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Original Research Article

A comparative study of the platelet augmentation potential of leaf extracts of *Psidium guajava* with *Carica papaya* in thrombocytopenic rats

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Abstract: The aim of this study was to evaluate the platelet augmentation activity of the leaf extracts of *Psidium guajava* and to compare it with that of *Carica papaya* and to explore the possible use of *Psidiumguajava* in cases of thrombocytopenia, especially in dengue fever. Thrombocytopenia was induced in Albino Wistar rats using Cyclophosphamide 25mg/kg s.c. from Day 1-3. Aqueous extracts of the leaves of *Psidium guajava* and *Carica papaya* were administered p.o to the treatment groups from Days 1-15. Platelet counts of the rats belonging to all the groups were done on Days 1, 4, 7 and 11 and clotting time was measured on Day 15. The data was collected and analyzed using GraphPad Prism 5. Leaf extracts of both *Psidium guajava* and *Carica papaya* were found to increase platelet counts significantly following Cyclophosphamide induced thrombocytopenia. There was no significant difference in the platelet augmentation potential between leaf extracts of *Psidium guajava* and *Carica papaya*. The clotting time of the groups treated with both lower and higher doses of both extracts were found to be near normal. Our study suggests a potential role of leaf extracts of *Psidium guajava* in improving the platelet counts in various thrombocytopenic disorders including a role in ameliorating the haemorrhagic complications of dengue fever.
Keywords: Platelet augmentation, Cyclophosphamide, *Psidium guajava*, *Carica papaya*, Thrombocytopenia.

INTRODUCTION

Dengue is a viral disease, spread by mosquitoes of the genus Aedes, primarily Aedes aegypti, which is today the most important arboviral disease worldwide in terms of morbidity, mortality and economic impact [1]. All four distinct dengue viruses (dengue 1-4) have Aedes aegypti as their principal vector, and all cause a similar clinical syndrome consisting of a sudden onset of fever, headache, retroorbital pain and back pain along with severe myalgia ("break-bone fever"). In rare cases, second infection with a serotype of dengue virus different from that involved in the primary infection leads to dengue haemorrhagic fever with severe shock. Laboratory findings include leukopenia, thrombocytopenia, and, in many cases, serum aminotransferase elevations [2].

Apart from dengue, many other viral and bacterial infections also result in thrombocytopenia and are the most common noniatrogenic causes of thrombocytopenia [3]. Thrombocytopenia is a rare blood disorder that affects the platelets of the blood and is characterized by low platelet count (100000 cells per mm of blood or less) and low platelet survival time. Other symptoms include a tendency to bleed excessively into mucous membranes, especially during menstruation [4]. Thrombocytopenia may result from decreased bone marrow production, sequestration, usually in an enlarged spleen; and/or increased platelet destruction. The various clinical conditions described under thrombocytopenia are-

1. Infection-induced thrombocytopenia
2. Drug-induced thrombocytopenia
3. Heparin-induced thrombocytopenia
4. Immune thrombocytopenic purpura
5. Inherited thrombocytopenia
6. Thrombotic thrombocytopenic purpura and
7. Hemolytic uremic syndrome [3].

According to a World Health Organization (WHO) fact sheet dated December 2008, 80 % of the population in some Asian and African countries depends on traditional medicine as their primary health care due to economic and geographical constraints [5]. Natural products have become the main source of test material in the development of antiviral drugs based on

traditional medical practices [6]. For treatment of emerging diseases, herbs are staging a comeback and herbal 'renaissance' is taking place all over the globe. The blind dependence on synthetics is over and people are returning to the naturals with the hope of safety and security.

Recently, *Carica papaya* (commonly known as Papaya) leaves have been successfully employed in folk medicine for the treatment of dengue infections with haemorrhagic manifestations, using suspensions of powdered leaves in palm oil. *Carica papaya* L. 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 [7]. *Carica papaya* leaves contain various phytoconstituents like saponins, tannins, cardiac glycosides and alkaloids. The alkaloids present include carpaine, pseudocarpaine and dehydrocarpaine I and II. These constituents can act on the bone marrow, prevent its destruction and enhance its ability to produce platelets. Moreover, it can also prevent platelet destruction in the blood and thereby increase the life of the platelet in circulation [8].

Psidium guajava, commonly known as Guava, belonging to family Myrtaceae is also a potential anti-dengue drug [9]. It is an evergreen shrub or small tree indigenous to Mexico, the Caribbean and Central and South America. It is cultivated widely in tropical and subtropical regions around the world. *Psidium guajava* leaf extract has been tested in vitro and showed to inhibit the growth of dengue virus [10]. Water boiled with guava leaves was used to avoid bleeding in dengue haemorrhagic fever, and increased platelet counts to 100000/mm3 within a period of approximately 16 hours [11]. *Psidium guajava* ripe fruit or juice has healing properties in cases of dengue fever by improving the declining levels of platelets [12].

Cyclophosphamide was used as toxicant in the current study because of its capacity to induce stable thrombocytopenia [13]. Cyclophosphamide is an alkylating agent having cytotoxic and radiomimetic actions which leads to thrombocytopenia but is less damaging to platelets [14].

The aim of this study was to make a comparative analysis of the ability of the leaf extracts of *Carica papaya* and *Psidium guajava* in increasing platelet counts to explore the possible use of *Psidium guajava* in cases of thrombocytopenia, especially in dengue fever.

