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Original Research Article

A randomized, double-blind, placebo-controlled, proof of concept study to assess the safety and efficacy of *Carica papaya* and *Tinospora cordifolia* leaf extract (Thrombobliss) in subjects undergoing chemotherapy treatment and subjects with systemic microbial infection and subsequent reduction in platelet count

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ABSTRACT

Background: Thrombocytopenia is defined as persistent decrease in the number of blood platelets. Thrombocytopenia usually serves as symptom for identifying hematologic malignancies, infectious diseases, auto-immune disorders and as common side effect of about 200 different disease types. There was neither a specific treatment available for thrombocytopenia, nor to increase the platelet count leading to mortality. Current methods are less effective and have their limitations. This study was conducted to prove that *Carica papaya* and *Tinospora cordifolia* leaf extract can increase the platelet count in study subjects with dengue, other microbial infections.
Methods: Totally 250 patients were randomized, subjects within inclusion criteria and exclusion criteria were randomly assigned, in a 1:1 ratio, to receive orally thrombobliss in a dose of 5 ml syrup twice daily or placebo for five consecutive days. Platelet count was monitored every day.
Results: The result showed that there is increase in the platelet count from day 2 in median platelet data and more evident after 72 hours seen in both mean and median platelet count. Even in the cancer patients undergoing chemotherapy, there has been significant increase in platelet count. The statistical analysis of the data showed that there is a significant increase in platelet count and is statistical significant $p<0.05$.
Conclusions: This study showed that novel combination of *Carica papaya* and *Tinospora cordifolia* leaf extract effectively increases platelet count and can be used to treat thrombocytopenia. It is also evident that this combination is safe and effective in treating thrombocytopenia patients due to dengue and other microbial infection.

Keywords: Thrombocytopenia, *Carica papaya*, *Tinospora cordifolia*, Platelet count

INTRODUCTION

Thrombocytopenia

Thrombocytopenia, a hematological disorder characterized by abnormally low platelet count in the blood stream. There are about 150,000 - 450,000/µl platelet in peripheral circulation of blood. The average life of them is 8 - 10 days. Nearly 2/3 platelet is in blood and 1/3 is in spleen. Cases with platelet count between 70 and 150 × 10^3 per µL are considered to be mild (70 to 150 × 10^9 per L) and severe if less than 20 × 10^3 per µL (20 × 10^9 per L).[1] Thrombocytopenia caused by three main causes: Decreased platelet production from bone marrow, increased platelet destruction and platelet splenic sequestration.[2] Different causative agents causes various thrombocytopenia such as ITP, drug-induced thrombocytopenia, hepatitis C virus, HIV induced, dengue, chikungunya, malaria, etc. Infections may cause thrombocytopenia due to increased peripheral platelet consumption or by direct bone marrow suppression. Recent studies found that 14 percent of hospitalized patients with influenza A (H1N1) virus reported to have thrombocytopenia.[3] Platelet counts usually recover in patients with a self-limited viral infection.[4]

During the critical phase of dengue, malaria, chikungunya that thrombocytopenia is characterized by a decrease in platelet count below 100000 permm^{3}from the baseline.[5]

Different medications responsible for platelet interference are nitrates, beta-lactam antibiotics, beta-blockers, nitrates, tricyclic antidepressants; etc.[6] Heparin exposure can also lead to life-threatening thrombocytopenia.[7] Immune-mediated disorders (e.g. ITP, DITP) and inherited disorders (e.g. Bernard-Soulier, TAR syndrome) can lead to isolated thrombocytopenia. Prolonged thrombocytopenia and absence of relative increase in the platelet count were also associated with a greater risk of mortality.[8]

