



DEVELOPMENT AND EVALUATION OF ANTI-INFLAMMATORY GEL CONTAINING ZINGER OFFICINALE .

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ABSTRACT:

A gel is a solid or semisolid system of at least two constituents, consisting of a condensed mass enclosing and interpenetrated by a liquid. Gels and jellies are composed of small solids dispersed in a relatively large amount of liquid, yet they possess more solid-like than liquid-like character. The characteristic of gel and jelly is the presence of some cutaneous structure, which provides solid-like properties. The gel consists of a natural or synthetic polymer forming a three-dimensional matrix throughout a dispersion medium or hydrophilic liquid. After application, the liquid evaporates, leaving the drug entrapped in a thin film of the gel-forming matrix physically covering the skin. Gel formulations prepared with Carbopol 934 and Carbopol 940 showed good homogeneity, no skin irritation, stability, and anti-inflammatory activity. Formulation F2 with 5% extract and 4% Carbopol 940 showed the best formulation with significant anti-inflammatory activity.

Keywords : Ginger gel, carbopol, anti-inflammatory Activity, gel formulations

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INTRODUCTION

Gel:

A gel is a solid or semisolid system of at least two constituents, consisting of a condensed mass enclosing and interpenetrated by a liquid. Gels and jellies are composed of small amounts of solids dispersed in relatively large quantities of liquid, yet they possess more solid-like than liquid-like characters. The characteristic of gel and jelly is the presence of some cutaneous structure, which provides solid-like properties.

Gels have better potential as a vehicle to administer drugs topically than ointment because they are non-sticky and require low energy during formulation.

The gel consists of a natural or synthetic polymer forming a three-dimensional matrix throughout a dispersion medium or hydrophilic liquid. After application, the liquid evaporates, leaving the drug entrapped in a thin film of the gel-forming matrix physically covering the skin.



MATERIALS AND METHODS

PREPARATION OF EXTRACT:

100 grams of ginger powder was soaked in 500 ml of methanol in a conical flask covered with aluminum foil. This process was performed in duplicates. This mixture was allowed to macerate for 4-5 days with interval shaking. After maceration, the mixture was subjected to filtration. The filtrate was collected in china dishes upto 60%. This was allowed to evaporate for a week until dry extract was obtained.

PREPARATION OF PHOSPHATE BUFFER (7.4 pH):

Saline pH 7.4, Phosphate-buffered: Dissolve 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate, and 8.0 g of sodium chloride in sufficient water to produce 1000 ml. Adjust the pH if necessary.

POLYMERS: carbopol 934, carbopol 940.

SOLVENTS: Triethanolamine, Methanol, Distilled water.

PERMEATION ENHANCERS: propylene glycol.

PRESERVATIVES: Sodium benzoate.

PHYTOCHEMICAL SCREENING:

Qualitative Phytochemical Screening of Ginger Extract :

Phytochemical screening was carried out for Methanolic extract of ginger using standard procedures.

Test for tannin: 0.5 g of plant extract was mixed with 2 mL of water and heated in a water bath. The mixture was filtered, and 1 mL of 10% FeCl₃ solution was added to the filtrate. A blue-black solution indicates the presence of tannin.

Test for flavonoid: 5 mL of distilled water and about 0.2 g of plant extract were mixed thoroughly. And 1 mL of 1% AlCl₃ solution was added and shaken. A light yellow precipitate indicates the presence of flavonoids.

Test for phenol: About 0.5 g of plant extract was added to 1 mL of 10% FeCl₃ solution. A deep bluish-green coloration was an indication of the presence of phenol.

Test for saponin: About 0.2 g of plant extract was shaken with 4 mL of distilled water and then heated to boil in a water bath. The creamy mass of tiny bubbles (Frothing) shows the presence of saponin.

Test for reducing sugar: 2 mL of distilled water and 0.2 g of plant extract were mixed and thoroughly shaken in a test tube. 1 mL each of Fehling solution A and B were added to the mixture. A brick-red precipitate at the bottom of the test tube confirms the presence of reducing sugar.

Test for chalcone: 0.2 g of plant extract and 2 mL of 1% ammonium hydroxide were mixed. The appearance of reddish color shows the presence of chalcone.

