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Evaluation of *Equisetum hyemale* and *Euphorbia hirta* leaf extracts in increasing platelet count of sprague dawley rats

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Abstract

Insufficient platelet production emerged as a global problem and result in a wide variety of health problems. This study aimed to evaluate an alternative to combat low platelet count. The efficacy of *Equisetum hyemale* and *Euphorbia hirta* in increasing the platelet count was assessed using experimental pre-test and post-test design consisting of five treatments. Aspirin-induced thrombocytopenia was the model used in Sprague Dawley rats to determine the extracts' effect in the platelet count within 11 days. On the 4th day to 11th day, the plant extracts showed a significant difference with the negative control. Further, in combination or alone, both plant extracts have no difference compared to the positive control. Hence, the results show that the plant extracts possess platelet-increasing property. The study recommends examining hematology and histopathology of various organs for any side effects.

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Introduction

Platelets are essential in the formation of blood clots to prevent bleeding. The relative decrease of platelet in the blood is termed thrombocytopenia. It is commonly seen in cases of bone marrow problems and platelet destruction. The bone marrow may not produce enough platelets due to aplastic anemia, Vitamin B-12 deficiency, folate deficiency, iron deficiency, viral infections (Covid-19, Dengue, HIV), exposure to chemotherapy, radiation or toxic chemicals, consuming too much alcohol, cirrhosis, leukemia, and myelodysplasia (Azzam *et al.*, 2013; Govindappagari *et al.*, 2019; Bockmann *et al.*, 2020; Taylor *et al.*, 2020). A low platelet count can also be a result of the body destroying too may platelets. This can be due to the side effects of certain medications, include diuretics and anti-seizure medications. It can also be a symptom hypersplenism, pregnancy, a bacterial infection in the blood, idiopathic thrombocytopenic purpura, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, and disseminated intravascular coagulation (Nurden and Nurden, 2014; Zhao *et al.*, 2016; Harde *et al.*, 2019; Sasamori *et al.*, 2020).

Because of its frequent occurrence, many drugs are developed to treat and prevent low platelet count. Several clinical studies have been carried out for years for its possible treatment (Barsam *et al.*, 2011; Linkins *et al.*, 2012; Ghanima *et al.*, 2012; Kang *et al.*, 2015; Wei *et al.*, 2016; Padmanabhan *et al.*, 2017). It includes glucocorticoids given intravenously or by mouth, and immunoglobulin administered intravenously. These medications have known side effects, expensive, and the treatment usually requires patients to be hospitalized. Thus, the researchers conducted this study to evaluate an herbal alternative that is accessible and inexpensive.

Equisetum hyemale and *Euphorbia hirta* are widely grown in India, Australia, and the Philippines. Both are employed in folk medicine to stop bleeding, heal ulcers and wounds, and increase platelet count of dengue patients. This study aims to evaluate the effect of the leaf extracts on platelet count in thrombocytopenia-induced animal models and compare them to commercially available drugs.

Materials and methods

Collection and preparation of plant materials

Freshly harvested leaves of *E. hyemale* and *E. hirta* are collected at Tuguegarao City, Philippines, using sterile gloves. The samples are washed with distilled water and dried at 60°C for three consecutive days until constant weight. The dried plant materials were subjected to grinding through mortar and pestle and poured in a blender to make them finer. The plant powders were then stored and sealed in plastic bags in a cool dark place.

Acute oral toxicity study

Organization for Economic Cooperation and Development (OECD) Guideline No. 420 for fixing the dose for biological evaluation was adopted to determine acute oral toxicity of the aqueous extracts. The LD_{50} fractions fall under category four values of the guidelines with no signs of acute toxicity at doses of 2000 mg/kg. A total of fifteen rats, divided into three groups, received a single oral dose of 50, 75, and 100 mL/kg (four grams of powder in 250 mL of distilled water reduced to 25 mL). All rats did not show any signs and features of toxicity or mortality.

Animal preparation

Eight to fifteen week–old healthy adult female Sprague Dawley rats weighing around 300 grams were used. The test animals are housed at the Philippine Institute of Traditional Alternative Health Care laboratory, where they are allowed to acclimatize under standard conditions 20±1°C following the OECD Guideline 401. They were kept at 12:12 light and dark cycle in polypropylene cages for 14 days before the experiment. Animals were fed with standard rodent diet and provided water *ad libitum*.

