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Original Article

Computational Study and Evaluation of Lidocaine and Nitroglycerin Ointment for External Hemorrhoid Treatment

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ABSTRACT

Background and Objectives: Hemorrhoids are defined as swollen veins in the anus and lower rectum. Hemorrhoids are a common medical problem and have a negative impact on people's lives. The drugs that are available on the market are limited, and therefore, the objective of the present research is to develop and evaluate a lidocaine and nitroglycerin ointment combination for enhanced external hemorrhoid treatment. **Materials and Methods:** Five formulations of 0.1% lidocaine and 0.1% nitroglycerin ointments (F1-F5) were developed using hydrocarbon bases (B1 and B2), hydrophilic petrolatum B3, hydrophilic B4, and emulsifying base B5. Different physicochemical parameters were conducted to evaluate the quality of the studied formulations. **Results:** Cracking was observed for F1 and F2 and phase transitions were noticed for F4 and F5. While, the hydrophilic petrolatum base formulation F3 showed the best physical stability up to one month with high uniformity with a drug content of 107.5% and a calibration curve R=0.9999. Docking study confirmed the activity of lidocaine and nitroglycerin towards hemorrhoids through conventional hydrogen bonds with target proteins 6s3a and 6t3w, respectively. F3 was tested by nine patients who suffered from external hemorrhoids for seven days. Patients reported a decrease in hemorrhoid grain sizes with excellent tolerability. **Conclusion:** The developed single formulation of two different drugs, lidocaine and nitroglycerin, has synergistic action and is well-tolerated treatment option for external hemorrhoids, as it not only decreases the hemorrhoid symptoms (pain and itchy) but also eliminates the side effects like headache that arise when the drugs are administered alone.

Keywords: Hemorrhoids, Nitroglycerin, Lidocaine, Formulation, *In silico*, Physicochemical.

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INTRODUCTION

Hemorrhoid is considered the fourth most common gastrointestinal diagnosis and has a severe impact on a patient's daily life. It accounts for more than 2.2 million outpatient visits each year.[1] Symptoms of hemorrhoids appear when the anal cushions (supporting tissues) and hemorrhoids veins are stretching and enlargement during the bowel movement (defecation), lifting heavy weight, pressure on the pelvic, or pregnancy. This venous enlargement converts the normal cushions to abnormal hemorrhoids, in another term causing venous dilatation.[2,3] Hemorrhoids are classified according to where they are located and how much they prolapse. Hemorrhoids may develop inside the rectum (internal hemorrhoids arise above the dentate line), outside the anus (external hemorrhoids arise below the dentate line), or mixed (interno-external) hemorrhoids, which can arise on either side of the dentate line.[4]

The therapeutic strategies for hemorrhoids begin with dietary and lifestyle moderation, followed by medication and hemorrhoidectomy.[5] Several kinds of drugs are available on the market to treat hemorrhoids, such as steroids (hydrocortisone, prednisolone, and diflucortolone valerate) and local anesthetics (lidocaine). Tjandra *et al.*, reported that by using 0.2% glyceryl trinitrate topical ointment for grade-1 hemorrhoids, the pressure on the anal canal is significantly decreased and thus relieved the hemorrhoid symptoms. However, 43% of patients suffered from headaches while being treated.[6] Therefore, an alternative concept to treat hemorrhoids should be taken into account based on increasing the efficiency of the drugs and eliminating the side effects and symptoms associated with these drugs. Scientists have recommended some drug combinations to promote the treatment of hemorrhoids. Kestránek J. and Lorenc Z *et al.*, provided a clinical overview of the efficacy of tribenoside (an

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anti-inflammatory drug) and lidocaine ointment on women who are pregnant and postpartum. They demonstrated that this combination relieves symptoms of hemorrhoid disease, is fast-healing, and is safe to administer.[7,8] Malinverno M. *et al.*, (2023) studied the molecular mechanism of wound healing, and they established that the presence of tribenoside in this combination stimulates cell migration and inhibits the toxicity of reactive oxygen species.[9] Perrotti *et al.*, reported that the drug combination of 3% nifedipine (calcium channel blocker medication) and 1.5% lidocaine treats patients who have had an open hemorrhoidectomy.[10,11] Pharmacokinetic studies were also carried out by several scientists, and they established that a single dose of this combination (nifedipine and lidocaine) is absorbed in the blood stream quickly and in a small quantity, which gives it the advantage of being tolerated and safe to use without serious drug complications.[11,12]

To the best of our knowledge, the drug combinations based on the treatment of hemorrhoids are very limited on the market. Therefore, new formulations based on the combination of lidocaine and nitroglycerin (a vasodilator medication) are prepared in the current research and employed to treat hemorrhoid disease.

