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Research Article

Platelet Augmentation Activity of Selected Philippine Plants

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Abstract

Various plants in the Philippines are currently being used to increase platelet counts in thrombocytopenic disorders including dengue hemorrhagic fever but the efficacy of such claims is uncertain. The present study investigated the platelet augmentation activity of *Carica papaya* L. (Family- Caricaceae), *Ipomea batatas* (L.) Lam (Family-Convolvulaceae), *Althernanthera sessilis* (L.) R. Brown ex De Candolle (Family-Amaranthaceae), *Euphorbia hirta* (L.) (Family-Euphorbiaceae) and *Momordica charantia* L. (Family-Cucurbitaceae) as potential therapy for thrombocytopenia. Plant extracts were screened for phytochemical constituents. Platelet reduction on Sprague-Dawley rats was induced by oral administration of 0.083 mg/kg body weight of anagrelide. A solution of the lyophilized aqueous plant samples were administered for 9 days. Pre-and post-treatment blood samples for platelet counts were taken on the tenth day. Results showed that all the plant extracts tested were positive for glycosides. Extracts from *Carica papaya* (p=0.0002), *Ipomea batatas* violet variety (p=0.0070) and green variety (p=0.0000), *Alternanthera sessilis* (p=0.0001) and *Euphorbia hirta* (p=0.0489) have significant platelet augmentation (p<0.05) activity. Only *Momordica charantia* extract failed to show significant platelet increasing activity (p=0.1014). The percentage increase of mean platelet counts after reduction with anagrelide were as follows: *Carica papaya* group (125.87%), *Ipomea batatas* green variety group (107.88%), *Ipomea batatas* violet variety group (106.07%), *Althernanthera sessilis* group (93.17%) and *Euphorbia hirta* group (80.92%). These results suggest that extracts from *Carica papaya*, and *Ipomea batatas* green and violet variety, *Althernanthera sessilis* and *Euphorbia hirta* may be used as potential supportive treatment for thrombocytopenic disorders.

1. INTRODUCTION

Imbalances in normal platelet counts are typically caused by genetic disorders or fatal diseases such as dengue hemorrhagic fever (DHF). Inability to provide supportive treatment such as platelet transfusions to correct decrease in platelet counts often proves fatal to patients. In most rural areas, a health care institution is often inaccessible and most patients cannot afford the high cost of hospitalization. In lieu of this, herbal medicines are often used as alternative treatment. Several decoctions of herbal plants taken by patients are purportedly used to address platelet imbalances due to DHF. With the increasing demand for other treatment options for thrombocytopenic disorders aside from platelet transfusion, this study is conducted to investigate various plants that have been reported for their pharmacological activities such as *Carica papaya*[1-5], *Ipomea batatas*[6-8], *Alternanthera sessilis*[9,10], *Euphorbia hirta*[11,12] and *Momordica charantia*[13-15], which may have the property to increase platelet counts.

2. MATERIALS AND METHODS

2.1 Collection and Processing of Plant Material

Plant specimens were collected from February to April 2011. All plant samples were taken in various regions in the Philippines. *Carica papaya* leaves were taken from San Pablo, Laguna, while the leaves of *Momordica charantia* were collected from Cotabato. *Ipomea batatas* green and violet variety and the whole plant samples of *Euphorbia hirta* L. were provided by the faculty members of the College of Health Sciences (Pharmacy), Mariano Marcos State University, Ilocos Norte while *Alternanthera sessilis* were collected from Dao, Capiz. A herbarium specimen was submitted to the National Museum of the Philippines and authenticated by Dr. Wilfredo F. Vendivil, Chief of Botany Division with control number 672-675 and 485. The freshly collected leaves of *Althernanthera sessilis*, (L.) R. Brown ex De Candolle Family-Amaranthaceae, *Carica papaya (*L.) Family-Caricaceae, *Momordica charantia (*L). Family-Cucurbitacea*, Euphorbia hirta* (L.) Family-Euphorbiaceae and *Ipomea batatas* (L.) Lam Family-Convolvulaceae were washed thoroughly to remove dirt and were dried under the shade for three days. The dried samples were powdered using the Bauknecht 80 Milling Apparatus and sieved through mesh number 80. The recovery rate of powder was 10%. The recovered material was stored in a plastic bag and sealed securely to render it airtight. The weights before and after drying, powdering, and sieving were determined to monitor the yield after each process involved in the investigation. The powdered plant samples were used for extraction process. Fifty grams of each of the powdered sample was extracted by percolation using distilled water as the solvent. The extracts were then dried by lyophilization in the Philippine General Hospital. Lyophillized extracts were weighed, labeled properly, stored in amber bottles and placed in ziplock plastics and kept in refrigerator.