AIMS AND OBJECTIVES

(1)To evaluate the ability of *Psidium guajava* leaf extracts in increasing the platelet count in cyclophosphamide induced thrombocytopenic rat model.

(2)To compare the platelet augmentation activity of *Psidium guajava* and *Carica papaya* leaf extracts in cyclophosphamide induced thrombocytopenic rat model.

MATERIALS AND METHODS

This study was undertaken for a period of two months from February, 2016, in the Department of Pharmacology, Gauhati Medical College, Guwahati after due approval from the Institutional Animal Ethics Committee (MC/05/2015/45). Albino Wistar rats (100-125g) of either sex were used for the study. They were housed under standard conditions of temperature and light and were fed with standard diet and water ad libitum. Rats belonging to the extremes of age, diseased and pregnant rats were excluded from the study. Aqueous extract of leaves of *Carica papaya* and *Psidium guajava* were used. Acute toxicity study was carried out according to the OECD 425 guidelines to determine the doses. A total of 36 animals were taken and then divided into six groups comprising of six animals each, as follows- Distilled water, Cyclophosphamide only, Cyclophosphamide + *Psidium guajava* leaf extract (PGLE) 150 mg/kg, Cyclophosphamide + *Psidium guajava* leaf extract (PGLE) 300 mg/kg, Cyclophosphamide + *Carica papaya* leaf extract (CPLE) 400 mg/kg and Cyclophosphamide + *Carica papaya* leaf extract (CPLE) 800 mg/kg. Cyclophosphamide 25 mg/kg was administered i.s. to Groups II-VI daily from Day 1-Day 3 and the leaf extracts were administered p.o. to the corresponding groups from Day 1-Day 15. Blood was collected from the tip of the tail after subjecting the animals to light anesthesia using ether and the following parameters were examined-

- Platelet count, using automated cell counter Coulter Act-Diff (on Day 1, 4, 7 and 11)
- Clotting time by capillary method (on Day 15)

The data was collected and analyzed using GraphPad Prism 5.

RESULTS

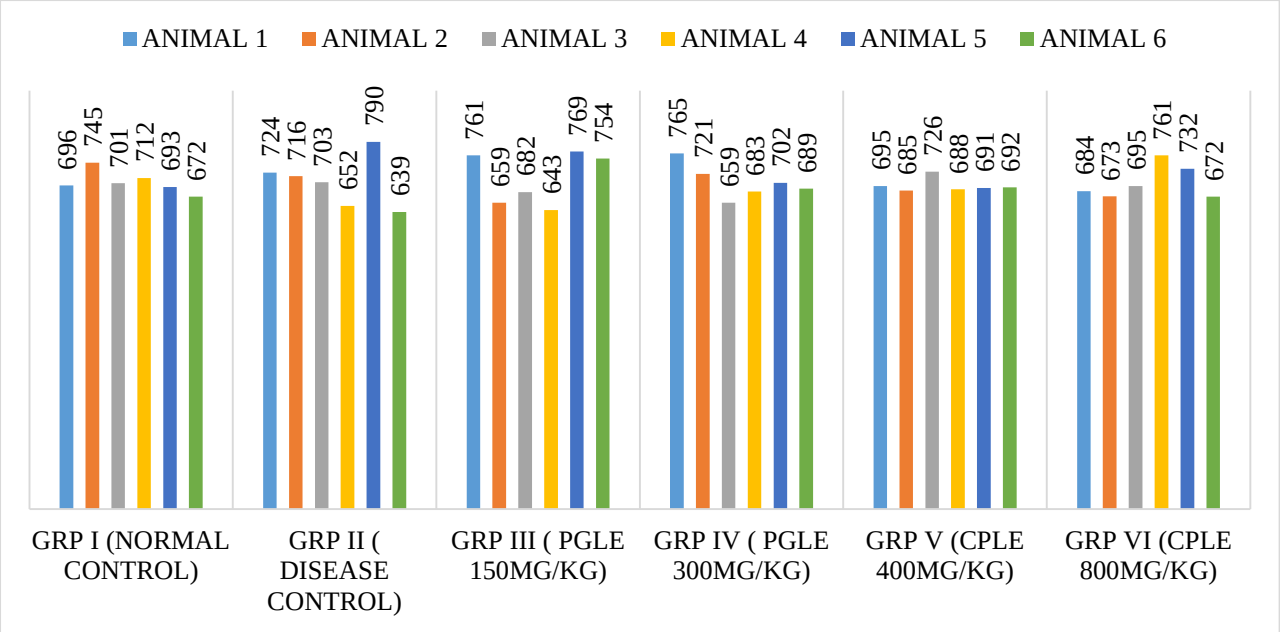

Fig 1(A): Platelet counts of all six groups on Day 1 (in 10^3/µL)

