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Original Research Article

Formulation and evaluation of a phenylephrine gel for bleeding control: An *in vivo* study in rabbits

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Abstract

Purpose: *To develop and characterize a formulation of phenylephrine gel, and to compare the hemostasis time of the developed gel with that of the control and a standard commercial hemostatic sponge.*

Methods: *A phenylephrine gel (1 % w/w) was prepared with Carbopol 940 (1.5 % w/w), hydroxypropyl methylcellulose (HPMC) K15M (1 % w/w), propylene glycol (5 % w/w), and distilled water. The prepared gel was assessed in terms of appearance, homogeneity, pH, viscosity and physical stability. A randomized, controlled experimental design was used with 30 rabbits divided into five groups (normal control, heparin control, placebo gel, phenylephrine gel, and commercial hemostatic sponge) of 6 rabbits each. Hemostatic activity was assessed by measuring bleeding time in each rabbit following the assigned treatment.*

Results: *The formulated phenylephrine gel had the physicochemical properties of transparency, good homogeneity, pH 6.40 and viscosity of 11,960 cP, and no phase separation was observed. Bleeding time was found to be shorter in the phenylephrine gel group (30.0 s) than in the other groups. When compared with the heparin control (501.0 s) and standard treatment (157.8 s), bleeding time was significantly decreased with the use of phenylephrine gel ($p < 0.001$). There was also a reduction in bleeding time with standard treatment as compared to the heparin control ($p < 0.001$).*

Conclusion: *The formulation of 1 % phenylephrine gel had acceptable physicochemical stability and provided rapid and effective hemostasis. The results suggest the possibility of using phenylephrine gel as a fast-acting topical hemostatic agent.*

Keywords: *Bleeding control, Hemostatic gel, Phenylephrine HCl*

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INTRODUCTION

Uncontrolled bleeding is a significant clinical problem and one of the most common causes of preventable death after severe trauma [1]. It can also complicate surgical procedures, increasing blood loss, transfusion requirements, and postoperative morbidity [2]. Topical hemostatic agents facilitate local bleeding control in emergency medicine, surgery, dentistry, and wound care, particularly when rapid hemostasis is required [3]. However, limitations such as variable tissue adherence, delayed hemostatic action, potential immunogenicity, and cost may

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restrict the suitability of some products, highlighting the need for effective and accessible alternatives [4].

Phenylephrine hydrochloride is an α_1-adrenergic receptor agonist that induces vasoconstriction by contracting vascular smooth muscle and reducing local blood flow [5]. This mechanism supports its potential application as a topical hemostatic agent. Phenylephrine is widely used in ophthalmic and nasal formulations and is also incorporated into topical and rectal preparations for the temporary relief of hemorrhoidal symptoms and anorectal discomfort [6,7]. Topical gels offer practical advantages for localized drug delivery, including ease of application and prolonged contact with the application site [8].

Hydroxypropyl methylcellulose (HPMC) and Carbopol are commonly used gelling agents in topical pharmaceutical formulations [9]. Their combination may influence gel consistency, rheological properties, and drug retention [10]. There is a limited amount of research focused on developing a topical phenylephrine gel formulation optimized for hemostatic use. Therefore, this study aimed to formulate a phenylephrine hydrochloride gel using HPMC and Carbopol as gelling agents, evaluate its physicochemical properties, and assess its hemostatic activity in a rabbit bleeding model.

EXPERIMENTAL

Animals

Male healthy New Zealand White rabbits (aged 8 to 12 weeks), weighing 1 to 1.5 kg, were obtained from the Animal House Centre, University of Science and Technology, Sana'a, Yemen. Under controlled conditions, they were provided with free access to feed and water; ambient temperature and humidity levels were controlled and a light/dark cycle of 12 hours was established.

Chemicals

Phenylephrine hydrochloride was obtained from Sheba Pharma, Sana'a, Yemen. HPMC, Carbopol 940, propylene glycol and sodium hydroxide were purchased from Alkhadra' Laboratories (Sana'a, Yemen).

Commercial hemostatic sponge was obtained from Ningbo Lifecare Medical Instrument Co., Ltd. (Ningbo, Zhejiang, China). Heparin was supplied by PAN Pharma (Luitré, France). Ketamine and xylazine were sourced from Sigma-Aldrich (Darmstadt, Germany).

