



Platelet Augmentation Potential of Polyherbal Formulation in Cyclophosphamide-Induced Thrombocytopenia in Wistar Rats

Girish Sailor¹, Komal Hirani², Ghanshyam Parmar³, Rajesh Maheshwari³, Rupa Singh³, Avinash Kumar Seth³

¹ Bhagwan Mahavir College of Pharmacy, Vesu, Surat, Gujarat, India

² Vital Care Pvt Ltd, Por-Ramangamdi, Vadodara, Gujarat, India

³ Department of Pharmacy, Sumandeep Vidyapeeth, Piparia, Waghodia, Vadodara, Gujarat, India

Corresponding author: Girish Sailor, Bhagwan Mahavir College of Pharmacy, BMEF Campus, Bharthana Road, Vesu, Surat-395017, Gujarat, India; E-mail: sailorgirish@gmail.com; Tel.: +919727747461

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Abstract

Introduction: Thrombocytopenia is a condition characterized by abnormally low levels of thrombocytes, also known as platelets, in the blood. Several medicinal plants possess curative and protective effect against thrombocytopenia associated with diseases or drugs.

Aim: In the present study, we have investigated the platelet augmentation activity of polyherbal formulation (VITA PLAT Capsule) in cyclophosphamide-induced thrombocytopenic rat model.

Materials and methods: Twenty-four albino Wistar rats were divided into four groups. Thrombocytopenia was induced in the rats by administering cyclophosphamide (25 mg/kg, i.p.) for three days to all the groups except normal controls. The test groups were given orally a polyherbal formulation suspended in normal saline for 14 days. Blood was withdrawn from the retro-orbital plexus of the rats on days 1, 7, and 14 of study to determine platelet counts in all groups. Clotting time and bleeding time were determined on the last day of study. Data were collected and analyzed using GraphPad Prism 8.

Results: The results showed that the polyherbal formulation treatment could significantly ameliorate platelet count in thrombocytopenic rats in the initial as well as in the later phase. The total WBC count was also improved during later phase in test groups. However, there is no significant difference between clotting time and bleeding time in all groups.

Conclusions: Our study suggests a potential role of this formulation in the augmentation of platelet counts in various thrombocytopenic disorders including a role in ameliorating the haemorrhagic complications of dengue fever.

Keywords

cyclophosphamide, platelet augmentation, polyherbal formulation, thrombocytopenia

INTRODUCTION

Thrombocytopenia is a condition associated with a lower production of platelets than the normal numbers in the bone marrow and is often multifactorial.¹ The physiological range for thrombocytes in normal healthy human is $150\text{--}400 \times 10^9/L$

L of blood. Thrombocytopenia causes few signs or symptoms with platelet counts in the range of $50\text{--}100 \times 10^9/L$ that represent moderate-to-severe thrombocytopenia. Thrombocytopenia is often associated with certain diseases such as dengue, idiopathic thrombocytopenic purpura (ITP), malignancy, chikungunya, aplastic anaemia, drug induced throm-

bocytopenic purpura, and haemolytic uremic syndrome.² Treatment of thrombocytopenia is largely dependent upon the cause and severity of the condition.³ Normally, the first line of treatment includes use of corticosteroids, immunoglobulins and splenectomy; this treatment, however, is not effective for 25 to 30% of the patients with chronic idiopathic thrombocytopenic purpura (ITP).⁴

In India, dengue is endemic in almost all states and is the leading cause of hospitalization.^{5,6} It destroys the immune system and causes headache, inflammation, hypertension, mental disorders, and thrombocytopenia. Death occurs due to its adverse effects on liver and excessive bleeding. As there is currently no effective vaccine for dengue, common people resort to traditional medicine to cure it using a specific plant that has shown to be able to increase the platelet count and to promote hepatoprotection against its devastating virus.⁷

