RESEARCH ARTICLE

Formulation and Evaluation of Haemocoagulase Gel for Dental Pulp- an *In-vitro* study

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ABSTRACT

This study aims to develop a novel hemostatic gel of hemocoagulase, a snake venom-derived serine protease enzyme, by incorporating it into the matrix of bioadhesive polymers to prevent damage due to hemorrhage. Haemocoagulase was combined with polymers Carbopol 940 and HPMC K 100, and these formulations were evaluated for physical properties, efficacy, safety and stability. Batches F3 and F6 were selected for further studies on animal models, which were found to be safe without any skin irritation effect and effective as hemostats with blood clotting times of 1.24 ± 0.007 min and 1.36 ± 0.014 min, respectively, in a rat liver incision model. From this study, it can be suggested that the combination of hemocoagulase with bioadhesive polymer can provide a novel hemostatic tool for controlling bleeding.

Keywords: Hemostatic gel, Hemocoagulase, Botroclot, Physical barrier, Blood coagulation.

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INTRODUCTION

Blood is viscous, dense, and fluid; connective tissue can be referred to as the fluid of life, as it helps in the transport of nutrients, enzymes and gases such as CO_2 and O_2 and the regulation of water, acid-base balance and body temperature.[1] The normal blood clotting time is 3 to 8 minutes in an average healthy adult.[2] In the case of small injury or damage to blood vessels, the body starts its normal hemostasis process and clots blood by the mechanism of initial hemostasis, which forms platelet plugs, followed by a second level of hemostasis by developing fibrin polymers, which helps stabilize platelet plugs.[3] However, there are many conditions, such as thrombocytopenia, diabetes, leukemia, hemophilia, and von Willebrand disease, where the normal hemostatic balance of the body is affected and a small injury to blood vessels may lead to excessive bleeding.[4] Uncontrolled bleeding is a very serious condition because it increases the risk of sudden ischemic events, complicates surgical conditions, increases hospital stays and treatment costs and may lead to multiple organ failure, morbidity and mortality.[5-7] Conditions such as trauma, accidents, battlefields, people undergoing anticoagulant therapy, and oral, cardiac and gynecological surgeries also require hemostat to prevent blood loss.[8,9]

To overcome these problems and prevent blood loss, hemostatic agents are used. Hemostats are materials that help in blood coagulation and prevent hemorrhage.[10] They are categorized into three main types: active, passive and others.[11] The active agents are those that themselves participate in the coagulation cascade and facilitate blood clotting; they are quite expensive but highly effective. Commonly used active hemostatic agents are fibrin, hemocoagulase, etc.[11] Passive agents are those that create a barrier to halt blood flow and provide a platform for platelet aggregation. These agents are widely available and have low cost. The commonly used passive agents are gelatin, polysaccharides, collagen and cellulose.[12] Other agents include sealant, combination products of active and passive agents, adhesives, etc.[13]

This study was performed by using hemocoagulase, a snake venom-derived serine protease enzyme with thrombin-like activity, isolated from the snakes *Bothrops atrox, B. jararaca and B. atrox majooni*.[14] It was discovered in 1936 and commercially produced by recombinant expression in *Pichia pastoris*.[15] It has procoagulant activity and facilitates blood coagulation by cleaving fibrinopeptide at Arg16-Gly17 and gives DesA fibrin monomer, which joins together to form fibrin, an important factor in the coagulation cascade.[16] Hemcoagulase is also effective in the presence of anticoagulants such as heparin and antithrombins.[14] Hemocoagulase is widely used in many medical conditions and available in solution form, such as botroclot and clotase, and in injectable forms, such

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as Botropase, Troylase and Reptilase, in the Indian market.

The most widely used formulation of hemocoagulase for topical conditions is in solution form, which is easily washed out with a heavy flow of blood; hence, localization at the site of action is not possible, which limits its efficiency as a hemostatic material. To avoid this problem, hemocoagulase formulations are prepared in gel form using bioadhesive polymers. This bioadhesive gel of hemocoagulase will have the advantage of localization at the site of action, providing a physical barrier for platelet aggregation and blood flow along with the active hemostatic action of hemocoagulase.[17]

The bioadhesive hemostatic gel was formulated by using polymers Carbopol 940 and HPMC K 100 due to their properties, such as high availability, a wide range of viscosity, good bioadhesive and biodegradability.[18-20] Due to the unavailability of hemocoagulase, a marketed Botroclot formulation by Juggat Pharma, Bangalore, Karnataka, is used as a source of hemocoagulase. The hemostatic gel was formulated and evaluated for its physical characteristics, safety and functional efficiency in, *in-vitro* and *in-vivo* for stability conditions. The study was carried out to assess the effectivity of the combination product as hemocoagulase with bioadhesive polymer in clotting blood by both active and passive mechanisms and to develop a novel effective hemostatic material.

MATERIALS AND METHODS

Materials

Botroclot solution from Juggat Pharma, Bangalore, Karnataka, was purchased and used as a source of haemocoagulase. The gelling agents Carbopol 940 and HPMC K 100 were obtained from M.V. P's College of Pharmacy, Nashik.

