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Original Article

Safety and Efficacy Study of Zyborica™ Tablet (*Carica papaya* Leaf Extract) for Thrombocytopenia Associated with Dengue Fever: A Randomized Double Blind Placebo Controlled Trial

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ABSTRACT

Objective: The present work was to evaluate the safety and efficacy of Zyborica™ Tablet, *Carica papaya* leaf extracts in low platelet count of (30,000 /µL to 150,000/ µL) patients reported with dengue fever. **Experimental Approach:** A total of 60 evaluable patients who met all the inclusion criteria and did not meet any of the exclusion criteria were randomized into Zyborica™ and placebo arms in 1:1 ratio. Eligible patients were enrolled post consenting to participate in the study, on an institutional ethics committee approved informed consent form on Day 1. Patients were instructed to administer either Zyborica™ or Placebo oral tablets 1100 mg thrice a day for five consecutive days (Day 2 to Day 6). Patient blood samples were collected from Visit 1 (Day 0) through Visit 5 (Day 6) for measuring their RBC, WBC, platelets, hematocrit values. Together with improvement in platelet count, Zyborica's efficacy in Dengue fever patients was evaluated. Safety and tolerability were assessed through the vitals and adverse effects. **Findings:** Zyborica™ demonstrated statistically significant change ($p<0.01$) in the platelets not only on Day 6 when compared to their respective Day 1 values but also when compared to that of Placebo. Zyborica showed no Adverse Events and no clinically significant changes in vital parameters and proved to be completely safe and tolerable when administered orally. **Conclusion: Zyborica™** Tablet has been found to have good patient compliance and also very effective in the improvement of decreased platelet count and other blood parameters in dengue patients, demonstrating its positive clinical effects in thrombocytopenia conditions.

KEYWORDS: Zyborica™, platelet, safety, tolerability, efficacy.

1. INTRODUCTION

Dengue is an acute viral infection with potential fatal complications. The incidence of dengue has grown dramatically around the world in recent decades. World health organization (WHO) currently estimates there may be

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50–100 million dengue infections and half a million dengue hemorrhagic fever (DHF) worldwide every year, with an average case fatality rate of around 5% [1, 2]. India had the highest burden of the disease, accounting for about a third of the world's cases. 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, transition of the febrile to the febrile phase of the illness, is the critical phase, and if left unattended or untreated it can lead to mortality.

Carica papaya is a member of the Caricaceae and is a dicotyledonous, polygamous, and diploid species [3]. *C. papaya* leaves have been used in folk medicine for centuries. Recent studies have shown its beneficial effect as an anti-inflammatory agent, for its wound healing properties [4], antitumor as well as immune modulatory effects [5] and as an antioxidant [6]. 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. Safety studies based on OECD guidelines for acute, sub-acute, and chronic toxicity were conducted on *C. papaya* extract and showed that it was found to be safe for human consumption [7]. *C. papaya* leaf extract has attracted attentions because of the promising results shown from the various studies conducted in South East Asian countries on the effectiveness of *C. papaya* leaf extract on increasing the platelet counts in patients with thrombocytopenia associated with dengue. There are no approved vaccines or drugs against dengue; 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.

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 /µl, 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 (thrombopoietin) agonists and mimetics like Eltrombopag and Romiplastim 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 existing scenario, 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 [8, 9]. The evolution of *Carica papaya* leaf extract in the management of thrombocytopenia associated with dengue is significant as it would be better & viable option in fever associated with thrombocytopenia.

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.

2. METHODS

Objective and Rationale: In view of existing data in the treatment of thrombocytopenia and as there is no existing clinical data on a single indigenous formula like **Zyborica™**, *Carica papaya* leaf extracts, and the present study was initiated to evaluate the safety and efficacy of Zyborica™ in dengue patients reported with thrombocytopenia.

Ethics: The study was conducted in Pristine Hospital & Research Centre Pvt Ltd in Bangalore. Institutional Ethics Committees provided favorable opinion for the conduct of this study. No further changes or amendments were made to the approved protocol and was executed in its complete form. This study was conducted in accordance with the clinical research guidelines established by the Drugs and Cosmetics Act, 1940 of India, Drugs and Cosmetics Rules, 1945 of India, Ethical Guidelines for Biomedical Research on Human Participants, 2006 of Indian Council of Medical Research (ICMR) in India, the principles enunciated in the Declaration of Helsinki (Edinburgh, 2000) and the ICH harmonized tripartite guideline regarding Good Clinical Practice (GCP).