Test for glycoside: 0.2 g of plant extract and 2.5 mL of dilute sulphuric acid were mixed and boiled for 15 minutes, cooled, and neutralized with 5 mL each of Fehling solution A and B. The formation of a red brick precipitate confirmed glycoside.

Acidic test: 0.2 g of plant extract and sufficient distilled water were mixed and warmed in a hot water bath and cooled. A wet litmus paper was dipped inside the solution.

Test for volatile oil: 0.2 g of plant extract and 2 mL of ethanol were mixed, and a few drops of ferric chloride solution was added. A green coloration indicates volatile oil.

Test for amino acid (protein): 0.2 g of plant extract and 5 mL of distilled water were mixed and left for three h. The mixture was later filtered. To 2 mL of the filtrate, 0.1 mL million reagent was added. A yellow precipitate indicates the presence of protein (amino acid).

Test for phlobatannins: 0.2 g of plant extract and 2 mL of 10% aqueous hydrochloric acid solution were mixed and boiled. A deposition of a red precipitate indicates the presence of phlobatannins.

Test for anthraquinones: 0.2 g of plant extract and 5 mL of chloroform were mixed and shaken for 5 minutes. The mixture was filtered. 2.5 mL of 10% ammonium hydroxide was added to the filtrate. A bright pink, red, or violet color at the upper layer indicates free anthraquinones.



Test for steroids (Salkowski test): 0.2 g of plant extract and 2 mL of chloroform were added, and 2 mL of concentrated sulphuric acid was added to form a layer. The formation of a violet/blue/green/reddish-brown ring at the interface indicates the presence of a steroidal ring.

IN-VITRO ANTI-INFLAMMATORY ACTIVITY (HRBC METHOD)

Blood was collected from a healthy human volunteer and mixed with an equal volume of Alsvers solution. This blood was centrifuged at 3000 rpm. Packed cells were washed with isosaline, and 10% suspension was made. Various concentrates of extracts were prepared using distilled water, and to each concentration 1ml phosphate buffer, 2 ml hypo saline 0.5 ml HRBC suspension was added and subjected to incubation at 37°C for 30 minutes. After incubation, the mixture was centrifuged at 3000 rpm for 20 minutes, and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 281.4 nm.

$$\% \text{ of hemolysis} = (\text{optical density of test} / \text{optical density of control}) \times 100$$

$$\% \text{ of protection} = 100 - (\text{optical density of test} / \text{optical density of control}) \times 100$$

COMPOSITION:

Blank:

Phosphate buffer -1 ml, Hyposaline -2 ml, Normal saline -0.5 ml.

Control:

Phosphate buffer – 1 ml, Hyposaline -2 ml, HRBC suspension – 0.5 ml.

Standard drug:

Phosphate buffer – 1 ml, Hyposaline – 2 ml, standard drug –1 ml (50-250 mg/ml),

HRBC suspension – 0.5 ml.

Test:

Phosphate buffer – 1ml, Hyposaline – 2 ml, plant extract – 1ml, HRBC suspension -0.5 ml.

STANDARD GRAPH:

100 mg of crude extract was dissolved in a little volume of methanol and diluted to 100 ml in a volumetric flask to get a concentration of 1000 micrograms/ml. This was treated as a stock solution. Various aliquots of stock solution were diluted further to obtain different concentrations. Resultant solutions were scanned for wavelength in the 200-400 nm range using a UV- spectrophotometer.

Preparation of calibration curve: Aliquotes of the stock solution of ginger extract (1000 micrograms/ml) were pipetted out into a series of 10 ml volumetric flasks and diluted with methanol to get a final concentration of 20-100 micrograms/ml. The absorbance of the resultant solutions was measured at 281.4 nm.