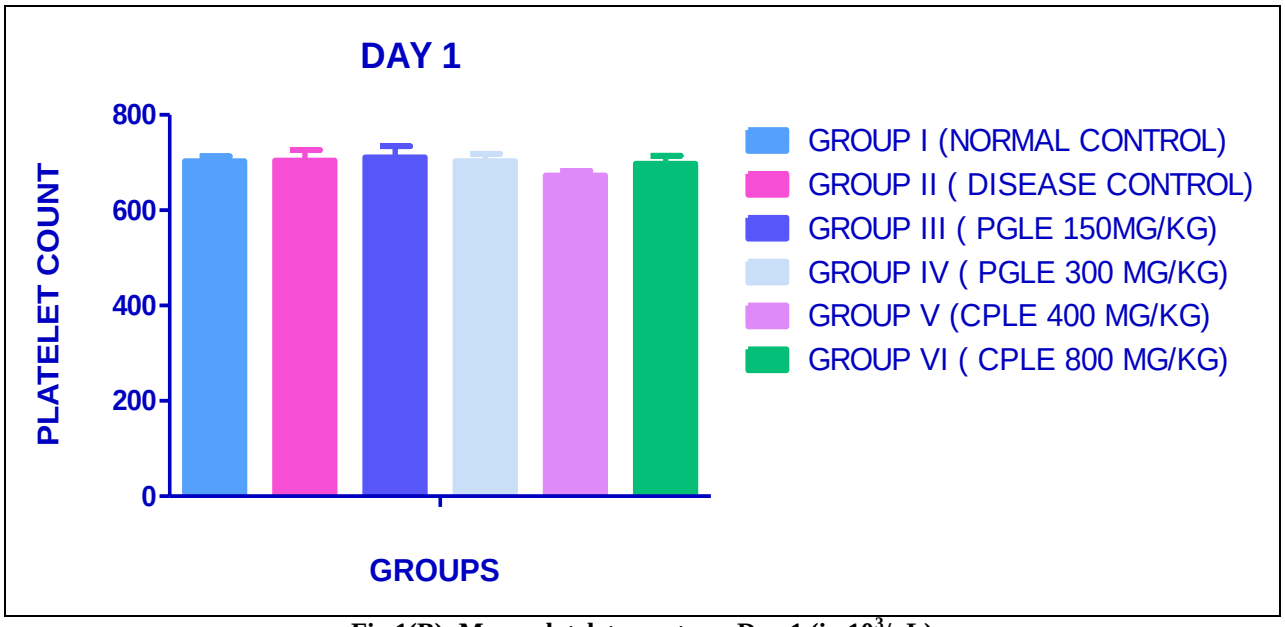

Fig 1(B): Mean platelet counts on Day 1 (in 10^3/µL)

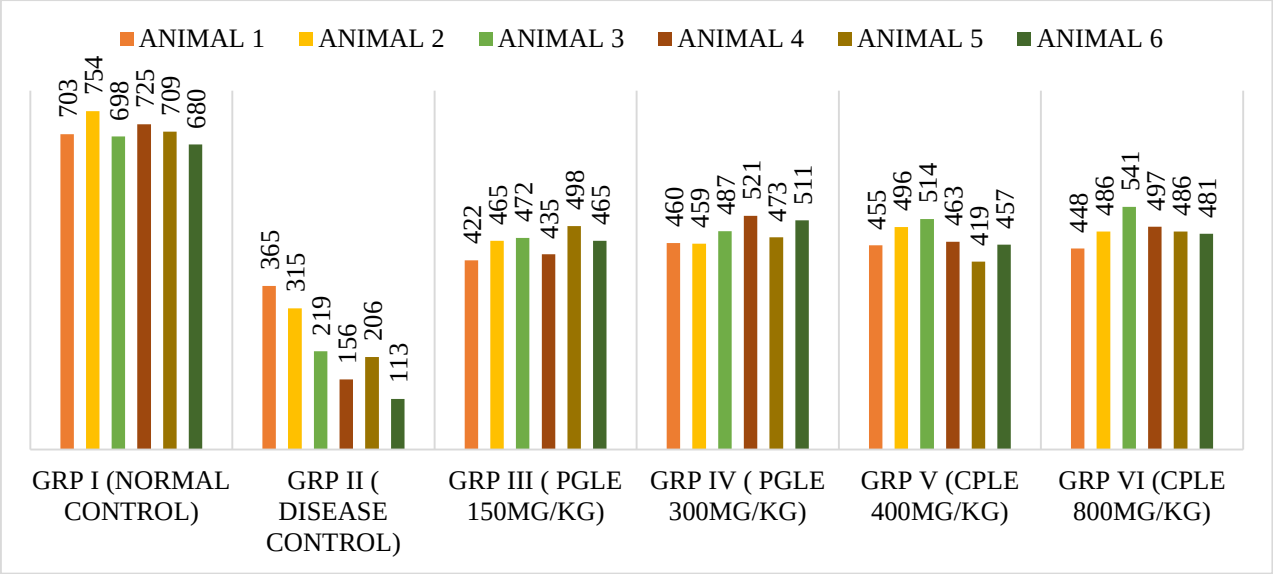

Fig 2(A): Platelet counts of all six groups on Day 4 (in 10^3/µL)