Formulation of gel base

A two-step mixing process was used to create the gel base for phenylephrine hydrochloride. First, Solution A was prepared by adding 1 % w/w HPMC to 20 mL of distilled water at room temperature and stirred using a magnetic stirrer at 1,200 rpm for 2 hours to ensure good dispersion of the polymer. The stirring was switched to manual mixing using a glass rod when the viscosity increased, and it was stirred for an additional 15 minutes until a smooth and homogeneous mixture free from lumps was obtained. Secondly, Solution B was prepared by dispersing 1.5 % w/w Carbopol 940 in distilled water (20 mL) and stirred for 2 hours at room temperature using a magnetic stirrer at 1,200 rpm. Manual stirring with a glass rod was used for complete dispersion of Carbopol as the viscosity increased with hydration. Finally, Solutions A and B were slowly mixed by hand stirring. The mixing was continued for 10 minutes to form a smooth and homogeneous gel base (Solution C) for incorporation of the active ingredient (Table 1).

The gelling agents (Carbopol 940 1.5 % w/w and HPMC K15M 1 % w/w) were selected based on published data showing that Carbopol–HPMC combinations were suitable for topical gel formulations and that the polymer composition affected the rheological properties and spreadability [10].

Formulation of phenylephrine hemostatic gel

Phenylephrine HCl (1 % w/w) was dissolved in propylene glycol (5 % w/w) with continuous stirring to ensure complete solubilization. The phenylephrine HCl solution (Solution D) was then dropped into the gel base (Solution C) with continuous stirring to allow the active ingredient to be incorporated gradually and evenly. Then 1 M sodium hydroxide solution (1 % w/w) was added dropwise with continuous stirring to adjust the pH. The mixture was stirred for 5 minutes to achieve a smooth and uniform distribution of phenylephrine hydrochloride, and the required amount of distilled water was added to make the final product weight 100 % w/w.

Table 1: Composition of phenylephrine HCl gel

Ingredient	Quantity (% w/w)
Phenylephrine HCl	1
Carbopol 940	1.5
HPMC K15M	1
Propylene glycol	5
Sodium hydroxide (NaOH)	1
Distilled water to	100

Evaluation of phenylephrine gel

Appearance and homogeneity test

Appearance and homogeneity of the prepared gel were determined by visual inspection following gel settling in the prepared containers. A small amount of the gel was spread as a thin layer on a clean and clear glass slide and viewed under proper lighting conditions for physical appearance, clarity, consistency, and any visible aggregates or phase separation [11].

pH measurement

The pH of the phenylephrine gel was determined using a digital pH meter (Hanna Instruments, HI 2211, USA). One gram of the phenylephrine gel was evenly distributed in 100 mL of distilled water. The pH of the dispersion was then measured by placing the glass electrode directly into the dispersion, and all pH determinations were made three times at room temperature [12].

Viscosity measurement

The viscosity of the phenylephrine gel was measured using a Brookfield viscometer (Model DV2T, Middleboro, USA). The required amount of the prepared gel was filled into the sample adapter and the measurement was performed at a constant temperature (25 ± 1 °C) with a fixed rotational speed (10 rpm) using spindle number 64. All measurements were made three times [13].

Assessment of hemostatic activity of phenylephrine gel

The hemostatic activity of the prepared phenylephrine gel was tested with a total of 30 rabbits. The animals were randomly assigned to 5 groups with six animals in each group (Table 2). To provide an adequate amount of anticoagulation, rabbits in Groups II – V received subcutaneous injections of heparin (100 – 150 IU per rabbit, with body weights ranging from 1 to 1.5 kg), administered 25 minutes before wound creation to ensure optimal anticoagulant activity during hemostatic assessment [14]. The animals were anesthetized using ketamine (35 mg/kg, IM) and xylazine (5 mg/kg, IM) to decrease pain and discomfort and were monitored throughout the study for signs of discomfort. Uniform incisions measuring 2 cm long and 1 mm deep were made on the left thigh of each rabbit by an anatomist using a sterile scalpel.

