

Effect of *Desmodium triflorum* on bleeding time: A pilot study

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Abstract

Natural herbs are popular in current society due to less side effects. *Desmodium triflorum*, is such a valuable plant and crushed leaves are applied on minor wounds to prevent bleeding by villagers. Anyway, the scientific investigations carried out on medicinal plants are fewer. Thus, the current study was launched in the Faculty of Indigenous Medicine to fill the gap to a certain extent and to find out the effect of *Desmodium* leaves on bleeding time. The students of the faculty were considered as the target group due to easy handling and narrow age limit. Bleeding time was tested with the less invasive Duke method. As the control, normal bleeding time of the selected group (n=16) was obtained. The same group was used for the test also, to overcome issues such as age gap, sex gap and health differences. In the test, the pricked site was applied with pure crushed leaves (25 mg) of *Desmodium*. Then, at every 30 seconds, the site was screened for bleeding with the blotting paper (after removing the plant materials). The bleeding time was obtained at the time of stoppage of bleeding. The average bleeding time of control and test was 71.50 and 39.50 seconds respectively and the difference was statically significant ($P < 0.05$) under the paired t test. It is obvious that the bleeding time has an effect from the crushed plant materials. Bleeding time is controlled mainly by the constriction of blood vessels and formation of platelet plugs in the wound area. The reduction of bleeding time by the plant could be due to its phytochemicals such as tannin, flavonoids which have astringent effect that reduce the blood flow to the area. Further, Anti prostaglandin property of phytochemicals reduce vasodilation. Thus, the study will be a platform for scientists to

conduct more investigations on the plant and develop a natural drug for wound bleeding.

Keywords: *Desmodium triflorum*, phytochemicals, astringent, anti-prostaglandin, bleeding time

Introduction

From ancient time, hand remedies have been used by villagers as emergency medicine in Sri Lanka. Among them *Desmodium triflorum* (*Heen Undupiyaliya*) has been commonly used in arresting fresh wound bleeding. Whole fresh plant is crushed and applied around the wound as a remedy to arrest bleeding. Though several such hand remedies are available, only a handful amount has been investigated scientifically to explore their effectiveness/mechanism of action. Thus, this *in-vitro* study was planned to find the effectiveness of *Desmodium triflorum* on bleeding. When a fresh wound is occurred, two body mechanisms come in to arrest bleeding. The damaged vessels constrict and try to reduce the blood flow toward the site. Formation of platelet plug also seal the damaged vessel. These two mechanisms can manage minor bleeding. Thus, how fast small vessels in the skin stop bleeding¹ is called bleeding time and the normal reference range, is 2-7minutes². The clotting cascade come into play later and stop bleeding completely by making a blood clot. In this *in-vitro* study, it is to test the effect of *Desmodium triflorum* on bleeding time, the Duke method was used here in the test to calculate bleeding time as it is less invasive, user friendly and commonly used in hematology. *Desmodium triflorum* which is in Fabaceae family is a small, perennial herb and leaves are small, alternate, stipulate and trifoliate.

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Stipules ovate in shape. Flowers are small, white or pink colored³ (Figure 1).



Fig. 1: Plant *Desmodium triflorum*

In extracts of *D. triflorum*, it has revealed the presence of alkaloids, flavonoids, proteins, phytosterols, saponins and tannins^{4,5}.

As the researchers, there was a research problem to know whether there is a true effect by *Desmodium triflorum* on decreasing bleeding time. Thus, the objective of the current study is to find out the effect of *Desmodium triflorum* on bleeding time.

Materials and methods

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The ethical approval was obtained from the ethic review committee of the Institute where the study was carried out.

This study designed as an experimental study and conducted in Physiology Laboratory, Faculty of Indigenous Medicine (FIM), University of Colombo, Sri Lanka and the time period was October 2022 to January 2023.

The students of the FIM were made involve in the study, due to the facts that the university students are young, less of hematological diseases, supportive and easy to handle.

Participant were selected randomly on lottery base method. The participants were acknowledged first regarding the study and the information forms were given, then the consent was obtained. They were given equal chances to ask questions for their further

knowledge. Investigators asked questions on the study to clarify that the participants understood about the research. The participants were informed that they would leave the study freely at any time if they would not wish to continue the research study.

