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Research Article

EVALUATION OF ACTIVITY OF *ALTERNANTHERA SESSILIS* LEAVES AQUEOUS EXTRACT ON PLATELET COUNT IN DRUG-INDUCED THROMBOCYTOPENIA IN ALBINO RATS

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ABSTRACT

Objectives: of Study: (1) To determine the platelet augmenting effect on drug-induced thrombocytopenia in rats. (2) To observe any adverse effects and mortality in the animals.

Methods: Albino rats of either sex of average weight 150–200 g are used. A total of 36 (n=36) rats were divided into VI groups of 6 each. Groups I, II, III received cyclophosphamide 25 mg/kg body weight and group IV, V, VI received 50 mg/kg body weight for 3 consecutive days respectively. Blood was withdrawn from the retro-orbital plexus on the 1st, 4th, 7th, and 11th day of study after subjecting the animals to light anesthesia using ether and platelet count determined by making peripheral smear.

Results: Platelet count: *Alternanthera sessilis* leaves aqueous extract at concentrations of 200 mg/kg and 400 mg/kg were found to significantly increase the platelet count in cyclophosphamide-induced rat model.

Conclusion: The present study demonstrated the platelet augmenting effect of *A. sessilis* leaves aqueous extract. Further detailed studies are required to establish its usefulness.

Keywords: *Alternanthera sessilis*, Thrombocytopenia, Albino rats, Dengue fever.

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INTRODUCTION

Platelets circulate in the blood for 7–10 days and play a critical role in hemostasis. Significant quantitative or qualitative platelet dysfunction results in many complications, including bleeding.

Thrombocytopenia is defined as a platelet count of <150 × 103/μL. It can result from decreased platelet production, increased platelet consumption or increased sequestration [1].

Among various etiologies, *Dengue* is highly prevalent, with a number of people getting infected and has high mortality. A remedy for this dreaded disease, however is still elusive and it has now become a major international public health problem. Dengue hemorrhagic fever is a potentially fatal complication, resulting in bleeding and thrombocytopenia where there is an abnormally low level of platelets in the body [2].

Apart from viral infections, a number of other causes are involved in decreasing the number of platelets in the blood as mentioned above. There are very few options for increasing the number of platelets in therapeutics. Few traditional medicinal plants are reported to have haematinic properties [3].

Alternanthera sessilis (*Amaranthaceae*) is a perennial herb with several spreading branches, bearing short petioled simple leaves and small white flowers, found widely in India especialy in southern part. This herb has been reported to be used as galactogog, hemetenic, febrifuge and in indigestion problems. The leaves were used in eye diseases, cut wounds and antidote to snake bite and skin diseases. In addition as it also has antiviral activity, which can address both decreased platelet count and viral infection, with thrombocytopenia like dengue fever [4]. One of the probable mechanism of hematinic action and of increasing platelet count may be due to its free radical scavenging activity and membrane stabilization which prevents peripheral destruction of platelets [5].

Hence, the present study is aimed to explore the activities of *A. sessilis* like anti-thrombocytopenic effect on platelet count in experimentally induced thrombocytopenia in rats.

Aims and objectives

1. To determine the platelet augmenting effect on drug-induced thrombocytopenia in rats
2. To observe any adverse effects and mortality in the animals.

METHODS

The present study was carried out in the Department of Pharmacology, S S Institute of Medical Sciences and Research Centre, Davangere, Karnataka, over a period of 2 years from July, 2017 to June, 2019.

Chemicals and drugs

1. Distilled water
2. Cyclophosphamide
   Cyclophosphamide 1 g anhydrous powder of Neon laboratories limited with trade name Phosmid-1000.
   Batch number: 29320,
   Mfg. date: December, 2018,
   Expiry date: November, 2019.
   1 g cyclophosphamide was reconstituted with 50 mL of sterile water.
3. Aqueous extract of *A. sessilis* leaves.
   10% solution of aqueous extract of *A. sessilis* leaves was used.

Source of data

Albino rats weighing 150–200 g of either sex which are inbred in Central Animal House of S.S. Institute of Medical Sciences and Research

Centre, Davangere were used. The study duration was 2 years, from July 2017 to June 2019. Animals were randomly housed as 3 rats per cage at an ambient temperature and humidity, with a 12 h light and 12 h dark cycle. The animals had free access to food and water, *ad libitu*. The study was approved by the Institutional Animal Ethical Committee. (Ref No: SSIMS and RC/IAEC/51/2015).