Ethical approval

All *in vivo* experiments were performed after approval by the Research Ethics Committee of the University of Science and Technology, Faculty of Medicine and Health Sciences, Sana'a, Yemen (Approval no. 1447/0083/UREC/UST). The *in vivo* experiments were carried out in compliance with the guidelines of the National Research Council (NRC) Guide for the Care and Use of Laboratory Animals [15].

Table 2: Grouping of rabbits to study hemostatic activity

Group	Description
I	Normal control group: Healthy rabbits with no heparin or hemostatic treatment.
II	Heparin control group: Rabbits were administered heparin subcutaneously at a dose of 100 – 150 IU.
III	Placebo gel group: Heparinized rabbits were treated with 0.5 g of a gel base lacking the active ingredient.
IV	Phenylephrine gel group: Heparinized rabbits were treated topically with 0.5 g of 1 % phenylephrine gel immediately after wound creation.
V	Standard treatment group: Heparinized rabbits were treated with a commercial hemostatic sponge.

Bleeding time measurement

Bleeding time (BT) was taken as the duration between making the wound and the time when the bleeding stopped. The measurement was performed in each treatment group to evaluate the hemostatic activity of the prepared phenylephrine gel [10].

Statistical analysis

Data were expressed as mean ± standard deviation (SD). IBM SPSS Statistics (version 26) was used for statistical analysis. One-way analysis of variance (ANOVA), followed by Tukey's HSD post hoc test, was utilized to determine the difference in bleeding time

between the groups studied. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Phenylephrine gel formulation

The phenylephrine hemostatic gel was formulated using a combination of 1 % HPMC and 1.5 % Carbopol. These concentrations were utilized to prepare a gel that has proper physicochemical properties for topical application.

Physicochemical evaluation of the gel

The gel was physically stable, with transparency and good homogeneity and showed no evidence of phase separation (Table 3). The pH value was 6.40 ± 0.16, which is within the acceptable pH range for topical and mucosal application. The viscosity was 11960 ± 25.2 cP, which indicates acceptable rheological characteristics for gel consistency and application.

Table 3: Physicochemical properties of phenylephrine gel

Parameter	Phenylephrine gel
Appearance	Transparent
Phase separation	No phase separation
Homogeneity	Good
pH value	6.40±0.16
Viscosity (cP)	11960±25.2

Data are expressed as mean ± SD (n = 3).

Hemostatic activity of the phenylephrine gel

The hemostatic efficacy of the prepared phenylephrine gel was evaluated by measuring the bleeding time in rabbits, and the results are illustrated in Table 4 and Figure 1. The statistical analysis results showed that there was a significant difference between the groups studied ($p < 0.001$), as shown in Table 5 and Figure 2. The phenylephrine gel had the most significant and marked decrease in bleeding time when compared to all other groups (30.0 ± 5.4 s; $p < 0.001$). This reduction was greater than the reduction that was seen with the standard commercial hemostatic sponge (157.8 ± 23.4 s; $p < 0.001$).

Table 4: Bleeding time of various treatment groups

Group	Bleeding time (s)
Normal control	191.4±28.8
Heparin control	501.0±41.4
Placebo gel	429.0±27.6
Phenylephrine gel	30.0±5.4
Standard treatment	157.8±23.4

Data are expressed as mean ± SD (n = 6)

Table 5: Comparisons of bleeding time between treatment groups

Comparison	p-value
Phenylephrine gel vs Heparin control	< 0.001
Phenylephrine gel vs Placebo gel	< 0.001
Phenylephrine gel vs Standard	< 0.001
Placebo gel vs Heparin control	< 0.004
Standard vs Heparin control	< 0.001

One-way ANOVA followed by Tukey's HSD post hoc test was used to compare bleeding times among groups, with statistical significance set at $p < 0.05$

DISCUSSION

The present study developed a phenylephrine hydrochloride gel intended for use as a topical hemostatic agent and tested its physicochemical characteristics and bleeding control activity in rabbits. The physicochemical evaluation showed that the prepared gel had the desired properties required for effective application via the topical route and therapeutic activity.

