The effect of external application of a paste of leaves of *Achyranthes aspera* L. on bleeding time: A pilot study

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Abstract

Usage of medicinal flora as home remedies is popular in the modern society due to economic benefits. *Achyranthes aspera* (*Apamarga, Karal heba*), is a valuable plant and a paste of leaves of it, is applied on minor wounds by Sri Lankans to prevent bleeding. As it is important to investigate the pharmacodynamic properties of the plant on wound bleeding, the current study was caried out to detect the effect of *Achyranthes aspera* leaves on bleeding time. The setting was the Faculty of Indigenous Medicine. The undergraduates of the faculty were considered as the target group as it was less cumbersome and to reduce age gap issues. Bleeding time was tested with less invasive Duke method. Normal bleeding time of the selected group (n=16) was obtained as the control. The same group was used for the test also and this was to overcome the issues such as age gap, sex gap and health differences. In the test, a paste of pure crushed leaves (25 mg) of the plant was applied to the pricked site. Then, at every 30 seconds, the site was observed for bleeding with the blotting paper (by removing the paste and touch the area with paper). The bleeding time was obtained at the time of stoppage of bleeding. The average bleeding times of control and test was 63.35 and 37.50 seconds respectively and the difference was statically significant ($P<0.05$) under the paired t test. Thus, this confirms that the bleeding time has been significantly reduced by the materials of crushed plant leaves. Bleeding time is regulated mainly by the formation of platelet plug and constriction of blood vessels in the wound area. Reduction of bleeding time by the plant material, could be due to the phytochemicals such as flavonoids, tannins, which possesses astringent effect with which reduce the blood flow to the wound area. Further, phytochemicals which have anti prostaglandin property also reduce the vasodilation. Thus, the study will be a platform for scientists to conduct more research on the Achyranthes and develop a novel drug for wound bleeding.

Keywords: *Achyranthes aspera*, anti-prostaglandin, astringents, bleeding time

Introduction

From the ancient time, medicinal plants have been used by villagers as home remedies in Sri Lanka. Among them *Achyranthes aspera* (*Apamarga*), has been commonly used in arresting bleeding from fresh wound. leaves of the fresh plant are crushed and applied as a paste on the wound. As there are a lot of plant are used as home remedies, it is necessary to carry out scientific studies to find the pharmacodynamic effect of these plants. Thus, this study was carried out to find the pharmacodynamic effect such as the effect of *Achyranthes aspera* on bleeding time.

Formation of platelet plug which seal the damaged vessel and the constriction of blood vessel which reduce the blood flow to the area, are among them. These two mechanisms can manage minor bleeding.

How fast small vessels in the skin stop bleeding[1] is called bleeding time and the normal reference range, is 2-7 minutes[2]. The Duke method was used here to calculate the bleeding time as it is less invasive, user

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friendly and commonly used in hematology. *Achyranthes aspera* which is in Amaranthaceae family is a small, perennial plant with a few spreading branches. Leaves are opposite, lanceolate or ovate. Flowers are small, white or pale green[3].

In the leaves extracts of *Achyranthes aspera* (Figure 1), phytochemicals such as alkaloids, flavonoids, phenolic compounds, saponins and tannins[4.5] have been found. Hence, this study was conducted to find out whether there is a positive effect of *Achyranthes aspera* on decreasing bleeding via bleeding time.

Fig. 1: *Achyranthes aspera* (Flora of Sri Lanka, 2024)[6]

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 faculty 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 September 2022 to January 2023.

Students of the FIM were made involved in the study, due to the facts that the university students are young, supportive, less of hematological diseases, less age gap issues, and easy to handle.

As the study was a pilot study the sample size considered was 16 [12].

Participant were selected randomly on lottery base method. The participants were acknowledged first regarding the study and the information forms were given to them and the consent was obtained. Participants 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 have equal chance to leave the study freely at any time if they would not wish to continue the research study.

Exclusive criteria

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

Inclusive criteria

- Undergraduates Faculty of Indigenous Medicine, University of Colombo
- 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. University of Colombo. The plant leaves were cleaned, washed and air dried. It was crushed with a mortar and pestle.

Bleeding time test [2]

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

Control experiment

Fingertip of the participant was sterilized well with surgical sprite (70% Ethyl alcohol) and the area was air dried and punctured (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 range of bleeding time for Duke method is 1-3 minutes[7].

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 was started. Then, a small amount of crushed plant material (25 mg) was kept on the puncture site. Thereafter every 30 seconds, the crushed plant material was removed carefully and 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 35% and 65%. The average age of the study group was 28 years. Under sex distribution, the female party was predominant.

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

Table 1: Bleeding times 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	63.35 seconds	37.50 seconds	$P < 0.05$

According to the result there has been a great reduction of the bleeding time in the test group, relative to the control group and the value has been statistically significant. Thus, it is clear that the decrease of the average bleeding time of the control group is due to the effect of plant extract.

The arrest of bleeding is occurred due to the collective effect of four mechanisms such as vasoconstriction, formation of platelet plug, formation of platelet clot and fibrinolysis. Anyway, out of the mentioned mechanisms, the first two mechanisms assist in managing the bleeding of small wounds, as well as minor cuts and abrasions.

Central council for research in ayurveda & siddha, has found (in1996) that the *Achyranthes aspera* plant has anti-inflammatory activity[8]. This has been again proved by the researcher Abraham Fkru and collegues[9] in 2012. In addition, the plant has antibacterial activity also (destroy the bacteria who invade the wound)[10]. The saponin and vitamin C in the plant have the effect on collagen formation[5] which is useful for wound healing. Phytochemical studies have found that the plant has phenolic protein complexes which favor astringent effect[11]. The phytochemical, tanin found in the plant is the other astringent, which cause blood vessel constriction[4]. It has been found out that the plant has effect on increasing platelet count also[8].

Anyway, the current study has found that the plant extract reduces the bleeding time significantly. As the reduction of bleeding of small wound basically, occurs due to the formation of platelet plug and contraction of small blood vessels in the area. Previous finding on plant[8,9,10], support the outcome of current study mechanically. Thus, astringent effect of phytochemicals such as tannin, resin, phenolic compounds detected from the plant, constrict the blood vessels to reduce the blood flow to the wound area. The anti-prostaglandin property of the plant also reduces the vasodilation in the wound[9]. In addition, the increasing of availability of platelet in the area can make a platelet clot easily to reduce the bleeding. Moreover, the antibacterial effect and collagen formation effect indirectly improve the wound healing. This could be the reason for usage of particular plant extract by villagers for minor cut and abrasion since longtime.

Conclusion

The study proves that *Achyranthes aspera* crushed plant materials has a significant effect on the reduction of bleeding time of minor wounds hence the bleeding. this could be due to the astringent effect of phytochemicals such as tannin, resin, phenolic compounds found in the plant[8,9]. Hence it is obvious that the plant extract act basically via its astringent property for stoppage of bleeding and it promotes the heling of wound via other properties such as antibacterial and collagen formation proprieties.

The results of this study will help researchers to do more studies on the topic and detect more phytochemicals acting as hemostatic agents which have astringent properties/ vasoconstriction effects, hence 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 due to this is a pilot study. 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 is better to follow more than one method such as Ivy method and to justify the outcome of the study.

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