

ORIGINAL ARTICLE

A Multi-centric, Double-blind, Placebo-controlled, Randomized, Prospective Study to Evaluate the Efficacy and Safety of *Carica papaya* Leaf Extract, as Empirical Therapy for Thrombocytopenia associated with Dengue Fever

Prabhu Nagnathappa Kasture¹, KH Nagabhushan², Arun Kumar³

Abstract

Dengue is a rapidly expanding global health problem. Approximately 2.5 billion people live in dengue-risk regions with about 100 million new cases each year worldwide. The cumulative dengue diseases burden has attained an unprecedented proportion in recent times with sharp increase in the size of human population at risk. The management of dengue virus infection is essentially supportive and symptomatic and no specific treatment is available for increasing the fallen platelets, which have a significant role in causing the mortality of dengue patient. This study was conducted to evaluate the platelet increasing efficacy of *Carica papaya* leaf extract (CPLE) in patients with dengue fever (DF).

Aim: The administration of *Carica papaya* leaf extract should significantly increase the platelet count in cases of thrombocytopenia associated with dengue, preventing the patient to go in DHF or DSS conditions.

Material and Method: A Multi-centric, Double blind, Placebo controlled, Randomized, prospective study was conducted in 300 patients across 5 centres, to evaluate the Efficacy and Safety of *Carica Papaya* Leaf Extract, as empirical therapy for thrombocytopenia associated with dengue fever.

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.

This study has been registered in the clinical trial registry–India (CTRI Registration number: CTRI/2015/05/005806)

Results: The results indicate that CPLE had significant increase ($p < 0.01$) in the platelet count over the therapy duration, in dengue fever patients, confirming CPLE 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.

Editorial Viewpoint

- There is an increasing incidence of dengue and it is a major public health issue across the globe.
- *Carica papaya* leaf extract (CPLE) was given as empirical therapy 3 times a day for 5 days.
- Few side effects like GI intolerance were observed.
- CPLE is shown to increase expression of ALOX12 and PTAFR gene responsible for platelet production.

Introduction

Dengue has been named one of the most important emerging infections in 2014. The geographic region at risk for dengue has increased fourfold over the past three decades, unprecedented for a vector-borne disease.¹ DENV is an arthropod-borne flavivirus associated with both hemorrhagic fever and hemorrhagic shock.² The classical clinical presentation of DENV is characterized by abrupt onset of headache, myalgia and high fever, in addition to

Conclusion: 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.

arthralgia, retro-orbital pain and hemorrhagic manifestations. The DENV hemorrhagic fever is characterized by fluid leakage into the interstitium. These symptoms are commonly seen in many other infectious diseases, which complicates diagnosis.

Epidemiology

2.5 billion people live in DENV-endemic regions,² and roughly 400 million infections occur per year with a case fatality rate exceeding 5-20% in some areas. Over 100 countries are affected, including Europe and the United States.

DENV is endemic in many parts of Asia Pacific regions, and the DENV case frequency and fatalities are increasing, where the total number of DENV cases reported quadrupled between the 1980s and 2000-2007. Furthermore, in recent years, infections in many LMICs are increasingly noted in adults, leading to significant number of work days lost and increasing costs to society.³

According to the World Health Organisation, primary prevention is the most effective measure in dengue prevention and control since no vaccine is currently available.³

The preventive measures include use of insecticide sprays and elimination of all mosquito breeding grounds (areas of standing water are cleared, particularly in schools). While attempts at early diagnosis paired with prevention are helpful, the combined lack of effective treatment for dengue and increasing dengue transmission are worrisome.