Methods

Formulation of hemocoagulase gel

Six formulation batches were prepared by using hemocoagulase from Botroclot solution and the gelling agents carbopol 940 and HPMC K 100 at varying concentrations from 0.5 to 2%, as given in Table 1. The procedure used for gel formulation is given below:

- *Carbopol gel*

An accurately measured Carbopol 940 was soaked in a small quantity of water. This formed mass was then neutralized by using triethanolamine to a nearly neutral pH. Then, botroclot formulation containing hemocoagulase dissolved in WFI and preservative chlorhexidine gluconate was mixed continuously using a glass rod so a uniform gel could be formed.[21]

Table 1: Formulation of haemocoagulase gel

S. No.	*Formulation*	*Botrocloat (mL)*	*Carbopol 940 (gm)*	*HPMC K 100 (gm)*	*Distilled water (mL)*
1.	F1	50	0.5	-	5
2.	F2	50	0.75	-	5
3.	F3	50	1	-	5
4.	F4	50	-	0.5	5
5.	F5	50	-	0.75	5
6.	F6	50	-	1	5

- *HPMC K 100 gel*

An accurately weighed quantity of HPMC K 100 was soaked in a small quantity of distilled water warmed to 50°C. To this, botroclot solution was added containing hemocoagulase with preservative chlorhexidine gluconate. This mixture was then continuously stirred to form a uniform gel, and this formulated gel was then kept in a refrigerator overnight (hot/cold technique).[22]

Preparation of the gel takes place within a few seconds after the addition of the excipients.

The unique challenge in preparing this gel is to preserve the hemocoagulase property, which will be tested further in research. The gel preparation is currently unavailable which would add to the innovation and in actual practice in the future.

Evaluation

Drug-excipient compatibility

Study of the compatibility between drugs and excipients is very important, as the presence of incompatibility may affect the physical, chemical, and structural stability of formulations and decrease their functional efficiency. To avoid this, drug-excipient compatibility was evaluated by performing FTIR analysis of samples of Botroclot, Carbopol 940, HPMC K 100, Carbopol 940 with botroclot and HPMC K 100 with botroclot.

Appearance

The formulated batches of haemocoagulase gel were examined for appearance, such as color and clarity, by visual observation.[23]

Further tests such as pH, viscosity, and bio-adhesive strength are performed to determine the biocompatibility of the formulated gels in order to use them for further research planned in animal and/or human studies. Hence, there is no statistical analysis performed on their values, but the best suitable preparation will be used for further research.

pH

A digital pH meter(pHCal, Analab) was used to record the pH of all the formulations, which was first calibrated to pH 4 and 7 with standard buffer solution, and then readings were measured three times and expressed as the mean ± S.D.[24]

Viscosity

The viscosity of the formulations was recorded at room temperature by a Brookfield viscometer [LV (DV II + Pro)] with spindle no 64 at 2 rpm for Carbopol and 50 rpm for HPMC-containing gel.[24]

Bioadhesive strength

This test was carried out by a modified two-arm balance method with some modifications. The freshly prepared sheep's buccal mucosa was used from a slaughterhouse, cleaned and then attached to two glass slides with the help of glue. One glass slide was glued on a wooden platform, and the other

glass slide was fixed to the base of the left arm of the weighing pan in such a way that the mucosal skin attached to the glass slides faced each other. Accurately 0.5 g of prepared gel was placed between glass slides attached to goat skin mucosa. One hundred grams of weight was placed on the left arm of the balance to provide contact time for uniform spreading of gel between slides for about 5 minutes. After 5 minutes, 100 g of weight was removed from the left arm of the balance, and 10 g of weight was continuously added every minute in the right pan of the weighing balance until both glass slides separated.[25] The point of separation of both glass slides was marked, and the weight required for separation of glass slides was recorded. This experiment was performed in triplicate, and the bioadhesive strength was calculated in gm/cm^2 as follows:[26]

$$Adhesive\ strength = \frac{W}{A}, \quad (1)$$

where the adhesive strength was measured in gm/cm^2,

W is the weight required to detach slides from each other in gm,

A is the area of the glass slide in cm^2.

Spreadability

The spreadability test was performed by using a wooden apparatus with a pulley. A glass slide was glued to the wooden base. Accurately weighed 0.5 g of prepared gel was placed on that glass slide. The different glass slide was placed above it, which was tied to a weight holder through a pulley. On the glass slide, 100 gm of weight was placed for 5 minutes in order to apply preload to provide contact time and spread gel uniformly between the plates. After 5 minutes, 50 g of weight was loaded to the weight holder. Then, the time taken for the slide to move a specific distance was recorded. This procedure was carried out three times, and spreadability was calculated as follows:[24,27]

$$S = M\frac{L}{T}, \quad (2)$$

where S =spreadability of the gel formulation,

M = weight tide to upper slide in gm,

L = length of the glass slide in cm,

T = time required for plates to slide.

In-vitro blood clotting time

The *in-vitro* clotting time test performed using the Lee-white clotting time test (LWCT). Blood samples were collected from healthy individuals after obtaining informed consent in citrate dextrose tubes. One milliliter of blood sample was then added to eight different cleaned, dried and preheated (30°C) test tubes containing 0.1 mL 1 M $CaCl_2$ solution. Then, test materials such as marketed formulations and prepared formulation batches were added to test tubes individually. No test material was added to one test tube, which was referred to as the control. These test tubes were kept undisturbed for 30 seconds and then tilted at 45° regularly to observe blood clotting. Blood clotting was marked by reduced blood flow even after tilting the test tube, and the time was recorded. This test was also performed three times, and the average was calculated.[28-30]

