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Preventive effect of *Katuki* (*Picrorhiza kurroa* Royle ex Benth.) in experimentally induced thrombosis in Wistar rats

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Katuki is a traditionally used popular medicinal plant possessing broad-spectrum therapeutic action and diverse pharmacological properties, including antioxidant, anti-inflammatory, and hypolipidemic effects. However, its preventive effect on thrombosis has not been evaluated yet. The present study evaluated the preventive effect of a hydroalcoholic extract of *Katuki* (HAEK) in κ-carrageenan-induced rat tail thrombosis in male Wistar rats. The preventive effect of the test drug was assessed based on tail thrombus length and changes observed in the thrombosed tail, along with haematological parameters, Prothrombin time (PT), Activated partial thromboplastin time (APTT), clotting time (CT), and platelet count (PC). There was also a drug-only group, which received HAEK only to assess its effect on haematological parameters under physiological conditions without induction of thrombosis. Thirty-six male Wistar strain albino rats were randomly allocated to six groups (n=6/group): normal control, negative control, aspirin standard control (20 mg/kg), HAEK 200 mg/kg, HAEK 400 mg/kg, and HAEK 400 mg/kg drug-only. HAEK or aspirin was administered orally for 7 days before κ-carrageenan induction in the respective groups; the drug-only group received HAEK for 9 days and was not subjected to thrombosis induction. κ-carrageenan was injected intraperitoneally on the 7th day. Thrombus length was assessed at 24 and 48 h, and tail changes were observed for 10 day following thrombus induction. Haematological parameters were assessed and compared between the groups to evaluate the effect. At 24 h, thrombus length was 5.48±0.32 cm in the negative control, 3.00±0.28 cm with aspirin, 4.70±0.21 cm with HAEK 200 mg/kg, and 2.91±0.19 cm with HAEK 400 mg/kg. At 48 h, the corresponding values were 7.63±0.18, 4.01±0.26, 6.55±0.39, and 4.45±0.29 cm, respectively. HAEK 400 mg/kg significantly reduced thrombus length at both time points ($p<0.001$), whereas the reduction at 200 mg/kg was not significant. On day 9, PT, APTT, CT, and PC were 17.66±0.33 s, 29.16±0.70 s, 90.00±6.70 s, and 5.18±0.14 lakh/cumm, respectively, in the negative control; corresponding values in the aspirin group were 22.00±0.81 s, 35.16±0.70 s, 120.00±5.47 s, and 5.99±0.13 lakh/cumm, and in the HAEK 400 mg/kg group were 21.33±0.71 s, 33.33±0.76 s, 117.50±4.61 s, and 5.94±0.16 lakh/cumm. In the HAEK drug-only group, none of the measured haematological parameters differed significantly from the normal control. These findings support a preventive antithrombotic effect of HAEK in this experimental model without a detectable alteration of the measured haematological parameters under the non-thrombosed condition.

Keywords: Antithrombotic, Antiplatelet, Coagulation, Fibrinolytic, κ-Carrageenan, Prophylaxis

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Thrombosis is a pathological blood clot formation inside a blood vessel, which can lead to cardiac and vascular diseases[1]. Cardiovascular diseases are the major cause of morbidity and mortality[2]. Furthermore, stroke continues to be second-leading cause of death and as well as third-leading cause of combined death and disability in the world[3]. Given the substantial burden of thrombotic disorders**,** prevention of thrombus formation is an important strategy to reduce risk of thrombotic disorders**.** However currently available antithrombotic drugs are costly and have serious side effects, there is a need to explore safe antithrombotic drugs for preventing thrombotic disorders[4]. In such circumstances, herbal drugs may serve as potential alternatives to conventional medicine.

Katuki (*Picrorhiza kurroa*) is a well-known herb that exhibits a wide range of therapeutic actions. According to *Ayurveda* literature, *Kutaki* has got *Katu* (pungent), *Tikta* (bitter) *Rasa* (taste); *Laghu* (*light*), *Ruksha* (*rough*), *Sara* (*moving*), *Guna* (*properties*); *Katu Vipak*a (pungent metabolic transformation) and *Kaphapittahara* (alleviates *Kaphapitta*) properties.

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Also, it is said to possess *Bhedaneeya* (piercing through and expelling of waste metabolites), *Lekhaneeya* (adipolytic), *Shothahara* (anti-inflammatory), *Hridya* (good for the heart), and *Raktavikar nashak* (alleviate hematologic disorders) action. These properties suggest a potential pharmacological relevance in inflammatory, metabolic, and hematological disorders.

In recent times, *Katuki* has fascinated researchers due to its potential therapeutic benefits. Various biological activities have been reported for its extracts and isolated compounds, such as cardioprotective, antioxidant, hypolipemic etc[5-7]. A recent study on apocynin isolated from *Katuki* indicated its therapeutic benefits for inflammatory conditions, showing potential as a basis for developing a new anti-thrombotic medication[8]. Another study on *picroliv* exhibited that it could aid in restoring blood supply in ischemic conditions[9]. However, no studies have been done yet to evaluate whether *Katuki* has a potent preventive or inhibitory effect on thrombosis.