Nitroglycerin (1,2,3-propanetriol trinitrate) is a vasodilatory medication that has been extensively used to treat anal fissures by reducing the pressure on the internal anal sphincter muscle.[13] Nitroglycerin is metabolized by the liver to liberate Nitrous Oxide (NO) which is responsible for reducing the smooth muscular tone of blood vessels.[14] The precursor of nitric oxide is responsible for blocking the calcium channel in the symptomatic relief of hemorrhoids, therefore, leading to the relaxation of smooth muscle in a wide range of tissues.[5]

Therefore, the objectives of this research are to: 1) develop an efficient ointment with good tolerance by formulating five nitroglycerin/lidocaine ointments (F1-F5) using different bases, i.e., white base B1, simple ointment base B2, hydrophilic petrolatum base B3, hydrophilic B4, and emulsifying ointment base B5; 2) study the physicochemical properties of the prepared formulation (weight variation, uniformity, smoothness, spreadability, phase separation, pH tests, and drug content) and choose the best formulations for the clinical study; 3) investigate the computational model (PASS and molecular docking) for nitroglycerin and lidocaine by using target proteins 6s3a and 6t3w, respectively; and 4) examine the selected formulation of nitroglycerin/lidocaine ointment on volunteers who suffer from an external hemorrhoid disease.

MATERIALS AND METHODS

Chemicals and Reagents

Nitroglycerin, Lidocaine, Spirit, White wax, Propylene glycol, White soft paraffin, Sodium lauryl sulfate, Emulsifying wax, Beeswax, White petrolatum, Wool fat, Liquid paraffin, Hard paraffin, Cetostearyl alcohol, Methylparaben, and Purified water.

Techniques

Analytical balance, pH meter, Ultrasonic cleaner, Water bath, Dissolution test apparatus, and UV-vis spectrophotometer.

Preparation Methods

Preparation of white ointment (B1-hydrocarbon base)

White wax (0.6 g) was dissolved in water and gently heated in a water bath. Then, white petrolatum (11.4 g) was added to the white wax and warmed until liquefied. Finally, the mixture was lifted to cool with stirring until congealed.

Preparation of Simple Ointment (B2-hydrocarbon base)

In a round conical flask, wool fat (0.6 g) was dissolved in cetostearyl alcohol (0.6 g), and then hard paraffin (0.6 g) was added with stirring to form a homogeneous mixture. After that, white soft paraffin (10.2 g) was added, and the resulted mixture was warmed until liquefied. The mixture was contentiously stirred until it cooled.

Preparation of Hydrophilic Petrolatum (B3-anhydrous absorption base)

Stearyl alcohol (0.36 g) and white wax (0.9 g) were melted, and then beeswax (0.36 g) was added and stirred until all the solids were completely dissolved. Then, white petrolatum (9.78 g) was added, and the mixture was warmed until liquefied. Finally, the mixture was congealed at a constant rate of cooling.

Preparation of Hydrophilic Ointment (B4-absorption base, water-removable)

Stearyl alcohol (5 g) was added to white petrolatum (5 g) and warmed in a water bath to 75°C. Sodium lauryl sulfate (0.2 g), propylene glycol (2.4 g), methylparapen (0.008 g), and purified water (7.4 g) were mixed together and heated in a water bath up to the temperature 75°C. This mixture was poured into the stearyl alcohol solution, and finally, allowed to cool with stirring until congealed.

Preparation of Emulsifying Ointment (B5-emulsion base)

Emulsifying wax (3.6 g), white soft paraffin (5.4 g), and liquid paraffin (2.4 g) were melted together in a water bath and then left to stir until reached room temperature.

Physiochemical Study

Quality assurance is a crucial step to confirm the safety and pharmaceutical efficiency of the prepared drugs. Different physicochemical parameters (weight variation, uniformity,

smoothness, spreadability, phase separation, pH tests, drug content, and *in vitro* diffusion study) were conducted to validate the stability and quality of all prepared formulations. The physicochemical analysis was carried out according to USP procedures.[15]

Weight Uniformity of the Nitroglycerin and Lidocaine

The uniformity test is a pharmaceutical analysis to confirm that the active pharmaceutical ingredient is distributed uniformly throughout the ointments, tablets, and capsules. The weight uniformity was measured by individually weighing 7 units in separated containers, and then the average and the error proportion were calculated.

pH test

pH test is used to measure the acidity of the ointment. The pH test was carried out in triplicate by inserting the pH meter electrode inside the topical formulations (at room temperature (24°C) and at human body temperature (37°C)) and then leaving until attaining equilibrium before collecting the results.