Procedure

A total of 36 (n=36) Albino rats were grouped into 6 groups with 6 rats in each group. A rat model of thrombocytopenia induced by cyclophosphamide by Kristiana *et al*. is used. Cyclophosphamide was used to induce thrombocytopenia as it can induce safe and stable thrombocytopenia (Two doses of cyclophosphamide are taken to compare the degree of thrombocytopenia induced and the platelet augmenting activity of the test drug). Group I was taken as control and received cyclophosphamide 25 mg/kg s.c for three consecutive days. Group II and III received cyclophosphamide 25 mg/kg s.c for three consecutive days plus drug (aqueous extract of A. sessilis) 200 and 400 mg/kg p.o, respectively, for 15 days. Group IV was taken as the second control and received cyclophosphamide 50 mg/kg s.c for three consecutive days. Group V and VI received cyclophosphamide 50 mg/kg s.c for three consecutive days plus drug (aqueous extract of A. sessilis) 200 and 400 mg/kg p.o, respectively, for 15 days [6].

1. CYCLOPHOSPHAMIDE (25 mg/kg) [6]

All animals will be given cyclophosphamide (25 mg/kg s.c) for first 3 days.

- GROUP I- (control group) distilled water (1 mL p.o)
- GROUP II- *A. sessilis* leaves aqueous extract (200 mg/kg) [7] for15 days
- GROUP III- *A. sessilis* leaves aqueous extract (400 mg/kg) [7] for15 days.

2. CYCLOPHOSPHAMIDE (50 mg/kg) [6]:

- GROUP IV- (control group) distilled water (1 mL p.o)
- GROUP V- *A. sessilis* leaves aqueous extract (200 mg/kg) [7] for15 days
- GROUPVI – *A. sessilis* leaves aqueous extract (400 mg/kg) [7] for 15 days.

Inclusion criteria

1. Albino rats of either sex weighing between 150 and 200 g
2. Healthy animals with normal activity.

Exclusion criteria

1. Albino rats <150 g or >200 g
2. Pregnant rats
3. Animals previously used in other experiments.

Duration of study

From July, 2017 to June, 2019 (2 years).

Specimen collection

Blood was withdrawn from retro-orbital plexus using heparinized capillaries to prevent platelet activation on the 1st, 4th, 7th and 11th day of dosing, after subjecting the animals to light anaesthesia using ether and platelet count was determined by making peripheral smear [6]. Prepration of aqueous extract of *A. sessilis* leaves.

Plant authentication was done by morphological analysis, i.e., identifying the plant by examining its physical characteristics like leaves and stem and comparing it with the known reference species by the botanist and other experts.

Extraction was done in the Department of Pharmacognosy, Bapuji College of Pharmacy, Davangere. The plant leaves were shade dried for 60 days at room temperature and pulverized to powder in a mechanical grinder.

Soxhlet extraction method is used, Soxhlet extractor is used for this purpose. This extractor is provided with a siphoning system. Dry powdered *A. sessilis* leaves are placed in a porous cellulose thimble. The thimble is placed in the extraction chamber which is suspended above a flask containing the solvent (distilled water is used as solvent) and below a condenser. The flask is heated and the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the sample and soaks the materials and extracts the constituents. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask and the process is continuously repeated till the extraction is complete. At the end of the extraction process, which lasted few hours, the flask containing the solvent and extract is removed. Finally, the solvent in the flask is evaporated.

RESULTS: STATISTICAL ANALYSIS

Descriptive analysis that includes mean, standard deviation and range value were found for each group and used for analysis. One-way of variance was used for simultaneous multiple group comparison followed by Turkey's post hoc analysis for group wise analysis. Significance is established for a probability value $p<0.05$ and is considered highly significant when $p<0.001$.

Comparison between Groups I, II and III (Table 1 and Graph 1)

On day 1, there was no significant difference between the basal platelet counts in all 3 groups. On day 4, there was significant (<0.05)) difference in platelet count between control (group I) and test drug-treated groups (group II, III). On days 11 and 14, there was a highly significant (<0.001) difference in platelet count between control and test drug-treated groups.