Plants exhibiting anti-inflammatory, hypolipidemic, antioxidant, antiplatelet and cardio protective effects have been suggested as promising candidates for thrombosis prevention[10]. Therefore, based on the explored biological activity for its extracts in the current era and properties mentioned in *Ayurveda*, along with the above rationale, *Katuki* was found to be a suitable drug for the present research.

HAEK was used as an experimental preparation for present study to extract phytoconstituents across a broader polarity range and facilitate pharmacological evaluation, supported by its prior use in pharmacological investigations[5-7]. Hence, the aim of the research was to evaluate the preventive impact of *Katuki* in κ-carrageenan-induced rat tail thrombosis model.

Materials and Methods

Plant material collection, identification, authentication and processing

Rhizomes of *Katuki* were procured from Minor Forest Produce Processing and Research Centre (MFP-PARC), Bhopal. Plant material was authenticated by the Drug Authentication Committee of the Institute. The voucher specimen (kls/phar/278) was preserved in the Pharmacognosy museum of Pt. Khushila Sharma Government Ayurveda College and Institute, Bhopal, Madhya Pradesh. After authentication, Rhizome was washed with water, dried and then crushed into a coarse powder. Powder was stored in an airtight container for further use.

Pharmacognostical evaluation

Macroscopic examination of the drug was performed in the Pharmacognosy Laboratory, Pt. Khushilal Sharma Government Ayurveda College and Institute, Bhopal. Microscopic examination and physicochemical evaluation were carried out at the Drug Testing Laboratory, Amkho, Gwalior, Madhya Pradesh.

The raw drug was examined in daylight and also by a dissecting microscope for various macroscopical characters. The microscopic evaluation of the drug, including histological evaluation and powder microscopy along with preliminary physicochemical analysis was done as per API[11].

Preparation of plant extract

Hydroalcoholic extract of *Katuki* was prepared by using water and ethanol in 1:1 ratio with the help of a Soxhlet extractor. 25 g coarse powder of *Katuki* rhizome was taken with 250 mL solvent for extraction. Extraction was carried out continuously until the solvent circulating through the sample turned colorless. The solvent was removed using a hot water bath to get a concentrated extract. The yield was computed as a percentage. (Weight of extract/Weight of Sample× 100=Total yield of extract (%)). The yield was 20%. The process of extraction was repeated 4 times to obtain a sufficient amount of extract for further HPTLC study and experimental study.

High Performance Thin Layer Chromatography (HPTLC)

HPTLC analysis of HAEK was done at the Research Laboratory of Anchrom Enterprises (I) Pvt. LTD., Mumbai. by using the CAMAG HPTLC system, CAMAG Visualizer, and the latest CAMAG vision CATS software. The procedure was performed with reference to the HPTLC Association method.[12].

Sample preparation

The sample extract was weighed as 100 mg and dissolved in 10 mL of HPLC grade methanol, the mix was sonicated for 10 min then centrifuged at 2500 RPM for 10 min and the supernatant thus formed was used for application.

Chromatographic conditions

HPTLC analysis was carried out using TLC silica gel 60 F254 plates (Merck, product no. 1.05554.0007) as stationary phase. Ethyl acetate: methanol: water (82:10:8, v/v/v) was used as the mobile phase for

chromatographic development. After 20 min of mobile phase saturation in developing chamber, the plates were developed to a distance of 70 mm. The plates were derivatized using 10% methanolic sulphuric acid for visualization of the separated constituents.

Pharmacological study

Housing of animals

Thirty-six Wistar strain male albino rats weighing between 180 to 220 g and aged 8-12 weeks were used in the study. Following ethical approval from Institutional Animal Ethical Committee (IAEC), vide letter No. PH/IAEC/VNS/2K22/33; Animals were procured from the Animal House of VNS Institute of Pharmacy, Bhopal, following CPCSEA guidelines. The animals were acclimatized for 7 days before the experiment. Animals were housed in polypropylene cages with husk bedding. Each cage was labeled with the group name, animal number, procurement date, sex, and individual rats were marked with picric acid for identification. Standard laboratory conditions were maintained at 21-22°C, 40%-70% humidity and a 12-h light/dark cycle. Bedding was changed on alternate days, and cages were changed weekly. The rats were fed a pelleted rodent diet and drinking water supplied in polypropylene bottles with stainless steel sipper tubes.

Acute toxicity study

An acute toxicity study was carried out for the hydroalcoholic extract of *Katuki* (HAEK) as per the OECD guidelines-423 (Acute Oral Toxicity). Animals were fasted and weighed before dosing. Three male Wistar rats were given a single dose of Hydroalcoholic extract (Ethanol: Distilled water-1:1) of *Katuki* 2000 mg/kg by oral route. All the animals were observed for mortality and general behaviour for 14 days.

Experimental study

The present study was conducted in the Rat tail thrombosis model. κ-carrageenan was used to generate rat tail thrombosis in this study because of rationality and simplicity of this model. This model enables the observation and measurement of the progression of thrombosis visually, continuously, and accurately[13].