Spreadability and Irritation

The spreadability and irritation were measured by spreading 1.68 g of the formulation on the volunteers' skin, and then the observations were recorded.

Accelerated Stability Test

The purpose of the stability test is to confirm that the pharmaceutical products remain stable with high quality under different conditions, such as heat and humidity, during storage in the market. In this test, the ointments are subjected to stress at different temperatures, refrigerated, and finally analyzed simultaneously. The ointments F1-F5 are stressed at 25°C, 37°C, and 45°C for 30 days, and the total heat input required to cause product failure is measured. Further, the moisture, pH, and package were also subjected during the test. It is important to mention here that product failure occurs due to degradation reactions caused by oxidation and hydrolysis, which have effects on pharmaceutical activities.[16]

Drug Content Study

0.005 mg of nitroglycerin HCl ointment (equivalent to 0.0033 mg nitroglycerin); and 0.2 mL lidocaine (equivalent to 0.0133 mL) were accurately weighed. Separately, 1.33 mg of ointment and 4 mL of lidocaine ointment were put in the 100 mL volumetric flask and dissolved with 60 mL of 0.1 N HCl. The mixtures were sonicated for 10 min, mechanically shaken for 30 min, and the volume was made up to 100 mL. Filtration was performed to remove any insoluble materials. The absorbance of nitroglycerin and lidocaine was measured in triplicate at 220 nm and 230 nm, respectively, and finally, the percentage drug content was calculated. The test was performed on placebo, fresh, and conditioned nitroglycerin and lidocaine ointment.

In vitro Diffusion Study (Release of Ointment)

To estimate the diffusion of the drugs through the biological cells, an *in vitro* diffusion study was carried out. The drug release experiment was accomplished using the USP diffusion apparatus 2, basket method, at 50 rpm via a constant automatic monitoring system in the acidic medium. However, for topical ointments, the acidic conditions enhance the drug's solubility and permeability, thus improving its absorption into the affected area. To optimize nitroglycerin release, hydrochloric acid is used while keeping the temperature at 37±0.5°C. The samples were withdrawn at time intervals (5, 15, and 30 min) and the absorbance was automatically recorded with UV-vis spectrophotometry at λ_{max} 220 nm. The experiment was performed in triplicate, and the percentage of drug release from the ointment was determined as a function of time.

Calibration Curve

The calibration curve is performed to determine the nitroglycerin and lidocaine concentrations. Standard stock solutions containing 0.0101 mg/mL of nitroglycerin and 0.01 mg/mL of lidocaine were prepared in 0.1 N of HCl. From the standard solution,

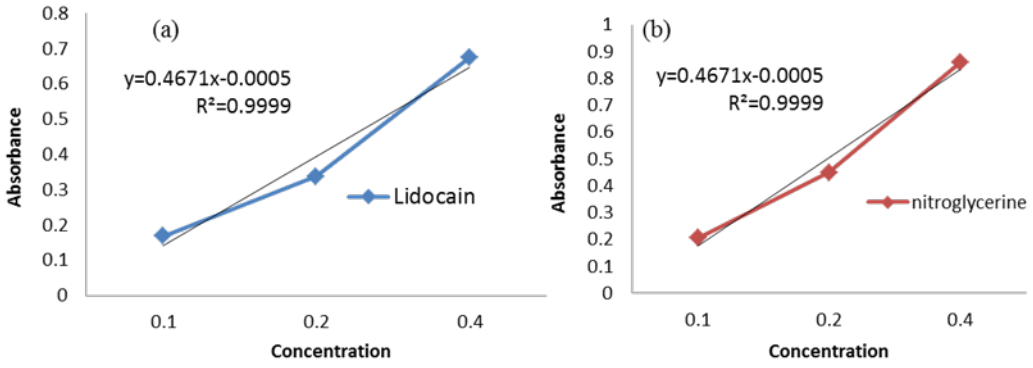

Figure 1: Calibration curve of a) lidocaine, and b) nitroglycerine.

three different nitroglycerin and lidocaine concentrations were prepared (0.1, 0.2, and 0.4 N) by dilution with water. The absorbance was measured using a UV-vis spectrophotometer at λ_{max} 220 nm for nitroglycerin and 230 nm for lidocaine in triplicates. After plotting the linear line, R^2 was found to be 0.9999 (Figure 1) for both nitroglycerin and lidocaine.