The clear and excellent homogeneity of the gel suggests that phenylephrine is evenly distributed across the formulation, which is essential for ensuring that the drug is delivered consistently and effectively. The clarity and smooth texture of the gel suggested that HPMC and Carbopol 940 at the concentrations used were effective in creating a physically stable and visually acceptable semisolid formulation, similar to that previously reported using other concentrations of these polymers [16]. The gel had a viscosity of 11,960 cP at ambient temperature, which indicates the semisolid consistency of the gel and allows it to be applied topically as a gel. This viscosity is enough to allow the gel to adhere to the wound and to prevent any run-off, thus facilitating prolonged contact with the tissue and the effectiveness of phenylephrine hemostasis.

The results are in accordance with other HPMC–Carbopol-based gels in which moderate viscosity resulted in ease of spreadability and maintained the structural integrity of the gel at the application site [9,10]. Also, no phase separation was observed during the period of evaluation, suggesting that the prepared gel was physically stable and had a uniform distribution of the drug. The same results have been noted in earlier research [16]. The phenylephrine gel had the highest hemostatic efficacy and decreased bleeding time of heparinized rabbits significantly when compared with the placebo and the standard commercial hemostatic sponge.

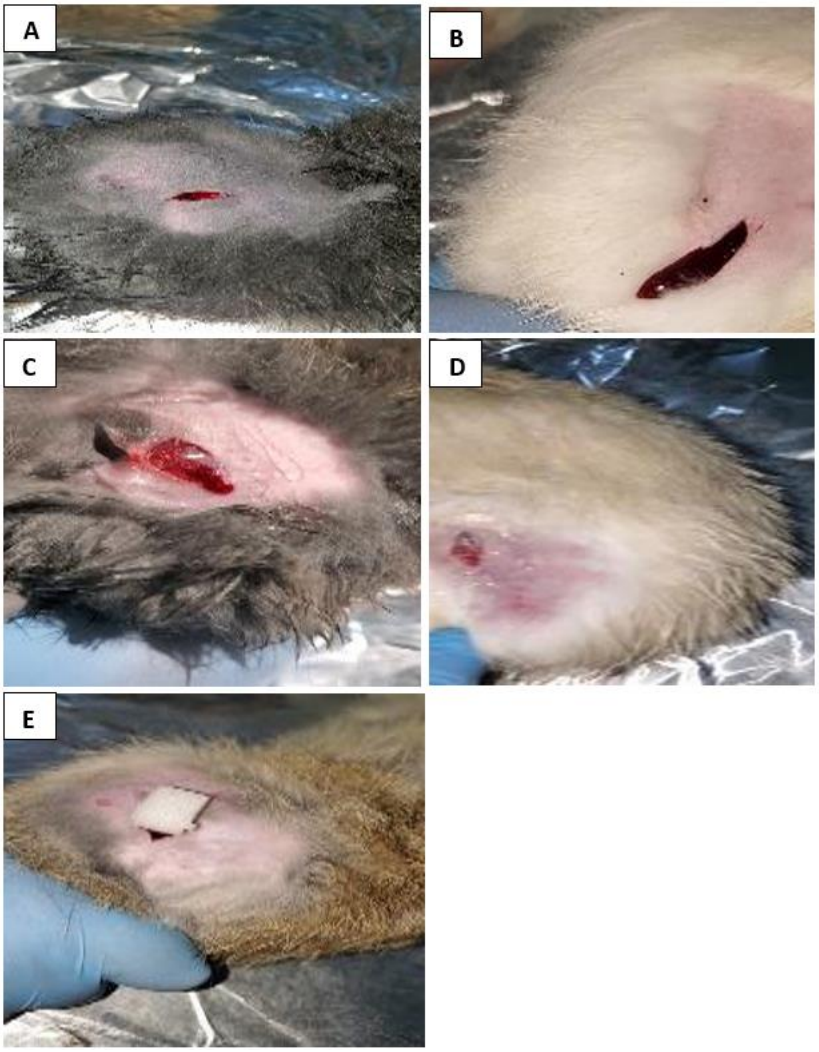

Figure 1: Representative photographs showing bleeding-control outcomes in rabbits. A: Normal control (191.4 s); B: Heparin control, showing prolonged bleeding time (501.0 s); C: Heparinized rabbit treated with a placebo gel, showing limited bleeding control (429.0 s); D: Heparinized rabbit treated with a phenylephrine hydrochloride gel (1 % w/w), showing significant bleeding reduction (30.0 s, $p < 0.001$); and E: Heparinized rabbit treated with a commercial hemostatic sponge, showing significant bleeding reduction (157.8 s, $p < 0.001$)

