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Effect of kohiladi decoction on clotting of blood

K.R.Weerasekera, E.R.H.S.S.Ediriweera, M.S.P. Cooray Vidyashekhera, J.R.A.C.Jayakody[1] and W.D.Ratnasooriya[1,2]

Department of Diagnosis and Treatment, Institute of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka.

[1] Department of Zoology, University of Colombo, Colombo, Sri Lanka.

[1,2] Corresponding author: e- mail : wdr@zoology.cmb.ac.lk

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Abstract

The study examined the effect of kohiladi decoction (consisting of tubers of *Lasia spinosa*, Linn, entire plant of *Centella asiatica* Linn, seeds of *Vigna radiate* Linn, bulbs of *Allium cepa* Linn and dried fish of *Katsuwonus pelamis*), a decoction frequently recommended for bleeding piles by Sri Lankan traditional and Ayurvedic physicians, on clotting of blood. This was tested both *in vitro* (using goat blood and human blood) and *in vivo* (using rats). The results show that 1 mg/ml concentration of decoction prolonged the calcium induced clotting time significantly (goat blood by 55% and human blood by 76%) in *in vitro*. In contrast, a 380 mg/kg/day dose of decoction had no effect on clotting when given as a single dose but shorten the clotting time when given for 2 or 3 days. The decoction also had moderate antioxidant activity when determined by thiobarbituric acid reaction substances assay. It is concluded that kohiladi decoction has anticlotting action *in vitro* and proclotting activity *in vivo*.

Key words: kohiladi decoction, clotting, anticlotting, haemorrhoids

1. Introduction

Haemorrhoids is a major affliction that affect the human society. Haemorrhoids occur in about half the population over 50 years and men are more frequently affected than women (Cuschieri *et al.*, 1982). The main symptoms of the haemorrhoids are bleeding, prolapse, discharge and pain (Mann *et al.*, 1997). In Western medicine haemorrhoids are treated by several methods. These include chemotherapy, reduction of sphincteric pressure, fixation treatment and surgical treatment (Cuschieri *et al.*, 1982). In Sri Lanka, in traditional and Ayurvedic medicine application of herbal oils and pastes, chemical cauterization (kshara sutra) and several decoctions are used in the treatment of haemorrhoids (Kumarasingha,

1981). Kohiladi decoction is one decoction that is recommended frequently in the treatment of bleeding piles. This decoction consists of tubers of *Lasia spinosa* Linn (Family: Araceae, kohila in Sinhala), entire plant of *Centella asiatica* Linn (Family: Umbeliferae, gotukola in Sinhala), seeds of *Vigna radiate* Linn (Family: Leguminosae, muneta in Sinhala), bulbs of *Allium cepa* Linn (Family: Liliaceae, ratulunu in Sinhala) and dried fish of *Katsuwonus pelamis* (Family: Scombridae, commonly known as umbalakada in Sinhala and maldive fish in English). It is possible that this kohiladi decoction acts by promoting blood clotting and inducing anti inflammatory and analgesic actions. In addition, it may also enhance blood circulation. However, these potential mechanisms have not been scientifically investigated, as yet

The aim of this study is to examine whether kohiladi decoction has any promoting activity on clotting on blood. This was tested both *in vivo* using rats and *in vitro* using goat and human blood.

2. Material and methods

2.1 Experimental animals

Healthy adults cross bred male albino rats purchased from Medical Research Institute in Colombo, were used as experimental animals. These animals were kept in standardized animal house condition (temperature 28 - 31° C, photoperiod approximately 12 h natural light per day, relative humidity: 50-55%) with free access to palleted food (Vet House Ltd., Colombo, Sri Lanka) and tap water.

2.2 Purchasing and authenticated of ingredients

All the five ingredients of the decoction were purchased from the open market at Rajagiriya in August 2003 and were authenticated by Dr. Damayanti Vitanage, the Head, Department of Meteria Medica, Institute of Indigenous Medicine, University of Colombo. Voucher specimens (MM 23) were deposited at Department of Meteria Medica, Institute of Indigenous Medicine.

2.3 Preparation of hot water extract

The decoction was prepared according to Sri Lankan Ayurvedic Pharmacopeia (Jayasinghe, 1975). All the ingredients were washed separately using running tap water for 10 minutes and shade dried for 3 hours. Twelve grams of each of the ingredients was cut into small pieces (2-3 cm length) except the seeds of *V. radiate* and chopped using mortar and pestle. These were slowly boiled (about 6 hours) in 1920 ml of tap water until the volume was reduced to 240 ml. The resulting decoction was filtered and evaporated until a dark brown semisolid (yield; 44.75% w/w) was formed. This was stored in a refrigerator(at 4°C) until used. At the time of

use this semi solid was dissolved in distilled water (DW) to obtain with required dosages.