Computational Study

Biological Activity Predictions Using PASS

PASS was performed via the method described by Al-Madhagi *et al.*[17] The PASS web tool estimates the pharmacological effects, such as receptor binding, enzyme inhibition, and toxicities of the molecules. It comprehends the biological active spectra via the 2D structure of molecules. PASS software compares the activity of a new drug with a known biological active compound that is saved in the database. PASS software was utilized to estimate the biological activities of the studied compounds (www.way2drug.com). The biological activity range (Pa-Pi, active and inactive) for nitroglycerin and lidocaine was assessed using the structure-activity relationship database (SARBase).[18] From the interpretation prediction scale (0-1), it was established that the higher values recorded for Pa-Pi, ensure the potential biological activity of the drug and vice-versa.[19]

Molecular Docking

The molecular docking was carried out by using molecular docking software. After the docking process was completed, the compounds were ranked depending on the RMSD values. They differ in the manner which atoms are coordinated in distance (RSMD lower bound and RSMD upper bound). Root mean square deviation values represent the differences between the observed confirmation of the studied drug and the predicted values in the database. A lower root mean square deviation value indicates that the prediction model is identical and similar to the studied values. Hence, the studied compounds exhibit pharmacological activities. Meanwhile, higher values represent inconsistency between the predicted and observed values. Each ligand was first ranked depending on the binding affinity, then filtrated based on the binding at the target active site. Then, the binding affinity between each compound and the protein was calculated in kcal/mol. A higher binding affinity value indicates that the target compound is strongly binding to the protein. Additionally, incorporating a 2D-binding map to the molecular docking provides visual representations of the exact interactions between ligands and target proteins. 2D-binding map incorporates the molecular docking to be formed for each ligand conformation using protein-ligand interaction (https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index). Hence, the binding affinity and 2D-binding maps provide information about ligand-protein interactions. The ligands that bind with the target amino acid with favorable binding affinity in comparison with standard ligands present in the crystal structure are chosen as a hit.

Clinical Study

For the clinical trials, the inclusion criteria include patients who were diagnosed with external hemorrhoids. The diagnosis of an anal fissure was performed after taking the patient's history and performing a physical examination. Nine volunteers applied the F3 formulation (the nitroglycerin ointment concentration was 0.1%), and all of them agreed to participate in this study, provided informed consent, and signed up to use the medication as directed. The medication was taken for seven days, applied twice a day to the affected area, and the therapeutic efficacy based on improving the hemorrhoids symptoms (such as anal itching, burning, bleeding, pain, and hemorrhoids size) was compared before and after the administration of the medication.

RESULTS AND DISCUSSION

Five topical formulations (F1-F5) have been prepared using several bases (white base B1, simple ointment base B2, hydrophilic petrolatum base B3, hydrophilic B4, and emulsifying ointment bases B5) that are expected to have an effective efficacy against hemorrhoids. The composite dressings were loaded with 1.68 g of nitroglycerin and 1.5 mL of lidocaine.

Photochemical Evaluation Study

Physical Stability Test of Bases and Ointment Formulations

The physical stability test of the ointments was conducted to ensure and select the most stable formulation. Stability tests are routine procedures performed on drugs that are employed at various stages to get the best quality of development drug. Real-time stability testing is demonstrated to run for a long time to ensure a significant degradation that occurs under the recommended storage conditions. The duration time of the test depends on the stability of the pharmaceutical drug, which should be long enough to clearly indicate that there is no detected degradation. The stability test was carried out by storing the B1-B5 bases and F1-F5 formulations at temperatures of 25°C, 37°C, and 45°C and humidity of 80 for up to one month. The results of the physical stability test of bases and ointment formulations showed that F3 was sustained and physically stable with no change in its appearance over a 30-day period. Meanwhile, color changes, phase separation, and cracking occurred for the formulas F1, F2, F4, and F5, respectively. This indicates poor stability for formulations F1, F2, F4, and F5. It is important to mention here that the phase separation observed at F4 may contribute to the external water phase. This phase leads to chemical stability problems, drying, and enhanced microbial growth.[20,21] After the physical stability evaluation of the F1-F5 formulations, as expected, the formulation with hydrophilic petrolatum base F3 showed the best physical stability, hence it was selected for further investigation.

Formulation with the hydrophilic petrolatum base stayed stable over different ranges of temperatures. Further, ointments are usually formulated in emulsion forms (water and oil), and the hydrophilic petrolatum base stabilizes these emulsions by binding the oil and water together, thereby preventing the occurrence of the separation phase.

Content Uniformity of Ointment

The average content and proportional error for 7 samples of nitroglycerin and lidocaine were calculated as follows:

Weight uniformity of nitroglycerin

$$\text{Avarage } (AV) = \frac{\text{Total weight of ointment}}{\text{Number of ointment}} = \frac{104.93}{7} = 14.99$$

$$Error\ proportion = \text{AV} \times \frac{5}{100} = 14.99 \times \frac{5}{100} = 0.7495$$

The range (g)=15.7395-14.2405.

