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Formulation And Comparative Evaluation of Semisolid Dosage Forms Of Natural Agents(Camphor and Menthol) For Muscle Spasms

P. Anusha, P. TripuraSundari

Department of Pharmaceutics, RBVRR College of pharmacy Affiliated to Osmania University, Barkatpura, Hyderabad-500027, India

ABSTRACT

A muscle spasm, or muscle cramp, is an involuntary contraction of a muscle. Muscle spasms occur suddenly, usually resolve quickly, and are often painful. The use of NSAIDs has been routine in the management of muscle spasm. Although effective at reducing pain and inflammation. NSAIDs may not be appropriate to use frequently or longer time due to their known side effects. Natural agents like camphor and menthol are having analgesic activity as well as each active individual ingredient has its own medicinal value. For greater and effective results we are using combination of camphor and menthol. Hydrophilic and hydrophobic ointments were prepared by taking 25mg, 50mg and 100mg of each agent as combination of camphor-menthol. All the prepared formulations were evaluated for pH, spreadability and diffusion studies. The selected formulations were evaluated for In-vivo studies in comparison with marketed preparations. The finalized preparation was kept for stability studies according to ICH guidelines.

Keywords: ICH guidelines, Natural Agents

*Corresponding Author Email: anushapallerla4@gmail.com

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INTRODUCTION

Muscle spasm:

A muscle spasm, or muscle cramp, is an involuntary contraction of a muscle. Muscle spasms occur suddenly, usually resolve quickly, and are often painful. A muscle spasm is different than a muscle twitch. A muscle twitch, or fasciculation, is an uncontrolled fine movement of a small segment of a larger muscle that can be seen under the skin. Muscle spasm is one of the major factors responsible for chronic pain, it characterizes several pathologies of loco-motor apparatus as well as inflammatory rheumatic and degenerative orthopedic pathologies. When it affects joints, they cause not only pain but also rigidity, which reduces joint mobility & flexibility in affected part. Muscle contractures are also characterize several pathologies of locomotor apparatus and are one of the main factors responsible for persistence of pain associated of these pathologies. Cramps are of form of muscle spasm that are abrupt in nature and last for minutes at a time.

For this reason the study of molecule endowed with muscle relaxant and antispasmodic properties is important. Muscle relaxants are used in the management of musculoskeletal and neuro-muscular disorders. There are mainly two types, centrally acting relaxants and directly acting relaxants.

The word Topical is derived from the Ancient Greek topos (plural: topoi), meaning “place” or “location”. In the past two decades, transdermal drug delivery has moved from a clinical reality to the point where it represents a viable diagnostic tool for non invasive diagnosis.

MATERIALS AND METHOD

Materials used :

Drugs: Camphor, Menthol

Hydrophillic base : soft paraffin, propylene glycol, steryl alcohol, sodium lauryl sulphate, water.

Hydrophobic base : white soft paraffin, woolfat, lanolin, cetosteryl alcohol Preservatives: methyl paraben, propyl paraben.

FORMULATION TABLE:

Formulations prepared by employing camphor and menthol in hydrophilic bases

Table 1: Formulations of camphor – menthol in hydrophilic base

Ingredients	F1	F2	F3
Camphor + Menthol each	25mg	50mg	100mg
White soft paraffin	1.25mg	1.25mg	1.25mg
Propylene glycol	0.621ml	0.621ml	0.621ml
Steryl alcohol	1.25mg	1.25mg	1.25mg
Sodium lauryl sulphate	0.15mg	0.15mg	0.15mg
Distilled water	1.85ml	1.85ml	1.85ml
Methyl paraben	0.25g	0.25g	0.25g

Propyl paraben	0.15g	0.15g	0.15g

Table 2: Formulations of camphor – menthol in hydrophobic base

Formulations prepared by employing camphor and menthol in hydrophobic bases.

Ingredients	**J1**	**J2**	**J3**
Camphor + Menthol each	25mg	50mg	100mg
White soft paraffin	4.25mg	4.25mg	4.25mg
Lanolin	0.25mg	0.25mg	0.25mg
Paraffin wax	0.25mg	0.25mg	0.25mg
Ceto steryl alcohol	0.25mg	0.25mg	0.25mg
Methyl paraben	0.25g	0.25g	0.25g
Propyl paraben	0.15g	0.15g	0.15g

Procedure for preparation of ointment:

Different formulations were prepared by using hydrophilic bases and hydrophobic bases. The hydrophilic bases were prepared by melting the steryl alcohol and white soft paraffin at 70 °c. Continue heating until the temperature of the mixture is about 75°c. Add propylene glycol, sodium lauryl sulphate, methyl paraben, propyl paraben to the water and heat to 75 °. Add aqueous phase to the oily phase with continuous stirring. Switch off the heating and stir continuously until the mixture has congealed.

Different Formulations namely F1, F2 and F3 were prepared by using hydrophilic base The hydrophobic bases were prepared by melting the Hard paraffin and cetosteryl alcohol on water bath. To this incorporate white soft paraffin, lanolin. stir until the ingredients are melted. Different Formulations J1, J2 and J3 were prepared using hydrophobic base. To the hydrophillic base and hydrophobic base camphor and menthol were added separately and dissolved at 70 °c in water bath stir continuously until the mixture has congealed.

Construction of calibration curve of camphor in pH 6.4 Phosphate buffer:

Preparation Of Buffer (pH 6.4 Phosphate buffer):

6.8 g of potassium dihydrogen orthophosphate were weighed and taken in a 1000ml volumetric flask and make the volume to 1000ml with distilled water. 11.6ml of 0.2 M sodium hydroxide is added, to get pH 6.4 phosphate buffer.

Preparation of Standard Stock Solution (1000µg/ml or 1mg/ml):

10mg of camphor drug was weighed and transferred into a 10ml volumetric flask, dissolved in ethanol and volume was made upto the mark with phosphate buffer.