Chemical and drug

HAEK was prepared at the phytochemistry laboratory of Pandit Khushilal Sharma Government Ayurveda Institute, Bhopal. Aspirin was purchased from the CDH company. HAEK extract (dose 200 mg/kg, 400 mg/kg) and Aspirin (dose-20 mg/kg) were dissolved in distilled water and given orally to the rats in respective groups. Kappa carrageenan was obtained from Tokyo Chemical Industry (TCI) company.

Selection and grouping

Those rats having a minimum 15 cm tail length were only included in the study. Rats under any other experimental study group or unhealthy rats were excluded from the study. After acclimatization, 36 rats were randomly divided into 6 groups, each with 6 rats (Table 1). There was also a drug-only group in which only drug HAEK was administered to evaluate the effect on hematological parameters without induction of thrombosis.

The tail length of all animals in groups 2 to 5 was measured and evaluated before the experiment. Tail length values were analysed by statistical analysis. There was no statistical difference between the tail length in all study groups at $p < 0.05$.

κ-carrageenan (k-crg) induced rat tail thrombosis model

Before the commencement of the study, κ-carrageenan dosage of 10 mg/kg was administered intraperitoneally in 2 rats to determine and stabilise the optimal rat tail thrombosis model. To obtain the optimum model, κ-carrageenan dose, environmental temperature, and infarction time were tested and selected in the initial study. The 21-22°C temperature

Table 1 — Study design

Group number	Groups	Intervention		No. of animals
		Drug dosing (oral route)	Thrombus induction (Intraperitoneal injection) (on 7th day after last drug dosing)	
1	Normal control	No drug	No thrombus induction	6
2	Negative control	No drug	k-Crg (10 mg/kg i.p.)	6
3	Standard control	Aspirin (20 mg/kg p.o.) for 7 days	k-Crg (10 mg/kg i.p.)	6
4	HAEK (200 mg/kg)	HEAK (200 mg/kg) for 7 days	k-Crg (10 mg/kg i.p.)	6
5	HAEK (400 mg/kg)	HEAK (400 mg/kg) for 7 days	k-Crg (10 mg/kg i.p.)	6
6	HAEK (drug-only)	HEAK (400 mg/kg) for 9 days	No thrombus induction	6

(HAEK - hydroalcoholic extract of Katuki ; κ-Crg - κ-carrageenan)

was fixed for developing thrombosis model. The κ-carrageenan at dose of 10 mg/kg BW ensured the appearance of thrombus and a suitable thrombus length in the tail of all the carrageenan-treated group. After 4 h of injection, a slight infarction in the tip end of the rat tail, followed by redness, was observed in the tail. A thrombus appeared as a red-wine colour in the tip of the tail. This change is visible and easy to measure. The length of the thrombus increased, and the boundary of the thrombus in tails became clearer with the passage of time. The thrombosis model was established successfully over a period of 24 h.

Assessment of thrombus length

In the experiment, thirty minutes after the final dosing with various drugs, each rat in groups 2 to 5 was injected with 10 mg/kg BW κ-carrageenan dissolved in physiological saline intraperitoneally. Rats were monitored for the manifestation and appearance of thrombosis. Thrombus length was observed, measured by using a transparent ruler, and images were captured at 24 and 48 h. Thrombus length was also measured at 72 h to evaluate any changes in the thrombus length.

Visual assessment of the tail

Tails of all animals were observed for 10 days after κ-carrageenan injection.

Assessment of hematological parameter (PT, APTT, PC, CT)

Hematological parameters were assessed and compared on 9th day of the study. The blood sample of each animal was collected in EDTA vials for platelet count and in sodium citrate vials for PT and APTT tests and sent to Nidan Pathology Lab, Kolar Road, Bhopal, for investigation. The clotting time test was performed by using the Glass slide method. A drop of blood was collected from each animal and put onto a glass slide. Every 15 seconds, using a sterile lancet, the blood column was pulled upward from the glass slide. The process was repeated until the appearance of a fibrin thread.

Study protocol

The study protocol is summarized in the given flowchart (Fig. 1).

Statistical analyses

Statistical analyses were done using GraphPad Prism version 5.0. Data obtained from the experiments were expressed as the mean ± S.E.M. Statistical differences between groups were calculated by a one-way ANOVA, followed by Tukey's multiple comparison tests. p-value less than 0.05 was considered statistically significant.