Volume uniformity of lidocaine

$$\text{Avarage } (AV) = \frac{\text{Total weight of ointment}}{\text{Number of ointment}} = \frac{31.5}{7} = 27.64$$

$$Error\ proportion = AV \times \frac{2.5}{100} = 27.64 \times \frac{2.5}{100} = 0.69$$

The range mL=28.33-26.95.

The % average content was found between 98-100% and within the limit 90-109%, therefore the formulation pass the uniformity of content test.

Spreadability

The study revealed that F3 ointment, which contains a hydrophilic petrolatum base, spreads easily without phase transition, and no irritation was observed, as recorded by the volunteers. Again, thanks to the hydrophilic petrolatum base, which permits water incorporation with the ointment, and thus it becomes less greasy and more easily spreadable.

pH test

At the human body temperature (37°C), the pH value was found to be 6.58. Kartini, Winarjo *et al.*, reported that for a proper topical ointment, the pH value should range between 4.2 and 6.5.[22]

Drug Content

To calculate the percentage of drug content of 0.25 mg/mL nitroglycerin, the obtained nitroglycerin absorbance (0.207 nm) was compared with the standard nitroglycerin solution (0.1 mg/mL; Abs. 0.77 nm) based on the following formula:

$$\%\text{ of drug content} = \frac{\text{Absorbance of nitroglycerin}}{\text{Absorbance of standard}} \times \frac{\text{Concentration of standard}}{\text{Concentration of nitroglycerin}} \times 100$$

$$\%\text{ of drug content} = \frac{0.207}{0.77} \times \frac{0.1}{0.25} \times 100 = 107.5\%$$

The drug content percentage of nitroglycerin was found to be 107.5% which met the USP limitation (90%-110%).

In vitro Diffusion Studies (Release of Ointment)

In vitro diffusion studies provide crucial insights into the drug release profile of ointment. It helps optimize formulation, predict bioavailability, and support pharmacokinetic modeling. The faster dissolution may help to achieve the effective therapeutic activity of drugs by attaining a faster onset of action. The calibration curve with $R^2 = 0.999$, indicates the effectiveness of the selected ointment F3. In addition, *in vitro* diffusion studies show the highest drug release of F3 was found after 30 min (4.32%), whereas the lowest after 5 min (2.40%).

In silico Study

To estimate the pharmaceutical activity of drugs, *in silico* drug design experiments (PASS and molecular docking) are utilized. *In silico* analysis predicts the types of protein-ligand interactions (such as hydrogen bonds, Van der Waals, alkane bonding, and others) and the target binding.

Prediction of Biological Activity Using PASS

Tables 1 and 2 summarize the PASS of the general activities' spectra and the prediction of biological activity for nitroglycerin and lidocaine. The nitroglycerin gave various prediction values ranging 0.80% <Pa<0.99%. The PASS prediction revealed that nitroglycerin showed a high prediction activity value as a vasodilator (Pa=0. 85), nitric oxide donor (Pa=0.98), and anti-anginal (Pa=0.99%.). In addition, PASS predicts another mechanism as analgesic and anti-inflammatory, with Pa=0.86 and 0.87, respectively. Nitroglycerin gave a prediction value of Pa=0.958 and 0.829 as myocardial ischemia and Cyclooxygenase 3 inhibitor, respectively.

On the other side, lidocaine gave varied prediction values, ranging 0.64%< Pa<0.82. The PASS prediction of lidocaine confirmed the anesthetic and calcium channel (voltage-sensitive) activities with Pa=0.79 and 0.70, respectively. Additionally, lidocaine shows a low prediction of 0.632 as a general pump inhibitor.

Molecular Docking

Validation of Docking Parameter

In order to validate nitroglycerin docking analysis, lidocaine re-docked was posed into the active site (binding pocket) of the selected protein 6s3a to get the docked pose and root mean square deviation. Figure 2 shows that lidocaine binds by conventional hydrogen bond at CYS179A.

Molecular Docking of Nitroglycerin with the Selected Protein 6t3w

Molecular docking of nitroglycerin was carried out with 6t3w to identify and understand the binding type of nitroglycerin with

Table 1: PASS general activities spectrum for nitroglycerin.