Pathology

Dengue symptoms usually begin 4 - 7 days after the mosquito

bite and typically last for 3 - 10 days. Infected patients deprived of medication, may develop capillary leakage near or at the end of the febrile phase which progresses to DHF (characterised by polyserositis, pleural effusion and haemoconcentration). At this stage if patients do not receive intravascular fluid resuscitations it progresses to DSS and finally death of the patients.^{4,5}

The capillary leakage is mainly due to increase in vascular endothelial cell permeability and thrombocytopenia.^{4,5} The mechanism behind the platelet reduction is not yet clear till date due to lack of suitable animal model studies.⁵ There are two mechanisms causing thrombocytopenia. DENV induced bone marrow suppression decreases the platelet synthesis and leads to thrombocytopenia.⁴ Immune-mediated clearance of platelets also causes thrombocytopenia.^{4,5} In this mechanism, anti-platelet antibodies clears the virus attached platelets via complement activation and also inhibits ADP-induced platelet aggregation.^{4,9}

Management of Dengue Fever

Currently there is no specific treatment for DENV, recent hopeful vaccine candidates have just been deemed ineffective,¹⁰ and there is no prediction of complete vector control. However, rapid diagnosis followed by targeted vector control efforts decrease DENV transmission, and early detection followed by supportive care is reported to potentially decrease mortality rates from 5-20% to less than 1%.¹¹⁻¹³

There is no specific treatment for dengue; intensive supportive care is the most important

aspect of management. The thrombocytopenia which usually happens in the defervescence stage of the illness is the critical phase, and if left unattended or untreated it can lead to mortality.

Till now there is no approved vaccine or drug against dengue virus, therefore there is an urgent need of development of alternative solutions for dengue. Several plants species have been reported with anti-dengue activity. Recently, the use of alternative medicine and the consumption of plant materials have increased in many countries in the world, mostly because plant-derived drugs and herbal formulation are commonly considered to be less toxic and less side effects than the synthetic ones.

Challenges and Obstacles

Attempts to develop an antiviral agent for dengue have met several hurdles. Dengue is caused by four distinct serotypes which often undergo mutations.¹⁴ Like in other ribonucleic acid (RNA) viruses, these mutations are due to the error-prone nature of RNA polymerase, which results in the formation of quasispecies. It is currently unclear which viral genome results in a higher viral titre.¹⁵ An antiviral would have to be effective against all the serotypes.¹⁷

A lot of hope rests on the development of effective vaccines, many of which are undergoing clinical trials.¹⁸ Besides vaccines, every other possible treatment including traditional medicines are being investigated to test their usefulness in controlling this problem.¹⁶

Several studies have been conducted to determine the usefulness of herbal medicine in curing dengue. Researchers have indicated that the juice of the leaves of the *Carica papaya* plant from the family Caricaceae helps to increase the platelet levels and have demonstrated definitive beneficial effects in these patients.¹⁸⁻²⁰

Table 1: Sex distribution between Caripill and placebo groups

Sex	Caripill Count (%)	Placebo Count (%)	Total Count (%)
Female	63 (42)	71 (47.3)	134 (44.7)
Male	87 (58)	79 (52.7)	166 (55.3)
Total	150 (100)	150 (100)	300 (100)

P not significant

Aim

The administration of Carica papaya leaf extract should significantly increase the platelet count in cases of thrombocytopenia associated with dengue, preventing the patient to go in DHF or DSS conditions.

Material and Methods

A Multi-centric, Double blind, Placebo controlled, Randomized, Prospective study was conducted in 300 patients across 5 centres', to evaluate the Efficacy and Safety of Carica Papaya Leaf Extract, as empirical therapy for thrombocytopenia associated with dengue fever.

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 entire five centre's Ethics committee approved the protocol and the trial was registered under clinical trial registry system of India (CTRI Registration no : CTRI/2015/05/005806).

Inclusion Criteria

1. Male and female patients above 18 years and below 60 years old,
2. Patients who are confirmed to have DF or DHF grade I and II by NS1 antigen test,
3. Patients having thrombocytopenia with at platelet count between 30,000 /micro litre to 100,000/micro litre,

Table 2: Age distribution between Caripill and placebo groups

Age (yrs)	Caripill Count (%)	Placebo Count (%)	Total Count (%)
18 - 25	16 (10.7)	18 (12)	34 (11.3)
26 - 35	47 (31.3)	49 (32.7)	96 (32)
36 - 45	55 (36.7)	54 (36)	109 (36.3)
46 - 55	32 (21.3)	29 (19.3)	61 (20.4)
Total	150 (100)	150 (100)	300 (100)

4. Patients with a baseline alanine transaminase (ALT) level of not more than 3 times of the upper limit of the normal range (not more than 165 U/L),
5. Patient who is willing to give informed consent to participate in study.

