Research Article

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EVALUATION OF THE HAEMOSTATIC EFFECT OF PANCHAVALKALA KASHAYA AND AQUEOUS EXTRACT IN WISTAR RATS

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ABSTRACT

The effect of Panchavalkala group of drugs on blood coagulation has not been scientifically tested despite it has mentioned in Ayurveda by Acharya Sushruta. The current study investigates the effect of Panchavalkala kashaya and Panchavalkala aqueous extracts on Wistar rats by tail tip amputation method. Amount of bleeding and Haemostatic time is observed immediately after tail tip amputation in heparinised and non- heparinised groups. The data was analysed using Kruskal Wallis test. In non-heparinized category of Wistar rats proved that both Panchavalkala kashaya and Panchavalkala aqueous extract have highly significant haemostatic effect in the test animals. Panchavalkala aqueous extract was more effective in reducing haemostatic time as well as the amount of bleeding in heparinised Wistar rats when compared to Panchavalkala kashaya, which did not show any significant haemostatic effect in the heparinized test animals.

Keywords: Haemostatic, Panchavalkala kashaya, Panchavalkala aqueous extract.

INTRODUCTION

Haemostasis is a process to prevent and stop bleeding, to keep blood within a damaged blood vessels and it is the first stage of wound healing[1]. In Ayurveda, Acharya Sushruta has explained various haemostatic measures which are Santhana, Skandana, Pachana and Dahana along with the protocol of using above methods. Among these Santhana (uniting) is the first line of treatment of haemorrhage during surgery followed by Skandana (coagulation of blood). If the bleeding is still not controlled, then Pachana (ripening or digestive measures) and Dahana karma (thermal cauterisation) should be done[2]. One among the drug mentioned by Acharya Sushruta for Skandana karma with Seetha veerya (cold potency) is Panchavalkala. Panchavalkala group of drugs include, Nyagrodha (*Ficus benghalensis*, Moraceae family), Udumbara (*Ficus glomerata*, Moraceae family), Aswatha (*Ficus religiosa*, Moraceae family), Plaksha (*Ficus lacor*, Moraceae family), Parisha (*Thespesia populnea*, Malvaceae family). Haemostasis is of critical importance among all surgical procedures which is essential technique for minimizing blood loss during surgery and management of bleeding wounds. Among the four basic haemostatic method mentioned by Acharya Sushruta, Skandana is the method to be analysed for its practical applicability in managing superficial bleeding wounds.

MATERIALS AND METHODS

Materials: Animal- Albino Wistar rats of either sex weighing 150–200 g were maintained under normal laboratory condition of humidity (50%), temperature (23±2 ^{0}C), and 12-hour light/dark cycle, allowed free access to food and water. Drugs: 1. Injection Ketamine hydrochloride and Xylazine were purchased commercially. 2. Injection Heparin sodium solution (640 IU/kg), 3. Normal saline, 4. Panchavalkala kashaya, 5. Panchavalkala aqueous extract, 6. Scalpel with blade, 7. 1 ml syringe, 8. Povidone-iodine solution, 9. Surgical spirit, 10. Ependorf.

Setting: Department of Research and Development Pankajakasthuri Ayurveda Medical College and Post Graduate Centre, Biochemistry lab under Pankajakasthuri Herbal Research Foundation, Kattakada, Thiruvananthapuram, Kerala, India. Animal house under (2093/PO/ReRcBi/S/20/ CPCSEA) under Pankajakasthuri Herbal Research Foundation, Kattakada, Thiruvananthapuram, Kerala, India

Ethical Approval: The experimental protocol of the present study was reviewed thoroughly and approved by Institutional Ethical Committee of Pankajakasthuri Ayurveda Medical College and Post Graduate Centre with reference number PKAMC/ IEC/64/2020 and by the Institutional Animal Ethical Committee of Pankajakasthuri Herbal Research Foundation, Killy, Kattakada, Thiruvananthapuram, Kerala, India (Reg. No. 2093/ PO/ReRcBi/S/20/CPCSEA) with reference no. PKAMC/IAEC/ NOC/04/2021 and animals were maintained as per the guidelines of the CPCSEA, India.

Method of trial drug preparation: Drugs used in the preparation of trial drugs are; Nyagrodha (*Ficus bengalensis)*, Udumbara (*Ficus glomerata)*, Aswatha (*Ficus religiosa*), Plaksha (*Ficus lacor)*, Parisha (*Thespesia populnea*). Fresh barks of the above drugs were collected with help of local herbalist. The barks were chopped into small pieces, shade dried at ambient temperature, and stored in airtight container. The powders from each bark were taken in equal proportion for Kashaya (decoction) and extract preparation. Panchavalkala kashaya was prepared by boiling

coarse powder of drugs in 16 times water and reduced to 1/4th and strained using cloth[3]. For extract preparation 5 g of drug was macerated with 100 ml of water in a closed flask for 24 hours, shaking frequently during 6 hours and was allowed to stand for 18 hours. The extract obtained was taken in a centrifuge tube and centrifuged at 13,000 rpm for 15 minutes in a refrigerated centrifuge machine. The supernatant was filtered through Syringe filter (pore size, 0.45 µm) after filtering with filter paper[4]. To prepare pre-determined concentration the Panchavalkala aqueous extract was lyophilised. 1mg/ml concentration was taken for the study.

