP, Leukaemia or Hemophilia
- f) Serum Creatinine is more than 1.4 mg/dl (if female) or 1.5mg/dl (if male)
- g) Patient has participated in another trial within past one month
- h) The investigator can exclude patient at his/her discretion depending upon the condition of the patient.

Diagnosis of DF or DHF:

A clinical diagnosis of DF and DHF was made by the clinician based upon the patient's presentation and blood

investigations. A rapid dengue test (NS1 Ag) was used to confirm the dengue case.

Treatment of the Subjects:

Once, current dengue infection was confirmed, a baseline investigation of platelet count was done. Patients in the intervention group received 1100 mg t.i.d of *Carica Papaya* leaf extract tablet for 5 days, in addition to receiving the standard management of Dengue. The controls received the standard management only. The platelets of both the groups were monitored daily.

Results

All the 30 subjects enrolled completed the study. These subjects were diagnosed as dengue cases by NS1 antigen test. After administering the tablet *Carica papaya* leaf extract (Caripill) to the study group (n=14) every day platelets of both the groups were monitored.

Demographic Characteristic:

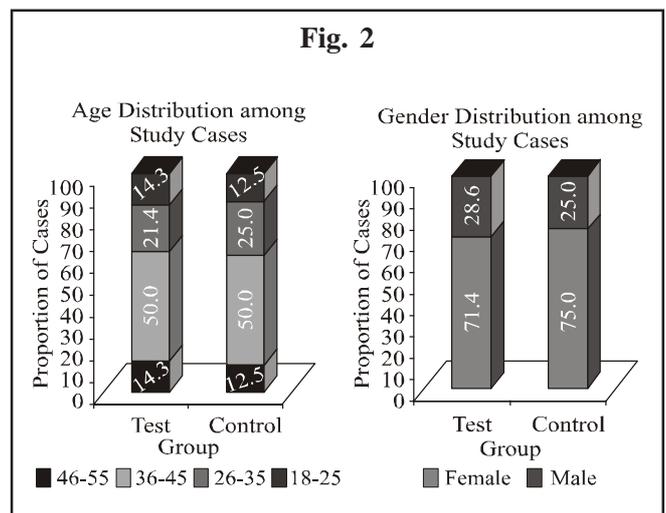
Distribution of sex of the subjects in the two groups did not show any difference, men were more than women, most subjects infected are in the age of 26-35 years old followed by 36-45 years old (Table 1). This might be related with the higher exposure of subjects to the dengue vector in the working place or during travelling periods.

Variables	Subjects (%) n=30
Sex :	
Male	22 (73.34)
Female	08 (26.66)
Age (years):	
18 - 25	04 (13.33)
26 - 35	15 (50.00)
36 - 45	07 (23.34)
46 – 55	04 (13.33)

Above data states that, 71.4% of cases among Test group were of the age 26 – 45 years which was comparable to 75.0% of cases among Control Group and the difference was not significant.

Age Groups (Yrs)	Test		Control	
	No.	%	No.	%
18 - 25	02	14.3	02	12.5
26 - 35	07	50.0	08	50.0
36 - 45	03	21.4	04	25.0
46 – 55	02	14.3	02	12.5
Total	14	100.0	16	100.0

By Chi- Square Test p > 0.05, Not Significant

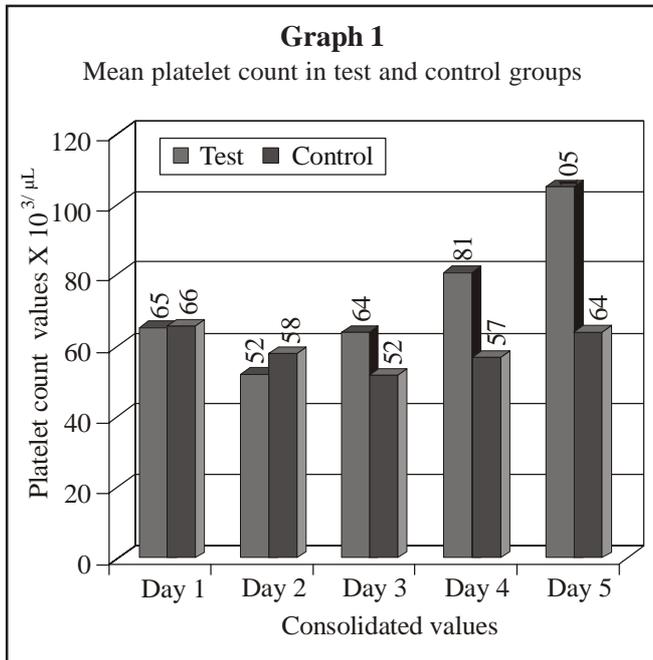


Above data reveals that, 71.4% of cases among Test group were male which was comparable to 75.0% of males cases among Control Group and the difference was not significant.