Comparison between Groups I and II (Table 2 and Graph 2)

On day 1, there was no significant difference between the basal platelet count in both groups. On day 4, there was a significant (<0.05)) difference in platelet count between the control and test drug-treated group. On days 11 and 14, there was a highly significant (<0.001) difference in platelet count between control and test drug-treated group.

Comparison between Groups I and III (Table 3 and Graph 3)

On day 1 there was no significant difference between the basal platelet counts in both groups. On day 4, there was a significant (<0.05) difference in platelet count between control and test drug-treated

Table 1: Statistical analysis showing comparison of platelet counts between Group I, II, and III

Parameters	Mean	SD	p-value	Significance
Day 1 (Baseline reading)				
Group I	329,166.67	55,715.049	0.936	NS
Group II	325,000.00	108,397.417		
Group III	341,666.67	73,598.007		
Day 4				
Group I	120,000.00	44,158.804	0.001	S
Group II	266,666.67	60,553.007		
Group III	233,333.33	60,553.007		
Day 7				
Group I	87,500.00	37,914.377	0.0001	HS
Group II	245,833.33	40,052.049		
Group III	200,000.00	54,772.256		
Day 11				
Group I	87,500.00	37,914.377	0.0001	HS
Group II	233,333.33	40,824.829		
Group III	183,333.33	40,824.829		

HS (p<0.0001), S (p<0.005), NS (p>0.005). HS: Highly significant, SD: Standard deviation, S: Significant, NS: Not significant

Table 2: Comparison of platelet counts between Group I and II

Parameters	Mean	SD	p-value	Significance
Day 1				
Group I	329,166.67	55,715.049	0.935	NS
Group II	325,000.00	108,397.417		
Day 4				
Group I	120,000.00	44,158.804	0.001	S
Group II	266,666.67	60,553.007		
Day 7				
Group I	87,500.00	37,914.377	0.0001	HS
Group II	245,833.33	40,052.049		
Day 11				
Group I	87,500.00	37,914.377	0.0001	HS
Group II	233,333.33	40,824.829		

HS: Highly significant, SD: Standard deviation, S: Significant, NS: Not significant

Table 3: Comparison of platelet counts between Group I and III

Parameters	Mean	SD	p-value	Significance
Day 1				
Group I	329,166.67	55,715.049	0.747	NS
Group III	341,666.67	73,598.007		
Day 4				
Group I	120,000.00	44,158.804	0.005	S
Group III	233,333.33	60,553.007		
Day 7				
Group I	87,500.00	37,914.377	0.003	S
Group III	200,000.00	54,772.256		
Day 11				
Group I	87,500.00	37,914.377	0.002	S
Group III	183,333.33	40,824.829		

SD: Standard deviation, S: Significant, NS: Not significant

groups. On days 11 and 14, there was a significant (<0.05) difference in platelet count between control and test drug-treated group (group III).

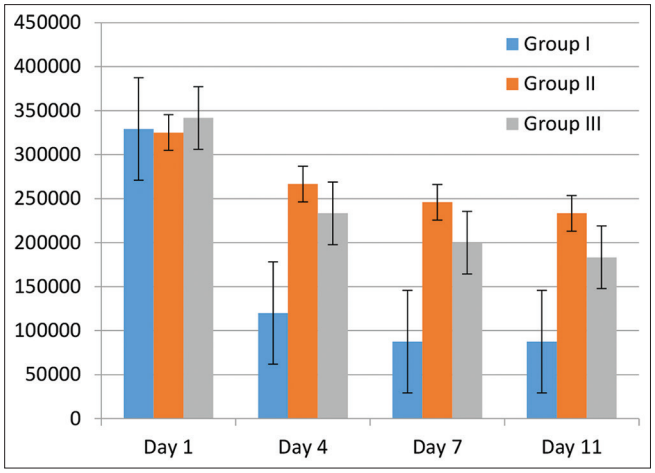
Comparison between Groups II and III (Table 4 and Graph 4)
When both the test groups with two different doses of drug, that is, group II (200 mg/kg) and group III (400 mg/kg), were compared, there was no significant difference in both platelet count throughout the experiment.

