

Utilization of *Glycine max* (Soybean) in Depleting the Platelet Count of *Sprague dawley*

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Abstract

Thrombocytosis in a minimal normal range deviation is not clinically significant but having excessive proliferation and production causes serious complications such as stroke, heart attack, or clot in blood vessels which can be very painful (Cleveland Clinic, 2022). Medication for thrombocytosis associated conditions are available but common drugs are not made for direct platelet reduction. It was recently debated that at least five years, the side effect of these specific available drugs will outweigh its benefits which is alarming for those who need consistent intake due to gene-linked essential thrombocytosis (Walzer, M., 2019). Philippines, a developing country has a culture of home medication and are after over-the-counter drugs without prescriptions. The society's culture is not proactive in terms of hospitalization and checking up on times of illnesses, but reactive if conditions get worse. In this study, the researcher aimed not to manufacture a pharmaceutical drug, but to appeal to organic consumption to one of abundant iron source food in the country to reduce platelet count which can easily incorporate in the diet and lifestyle of Filipinos. Thus, this study utilized 75% *Glycine max*(soybean) in depleting the platelet count through the diet of *Sprague dawley*. Platelets are observed in first and second observation with three (3) day interval. The study concluded that effect was only significant in the second observation (6th day) since iron absorption as the main metabolite of interest in *Glycine max* takes 3 days to be absorbed by *Sprague dawley* (Huebers, H.A et al., 1990).

Keywords: *Glycine max*, *Sprague dawley*, Platelet Count, Thrombocytosis, First observation, Second observation

1. Introduction

In the case that we cut ourselves or have any other bleeding, platelets and coagulation factors are the components that aid in the blood clotting process. However, under certain circumstances, the platelet count might drop/rise significantly, which can be detrimental to general health. This study is focused on providing possible treatment to cases associated to thrombocytosis which is an increased proliferation and production of platelet count thus are lethal in the systemic activity. According to Cleveland Clinic (2022), thrombocytosis in a minimal normal range deviation is not clinically significant but having excessive proliferation and production causes serious complications such as stroke, heart attack, or clot in blood vessels which can be very painful. This is commonly associated with individuals who have genetic defects; Erythromelalgia is a condition experienced by patients who have essential thrombocytosis, characterized by a pain, numbing, or tingling sensation of hands and feet. In severe cases, thrombocytosis can cause heart attack since blood is becoming too sticky and the nature of platelets are associated with clotting as it aggregates and blocks blood vessels.

According to MayoClinic, 2022, prescriptions for these conditions are available such as

hydroxyurea (Droxia,Hydrea), anagrelide(Agrilyn) or interferon alfa (intron A). Moreover, plateletpheresis can be a choice during emergency situations but is temporary. This is not an issue until uncovering each of its side effects for the fact that these are still drugs and are not made for direct platelet alteration. In an argument of MedlinePlus (2021), hydroxyurea affects the bone marrow by severely inhibiting its proliferation which may result in bleeding status and to a worse extent affecting other blood cells. According to Goodrx, a company that manufactures anagrelide (agrilyn), the aforementioned is a common antiplatelet drug but has a lot of interaction with many medications, this would mean not applicable for people having pharmaceutical therapy. In addition to this, aspirin a common over the counter blood thinner, is widely used up to these days which is linked to Reye's syndrome.

Throughout history, soybeans were only regarded as one of the primary ingredients required to produce tofu and other cuisines from upscale serving to native dishes such as *taho*. Soybean-based food in the Philippines is bountiful. Soybeans appeared to be a good source of nutritional iron (Kolb et al., 2003). A study conducted by Park et al., (2012) had observed an increase in thrombopoiesis among women with iron deficiency anemia. The severity of iron deficiency anemia will induce thrombocytosis and on the other hand, iron supplements will eventually act as anti-thrombotic. The (6th Edition) Rodaks Hematology Clinical Principles and Applications also asserts that thrombocytosis is a laboratory diagnosis of iron deficiency anemia, and much is observed if the disease is due to chronic bleeding. Iron was proved to have an inverse relationship with platelet production. Hence, this study will test if iron supplementation of *Glycine max* will decrease thrombopoiesis on the opposite trend. Generally, the study attempted to determine the efficacy of utilizing *Glycine max* (Soybean) in depleting the platelet count of *Sprague dawley*.

Specifically, the study aimed to answer the following questions:

1. What is the difference between the average platelet count of *Sprague dawley* in the Control Group and Experimental Group after the:
 - 1.1. First observation (3-day treatment)
 - 1.2. Second observation (6-day treatment)
2. What is the final platelet count status of *Sprague dawley* in the Control Group and Experimental Group?
3. Is there a major difference between the final platelet status of *Sprague dawley* in the Control Group and Experimental Group from the normal value?
4. Is soybean diet-complement effective as antithrombotic agent?

2. Method

2.1 Research Design

The purpose of this study is to determine the efficacy of utilizing *Glycine max* (Soybean) in depleting the platelet count of *Sprague dawley*. The study utilized an experimental research design which is necessary to be conducted in the laboratory and can only be proven tested in such a manner. Rats as a sample of the study are ensured raised in the same external conditions given that all of the samples included in the study are in good health and with no underlying diseases prior to subjecting them for the experiment. Samples used are adults with the same age. Moreover, a two grouped research analysis was applied dividing the sample into a control group and experimental group which will undergo two (2) times monitoring of platelet status with three (3) day intervals. All of these procedures are rightly fitted under experimental research.