Skin irritation test

This test was performed after the approval of the Institutional Animal Ethics Committee, Approval No. (IAEC/Dec2021/06) as per the guidelines.[31] This test was carried out on male Wistar rats (220–240 kg) divided into four groups (n = 6). The rats were shaved 24 hours before the experiment. After this, test materials such as the marketed formulation (Botroclot) and F3 and F6 formulations were coated to the shaved area and covered with cotton gauze material and nonirritant adhesive tape to retain it for the complete test period. One group of animals was not treated with any test material and was referred to as the control group. After four hours, the dressing was removed, and observations were recorded for the occurrence of any sign of eczema or edema. Similar observations were also recorded at intervals of 1, 24, 48 and 72 hours. According to the Draize dermal skin irritation scoring system, the grade of irritation was checked, and the primary irritation index was calculated[32] using the following formula:

$$PII = \frac{\sum(Erythema\ at\ 1,\ 24,\ 48\ and\ 72\ hrs)}{No.\ of\ test\ sites \times 4\ scoring\ interval} + \frac{\sum(Oedema\ at\ 1,\ 24,\ 48\ and\ 72\ hrs)}{No.\ of\ test\ sites \times 4\ scoring\ interval} \quad (3)$$

The study is conducted as per the ARRIVE guidelines.

In vivo blood clotting time

This test was carried out upon approval from the Institutional Animal Ethical Committee and performed on male Wistar rats (220–240 g) divided into four groups according to the treatment given. The animals were anaesthetized with chloroform, and a vertical incision was made on the dorsal side of the rat, cutting through the skin and muscle layer to expose the liver. A small transverse incision was taken on the left lobe of the exposed liver to bleed heavily. To the injury site, test materials such as marketed formulation (Botroclot), F3 and F6 formulations were applied. No treatment was given to the control group. The injured site was continuously monitored, and the time required for blood clotting was recorded by using a stopwatch. After observation of reduced blood flow and the occurrence of blood clots, the injury was cleaned with cotton gauze to confirm the clotting of blood.[16,17]

Stability test

The stability of the test formulation was determined according to ICH guidelines on optimized batches F3 and F6. The stability study was carried out at room temperature (35°C) for a duration of two months and pH, viscosity, bioadhesive strength and *in-vitro* blood clotting time were evaluated at three time points.[33,34]

Statistical analysis

The results of in vivo blood clotting time were analyzed with the help of one-way and two-way analysis of variance (ANOVA) by Tukey's and Sidak's multiple comparison test.

RESULTS AND DISCUSSION

Drug-Excipient Compatibility

FTIR spectra of marketed formulation Botroclot and gelling agents, i.e., carbopol and HPMC were compared with carbopol

and HPMC gel containing botroclot solution. Botroclot formulation shows two peaks in the IR spectra at 3347.1 and 1838.7 of the functional groups OH and –C=O, respectively. Similarly, Carbopol 940 shows peaks for –OH, -C-H, C=O, CH_2, and C-O at 3123.5, 2937.1, 1899.7, 1449.2, and 118.2, and HPMC K 100 shows peaks for –OH, -CH, CH2, and C-O at 3438.5, 2898.1, 1453.7, and 1051.1, respectively. These observations were compared with the IR spectra of carbopol and HPMC gel, which show peaks of OH, C=O, CH_2, and C-O at 3339.7, 1838.3, 1405.2, and 1095.8 and 3354.8, 1838.3, 1453.7, and 1058.8, respectively. No significant change was observed in the IR spectra or peak intensity. This suggests that the main drug and excipients are compatible with each other; the drug is only physically entrapped in a gel matrix, and this matrix does not affect any properties of the drug.

Appearance

All the formulation batches were formulated and observed as clear and colorless gels, as shown in Figure 1.

pH

The pH of the formulated gel batches was observed to be in the range of 6.41 to 6.92, as given in Table 2. All the batches show nearly neutral pH, and hence, they can be used topically or in body cavities without causing any irritation or damage to body cells and tissues. The pH of blood is approximately 7.4, which is slightly alkaline, and a decrease in pH to a slightly acidic environment may stimulate blood coagulation;[35] hence, the slightly acidic pH of the formulation may facilitate rapid blood coagulation.

Viscosity

Viscosity observations are given in Table 2. Formulations containing carbopol 940 exhibit greater viscosity and greater consistency than formulations containing HPMC K 100. It was noticed that the viscosity of the formulation increases with an increase in the concentration of gelling agents. Formulation F3 shows the highest viscosity of 38512 centipoises. Better viscosity helps in maintaining a good conformal structure of gel at the place of application and remains there for more time.

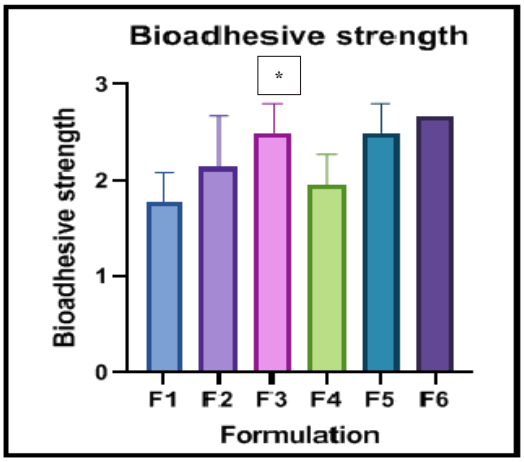
Figure 1: Gel formulation A) carbopol gel; B) hpmc k 100 gel

Bioadhesive strength

The bioadhesive capacity of the formulation is very important for hemostatic action, as this property helps in the localization of formulation at the site of action, creates a physical barrier for the entrapment of blood cells, exerts pressure to stop the flow of blood and is retained at the site of action for a longer duration even in the presence of heavy blood flow.[36] The adhesive strength of the formulations was found to be in the range of 1.778 ± 0.252 to 2.667 gm/$cm^{2,}$ as given in Table 2. It was seen that as the concentration of gelling agent increased, the bioadhesiveness of the formulation also increased. Formulations F4, F5, and F6 containing HPMC K 100 show greater adhesivity than formulation batches F1, F2, and F3 containing carbopol 940. Batches F3 and F6 show the highest adhesive strength among formulations having the same gelling agents. Statistical analysis of the ANOVA results suggested that a slightly significant difference existed between the adhesive strength of different formulations (p <0.0413) (Figure 2).