Subject Information and Consent: Written and oral information about the study in a language understandable by the subject was provided to all subjects. Each subject was informed by the investigator, prior to the screening evaluation, of the purpose of this clinical trial, including possible risks and benefits and documented the informed consent process in the subject's chart. Ample time was provided for each subject to decide whether to participate in the study and all the questions and clarifications regarding the study were clarified by the investigator.

Study Design and Selection of Study Subjects: This was a double blinded study design; subjects who met all of the inclusion criteria and did not meet any of the exclusion criteria were included in the study. 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 platelet count of 30,000/µL to 150,000/µL; 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.Patients who are willing to give informed consent to participate in the study. Exclusion criteria: 1.Patients with Dengue hemorrhagic fever grade III and IV, 2. Patients with platelet count less than 30000/micro litre, 3. Pregnant or lactating women, 4. Patients who have received blood or blood products transfusion during the current illness, 5.Patients with idiopathic 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. A total of 64 patients were screened, 61 of them who met all the inclusion criteria and did not meet any of the exclusion criteria were enrolled and out of which 60 completed the study. The first patient was enrolled on 23 Jul 2018 and the last patient completed the study 07 Dec 2018.

Methodology and Study Procedures: Patients who fulfilled the eligibility criteria were included into the study, and were advised to administer the study medication (Zyborica™) orally 3 tablets daily, half an hour after meals for 5 consecutive days, in addition to receiving the standard management of dengue. Safety and tolerability were assessed through the adverse effects. Efficacy was assessed through measurement of platelet count at every visit, starting from Day 1 through Day 6; changes in RBC, WBC, Hematocrit values from the baseline levels till the end of therapy were also measured as secondary objectives of the study. A protocol violation was defined as any change, deviation, or departure from the study design or procedures of protocol or deviation from standard operating procedures, Good Clinical Practices (GCPs).Data was captured using case report forms (CRF). All the fields in the CRF were filled. Any missing fields were explained. All entries were legible and on the CRF of every subject, no information was provided that reveal the identity of the patient. Only the code numbers (screening numbers, patient ID) were written on the paper based CRF. The first patient was enrolled on 23 Jul 2018 and the last patient completed the study on 07 Dec 2018.

Safety and efficacy outcomes:

The safety outcomes were vital measurements, adverse events and non-tolerability to the study investigational product. Efficacy outcomes were increase in low platelet count when compared to their respective screening visit/Day 1 values and also placebo group values. Improvement in RBC, WBC and Hematocrit values were also considered as efficacy outcomes.

Statistical Analysis:

Statistical comparisons were evaluated at the 5 % ($p<0.05$) significant level. Comparison of means between treatment groups was performed by two tailed ANOVA.

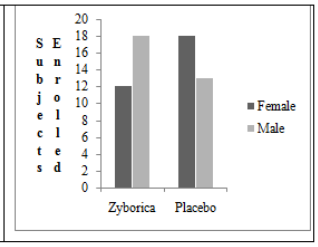
3. RESULTS AND DISCUSSION

The main concept in treating thrombocytopenia is to eliminate the underlying problem, whether that means discontinuing suspected drugs that cause thrombocytopenia, or treating underlying sepsis. Corticosteroids, intravenous immune globulin, and splenectomy remain mainstays of treatment; however, newer therapies including rituximab and the thrombopoiet in receptor agonists are remodeling conventional treatment algorithms. Immune suppressant medications and cytotoxic drugs continue to be used in patients with severe and chronic refractory ITP with some success; however, estimates of the effect of these and other treatments are limited. In severe cases and associated with bleeding platelet transfusion is recommended.