Preparation of Sub Stock Solution(100µg/ml):

1ml of above stock solution was taken in a 10ml volumetric flask and make the volume upto the mark with buffer (6.4phosphate buffer).

Preparation of Series of Standards:

From the above stock solution 2,4,6,8,10ml was transferred into separate 10ml volumetric flask and the volume was made up with buffer. This gives 20, 40,60,80,100 µg/ml respectively. The absorbance of the solutions was measured at 290nm using UV-Visible spectrophotometer. A graph was plotted by taking concentration on X-axis and absorbance on Y-axis.

Table 3: Standard curve of Camphor in pH 6.4 phosphate buffer

Concentration(µg/ml)	Absorbance
0	0
20	0.028
40	0.054
60	0.076
80	0.103
100	0.126

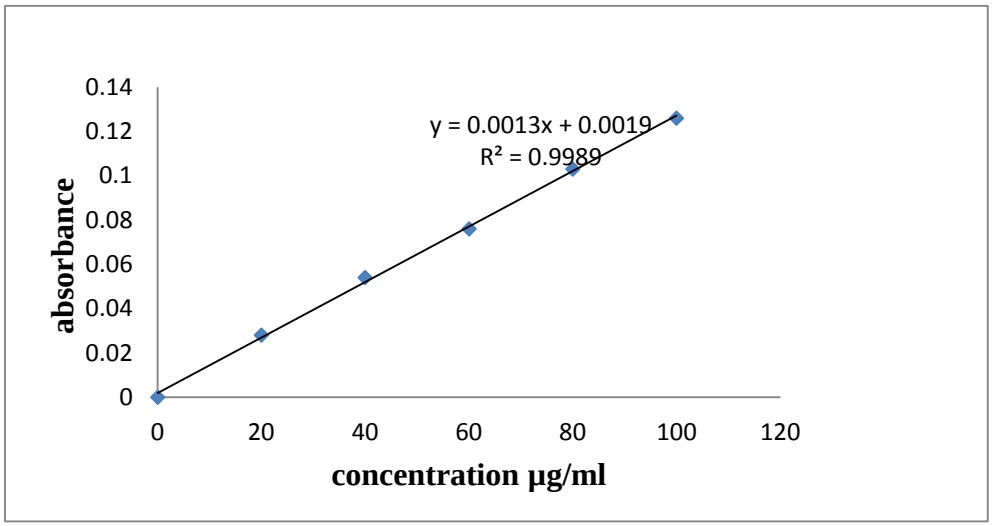

Figure 1: standard plot for camphor

Construction of calibration curve of menthol in pH 6.4 Phosphate buffer:

PREPARATION OF STANDARD STOCK SOLUTION (1000µg/ml) :

10mg of menthol drug was weighed and transferred into a 10ml volumetric flask, dissolved in ethanol and volume was made upto the mark with6.4 phosphate buffer.

PREPARATION OF SUB STOCK SOLUTION(100µg/ml):

1ml of above stock solution was taken in a 10ml volumetric flask and make the volume upto the mark with buffer (6.4phosphate buffer).

PREPARATION OF SERIES OF STANDARDS:

- Volumes of 0.2 ,0.4,0.6,0.8,10 mL of menthol solution were transferred in dry beaker and a final volume of 4.0 mL was adjusted with ethanol.

- mL of 1% salicylic aldehyde solution and 10 mL of concentrated sulphuric acid solution were added carefully, drop by drop.
- Then the beaker were gently stirred to mix the solution and let it stand for 30 min, to reach the room temperature.
- The absorbance was measured at 510 nm, in a 1.0 cm glass cell against a blank solution prepared similarly, but without menthol.

Table 4: Standard curve of Menthol in pH 6.4 phosphate buffer

Concentration(µg/ml)	Absorbance
0	0
1.33	0.075
4.	0.180
6.65	0.291
9.3	0.481
12.0	0.520

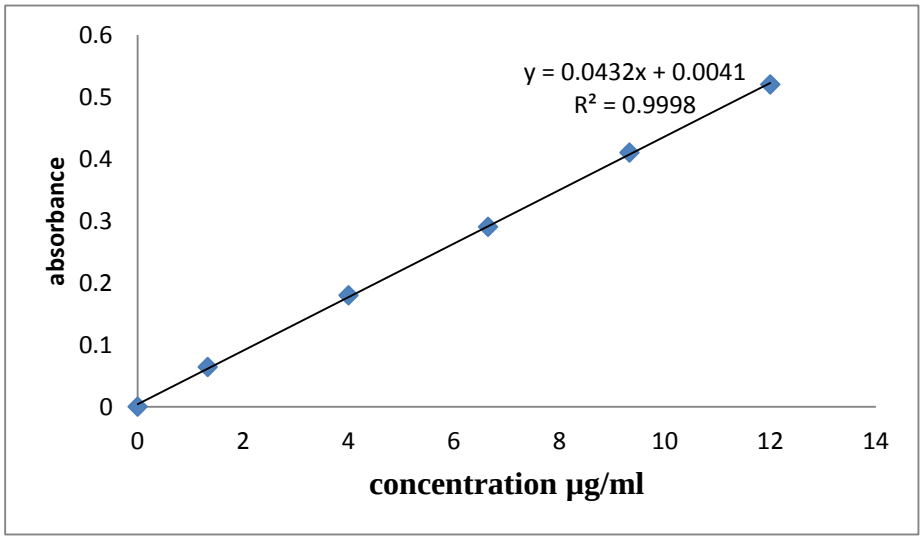

Figure 2: Standard plot for menthol

EVALUATION TESTS:

Physical Examination:

The Prepared ointment formulations were inspected visually for their colour, homogeneity, consistency.

Homogeneity:

All the developed ointments were tested for homogeneity by visual inspection. They were tested for their appearance with no lumps.