FORMULATION:

INGREDIENTS	F1	F2
Ginger extract	2.5 grams (5 %)	2.5 grams (5 %)



Carbopol 934	2 grams(4%)	-
Carbopol 940	-	2 grams(4%)
Polyethelene glycol	10 ml	10 ml
Triethanolamine	q.s	q.s
Sodium benzoate	q.s	q.s
Distilled water	Upto 50 ml	Upto 50 ml

The Ginger gel was formulated by the dispersion method. First, the required amount of carbopol was taken and sprinkled in 3 mL of water in a beaker. The beaker was kept aside for 15 minutes for the carbopol to swell. Later, a weighed amount of propylene glycol and ginger powder was added to the beaker and stirred utilizing a mechanical stirrer for 1 hour. Alternatively, the slurry of ginger powder in propylene glycol can also be prepared and incorporated into the carbopol mixture's beaker. Once the ginger powder was evenly dispersed, triethanolamine was added to adjust the pH to 7. Next, sodium benzoate was added to it, and a sufficient quantity of distilled water was added to get the ginger gel.



940(4%)

934(4%)

EVALUATION:

PHYSICAL APPEARANCE:

The physical appearance of the formulation was checked visually which comprised.

Color - The color of the formulations was checked out against white background.

Consistency-The consistency was checked by applying on skin.

Homogeneity -The homogeneity was observed visually .

Feel on the skin - No Irritation

pH:

pH of the formulated gel was determined by using pH meter. In this method, gel was dispersed in purified water. The electrode was washed with double distilled water, dried by tissue paper and calibrated before use with standard buffer solution at 4.0, 7.0, 9.0. The pH measurements were done in triplicate and average values were calculated ¹⁰.

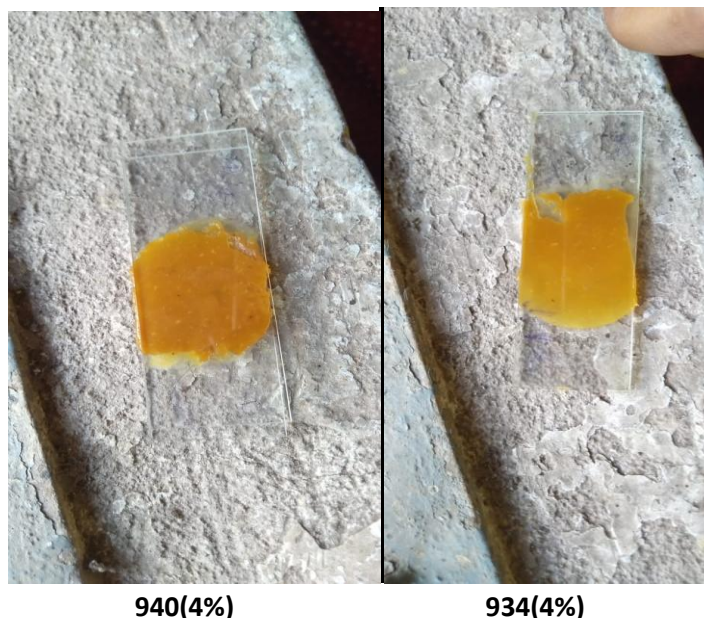
VISCOSITY:

Viscosity of gel was determined by using Brookfield rotational viscometer at 5,10,20,30 and50 rpm. Each reading was taken after equilibrium of the sample at the end of two minutes. The samples were repeated three times ¹².

SPREADABILITY:



The spreadability studies were carried out using 1gm of the gel on the butter paper. This was then placed between two parallel tiles with an upper plate bearing a weight of 1 kg. The spreading diameter of the gel was recorded as spreadability. The average diameter of the circle after the spreading of the gel was determined .



DRUG CONTENT:

The assay of the drug in the gel was performed by extracting the ginger constituents from 1gm of ginger gel with 25 mL of phosphate buffer (pH-7.4) for 15 minutes. The resultant mixture was filtered through a membrane filter, having pore size 0.45 µm. The absorbance of the filtrate was determined spectrophotometrically at 281.4 nm (Shimadzu UV-VIS spectrophotometer) after appropriate dilution with phosphate buffer pH 7.4. The above assay was performed in triplicate. The same procedure was carried on a blank reference gel. The concentration of the novel drug (ginger) was estimated from the calibration curve deduced above.