Aspirin induction on Sprague Dawley rats

Aspirin was administered intraperitoneally to induce thrombocytopenia in Sprague Dawley Rats. The administration was done accurately on the lower left quadrant where the least damage to the organs is

inflicted. According to Dejana *et al.* (2008), Aspirin (100 mg/kg) causes thrombocytopenia two hours after administration.

Plant extract administration

Twenty-five rats were segregated into five groups. Treatment A is distilled water (negative control), treatment B is 100% *E. hirta* aqueous extract, treatment C is 50% *E. hirta* and 50% *E. hyemale* aqueous extracts, treatment D is 100% *E. hyemale*, and treatment E is a steroid (positive control). The aqueous extracts were prepared by adding four grams of plant powder in 250 mL of distilled water. The administration was made using an oral gavage system.

Analysis of blood samples

Blood samples were collected through tail snipping adopted from the policy approved by the Institutional Animal Care and Use Committee (IACUC). Approximately 25 micrograms of blood were collected into potassium EDTA-coated tubes on Day 1, 4, 7, and 11. Platelets were manually counted from smeared slides using a platelet count estimate.

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine if there is a significant difference in the baseline platelet count before the intervention. It is also used to determine the significant difference among groups after treatment administration and further analyzed using Tukey Multiple Comparisons posthoc analysis. Statistical analysis was performed using SPSS version 20 software at 0.01 level of significance.

Results and discussion

In evaluating the effect of extract treatments, the actual platelet counts of the Sprague Dawley rats were counted at Day 0 (baseline), Day 4 (after three days of aspirin), Day 7 (after three days of treatment administration), and Day 11 (after seven days of treatment administration). Table 1 shows that on Day 4, it is evident that the platelet count of all test animals declined due to aspirin administration for three consecutive days. On Day 7, the platelet counts increased in different treatment after administration. For the group that received distilled water (Treatment A), it only increased between 30-108.

Table 1. Platelet count in the treatment groups.

Treatment	Replicate	Platelet Count (x10^9 cells)			
		Baseline (Pre-test results)	Day 4 (after 3 days of aspirin)	Day 7 (after 3 days of treatment administration)	Day 11 (after 7 days of treatment administration)
A (Distilled Water)	1	685	262	370	405
	2	565	367	402	496
	3	715	378	441	464
	4	658	471	501	564
	5	754	372	419	511
B (100% *E. hirta* aqueous extract)	1	668	370	602	842
	2	675	287	578	887
	3	716	258	625	818
	4	549	305	678	855
	5	752	287	527	810
C (50% *E. hirta* and 50% *E. hyemale* aqueous extract)	1	682	492	608	723
	2	782	301	632	795
	3	658	299	587	722
	4	673	375	549	698
	5	768	335	601	758
D (100% *E. hyemale* aqueous extract)	1	592	461	712	859
	2	668	369	681	877
	3	565	287	625	842
	4	551	315	588	649
	5	659	382	592	833
E (Steroids)	1	702	440	602	711
	2	693	372	617	979
	3	506	451	625	766
	4	633	305	714	896
	5	563	436	627	843

On Day 11, the platelet counts are higher than the baseline except for the group that received only distilled water. This implies that the test animals have started to recover from the aspirin-induced thrombocytopenia by increasing platelet production. This means that the extract treatments (Treatment B-D) and steroid (Treatment E) effectively increased the platelet count.

Table 2. One-way analysis of variance for the change in the platelet count before giving the treatment.

Type	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	37841.440	4	9460.360	1.946	0.142
Within Groups	97205.600	20	4860.280		
Total	135047.040	24			

Multiple mechanisms mediated by many active principles in the leaf extracts may be responsible for raising the blood cell counts. *E. hyemale* leaves contain polyphenolic compounds that treat or prevent platelet aggregation (Mekhfi *et al.*, 2004). Several studies suggest that *E. hyemale* helps minimize internal or external bleeding, excessive menstruation, and treat uncontrolled bleeding of the nose, lung, or stomach (Li *et al.*, 2012; Lin *et al.*, 2014). *E. hirta* is reported to contain polyphenols, tannins, and flavonoids (Rehman *et al.*, 2018). Tannins have complex-forming properties that may contribute to the positive effects during retraction events in the release of individual pro-platelets (Saraf and Kavimandan, 2017). Flavonoids have anabolic impacts which may be responsible for the stimulant effect on blood production (Songlin *et al.*, 2009). *E. hirta* is known to stop internal hemorrhaging, alleviate dengue fever, and improve healing mechanisms (Parvaiz and Javaid, 2013; de Guzman *et al.*, 2016; Perera *et al.*, 2018).