Result

Pharmacognostical study

During macroscopic study following characters were identified in drug sample: Shape- straight or slightly curved, subcylindrical; size- up to 8 cm long, 4 to 8 mm thick; outer surface- grey or creamish brown in colour, having impression of round root

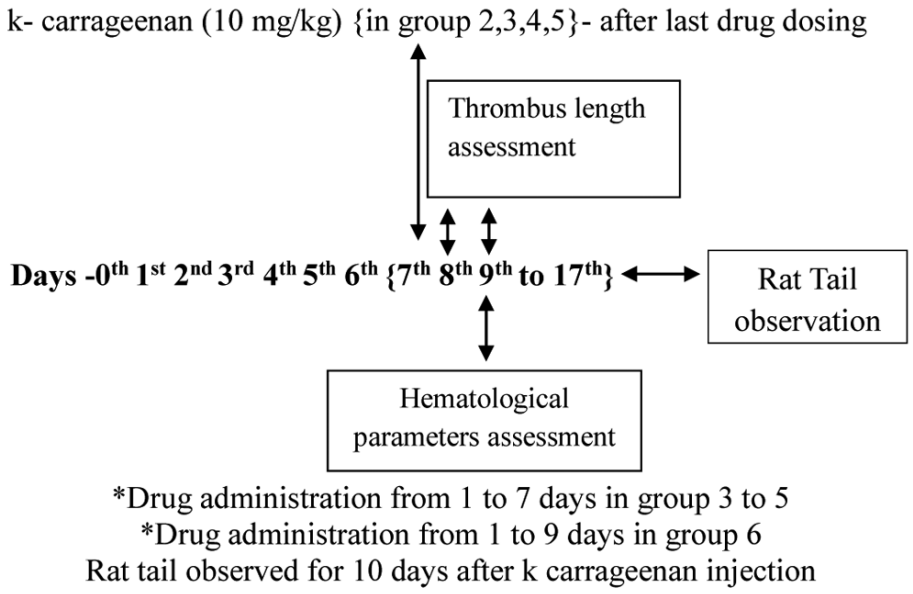


Fig. 1 — Study protocol

scars and scales; fracture- short; touch- rough due to wrinkles and scars of roots and bud scales; odour- faint and disagreeable; taste- bitter and long lasting.

Microscopical examination (Transverse section of rhizome) showed many layers of cork consisting of tangentially elongated cells; cortex single layered, one or two small vascular bundles present in cortex; secondary phloem composed of phloem parenchyma and a few scattered fibres; cambium 3-4 layered; secondary xylem consists of vessels, tracheid's, xylem fibres and xylem parenchyma, tracheid's long, thick-walled; xylem parenchyma thin-walled and polygonal in shape; centre occupied by a small pith consisting of thin-walled cells; simple round to oval, starch grains abundantly present in all cells.

Microscopic examination of the raw drug dusty grey powder showed cells with brown content, a group of fragments of cork cells, pitted vessels, and single round to oval starch grains. Result of physicochemical study is mentioned in (Table 2).

HPTLC Profile

The result of the HPTLC profile is given in the (Fig. 2 and Graph 1).

Acute toxicity study

An acute toxicity study revealed the safety of the hydroalcoholic extract. There was no mortality and no toxic reactions observed at tested doses of 2000 mg/kg up to14 days.

Table 2 — Physicochemical parameters

Sr.	Test parameters	Result
1.	Foreign Matter	free from observable signs of mold growth, sliminess, stones, rodent excreta & insects.
2.	Total ash	4.98%
3.	Acid-insoluble ash	0.96%
4.	Water-soluble ash	0.37%
5.	Alcohol soluble extractive	10.3%
6.	Water-soluble extractive	33.62%
7.	pH value	5.6%
8.	Moisture content	10.3%

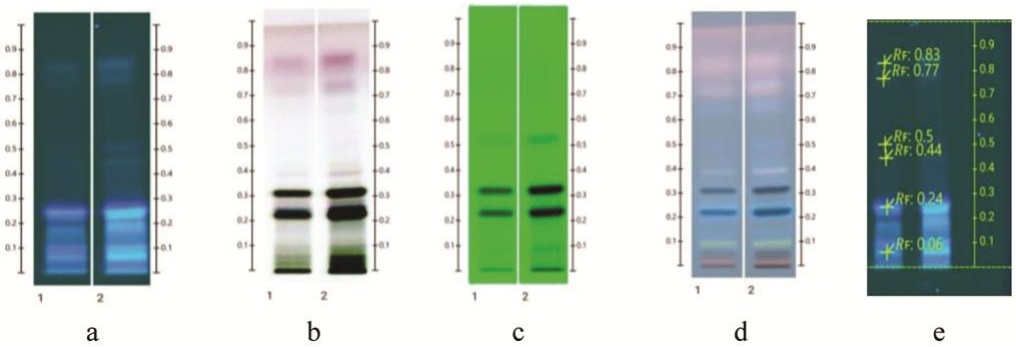
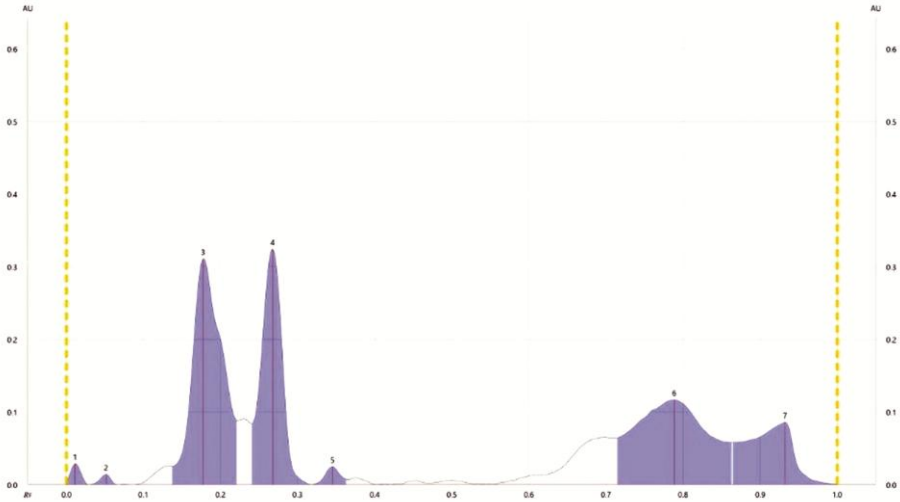