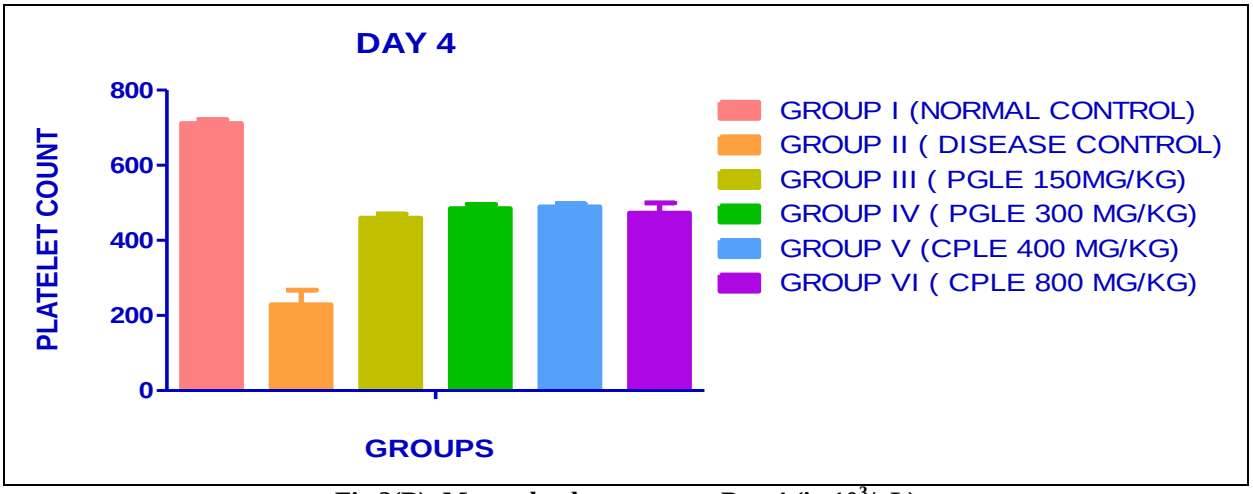

Fig 2(B): Mean platelet counts on Day 4 (in 10^3/µL)

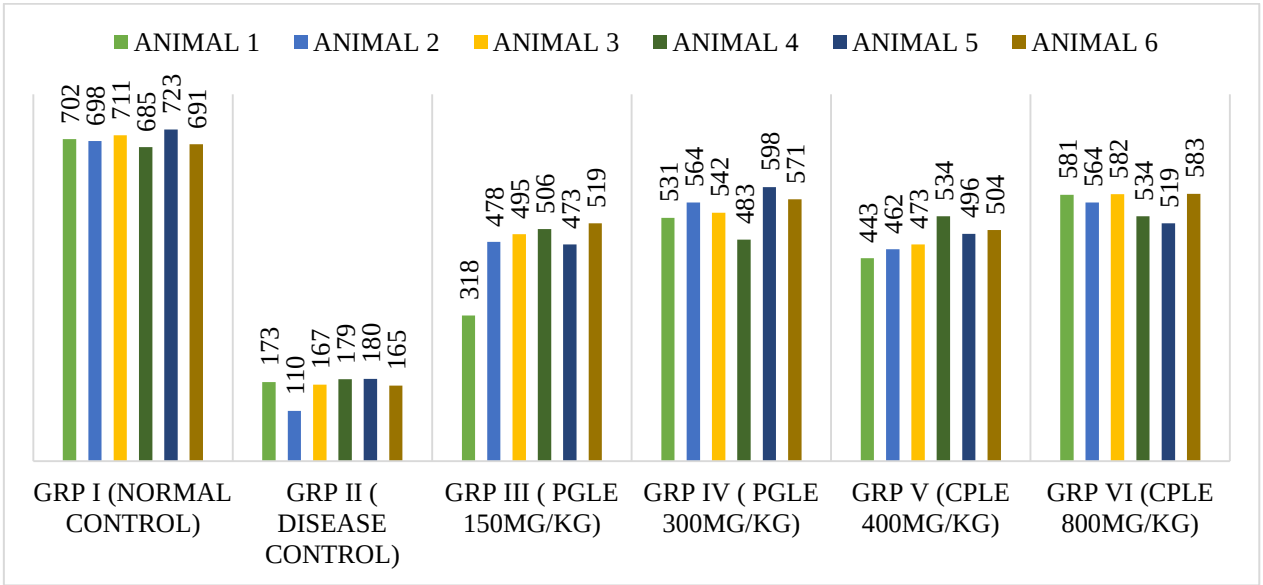

Fig 3(A): Platelet counts of all six groups on Day 7 (in 10^3/µL)

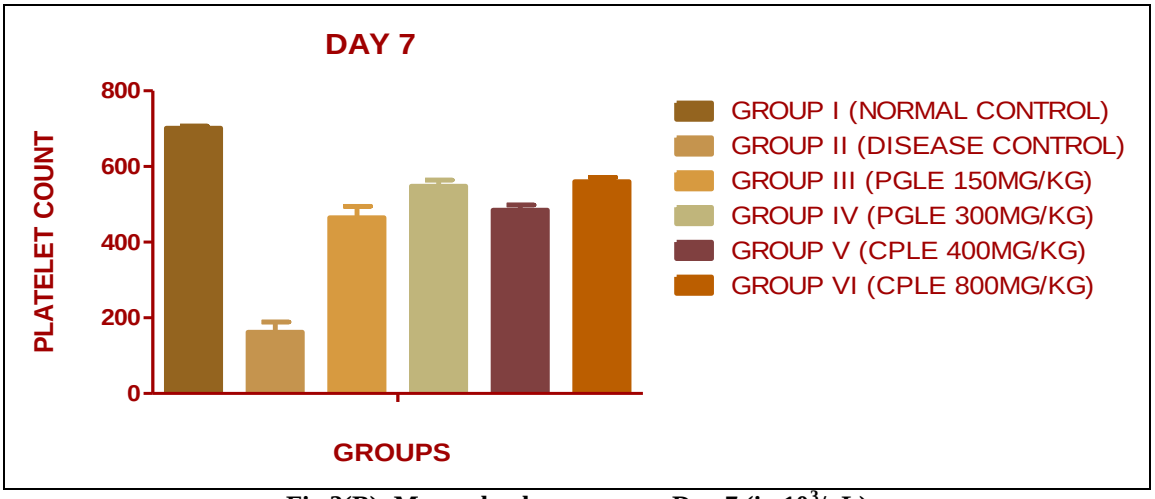

Fig 3(B): Mean platelet counts on Day 7 (in 10^3/µL)