Exclusive criteria

- The participants who had been on warfarin, Aspirin, NSAIDs, or Alcohol for last 7 days prior to the test
- Participants with hemophilia

Inclusive criteria

- Participants who were healthy and wished to participate in the study

Selection and preparation of plant extract

The selected plant was identified and authenticated with the Department of *Dravyaguna vignana* in the FIM. The total plant was cleaned, washed and air dried. It was crushed with a mortar and pestle.

Bleeding time test²

Both test and control were done on the same individual to avoid issues such un -machining of age, sex and health condition in the two groups.

Control experiment

Fingertip of the participant was sterilized well with surgical sprite (70% Ethyl alcohol) and the area was dried and puncture (non - deep, up to 3mm) with a disposable non expired Lancet. The wound was swabbed with filter paper every 30 seconds until no more blood was absorbed. The total time since the puncture to no more blood stain was seen in filter paper was considered as bleeding time. Normal reference rage of bleeding time for Duke method is 1-3 minutes⁶.

Test experiment

Fingertip of the participant was sterilized well with surgical sprite (70% Ethyl alcohol). The area was dried and puncture (non – deep / up to 3mm) with a disposable non expired Lancet. The first blood drop of the wound was swabbed with filter paper and counting time started. Eventually, a small amount of crushed plant material (25 mg) was kept in the

puncture site. Then, after every 30 seconds, the crushed plant material was removed carefully, blood was wiped out with filter paper and replaced the material quickly. Thus, time was counted until no stain of blood was seen in the filter paper.

Statistical analysis

Paired t test of the SPSS statistical package was utilized to make a statistical comparison between control and test results of bleeding time.

Results and Discussion

In this pilot study, 16 students were tested and the distribution of male and female were 37% and 63%. The average age of the study group was 28 years. Under the sex distribution of the study group, the male was predominant.

The bleeding time of test group and control group is shown in Table 1.

Table 1: The bleeding time of test group and control group

Test	Average time value of the control group (n = 16)	Average time value of the test group (n = 16)	Statistical Significance
Bleeding time	71.50 seconds	39.50 seconds	P < 0.05

According to the result, the bleeding time was significantly reduced in the test group compared to the control group, and this result was statistically significant. Therefore, it is clear that the decrease in bleeding time in the control group is due to the effect of the crushed plant materials.

The arrest of bleeding is a result from a combination of four mechanisms: vasoconstriction, platelet embolism, platelet clot formation, and fibrinolysis. Previous research has found that *Desmodium triflorum* has several active components, such as, Alkaloids, Flavonoids, Saponins and Tannins^{7,8}. Among them, Tanins are vaso protective, they limit the fluid loss and promote regeneration of tissues in *Manohara & Munasinghe, Desmodium triflorum on bleeding time*

case of superficial wounds or burns⁹. Tannins also precipitate proteins to form vascular plugs¹⁰. Flavonoids have an anti-hemorrhagic effect due to stabilization of capillary integrity¹¹, it triggers platelet aggregation also. Thus, it is obvious that phytochemicals in the plant help constrict the blood vessels to reduce bleeding. More overly phytochemicals inhibit the formation of prostaglandin, which occurs during vessel wall injury. Prostaglandin is responsible for vessel relaxation and increase in bleeding¹². More overly, Saponin in *Desmodium* is effective in stimulating the production of collagen, which is important for wound healing⁽¹³⁾. When considering the previous research studies mentioned above, it is clear that the plant contains phytochemicals and its effect on reducing bleeding has occurred due to their assistance. Thus, this could be the reason for using this plant by villagers to arrest bleeding from minor wounds for a long time.

Conclusion

Desmodium triflorum crushed plant materials has an effect on the reduction of bleeding time of minor wounds. The results of this study will help researchers to do more studies on the topic and detect the phytochemicals acting as hemostatic agents which have astringent properties/ vasoconstriction effects to develop a drug for bleeding disorders. Moreover, the outcome of the study could be limited by the number of participants, as well as on the technique and chemicals which were used. The present study has been carried out with a limited number of participants such as 16. This could limit the outcome of the research. Thus, it's better to improve the research with a greater number of participants. Further, in the study Duke method has been used to detect the bleeding time. it's better to follow more than one method such as Ivy method and to justify the outcome of the study.

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