Fig. 2 — Showing the result of HPTL, Where (a) Plate after development under fluorescent light (366 nm), (b) Plate after derivatization under white light, (c) Plate after development under UV light (254 nm), (d) Plate after derivatization under fluorescent, (e) Plate after development under fluorescent light (366 nm)



Graph 1 — Chromatogram of derivatized plate under 540 nm visible light

Experimental study

Effect of HAEK on the rat tail thrombosis model

Tails of animals were observed for 10 days after κ-carrageenan injection. Redness in the tail was observed 6 h after injection. A thrombus was seen at the tip of the tail as red wine colour appeared at 24 h in all animals given κ-carrageenan. Thrombus length was extended up to 48 h. Thrombus length was measured and photographed at 24 h and 48 h, respectively, after κ-carrageenan injection (Table 3; Fig. 3a-d & Fig. 4a-d). HAEK 400 mg/kg and aspirin significantly reduced thrombus length at 24 and 48 h compared with the negative control ($p<0.001$). HAEK 200 mg/kg produced a non-significant reduction.

The rate of inhibition in thrombus length was calculated in comparison to the negative control group. The rate of inhibition of thrombus was highest in the HAEK (400 mg/kg) treated group and the Aspirin group, respectively, at 24 h and 48 h. Values

Table 3 — Values of tail length, Thrombosis length, Rate of inhibition of thrombosis length

Group	Tail full length (cm)	Thrombosis length (cm)		Rate of inhibition (Thrombus length)	
		24 h	48 h	24 h	48 h
Normal control	-	-	-	-	-
Negative control (k Crg)	15.53±0.14	5.48±0.32	7.63±0.18	-	-
Standard (Aspirin+k Crg)	15.55± 0.14	3.00± 0.28[a]	4.01±0.26[a]	45.25%	47.44%
HAEK 200 mg/kg+k Crg	15.48±0.14	4.70±0.21[d,A]	6.55±0.39[d,A]	14.23%	14.15%
HAEK 400 mg/kg+k Crg	15.58±0.17	2.91±0.19[a,D]	4.45±0.29[a,D]	46.89%	41.67%
HAEK 400 mg/kg (Drug only)	-	-	-	-	-

Values are expressed as mean ± standard error of mean, for 6 animals in each group. (Tail length, Thrombosis length)
Comparison: Negative control with Aspirin, HAEK (200 mg/kg) and HAEK (400 mg/kg) treated groups: (*** - a, ** - b, * - c, ns – d), Where (Highly significant - p<0.001 ***; Moderately significant - p<0.01 **; Significant - p<0.05 *; ns - non-significant p>0.05).
Aspirin treated group with HAEK (200 mg/kg) and HAEK (400 mg/kg): (*** - A, ** - B, * - C, ns – D)
Values are expressed as percentage for Rate of inhibition of thrombosis