Exclusion Criteria

1. Patients with Dengue hemorrhagic fever grade III and IV,
2. Patients with platelet count less than 30,000/micro litre,
3. Pregnant or lactating women,
4. Patients who have received blood or blood products transfusion during the current illness,
5. Patients with thrombocytopenia Purpura (ITP), Leukemia, Hemophilia,
6. Patients who have a serum ALT level 3 times higher than the upper limit of the normal range (>165 U/L),
7. Impaired renal function with serum creatinine >1.5 mg/dl (males) and >1.4 mg/dl (females),
8. Participation in another trial with an investigational drug within 1 month prior to this trial,
9. Hypersensitivity to any of the components of the formulation,
10. The presence of any other condition that leads the investigator to conclude that the patient is inappropriate for inclusion in this clinical study.

Intervention and Duration

All the study subjects were managed with the standard protocol/guidelines therapy for

Dengue fever. In addition to the standard management protocol, after randomization in two groups, the study/intervention group received Carica Papaya leaf extract (Tablet Caripill) per orally with a strength of 1100 mg tablet, three times a day for 5 days and the control group received Placebo for the same frequency and duration as the intervention group. All the subjects were followed daily for 5 days.

Results

All the 300 subjects enrolled were diagnosed as dengue cases by NS1 antigen test. After administering the tablet Carica papaya leaf extract (Caripill) to the intervention group (n=150) and placebo to the control group (n=150); every day platelets of both the groups were monitored.

- 1.1 Demographic Characteristics: Distribution of sex of the subjects in the two groups did not show any difference (Table 1), men were more than women, most subjects infected are in the age of 26-45 years old and the distribution in both the groups did not show any difference (Table 2).

Majority of the cases were febrile (99%) in both the groups and many of them had associated headache and muscular pain as predominating symptoms (78.3% and 93.7% respectively). The other symptoms observed in the study subjects were rash (30.3%), joint pain (67%), retro orbital pain (55.3%) and vomiting (19.3%).

- 1.2 Primary outcome variables: In this study, Platelet Count was considered as the primary outcome whereas WBC, RBC and Hematocrit are considered as secondary end points. Non parametric test – Friedman's test was used to compare difference between different time points in both the groups.

Tables 3 to 6 gives the number of subjects available in each time point

Table 3: Platelet minimum, maximum and median values for both the groups

Platelet count	Caripill				Placebo			
	N	Min	Max	Median	N	Min	Max	Median
Baseline	150	32,500	97,500	52,543	150	35,000	94,500	51,850
Day 1	150	32,000	94,000	48,000	150	32,000	94,000	45,345
Day 2	150	37,000	98,000	59,500	150	31,500	95,000	49,437
Day 3	150	60,500	1,22,000	88,897	148	32,500	95,000	55,633
Day 4	149	80,000	1,48,500	1,02,579	148	35,000	96,500	64,582
Day 5	147	98,500	2,18,500	1,55,886	145	35,000	98,500	70,528

