

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Potential Benefits of *Boerhavia diffusa* Leaves Extract for the Management of Thrombocytopenia in Sprague Dawley Rats.

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ABSTRACT

Heparin induced Thrombocytopenia was taken as the model to study the response of the ethanolic extract of *Boerhavia diffusa* in the management of Thrombocytopenia in Sprague dawley rats. The evaluation parameters included platelet count, bleeding time and clotting time. Thrombocytopenia is closely associated with heparin therapy and also sometimes with certain other haematological disorders or conditions related. From this research we could establish that *Boerhavia diffusa* leaves extract was much beneficial in the treatment and management of the same and therefore can be considered as a very good choice of therapy. Owing to the fact *Boerhavia diffusa* is non-toxic at pretty high doses as well, it can be considered as a safe choice of drug as well.

Keywords Thrombocytopenia, Heparin, *Boerhavia diffusa*, Platelets

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INTRODUCTION

Thrombocytopenia is the medical term for a low blood platelet count. Platelets (thrombocytes) are colorless blood cells that play an important role in blood clotting. Thrombocytopenia often occurs as a result of a separate disorder, such as leukemia or an immune system problem, or as a medication side effect. Thrombocytopenia may be mild and cause few signs or symptoms[2]. When the blood has too few platelets, mild to serious bleeding can occur. Bleeding can occur inside the body (internal bleeding) or underneath your skin or from the surface of your skin (external bleeding).

Dengue is a viral disease characterized by rapid fall in the platelet count and in the present time it is rapid task to cure dengue after the diagnosis in later stage. It is caused by Flavivirus which is spread by *Aedes aegypti* mosquito which is also known as Tiger mosquito. There is too much of death that has been reported since decades. Allopathy and several other treatments are effective but curing slowly and high dose of antibiotics may lead some side effects [3]

Heparin-induced thrombocytopenia (HIT) is a complication that is very often associated with heparin therapy. There are two types of HIT. Type 1 HIT which can be observed within the first 2 days after exposure to heparin, and the platelet count normalizes subsequently. Type 1 HIT is a nonimmune disorder that is caused merely due to direct effect of heparin on platelet activation.[4] Type 2 HIT is an immune-mediated disorder that usually occurs 4-10 days after exposure to heparin. [4]

Boerhaavia diffusa (Family- Nyctaginaceae), has drawn a lot of attention due to immunomodulatory effects [5], anti-lymph proliferative activity, anti-viral activity [6] and anti-bacterial activity. The plant is also used in anaemia [7][8], blood purifier[9], wound healing [10], anti-inflammatory [11].This is also used to stop bleeding [12]. This plant also possesses diuretic, cardiotonic [13], antioxidant [14], antihemorrhagic [15], antispasmodic [16], antimicrobial [17], cytotoxic and anticancerous activity.

MATERIALS AND METHODS

Experimental Animals

Male Sprague dawley rats weighing between 150±50g were housed at 25° ± 5°C in a well-ventilated animal house under 12:12 hour light dark cycle. The experimental protocol was approved by the Institutional Animal Ethics Committee. The animals were maintained under standard conditions in an animal house approved by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).All the rats were provided with normal pellet diet and water ad libitum, prior to the dietary manipulation. The Institutional ethical committee approved the experimental protocol no. KCP/IAEC-0004/2013-14.

Plant Extract

The alcoholic extract of *Boerhaavia diffusa* L was obtained from Green Chem Herbal extracts And Formulations, Bangalore.

Induction of Thrombocytopenia

Heparin induced Thrombocytopenia [18]

Subcutaneous injection of low molecular weight Heparin, at the dose of 2000 IU/kg was injected to the rats daily for 10 days.

Experimental Protocol for Thrombocytopenia

Group 1: Normal control

Group 2: Heparin Control

Group 3: Low dose of Alcoholic extract of *Boerhavia diffusa* (200 mg/kg p.o.)

Group 4: Intermediate dose of Alcoholic extract of *Boerhavia diffusa* (300 mg/kg p.o.)

Group 5: High dose of Alcoholic extract of *Boerhavia diffusa* (400 mg/kg p.o.)