Determination of pH:

50mg of Ointment sample was taken in 100 ml dry beaker, 50 ml water was added to it. Beaker was heated on water bath maintained at about 60ºC to 70ºC for 10 minutes, cooled to room temperature. The pH of water extract was measured by using pH meter. The pH measurements were done by using a digital type pH meter by dipping the glass electrode into the ointment formulation.

Spreadability:

The spreadability is expressed in terms of time in seconds taken by two slides to slip off from ointment, placed in between two slides under the direction of certain load. Lesser the time taken for separation of two slides, better the spreadability of ointment. The spreadability was calculated by using the following formula S=M.L/T

Where, M = weight tied to upper slide L = length of glass slides T = time taken to separate the slides.

In-Vitro Release Studies of Prepared ointment

In-vitro drug release was performed using franz diffusion cell. Dialysis membrane was previously soaked in the respective dissolution medium overnight and used as the permeation membrane. Phosphate buffer pH 6.4 was placed in receptor compartment.

An accurately weighed quantity (250mg) of the formulated ointment was uniformly spread on the dialysis membrane was placed in donor compartment. One side of the cellophane membrane was kept in contact with the medium i.e., Phosphate Buffer pH 6.4. The medium was constantly agitated using a magnetic stirrer and the temperature was maintained at a 37 ± 1 °C throughout the operation. Samples of 1 ml volume were then withdrawn from the receptor compartment at intervals of 5 minutes over a period of 1 hour and the amount withdrawn was replaced with fresh volume of the medium. The samples withdrawn were then analysed for the amount of Drug released by UV spectrophotometric method by measuring the absorbance of the samples at particular wavelength against Phosphate Buffer pH 6.4 taken as blank.

Test for rate of penetration:

Using flow-through diffusion cell or microdialys method; the rate of penetration of the preparation can be estimated. Animal or human skin of definite area should be collected and tied to the holder present in a diffusion cell. The diffusion cell is placed in a fluid bath.

Measured quantity of the preparation is applied over the skin and the amount of drug passed into the fluid is measured at regular intervals by analyzing the aliquots of fluid using a spectrophotometer.

***IN VIVO* studies:**

Evaluation of analgesic activity:

In the present investigation, analgesic activity of camphor-menthol, were studied by using Eddy's hot plate method and tail flick method using diclofenac sodium as standard drug.

Hot plate induced analgesia:

The animals were placed individually in hot plate regulated at a temperature (55±2 °C) before the treatment and its reaction time was determined. After noting the initial reaction time, the treatment was given to each mouse.

Then the mice were placed on the Eddy's hot plate under regulated temperature and the licking of the forepaws was recorded as the hot plate latency with the help of a stop-watch. The mice were divided into four groups with (n=6) and treated with the respective solutions as given below

Group-I: served as control (hydrophobic) .

Group-II: served as control (hydrophilic)

Group-III: served as standard & received diclofenac .

Group-IV: served as camphor-menthol hydrophobic.

Group-V: served as camphor-menthol hydrophilic.

Tail flick method using immersion of tail:

Mice of 100mg weight were used. Rat is placed into cage in such a way that their tail hangs freely. Distal 5cm of mice is marked and immersed into a cup of warm water (55^{o}c) for 15 seconds. The reaction time is determined periodically after administration of test drug.

Stability studies:

The stability studies were carried out in all formulations at temperature 45°C for 3 months. All the evaluation parameters i.e. pH, viscosity, spreadability, consistency and phase separation were studied at different time intervals i.e., 15, 30, 60 and 90 days.

RESULTS AND DISCUSSION

Physical Appearance:

Table 5: Hydrophilic formulations T**able 6: hydrophobic formulations**

S. No	Formulation Code	Colour	Homogeneity	Consistency	Formulation Code	Colour	Homogeneity	Consistency
1	J1	White	Good	++	F1	White	Good	++
2	J2	White	Good	+++	F2	White	Good	+++
3	J3	White	Good	++++	F3	White	Good	++ ++

Ointment formulations were white viscous preparation with a smooth homogeneous texture.

All the prepared formulations were observed for physical appearance and they were graded based on their appearance. Formulations F3 and J3 have shown good appearance when compared to other prepared formulations.

Determination of pH:

Table 7: pH of hydrophobic formulations

S. No.	Formulation Code	pH
1	J1	7.15±0.4
2	J2	6.7±0.9
3	J3	6.35±0.20

The pH of the ointment solution was measured with the help of pH meter. 0.5g of ointment was dissolved in 50ml of distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate.

Table 8: pH of hydrophilic formulations

S. No.	Formulation Code	pH
1	F1	6.46±0.3
2	F2	6.25±0.1
3	F3	7.19±0.6

All the preparations were checked for pH and observed that all formulation are within the prescribed range of pH for topical dosage forms.

Spreadability:

Pharmaceutical semisolid preparations include ointments, cream, emulsion, gel, and rigid foams. Spreadability of the formulations was determined by measuring the spreading diameter of 1gm of sample between two horizontal glass plates after one minute. The standard weight applied to the upper plate was 25gm. Each formulation was tested.

Table 9: Spreadability of hydrophilic formulations

S. No.	Formulation Code	Spreadability
1	F1	12gm.cm/sec
2	F2	11gm.cm/sec
3	F3	12.5gm.cm/sec

Table 10: Spreadability of hydrophobic formulation

S. No.	Formulation Code	Spreadability
1	J1	20gm.cm/sec
2	J2	16.25gm.cm/sec
3	J3	14gm.cm/sec

All the formulations were checked for their spreadability property. Among all F3 and J1 has shown better spreadability. The spreadability ranged from 11-20gm.cm/sec. Among all F3 and J1 has shown better spreadability.

Table 11: % Drug content of hydrophilic formulation

Formulation code	Camphor	Menthol	% drug content
F1	9.5	7.75	34.5%
F2	33	29.5	62.5%
F3	89	80	84.5%

Table 12: % drug content of hydrophobic formulation

Formulation code	Camphor	Menthol	% drug content
J1	7.25	6	26.5%
J2	20.75	19.5	40.25%
J3	78	67	72.5%

Drug content:

Percentage drug content estimation of various prepared formulations was done by UV spectrophotometer. The absorbance was measured and percentage drug content was calculated. Percentage -drug content of various formulations was found to be in the range of 26.5% to 84.5%.