Table 4: WBC minimum, maximum and median values for both the groups

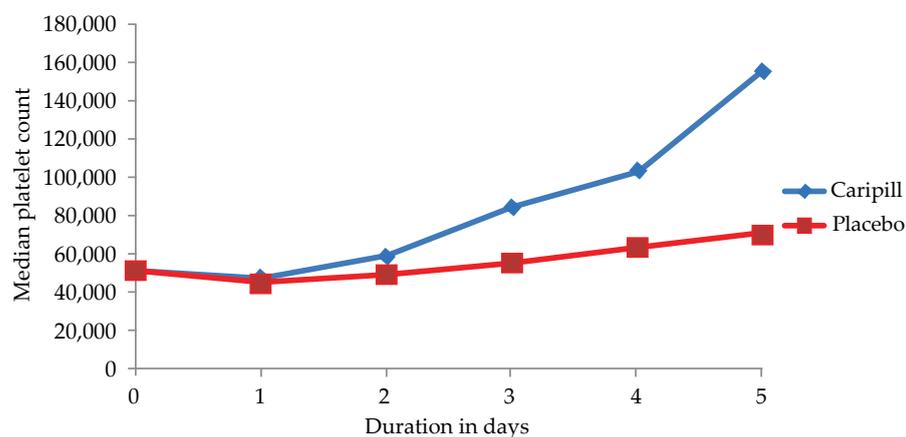
WBC	Caripill				Placebo			
	N	Min	Max	Median	N	Min	Max	Median
Baseline	150	2.90	8.20	4.30	150	2.70	8.10	4.23
Day 1	150	2.90	7.90	3.90	150	2.70	7.20	4.00
Day 2	150	3.30	8.70	3.90	150	2.90	7.70	3.80
Day 3	150	3.90	9.90	4.20	148	3.20	7.90	3.90
Day 4	149	4.10	9.90	5.80	148	3.80	7.90	4.50
Day 5	147	4.50	9.90	7.90	145	3.80	8.10	4.90

Table 5: RBCs minimum, maximum and median values for both the groups

RBC	Caripill				Placebo			
	N	Min	Max	Median	N	Min	Max	Median
Baseline	150	3.70	5.60	4.15	150	3.58	4.60	4.10
Day 1	150	3.80	5.50	4.40	150	3.60	4.60	4.35
Day 2	150	4.00	5.60	4.60	150	3.80	4.80	4.43
Day 3	150	4.10	5.60	4.64	148	3.80	4.80	4.57
Day 4	149	4.10	5.60	4.70	148	3.90	5.00	4.67
Day 5	147	4.10	5.70	4.72	145	3.90	5.10	4.68

Table 6: Hematocrit (%) minimum, maximum and median values for both the groups

Hematocrit	Caripill				Placebo			
	N	Min	Max	Median	N	Min	Max	Median
Baseline	150	45	65	54	150	42	67	55
Day 1	150	44	67	52	150	42	65	53
Day 2	150	40	55	47	150	41	60	49
Day 3	150	40	58	42	148	41	60	45
Day 4	149	38	45	40	148	40	58	42
Day 5	147	35	42	38	145	40	54	40

**Fig. 1: Comparison of platelet count between two groups during different timepoint**

for both the group, also minimum, maximum and median values are presented. Median values are presented in Figures 1 to 4.

Above data reveals that at baseline, median Platelet Count was 52.54 among test group, which was comparable with 51.85 among control group and the difference was not statistically significant.

After the treatment at the end of Day 5, the median Platelet Count showed a significant increase in the intervention group and an insignificant rise in the Control group as compared to baseline values. If compared in both groups, change was more in the intervention group than Control group and the difference was statistically significant ($p < 0.01$).

The above data indicates no significant difference in the baseline values of WBC in both the groups, however at the end of treatment the WBC in the test group was significantly increased and comparing with the control group this increase was found to be statistically significant ($p < 0.05$).

The data in the table above indicates similar baseline values of RBC in both the groups and the values in both the groups had increased marginally which was not statistically significant. The difference in the RBC values of both the groups at the end of the treatment was not significant statistically ($p = 0.625$).

There was no significant difference in the hematocrit values in both the groups at the baseline and no significant difference values in them were observed even at the end of the treatment too. The median values of both the group showed a declining trend at the end of day 5 as compared to the baseline however this decrease was not statistically significant ($p = 0.378$).