Epidemiology

The incidence rates for immune thrombocytopenia (ITP) are as follows: in the US there are 66 adult cases and 50 childhood cases per 1,000,000 per year; in Denmark and England there are 10 to 40 cases per 1,000,000 per year; and in Kuwait there are 125 cases per 1,000,000 per year[9]. In another study, 3771 incident ITP patients were identified. Incidence was 2.9/100 000 person-years, with peaks among children and in those >60 years of age. The incidence of thrombocytopenia was lower in overseas Caribbean French departments and among Afro-American people. Persistence or chronicity occurred in 36% of children compared with 67% of adults in north-south gradient in mainland France, 18% of ITPs were secondary among adults while the other causes were malignancy (10.9%) and myelodysplastic syndromes (2.3%). Severe gastrointestinalor central nervous system bleeding at ITP onset was rare (<1%).[10] In India, Prevalence of thrombocytopenia in antenatal was 8.8%. Gestational thrombocytopenia was seen in 64.2%, obstetric in 22.1% and medical in 13.68% cases.[11]

Different treatments for Thrombocytopenia

Understanding the pathophysiology of the disease can help in successfully treating thrombocytopenia. Many mechanisms that happen during any syndromes or diseases may lead to thrombocytopenia. So, prompt investigation and identification may be crucial and sometimes life-saving as in TTP, heparin-induced thrombocytopenia, acute leukemia or even severe ITP.

First treatment approach is the direct treatment of the root factor causing thrombocytopenia.[12] Corticosteroids are considered first-line treatment and increase platelet counts usually within one week of initiation, with the recommended prednisone dose 1 mg/kg/day orally for up to 21-28 days.[13,14] More prominent platelet responses have been reported with repeated pulses of high-dose dexamethasone 40 mg daily for 4 days.[15] Also, initial treatment can be done with intravenous immunoglobulin or intravenous anti-D (Rho [D] immune globulin) with or without steroids.[17] The initial intravenous immune-globulin dose of either 0.4 g/kg/day for up to 5 days has been found to be effective.[16]

In case of severe bleeding with unknown root cause of thrombocytopenia, platelet transfusion is carried out. Platelet transfusion will help in solving blood loss during or after surgery. While the fear of sensitization has dominated restriction of platelet transfusion, the availability of leuko-reduction has greatly decreased this risk. High risk of marrow failure or a high risk of acute leukemia limits the option of allogeneic stem cell transplantation for inherited thrombocytopenia.[17]

With most chemotherapy agents, rarely blood count occurs 7 to 10 days after chemotherapy and recovery over 2 to 3 weeks.[18]

The most effective second-line treatment option is splenectomy. The initial response rate is perhaps 80-85% with a 5-year response rate of 60-65%.[19] All the above treatments pose their own limitations and risks. They are indeed difficult to proceed on as few are costlier methods and patients need time to get adopted. So, herbal treatments were preferred. Papaya extract no doubt offers a cheap and possibly effective treatment for thrombocytopenia. However, currently, it is also necessary to evaluate that safety and efficacy of the leaf extract. Thus, if proved to be effective, this plant could effective in treating thrombocytopenia.

Carica papaya and Tinospora cordifolia

Carica papaya is a member of the Caricaceae and is a dicotyledonous, polygamous, and diploid species.[20] It is cultivated in many tropical countries such as Bangladesh, India, Indonesia, Sri Lanka, the Philippines, and the West

Indies including Malaysia.[21] Recent studies have shown its beneficial effect as an anti-inflammatory agent, for its wound healing properties, antitumor as well as immune-modulatory effects and as an antioxidant.[22-24] A toxicity study (acute, sub-acute, and chronic toxicity) conducted on *Sprague Dawley* rats administered with *Carica papaya* leaves juice (CPLJ) of the sekaki variant revealed that it was safe for oral consumption.[25] They have been used in tropical and sub-tropical regions of the world to counter dengue (thrombocytopenia) and ITP. Few studies and trials have been conducted on the effect of papaya leaf extracts on thrombocytopenic condition.