The monitoring of platelet count clearly indicated that the change in the Platelet count is faster in the intervention

Gender	Test		Control	
	No.	%	No.	%
Male	10	71.4	12	75.0
Female	04	28.6	04	25.0

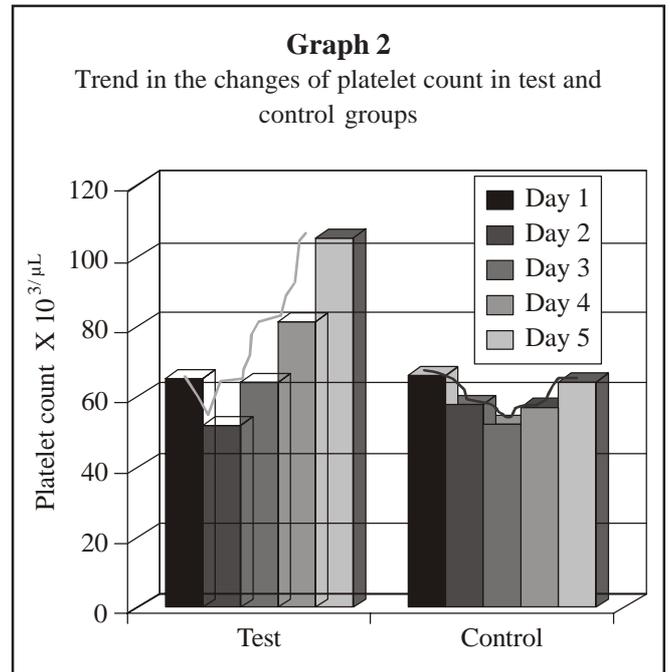
By Chi- Square Test p > 0.05, Not Significant



group as compared to the control group (**Graph 1**).

The **Graph 2** below clearly indicated a rising trend (steep) in the platelets of the study group, whereas in the control group there was a gradual rise (shallow) on 4th and 5th day.

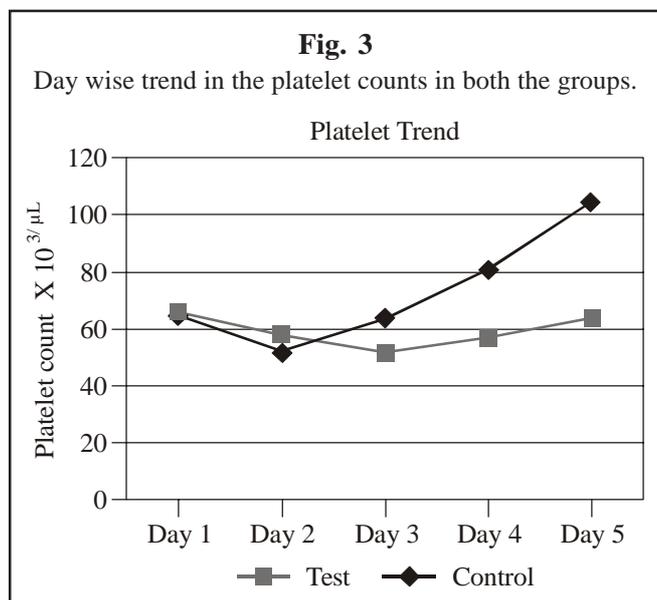
In the study group there was a significant increase in the platelet count after 2nd day onwards which was very much evident at the end of 5th day ($p < 0.003$).



The trend line is 'J' shaped where 2nd day onwards a steep rise was observed in the control group. When compared to the control group rise which showed as shallow rise increasing gradually only on 4th and 5th day.

- Above data reveals that at baseline, mean Platelet Count was 64.79 among test group, which was comparable with 65.94 among control group and the difference was not statistically significant.

Comparison of changes in mean platelet count between the groups:			
Duration	Mean Platelet Count (X ± SD)		
	Test (N = 14)	Control (N = 16)	Comparison (p value)
Day 1	64.79 ± 20.86	65.94 ± 17.79	0.956 (NS)
Day 2	51.86 ± 18.19	58.19 ± 16.19	
Day 3	64.14 ± 16.27	53.69 ± 19.02	
Day 4	80.71 ± 20.12	58.69 ± 19.86	
Day 5	104.71 ± 30.57	66.63 ± 22.49	
Difference (Day 1 – 5) (p value)	*39.92 ± 38.51 (0.002)	00.69 ± 24.75 (0.913) NS	*0.003
By Student t Test *Significant NS = Not Significant			



- After the treatment at the end of Day 5, mean Platelet Count showed a significant increase of 61.6% in Test group and an insignificant rise of 1.0% in Control group from baseline. If compared change was more in Test group than Control group and the difference was statistically significant.

This line **Graph 3** indicates rise in the platelet count at the point of defervescence which is supposed to be critical. The wide gap implies a significant rise in the study group as compared to the control group.

Discussion

From the various reports published in scientific literature, it appears that *C. papaya L.* leaf extract does have beneficial properties in dengue. It has been shown to bring about a rapid increase in platelet count.

It is shown that the subjects in the intervention group that received CPLE (Caripill) can reach faster and higher increase in platelet count compared to the control group.

This result are similar to those reported by Sathasivam et al. (2009) that CP leaves extract can increase platelet count in mice, and also in dengue fever patient as reported by Ahmad et al. (2011), Hettige S. (2008), Yunita F (2012) and soobitha subenthiran (2013).

There were few adverse events reported related to GI disturbances like nausea, vomiting which were similar in

both the groups and not related to drugs displaying the tolerability and safety of CPLE (Caripill).

The finding of this pilot study corroborates the claim that the *Carica Papaya* leaf extract tablet consumption during the course of dengue infection has the potential to induce the rapid production of platelets. This was clearly demonstrated by the significant increase in the mean platelet count.

Conclusion

Papaya extract no doubt offers a cheap and possibly effective treatment for dengue.

Various clinical and preclinical studies conducted have demonstrated a positive effect in dengue cases with thrombocytopenia. The current pilot study also demonstrates the same positive beneficial trend in increasing the platelets significantly. However, Large scale randomized clinical trials are necessary to further establish its pivotal role in the management of dengue.

Limitations

Since this was a pilot study to assess the effectiveness of *Carica papaya* leaf extract on platelets, other parameters were not monitored.

It being a pilot study the sample size may not be representative and larger studies should be conducted for representation purpose.

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