All these above mentioned treatment options have their own advantages and disadvantages. Some cons include severe toxicities and immunosuppressant effects of corticosteroids and severe patient noncompliance in platelet transfusion. There are few drugs available in the market eg. Romiplastin, Eltrombopag, associated with many adverse effects and it may not be affordable to all. The transfusion needs institutionalization which would incur cost, asks for provision of infrastructure which may not be available at all places making it inaccessible and unaffordable to many of the patients. Therefore, consideration should be given to alternate therapies to combat the low platelet count which is relatively free from the toxic side effects of the drugs. [10-14]

Table 1: Patient demographics on screening visit

Item	Mean values
Age (yrs)	62.14
Height (feet)	5.28
Weight (kgs)	71.6
Smoking (Yes)	12
Alcohol (Yes)	9



Almost similar number of male and female patients participated in the study, with female participants on a bit higher side. Average age, height and weight of the patients is depicted in Table 1. Sequence of activities performed during every visit is illustrated in Table 2. Approximately 12% of the enrolled subjects were smokers and 9% were alcoholics for at least 3 years prior to participation of the study. Vital signs like temperature, pulse rate, heart rate, and blood pressure were measured and recorded from screening till last visit, Day 6. Combined with vital parameters, adverse events and tolerability, the product Zyborica exhibited a very good safety profile with no adverse events and was found to be well tolerated by all the study subjects. No statistical

significant changes observed in other vital parameters but the decreased platelet count on the screening visit was found to have an increasing trend from the Day 4 itself. However, it reached statistical significance ($p<0.05$) from Day 3 until Day 6 (Fig. 1). Respective WBC, RBC and HCT final visit (Day 6) values when compared to their respective screening visit (Fig. 2 to Fig. 4) were found to be statistically significant ($p<0.01$). No changes were observed in the LFT and RFT tests. Compliance with study supplement was reviewed at each visit by examination of the returned used bottles. All accountability records were incorporated into the investigator's study file. The patient compliance to the study product was almost 98% (data not shown).

Table 2: Schedule of Assessments/Evaluations

Activities	Screening (Day 1)	Day 2	Day 3	Day 4	Day 5	Day 6
	Visit 0	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Eligibility criteria	X					
Informed consent	X					
Demographic data	X					
Medical history	X					
Physical examination	X					
Vital signs	X	X	X	X	X	X
ECG	X					X
Urine Pregnancy test	X					
Platelet count	X	X	X	X	X	X
Hematocrit	X	X	X	X	X	X
RBC	X	X	X	X	X	X
WBC	X	X	X	X	X	X
LFT	X					
RFT	X					
Randomization	X					
IP dispensing and reconciliation	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X

Small sample size with no power calculation was one of the limitations of this study. The percentage of herbal extract of Zyborica is proprietary and confidential information of MiLab Life Sciences (P) Ltd, hence they are not disclosed here. Nevertheless, the final product that was used for the trial has passed all the required QC (Quality Control) procedures. All the investigational products used in this study were from three different batches manufactured in a GMP compliant plant in India during the period of February to May 2018 with a shelf life of 3 years. The results indicate that there were no batch to batch variations and the product exhibited consistently good results throughout the clinical trial study duration. The results of overall investigator and patient global improvement, one of the subjective efficacy assessments, were overwhelming.

Mechanism of Action - Certain genes have been shown to influence platelet production and platelet aggregation, namely, the **Arachidonate 12-lipoxygenase (ALOX 12)** also known as the Platelet-type Lipoxygenase as well as the **Platelet-Activating Factor Receptor (PTAFR)**. An increase in activity of these genes is required for platelet production and activation. The **ALOX 12** gene is strongly expressed in megakaryocytes and has been known to be responsible for the **12-Hydroxyeicosatetraenoic acid (12-HETE)** production of platelets [15]. The **PTAFR** gene was been found to be expressed in megakaryocytes indicating that it could be a precursor for platelet production in addition to its well-known role in platelet aggregation.

ALOX 12 is known to be associated with increased megakaryocyte production as well as its conversion to platelets through **12-HETE** mediated pathway which in turn leads to increased platelet production [16].

These results are similar to those reported by Sathasivam et al. that CP leaves extract can increase platelet count in mice, and also in dengue fever patient as reported earlier [17].