Pa	Pi	General activity	Pa	Pi	General activity
0.996	0.002	Antihypertensive	0.884	0.003	Spasmolytic, urinary
0.992	0.001	Anti-anginal	0.880	0.001	Angiogenesis stimulant
0.984	0.000	Nitric oxide donor	0.878	0.004	Analgesic
0.971	0.000	Cyclic GMP phosphodiesterase inhibitor	0.864	0.005	Anti-inflammatory
0.965	0.003	Anti-ischemic	0.858	0.004	Vasodilator
0.958	0.002	Myocardial ischemia treatment	0.841	0.013	Acrocylindropepsin inhibitor
0.939	0.001	Guanylate cyclase stimulant	0.841	0.013	Chymosin inhibitor
0.935	0.004	Adrenaline antagonist	0.841	0.013	Saccharopepsin inhibitor
0.933	0.004	Antiadrenergic	0.829	0.002	Cyclooxygenase 3 inhibitor
0.928	0.004	Analgesic, non-opioid	0.825	0.009	Feruloyl esterase inhibitor
0.903	0.001	CF transmembrane conductance regulator agonist	0.833	0.028	Membrane integrity agonist
0.885	0.004	Vasodilator, coronary	0.808	0.004	Ophthalmic drug
0.826	0.024	Ubiquinol-cytochrome-c reductase inhibitor	0.816	0.014	Sugar-phosphatase inhibitor

Table 2: PASS general activities spectrum for lidocaine.

Pa	Pi	General activity	Pa	Pi	General activity
0.826	0.011	Gluconate 2-dehydrogenase (acceptor) inhibitor	0.757	0.051	Phobic disorders treatment
0.792	0.004	Anesthetic	0.709	0.007	Trimethylamineoxide aldolase inhibitor
0.772	0.004	Anti-hypoxic	0.702	0.005	Calcium channel (voltage-sensitive) activator
0.764	0.003	Anesthetic local	0.702	0.008	Sulfite reductase inhibitor
0.762	0.005	Spermidine dehydrogenase inhibitor	0.694	0.002	Sodium channel blocker class Ib
0.766	0.009	Alkane 1-monooxygenase inhibitor	0.704	0.014	Membrane integrity antagonist
0.747	0.005	CYP2C11 substrate	0.716	0.035	Anti-seborrheic
0.749	0.009	Proteasome ATPase inhibitor	0.675	0.019	Venombin AB inhibitor
0.741	0.005	Phenol O-methyltransferase inhibitor	0.663	0.010	Gamma-guanidinobutyraldehyde dehydrogenase inhibitor
0.730	0.008	Spasmolytic, urinary	0.668	0.026	Pseudolysin inhibitor
0.727	0.012	2-Hydroxyquinoline 8-monooxygenase inhibitor	0.648	0.012	Endopeptidase So inhibitor
0.720	0.005	(R)-Pantolactone dehydrogenase (flavin) inhibitor	0.643	0.013	Fructose 5-dehydrogenase inhibitor
0.719	0.007	Amine dehydrogenase inhibitor	0.649	0.018	Polyamine-transporting ATPase inhibitor
0.835	0.008	Taurine dehydrogenase inhibitor	0.661	0.031	Fusarinine-C ornithinesterase inhibitor
0.643	0.028	Anti-dyskinetic	0.640	0.013	Gastrin inhibitor
0.630	0.007	Nicotinate dehydrogenase inhibitor	0.682	0.058	Membrane integrity agonist
0.634	0.019	Superoxide dismutase inhibitor	0.632	0.018	General pump inhibitor

the target protein via hydrogen bonds, hydrophobic interactions, and electrostatic interactions. Figure 3 demonstrated that nitroglycerin binds to a different active site of 6t3w by 1) conventional hydrogen bonds binding with CYS179A, GLU313A, ARG317A, SER136A, SER233A, THR231A, and VAL232A and 2) water bridges binding with VAL137A, and ARG240A.

Preliminary Clinical Study

In vivo hemorrhoid action was evaluated in nine volunteers who had suffered from external hemorrhoids. The volunteers received the active formulation F3, which consisted 0.1% nitroglycerin and 0.1% of lidocaine. The drug was applied twice a day to the affected area for seven days. The participants' ages were between 19-33 years old. After the medication, hemorrhoids recovery is found in 85.7% of the tested patients (Table 3). In terms of efficacy, F3 displayed excellent medication compliance by reducing pain and grain size with almost no adverse effects (Table 3). The clinical examination supports the PASS prediction that, nitroglycerin has an anti-inflammatory effect and improves the local microcirculation and vascular tone. Additionally, the nitric oxide donor and vasodilation of blood vessels result in a relaxation in the muscles. These activities were examined by the observation of decreasing the pain and grain size of hemorrhoids. On the other side, lidocaine confirmed the anesthetic activity by fast relief of pain and itching of hemorrhoid symptoms. Interestingly, PASS prediction revealed that a single dose of the nitroglycerin+lidocaine combination has a synergic action to decrease hemorrhoid symptoms.

