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A randomized, open-label, controlled, parallel-group comparative study of two varieties of *Carica papaya* leaves in dengue-induced thrombocytopenia

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ABSTRACT

Background: Dengue-induced thrombocytopenia can be life-threatening. However, supportive and symptomatic treatment is the current management protocol. The leaf extract of papaya has shown promising results in improving the platelet count. There are many varieties of *Carica papaya* such as Washington, Honeydew, Coorg honeydew, Solo, Surya, Red lady, CO-1, CO-3, CO-5, CO-6. **Aims and Objectives:** The aim is to evaluate the efficacy and safety of two varieties ("Red Lady" and "Solo") of *C. papaya* fresh leaf extract in patients diagnosed with dengue-induced thrombocytopenia. **Materials and Methods:** The study was conducted for 2 months, and 18 dengue-confirmed inpatients with thrombocytopenia (platelet count <1,50,000/μL) were enrolled. The study participants were grouped into the standard "Control" group, "Solo" group who received fresh papaya leaf extract "Solo" and "Red Lady" group who received fresh papaya leaf extract of "Red Lady." About 30 mL of leaf extract was given thrice daily for 5 days. **Results:** The mean change from the baseline value of platelets was found to be 59,168/μL for the control group and 57,000/μL for Red Lady, and 93,167/μL for the solo group. The study showed a positive trend with improvement in platelet count in "Solo" Variety group when compared to "Red Lady" group and the control group. The safety results showed that both the papaya leaf extracts have a favorable safety profile. **Conclusion:** There was an improvement in platelet count in "Solo" variety group when compared to "Red Lady" and the control group. However, the results were not statistically significant. No adverse events were observed during the study.

Key words: *Carica papaya*; Dengue fever; Thrombocytopenia; Platelets

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INTRODUCTION

Dengue is one of the highest and most rapidly spreading vector-borne viral diseases with high mortality rate.[1] Southeast Asia is the region that contributes to more than half of the global burden of dengue, and India is one among the 30 most highly endemic countries in the world. From 2015 to 2019, dengue cases in Southeast Asian region increased by 46% (from 451,442 to 658,301).[2]

Thrombocytopenia is common in dengue and can occur with vascular leakage, shock, and organ impairment, which can result in death.[3] The most common reason for thrombocytopenia is poor production of platelets by the bone marrow, minimal survival of platelets, and sequestration of platelets in the spleen and liver.[4] However, for such a dreadful disease, supportive and symptomatic treatment is the current management protocol.[5,6] Because of highly mutant serotypes of the flavi virus, the

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development and approval of a specific drug are still on the horizon.[7-9] Dengue vaccine development has still been a challenging task due to the existence of four antigenically distinct dengue virus serotypes, each capable of eliciting cross-reactive and disease-enhancing antibody response against the remaining three serotypes.[10] The live-attenuated chimeric yellow fever/tetravalent dengue vaccine has been licensed in several countries but has shown low efficacy in children and dengue-naïve individuals.[11]

Many studies have been conducted to unearth other effective therapeutic agents for dengue-induced thrombocytopenia. The leaves of *Carica papaya* or papaya pear, which is widely grown in the tropical and subtropical regions, are traditionally used and evaluated in multiple studies in dengue and the results have been found to be promising.[12-14] *C. papaya* leaf extract, when proven effective and made part of disease management protocol, is a valuable incorporation to the existing symptomatic management protocol since it is economical, easily grown, and abundantly available in India and other Southeast Asian Regions.

Papaya belongs to the family "*Caricaceae*". The leaves of papaya have been shown to contain many active components such as papain, carpaine, benzyl glucosinolates, latex, papain, zeaxanthin, choline, vitamins A, B, C, E, and K, folate, pantothenic acid, zeaxanthin, lycopene, lutein, magnesium, copper, calcium, and potassium.[15,16] The papaya leaf extract has been related to anti-inflammatory and antiviral activity.[17] There are wide varieties of *C. papaya*. Few of the varieties which are grown in India are Washington, Honeydew, Coorg honeydew, Solo, Surya, Red lady, C0-1, CO-3, CO-5, C0-6.[18,19]

This study compared and evaluated the efficacy and safety of two of these many varieties of *C. papaya* fresh leaf extract, i.e., "Red Lady" and "Solo" which are available locally with the standard control group. As per our knowledge, this study is the first of its kind to compare the efficacy and safety of different varieties of papaya fresh leaf extract in dengue-induced thrombocytopenia.