Spreadability

The spreadability is a very important property of gel, which helps in quick and even spreading of formulation on the site of action. It was found to be in the range of 11.589 ± 0.761 to 7.436 ± 0.280 gm.cm.$sec^{-1,}$ as shown in Table 2, suggesting



(p <0.0413) F3*

Figure 2: Bioadhesive strength

Table 2: PH, viscosity, adhesive strength, spreadability of formulation

Formulation	*pH*	*Viscosity (cP)*	*Adhesive strength (gm/cm^2)*	*Spreadability (gm.cm.sec^{-1})*
F1	6.66 ± 0.008	36052	1.778 ± 0.252	11.589 ± 0.761
F2	6.77 ± 0.045	38452	2.133 ± 0.435	8.367 ± 0.536
F3	6.58 ± 0.066	38512	2.489 ± 0.251	7.436 ± 0.280
F4	6.41 ± 0.124	1158	1.956 ± 0.251	12.639 ± 0.664
F5	6.66 ± 0.352	7174	2.489 ± 0.251	11.853 ± 0.716
F6	6.92 ± 0.041	9313	2.667 ± 0	8.932 ± 0.710

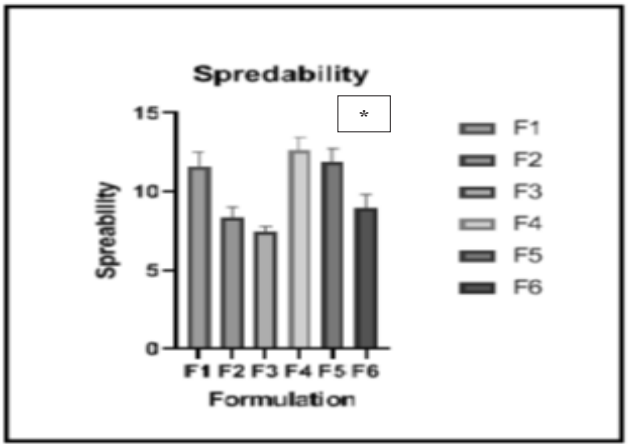


Figure 3: Spreadability of gel ($p < 0.0001$)F6*

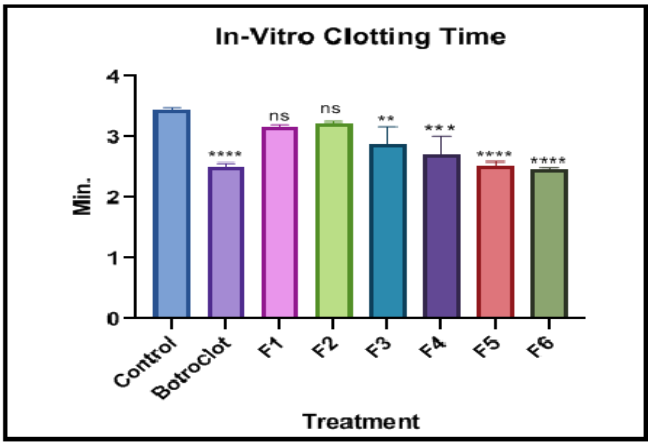


Figure 4: In-vitro blood clotting time ($p < 0.0001$)

good spreadability of formulations, which reduces with an increase in the concentration of the gelling agent. Statistical analysis of spreadability by one-way ANOVA suggested that a significant difference existed between the spreadability of formulations ($p < 0.0001$) (Figure 3).

In-vitro blood clotting time

The *in-vitro* blood clotting time was calculated by the Lee-White method and is given in Table 3. The normal blood clotting time was 3.447 ± 0.034 min. All other test materials were seen to be effective in clotting blood at a time less than that required for normal blood to clot. Botroclot effectively clotted blood in 2.507 ± 0.047 minutes. The formulations with HPMC K 100 were found to be more effective in clotting blood than formulations with Carbopol 940. The order of effectiveness of formulations as hemostats in clotting blood was found to be F6 > Botroclot > F5 > F4 > F3 > F1 > F2> Normal blood. The statistical analysis of *in-vitro* clotting time by ANOVA, as given in Figure 4, suggests that all the formulations effectively clot blood at a significantly faster rate except for formulations F1 and F2 (p <0.0001) (Figure 4).

Table 3: In-vitro blood clotting time

Treatment	*Blood clotting time (minutes)*
Control	3.447 ± 0.034
Botrocloat	2.507± 0.047
F1	3.173 ± 0.025
F2	3.227 ± 0.020
F3	2.897 ± 0.224
F4	2.706 ± 0.244
F5	2.537 ± 0.045
F6	2.467 ± 0.025

Skin irritation test

This test was performed according to OECD guidelines on Wistar rats (n = 6, 220–240 gm) to evaluate the safety of the formulation and check if the formulation caused any irritation to the skin, as the skin irritation effect of gel may worsen the injury or wound and lead to delayed blood coagulation. No sign of the presence of any skin irritant condition, such as eczema or edema, was found when observed for 4 days, as shown in Figure 5. According to the Draize skin irritation grading system, a grade value of 0 was given for each group of rats, and the primary irritation index (PII) was found to be 0 for all