Comparison between Groups IV, V and VI (Table 5 and Graph 5)
On day 1, there was no significant difference between the basal platelet counts in all 3 groups. On day 4, there was a significant (<0.05) difference in platelet count between control (group IV) and test drug-treated groups (group V, VI). On day 11 and 14 there was highly significant (<0.001) difference in platelet count between control and test drug treated groups.

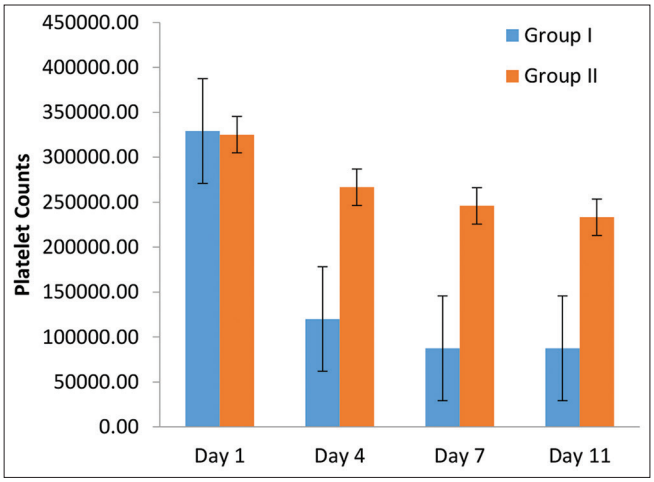
Comparison between Groups IV and V (Table 6 and Graph 6)
On day 1, there was no significant difference between the basal platelet counts in both groups. There was no significant difference in platelet count between control and test drug-treated group (group V) on day 4. On days 11 and 14, there was a significant (<0.05) difference in platelet count between control and test drug-treated group.

Comparison between Groups IV and VI (Table 7 and Graph 7)
On day 1, there was no significant difference between the basal platelet count in both groups. On days 4, 11 and 14, there was highly significant (<0.001) difference in platelet count between control (group IV) and test drug-treated group (group VI).

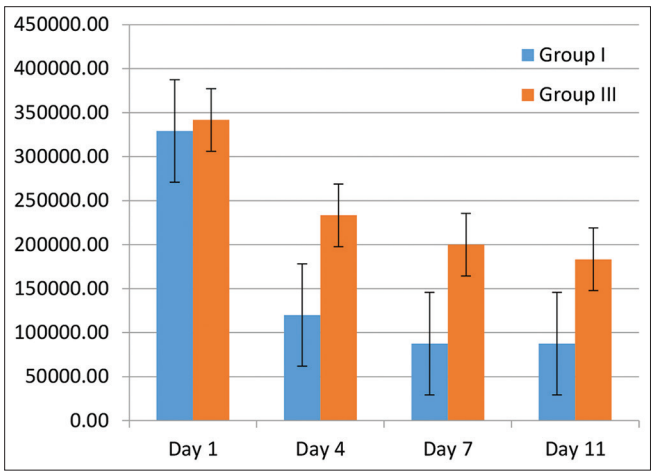
Comparison between Groups V and VI (Table 8 and Graph 8)
When both the test groups with two different doses of drug that is, group V (200 mg/kg) and group VI (400 mg/kg) were compared, there was no significant difference in both platelet count and clotting time throughout the experiment.



Graph 1: Comparision of platelet counts between Group I, II and III



Graph 2: Comparision of platelet counts between Group I and II



Graph 3: Comparision of platelet counts between Group I and III

DISCUSSION

Thrombocytopenia is a hematological abnormality due to a decrease in circulating platelets resulting in spontaneous bleeding. Various causes results in platelet destruction as mentioned earlier; among them infection induced thrombocytopenia, especially dengue is an important public health concern, treatment for which is still elusive. The present study

Table 4: Comparison of platelet counts between Group II and III

Parameters	Mean	SD	p-value	Significance
Day 1				
Group 2	325,000.00	108,397.417	0.762	NS
Group 3	341,666.67	73,598.007		
Day 4				
Group 2	266,666.67	60,553.007	0.363	NS
Group 3	233,333.33	60,553.007		
Day 7				
Group 2	245,833.33	40,052.049	0.129	NS
Group 3	200,000.00	54,772.256		
Day 11				
Group 2	233,333.33	40,824.829	0.060	NS
Group 3	183,333.33	40,824.829		

