Pharmacological Research
Pro blood clotting activity of *Scoparia dulcis* in rats

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Abstract

*Scoparia dulcis* Linn (Family: Scrophulariaceae, Sinhala: WalKoththamalli) is a perennial herb growing in many tropical countries including Sri Lanka. Traditional Physicians in rural down south areas apply crushed *S. dulcis* plant on cuts and bruises to stop bleeding. *S. dulcis* may also have Rakta Sthambhana property. The study on effect of decoction (water extract) of *S. dulcis* on blood clotting time in rats was carried out to investigate this. Two groups of rats, 12 males and 42 females were used in this experimental study. Forty-two female rats were assigned into seven equal groups (n = 6/gp). Different doses of DE (25, 50, 100, 1000, 1500 mg/kg) (group 1-5) or 2 ml of distilled water (DW) (group 6) were orally administered. 0.1 ml of vitamin K was injected intramuscularly (group 7) as reference drug to seventh the group. Twelve male rats were assigned into two equal groups (n = 6/gp), 2 ml of distilled water (DW) and doses of DE (1500 mg/kg) were orally administered. Clotting time was determined on the Days 1, 2, and 7 using Lee and White method. In the DE treated groups with all doses, there was no reduction in clotting time on the Day 1 but a significant reduction of clotting time (P < 0.05) was observed on the Days 2 and 7. In the group treated with vitamin K, there was no significant reduction in clotting time on Day 1 or 2, but there was a significant reduction in clotting time on Day 7. It is concluded that *S. dulcis* has proclotting activity (rakthasthambhana property) and this was faster than vitamin K.

Key words: Clotting time, Rakta stambhana, Scoparia dulcis

Introduction

*Scoparia dulcis* Linn (Family: Scrophulariaceae, Sinhala: WalKoththamalli) is perennial herb growing in many tropical countries including Sri Lanka.¹ Traditional physicians in Sri Lanka use this in treatment of diabetes mellitus and in unspecified urinary diseases. Interestingly, some traditional physicians in rural down south areas of the country recommend to apply crushed *S. dulcis* plant on cuts and bruises to arrest bleeding. However, validity of this recommendation is not scientifically tested. Therefore, this study was undertaken in order to test and scientifically confirm the validity of this recommendation in traditional medicine.

Materials and Methods

Collection of herbs and preparation of decoction

Fresh *S. dulcis* plants were obtained from paddy fields of Kaluthara in Sri Lanka. The specimen identity was authenticated in Department of Botany, University of Colombo, Sri Lanka. A voucher specimen (SN-32) has been kept in the museum of the Department of Zoology, University of Colombo, Sri Lanka.

The fresh herb was cut into small pieces and 120 g of these were added to 1920 ml distilled water (DW) and boiled for 10-12 h, until the volume of DE was reduced to 240 ml. This was filtered and the filtrate was gradually evaporated at 30 ± 2°C under partial vacuum until the Volume was 24 ml (yield: 2% w/w). The resulting extract was freeze-dried.

Animals

Healthy adult male and female Wistar rats (200-250 g) were used and kept under standardized animal house condition with free access to water and pelleted food (Vet house Ltd, Colombo, Sri Lanka). All the experiments were conducted in accordance with the internationally accepted laboratory animal use and care and guide lines and rules of the faculty of Science, University of Colombo, Sri Lanka for animal experimentation.

Evaluation of reduction of clotting time in vivo

Two groups of rats, male and female, were used in this experimental study. 12 male rats were randomly divided into two groups and treated orally for 7 days in the following manner 1 (n = 6) 2 ml DW, 2 (n = 6) 1500 mg/kg DE. The clotting time was measured 3 h after the treatment on Days 1,
2, and 7. Males were treated only with the highest dose which showed maximum clotting activity in female rats.

36 female rats were randomly assigned into six groups and treated orally for 7 days in the following manner: 1 (n = 6) 2 ml DW, 2 (n = 6) 25 mg/kg DE, 3 (n = 6) 50 kg/kg DE, 4 (n = 6) 100 kg/kg DE, 5 (n = 6) 1000 kg/kg DE, 6 (n = 6) 1500 kg/kg DE. These dosages were estimated, basing the calculation upon a human equivalent dose. The clotting time was measured in each group 3 h after the treatment Days 1, 2, and 7.

The reference drug, 0.1 ml of vitamin K (Konakion, E. Hoffmann. La Roche Ltd, Basel, Switzerland) was injected intramuscularly (most common method therapeutically used to arrest bleeding quickly) to 6 female rats in group seven and clotting time was measured on the Days 1, 2, and 7.