Aims and objectives

The aim is to evaluate the efficacy and safety of two varieties of ('Red Lady' and 'Solo') of C. Papaya fresh leaf extract in patients diagnosed with dengue-induced thrombocytopenia.

MATERIALS AND METHODS

The study was designed as an open-label, controlled, parallel-group comparative study assessing the efficacy and safety of two different varieties of *C. papaya* leaves in patients with dengue-induced thrombocytopenia. The study protocol was reviewed and approved by the Institutional Ethics Committee (dated: 28 May, 2018). The protocol was also submitted to the Indian Council of medical research under Short Term Studentship Programme 2018 and was accepted. Written informed consent in agreement with the Declaration of Helsinki was obtained from all enrolled individuals. The study was duration-based study and was conducted for 2 months. The patients were recruited from July 1, 2018, and September 1, 2018.

Inclusion and exclusion criteria

Patients who were willing to participate and comply with the procedures of the study, male, non-pregnant, non-lactating female patients between the age of 18–60 years, confirmed to have Dengue fever (Positivity of dengue NS1 antigen and/or presence of serum IgM/IgG) with a platelet count of <1,50,000/μL, patients with a baseline alanine transaminase (ALT) level of not more than three times of the upper limit of the normal range (not more than 165U/L) were included in the study.

Patients who were diagnosed with Dengue hemorrhagic fever, patients with a platelet count <20000/mL, thrombocytopenia with active bleeding, patients who have received blood or blood product transfusion during the current illness, Patients with idiopathic thrombocytopenic purpura, Leukemia, Hemophilia or any other bleeding diathesis, Other cases of fever with thrombocytopenia like malaria, brucellosis, leptospira, and enteric fever and who have developed Hepatitis with a serum ALT level 3 times higher than the upper limit of the normal range (>165 U/L), impaired renal function with serum creatinine >1.5 mg/dL (males) and >1.4 mg/dL (females) and patients with previous history of allergy to papaya were excluded from the study.

After screening, baseline tests – complete blood count, platelet count, hematocrit, Liver function tests, Renal function tests were performed. The study participants were grouped into "Control" group who received Routine supportive treatment, "Solo" group received fresh papaya leaf extract of the variety "Solo" along with routine supportive treatment, and "Red Lady" group received fresh papaya leaf extract of the variety "Red Lady" along with routine supportive treatment. After screening and enrolment, patients were given freshly extracted papaya leaf Juice 30 mL thrice daily for 5 days. The vital signs, platelet count, and hematocrit were monitored daily. The patients were closely monitored for any adverse effects. After discharge, patients were followed up on the 10th day.

The fresh papaya leaves were sourced from the farmers in the locality with support from the Horticulture department.

Two different farmers provided csupply of Red lady and Solo papaya leaves. Figure 1 shows two varieties of papaya trees from which the leaves were obtained.

Method of fresh papaya leaf extraction followed

Fresh, healthy mature papaya leaves from a fruit-bearing tree were obtained on a daily basis.

The leaves were washed thoroughly with running tap water and were chopped into small pieces, excluding the main stem (the small stems in the leaves were not removed). 50 g of papaya leaves were weighed. 50 mL of boiled and cooled water and 25 g of sugar were added. The above mixture was hand-pounded well for 15 min till uniform pulp was made with a mortar and pestle. This pulp was mixed well and kept for about 30 min. It was squeezed by a clean hand to get the papaya leaf extract (No sieve was used to extract the juice). Whenever necessary, the preparation was stored for not more than 24 h in the lower compartment of the refrigerator (+4°C). The bottle was shaken well before the preparation was to be given to the patient. The treatment groups were given papaya leaf extract of the respective variety – 30 mL 3 times a day before meals. Few sips of cold water was allowed to be taken immediately after the Papaya leaf extract, to overcome the bitter taste.[20] Along with three bottles, an additional bottle of papaya leaf extract was dispensed to avoid discontinuity in case of accidental spillage. 50 g packet of sugar was dispensed every time for the patient to consume in case of nausea due to bitterness of the extract.

An increase in the platelet count from baseline level to the end of therapy was considered as the primary outcome. Change in the hematocrit levels from the baseline levels to the end of therapy and comparison of incidence and rate of adverse events that occurred between the two arms to evaluate the safety and tolerability were considered secondary outcomes for the study.

Figure 1: Papaya trees – two varieties used in the study

By using gender as a stratifying variable, stratified randomization was initially planned to allocate patients into three groups. However among 18 patients enrolled, all were male except one. Hence, stratified randomization with gender as a variable was not performed.