Method of *in vivo* study: The test animals were categorized into two with three groups each, each group had 6 Albino Wistar rats. The first category was Wistar rats without any pre-treatments with 3 groups viz: A1 (control), B1(Trial with Panchavalkala kashaya), C1 (Trial with Panchavalkala aqueous extract). The second category was designed to evaluate the effect of trial drugs in Wistar rats under anticoagulant therapy which included three groups (A2, B2, C2) of Wistar rats pre-treated with equal volume (0.25 mL) of injection heparin sodium IM (640 IU/kg) 3 times a day for 3 consecutive days. On day 3, the rats were anesthetized by Intra-peritoneal injection with a mixture of ketamine hydrochloride (75 mg/kg) and xylazine (10 mg/kg). The method was adopted from Sogut O et.al (2015). The bleeding assays were performed via tail tip amputation method[5]. The rats were placed in the prone position, and a distal 10mm segment of the tail was marked and prepared using povidone-iodine solution followed by surgical spirit. After the part preparation, distal 10 mm segment of tails were amputated transversely in test animals using a scalpel. This was immediately followed by irrigation with 1ml Normal saline in Control groups A1 and A2, with Panchavalkala aqueous extract in groups B1 and B2 and with 1ml Panchavalkala kashaya in groups C1 and C2. The blood was collected in pre-weighed ependorfs. Haemostatic time and Amount of bleeding were evaluated and monitored for 20 minutes to detect rebleeding. Haemostatic time is defined as the interval (minute) between the start of bleeding (i.e., cutting off the tail tip) and the achievement of haemostasis and was measured using a stopwatch. To determine the Amount of bleeding an ependorf were weighed before and after the procedure using a precision laboratory balance, and the difference in weight was used as a measure of the amount of bleeding[5]. Post- operative care was done using standard wound care methods.

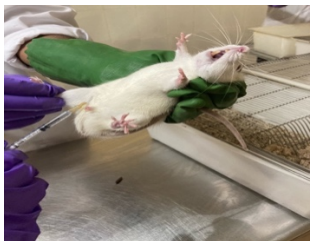

Figure 1: Anesthetization of Rats

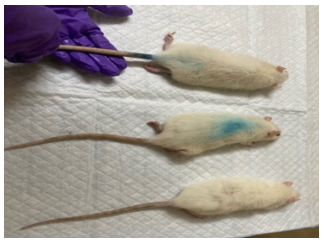

Figure 2: Surgical site preparation

Figure 3: Marking distal 10 mm segment

Figure 4: Amputation with Scalpel

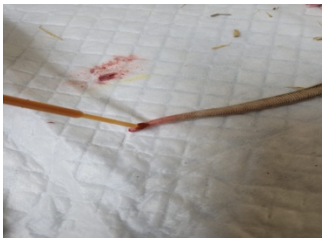

Figure 5: Irrigation with trial drug

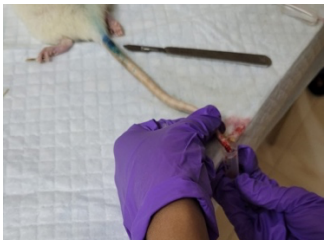

Figure 6: Collection of blood in ependorf

RESULT

In non-heparinised groups the mean Amount of bleeding of group A1 is 178.78 ± 98.73 (mg), group B1 is 56.98 ±27.13 (mg) and group C1 is 15.23± 13.07 (mg). The P-value showed that there was significant reduction in amount of bleeding in group C1 compared to group A1 and B1. From this it can be inferred that both interventions i.e. Panchavalkala kashaya and Panchavalkala aqueous extract are effective in reducing the amount of bleeding in non-heparinised rats. Among them Panchavalkala aqueous extract was highly significant in reducing the amount of bleeding.

Table 1: Kruskal Wallis Test – Effect of intervention on Amount of bleeding in Non-Heparinised Groups

Pairwise Comparisons (Kruskal Wallis Test)	Test Statistic	Std. Error	Std. Test Statistic	P value	Adj. Sig.[a]
Group A1- Group B1	12.000	6.083	1.973	0.049	0.728
Group A1- Group C1	20.667	6.083	3.398	0.001	0.010
Group B1 - Group C1	8.667	6.083	1.425	0.154	1.000

In heparinised group the mean amount of bleeding of group A2 is 558.36 ± 314.94 (mg), group B2 is 134.66± 95.65 (mg) and C2 is 81.35± 46.85 (mg). The p-value showed that there is significant reduction in amount of bleeding in trial group C2 when compared to Group A2 (control) and from this it can be inferred Panchavalkala aqueous extract is effective in reducing amount of bleeding among heparinised groups of rats. And Panchavalkala kashaya did not show any significant haemostatic effect in the heparinized test animals.