Table 4: Staility test of formulations

Evaluation test	*Formulation*	*Time interval (Days)*		
		0	*60*	*7*
Appearance	F3	Clear, Colourless	Clear, Colourless	Clear, Colourless
	F6	Clear, Colourless	Clear, Colourless	Clear, Colourless
pH	F3	6.58 ± 0.066	6.51 ± 0.012	6.49 ± 0.053
	F6	6.92 ± 0.041	7.04 ± 0.070	7.10 ± 0.033
Viscosity (cP)	F3	38512	38562	38565
	F6	9313	8753	8749
Bioadhesive strength (gm/cm^2)	F3	2.489 ± 0.251	2.844 ± 0.251	3.022 ± 0.251
	F6	2.667 ± 0	2.311 ± 0.251	2.311 ± 0.251
In-vitro clotting time (min.)	F3	2.897 ± 0.224	3.243 ± 0.029	3.297 ± 0.049
	F6	2.467 ± 0.025	2.913 ± 0.012	3.083 ± 0.017

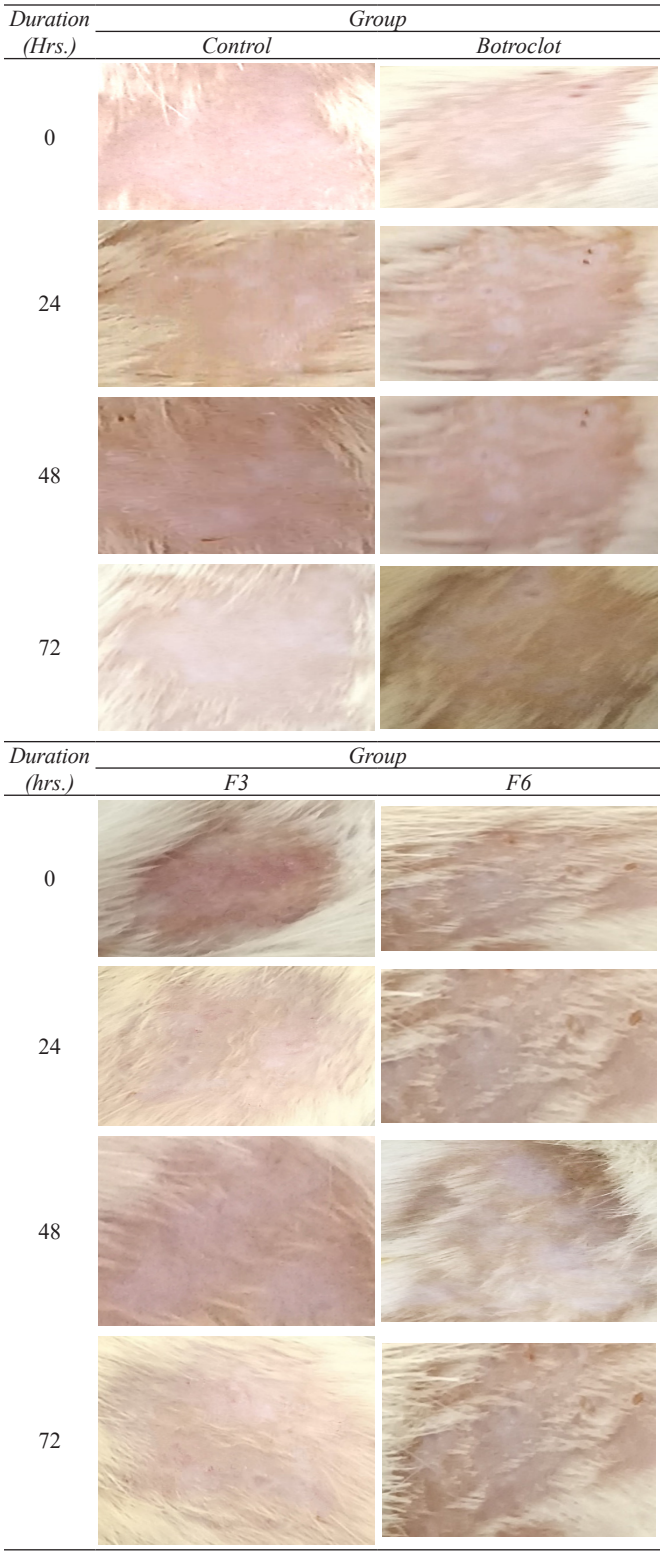
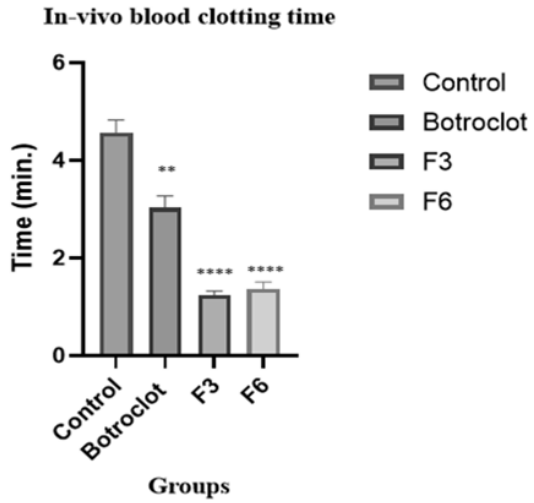


Figure 5: Skin-irritation test

the groups of animals, suggesting the safety and nonirritancy of the gel formulations.