2.2 Phytochemical Screening

The presence of various phytochemical constituents in the extract was determined using standard screening tests for pH, tannins, glycosides, flavones, flavonoids, reducing substances, alkaloids, plant acids and saponins.

2.3 Platelet Determination

Thirty six, 6-8 weeks old Sprague-Dawley rats (18 female and 18 male) were procured from the Philippine Food and Drug Administration. They were acclimatized for two weeks and assigned in individual cages with free access to food and water. The protocol

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was approved by the Institutional Animal care and Use Committee (IACUC) of the National Institutes of Health.

Platelet baseline count was determined by extracting 0.5ml of blood from the saphenous vein of the rats. The blood was collected using heparinized tubes and was placed in vacutainers containing EDTA. Collected blood was submitted to the University of the Philippines Diliman, Veterinary Teaching Hospital for platelet counting. Platelet reduction was then induced by oral administration of 0.083mg/ kg body weight of Anagrelide (Agrylin) which was given for 15 days. The platelet inhibitor Anagrelide (Agrylin®) was procured from Globo Asiatico. The reference range was used for normal platelet count was 500-1,300 x10^3/µL. Anagrelide was dissolved in distilled water and placed in a sonicator for 15 minutes.

The doses given were 0.2mg/ml and 1mg/ml. For platelet counts of rats which did not still respond well to the previous doses of 0.2 mg/ml, the dose of 1mg/ml was finally given after 3rd extraction of blood.

Platelet counts were collected five times including the baseline count. Decrease in platelets was monitored every three days for 15 days. When the decrease in platelets was established, aqueous solution of test samples was administered for 9 days. Water was used as negative control. On the 9th day cardiac puncture was performed on anesthetized rats to obtain the blood samples for platelet count. The values were based from the University of Minnesota Reference Values for Laboratory Animals-Normal Hematology Values[16].

2.4 Data Analysis

The percent difference of the platelet counts of the consecutive blood collections were computed and tabulated to determine their percent decrease and increase from the platelet baseline. Percent difference was computed by dividing the current platelet count to the platelet baseline then multiplied by 100. Data were taken as mean and standard deviation and analyzed through descriptive and analytical statistical treatment (paired t test). All analyses were conducted using Statistical Package for the Social Sciences (SPSS v. 15) and a p value less than 0.05 were considered as significant.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

The phytochemical screening was done to determine the possible constituents of the aqueous extract of the plant samples as reference for future studies. Based on the results, all of the plant samples have glycosides.

The aqueous plant extract of *Carica papaya* was shown to contain saponin glycosides, reducing substances, plant acids, saturated triterpenes and plant acids. *Momordica charantia,* on the other hand, contained tannins, saponin glycosides, reducing substances, plant acids and saturated triterpenes. The extract of violet *Ipomea batatas* leaves was found to have tannins, saponin glycosides, reducing substances, plant acids, saturated triterpenes and flavonoids while the green variation of *Ipomea batatas* leaves tested positive for tannins, saponin glycosides, plant acids, saturated triterpenes and flavones. The leaves of *Euphorbia hirta* was also found to have tannins, glycosides, reducing agents, plant acids, and flavonoids. Lastly, the leaves of *Alternanthera sessilis* were positive for tannins and glycosides.

3.2 Platelet Augmentation

Table 1 shows the differences of the mean platelet counts of the platelet counts at baseline, after administration of anagrelide and after treatment with the plant extracts.

Results of the paired t-test showed that the platelet increase on all treatment groups were found to be significant $p \leq 0.05$ except for the rats treated with *Momordica charantia* extract (p=0.1014).

Highest percentage increase of mean platelet counts was found with *Carica papaya* (125.8732%), followed by *Ipomea batatas* green variety (107.8844%), *Ipomea batatas* violet variety (106.0711%). *Alternanthera sessilis* showed 93.1777% while *Euphorbia hirta* increased mean platelet counts by 80.92363%. *Momordica charantia* showed the lowest percentage mean platelet count increase with 60.4797%.