Table 2: Kruskal Wallis Test –Effect of intervention on Amount of bleeding in Heparinised Groups

Pairwise Comparisons (Kruskal Wallis Test)	Test Statistic	Std. Error	Std. Test Statistic	P-value	Adj. Sig.[a]
Group A2 - Group B2	11.167	6.083	1.836	0.066	0.996
Group A2 - Group C2	16.167	6.083	2.658	0.008	0.118
Group B2 - Group C2	5.000	6.083	0.822	0.411	1.000

In non-heparinised groups the mean haemostatic time of group A1 is 106.33 ± 14.52 (sec), group B1 is 50±39.83 (sec) and C1 is 39.83±5.03 (sec). The p- value showed that there was significant reduction in haemostatic time in both trial groups treated with Panchavalkala kashaya and Panchavalkala aqueous extract.

Table 3: Kruskal Wallis Test –Effect of intervention on Haemostatic Time in Non-Heparinised Groups

Pairwise Comparisons (Kruskal Wallis Test)	Test Statistic	Std. Error	Std. Test Statistic	P-value	Adj. Sig.[a]
Group C1-Group B1	3.667	6.078	0.603	0.546	1.000
Group C1-Group A1	19.000	6.078	3.126	0.002	0.027
Group B1 - Group A1	15.333	6.078	2.523	0.012	0.175

In heparinised groups the mean haemostatic time of group A2 is 173.66 ± 69.07, group B2 is 95.33 ± 21.95 (sec) and group is C2 is 72.33±23.76 (sec). The p- value showed that there was significant reduction in Haemostatic time in Group C2 treated with Panchavalkala aqueous extract compared other two groups.

Table 4: Kruskal Wallis Test –Effect of intervention on Haemostatic Time in Heparinised Groups

Pairwise Comparisons (Kruskal Wallis Test)	Test Statistic	Std. Error	Std. Test Statistic	P -value	Adj. Sig.[a]
Group C2- Group B2	7.333	6.078	1.207	0.228	1.000
Group C2-Group A2	17.000	6.078	2.797	0.005	0.077
Group B2-Group A2	9.667	6.078	1.590	0.112	1.000

DISCUSSION

This study helped to evaluate the primary process of haemostasis ie vasoconstriction of vascular injury through reflex neurogenic mechanisms along with secondary hemostasis. The first line of action in raktasthambhana (haemostasis) is Skandana and Santhana which is done by Kashaya rasa (Astringent taste) and sheeta veerya (cold potency) drugs. All the drugs among the Panchavalkala have kashaya rasa and sheeta veerya. Kashaya rasa and sheeta veerya helps for vasoconstriction and clotting of blood, which forms the primary response to bleeding. Kashya rasa have Stamabhana (stasis) property and show static action on discharge. The mode of action of the Sandhana (uniting) procedure can be understood as a vasoconstriction and repairing of the damaged blood vessels. Broadly it can be used in capillary hemorrhages and tropical hemorrhages. The Skandana procedure helps in clotting of the blood[6]. Phytochemical analysis of Panchavalkala aqueous extract showed the presence of flavonoids, alkaloids, tannins, glycosides, and saponins. *In vivo* effects on thrombin of *Paris fargesii var. brevipetala* saponins activity shows Haemostatic effect[7].

In addition, Panchavalkala group of drugs has established antimicrobial effect[8] as well as wound healing properties[9]. Ficus plants from Panchavalkala are of kashaya rasa, sheeta virya and kapha, pittadoshahara and Panchavalkala is said to be effective especially in gynaecological disorders[10]. Panchavalkala kashaya given as uttara Vasti (vaginal douche) in Dysfunctional Uterine bleeding was effective as an emergency management in promoting haemostasis[11]. Panchavalkala has been proven effective for preoperative preparation of surgical sites, with support from toxicity studies conducted through topical application[12]. In this animal study, the trial drugs showed the ability to promote haemostasis, which is the initial phase of wound healing. Also, Panchavalkala group of drugs has proven wound healing effect and they offer added benefit of hemostasis[13].

CONCLUSION

The *in vivo* study conducted in non-heparinized category of Albino Wistar rats proved that both Panchavalkala kashaya and Panchavalkala aqueous extract have highly significant haemostatic effect in the test animals. The study also proved that both the trials groups are equally effective in reducing haemostatic time as well as the amount of bleeding in Wistar rats.

The *in vivo* study conducted in heparinized category of Albino Wistar rats proved that only Panchavalkala aqueous extract has significant haemostatic effect in the test animals. The study also proved that Panchavalkala aqueous extract was more effective in reducing haemostatic time as well as the amount of bleeding in heparinised Wistar rats when compared to Panchavalkala kashaya, which did not show any significant haemostatic effect in the heparinized test animals.

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