SD: Standard deviation, NS: Not significant

Table 5: Comparison of platelet counts between Group IV, V and VI

Parameters	Mean	SD	p-value	Significance
Day 1				
Group IV	283,333.33	81,649.658	0.332	NS
Group V	341,666.67	106,848.803		
Group VI	358,333.33	73,598.007		
Day 4				
Group IV	141,666.67	37,638.633	0.003	S
Group V	250,000.00	77,459.667		
Group VI	291,666.67	66,458.007		
Day 7				
Group IV	75,000.00	22,360.680	0.000	HS
Group V	233,333.33	51,639.778		
Group VI	233,333.33	60,553.007		
Day 11				
Group IV	27,500.00	17,535.678	0.000	HS
Group V	208,333.33	73,598.007		
Group VI	191,666.67	58,452.260		

HS: Highly significant, SD: Standard deviation, S: Significant, NS: Not significant

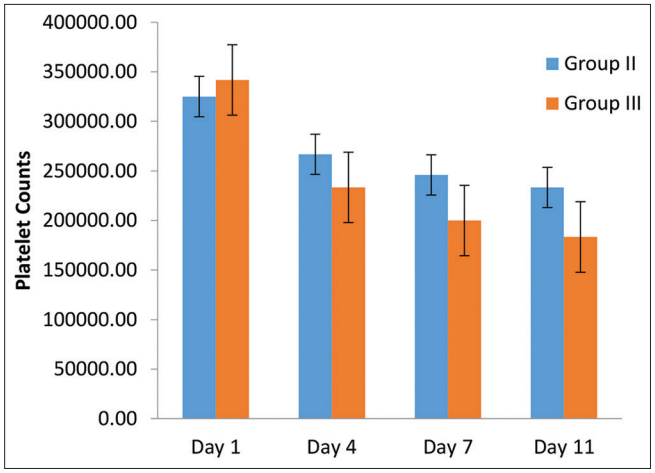
Table 6: Comparison of platelet counts between Group IV and V

Parameters	Mean	SD	p-value	Significance
Day 1				
Group IV	283,333.33	81,649.658	0.315	NS
Group V	341,666.67	106,848.803		
Day 4				
Group IV	141,666.67	37,638.633	0.017	NS
Group V	250,000.00	77,459.667		
Day 7				
Group IV	75,000.00	22,360.680	0.000	HS
Group V	233,333.33	51,639.778		
Day 11				
Group IV	27,500.00	17,535.678	0.001	S
Group V	208,333.33	73,598.007		

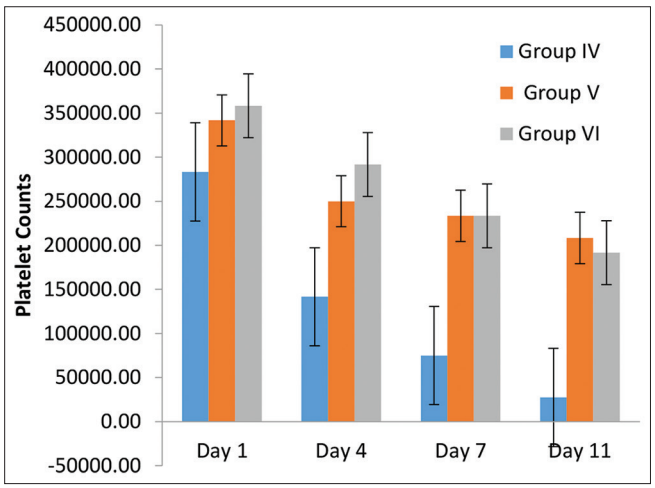
HS: Highly significant, SD: Standard deviation, S: Significant, NS: Not significant

focuses on the platelet augmenting effect of aqueous extract of *A. sessilis* leaves on cyclophosphamide-induced thrombocytopenia in albino rats. *A. sessilis* is a perennial vegetative herb used widely in south India and known for its haematinic [3] and anti-oxidant activity [5] and hence this plant was selected in the present study to evaluate its effect on platelets.