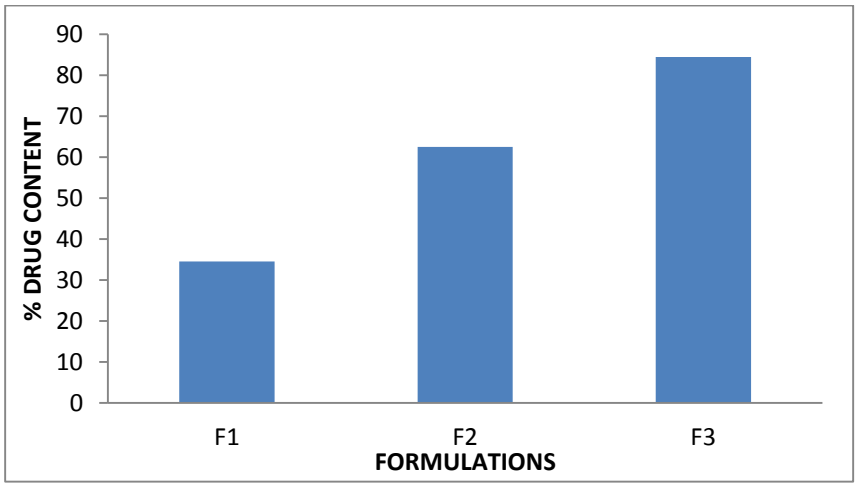

Figure 3: % Drug content of hydrophilic formulation

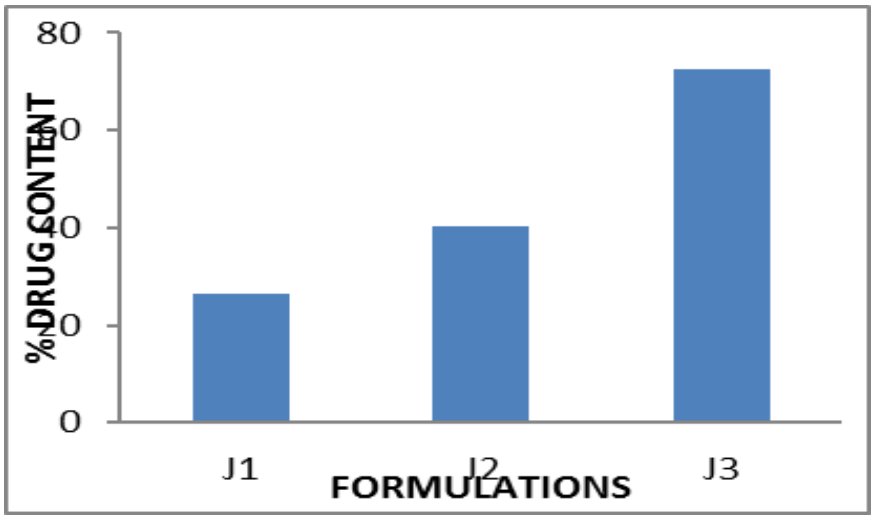

Figure 4: % Drug content of hydrophobic formulation

INVITRO DRUG RELEASE OF CAMPHOR FROM HYDROPHILLIC FORMULATIONS:

Table 13: *In vitro* drug release of camphor from prepared formulation

Time	F1	F2	F3
0	0	0	0
5	7	8.2	10.7
10	10	11	15
15	13	16	22
30	16	20	31
45	19	27	39
60	21.3	32	51

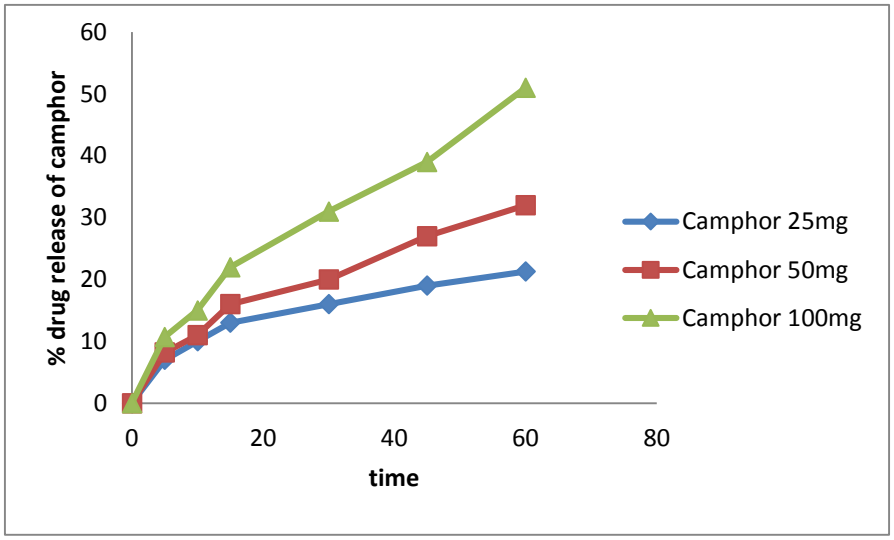

Figure 5*: In vitro* drug release of camphor from prepared formulation

In vitro drug release of camphor was obtained from the prepared hydrophilic formulations. In that, F3 showing good drug release than other formulations.