Tinospora cardifolia belongs to family Menispermaceae. It contains many useful effects such as antioxidant, antineoplastic, hepato-protective, hupolipidemic and immunological properties. It is a good immune-stimulant. It helps in proliferation of immune cells and also treats the affected liver cells.[26] This study has been carried out with phyto formulation syrup that contains 375 mg *Carica papaya* and 125 mg *Tinospora cardifolia* leaf extracts *(ThromboBliss)* that can be used to treat diseases associated with thrombocytopenia e.g. dengue, chikungunya, malaria, immune related thrombocytopenia, etc. The objectives of this study is to evaluate the safety and to determine the efficacy of *Carica papaya* and *Tinospora cordifolia* extract (Thrombobliss) in enhancing the platelet count in thrombocytopenia caused due to infections and in any active cancer subjects who are undergoing chemotherapy.

METHODS

This was a randomized, double-blind, placebo-controlled, multi-centre, parallel-group study of *Carica papaya extract and Tinospora cordifolia* leaf extract (Thrombobliss) in subjects with systemic microbial infection and thrombocytopenia. After randomization, subjects who meet all inclusion criteria and no exclusion criteria were randomly assigned, in a 1:1 ratio, to receive thrombobliss in a dose of 5mL syrup twice daily or a matching placebo for five consecutive days. These doses were administered orally. Totally 300 subjects were screened and 250 patients were randomized. Blood samples for assessing blood routine, platelet count, PT-INR and other safety laboratory tests were obtained between baseline assessments and the end of the study procedures. Safety-related assessments were to include reports of adverse events and clinical laboratory test results. Treatment was for five days and 5 ml dosage was administered orally at a time span interval of 12 hours plus one day follow up was done as a check.

Subject eligibility criteria

Inclusion criteria

All patients of age between 18 to 75 and with clinical objective evidence of bacterial or viral infection with white blood cell (WBC) count greater than >12,000/mm^3 or less than < 4,000/mm^{3}and Platelet count in the range of greater than >20,000/mm3 to less than <150,000/mm^3 and with Fever with core temperature of <36ºC or >38ºC were included in the study. Cancer patients having symptomatic and/or progressive cancer that was previously treated with at least 1 and no more than 3 prior lines of therapy, ECOG performance status score ≤2 and subject undergoing chemotherapy treatment with proven diagnostic assessment of thrombocytopenia (platelet count <1,00,000/mm^3 and >50,000 mm^3/µL) without platelet transfusion in the preceding 14 days and Subjects with Coagulopathy characterized by elevated PT-INR >1.20 were also included in the study.

Exclusion criteria

The patients were excluded from the study if the age is <18, body weight ≥175 kg, pregnant or breastfeeding ladies. Patients known allergy to herbal compounds or any components of the drug product or previous treatment with the drug were excluded from the study. Patients who have undergone intra-thoracic or intra-abdominal surgery, history of head trauma, spinal trauma, or other acute trauma with an increased risk of bleeding, cerebral vascular accident (CVA) or intra-cerebral arterio-venous malformation (AVM), cerebral aneurysm, or mass lesions of the central nervous system or melena, hematemesis, hemophilia, deep-vein thrombosis or pulmonary embolism, history of solid organ transplantation, allogeneic bone marrow transplantation, or stem cell transplantation, Severe renal failure were also excluded from the study. Patients were also excluded if there was a need of anticoagulants, antiplatelet agents, anti-thrombotics and thrombolytics during the treatment period.

RESULTS

Totally 300 patients were screened and 250 patients were enrolled in the study as they fulfil inclusion criteria. Patients were randomised and are split in to two groups of ratio 1:1. One group is the control group which received placebo (n=125) and the other group is the intervention group which received the drug thrombobliss (n=125). The platelet count was monitored every day. The study period was about five days and a follow up at 15th day.

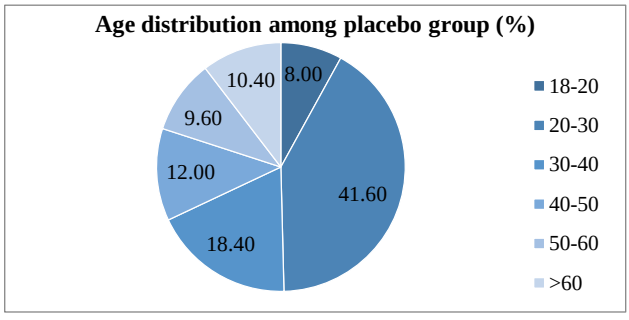

Figure 1: Age distribution of subjects among placebo group.