In the present study, we used cyclophosphamide, an antineoplastic and immunosuppressive agent, as a toxicant because of its capacity to induce stable thrombocytopenia.⁸ Intraperitoneal injections of cyclophosphamide (25 mg/kg/day) for three consecutive days have been found to be an uncomplicated, viable and stable thrombocytopenia model for rats.⁹

According to Ayurveda, a combination of drugs is used to improve the desired action and eliminate unwanted side effects. A polyherbal formulation (PHF) is composed of several medicinal plants (Table 1) that are known to exert a curative, protective effect against different types of thrombocytopenia associated with diseases or drugs as single or in other combinations.¹⁰⁻¹⁵ However, no preclinical evaluation has been performed yet to investigate the platelet boosting effect of PHF. Hence, the present preclinical study was performed to evaluate the platelet augmentation activity of a herbal formulation in cyclophosphamide-induced thrombocytopenia in rats to explore the possible use of PHF in cases of thrombocytopenia, especially in dengue.

MATERIALS AND METHODS

Drugs and chemicals

Cyclophosphamide injection (Endoxan) was purchased from local pharmacy. Polyherbal formulation (VITA PLAT Capsule) was provided by Vital Healthcare, Vadodara.

Table 1. Composition of VITA PLAT Capsule

Ingredients	Reference	Part used	Botanical name	Qty
Papaya extract	Aryabhishak	Leaf	<i>Carica papaya</i>	250 mg
Patha extract	Bhavaprakash	Root	<i>Cissampelospareira</i>	75 mg
Punarnava extract	Bhavaprakash	Whole plant	<i>Boerhaviadiffusa</i>	75 mg
Guduchisatva	Bhavaprakash	Stem	<i>Tinosporacordifolia</i>	75 mg
Neem extract	Bhavaprakash	Bark	<i>Azadirachtaindica</i>	50 mg

The diagnostic kits used in the study were procured from Transasia Bio-Medicals Ltd., Mumbai. All other chemicals and reagent used in the study were of analytical grade.

Experimental animal

The study was approved by the Institutional Animal Ethics Committee (IAEC) of Sumandeep Vidyapeeth, Vadodara and all the animal experiments and protocols were performed as per the guidelines issued by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The experiment was carried out on healthy adult albino Wistar rats of either sex weighing 200-250 g. Rats were housed in polypropylene cages, maintained under standardized condition (12-h light/dark cycle, 24°C, 35 to 60% humidity) and allowed free access to diet and purified drinking water.

Cyclophosphamide induced thrombocytopenia

Albino Wistar rats (200–250 g) of either sex were divided into 4 groups, each of 6 rats. Group I received 0.5% CMC (1 ml/kg, p.o.) from day 1 to day 14 and served as normal control. Group II received cyclophosphamide (50 mg/kg, i.p) from day 1 to day 3 and thereafter 0.5% CMC (1 ml/kg, p.o.) from day 4 to day 14 and served as a toxic control group. Similarly, groups III and IV received cyclophosphamide (50 mg/kg, i.p) from day 1 to day 3 and thereafter PHF, 200 and 400 mg/kg, p.o., respectively, for 14 days and served as treatment groups. All the test formulations were administered as a suspension in 0.5% of CMC. The animal equivalent dose of PHF was calculated from the human therapeutic dose of product label claim (2.1 g/day).¹⁶

Blood samples were withdrawn 1 h before starting of the experiment and also on days 7 and 14 of the experiment after administration of PHF. The blood was collected from the retro-orbital plexus of rats under light anesthesia, using glass capillaries and stored with disodium ethylenediaminetetraacetate for estimation of biochemical parameters. Total platelet count (PC), total leucocyte count (TLC), differential leucocyte count (DLC) including neutrophils, lymphocyte, monocyte, eosinophil and basophil were measured.¹⁷ The usual activities, fur lustre, amount of diet and drinking, and change of weight and mortality rate of the rats were observed closely. The animals were weighed every day for 14 days.