Discussion

Thrombocytopenia is one of the associated conditions in dengue cases and can lead to DHF further

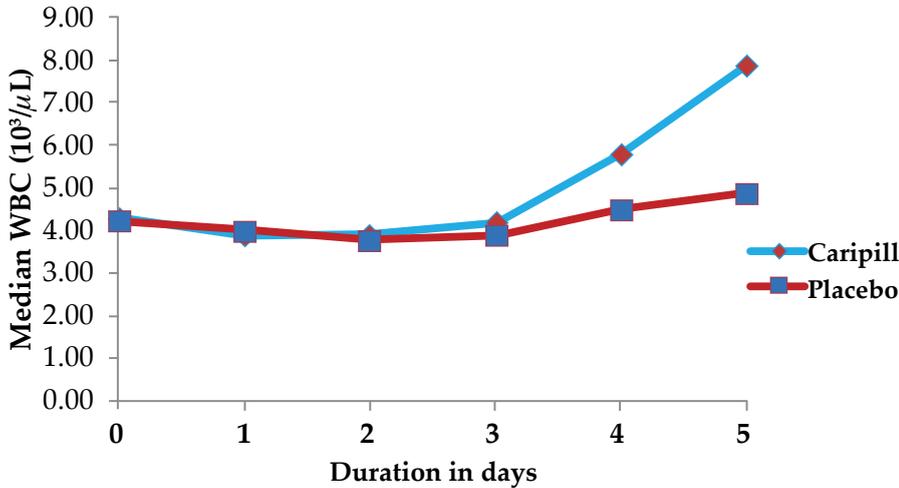


Fig. 2: Comparison of WBC between two groups during different time point

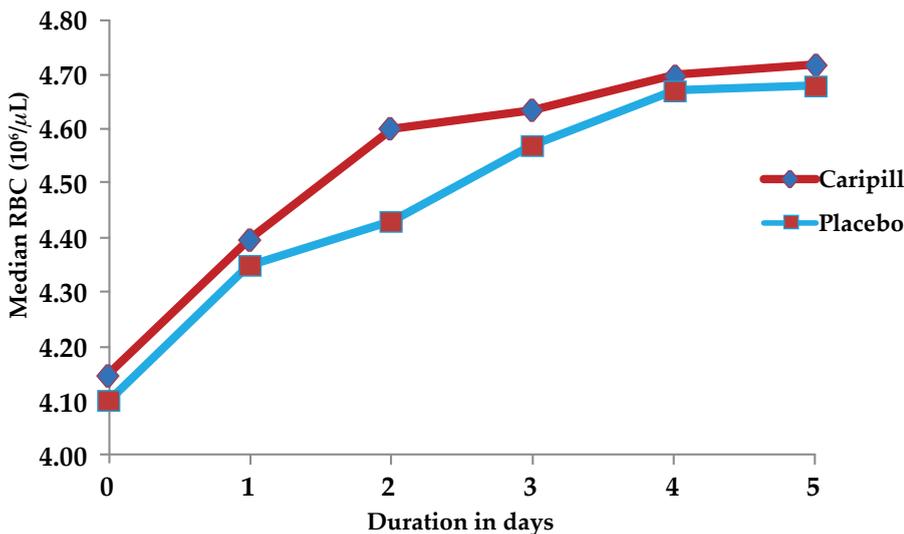


Fig. 3: Comparison of RBC between two groups during different time point

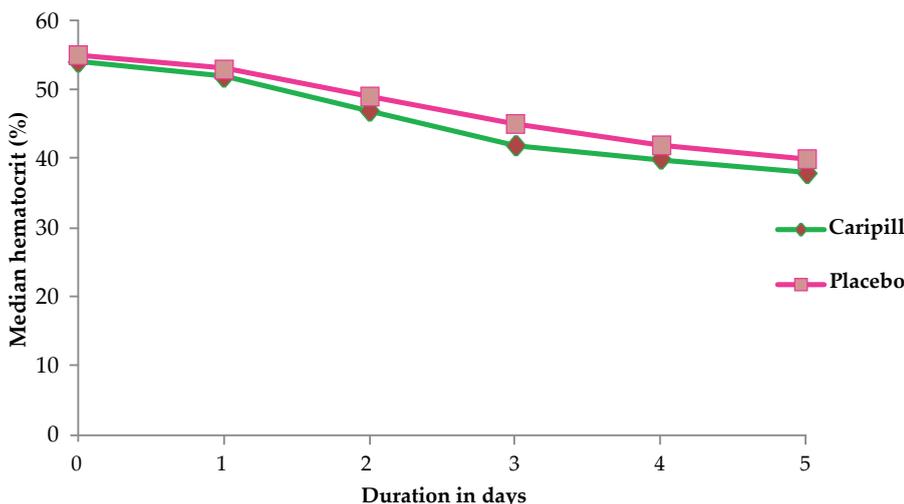


Fig. 4: Comparison of hematocrit between two groups during different time point

aggravating the shock leading to a fatal state.

In this study we observed a significant increase in the platelets in the intervention group which reaffirmed the results of our earlier pilot study. It is demonstrable that the subjects in the intervention group that received CPLE (Caripill) can reach faster and higher increase in platelet count as compared to the control group. Additionally, there was significant increase in the WBC counts in the intervention group as compared to the control group from the baseline values.

The results are corroborative 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 our own pilot study by Gowda et al,¹⁹ and Subenthiran.¹⁴

Carica papaya leaf extract (CPLE) is shown to increase the expression of Arachidonate 12-lipoxygenase (ALOX 12) and Platelet-Activating Factor Receptor (PTAFR) gene responsible for platelet production by FC=15 and FC=13.42 respectively.¹⁴

The WBC values in both the groups showed an increasing trend after third day onwards and can be correlated to the declining viraemia at this point of time. The increase in the WBC in the intervention group was significant as compared to the control group indicative of CP leaf extract may have stimulating action at the myeloid stem cells in the bone marrow. Nwangwa EK et al (2013) in their preclinical study documented an increase in the WBC counts with the administration of Carica Papaya leaf juice in mice, and clinical studies by Siddique et al,²⁰ corroborated the same in humans.

There were few adverse events reported related to GI disturbances like nausea (26), vomiting (17) which were distributed similarly in both the groups and not related to drugs displaying the tolerability and safety of CPLE (Caripill).