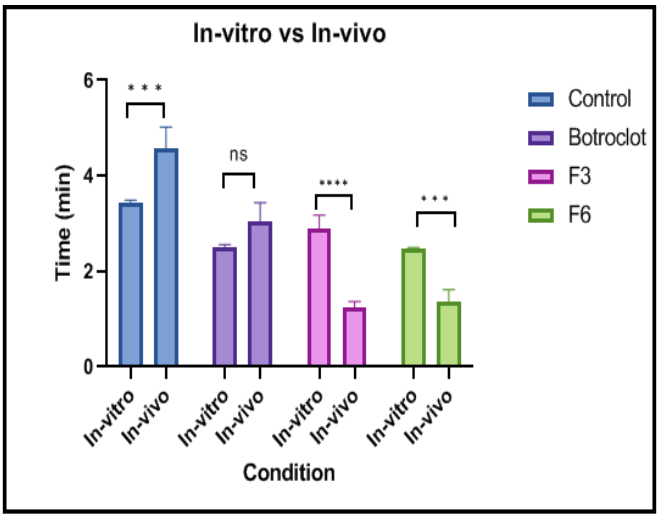
In-vivo blood clotting time

An *in-vitro* clotting time test was performed after approval from the IAEC Committee on four groups of rats, as shown in Figure 5. The blood clotting time was measured and is given in Table 4. The blood flowing from liver injury clots in 4.43 minutes in the case of the control group where no treatment was provided. In the case of botroclot, bleeding stops in 3.17 minutes, indicating its ability to clot blood rapidly. However, the marketed solution washes out with blood flow, reducing its efficiency as a hemostatic agent in heavy bleeding conditions. However, blood clots within 1.24 and 1.36 minutes when the injury was treated with the F3 and F6 formulations, indicating their effectiveness in clotting blood rapidly by the combination of both active and passive mechanisms. Statistical analysis of the in vivo blood clotting time by ANOVA suggests that formulations F3 and F6 clot blood at a significantly faster rate than the marketed solution, as shown in Figure 6 (p <0.0001).



TIME ($p < 0.0001$)

Figure 6: In-vivo blood clotting



Clotting time ($p< 0.0001$

Figure 7: In-vitro vs in-vivo blood

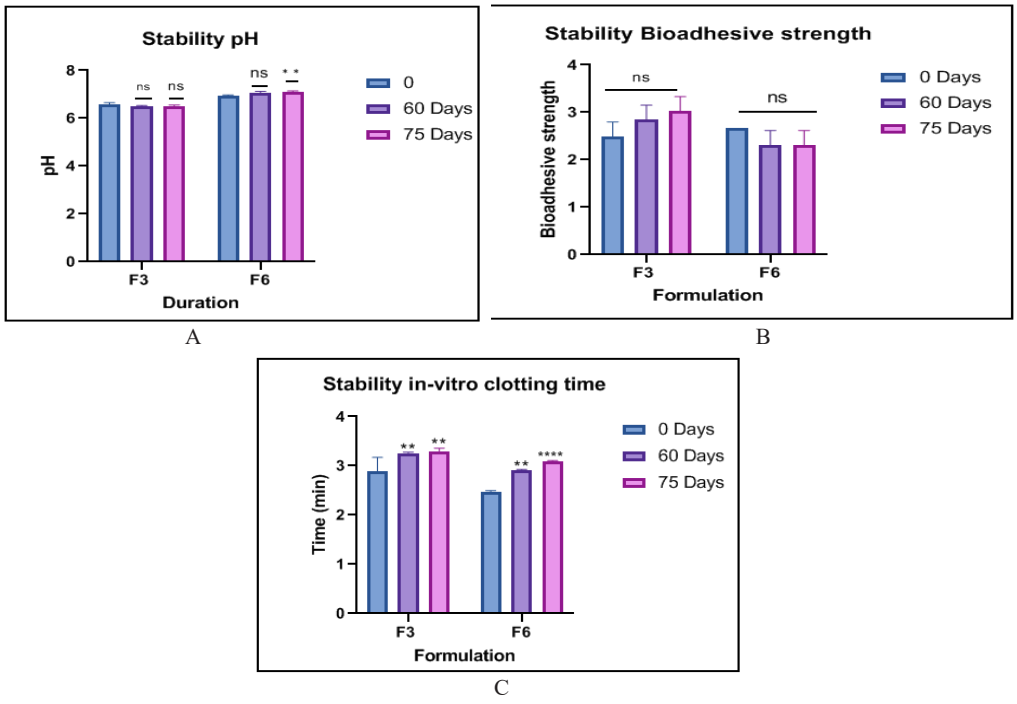


Figure 8: stability of A) ph, B) bioadhesive strength C) in-vitro clotting time

In-vitro and *in-vivo* blood clotting time

The *in-vitro* and *in-vivo* blood clotting times were compared and analyzed statistically by two-way ANOVA, as shown in Figure 7. Significant differences exist between the efficiency of the F3 and F6 formulations in clotting blood under *in-vitro* and in vivo conditions, which suggests that gel formulations halt the flow of blood by combining the active mechanism of hemocoagulase and the passive mechanism by creating a physical barrier. Blood clots at a faster rate under in vivo conditions than under *in-vitro* conditions, which explains the importance of the physical barrier in stopping the flow of blood.

Stability

Batches F3 and F6 were evaluated for stability for a duration of 2 months at three-time points by measuring pH, viscosity, bioadhesive strength and *in-vitro* clotting time and further analysed statistically. From the stability study, it was seen that the F3 formulation showed no change in pH or bioadhesivity and a slight change in viscosity and *in-vitro* blood clotting time. However, F6 formulations showed no change in bioadhesivity, slight changes in pH and *in-vitro* blood clotting time and significantly changed viscosity. The stability results were further analyzed by ANOVA, as shown in Figure 8. This suggests that formulation F3 is quite stable for a period of two months, whereas formulation F6 undergoes slight instability.