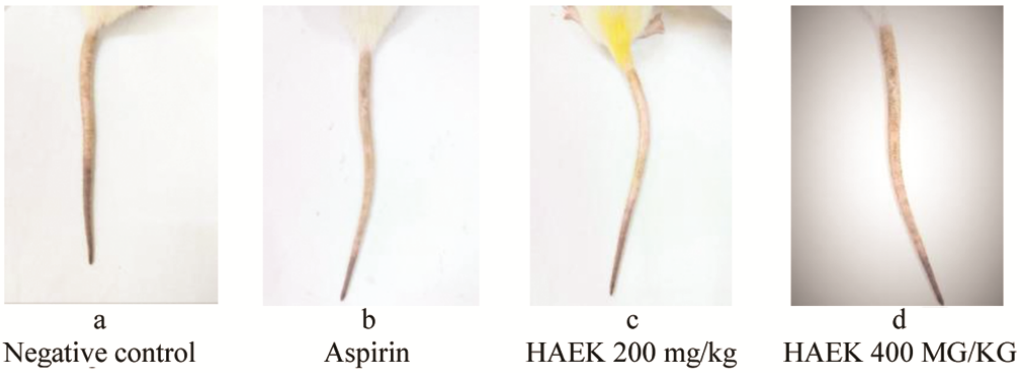


Fig. 3 — (a-d) Visual assessment of thrombus length at 24 h

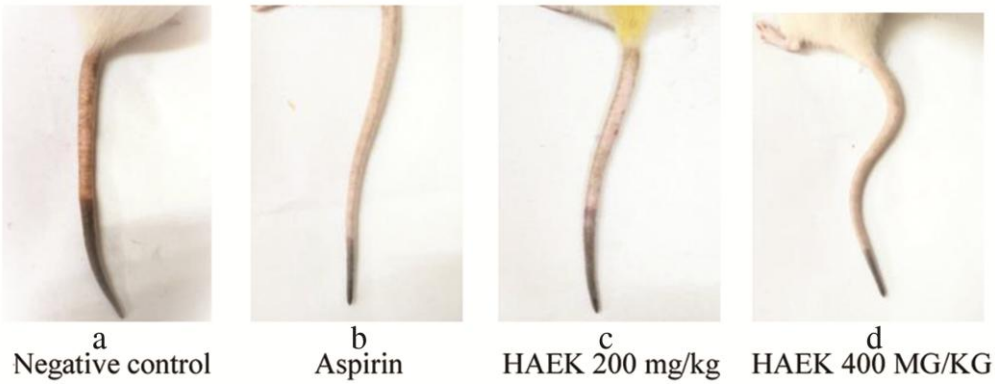


Fig. 4 — (a-d) Visual assessment of thrombus length at 48 h

of ROI are expressed as apercentage and shown in the following table (Table 3).

Visual assessment of the tail

At 72 h after thrombus induction, thrombus length was not increased but became darker and changed to black colour due to necrosis. This change was more clearly visible in the negative control group. The tail started shedding in some animals. Tail in animals of the negative control was shed off within 4 to 6 days after κ-carrageenan injection, while in the treated group, it was shed from 7 to 10 days in all animals after κ-carrageenan injection. Tail was persisted along with thrombus in the HAEK (400 mg/kg) treated group, while in the HAEK (200 mg/kg) treated group, it was shed off from 7to 10 days after κ-carrageenan injection. Photographs of the visual assessment are shown in the figure (Fig. 5a-d).

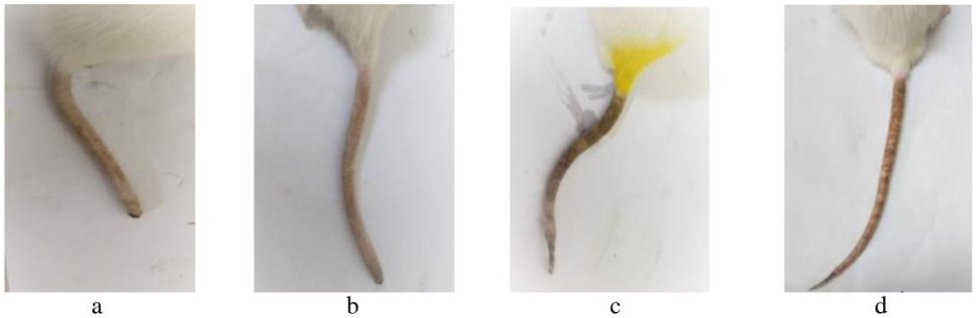
Effect of HAEK on hematological parameters

Assessment was done based on post thrombus induction hematological parameters by between group comparison, rather than by comparing BT and AT values within the same group; as this was preventive efficacy evaluation study. The effect of HAEK on APTT, PT, CT, and PC in animals is shown in (Table 4). The negative control group showed a significant decrease in APTT, PT, CT ($p<0.001$), and PC ($p<0.05$) when compared to the normal control group. The animals given HAEK (400 mg/kg+kcrg) and Aspirin showed a significant increase in APTT, PT, CT, and PC value in comparison to the negative control group. While the HAEK (200 mg/kg +k crg) treated group increased all values in comparison to the negative control group but the difference was not statistically significant. In a group that received the drug only, showed no statistical difference in all values of PT, APTT, CT, and PC as compared to the normal control group. Values of APTT, PT, CT, and PC in 6 groups on 9th day of study are given in the (Table 4).

Discussion

Pharmacognostical study

Macroscopic, microscopic, physicochemical and HPTLC findings provided pharmacognostic data



a Negative control (4th day) b Aspirin (7th day) c HAEK 200 mg/kg (7th day) d HAEK 400 mg/kg (10th day)

Fig. 5 — (a-d) Visual assessment of necrosis and tail length (Days are taken into consideration after induction of thrombosis)

Table 4 — Values of APTT, PT, CT and PC in 6 groups on 9th day of study

Group	PT(s)	APTT(s)	CT(s)	PC(lakh/cumm)
Normal control	23.33±0.88	37.33±0.33	132.50±4.61	6.26± 0.19
Negative control (k Crg)	17.66±.33***	29.16±0.70***	90.00± 6.70***	5.18± 0.14**
Standard (Aspirin+k Crg)	22.00±0.81^{b}	35.16±0.70^{a}	120.00±5.47^{b}	5.99± 0.13^{c}
HAEK 200 mg/kg+k Crg	20.00±.57^{d,D}	31.83±0.74^{d,B}	107.50±.61^{d,D}	5.69±0.18^{d,D}
HAEK 400 mg/kg+k Crg	21.33±0.71^{b,D}	33.33±0.76^{a,D}	117.50±4.61^{c,D}	5.94±0.16^{c,D}
HAEK 400 mg/kg (Drug only)	23.83±0.70^{ns}	37.50±0.42^{ns}	135.00±6.70^{ns}	6.20±0.19^{ns}