**Evaluation of calcium induced of blood clotting time in goat blood in vitro**

Goat blood was collected from the slaughter house, Dematagoda, Colombo into autoclaved citrated bottles (using 3.2% Sodium citrate solution).[10] 25 mg DE/1 ml DW, 50 mg DE/1 ml DW, 100 mg DE/1 ml DW and 1 ml of saline were separately added to four different samples of 4 ml of citrated goat’s blood. These were kept for 15 min. From all of the above samples 1 ml in each was drawn and 200 μl of 0.2% of CaCl₂ solution was added. Then clotting time was measured using Lee and White method as described by Ratnasooriya et al.[10]

**Evaluation of the mechanism of action**

In an attempt to evaluate the mechanism/s of the action, 12 male rats were assigned into two groups and treated for two days in the following manner 1 (n = 06) 2 ml DW and 2 (n = 06) 1500 mg/kg DE. On the second day, 3 h after treatment 4 ml of blood were taken by an intra cardiac puncture and tested for thrombin time (TT), prothrombin time (PT), activated prothrombin time (APTT), and platelet count.[2]

**Phytochemical screening of S. dulcis**

The DE was subjected to qualitative testing for alkaloids, flavanoids, phenols, coumarins, steroids, saponins, tanines, amino acids and peptides as described by Farnsworth.[3]

**Statistical analysis**

Data are given as mean ± SEM. Statistical comparisons were made using the Mann-Whitney U-test. Significance was set at \( P < 0.05 \).

**Results**

Phytochemical analysis of DE showed the presence of coumarins, phenols, saponins, tanines, amino acid and peptides. Effects of orally administered decoction of S. dulcis with different doses and distilled water, effect of intramuscularly administered Vitamin K on clotting time in rats are given in Table 1 and Figures 1 and 2. Effects of decoction of S. dulcis on clotting time with goat’s blood are given in Table 2. Effects of oral administration decoction of S. dulcis (1500 mg/kg) on TT, PT, APTT, and platelet count of female rats are given in Table 3.

**Discussion**

The results of this study show paradoxical effect of S. dulcis on clotting time of blood. In in vitro studies it shorten the calcium induced clotting time of goat blood with the doses of 25 and 50 mg/kg DE and prolonged the calcium-induced clotting time of goat blood 100 mg/kg DE. However, effects of clotting time were not dose dependent. This may indicate lack of receptor mediation in including the observed effects on clotting. Clotting of blood involves in two pathways: The extrinsic and intrinsic.[4] The extrinsic pathway usually produces clot in as little as 15 s, while the intrinsic pathway requires 2-6 min.[5] Since both proclotting and anticlotting effects of the S. dulcis are seen in minute range it is likely that the intrinsic pathway is affected.

The decoction of S. dulcis showed antioxidant activity in vitro.[9] Antioxidants are known to exert anti clotting effects in mammalian blood.[3] It s possible that the antioxidants activity of the S. dulcis could be one of the mechanism via S. dulcis induced anti-clotting effects in vitro with the dose of 100 mg/kg DE. In addition, flavonoids prevent platelet aggregation and thereby reduce the risk of cardio vascular diseases.[6] This would be another benefit of S. dulcis.

### Table 1: Effect of orally administered decoction of Scoparia dulcis on clotting time in male/female rats (mean ± SEM) in vivo

| Rats     | Dose         | Day 1 Clotting time (s) mean ± SEM | Day 2 Clotting time (s) mean ± SEM | Day 7 Clotting time (s) mean ± SEM |
|----------|--------------|------------------------------------|------------------------------------|------------------------------------|
|          |              | Clotting time (s) mean ± SEM        | Clotting time (s) mean ± SEM        | Clotting time (s) mean ± SEM        |
| Male     | 2 ml DW      | 115 ± 5 sec                         | 115 ± 12.1                          | 120 ± 11                           |
|          | 1500 mg/kg DE| 115 ± 9.2                           | 70 ± 6.3*                           | 70 ± 10*                           |
|          | 2 ml DW      | 115 ± 12.1                          | 130 ± 6.3                           | 120 ± 13.4                         |
|          | 25 mg/kg DE  | 115 ± 9.2                           | 70 ± 6.3*                           | 85 ± 2.1*                          |
|          | 50 mg/kg DE  | 105 ± 12.8                          | 80 ± 6.3*                           | 80 ± 6.3*                          |
|          | 100 mg/DE    | 115 ± 9.2                           | 90.2 ± 0.1*                         | 85 ± 2.1*                          |
|          | 1000 mg/DE   | 115 ± 9.2                           | 85 ± 5*                             | 80 ± 6.3*                          |
|          | 1500 mg/kg   | 115 ± 9.2                           | 45 ± 6.7*                           | 85 ± 2.1*                          |
|          | 0.1 mlVit.K/Kg | 115 ± 10                           | 120 ± 10                            | 65 ± 5*                            |

\( P < 0.05 \) significant
Ediriweera, et al.: Pro blood clotting activity of *Scoparia dulcis*.