Determination of clotting time

At 14 days, a glass capillary (inner diameter=0.9~1.1 mm, length=10 cm) was inserted into one eye of rat. Then the capillary was removed and placed on the desk horizontally until the blood full filled it. Every 30 seconds the capillary was broken and stretched gently to see if there were fibrin threads of blood at the breakage point. The time from bleeding to the fibrin threads was recorded.

Determination of bleeding time

Bleeding time was determined by a modified Duke method. The animal was kept in a rat restrainer with the tail exposed out. The animal tail was cleaned using hot water, rectified spirit, and its tip was punctured using a sterile needle and blotted on Whatman® filter paper until bleeding stopped. Bleeding time was recorded in seconds.

Statistical analysis

Values were expressed as mean \pm SEM and data were analyzed using one-way ANOVA followed by the Dunnett's Multiple Comparison Test at 95% confidence interval using GraphPad Prism (version 8; Graph- Pad Software Inc., San Diego, CA, USA). The significance level was set at $p < 0.05$.

RESULTS

Effects of PHF on platelet count

The present study was carried out to evaluate platelet augmentation potential of commercially available capsule VITA PLAT on cyclophosphamide-induced thrombocytopenia in rats. We found that the mean platelet count

was not significantly different between all the groups prior to the induction of thrombocytopenia (Fig. 1). However, platelet count was significantly ($p < 0.01$) decreased in the toxic control group as compared to normal control at day 7, which indicates successful induction of thrombocytopenia by cyclophosphamide. It was also observed that pretreatment of animals with PHF at a dose of 200 and 400 mg/kg protected the drastic reduction of platelet count as compared to the toxic control group. Additionally, on continuation of animal treatment with PHF, we found a significant ($p < 0.001$) increase in platelet count in comparison with the toxic control group (Fig. 1).

Effects of poly-herbal drug on total WBC count

Total WBC significantly ($p < 0.001$, $p < 0.05$) decreased in the toxic control group as compared to normal controls on days 7 and 14. Treatment with PHF (200 or 400 mg/kg) caused no significant differences in the total WBC on day 7, but significantly increased ($p < 0.001$) the total WBC on day 14 as compared to CP treated rats (Fig. 2).

Bleeding time and clotting time

The bleeding time and clotting time at day 14 showed no significant differences between the groups. This may be due to improvement in the platelet count of the treated as well as non-treated groups (Figs 3, 4).

DISCUSSION

Thrombocytopenia is a condition characterized by abnormally low levels of thrombocytes in the blood.¹⁸ Low platelet counts, or thrombocytopenia, can be caused by a variety of reasons. In general, these can be either a decre-