Values are expressed as mean ± standard error of mean, for 6 animals in each group.
Comparison: Normal control with negative control and HAEK (Drug only) group: (Highly significant - $p<0.001$, ***; Moderately significant - $p<0.01$, **; Significant – $p <0.05$, *; ns - non-significant $p>0.05$).
Negative control with Aspirin, HAEK (200 mg/kg) and HAEK (400 mg/kg) treated groups: (*** - a, ** - b, * - c, ns – d)
Aspirin treated group with HAEK (200 mg/kg) and HAEK (400 mg/kg): (*** - A, ** - B, * - C, ns – D)
(s-second)

supporting the identity and quality of the *Katuki* rhizome used in the study.

Physicochemical parameter

The physicochemical parameters supported the quality and suitability of the authenticated *Katuki* rhizome for extraction

HPTLC

HPTLC analysis revealed six bands at *R*f values of 0.06, 0.24, 0.44, 0.50, 0.77 and 0.83, indicated the presence of various separable constituents. This obtained bands under different visualization conditions provided qualitative fingerprint in present study. This fingerprint profile can support qualitative standardization in future studies.

Acute toxicity study

Prior to experiment Acute toxicity study was conducted to establish the preliminary safety of the specific HAEK preparation used in this study and to select safe experiment dose range. Based on the acute toxicity findings, 200 and 400 mg/kg doses ($1/10^{th}$ and $1/5^{th}$ of the tested dose) were selected.

Experimental study

In present study the HAEK drug-only group was included to evaluate the effects of HAEK alone on hematological parameters in non-thrombotic conditions. Therefore thrombosis was not induced in this group. The 7-day intervention period in the thrombosis-induced groups was adapted from a previously published study using the similar experimental thrombosis model[14]. HAEK and aspirin given for 7 consecutive days before κ-carrageenan induction to assess their preventive effects in different respective groups. In contrast, drug administration in HAEK drug only group was extended up to 9 days as no thrombosis was induced and hematological assessment was performed on day 9, at the same assessment point as the thrombosis-induced groups, with continued HAEK administration in HAEK drug only group.

Thrombus length

Kappa-carrageenan is commonly used to induce tissue inflammation and tail thrombosis in several animals including gmice and rats. By releasing inflammatory factors, it can cause endothelial cell damage and localized blood vessel inflammation which may lead to the formation of thrombus[15]. κ-carrageenan caused thrombosis in the tip of the tail after being injected intraperitoneally in animals.

Inflammation, especially in blood vessels, is considered to contribute to thrombosis[16]. Platelets adhere to the vessel wall to initiate thrombosis, which is then followed by aggregation of more platelet[17,18]. Additionally platelet responses are a strong stimulus to the essential clotting factors[19]. Hence, the shorter length of thrombosis in the HAEK-treated group might be due to affecting any of this path way of pathogenesis of thrombosis.

Visual assessment of the tail

At 72 h after thrombus induction, the thrombosed tail changed to black colour and also started shedding off in some animals after a few days due to necrosis. Tail shading was slower in the HAEK low dose treated group in comparison to the negative control and aspirin treated group, while tail length was persistent without shading in the high dose treated group. Hence, it can be interpreted that the HAEK-treated group was successful in slowing down the pathological changes in rat tail thrombosis, thereby showing its preventive effect.

Hematological parameter

PT, APTT, and CT were significantly shorter in the model group than in the normal control group. There was also a difference in the value of platelet count (PC) between the negative control group and the normal control group. These data suggest that rats in the negative control group were in a hypercoagulable stage. While the APTT is used to assess the activity of elements of the intrinsic and common pathway, PT is a useful test for assessing the activity of the factors of the extrinsic coagulation pathway[20]. PT indicates total quantity of prothrombin present in blood. When prothrombin and other Factors I, V, VII, and X are deficient it is extended. APTT is prolonged when inhibition of factors II, V, VIII, IX, X, XI, and XII occurs. CT is the time interval from the oozing of blood after injury till the formation of a clot[21]. Thus, the prolonged PT APTT and CT in the thrombus induced HAEK and aspirin-treated group in comparison to the negative control group suggest inhibition of any of the above discussed clotting factors.

Platelet count is also an important parameter to assess coagulation function in many studies[22]. There was a moderately significant reduction in platelet count at $p<0.01$ in the negative control group. The formation of tail thrombosis could deplete circulating platelets, resulting in reduction of platelets in the model group[14]. There was slight increase in platelets

count in HAEK (200 mg/kg) and HAEK (400 mg/kg) group with thrombus induction. It may be due to the inhibitory effect on thrombus formation in the treated group.