CONCLUSION

A hemostatic gel of hemocoagulase was formulated by using the gelling agent Carbopol 940 and HPMC K100. The formulated gel was then evaluated for physical and functional properties and stability. From the viscosity, bioadhesive strength and *in-vitro* blood clotting time test results, formulation batches F3 and F6 were considered to be optimized and further evaluated for skin irritation and in vivo clotting time tests. From the results of this test, both the F3 and F6 batches were found to be safe and effective in blood clotting compared to the marketed formulation of hemocoagulase. Hence, this formulation of hemocoagulase gel with bioadhesive polymers such as Carbopol 940 and HPMC K100 can be suggested as a novel and effective hemostatic tool in controlling heavy bleeding.

FUTURE PLANS

The same formulations will be used further for a pulp therapy-related study to assess hemocoagulase effect on rat teeth. And later in human teeth for Pulpotomy procedure.

AUTHOR CONTRIBUTIONS

- Swati Karkare- Conception, design, drafting of paper, revising, final approval.
- Nilima Thosar- Conception, design, revising and final approval.
- Milind Wagh- Design, analysis and interpretation
- Nikita Wagh- Analysis, Interpretation of data
- Vidyatai Jondhale-Analysis, Interpretation of data
- All authors agree to be accountable for all aspects of the work.

COMPETING INTERESTS

There are no conflicting interests.

FUNDING

This study did not receive any funding as the inhouse students of the institution conducted it. The institution provided all the materials, equipment, animals, and laboratory materials.

DATA AVAILABILITY

The data and material supporting this study can accessed through the corresponding author.