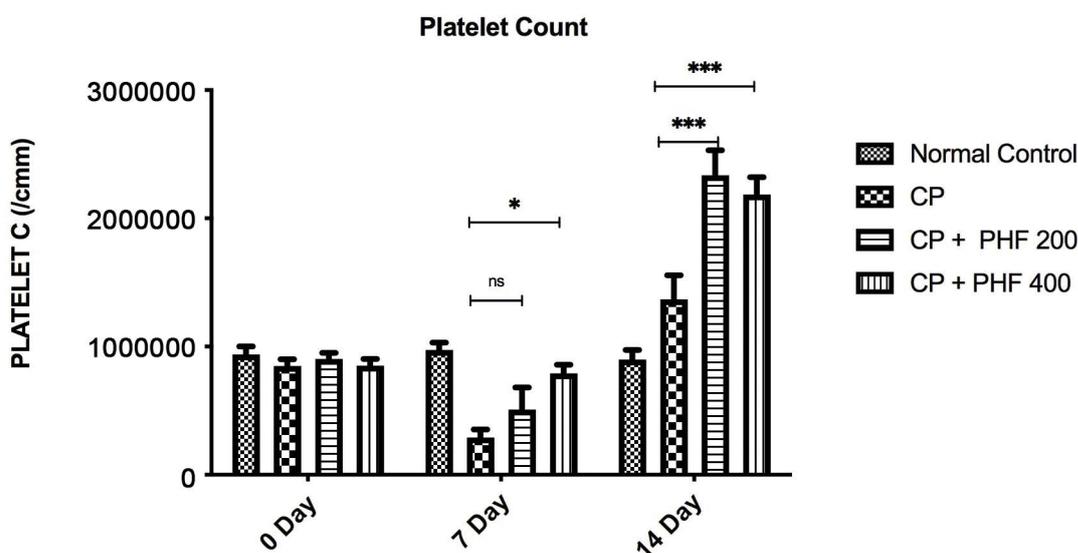


Figure 1. Effect of PHF on platelet count; values are expressed as mean \pm SEM ($n=6$); * $p < 0.05$, *** $p < 0.001$ compared to the CP group.

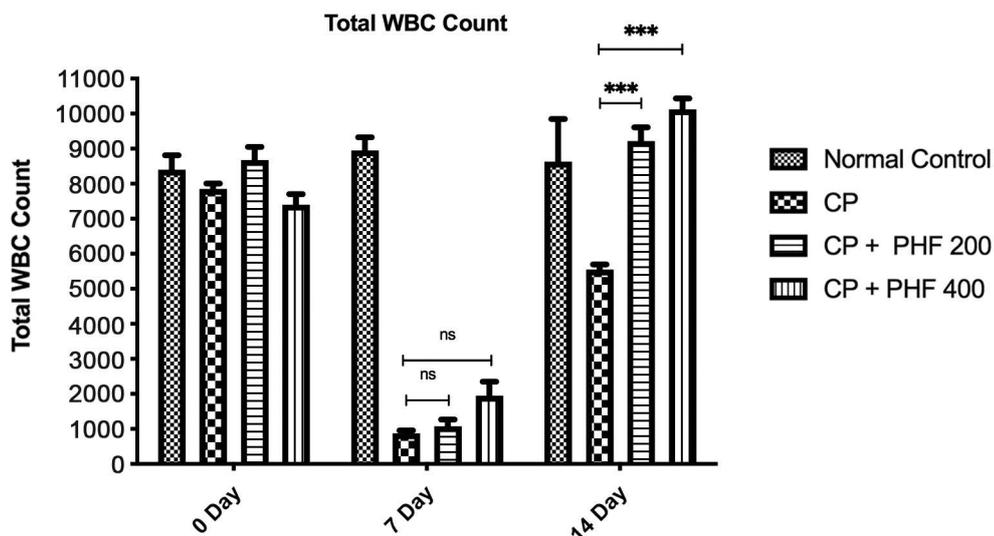


Figure 2. Effect of PHF on total WBC count; values are expressed as mean ± SEM (n=6); *** $p < 0.001$ compared to the CP group.