2.2 Locale of the Study

The study was conducted at Vet Central Lab Philippines where animal housing is available and safe to conduct. It is located at Unit B Ground Floor One Roxas Square #1 F Roxas, corner F. Blumentritt, San Juan, 1500 Metro Manila, deemed as an institution built for the medical concern of pets.

The study was subjected to contractual testing under registered veterinary doctors specializing in animal care and handling. The institution has laboratories with different sections as well as services offered; Hematology, Parasitology, Blood Chemistry, Routine test, Cytology, Allergy Test, Histopathology, Photomicrography, Microbiology, Laboratory Animal Research and more. The researchers appointed the locale in a practical and safest way to conduct considering other rationales behind its choice. The researchers find Vet Central Lab Philippines with appropriateness and utmost convenience.

2.3 Samples of the Study

The specie used in this study are *Sprague dawley* with a total sample of ten (10). The researchers utilized a non-probability purposive sampling as each sample in the population are being filtered and ensured raised in the same external conditions, given that all of the samples included in the study are in good health and with no underlying diseases. Furthermore, to ensure such a line of quality, a species of adult rats with the same age was desired. The goal of non-probability purposive sampling is to select participants for the sample who will accurately reflect the desired sample in a population. Five (5) rats were assigned for the control group which are the *Sprague dawley* sample number 1, 2, 3, 4, and 5 and other five (5) rats as the experimental group including the other half namely *Sprague dawley* sample number 6, 7, 8, 9, and 10.

2.4 Data Gathering and Procedures

Glycine max was procured from the local market and was been identified successfully by undergoing plant identification from the Bureau of Plant Industry Malate. Thus, the *Glycine max* samples were further subjected to phytochemical analysis from Department of Science and Technology - Taguig. *Sprague dawley* rat samples are procured from the institution of Vet Central Lab Philippines. Hence, a letter of request was sent to Bureau of Animal Industry for validation and to officially have a permit to conduct the study.

Saphenous vein technique is the blood extraction method used by the researcher to gather the data from the platelet count of rats. To interpret the result, the Final platelet count was drawn from the average of the First and Second observation result of each group and is compared to 436, 000- 844, 000 cu mm standard normal range. Treatment as antithrombotic are expected to lower the platelet count out of the normal range. Moreover, the first observation was treated with independent T test analysis with 0.05 confidence level between control and experimental group to determine its significant difference, same goes with second observation. Rat samples was separated into two groups; control group which received normal diet with usual laboratory food to omit possibilities of induced nutrient-deficiency blood complication. Experimental group on the other hand were supplemented with the modified food treated with 75% *Glycine max*. Water for consumption is demineralized water for both groups.

2.5 Data Analysis

Platelets were counted using formula for direct method of platelet counting. First and Second observation was statistically treated separately using Independent T-test. T-test for independent samples compares the means of two groups in order to determine whether there is statistical evidence that the associated population means are significantly different which as for this study the control group and experimental group. Lastly, the final platelet count which were drawn from the average of the two observations is interpreted with a remarked increase, within normal range, or decrease. Normal range used in the study is the standard normal value for *Sprague dawley*. $> 844,000$ cu mm is interpreted as Increase, 436,000- 844,000 cu mm is interpreted Within normal range, and $<436,000$ cu mm is interpreted as Decrease.

3. Results & Discussion

Table 1. The Platelet Count of Each Sample in Every Observation

	Sprague dawley	First Observation (cu mm)	Second Observation (cu mm)
Control Group	1	720,000	660,000
	2	535,000	490,000
	3	685,000	690,000
	4	560,000	625,000
	5	530,000	525,000
Experimental Group (Treatment)	6	620,000	510,000
	7	615,000	435,000
	8	520,000	445,000
	9	535,000	375,000
	10	650,000	530,000

Table 1 shows the difference of the First observation (3rd day) platelet count and Second Observation (6th day) platelet count of each group. Experimental group (sample 6-10) failed to decrease the platelet count in the first observation from the standard normal value but are way lesser count than the control group. In the Second observation, generally a consistent platelet count was maintained by control group and a marked significant decrease of platelet count was observed on the experimental group this time.

Table 2. Statistical Analysis for the Control Group vs. Experimental Group in the First and Second observation using Independent T-test

Source of Variation	P value	P value summary
First Observation	0.7148	Not significant
Second Observation	0.0195	Significant

Table 2 is the Independent T-test analysis for the difference of platelet count of control group versus experimental group in the first and second observation. First observation appeared to have a P-value of 0.7148 which is above the 0.05 confidence level which makes it not significant while the second observation resulted to a P-value of 0.0195 which is lower than the 0.05 confidence level which makes it significant.

Table 3. The difference between the Final Platelet Count of Control Group and Experimental Group

	Final Platelet Count (cu mm)	Interpretation
Control Group	602, 000	Within Normal Range
Experimental Group	524, 000	Within Normal Range

Table 3 shows the difference between the final platelet count of Sprague dawley in the Control Group and Experimental Group for comparison to the standard normal range of 436, 000- 844, 000 cu mm. Thus, the results show a decreased platelet count of group treated with 75% Glycine max but instill both are within the normal range.

4. Conclusion

Based on the data presented by the study, this study concludes that the first observation with 3 days consistent feeding of 75 % Glycine max have failed to decrease the platelet count of Sprague dawley. However, with 6 days consistent feeding of 75% Glycine max, a decrease count was observed. This result also conforms with the premise that 3 days is needed for rats to absorb iron metabolites form Glycine max (Huebers, H.A et al., 1990) and pursuant to this, metabolism and regulation of thrombopoiesis will just about to start, a main reason why decrease in platelet count was observable only at second observation (6th day).

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