Table 1: Mean platelet counts of the treatment groups/µL of blood

Group	Baseline	Anagrelide	Plant Extracts	% Increase	p value (α=0.05)
Momordica charantia	5303	1071	2710	60.4797	0.1014
Carica papaya	3216	-1200	4638	125.8732	0.0002
Alternanthera sessilis	6687	339	4969	93.1777	0.0001
Euphorbia hirta	5933	632	3313	80.92363	0.0489
Ipomea batatas- violet	4727	-210	3459	106.0711	0.0070
Ipomea batatas- green	4365	-382	4845	107.8844	0.0000

The table shows the percentage increase in mean platelet counts of all treatment groups and the obtained p values.

3.3 Discussion

Studies on herbal medicinal products for platelet augmentation are consistently increasing due to the limited supportive treatments available for thrombocytopenic disorders. As observed in this study, all of the plant extracts except for *Momordica charantia* showed significant increase in mean platelet counts of the Sprague-Dawley rats. Among the plant extracts tested for platelet augmentation activity, *C. papaya* showed the highest potential. Treatment with *C. papaya* consistently increased (125.8732%) the platelet counts of rats during the ten day treatment. The findings in this study are consistent with the results of other investigations involving *C. papaya*[1-3]. As shown in this investigation[2], platelet counts and the total white cell counts increased in all subjects within 24 hours after administration of *C. papaya* leaf extract. A more recent research[3] found that capsules prepared from *C. papaya* extracts showed significant increase in platelet counts in a randomized clinical trial consisting of 80 subjects. The *C. papaya* capsules were also believed to maintain the stability of the patients' hematocrit in normal levels. These results also contributed to shortened hospitalization of the dengue patients included in their study.

Treatment with *Ipomea batatas* green and violet variety also showed potential in increasing mean platelet counts with 107.8844% and 106.0711%, respectively. These two varieties of the sweet potato plant are already used in some areas in the Philippines as supportive treatment for platelet augmentation but such use is largely based on folkloric claims. There are only few published studies[6,7] regarding such activity to date.

Several studies were conducted on the pharmacological activity of *Alternanthera. sessilis*[9,10] but there was no particular study reported on its effect on platelet augmentation. However, previous findings showed its capacity to augment hemoglobin and bring to normal level other blood components[9]. Present investigation on this plant showed 93.1777% increase in platelet count which corroborated the previous investigation on its effect on blood components. Given the results of this study, it is recommended that more investigations be conducted on *Ipomea batatas* and *Alternanthera sessilis* to further support the evidence.

Unlike the *Ipomea batatas* and *Alternanthera sessilis*, there are numerous undocumented uses of *Euphorbia hirta* for platelet augmentation probably owing to its current prominence in the Philippines as an alternative supportive treatment for dengue hemorrhagic fever. This claim has been verified by the published report[11,12] and by the present study with mean platelet counts of 80.92363%. The increase in platelet counts is similar with the results obtained by the research conducted in 2012[11]. The results demonstrated that there is a significant increase in platelet counts and a decrease in bleeding and clotting times in ethanol-induced thrombocytopenic rats after administration of *Euphorbia hirta* decoction for 14 days. These findings warrant a careful review of other evidences regarding the use of *Euphorbia hirta* once these become available when the other studies are published.

Several traditional uses of *Momordica charantia* or bitter gourd[13-15] have been reported. However, its activity to increase platelets is nullified with the findings of this study. Among all plants tested for the potential to augment platelets, *Momordica charantia* showed the least percentage increase with 60.4797%. Paired t-test showed that this increase is not significant. Furthermore, there is no evidence to support the potential activity of the bitter gourd leaf extract as a treatment for thrombocytopenic disorders.

It is acknowledged that one of the limitations of this current study is that the mechanism of the platelet increasing property of the plant sample extracts is not elucidated. The primary aim of this study is

only to screen and compare the platelet increasing potential of the various plants tested. Further investigation is needed to isolate and determine the compounds responsible for platelet augmentation in the plants that showed potential activity to further elucidate the most probable mechanism of action. It is also recommended that the safety of the extracts of these plants for possible utilization as drugs be established.

4. CONCLUSION

It appeared in this study that *Carica papaya, Ipomea batatas g*reen and violet variety, *Alternanthera sessilis*, and *Euphorbia hirta* have significant platelet augmentation activity. This means that these plant extracts may possibly be used as supportive treatment for thrombocytopenic disorders and dengue hemorrhagic fever which may be an alternative to platelet transfusions. It is highly recommended that the phytochemical constituents responsible for such activity be identified and the possible mechanism of action of these plants in platelet augmentation be elucidated. The safety profile of these plant extracts should also be established prior to utilization.

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