INVITRO DRUG RELEASE OF MENTHOL FROM HYDROPHILLIC FORMULATIONS:

Table 14: *In vitro* drug release of menthol from prepared formulation

Time	**F1**	**F2**	**F3**
0	0	0	0
5	6.7	7.1	7.8
10	8	10	9.3
15	9.8	13.1	13
30	10	17.4	19
45	12	22.3	25
60	13.8	24	31

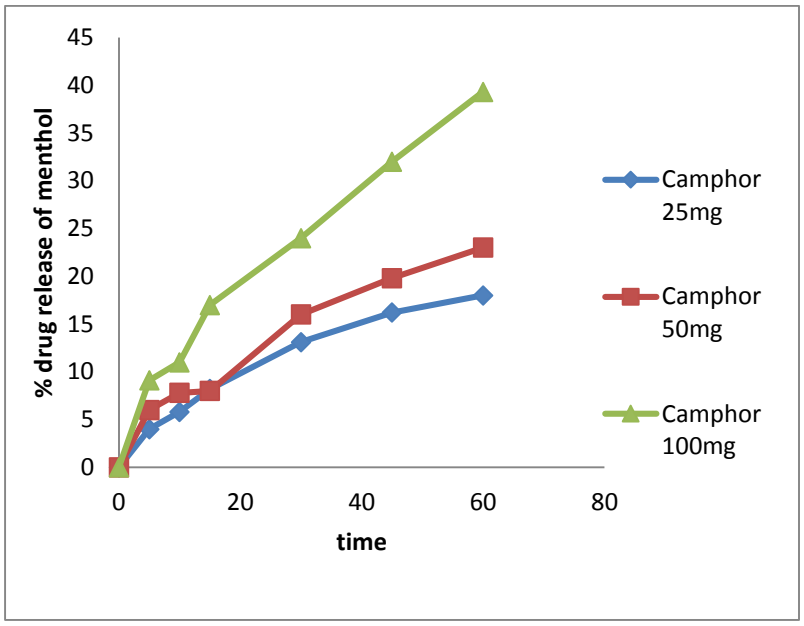

Figure 6*: In vitro* drug release of menthol from prepared formulation

In vitro drug release of menthol was obtained from the prepared hydrophilic formulations. In that, F3 showing good drug release than other formulations.

***INVITRO* DRUG RELEASE OF CAMPHOR FROM HYDROPHOBIC FORMULATIONS:**

Table 15: *In vitro* drug release of camphor from prepared formulation

Time	J1	J2	J3
0	0	0	0
5	4	6	9.1
10	5.8	7.8	11
15	8.2	8	17
30	13.1	16	24
45	16.2	19.8	32
60	18	23	39.3

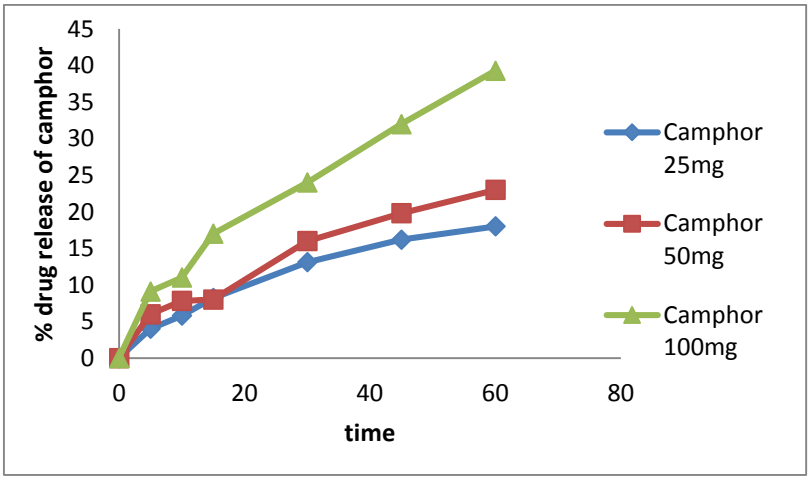

Figure 7*: In vitro* drug release of camphor from prepared formulation

In vitro drug release of camphor was obtained from the prepared hydrophobic formulations. In that, J3 showing good drug release than other formulations.

INVITRO DRUG RELEASE OF MENTHOL FROM HYDROPHOBIC FORMULATIONS:

Table 16: *In vitro* drug release of menthol from prepared formulation

Time	J1	J2	J3
0	0	0	0
5	3.5	4	7.4
10	5	6.2	9.3
15	6.1	8.3	12
30	8.2	9.6	17.3
45	8.9	11.7	21
60	9.9	13	28

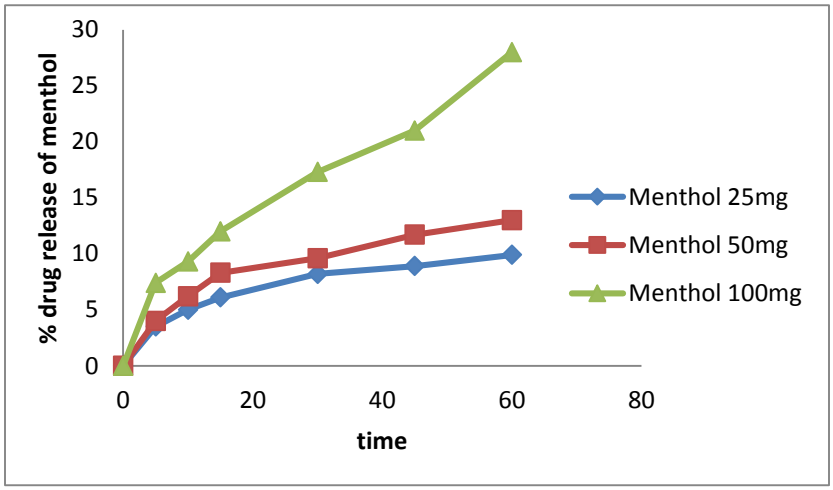

Figure 8: *In vitro* drug release of menthol from prepared formulation

In vitro drug release of menthol was obtained from the prepared hydrophobic formulations. In that, J3 showing good drug release than other formulations.