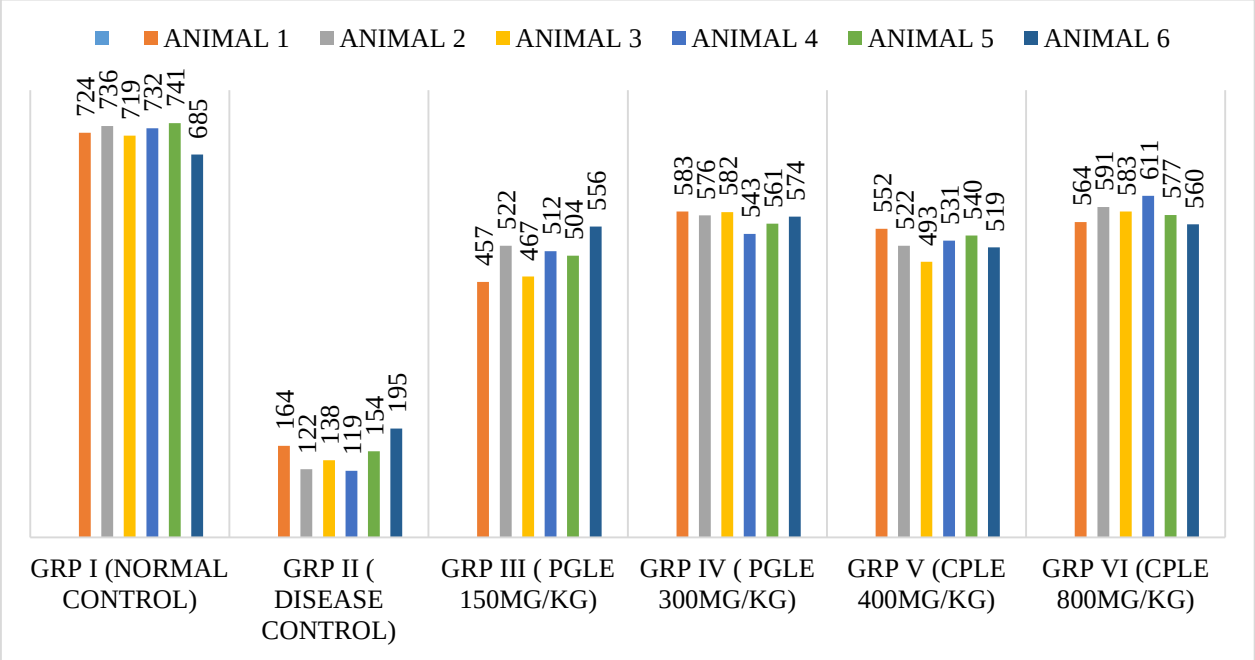

Fig 4(A): Platelet counts of all six groups on Day 11 (in 10^3/µL)

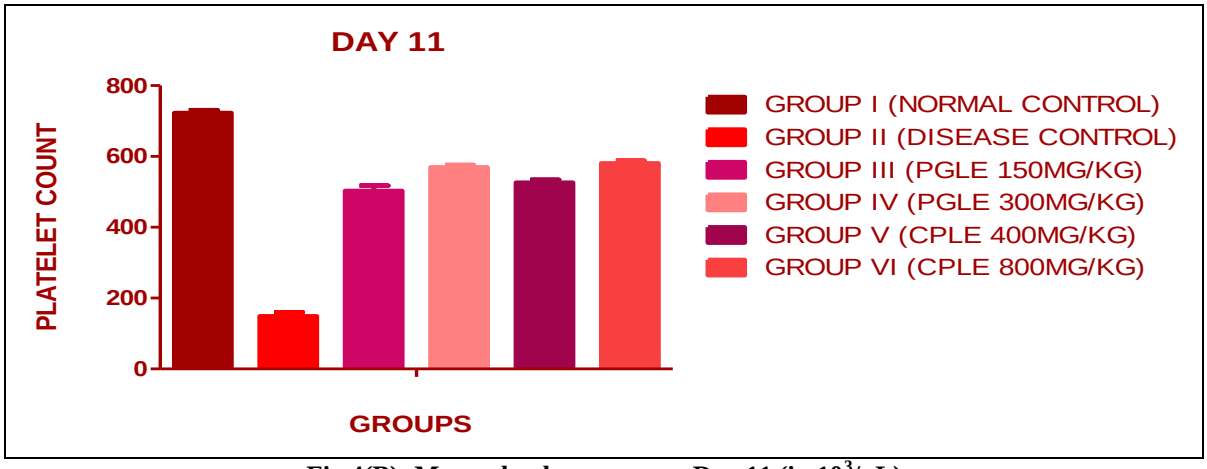

Fig 4(B): Mean platelet counts on Day 11 (in 10^3/µL)