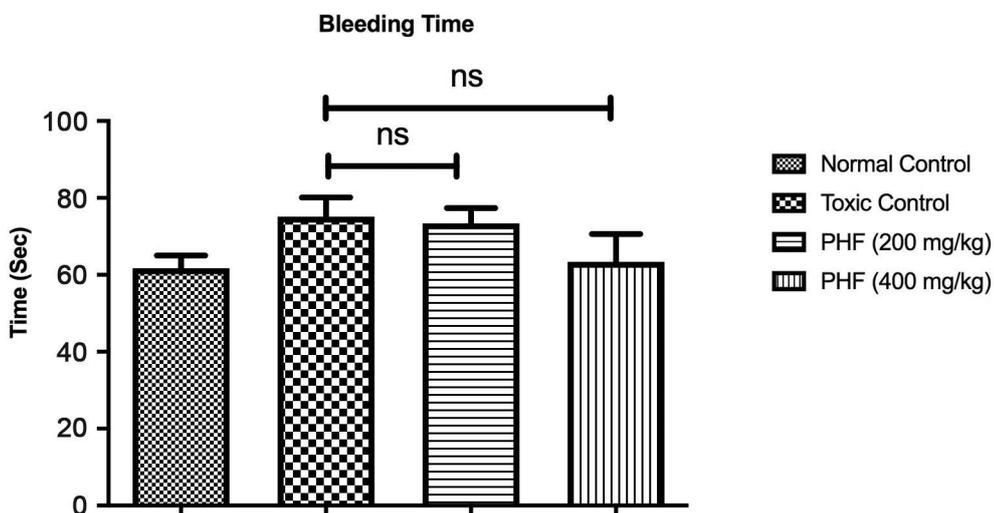


Figure 3. Effect of PHF on bleeding time; values are expressed as mean ± SEM (n=6).

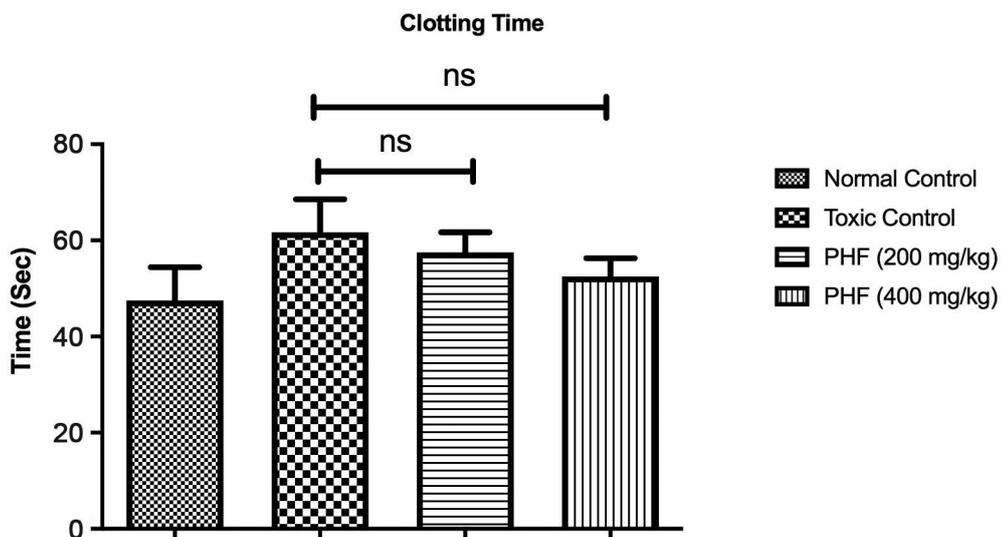


Figure 4. Effect of PHF on clotting time; values are expressed as mean ± SEM (n=6).

ased platelet production, or increased platelet destruction, or consumption, or it may be caused by taking drugs which have side effects of lowering the platelet count, or by conditions such as dengue, malaria, etc.^{18,19} In the present study, cyclophosphamide, an anticancer drug, is used to induce thrombocytopenia.^{20,21} The results show a significant reduction in platelet count in rats which received cyclophosphamide compared to normal rats due to its adverse effect.

In recent years, there has been a renaissance of herbal formulations due to their greater efficacy, lesser side effects, and better acceptability than modern drugs.^{22,23} Additionally, as a result of advances in science and better understanding of the PHF's phytoconstituents, newer combinations of herbal preparation were developed. This helped us to prepare formulations which work synergistically with the desired effect. VITA PLAT is a novel polyherbal formulation containing the medicinal plants that have been well known to be capable of treating thrombocytopenia.

The results of the *in-vivo* preclinical study indicate usefulness of this polyherbal formulation, VITA PLAT, in thrombocytopenia, which was confirmed by the significant increase in platelet count as compared to cyclophosphamide-treated animals. This particular effect of PHF may also be explained by the anti-thrombocytopenic effect of the constituent medicinal plants.¹⁵ The results also explained the protective effect of PHF during the initial critical phase (0-7 days) of thrombocytopenia induction using cyclophosphamide. Furthermore, a long-term (0-14 days) treatment with PHF shows that there is good improvement of platelet count as compared to that of diseased animal recovering from the thrombocytopenic condition. In addition, PHF treatment is also able to improve the total WBC which can be helpful in boosting the immunity. Hence, VITA PLAT can be used for a longer time in disease conditions in which there is a drastic decrease of the platelet count.