Demographic characteristics: Among the two groups, percentage of total male and female in placebo group is 52.8% and 47.2% respectively. Percentage of male and female in drug group is 44.8% and 55.2% respectively. Various age groups were participated in the study, amongst the highest percentage were in the age group of 20-30 years of age in both control group and intervention group (Figure 1 and 2). The drug thrombobliss is safe and no major side effects have been reported during the study.

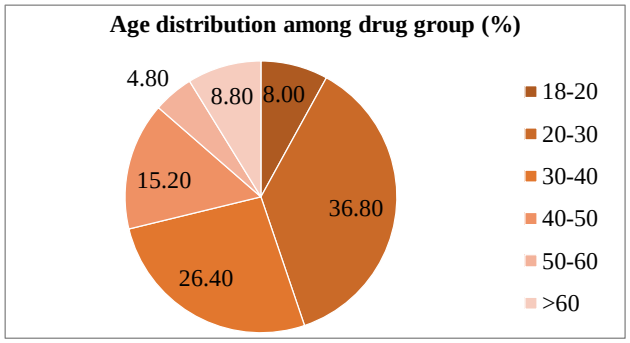

Figure 2: Age distribution subjects among drug group.

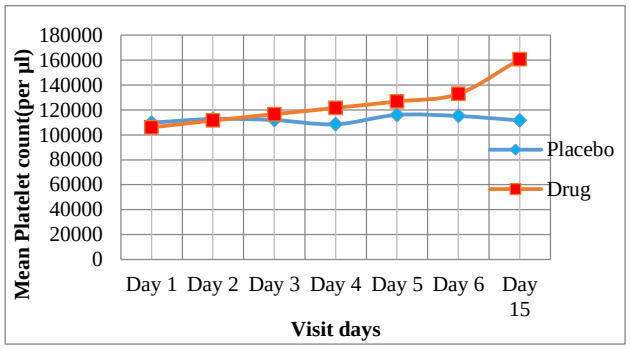

Figure 3: Comparison of mean platelet count at different end points.

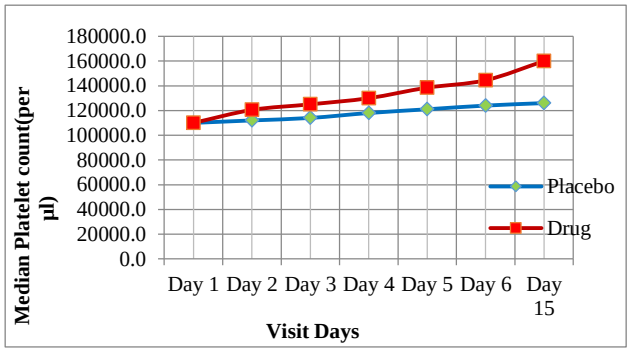

Figure 4: Comparison of median platelet count at different end points.

Primary outcomes: After the treatment, Both mean and the median data showed that there has been a significant increase in the platelet count after the administration of the drug thrombobliss compared to that of the placebo arm. The increase in the platelet count is seen to be increasing from day 2 in median platelet data and more evident after 72 hours as seen in both mean and median platelet count (Figure 3 and 4, Table 1 and 2) Even in the cancer patients who were undergoing chemotherapy, there has been significant increase in platelet count of the subjects who received drug. The statistical analysis of the data showed that there is a significant increase in platelet count and is statistical significant $p<0.05$.