REFERENCES

1. Sembulingam K, Sembulingam P. Essentials of Medical Physiology. 5th ed. Jaypee Brothers Medical Publishers (P) Ltd; 2010. 56–58 p.
2. Adhana R, Chaurasiya R, Verma A. Comparison of bleeding time and clotting time between males and females. Natl J Physiol Pharm Pharmacol. 2018;8(10):1388–90.Available at: doi: 10.5455/ijmsph.2018.06201417062018
3. Andrew GJ. Current understanding of Haemostasis. Toxicol Pathol. 2011;39(1):273–80.Available at: doi: 10.1177/0192623310389474
4. Palta S, Saroa R, Palta A. Overview of the coagulation system. Indian J Anaesth. 2014;58(5):515–23. Available at: doi: 10.4103/0019-5049.144643.
5. Stokes ME, Ye X, Shah M, Mercaldi K, Reynolds MW, Rupnow MFT, et al. Impact of bleeding-related complications and/or blood product transfusions on hospital costs in inpatient surgical patients. BMC Health Serv Res. 2011;11(1):135. Available at: doi: 10.1186/1472-6963-11-135.
6. Rossaint R, Bouillon B, Cerny V, Coats TJ, Duranteau J, Fernández-mondéjar E, et al. Management of bleeding following major trauma : an updated European guideline. Crit Care. 2010;14:1–29. Available at: doi: 10.1186/cc8943. Epub 2010 Apr 6.
7. Eikelboom JW, Hirsh J. Bleeding and management of bleeding. Eur Hear J Suppl. 2006;8:38–45. Available at: doi:10.1093/eurheartj/sul054
8. Ruangchainicom N, Mahardawi B, Sakdejayont W. Topical hemostatic agents from an oral-surgery perspective. J Oral Maxillofac Surgery, Med Pathol. 2021;33:249–55.Available at: doi.org/10.1016/j.ajoms.2020.11.007
9. Wright JD, Ananth C V., Lewin SN, Burke WM, Siddiq Z, Neugut AI, et al. Patterns of Use of Hemostatic Agents in Patients Undergoing Major Surgery. J Surg Res. 2014;186(1):458.Available at: doi: 10.1016/j.jss.2013.07.042
10. Budko E V., Chernikova DA, Yampolsky LM, Yatsyuk VY. Local hemostatic agents and ways of their improvement. IP Pavlov Russ Med Biol Her. 2019;27(2):274–85.Avialable at: doi.org/10.23888/PAVLOVJ2019272274-285
11. Kumar Mp S. Local Hemostatic Agents in The Management of Bleeding in Oral Surgery. Asian J Pharm Clin Res. 2016;9(3):35–41.A vailable at: https://journals.innovareacademics.in/index.php/ajpcr/article/view/11744
12. Zhong Y, Hu H, Min N, Wei Y, Li X, Li X. Application and outlook of topical hemostatic materials: a narrative review. Ann Transl Med. 2021;9(7):577–577. Available at:doi: 10.21037/atm-20-7160.
13. Pereira BM, Bortoto JB, Fraga GP. Topical hemostatic agents in surgery: Review and prospects. Rev Col Bras Cir. 2018;45(5):1–11. Available at:doi: 10.1590/0100-6991e-20181900.
14. CP M, P DN, TU VN. Effect of topical hemocoagulase therapy in wound healing. Int J Surg Sci. 2019;3(4):151–6.Available at: doi.org/10.33545/surgery.2019.v3.i4c.234
15. Choi SK, Kim CW, Kim JT, Seomun Y, Park MS, Kim CO. Coagulant Effect and Tolerability of Yeast-Produced Recombinant Batroxobin in Healthy Adult Subjects. Clin Drug Investig. 2018;38(9):829–35. Available at:doi: 10.1007/s40261-018-0673-x
16. Guo Y, Wang Y, Zhao X, Li X, Wang Q, Zhong W, et al. Snake extract-laden hemostatic bioadhesive gel cross-linked by visible light. Vol. 7, Sci. Adv. 2021.Available at: doi: 10.1126/sciadv.abf9635
17. Kumar VA, Wickremasinghe NC, Shi S, Hartgerink JD. Nanofibrous Snake Venom Hemostat. ACS Biomater Sci Eng. 2015 Dec 14;1(12):1300–5. Available at:doi: 10.1021/acsbiomaterials.5b00356
18. Safitri FI, Nawangsari D, Febrina D. Overview: Application of Carbopol 940 in Gel. Adv Heal Sci Reasearch. 2021;34:80–4. Available at:doi.org/10.2991/ahsr.k.210127.018
19. Tamasree Majumder, Gopa Roy Biswas, Sutapa Biswas Majee. Hydroxy Propyl Methyl Cellulose: Different Aspects in Drug Delivery. J Pharm Pharmacol. 2016 Aug 10;4(8). Available at: DOI:10.17265/2328-2150/2016.08.003
20. Chakraborti CK, Sahoo S, Behera PK. Role of different biodegradable polymers on the permeability of ciprofloxacin. J Adv Pharm Technol Res. 2014;5(3):140–6. Available at: doi.org/10.4103%2F2231-4040.137434
21. kumar Voleti V, Kumar Voleti V, Banu Shaik S, Konduru C, Peyam S, Kumar Yaramsetti C, et al. Formulation and development of dental gel containing clove oil for the treatment of human periodontal diseases. Vol. 3, Compr Phar. 2016;3(1):1-7
22. Aslani A, Malekpour N. Design, formulation, and physicochemical evaluation of periodontal propolis mucoadhesive gel. Dent Res J. 2016;13(6):484–93. Available at: doi: 10.4103/1735-3327.197037.
23. Upadhye K, Charde K, Dixit G, Bakhle S. Formulation and evaluation of herbal gel for management of mouth ulcers. Indian J Pharm Pharmacol. 2021;8(3):226–30. Available at:doi.org/10.18231/j.ijpp.2021.039
24. Chandira R., Pradeep, Pasupathi A, Bhowmilk D, Chiranjib, Jayakar B, et al. Design, Development and Formulation of Antiacne Dermatological Gel. J Chem Pharm Res. 2010;2(1):401–14.
25. Parhi R, Goutam SVS, Mondal S. Formulation and evaluation of transdermal gel of ibuprofen: Use of penetration enhancer and microneedle. Iran J Pharm Sci. 2020;16(3):11–32. Available at: doi.org/10.22037/ijps.v16.40382
26. Khullar R, Kumar D, Seth N, Saini S. Formulation and evaluation of mefenamic acid emulgel for topical delivery. Saudi Pharm J. 2012;20(1):63–7.Available at: doi: 10.1016/j.jsps.2011.08.001. Epub 2011 Aug 22
27. Garala K, Joshi P, Patel J, Ramkishan A, Shah M. Formulation and evaluation of periodontal in situ gel. Int J Pharm Investig. 2013;3(1):29–41. Available at:doi: 10.4103/2230-973X.108961
28. Nugraha SE, Suwarso E. In-vitro hemostatic activity of ethanol extracts of Beetroot (Beta vulgaris L .) in blood male albino rat. J Innov Innov Pharmacutical Biol Sci. 2018;5(1):34–6.
29. Ayodele OO, Onajobi FD, Osoniyi O. In-vitro anticoagulant effect of Crassocephalum crepidioides leaf methanol extract and fractions on human blood. J Exp Pharmacol. 2019;11:99–107. Available at: doi: 10.2147/JEP.S218261. eCollection 2019.
30. Wedasingha S, Isbister G, Silva A. Bedside Coagulation Tests in Diagnosing Venom-Induced Consumption Coagulopathy in Snakebite. Toxins (Basel). 2020;12(9):1–15.Available at: doi: 10.3390/toxins12090583
31. OECD 404. Guidelines for the Testing of Chemicals. Acute Dermal Irritation/Corrosion. OECD Guidel Test Chem Sect 4 Heal Eff. 2015;(July 28th):1–8. 2074-5788 (online) Available at: DOI: 10.1787/20745788

32. Gebrehiwot M, Asres K, Bisrat D, Mazumder A, Lindemann P, Bucar F. Evaluation of the wound healing property of Commiphora guidottii Chiov. ex. Guid. BMC Complement Altern Med. 2015;15(1):1–11.Available at: doi: 10.1186/s12906-015-0813-2
33. Joshua JM, Anilkumar A, Verjina CU, Vasudevan DT, Surendran SA. Formulation and evaluation of antiaging phytosomal gel. Asian J Pharm Clin Res. 2018 Mar 1;11(3):409–22.Available at: doi.org/10.22159/ajpcr.2018.v11i3.24257
34. Partha N, Snigdha P, Laxmidhar M. Formulation Development And In-Vitro Evaluation Of Dental Gel Containing Ethanol Extract Of Tephrosia Purpurea LINN. Int J Pharm Pharm Sci. 2016;8(8):132–41.
35. CROWELL JW, HOUSTON B. Effect of acidity on blood coagulation. Am J Physiol. 2019;379–82.
36. Pourshahrestani S, Zeimaran E, Kadri NA, Mutlu N, Boccaccini AR. Polymeric Hydrogel Systems as Emerging Biomaterial Platforms to Enable Hemostasis and Wound Healing. Vol. 9, Advanced Healthcare Materials. 2020. p. 1–52. Available from: doi: 10.1002/adhm.202000905. Epub 2020 Sep 16.