Evaluation Parameters

Platelet counting- by indirect method

A drop of blood was taken from the rat’s tail by tail snip method and peripheral blood smear was prepared. This smear was stained Leishman’s stain. It was then examined under 100X oil immersion lens in binocular microscope.

The platelets were counted along with the red cells until 1000 RBCs were counted. The platelet ratio was determined i.e. ratio of platelets to RBCs. RBC count was done in the counting chamber and red cells/mm³ of blood were found out. Then, consequently platelet count/mm³ of blood was estimated.

Clotting time

The clotting time was estimated by method of Lee and White. In this method, a drop of blood was placed on the dry glass slide. From the time of the drop being placed, the slide was tilted at an angle of 45⁰ at 30 seconds interval till there was no change in the shape of the blood drop on being tilted at an angle of 180⁰. This time was noted as the clotting time.

Bleeding time

It was determined by Duke’s method. Tail of the rat was pricked and the time was noted. The oozing blood was mopped with a filter paper at an interval of 30 seconds till the time bleeding stopped. This gave the bleeding time.

RESULTS AND DISCUSSION

Table 1 shows the details about the various evaluation parameters along with comparisons with the various groups.

Table 1: Evaluation parameters for Thrombocytopenia

	BLEEDING TIME (sec)	CLOTTING TIME (sec)	PLATELET COUNT (*10 ³ /mm ³)
NORMAL CONTROL	46.66 ± 2.47	135.83 ± 2.01	1249.5 ± 58.89
HEPARIN CONTROL	93.33 ± 1.05***	181.66 ± 1.05***	167.16 ± 4.63***
LOW DOSE OF EEBD (200 mg/kg p.o.)	61.66 ± 1.05*** ^a	160.83 ± 2.39*** ^a	424.5 ± 18.87*** ^a
INT. DOSE OF EEBD (300 mg/kg p.o.)	59.16 ± 1.54*** ^a	140.83 ± 0.83 ^a	588.33 ± 19.17*** ^a
HIGH DOSE OF EEBD (400 mg/kg p.o.)	57.5 ± 1.12*** ^a	161.66 ± 1.05*** ^a	388.67 ± 11.55*** ^a

EEBD= Ethanolic Extract of *Boerhaavia diffusa*

All values are represented as mean ± SEM, n=6, ***p<0.001 **p<0.01, *p<0.05, when all groups were compared with Normal control group.

^ap<0.001 ^bp<0.01, ^cp<0.05, when all groups were compared with Heparin control group.

During the induction of thrombocytopenia it was well observed that the rats had become very weak and non aggressive towards the pricking of needle. This may possibly indicate deteriorating health conditions of the experimental animals.

The values obtained from the Platelet count, bleeding time and clotting time show that the alcoholic leaf extract of *B. diffusa* at an intermediate dose of 300 mg/kg, showed the most promising results for the management of thrombocytopenia.

Also, while the treatment was given with the drug, there was a remarkable improvement that was observed as far as the physical characteristics were also concerned.

The most possible mechanism of action of the drug, for management of thrombocytopenia may be by inhibiting the activity of antithrombin or else by stopping the inhibition of the clotting factors X and IXa or it may be also through the increase in platelet count. The fact that *Boerhaavia diffusa* is a strong antifibrinolytic, may also contribute to the yield of positive results. The drug might be causing an increase in the platelet count by acting on the megakaryocytes or else may be acting on the platelet factors and thereby inducing more platelet production. Real mechanism of action is yet to be understood.

During the literature study of the drug, it was found that *Boerhaavia diffusa* has a very potent anti-viral activity. Keeping this view in mind we may also conclude that this therapy may also be proved to be useful in curing a serious illness Dengue Fever. This plant extract may be useful in both increasing the platelet count as well as curing the infection hence caused by the dengue causing virus.

CONCLUSION

Boerhaavia diffusa is one of the renowned medicinal plants used to treat large number of human ailments. The intermediate dose showed the most promising results for the management of thrombocytopenia. Based on results obtained we conclude that Alcoholic extract of *Boerhaavia diffusa* leaf can be used as effective treatment in the management of thrombocytopenia.

ACKNOWLEDGEMENT

We extend my sincere Thanks to Mr. R. Rajendran, CEO and Founder of Green Chem Herbal Extracts and Formulations, Bangalore, for providing us the drug extract.

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