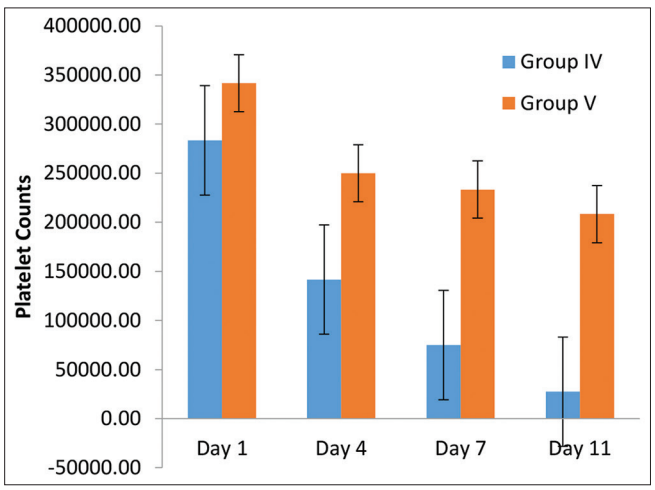
Two doses (25 mg/kg and 50 mg/kg) of cyclophosphamide was used to induce thrombocytopenia and to compare the degree of thrombocytopenia induced and to see the drug effect. Animals were subjected to light ether anaesthesia and blood was drawn from retro-orbital plexuses using heparinized capillaries to prevent platelet activation. On day 1, baseline readings were taken before drug administration and there was no significant difference in platelet counts of all groups. On day 4, there was a significant (<0.05) fall in platelet



Graph 4: Comparision of platelet counts between Group II and III



Graph 5: Comparision of platelet counts between Group IV, V and VI



Graph 6: Comparision of platelet counts between Group IVand V

counts below normal in control (Group I and Group IV) compared to test drug-treated groups (II, III, V, VI). On days 11 and 14, there was a highly significant difference (<0.001) in platelet counts between control and test drug-treated groups.

A. sessilis leaves contain various phytoconstituents like 2, 4-methylenecycloartanol and cycloeucalenol, choline, and oleanolic acid.

Saponins have been isolated from the leaves. Roots contain lupeol [17]. Young shoots contain protein and iron. It also contains 5-a -stigmasta-7-enol. The β-sitosterol possess potent anti-inflammatory by reducing the secretion of pro inflammatory cytokines and TNF. These constituents can act on the bone marrow, enhance its ability to produce platelets. Moreover, it can also prevent platelet destruction in the blood by its anti-oxidant free radical scavenging and membrane stabilization activity and thereby increase the life of the platelet in circulation [5,9].

As the herb is known haematinic and contains a rich source of minerals it may also support cell proliferation and maturation in bone marrow [3]. The plant also possess antiviral property and hence in viral infection induced thrombocytopenia it can act as antiviral and also improve platelet count [10]. It also as potent anti-inflammatory property and hence can be effective in immune thrombocytopenic purpura [11].

Carica papaya extract has been proved to be antithrombocytopenic and is used clinically in viral infection-induced thrombocytopenia. However, it is known to cause gastritis, vomiting, and hepatic necrosis in few cases. Moreover, it also lacks antiviral property. Hence, *A. sessilis* leaves

Table 7: Comparison of platelet counts between Group IV and VI

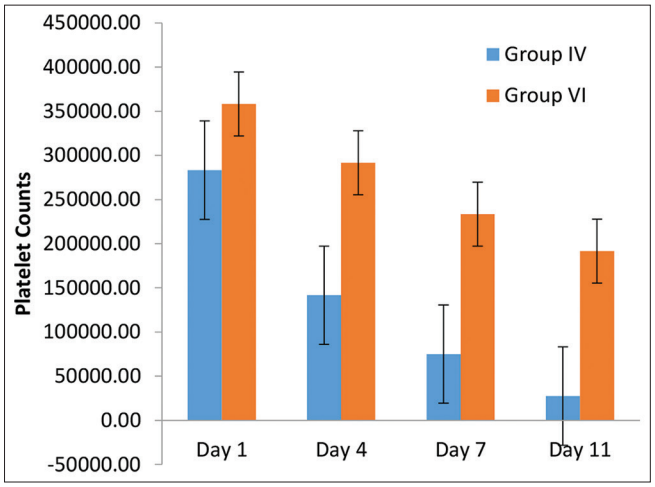
Group	Mean	SD	p-value	Significance
Day 1				
Group IV	283,333.33	81,649.658	0.126	NS
Group VI	358,333.33	73,598.007		
Day 4				
Group IV	141,666.67	37,638.633	0.001	HS
Group VI	291,666.67	66,458.007		
Day 7				
Group IV	75,000.00	22,360.680	0.001	HS
Group VI	233,333.33	60,553.007		
Day 11				
Group IV	27,500.00	17,535.678	0.001	HS
Group VI	191,666.67	58,452.260		