EX VIVO DRUG RELEASE OF CAMPHOR FOR HYDROPHILLIC FORMULATIONS:

Table 17: *Ex vivo* drug release of camphor from prepared formulation

Time	F1	F2	F3
0	0	0	0
5	13	11	14
10	17	13	21
15	24	21	29
30	29	34	35
45	32	40	47
60	36	57	68

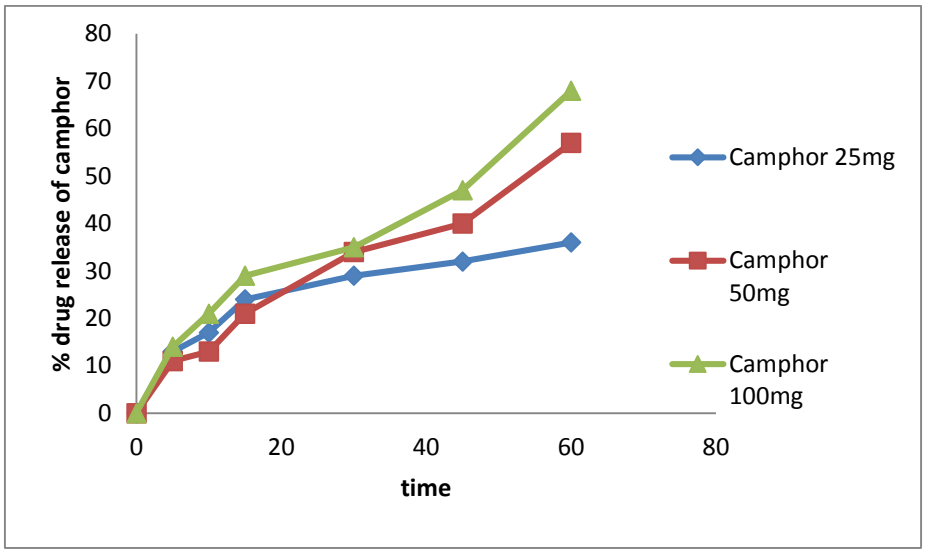

Figure 9: *Ex vivo* drug release of camphor from prepared formulation

Ex vivo drug release of camphor was obtained from the prepared hydrophilic formulations. In that, F3 showing good drug release than other formulations.

EX VIVO DRUG RELEASE OF MENTHOL FOR HYDROPHILLIC FORMULATIONS:

Table 18: *Ex vivo* drug release of menthol from prepared formulation

Time	F1	F2	F3
0	0	0	0
5	9.5	9	11
10	12.9	11	19
15	17.3	24	27
30	18	29	31
45	20	38	39
60	25	42	52

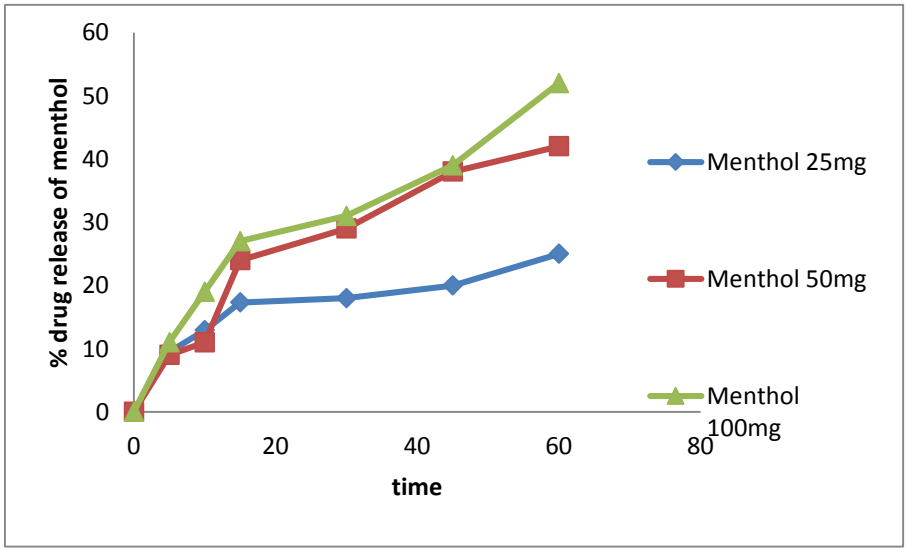

Figure 10: *Ex vivo* drug release of menthol from prepared formulation

Ex vivo drug release of menthol was obtained from the prepared hydrophilic formulations. In that, F3 showing good drug release than other formulations.

EX VIVO DRUG RELEASE OF CAMPHOR FROM HYDROPHOBIC FORMULATION:

Table 19: *Ex vivo* drug release of camphor from prepared formulation

Time	J1	J2	J3
0	0	0	0
5	17.1	19	26
10	24	25.3	33
15	37	31.4	47
30	44	45.7	54
45	47	67.2	69
60	52.2	70.1	82