Table 3. One-way analysis of variance for the change in the platelet count after giving the treatment.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	256538.280	4	64134.570	11.935	0.000
Within Groups	241814.200	45	5373.649		
Total	498352.480	49			

To verify that results are unaffected by the preliminary selection of rats, the researchers tested if the baseline platelet counts are the same for all rats. The result of the One-Way ANOVA with a level of significance of a=0.01 for baseline platelet count is shown in Table 2. At a 1% level of significance, there is no pairwise difference in the baseline platelet count among rats assigned to all the treatments. This support that all the rats have the same baseline platelet count before the treatments were given, making the results of the experiment reliable. This is critical in similar studies to determine if the treatments prevented the fall in platelet count, and the count is increased above baseline (Brown *et al.*, 2011; Saraf and Kavimandan, 2017; Yamashita *et al.*, 2018).

The researchers also used One-Way ANOVA to analyze platelet count change among rats per treatment (Table 3). The *p*-value is less than 0.01 which means that there is a significant difference in the platelet count after giving the treatment from Day 4 to Day 11. At a 1% level of significance, there is sufficient evidence that there is at least one significant pairwise difference in the change in the platelet count after giving the treatment from Day 4 to 7 and from Day 7 to 11. Tukey Multiple Comparisons Test was used to determine which among the treatments show significant differences with each other (Table 4). As seen, all the other treatments have substantial differences against the negative control, distilled water, for the platelet count change for all rats after giving the treatments. On the other hand, even

though the said treatments are better than the negative treatment, there is no significant difference among 100% *E. hirta*, 100% *E. hyemale*, 50% *E. hirta*, and 50% *E. hyemale*, and positive control, steroids. This suggests that the performances of these treatments to increasing the platelet count do not vary significantly.

Table 4. Tukey multiple comparisons test of treatments.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	p-value	99% Confidence Interval	
					Lower Bound	Upper Bound
A	B	-211.50000*	32.78307	.000	-324.9181	-98.0819
	C	-130.40000*	32.78307	.002	-243.8181	-16.9819
	D	-165.60000*	32.78307	.000	-279.0181	-52.1819
	E	-160.10000*	32.78307	.000	-273.5181	-46.6819
B	C	81.10000	32.78307	.115	-32.3181	194.5181
	D	45.90000	32.78307	.631	-67.5181	159.3181
	E	51.40000	32.78307	.525	-62.0181	164.8181
C	D	-35.20000	32.78307	.819	-148.6181	78.2181
	E	-29.70000	32.78307	.893	-143.1181	83.7181
D	E	5.50000	32.78307	1.000	-107.9181	118.9181

*significant at 0.01

A= Distilled Water; B=100% *E. hirta*; C=50% *E. hirta* and 50% *E. hyemale*

D=100% *E. hyemale*; E= Steroids.

This also means that the plant extracts are as active as the positive control in increasing the platelet count. *E. hyemale*, which is least studied for its platelet augmentation activity among the treatments, therefore, shows potential as an anti-thrombocytopenic agent. This plant is known for its antimicrobial, antioxidant, and anti-inflammatory activities (Jiang *et al.*, 2012; de Queiroz *et al.*, 2015; dos Santos Alves *et al.*, 2016). On the other hand, there are already three studies that investigated the platelet augmentation potential of *E. hirta* using different methodologies. Results confirmed the findings in this study that *E. Hirta* significantly increased platelet counts (Apostol *et al.*, 2012; Arollado *et al.*, 2013; Coloma *et al.*, 2014).

Conclusion

E. hyemale and *E. hirta* have significant platelet augmentation activity. These aqueous plant extracts are significantly different against the negative control, distilled water. Also, results showed no significant difference among 100% *E. hyemale*, 100% *E. hirta*, 50% *E. hyemale* and 50% *E. hirta*, and the positive control, steroids. This suggests that the performances of these treatments concerning increasing the platelet count do not vary significantly.

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