RESULT AND DISCUSSION:

PHYTOCHEMICAL SCREENING:

CONSTITUENTS	SOLVENT –EXTRACTS OF GINGER
	METHANOL
Flavonoids	–
Phenol	–
Tannin	–
Saponin	+
Anthraquinone	–
Volatile oil	+
Steroid	–
Glycoside	+
Reducing sugar	+
Phlobatannin	+
Aminoacids	+
Acid test	–
Chalcone	+

HRBC Results :

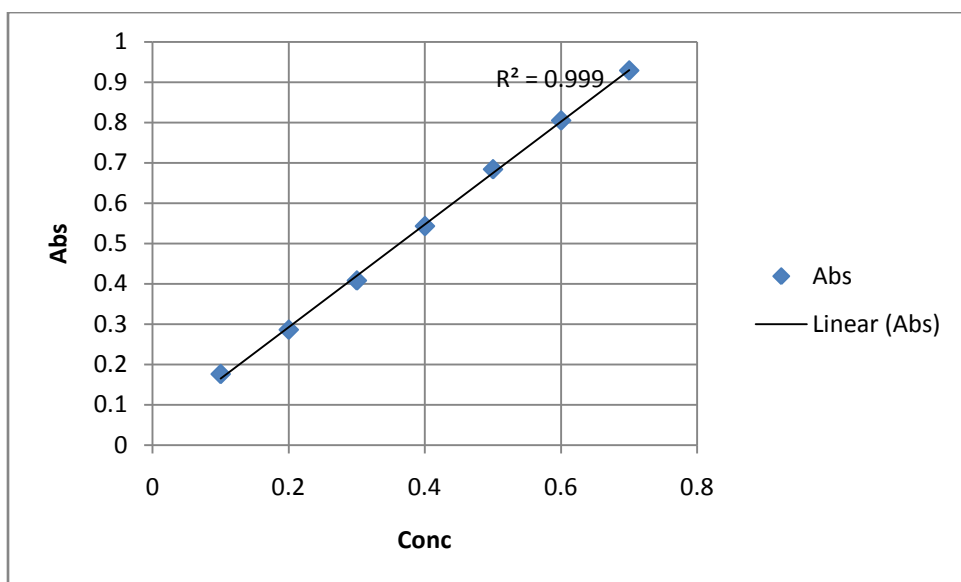


❖ % of hemolysis = (optical density of test /optical density of control)x100
 = $(0.012/0.04) \times 100 = 30$

❖ % of protection =100-(optical density of test/optical density of control)x100
 =100-(0.012/0.04)x100
 =100-30 =70

STANDARD GRAPH:

CONCENTRATION	ABSORBANCE
0.1	0.176
0.2	0.286
0.3	0.408
0.4	0.543
0.5	0.684
0.6	0.805
0.7	0.929



PHYSICAL APPEARANCE:

PARAMETER	F1	F2
COLOUR	Dark Brown	Dark Brown
CONSISTENCY	Smooth	Smooth
HOMOGENECITY	Homogenous	Homogenous
FEEL ON THE SKIN	No Irritation	No Irritation

pH :

S.NO	F1	F2
1.	4.07	5.2
2.	4.00	5.3
3.	4.02	5.3
AVERAGE	4.03	5.2



VISCOSITY:

F1 (cps)	F2 (cps)
4500	4600
4550	4700
4500	4600
AVERAGE =4516	4633

SPREADABILITY:

S.NO	F1	F2
1.	0.6	0.66
2.	0.62	0.68
3.	0.64	0.7
AVERAGE	0.62	0.68

DRUG CONTENT:

The content of drug per 1gm (50mg equivalent drug) ranged from 45mg to 48 mg which indicates efficient loading and uniform distribution of drug in the formulations.

CONCLUSION:

On the basis of the study, the data showed that the Ginger gel prepared from the dried methanolic extracts of ginger gave the significant anti-inflammatory activity when compared with standard Diclofenac gel. As phytochemical tests showed the presence of Glycosides, Reducing sugars, Volatile oils, Saponins, Phlobatannins, Amino acids, Chalcones in the methanolic extracts they might suppress the formation of prostaglandins and bradykinins or antagonize their action and exert its activity. The ginger gel showed its effect and can be useful for the treatment of local inflammation.

We conclude that from the values of pH, Viscosity, Spreadability, Drug content Ginger gel made with polymer carbopol 940 (F-II) was optimized based on the above parameters.

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