Additionally, the report of a previous study suggests that the times of bleeding and clotting can be increased in animals with thrombocytopenia.¹² However, the results of the present study did not show any significant differences in bleeding and clotting times between groups at 14 days. This may be due to the improvement in the platelet count of treated and non-treated groups.

CONCLUSIONS

Thrombocytopenia occurs in diseases such as dengue, malaria and others in which the first few days after onset of disease are very crucial for the patient as there is a rapid decline in the platelet count. Our study suggests that the polyherbal formulation exhibited an excellent protection effect in this initial critical period of platelet decline in the disease induced animals. Additionally, a long-term use of this formulation is also helpful in early recovery from the disease with low platelet count. The present study therefore

confirms that VITAL PLAT capsule shows great promise in managing both the initial and the later phases of any disease associated with thrombocytopenia.

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Потенциал роста тромбоцитов в многотравном составе при тромбоцитопении, индуцированной циклофосфамидом, у крыс линии Wistar

Гириш Сейлор¹, Комал Хирани², Ганшиям Пармар³, Раджеш Махешвари³, Рупа Сингх³, Авинаш Кумар Сет³

¹ Фармацевтический колледж Бхагвана Махавира, Везу, Сурат, Гуджарат, Индия

² Vital Care Pvt Ltd, Ваходара, Гуджарат, Индия

³ Факультет фармации университета „Sumandeep Vidyaapeeth“, Пинариа, Ваходия, Ваходара, Гуджарат, Индия

Адрес для корреспонденции: Гириш Сейлор, Фармацевтический колледж Бхагвана Махавира, Везу, Сурат, Гуджарат, Индия; E-mail: sailorgirish@gmail.com; Tel.: +919727747461

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Резюме

Введение: Тромбоцитопения – это состояние, характеризующееся аномально низким уровнем тромбоцитов, также известных как кровяные пластинки, в крови. Некоторые лекарственные растения обладают лечебным и профилактическим действием против тромбоцитопении, связанной с заболеваниями или лекарствами.

Цель: В настоящем исследовании мы исследовали активность роста количества тромбоцитов в многотравном составе (капсулы VITA PLAT) при тромбоцитопении, вызванной циклофосфамидом, на модели крыс.

Материалы и методы: Двадцать четыре крысы-альбиносы линии Wistar были разделены на четыре группы. Тромбоцитопению у крыс вызывали введением циклофосфамида (25 мг/кг внутривенно) в течение трёх дней всем группам, кроме контрольной. Опытным группам давали орально смесь многотравного состава, суспендированную в физиологическом растворе, в течение 14 дней. Кровь брали из ретроорбитального сплетения крыс на 1, 7 и 14 дни исследования для определения уровня тромбоцитов во всех группах. Время свёртывания и кровотечения определяли в последний день исследования. Данные были собраны и проанализированы с помощью GraphPad Prism 8.

Результаты: Результаты показали, что лечение смесью многотравного состава значительно улучшит уровень тромбоцитов у крыс с тромбоцитопенией как на начальной, так и на более поздней стадии. Общее количество лейкоцитов также улучшилось на более позднем этапе в экспериментальных группах. Однако не было значимой разницы между временем свёртывания и временем кровотечения во всех группах.

Заключение: Наше исследование показало потенциальную роль этого состава в повышении уровня тромбоцитов при различных тромбоцитопенических расстройствах, включая его роль в улучшении геморрагических осложнений при лихорадке денге.

Ключевые слова

циклофосфамид, рост тромбоцитов, многотравный состав, тромбоцитопения