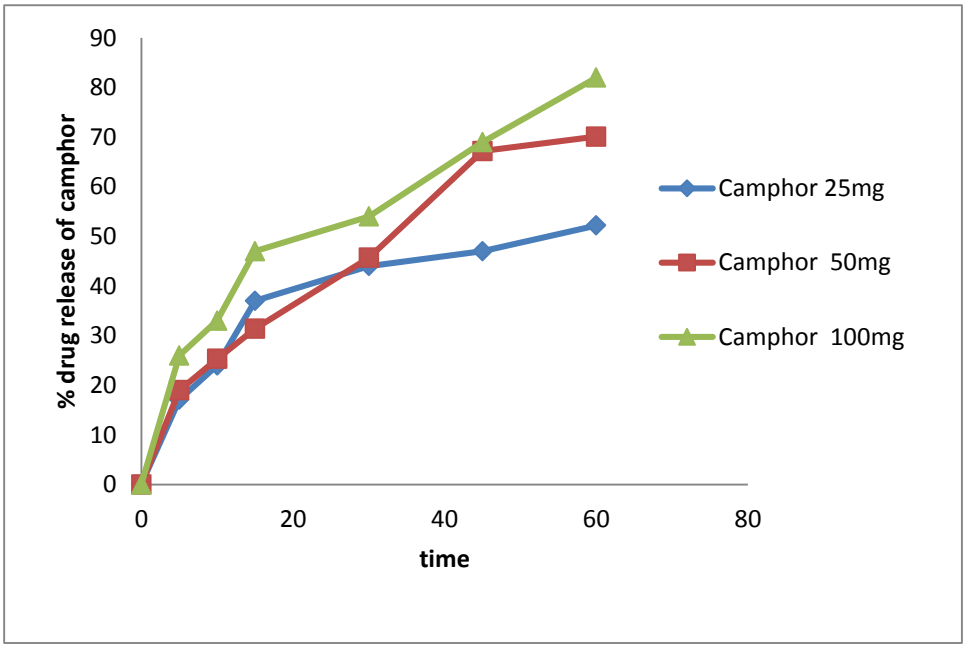

Figure 11: *Ex vivo* drug release of camphor from prepared formulation

Ex vivo drug release of camphor was obtained from the prepared hydrophobic formulations. In that, J3 showing good drug release than other formulations.

EX VIVO DRUG RELEASE OF MENTHOL FROM HYDROPHOBIC FORMULATION:

Table 20: *Ex vivo* drug release of menthol from prepared formulation

Time	J1	J2	J3
0	0	0	0
5	14.2	16.4	18
10	26	23.1	21.4
15	29	31.8	23.4
30	34	36.7	29.1
45	36.2	41.4	37.9
60	43.1	54.3	59.3

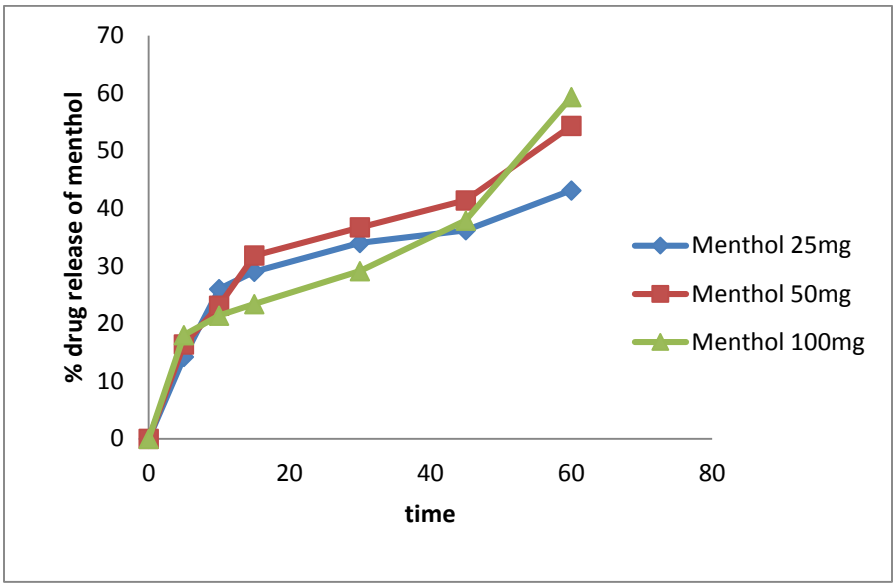

Figure 12: ***Ex vivo*** **drug release of menthol from prepared formulation**

Ex vivo drug release of menthol was obtained from the prepared hydrophobic formulations. In that, J3 showing good drug release than other formulations.

Compared to hydrophilic and hydrophobic, the hydrophobic base was showing good penetration properties and good drug release than hydrophilic base.

IN-VIVO **STUDIES FOR PREAPARED FORMULATIONS:**

The reaction latency on Eddy's hotplate increased significantly with topical application of standard, hydrophobic and hydrophilic formulations. The tail flick latency also increased significantly with standard, hydrophobic and hydrophilic formulations. The percentage increase in reaction latency on eddy's hot plate for standard, GROUP III, GROUP IV, GROUP V is 92%, 71%, 47% respectively. The percentage increase in tail flick latency for GROUP III, GROUP IV, GROUP V is 95%, 60%, 38% respectively. The topical application of hydrophobic formulation GROUP IV had shown analgesic activity comparable with that of marketed preparation in both eddy's hot plate method and tail immersion method.

Table 17: ***In vivo*** **studies for prepared formulations**

S.No	Groups	Reaction latency in on Eddy's hot plate(Seconds)	Tail flick latency in tail immersion test(Seconds)
1	Group I	$8.33_{\pm}$ 0.74	$7.78_{\pm}$ 0.82
2	Group II	$7.78_{\pm}$ 0.76	$5.78_{\pm}$ 0.69
3	Group III	$15_{\pm}$ 0.00$^{\#\#}$	$11.3_{\pm}$ 0.46$^{\#\#}$
4	Group IV	$14.3_{\pm}$ 0.51**	$12.5_{\pm}$ 0.34**
5	Group V	$11.5_{\pm}$ 0.38$^{\#}$	$8_{\pm}$ 0.11$^{\#}$

Values expressed in Mean ± SEM (n=6) $^{**}p < 0.001$ $^{*}p < 0.01$ significantly differs from hydrophobic control group; $^{\#\#}p < 0.001$ $^{\#}p < 0.01$ significantly differs from hydrophilic control group.