Nine (9) subjects in the test group reported to have rashes which were mild and self limiting. None of the subjects discontinued the treatment because of any adverse events. However 3 and 5 subjects in test and control group respectively did not turn up for follow up and treatments remained incomplete and therefore were considered as dropouts.

Twelve patients (8.3%) from the control group went for platelet transfusion as there was a fall in their platelets to the less than 20,000 /micro liter.

None of the patients in the test group required platelet transfusions and had a remarkable recovery with rapid increase in the platelets.

The above findings reinforce the similar findings of our earlier pilot study and reaffirm the platelet increasing property of *Carica papaya* leaf extract.

Conclusion

This randomized double blind study with a representative sample size reiterated the beneficial effect of *Carica papaya* leaf extract in thrombocytopenia associated with dengue.

Drastic fall of the platelets being one of the concerns in dengue cases, this novel option of *Carica papaya* leaf extract (Caripill) can be optimum offering which is simple, convenient, cost effective, safe and efficacious adjuvant in the treatment of thrombocytopenia.

Limitations

Severe cases of grade 3 and 4 were excluded and therefore the efficaciousness in these stages could not be evaluated. Further studies are recommended to evaluate the same and should be

conducted at tertiary care centre's.

In subjects undergoing platelet transfusion comparative study should be conducted to evaluate its effectiveness to bring down the transfusion volume and the rates.

Acknowledgement

We sincerely thank all the investigators viz. Dr. Ambanna Gowda, Dr. B R Ambedkar College, Bangalore; Dr. V C Srinivas Reddy, King George Hospital, Visakhapatnam, Andhra Pradesh; Dr. Poorna Prasad, Prashanth Hospital Bangalore; Dr. G Bharati, Rajiv Gandhi Institute of Medical sciences, Srikakulam, Andhra Pradesh; Dr. Vijay Kumar, Sri Venkateshwar Hospital, Bangalore; for their participation in this study and Dr. Arun Kumar, biostatistician for his statistical inputs.