2.4 Evaluation of Calcium induced blood clotting time in goat and human blood *in vitro*

Goat blood was collected from the slaughter house, Dematagoda, Colombo, and immediately citrated using 3.1% sodium citrate solution (Ratnasooriya and Ranatunga, 1975). Human blood was collected from the median cubital vein of healthy adult male and female volunteers using sterile conditions and was immediately citrated. Citrated blood and decoction mixtures were made in test tubes by mixing 4 ml of blood with 1ml of the decoction of a known concentration. The concentrations made were 1, 0.5, 0.25, 0.125, 0.0625 and 0.03125 mg/ml (dilutions were made by using 0.9% sodium chloride solution) (Ratnasooriya and Ranatunga, 1975). Citrated blood was mixed with 0.9% sodium chloride solution in a seventh test tube. Blood decoction mixture was pipette out in to a small, clean, dry glass tubes (1 cm diameter, 5 cm height); 0.2 ml of 2% Calcium chloride was added from a burette and the tubes were stoppard. The contents of each tube were mixed immediately by tilting and the tubes were tilted every 30 seconds until a fiom clot is formed (Ratnasooriya and Ranatunga, 1975). Experiments exactly similar to this were performed using human blood.

2.5 Evaluation of the blood clotting time of rats *in vivo*

Forty five male rats were randomly divided in to 5 equal groups (n=9/group). These rats were orally treated with different doses of decoction. Group 1 was orally treated with 95 mg/kg of decoction in 1ml, group 2 with 190 mg/kg of decoction in 1ml, group 3 with 285 mg/kg of decoction in 1ml, group 4 with 380 mg/kg of decoction in 1ml, group 4 with 570 mg/kg of decoction in 1ml and group 5 with 1ml DW. These treatments were done twice a day (9.00-9.30 h. and 15.30- 16.00 h) for 3 consecutive days. The highest dose selected was 15 times greater than the usual human recommended dose (Jayasinghe, 1975). Blood samples were collected from tails of these rats under light ether anesthesia 3 hours prior to treatment and day 1 post treatment using aseptic precautions and the clotting times were determined as described earlier.

In another experiment, 18 male rats were randomly divided in to 2 equal groups. Group 1 was treated for 2 consecutive days with 380 mg/kg of decoction in 1 ml (mid dose) and other with 1ml DW twice a day (9.00-9.30 h and 15.30-16.00 h). Blood was collected as described previously 3 hours before treatment and on the last day of treatment and day 1 post treatment and clotting times were determined.

2.6 Antioxidant activity

The experiment was carried out using thiobarbitiuric acid reaction substances assay as described by Dorman *et al.*, (1995). The vials containing the reagents were treated in triplicate with the extract so that the final concentrations of the kohiladi decoction in the vials become 0.075, 0.125 and 0.175 mg/ml. 100 μg/mc of butylated hydroxyl toluene (BHT) was used as the positive reference and DW was used in the control. The vials were mixed well and incubated at 95°C for 60 min and allowed to cool. 5 ml of butanol was added, mixed well and centrifuged at (1500×g). The absorbance of the butanol layer was measured at 532 nm and the antioxidant index was calculated as follows:

Antioxidant index = (1-T/C) × 100
T = absorbance of test, C = absorbance of control

2.7 Phytochemical screening

The kohiladi decoction was subjected to qualitative testing for alkaloids, flavonoids, phenols, coumarins, steroids, saponins, tanines and amino acids as described by Fransworth (Farnsworth, 1996).

2.8 Statistical analysis

Data are expressed as means ± SEM. Statistical comparisons were made using Mann-Whitney U- test. Significance was set at $P< 0.05$.

3. Results

3.1 Effect of kohiladi decoction on calcium induce blood clotting time of goat and human blood *in vitro*

As shown in Table 1, the highest dose of decoction significantly (P=0.0018) prolonged the calcium induce blood clotting time of goat blood (by 55%).

The highest dose of decoction also significantly increased (P=0.0001) the calcium induce blood clotting time of human blood (by 76%) (Table 2).

3.2 Effect of kohiladi decoction on blood clotting time in rats *in vivo*

As depicted in Table 3, 380 mg/kg dose of kohiladi decoction significantly (P=0.0013) shortened the *in vivo* clotting time of rat blood (by 37%). This dose of kohiladi decoction also shortened the clotting time (by 48%) significantly (P=0.0003) on day 1 post treatment when given consecutively for 2 days (Table 4).

3.3 Effect of kohiladi decoction on antioxidant activity *in vitro*

Kohiladi decoction showed a Linear dose dependent (r^2= 0.99;$p<0.05$) antioxidant activity as indicated by the antioxidant index. However, butylated hydroxyl toluene

(BHT) showed a higher antioxidant index than kohiladi decoction (Table 5). The dose dependent response relationship was linear (r^2= 0.99) and the EC_{50} for the antioxidant activity was 0.65 mg/ml kohiladi decoction.