**Table 2: Effect of decoction of *Scoparia dulcis* on clotting time with goat’s blood (mean ± SEM) in vitro**

| Dose       | Blood clotting time (mean ± SEM) (s) |
|------------|-------------------------------------|
| 1 ml saline| 165 ± 6.2                           |
| 25 mg/kg DE| 121.5 ± 3.4*                        |
| 50 mg/kg DE| 156 ± 7.1                           |
| 100 mg/DE  | 266.5 ± 12.8                        |

*P < 0.05 significant

**Table 3: Effect of oral administration decoction of *scoparia dulcis* (1500 mg/kg) on some blood parameters of female rats (mean ± sem)**

| Blood parameters          | Control (mean ± SEM) | Treated (mean ± SEM) |
|---------------------------|----------------------|----------------------|
| Platelet count            | 591888 ± 56883       | 62733 ± 71948        |
| Thrombin time (TT)        | 12.6 ± 0.4           | 12.4 ± 0.3           |
| Prothrombin time (PT)     | 14.8 ± 0.3           | 14.5 ± 0.7           |
| Activated prothrombin     | 31.1 ± 2.0           | 31.1 ± 1.3           |

*P < 0.05 significant

In conclusion, this study shows that decoction of *S. dulcis* possess coagulant activity in *in vitro* against goat blood with the dose of 25 mg/kg DE and anticoagulant activity in *in vitro* against goat blood with the dose of 100 mg/kg DE and coagulant properties *in vivo* against rats’ blood.

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स्कोपारिआ डर्सस की रक्तस्फोटन गतिविधि का प्रायोगिक अध्ययन

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स्कोपारिआ डर्सस (कुल—स्कोपूललेसी) एक बारहमासी बनसपति श्री लंका सहित कई उष्णकटिवत्तीय देशों में उगने वाली ओषधि है। पारंपरिक वैद्य दक्षिण के निचले इलाकों में शायद इस में रक्तस्फोटन गुण होने के कारण और धाव से निकलने वाले स्कट को रोकने के लिए इसका उपयोग करते थे। इसकी जांच के लिए चूहों में स्कोपारिआ डर्सस के क्राथ का प्रयोग रक्तस्फोटन समय पर प्रभाव देखने के लिए किया गया। इस प्रायोगिक अध्ययन में चूहों के दो समूहों में १२ नर एवं ४२ मादा चूहों का उपयोग किया गया। ४२ मादा चूहों को सात वर्ष का युग्म में (n=6/gp) देखे और डी.ई. की विशेष खुराक (२५, ५०, १००, १५०० मिली.ग्राम.) (समूह—१–५) वा २ मिली. आसुत जल मुख मार्ग से दिया गया (group-6)। सातवें समूह में ०.१ मिली. विटामिन – के, मास्पेशरी में री फरेंस्ट्रू ड्रग के रूप में दिया गया, जब कि १२ नर चूहों को दो समूहों में (n=6/gp), २ मिली. आसुत जल और डी.ई. की (१५०० मिली.ग्राम.) सुरक्षा मुख मार्ग द्वारा दी गयी। और विधियों का प्रयोग कर पहले, दूसरे और सातवें दिन रक्तस्फोटन समय क्षमता मिर्चियरिया की गयी। डी.ई. से इलाज किये-गये सभी समूहों में पहले दिन रक्तस्फोटन समय में कभी नहीं पायी गयी जब कि दूसरे, सातवें दिन महत्वपूर्ण कभी देखने को मिली। विटामिन-के-द्वारा इलाज किये-गये समूह में पहले और दूसरे दिन रक्तस्फोटन समय में महत्वपूर्ण कभी पायी गयी थी। अतः यह निष्कर्ष निकला कि स्कोपारिआ डर्सस में रक्तस्फोटन गुण है जो विटामिन – के की तुलना में अधिक था।