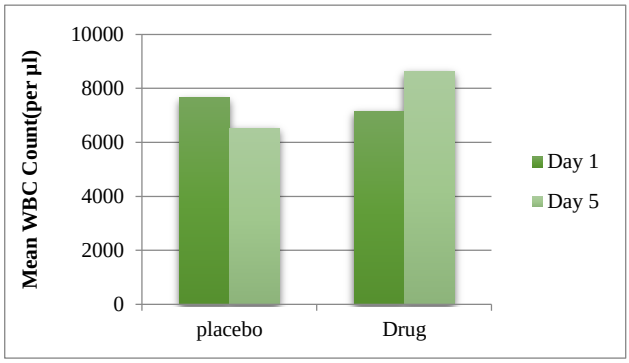

Figure 5: Comparison of mean WBC count at different end points.

Table 1: Platelet minimum, maximum and median value for placebo group.

Days	Number of subjects	Minimum value (per µl)	Maximum value (per µl)	Median Value (per µl)
1	125	28000	151000	110000
2	125	26000	161000	112000
3	125	29000	173000	114000
4	125	23000	184000	118000
5	125	26000	190000	121000
6	125	31000	193000	124000
15	125	26000	190000	126000

Table 2: Platelet minimum, maximum and median value for drug group.

Days	Number of subjects	Minimum value (per µl)	Maximum value (per µl)	Median Value (per µl)
1	125	25000	180000	110000
2	125	50000	180000	120500
3	125	50000	200000	125000
4	125	60000	200000	130000
5	125	55000	210000	138500
6	125	60000	230000	144500
15	125	85000	280000	160000

The WBC count values in the both the groups showed increasing trend and there was an increase in the WBC count in drug group as compared to placebo group after the treatment with thrombobliss, but the data are statistically insignificant $p<0.05$ (Figure 5).

The subjects have not received platelet transfusion and they have significant recovery. It is evident that thrombobliss is effective in increasing the platelet count in patients with systemic microbial infection and even in patients undergoing chemotherapy.

DISCUSSION

Thrombocytopenia occurs majorly in patient's incident with various dreadful bacterial and viral infections. There are no effective methods to treat thrombocytopenia.[27] Transfusions, growth factor injections and bone marrow transplant have their limitations. Management of thrombocytopenia is by drugs and blood products, both of which are costly. In this study, there has been significant increase in the platelet count in the intervention group (drug group) than the control group and it reaffirms the effectiveness of traditional use of *Carica papaya* in treating thrombocytopenia and *Tinospora cordifolia* in increasing immunity.[28]

Earlier studies have proved the effectiveness in treating dengue using *Carica papaya*.[29] In this study, it is found that the combination of *Carica papaya* and *Tinospora cordifolia* leaf extract to be effective in all treating all bacterial and viral infections related thrombocytopenia.

The results are similar to the animal studies that have been earlier carried out. It increases platelet count and is safe.[30]

Thrombocytopenia is a common issue in cancer treatment. Chemotherapy and radiation causes thrombocytopenia and other bleeding problems in certain cases. Due to these issues, chemotherapy is often delayed and treatment dose of chemotherapy is reduced which also increases the cost of treatment. Around 3000 dollars per event were spent to treat thrombocytopenia.[31] These disadvantages came be overcome to certain extent by the use of *Carica papaya* and *Tinospora cordifolia* leaf extract (Thrombobliss) as an adjuvant therapy to the patients undergoing chemotherapy. From the present study, it is evident that phyto extracts of *Carica papaya* and *Tinospora cordifolia* (Thrombobliss) enhance platelet in patients with infectious like Dengue and other infections associated with thrombocytopenia at a simple and cost effective manner.

CONCLUSION

The randomized, double-blind, placebo-controlled, study showed that novel combination of *Carica papaya* and *Tinospora cordifolia* leaf extract are effective in increasing platelet count in patients with thrombocytopenia. Thrombobliss is simple, cost effective and adjuvant therapy to treat patients with thrombocytopenia condition due to dengue and other microbial infection.

It is also evident that this combination is safe and effective even in patients undergoing chemotherapy and can be used to treat patients undergoing chemotherapy as adjuvant therapy for increasing platelet count.

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