The rapid healing observed in this combination may be attributed to the nitroglycerine, which increases the blood supply. Although the F3 formulation shows good activity, some side effects were observed, as light headache for the first two days of drug use for 14.3% of volunteers, which were eliminated during the treatment period. This is attributed to nitroglycerin, which is postulated as NO-donor that gives rise to the pathophysiologic mechanism of migraine pain.

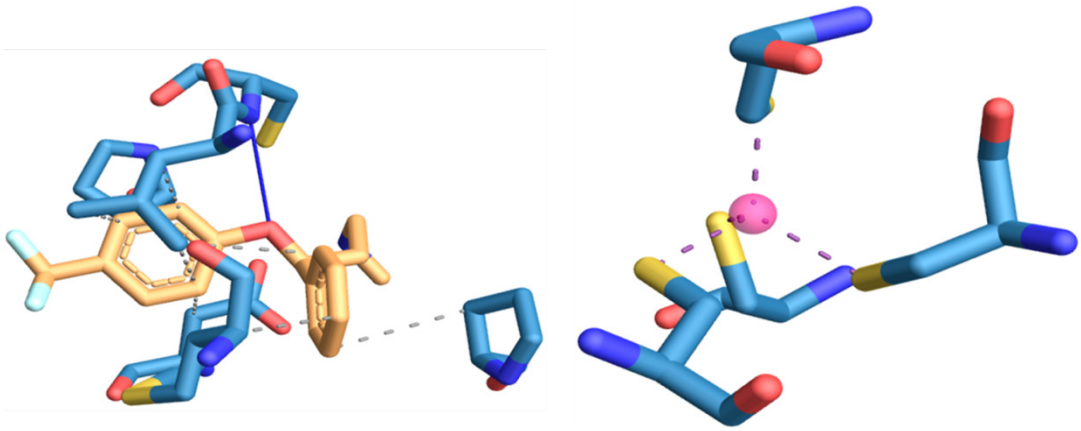

Figure 2: Schematic representation of binding interaction between lidocaine within the binding site of 6s3a crystal structure inhibitor.

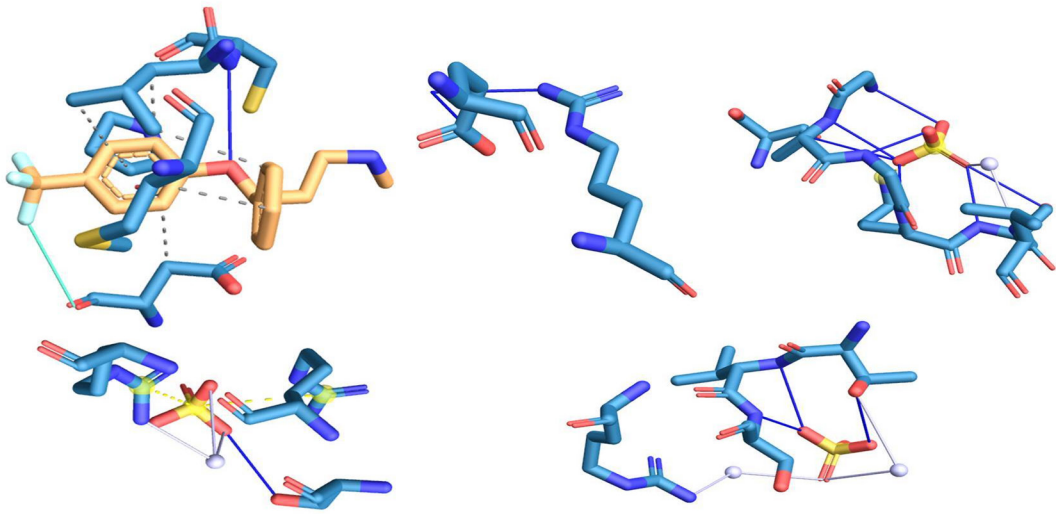

Figure 3: Schematic representation of binding interaction between nitroglycerin within the binding site of 6t3w crystal structure inhibitor.

Table 3: Results of the clinical study.

Sex	Age	Type	Response	Side Effect	Notes
Male	32	External	Yes	No	Melted pain with small size grain
Male	23	External	Excellent	No	No
Male	21	External	Excellent	No	Melted pain with small size grain.
Female	19	External	Excellent	No	Melted pain with small size grain.
Female	25	External	Excellent	No	Melted pain with small size grain.
Male	20	External	Not more	Yes	Light headache during the first two days of use of ointment Improvement after five days of commitment in the use of ointment.
Male	20	External	Excellent	No	Sensory improved.
Male	11	External	Excellent	No	Melted pain with small size grain.
Male	33	External	Excellent	No	Three days after the use of the ointment began not to disappoint the pain while sitting for long hours or when standing.