This suggests that HAEK might inhibit the formation of thrombosis by affecting PT, APTT, CT, and PC values.

Thrombogenesis can be controlled at different levels with antithrombotic drugs. However these drugs can also produce haemorrhagic effects, which may be direct or indirect; Therefore, evaluating, efficacy/safety ratios could be a useful metric[23]. Hence, to evaluate the effect of HAEK on physiological haematological parameters, a comparison was made between Drug-only group and the Normal control group. No significant difference in PT, APTT, CT, and PC values between the two groups. This result suggested HAEK did not show any abnormal effect on blood parameters.

Phytoconstituent

Extensive preclinical studies have shown that certain phytochemicals and plant-derived extracts have significant antithrombotic, antiplatelet, and fibrinolytic properties[24]. flavonoids, alkaloids, coumarins, polyphenols, saponins, furan derivatives, iridoid glycosides, sesquiterpenes, and aporphines are some of them[25]. *Picrorhiza kurroa* contains glycosides, iridoids, alkaloids, phenolics, terpenes, cucurbitacins[26-30], *ferulic acid*, etc.[31]. Hence, these phytochemicals may be responsible for its preventive effect in thrombosis in the present study. Acording to Guerrero *et al.* (2005, 2007) flavonoids may inhibit platelet function by binding to the TXA2 receptor[32,33]. Caffeic acid was shown to have antithrombotic properties via preventing platelet aggregation[34]. Ferulic acid given orally to mice decreased their risk of dying from pulmonary thrombosis and reduced clotting time[35]. In rats iridoid glycosides isolated from Gardeniae fructus exhibited antithrombotic action by preventing platelet aggregation and lowering arterial thrombus[36]. Some alkaloids showed antithrombotic and antiplatelet effects in an *in vivo* study[37]. On this account, all these phytoconstituents can contribute to the effect of HAEK in the present study.

Probable mode of action based on Ayurveda principles

Thrombosis in Ayurveda is indicated as *Grathita Rakta pitta* (coagulated blood) where coagulation of blood take place. In this situation blood is vitiated together with *Kaphadosha* and *Pitta dosha* and it result in obstructed blood flow[38,39].

Kutaki has got *Katu* (pungent), *Tikta* (bitter) *Rasa* (taste); *Laghu* (*light*), *Ruksha* (*rough*), *Sara* (*moving*), *Guna* (*properties*); *Katu Vipak*a (pungent metabolic transformation) and *Kaphapittahara* (alleviates *Kaphapitta*) properties. It also possesses *Lekhaniya, Bhedaniya, and Raktavikar hara* properties. *Tikta rasa* has *Lekhana, Kled- Meda Shoshana* (reducing moisture, *Meda Dhatu*) property. *Katu Rasa* has *Shonita sanghata bhedana (*splits/ fragments coagulated blood) property concept similar to fibrinolysis property. *Katu Vipak, Kaphapittahara and Raktashodhaka* (blood purifier) action also contribute to preventing *Rakta Dushti* (Blood disorders). *Sara Guna* of *Katuki* may be responsible for slowing down pathological changes in the tail by maintaining normal blood flow. An earlier study's findings suggest that *picroliv* may be developed as a therapeutic angiogenic drug to restore blood flow in condition like ischemia that involves insufficient blood flow, such as ischemia[9]. According to the modern concept, an imbalance between fibrin formation and fibrinolysis, besides hyper-fibrinogenemia, usually triggers thrombotic events[40]. So, it may be inferred that *Lekhana* and *Bhedana* actions can be helpful in fibrinolysis and thereby preventing imbalance and thrombosis. Hence, all these described properties and actions of *Katuki* might be responsible for the shorter length of thrombosis at 24 and 48 h in the present study, and also for persistent tail length till 10 days in the HAEK (400 mg/kg) group, and delayed pathological changes of thrombosed tail in the HAEK-treated group.

Conclusion

HAEK showed a preventive effect on thrombosis by reducing thrombus length and altering PT, APTT, CT, and PC Values in the κ-carrageenan-induced rat tail thrombosis model, Further studies evaluating coagulation factors, platelet aggregation, blood flow and histopathological changes are warranted to clarify the mechanism.

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Author Contributions

JPV: Conceptualising the study, drafting the manuscript, carrying out the experiment, Collecting data and performing statistical analysis. NU: Conceptualising the study, reviewed the manuscript and provided critical revisions, AM: Guided for performing the experiment. SM: Assisted in carrying out the experiment.

Conflict of Interest

There are no conflicts of interest.

Ethics Approval

The Ethical approval was acquired from Institutional Animal Ethical Committee (IAEC) of VNS Institute of Pharmacy, Bhopal, vide letter No. PH/IAEC/VNS/2K22/33. The study was carried out in compliance with the guidelines established by the Control and Supervision of Experiments on Animals (CPCSEA).

Use of Artificial Intelligence (AI)

Author did not use any AI tool for data interpretation and development of original manuscript.

Data Availability

The article contains all the data supporting the findings of this study.

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