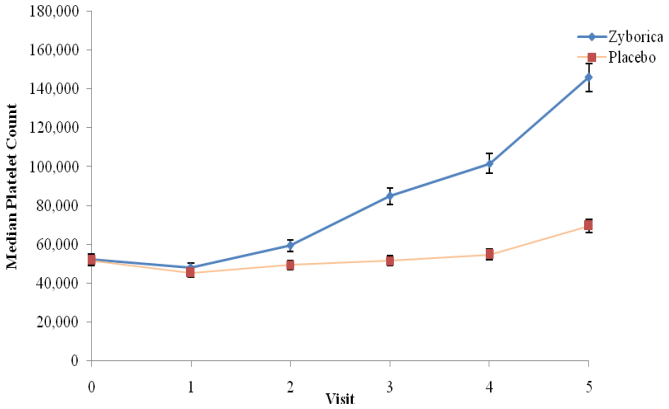


Fig 1: Comparison of Platelet Count between two groups at different time points

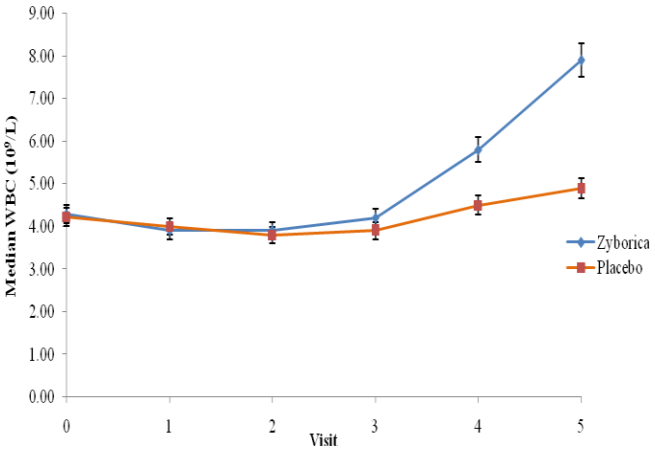


Fig 2: Comparison of WBC between two groups at different time point

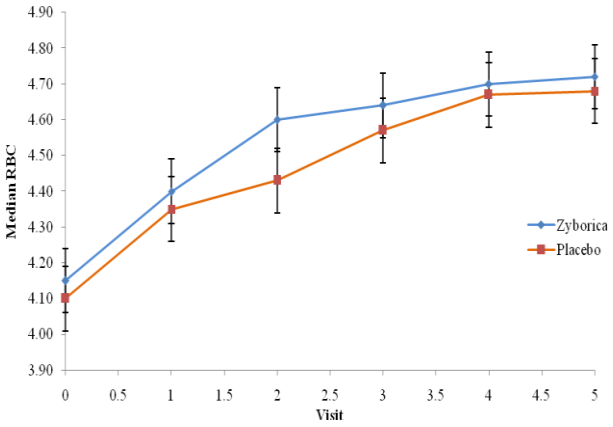


Fig 3: Comparison of median RBC between two groups at different time point

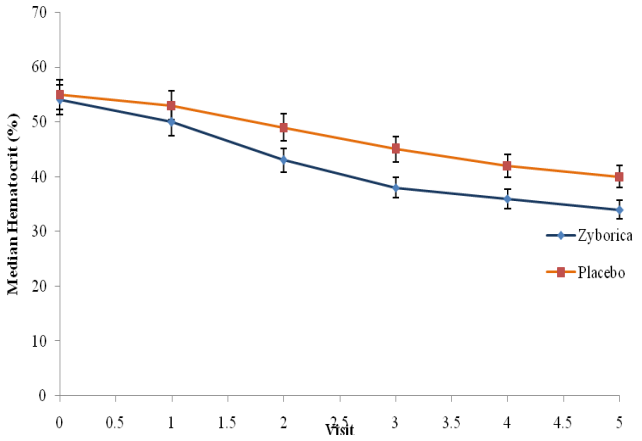


Fig 4: Comparison of Hematocrit between two groups at diffèrent time point

4. CONCLUSION

Zyborica™ tablets containing 1100 mg of *Carica papaya* leaf extract when administered thrice a day is found to be a trusted treatment option for thrompbocytopenia with very good patient compliance. This study has proved time and again the beneficial effects of *Carica papaya* leaf extract in increasing the platelet count, here in this study in dengue fever patients, when administered along with standard treatment of care.

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