RESULTS AND DISCUSSION

A total of 21 patients admitted as inpatients in the Department of General Medicine at Akash Institute of medical sciences and a research center were screened. Two patients were considered screen failures and the eligible 18 patient participants were randomly assigned to one of the three groups. One patient had active gastrointestinal bleeding; another patient had elevated liver enzyme levels. The third patient was excluded due to an associated severe lower respiratory tract infection. All the patients enrolled in the study were of Indian origin.

Table 1 provides the summary of age and gender of the patients. Patients age ranged between 21 and 59 years. The mean of the age for the Red Lady, Solo and control group were 48, 41.33 and 42.3 years respectively. Both the treatment groups and control groups were comparable with respect to the characteristics measured at baseline. No significant past medical and surgical history observed. The concomitant medications received were similar amongst the patients enrolled. The papaya therapy groups and control group were similar with respect to vital signs and physical examination at baseline and Day 5.

Platelet count and hematocrit data were taken from day 1, day 2, day 3, day 4 and day 5. Platelet count of day 1 and day 5 were considered as endpoints to compare the treatment groups. Since the data follows the normal distribution, an Independent student t-test is used for statistical analysis.

Table 2 provides a summary and analysis of change from baseline in mean platelet count. The mean platelet count

Table 1: Mean age and gender distribution

Study group	n	Mean age (years)	Gender	
			Male	Female
Red lady	6	48.0	6	0
Solo	6	41.33	5	1
Control	6	42.3	6	0

Table 2: Mean platelet count (in thousands/µL)

Study group/ Days of intervention	Day 1	Day 2	Day 3	Day 4	Day 5
Control	90833	101000	110666	124333	150000
Red Lady	87166	97500	105666	116166	144166
Solo	64833	80333	102666	121500	158000

for the groups Red Lady, Solo and Control at the baseline was 87166, 64833 and 90833 which was comparable. When compared to baseline, On Day 5, there was an increase in the mean platelet count in all three groups. However, the improvement observed in "Solo" variety group was apparent as the mean platelet count at the baseline was the least for "Solo" group.

Graph 1 provides the schematic representation of change from baseline in mean platelet count, which is consistent with the descriptive results shown in Table 3. It shows linear and greater increase of platelets in the "Solo" variety group. Whereas, in the "Red Lady" group, steady increase in platelet count is observed, but it trails below the "control" graph.

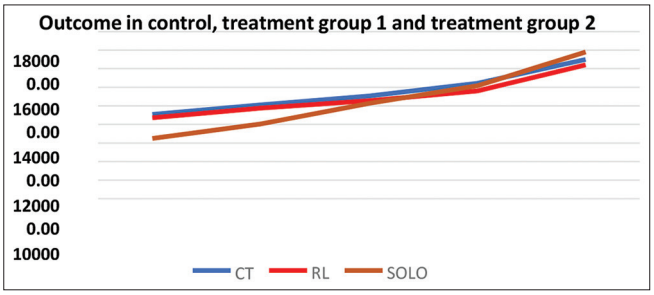
Table 3 summarizes - between the group analysis performed for the groups "Control" versus "Red lady." As the analyzing data consists of normally distributed interval-dependent variables for two independent groups "independent samples t-test" was used. The results indicate that there is no statistically significant difference between the mean platelet counts for "Control" and "Red Lady" groups.

Table 4 summarizes - between the group analysis performed for the groups "Control" versus "Solo." As the analyzing data consists of normally distributed interval-dependent variables for two independent groups "independent samples t-test" is used. The results indicate that there is a statistically significant difference between the mean platelet counts for "Control" and "Solo" groups on Day 1. (P=0.018). These results suggest that initiating the treatment with "Solo" variety at the earliest (immediately after diagnosis) would improve the outcome (Improvement in the platelet count).

Table 5 summarizes - between the group analysis performed for the groups "Red Lady" versus "Solo." As the analyzing data consists of normally distributed interval-dependent variables for two independent groups "independent samples t-test" is used. The results indicate that there is no statistically significant difference between the mean platelet counts for "Red Lady" and "Solo" groups.

To summarize, analysis performed to assess change from baseline in mean platelet count showed a linear and greater increase of platelets in the "Solo" variety group. Whereas, in the "Red Lady" group, a steady increase in platelet count is observed, but it trails below the "control" group. These results are suggestive of better efficacy of "Solo" variety over "control" and "Red lady" variety. The poorer performance of the Red lady variety hints that not all the varieties of papaya will favor improvement in dengue-induced thrombocytopenia.