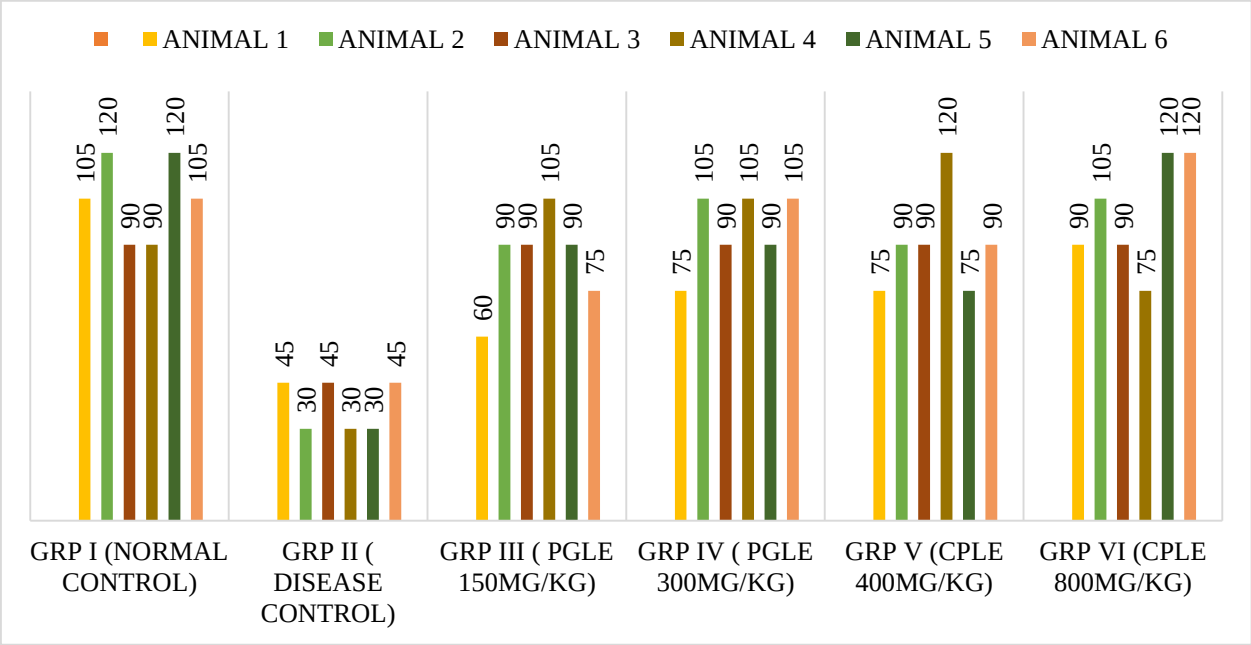

Fig 5(A): Clotting time of all six groups on Day 15 (in seconds)

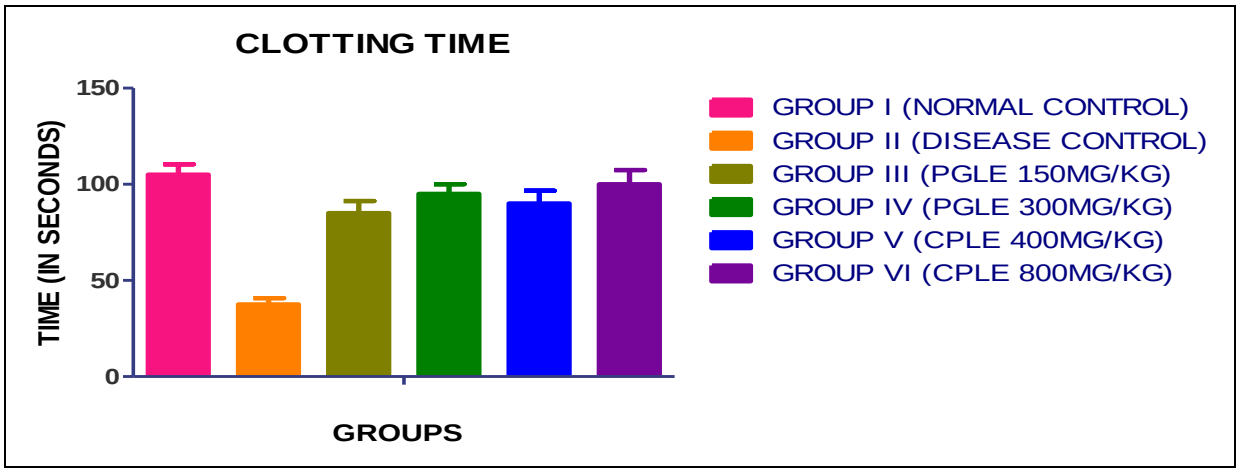

Fig 5(B): Mean clotting time on Day 15 (in seconds)

