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Short report

Haemostatic effect of fresh juice and methanolic extract of *Eupatorium ayapana* leaves in rat model

Aiyalu Rajasekaran[a,*], Muthusamy Kalaivani[a], Ganesan Ariharasivakumar[a]

[*a]*KMCH College of Pharmacy, Coimbatore, Tamil Nadu, India.*

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ABSTRACT

Eupatorium ayapana was tested for its haemostatic effect based on its ethanomedical survey. Fresh juice and methanolic extract was evaluated for its haemostatic effect in rat model using tail bleeding time and blood clotting time. The study revealed that both fresh juice and methanolic extract decreased the bleeding time and clotting time significantly at dose of 200 mg/kg and 50 mg/kg, respectively, which confirmed the use of Eupatorium ayapana in arresting blood bleeding traditionally.

1. Introduction

Medicinal plants are of important therapeutic aid for treatment of various diseases. It was estimated that about 20,000 species from various families are used as medicinal herbs [1]. Further 80% of world population depends on plant-based drugs [2]. In India, medicinal plants are used by all communities of people either directly as folk remedies or in indigenous system of medicine. According to National health expert, 2000 species of plants are used as medicine internally or externally in India for the management of diverse diseases such as catarrh, bronchitis, pneumonia, ulcers and diarrhea. Researchers now show more focus towards developing scientific data for folk medicine.

Eupatorium ayapana is a plant used as folk remedies for immediate arrest of bleeding of blood from wound in India. The plant is also used as folk medicines as cardiotonic, diaphoretic, emetic, hemostat, laxative, stimulant and tonic, haemostatic, antiseptic, cardiac stimulant, emetic, diaphoretic, laxative, various common cold, constipation, fevers, flu, pneumonia, yellow fever in countries like India, Peru, Bangladesh, Trinad, United states, Mauritius, Argentina etc. The antimicrobial activity of this plant was reported by Gupta et al [3]. The aim of the present study was to investigate the effect of Eupatorium ayapana on bleeding and clotting time using animal models.

2. Materials and Methods

2.1 Plant collection

Plant materials were collected from Forest research department, Coimbatore, India and identified after critical examination. The taxonomic identification of plant materials was confirmed by Botanical Survey of India (BSI), Southern circle, Coimbatore, Tamilnadu, India.

2.2. Preparation of fresh juice

The collected plant material was washed with distilled water, and the leaves were separated. Collected leaves were cut in to small pieces and crushed in Mechanical mortar with distilled water. The fresh juice was filtered off and transferred into a clean closed container.

2.3. Preparation of methanolic leaf extract

The collected plant material was dried under shade, and the leaves of the plant were separated from the stem, and ground in a grinder. The powdered, dried leaves of plant (200 g) were extracted with methanol by cold maceration for 48 h with occasional shaking and filtered through Whatman filter paper (No.1). The resulting filterate was concentrated to dryness and the yield was 5 % w/w.

2.4. Experimental animals

Male Wister rats weighing 250-450 g were used for the study. All rats were kept at room temperature in the animal house. The animal procedures were followed as per the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, rats were fed with standard laboratory food for one week in order to adapt to the laboratory conditions. This study was approved by the Institutional Animal Ethical Committee of KMCH College of Pharmacy, Coimbatore, India.

* Corresponding Author : Dr. Aiyalu Rajasekaran
Professor, KMCH College of Pharmacy, Coimbatore, Tamil Nadu, India.
Email: rsekaran2001in@yahoo.co.in

2.5. Experimental design

The animals were divided into seven groups (n=6): Group I was kept as control, without administration of drug, Group II- received oral administration of fresh juice at the dose of 50 mg/kg, Group III- received oral administration of fresh juice at the dose of 100 mg/kg, Group IV- received oral administration of fresh juice at the dose of 200 mg/kg, Group V- received oral administration of methanolic extract at a concentration of 50 mg/kg, Group VI- received oral administration of methanolic extract at the concentration of 100 mg/kg, Group VII- received methanolic extract at the concentration of 200 mg/kg orally.

2.6. Tail bleeding time

The bleeding time was determined using a modified tail cutting method, as described previously [4,5,6]. The rats were placed in a plastic cylinder with several openings from one of which the animal's tail emerged out. To avoid possible effect of anesthesia on blood vessel interplay and possible interaction between anesthetics and extracts to be investigated were not administered with anesthesia. The rats were maintained in room temperature (23 ± 1°C). Bleeding times were measured by transaction of tail; 2 mm from the tip using disposable surgical blade. The rat's tails were placed in isotonic saline solution pH 7.4 at 37°C immediately after injury. Bleeding time was noted from the moment transaction was done until bleeding stops completely and expressed in seconds. Bleeding time was noted at 0 min, 30min and 60min after administration of the fresh juice and metanolic extract of Eupatorium ayapana leaf.

2.7. Clotting time determination

The blood was collected in a capillary tube by retro-orbital puncture. A stop clock was started immediately and the time taken to form thread-like structure while breaking the capillary tube was noted in seconds.

2.8. Statistical analysis

Results were expressed as mean ± standard error of mean (SEM). Statistical analysis was carried out using Graphpad prism 5.

3. Results and Discussion

In the present study, the effect of Eupatorium ayapana (figure 1) on bleeding and clotting time was investigated. The fresh juice and methanolic leaf extract was used for evaluation using rat model.