HS: Highly significant, SD: Standard deviation, NS: Not significant

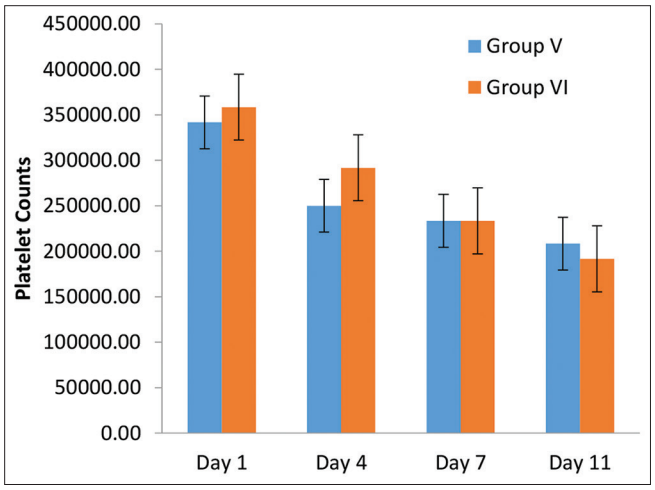
Table 8: Comparison of platelet counts between Group V and VI

Parameters	Mean	SD	p-value	Significance
Day 1				
Group V	341,666.67	106,848.803	0.760	NS
Group VI	358,333.33	73,598.007		
Day 4				
Group V	250,000.00	77,459.667	0.341	NS
Group VI	291,666.67	66,458.007		
Day 7				
Group V	233,333.33	51,639.778	1.000	NS
Group VI	233,333.33	60,553.007		
Day 11				
Group V	208,333.33	73,598.007	0.674	NS
Group VI	191,666.67	58,452.260		

SD: Standard deviation, NS: Not significant



Graph 7: Comparision of platelet counts between Group IVand VI



Graph 8: Comparision of platelet counts between Group Vand VI

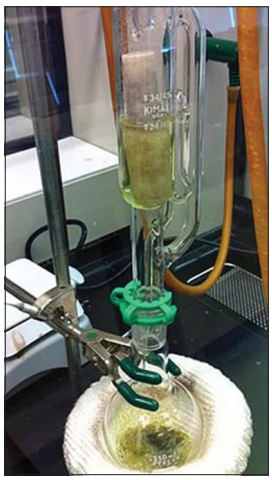


Fig. 1: Soxhlet extractor

Fig. 2: *Alternanthera sessilis* plant

extract which has both antiviral and platelet augmenting property can be helpful. Further studies are required to establish its safety.

A. sessilis leaves aqueous extract no doubt offers a cheap and possibly effective treatment for thrombocytopenia a. However, it is also necessary for further detailed studies to establish the benefits of plant. Large-scale randomized clinical trials in thrombocytopenia patients is necessary to establish its usefulness.

CONCLUSION

- *A. sessilis* leaves aqueous extract has shown *in vivo* platelet augmenting activity in cyclophosphamide-induced thrombocytopenia in Albino rats
- *A. sessilis* leaves aqueous extract effectively increased platelet count in both cyclophosphamide 25 mg/kg and 50 mg/kg induced thrombocytopenia
- Two doses of *A. sessilis* leaves aqueous extract (200 mg/kg and 400 mg/kg) did not show any difference in the degree of platelet augmentation that is there was no dose-dependent activity.

CONFLICTS OF INTEREST

Nil.

AUTHORS FUNDING

Nil.

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