References

1. Mayxay M, Castonguay-Vanier J, Chansamouth V, et al. Causes of non-malarial fever in Laos: a prospective study. *Lancet Glob Health* 2013; 1:e46-46e54.
2. Guzman MG, Halstead SB, Artsob H, et al. Dengue: a continuing global threat. *Nat Rev Microbiol* 2010; 8:57-16.
3. World Health Organisation: DENGUE: Guidelines For Diagnosis, Treatment, Prevention and Control. New Edition. (2009)
4. Lei HY, Yeh TM, Liu HS, et al. Immunopathogenesis of dengue virus infection. *J Biomed Sci* 2001; 8:377-388.
5. Martina BE, Koraka P, Osterhaus AD. Dengue Virus Pathogenesis: An Integrated View. *Clin Microbiol Rev* 2009; 22:564-581.
6. Funahara Y, Ogawa K, Fujita N, Okuno Y. Three possible triggers to induce thrombocytopenia in dengue virus infection. *The Southeast Asian J Trop Med Public Health* 1987; 18:351-355.
7. Huang KJ, Li SY, Chen SC, et al. Manifestation of thrombocytopenia in dengue-2 virus infected mice. *J Gen Virol* 2000; 81:2177-2182.
8. Schexneider KI, Reddy EA. Thrombocytopenia in dengue fever. *Curr Hematol Rep* 2005; 4:145-148.
9. Leong AS, Wong KT, Leong TY, Tan PH, Winnakrairot P. The pathology of dengue hemorrhagic fever. *Semin Diagn Pathol* 2007; 24:227-236.
10. Lei H, Huang K, Lin Y, et al. Immunopathogenesis of Dengue Hemorrhagic Fever. *American Journal of Infectious Diseases* 2008; 4:1-9.
11. Ghosh K, Gangodkar S, Jain P, et al. Imaging the interaction between dengue 2 virus and human blood platelets using atomic force and electron microscopy *J Electron Microscop* 2008; 57:113-8.
12. La Russa VF, Innis BL. Mechanisms of dengue virus-induced bone marrow suppression. *Baillieres Clin Haematol* 1995; 8:249-270. [PubMed: 7663049].
13. Garg P, Nagpal J, Khairnar P, Seneviratne SL. Economic burden of dengue infections in India. *Trans R Soc Trop Med Hyg* 2008; 102:570-7.
14. Subenthiran S, Choon TC, Cheong KC, et al. Carica papaya leaves juice significantly accelerates the rate of increase in platelet count among patients with dengue fever and dengue haemorrhagic fever. *Evidence Based Complement Alternat Med* 2013 616-737.
15. Kurosu T. Quasispecies of dengue virus. *Trop Med Health* 2011; 39:29-36.
16. Lee SH, Tang YQ, Rathkrishnan A, et al. Effects of cocktail of four local Malaysian medicinal plants (*Phyllanthus* spp.) against dengue virus 2. *BMC Complement Alternat Med* 2013; 13:192.
17. Senthilvel P, Lavanya P, Kumar KM, et al. Flavonoid from Carica papaya inhibits NS2B-NS3 protease and prevents Dengue 2 viral assembly. *Bioinformation* 2013; 9:1-7.
18. Sarala N, Paknikar SS. Papaya extract to treat Dengue: A novel therapeutic option? *Annals of Medical and Health Sciences Research* 2014; 4:320-324.
19. Gowda A. A pilot study to evaluate the effectiveness of carica papaya leaf extract in increasing the platelet counts in cases of dengue with thrombocytopenia. *Indian Medical Gazette* 2015; CXLIX:109-116.
20. Siddique O. Effects of Papaya Leaves on thrombocyte counts in dengue, *J Pak Med Assoc* 2014; 64:3.