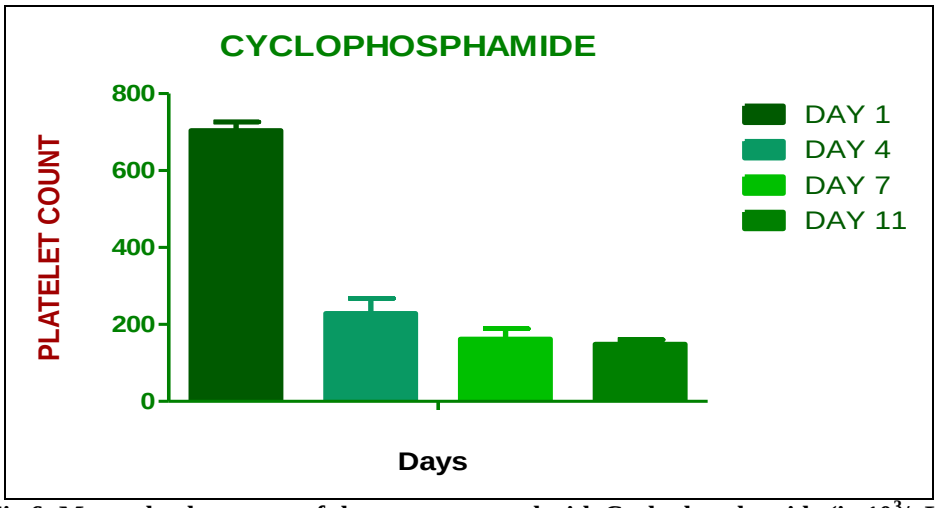

Fig 6: Mean platelet counts of the groups treated with Cyclophosphamide (in 10^3/µL)

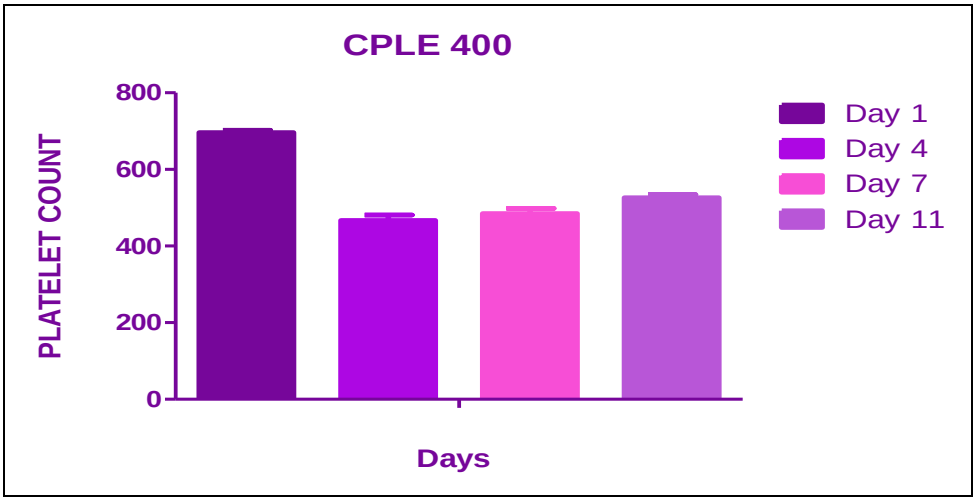

Fig 7: Mean platelet counts of the groups treated with *Carica papaya* leaf extract 400mg/kg (in 10^3/µL)

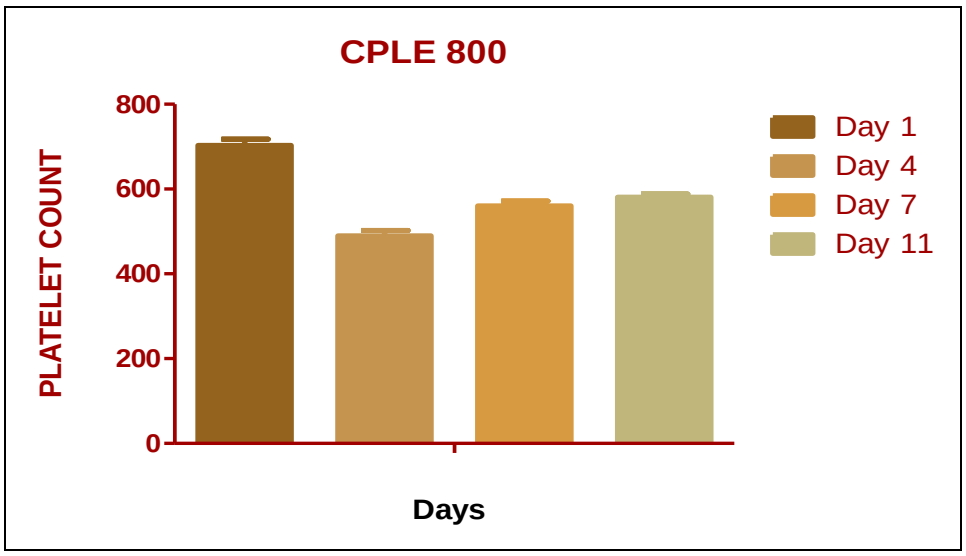

Fig 8: Mean platelet counts of the groups treated with *Carica papaya* leaf extract 800mg/kg (in 10^3/µL)