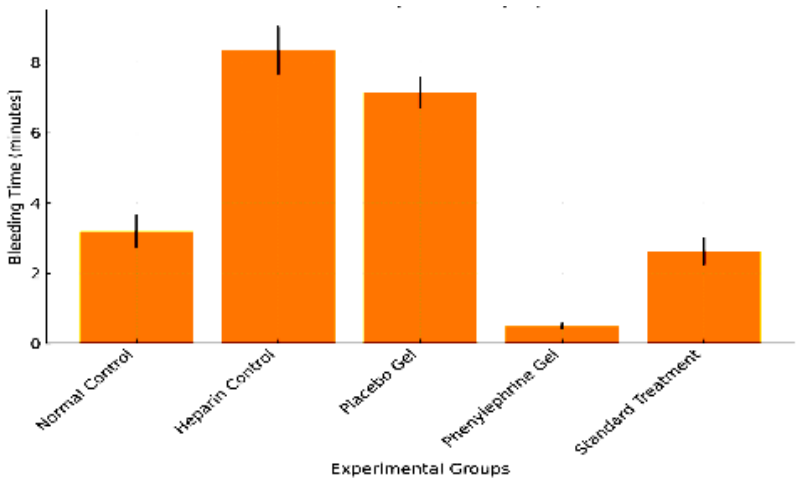

Figure 2: Comparisons of bleeding time between experimental groups

The enhanced effect is attributed to the pharmacological effect of phenylephrine as an α_1-adrenergic agonist, which causes local vasoconstriction and encourages rapid hemostasis. Phenylephrine has also been previously reported with similar vasoconstrictive properties in topical formulations for reducing superficial (skin) bleeding [17].

The concentration of 1 % w/w phenylephrine hydrochloride was chosen as it is a concentration that is found in other locally acting topical and nasal drug formulations for adults [5-7]. The α_1-adrenergic receptor activation by phenylephrine suggests a pharmacological reason to study the possibility of using it as a hemostatic agent for topical use. A gel formulation containing Carbopol 940–HPMC K15M may enhance local retention and contact time at the application site, but it is not necessarily expected to result in decreased systemic absorption unless tested. The results of the bleeding time test reflect the hemostatic properties of the formulation under the conditions of this present study in rabbits.

There are some limitations in this study. Only one concentration of phenylephrine hydrochloride (1 % w/w) and fixed concentrations of the gelling agents were investigated; further investigations are needed to assess the effect of the various concentrations and ratios of these gelling agents on the physicochemical properties and hemostasis of the gel. In addition, further investigations will be needed to evaluate the long-term stability, local tolerability, and safety of the formulation before it can be used in clinical trials.

CONCLUSION

Topical gel formulation of phenylephrine hydrochloride has been achieved by mixing HPMC and Carbopol 940 to obtain a smooth, transparent and homogeneous gel. The chosen polymer concentrations yielded the suitable viscosity and homogeneity. The gel had a physiologically acceptable pH level, making it suitable for topical hemostasis. The phenylephrine gel group had a significantly lower mean bleeding time (30.0 s, $p < 0.001$) when compared with the heparin control group (501.0 s) and the commercial hemostatic sponge group (157.8 s), demonstrating a greater reduction in bleeding time under the experimental conditions.

DECLARATIONS

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Author contributions

Abdullah H Maad and Abdullah H Al-Gamli helped conceive and design the study and drafted the manuscript. The formulation development and experimentation were performed by Abdullah H Al-Gamli, Ezz Al-dain Naji Qasem, and Khaled A Alhumaidha. Kamil K Atiyah Altameemi and Layth Abdullah Zainy were involved in data collection, data analysis and interpretation. All authors participated in critical revision of the manuscript for important content. All authors read and approved the final version of the manuscript.

Conflict of interest

There are no conflicts of interest.

Use of Artificial Intelligence

The authors confirm that AI-based tools were used solely for language editing and manuscript polishing purposes (e.g., grammar, phrasing, and clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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