Data on hematocrit were analyzed and was observed to be not significant. There were no adverse events observed



Graph 1: Change from baseline in mean platelet count

Table 3: Independent sample t-test control versus red lady

Mean platelet count	Groups	Mean	P-value
Day 1	Control	90833.33	0.777
	Red lady	87166.67	
Day 2	Control	101000.00	0.741
	Red lady	97500.00	
Day 3	Control	110666.67	0.728
	Red lady	105666.67	
Day 4	Control	124333.33	0.581
	Red lady	116166.67	
Day 5	Control	150000.00	0.659
	Red lady	144166.67	

Table 4: Independent sample t-test control versus solo

Mean platelet count	Groups	Mean	P-value
Day 1	Control	90833.33	0.018
	Solo	64833.33	
Day 2	Control	101000.00	0.102
	Solo	80333.33	
Day 3	Control	110666.67	0.674
	Solo	102666.67	
Day 4	Control	124333.33	0.880
	Solo	121500.00	
Day 5	Control	150000.00	0.576
	Solo	158000.00	

Table 5: Independent sample t-test red lady versus solo

Mean platelet count	Groups	Mean	P-value
Day 1	Control	87166.67	0.093
	Solo	64833.33	
Day 2	Control	97500.00	0.154
	Solo	80333.33	
Day 3	Control	105666.67	0.882
	Solo	102666.67	
Day 4	Control	116166.67	0.791
	Solo	121500.00	
Day 5	Control	144166.67	0.407
	Solo	158000.00	

during the study period. The patients were closely watched for any adverse events during the study period. Expected common side effects were rash, itching, pain abdomen, nausea, and vomiting. The initial rescue plan for the management of adverse effects was the withdrawal of the papaya extract and continuing with routine supportive treatment.

Limitations of the study

Restriction to a small geographical area and small sample size are the limitations of the study.

CONCLUSION

The aim of this clinical trial was to provide clinical data regarding the safety and efficacy of papaya leaf extract and the comparative efficacy of two different varieties in dengue-induced thrombocytopenia.

Sharma et al., investigated the antiviral activity of aqueous extract of *C. papaya* leaves against dengue virus and its effect on platelet augmentation by immunoblotting and flow cytometry. Papaya leaf extract significantly decreased the expression of the envelope and NS1 proteins in Virus-infected THP-1 cells. The marked decrease in intracellular viral load upon PLE treatment confirmed its antiviral activity.[21] Dharmarathna et al., investigated the potential role of fresh *C. papaya* leaf extract on hematological and biochemical parameters and toxicological changes in a murine model. Fresh papaya leaf extract (0.2 mL [2 g]/mouse) was given only to the test group (18 mice) and 18 mice acted as the control group. Moreover, the study showed that fresh *C. papaya* leaf extract significantly increased the platelet and RBC counts in the test group as compared to controls.[12] Vijeth et al., conducted a longitudinal study in 200 Dengue with thrombocytopenia patients. When followed up with daily platelet counts, there was an increase in platelet counts in the study group compared to the placebo group and on 3rd day there was a significant difference between both ($P=0.002$).[13] Fenny et al., conducted a randomized clinical trial in Indonesia with 80 subjects. These subjects were randomized into two groups, the control and intervention group who received two *C. papaya* leaf extract capsules thrice daily. *C. papaya* group showed significant increase in the platelet count ($P<0.05$), maintained stability of hematocrit in the normal level, shorten hospitalization ($P<0.05$) in dengue fever patients.[14]

The above-mentioned studies show that papaya leaf extract is effective in thrombocytopenia which is supported by the results of this study. As per the author's knowledge, the current study is first of its kind pilot study which evaluates the comparative efficacy of varieties of papaya leaf extracts in dengue-induced thrombocytopenia. The study is based on the thoughts that for commercial extraction of enzyme papaine from unripe papaya fruits, specific varieties like Co.2, Co.5 are preferred as the papaine yields are better.[22] The thromboprotecting active components in papaya leaf might also differ from one variety to another. This study shows that there is a difference in increase in the platelet counts. However, restriction to a small geographical area and low sample size are the limitations of this study. Further studies need to be conducted to evaluate the efficacy of "Solo" variety with a larger sample size to detect statistically significant differences. Furthermore, there is a need to further evaluate the efficacy of selected varieties of papaya leaf extract in dengue-induced thrombocytopenia.

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Authors Contribution:
SER- Design of the study, results interpretation, study conduct, review of literature; **VJ-** Concept, and design of the study, results interpretation, review of literature and preparing the first draft of the manuscript; **KS-** Study conduct, peer review; **SSG-** Peer review, revision of the manuscript.

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