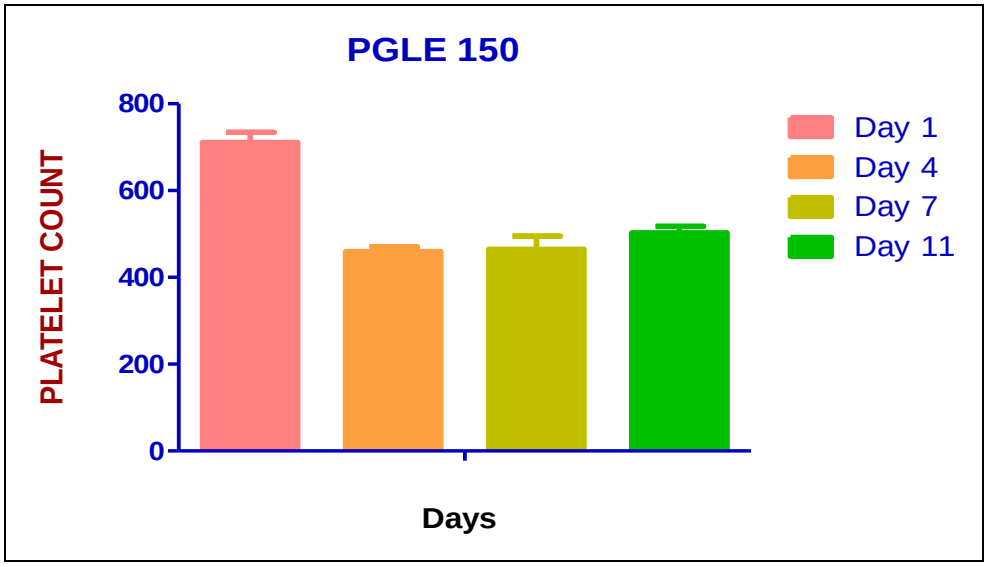

Fig 9: Mean platelet counts of the groups treated with *Psidium guajava* leaf extract 150mg/kg (in 10^3/µL)

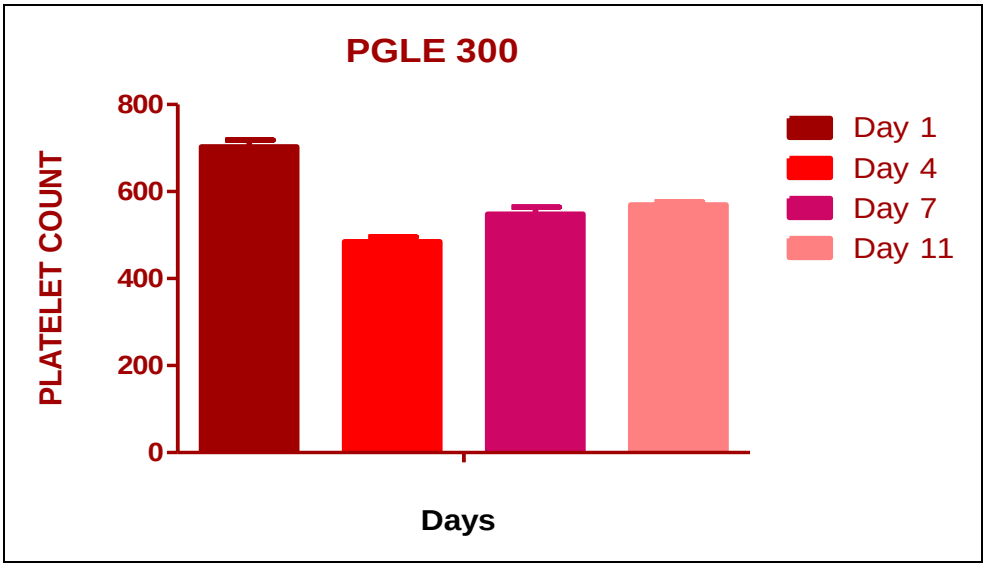

Fig 10: Mean platelet counts of the groups treated with *Psidium guajava* leaf extract 300mg/kg (in 10^3/µL)

DISCUSSION

Aqueous extracts of the leaves of both *Psidium guajava* and *Carica papaya* were found to have platelet augmentation potential. On Day 1, there was no significant difference between the platelet counts in the six study groups (P=0.2759). [Figure 1(A, B)] Cyclophosphamide, administered from Day 1 to Day 3, significantly decreased the platelet counts, which was more pronounced in the disease control group. [Figure 2(A, B)] The platelet counts were found to be significantly decreased on days 4, 7 and 11 in comparison to Day 1(P<0.05) but there was no significant difference in the counts between the three days i.e. days 4, 7 and 11 (P>0.05) in the disease control group. [Figure 6] The platelet counts improved significantly in the groups which were treated with *Carica papaya* leaf extracts from Day 4 onwards [Figure 3 (A, B) and Figure 4 (A, B)] and the group treated with higher dose (800 mg/kg) of the extract showed better improvement of platelet count. The platelet counts on Day 11 were significantly increased in comparison to Day 4. [Figure 7, 8] Significant increases in platelet counts were also observed in the groups treated with *Psidium guajava* leaf extracts. The group treated with higher dose of *Psidium guajava* leaf extract (300mg/kg) showed greater improvement in platelet count. [Figure 9, 10] There was no significant difference in the platelet counts between the groups treated with leaf extracts of *Carica papaya* and *Psidium guajava*. The clotting time in all the treatment groups improved significantly in comparison to the Cyclophosphamide-administered disease control group and it was observed to be near normal in the group treated with higher dose of *Carica papaya* leaf extract. [Figure 5 (A, B)]

CONCLUSION

Therefore our study found that leaf extracts of *Psidium guajava* have platelet augmentation potential almost similar to that of leaf extracts of *Carica papaya*. They were also found to improve the clotting time within two weeks of treatment. This suggests a potential role of the leaf extracts of *Psidium guajava* in improving the platelet counts in various thrombocytopenic disorders including a role in ameliorating the haemorrhagic complications of dengue fever. The platelet counts were not found to attain normal values but further studies are required with longer duration of treatment with these extracts to ascertain whether the increasing trend in platelet counts as observed in our study are sustained with longer treatment.

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