Figure 1. Eupatorium ayapana leaves

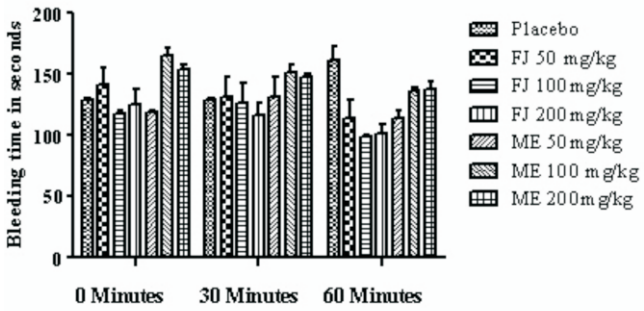
Table 1 and figure 2 summarizes the results of bleeding time experiment. Fresh juice after 60 minutes of administration showed significant decrease (P<0.01 and P<0.001) in bleeding time to 114, 99 and 102 seconds when compared to control (161 secs) at dose levels of 50, 100 and 200 mg/kg respectively. At 0 and 30 minutes also it showed reduction in bleeding time but not to at significant level.

Table 1. Effect of Eupatorium ayapana-Fresh leaf juice and methanolic leaf extract on tail vein bleeding time in rat model.

Treatment	Bleeding time (s)		
	0 Minutes	30 Minutes	60 Minutes
Control	129.5 ± 0.5	129±1	161.5 ±11.5
Fresh leaf juice 50 mg/kg	141.5±13.5^{NS}	131.5±16.5 ^{NS}	114 ± 15**
Fresh leaf juice 100 mg/kg	118.5±1.5 ^{NS}	126.5±16.5 ^{NS}	99 ± 1***
Fresh leaf juice 200 mg/kg	125 ±12.5 ^{NS}	117 ± 10 ^{NS}	102 ± 7***
Methanolic leaf extract 50 mg/kg	119 ± 0.5 ^{NS}	131.5±16.5 ^{NS}	113.5±6.5**
Methanolic leaf extract 100 mg/kg	165 ± 7*	152 ± 6 ^{NS}	137 ± 2 ^{NS}
Methanolic leaf extract 200 mg/kg	154 ± 4 ^{NS}	147 ± 2.5 ^{NS}	137.5 ±6.5 ^{NS}

P<0.01,*P<0.001, NS-Non significant.

Figure 2. Effect of treatment with Eupatoriam ayapana-fresh leaf juice and methanolic leaf extract on tail vein bleeing time in rat model



Similarly methanolic extract also showed significant reduction in bleeding time at the dose of 50 mg/kg at 60 minutes (P<0.01), but at 100 and 200 mg/kg dose levels it showed non significant decrease in bleeding time.

The clotting time obtained at all doses of Eupatorium ayapana extract and fresh juice were shown in table 2 and figure 3.The 60 min time taken for decrease in bleeding time may be for the absorption of orally administered fresh juice and methanolic extract of leaf.

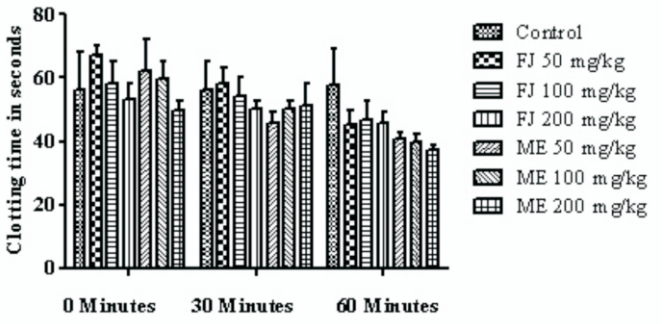
Theoretically impaired haemostatis will lead to impaired wound healing. Normal wound healing has been divided into four overlapping phases: (i) haemostasis, (ii) inflammation, (iii) proliferation and (iv) remodelling or resolution [7]. The haemostatic phase starts as soon as vessels are damaged. The significant reduction in bleeding time suggest that the Eupatorium ayapana leaf extract and fresh leaf juice have positive effect on haemostatic phase of wound healing, may possibly act on the integrity of blood vessel or involvement of platelets forming the haemostatic plug. Platelets are the blood cells involved in coagulation [8,9] or it may inhibit the formation of prostaglandin

Table 2. Effect of Eupatorium ayapana-Fresh leaf juice and methanolic leaf extract on blood clotting time in rat model.

Treatment	Bleeding time (s)		
	0 Minutes	30 Minutes	60 Minutes
Control	56.5±11.5	56±9	57.5±11.5
Fresh leaf juice 50 mg/kg	67.5±2.5^{NS}	58.5±4.5^{NS}	48.5±4.5^{NS}
Fresh leaf juice 100 mg/kg	58.5±6.5 ^{NS}	54.5±5.5 ^{NS}	47±6 ^{NS}
Fresh leaf juice 200 mg/kg	53.5±4.5 ^{NS}	50.5±2.5 ^{NS}	46±3 ^{NS}
Methanolic leaf extract 50 mg/kg	62.5±9.5 ^{NS}	46±3 ^{NS}	41±2 ^{NS}
Methanolic leaf extract 100 mg/kg	59.5±5.5 ^{NS}	50.5±2.5^{NS}	40±2 ^{NS}
Methanolic leaf extract 200 mg/kg	50±3 ^{NS}	51.5±6.5 ^{NS}	37.5±1.5^{NS}

P<0.01,*P<0.001, NS-Non significant.

Figure 3. Effect of treatment with Eupatorium ayapana-fresh leaf juice and methanolic leaft extract on clothing time rate model



by the vessel walls during injury. Prostaglandin released during injury is responsible for vessel relaxation, which leads to increase in bleeding of blood during injury [10]. Further studies on complete isolation and characterization of active constituent from Eupatorium ayapana and its mechanism of decrease in bleeding time are in progress.

Conflict of interest

Authors declare that there is no conflict of interest.

Acknowledgement

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