3.4 Pytochemical analysis

Phytochemical analysis of kohiladi decoction showed the presence of coumarins, flavonoids and amino acids.

Table 1. Effect of kohiladi decoction on the calcium induced blood clotting time of goat blood *in vitro* (mean ± SEM, n = 6)

	Control	Concentrations of decoction (mg/ml)					
		1	0.5	0.25	0.125	0.0625	0.03125
Time (min)	2.2±0.9	3.4±0.4*	2.5±0.3	2.6±0.3	2.3±0.3	2.5±0.2	2.1±0.2

(as compared with control ; *P< 0.05)

Table 2. Effect of kohiladi decoction on the calcium induced blood clotting time of human blood *in vitro* (mean± SEM, n= 15)

	Control	Concentrations of decoction (mg/ml)					
		1	0.5	0.25	0.125	0.0625	0.03125
Time (min)	3.0±0.1	5.3±0.2*	3.1±0.5	2.8±0.2	2.5±0.1	2.5±0.2	2.4±0.2

(as compared with control ; *P<0.05)

Table 3. Effect of kohiladi decoction on the blood clotting time *in vivo*- day 1 post treatment (means ± SEM, n=9)

	Control	Concentrations of decoction (mg/ml)				
		95	190	285	380	570
Time (min)	1.52±0.15	1.44±0.17	1.56±0.1	1.56±0.15	0.56±0.06*	1.61±0.08

(as compared with control; *P< 0.05)

Table 4. Effect of kohiladi decoction on the blood clotting time in rats as determined on treatment day 2 and day 1 post treatment (mean ± SEM, n=9)

	Day 2 of treatment	Day 1 Post treatment
Control Time (min)	1.58±0.15	1.27±0.08
Treated Time (min)	1.39±0.11	0.61±0.11*

(as compared with control; *P< 0.05)

Table 5. Effect of antioxidant activity of kohiladi decoction *in vitro*

Doses	AI (Anti oxidant index) %
Control	0
BHT	66.0 ± 4.3
Drug (kohiladi decoction)	
0.075mg/ml	47.88 ± 4.7
0.125mg/ml	39.87 ± 1.6
0.175mg/ml	29.95 ± 1.7

EC_{50} = 0.65 mg/ml, r^2 = 0.99, P < 0.05

4. Discussion

The results of this study show paradoxical effects of kohiladi decoction on clotting of blood. *in vitro* studies it prolonged the calcium induced clotting time of both of goat and human blood. In contrast, *in vivo* studies it shortens the clotting time of rat blood. However, in none of these systems the effects on the clotting time was dose-dependent. This may indicate the lack of receptor mediation in inducing the observed effects on clotting. Clotting of blood involves two pathways: the extrinsic and intrinsic (Laurence and Bonnett, 1980). The extrinsic pathway usually produces clot in as little as 15 seconds, while the intrinsic pathway requires 2-6 minutes (Guyton and Hall, 1984). Since both proclotting and anticlotting effects of the decoction are seen in minute range it is likely that the intrinsic pathway is affected.

The decoction showed moderate antioxidant activity *in vitro*. Antioxidants are known to exert anticlotting effects in mammalian blood (Felten, 2004). It is possible

that the antioxidant activity of the decoction could be one of the mechanisms via decoction induced anti-clotting effects *in vitro*. This may be precipitated by the flavonoids which are present in the decoction: flavonoids are powerful antioxidants (Desai, 2004). In addition, flavonoids prevent platelet aggregation and thereby reduce risk of cardiovascular diseases (Desai, 2004). This would be a yet another benefit of the kohiladi decoction.

In vivo studies, single day treatment (380 mg/kg/day) failed to induce any effect on clotting of rat blood. On the other hand, two and three day treatments with the same dose shortened the clotting time markedly and significantly. This suggests that a metabolite/s of the decoction is responsible for the shorting of the clotting time. In agreement with this finding usually the arrest of bleeding in haemorrhoids patients treated with the kohiladi decoction usually occurs around third day of treatment (personal observations of two of the authors; K.R.W., E.R.H.S.S.E) Alternatively, these observations indicate that the kohiladi decoction may promote the synthesis of clotting factors produced in the liver, similar to vitamine K (Katzung, 1984). However, this seems unlikely because the decoction contained coumarins and coumarin anticoagulant drugs such as warfarin sodium is known to act by inhibiting the synthesis of vitamin K dependent clotting factors (Katzung, 1984). We have observed that kohiladi decoction induces tonic contractions in isolated rat carotid arterial preparations (unpublished observations). It is possible that both clotting promoting activity and vasoconstriction action of the decoction be involved in arresting bleeding in haemorrhoids patients. Further studies are required to elucidate precise mode of action on the kohiladi decoction.

In conclusion, this study shows, for the first time, that kohiladi decoction possess anticoagulant activity in *in vitro* (against goat and human blood) and coagulant properties *in vivo* (against rat blood).

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