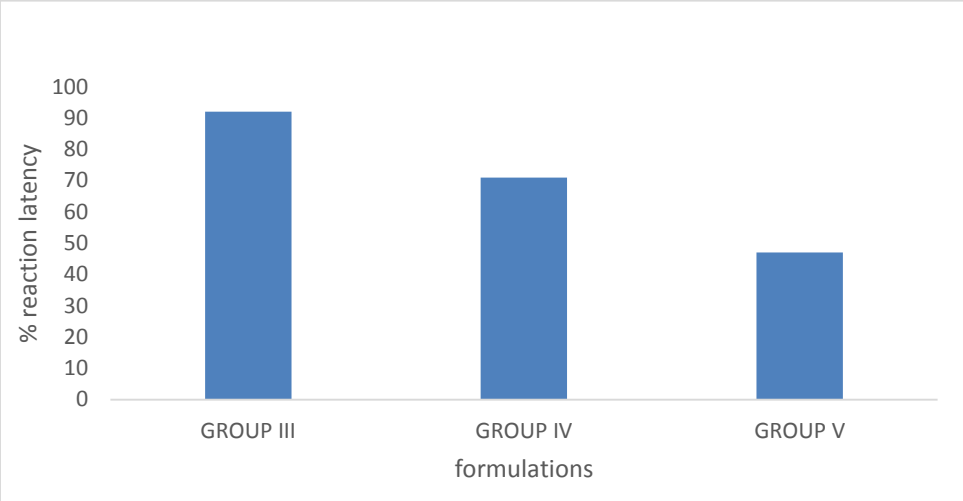

Figure 9: Reaction latency on eddy's hot plate

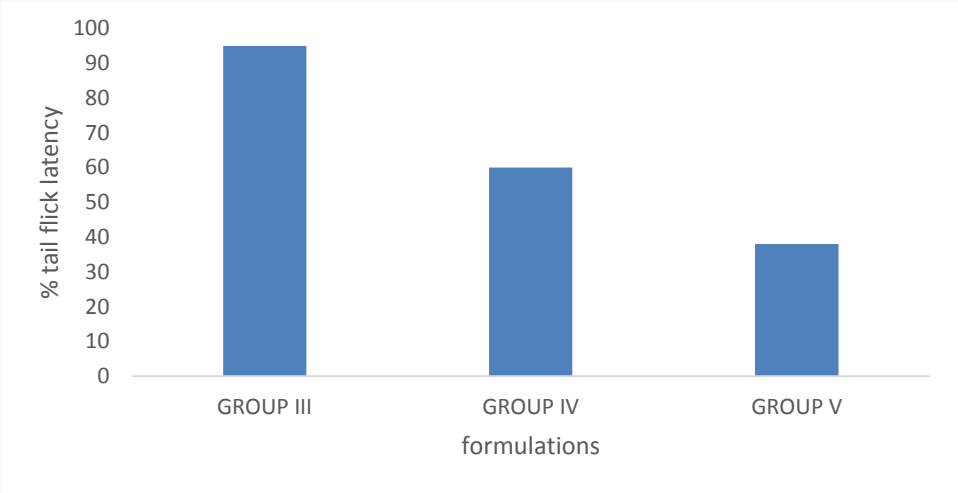

Figure 10: Reaction latency on tail flick method

GROUP 1:control hydrophobic GROUP 2:control hydrophilic

GROUP 3:standard GROUP 3:standard

GROUP 4: camphor-menthol hydrophobic GROUP 5: camphor-menthol hydrophilic

In-vivo studies were conducted for both hydrophilic and hydrophobic bases, In Comparison with hydrophilic formulations the hydrophobic formulations are showing good analgesic activity. Among all group 4 is showing better analgesic activity.

Stability studies:

The prepared formulations were packed in aluminum collapsible tubes (5 g) and subjected to stability studies at 45 ^{0}C /75% RH for a period of 3 months using stability chamber.

Among all formulations, J3 and F3 had shown better results in physical appearance, consistency, homogeneity, pH, spreadability, drug content, drug release studies. That's why, these two formulations were selected for stability studies.

Table 18: Physical appearance, homogeneity, consistency of camphor & menthol from F3 & J3

Physical Appearance												
Formulation Code	**Colour**				**Homogeneity**				**Consistency**			
	0 month	**1 month**	**2 month**	**3 month**	**0 month**	**1 month**	**2 month**	**3 month**	**0 month**	**1 month**	**2 month**	**3 month**
F3	White				Good				++ ++			
J3	White				Good				++++			

Table 19: pH of camphor & menthol from F3&J3

	pH			
Time	**0 months**	**After**		
		1 month	**2 months**	**3 months**
J3	6.35± 0.03	6.47	6.45	6.46
F3	6.53± 0.04	6.52	6.55	6.57

Table 20: spreadability of camphor & menthol from F3&J3

S. No.	**Formulation Code**	**Spreadability**			
		O month	**1 month**	**2 month**	**3 month**
1	F3	12.5gm.cm/sec	12.3 gm.cm/sec	12 gm.cm/sec	12 gm.cm/sec
2	J3	14gm.cm/sec	14 gm.cm/sec	14.1 gm.cm/sec	14.1gm.cm/sec

Table 21: combined %drug content of camphor & menthol from F3&J3

Formulation code	**% drug content**			
	0 month	**1 month**	**2 month**	**3 month**
F3	84.5%	84%	83%	83%
J3	72.5%	72%	70%	70%

Table 22: combined% drug release of camphor & menthol from F3&J3

% drug release				
		After 1month	After 2 months	After 3 months
Formulations	0 months	40^{o}c/ 75% RH	40^{o}c/ 75% RH	40^{o}c/ 75% RH
J3	81	79	78	78
F3	69	65	63	62

Parameters like pH and % drug release were evaluated for the samples kept for stability at each month and the results are tabulated. From the stability studies it is clear that the formulated preparations showed no significant changes in physical appearance, consistency, homogeneity, pH, spreadability, drug content, drug release which indicates that the preparations were stable.

CONCLUSION

Different formulations were prepared by using hydrophilic and hydrophobic bases by employing the dose of the natural agent at 25mg, 50mg and 100mg. All the prepared formulations were evaluated for pH, homogenicity, drug content, in-vitro studies, ex-vivo studies and in-vivo studies. Among all the preparations hydrophobic bases were showed the better effect when compared to hydrophilic base. Among all the prepared formulations of F3 and J3 (camphor-menthol100mgeach) is showing better results and comparable with marketed preparation.

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