LIMITATIONS

In this study, our focus was a preliminary clinical investigation of the nitroglycerin+lidocaine combination on the external hemorrhoids. Further studies are required about the potential side effects, safety, and efficacy in large individuals for a long period to determine its therapeutic and potential clinical applications.

CONCLUSION

In this study, a successful and fruitful attempt to prepare a single dose contains a nitroglycerin and lidocaine combination formula for hemorrhoid treatment. Five formulations (F1-F5) were prepared by using different bases (white base B1, simple ointment base B2, hydrophilic petrolatum base B3, hydrophilic base B4, and emulsifying base B5). To confirm the quality and stability of the studied formulation, different physicochemical parameters, including physical stability, homogeneity, pH, spreadability, drug content, and an *in vitro* diffusion test, were conducted in this study. The results show that F3, which contains hydrophilic petrolatum base B3, displays excellent physical stability for up to one month without showing any cracking, color change, or phase transition. Additionally, F3 spreads easily on the skin without any signs of irritation. While, cracking, color change, and phase transitions were observed in F1, F2, F4, and F5. Thus, F3 was selected for further investigation. The percentage drug content along with the calibration curve were found to be 107.5% and 0.9999, respectively. Furthermore, *in vitro* drug diffusion was carried out in hydrochloric acid, and the best drug released of F3 was 4.5% after 30 min, whereas the lowest was determined after 5 min (2.40%). The PASS prediction revealed that nitroglycerin can be used as an analgesic, anti-inflammatory, vasodilator, and nitric oxide donor with possible activity of 87.8%, 86.4%, 85.8%, and 98.4%, respectively. Furthermore, lidocaine showed the anesthetic and calcium channel (voltage-sensitive) activities with Pa =0.79 and 0.70, respectively. Interestingly, PASS prediction revealed that a single dose of the nitroglycerin+lidocaine combination has a synergistic mechanism of action to decrease hemorrhoid symptoms. In addition, the docking study also revealed conventional hydrogen bonds and water bridges binding interaction types between nitroglycerin and the target protein 6t3w. Based on the results, a clinical study was carried out by examining the prepared formula with a concentration of 0.1 nitroglycerin and 0.1% lidocaine for external hemorrhoid treatment. Nine volunteers who suffered from external hemorrhoids were enrolled in this study and took the drug twice a day for seven days. Pregnant women and patients with internal hemorrhoids were excluded. Patients reported an excellent recovery from external hemorrhoids treatment by eliminating hemorrhoid symptoms such as itchy, bleeding, and pain with deceasing in the grain size. Further, a slight headache was observed in the first two days, which was eliminated during the treatment period. It can be concluded that the synergic dual mechanisms of the nitroglycerin+lidocaine combination sufficiently decrease the different symptoms of hemorrhoids, along with providing very good tolerability and safety during the treatment period. Further investigations through *in vivo* studies would be recommended and encourage a large number of participants to enroll to support further clinical trials of the nitroglycerin and lidocaine combination as an anti-hemorrhoid drug.

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ETHICAL APPROVAL

Ethical clearance and approval of the study protocols obtained from the Ethical Research Committee of Yemeni Jordanian University, Faculty of Health Science in 5-2-2022 Reference No. YJU-22022 and the study followed the ethical principles in formulation, quality control, and biological evaluation on human volunteers. In terms of clinical trials, all volunteers were informed about the research and the drug to be used, then they were asked to sign a written informed consent for participation in the study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed to the study conception and design. Wafa M. Al-Madhagi: Supervision and soft wear provider for molecular docking, Arwa Alshargabi: Formal analysis and scientific writing (the original draft, review, and editing), and Abdulkarim K. Y. Alzomor: Methodology and quality control provider. All authors read and approved the final manuscript.

ABBREVIATIONS

PASS: Prediction of Activity Spectra for Substances; **UV-vis:** Spectrophotometry Ultraviolet–visible spectroscopy; **RMSD:** Root Mean Square Deviation.

SUMMARY

Five formulations of 0.1% lidocaine and 0.1% nitroglycerin ointments (F1-F5) were developed using hydrocarbon bases (B1 and B2), hydrophilic petrolatum B3, hydrophilic B4, and emulsifying base B5. The hydrophilic petrolatum base formulation F3 showed the best physical stability up to one month with high uniformity with a drug content of 107.5% as compared with other formulations. Docking study confirmed the activity of lidocaine and nitroglycerin towards hemorrhoids through conventional hydrogen bonds with target proteins 6s3a and 6t3w, respectively. Clinically, treatment with F3 decrease in hemorrhoid grain sizes with excellent tolerability.

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