Research Article

Development and evaluation of some novel polyherbal anti-inflammatory and analgesic formulations of some Indian spices

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ABSTRACT

Background: Inflammation is linked with a broad range of infectious and non-infectious diseases; hence, also considered on root of the all diseases. The crucial role of inflammatory processes makes possible evaluation research focusing on the treatment of inflammation. **Aim:** The present study was aimed to develop and evaluate some novel polyherbal anti-inflammatory and analgesic formulations of some Indian spices. **Materials and Methods:** Rhizome of Zingiber officinale. *C. longa*, bulb of A. sativum, and fruit of *C. annuum* were collected locally. Animals were procured from the institute and the protocol adhered to the IAEC guidelines. Herbal drugs were extracted using soxhelet apparatus. gel, cream, and ointment formulations were prepared using different concentrations of the herbal compounds. The formulations were then physically evaluated for pH, viscosity, spreadability, and stability. Acute toxicity studies were also performed in the animals. Anti-inflammatory activity was evaluated using paw edema method, cotton-pellet-induced granuloma method, and ultraviolet erythema induction. Tail flick, hot plate, and tail immersion methods were used for evaluation of analgesic activity. Diclofenac sodium was used as standard drug. Data were presented as mean ± standard deviation wherever applicable. For continuous variables, Student's t-test was used to differentiate mean difference. For comparison between more than 2 groups, one-way analysis of variance was used followed by post hoc analysis. $P < 0.05$ was considered significant. Statistical analysis was performed using SPSS trial version 21. **Results:** In acute toxicity studies, the animals could tolerate a dose >2000 mg/kg body weight with no mortality. Our study results showed that gel, cream, and ointment formulations in all polyherbal combinations significantly inhibited paw edema, cotton pellet-induced granuloma, and formation of erythrema. However, the activities were highest for gel formulation. These results supported that the polyherbal formulations significantly exhibit anti-inflammatory and analgesic activities. **Conclusion:** Gel formulations among prepared topical formulations showed the highest activity and also exhibited nonstaining, good spreadability, and patient compliance. Topical formulations containing spices can be used in chronic inflammatory and pain conditions and devoid of side effects.

Keywords: Analgesic activity, anti-inflammatory activity, polyherbal formulation

Introduction

Inflammation can be classified as acute or chronic inflammation this has been called "King of Human Miseries."[1]

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Most of the so-called nonsteroidal anti-inflammatory agents have also analgesic activity. Lim and Guzman (1968) differentiated between antipyretic analgesics causing analgesia by blocking impulse generation at pain receptors in the periphery while the narcotic analgesics block synaptic transmission of impulses signaling pain in the central nervous system.[2] An old but excellent survey on methods being used to test compounds for analgesic activity has been provided by Collier (1964).[3]

A systematic study of anti-inflammatory effects and use of formaldehyde-induced arthritis and croton oil-induced granuloma

pouch in rats as the experimental models of inflammation such as carrageenan induced paw edema in rats, cotton-pellet-induced granuloma in rats, and Freund's complete adjuvant induced arthritis.[4,5] Ginger has staring potential for treating a number of ailments. Generation of free radicals or reactive oxygen species during metabolism beyond the antioxidant capacity of a biological system results in oxidative stress,[6] which plays an essential role in heart diseases, neurodegenerative diseases, cancer, and in the aging process.[7]The bioactive molecules of ginger like gingerols have shown antioxidant activity in various modules.[8]

Curcuma longa Linn. commonly known as turmeric, is a perennial plant belonging to the family Zingiberaceae. It is a common ingredient in many health supplements in Asia, being used in various therapeutic applications such as blood purifying, wound healing, and inflammatory disorders and holds a prominent position in traditional Indian medicinal system.[9]

Curcuminoids are potent anti-inflammatory agents working through multiple mechanisms, viz., suppression of the activation of nuclear factor-kappa B and inhibition of cyclooxygenase (COX-2). The inhibition of COX-2 is a major mechanism behind the anti-inflammatory activity.[10,11]

Garlic (*Allium sativum* L.) is a pungent spice with a long history of use throughout the world for both its culinary and therapeutic properties.[12]

The garlic and its various forms reduce cardiovascular risk, including abnormal plasma lipids, oxidized low-density lipoproteins, abnormal platelet aggregation, and a high blood pressure.[13]

Rhizome of *Zingiber officinale* (*Zingiberaceae*) and *C. longa* (*Zingiberaceae*), bulb of *A. sativum* (*Amaryllidaceae*), and fruit of *Capsicum annuum* (*Solanaceae*) were selected for the study. Polyherbal formulations of various concentrations were prepared using extracts of herbal drugs and anti-inflammatory as well as analgesic activity was evaluated.

Materials and Methods

Plants material

The rhizome of *Z. officinale* (zingiberaceae) and *C. longa* (*Zingiberaceae*), bulb of *A. sativum* (*Amaryllidaceae*), and fruit of *C. annuum* (*Solanaceae*) were collected from the local region of Buldhana district.

Drugs and chemicals

All drugs and chemicals were of analytical grade. Diclofenac sodium was used as a standard drug in this study as a topical formulation.

Animal

Animals were procured from Anuradha College of Pharmacy, Chikhli, Buldhana, and used in this study. The experimental design was approved by Institutional Animal Ethical Committee, and the study was performed according to the committee for the purpose of Control and Supervision of Experiments on Animal guidelines for the use and care of animals.

Extraction of herbal drugs

Extraction of herbal drugs by the successive solvent system was performed using following methods.

Extraction method

Successive solvent extraction was done using Soxhlet apparatus.

Fresh parts were cleaned, washed with deionized water, sliced and dried in the sun for 1 week and again dried at 50°C in a hot air oven for 6 h. Dried parts were cut in small pieces, powdered by electronic mill fixed gram of sample were taken into a thimble and placed in a Soxhlet apparatus, were set up with various solvent from non-polar to polar. 250 ml of solvent was added and extracted according to their boiling point for 7 h. The solvents used were chloroform (BP = 61°C). Ethyl acetate (BP = 77°C), methanol (BP = 65°C), and acetone (BP = 56.53°C). After completion of extraction, the extract was then cooled, concentrated using rotary evaporator get a crude dried extract. Each raw sample was extracted by the same method and yield was calculated.[14] Following up and down method it was found that all extract does not produce any toxic effect at a maximum dose of 2000 mg/kg. For formulation purpose, the methanolic extracts are used.

Preparation of different formulations

Preparation of gel formulation[15]

The gel was prepared with four formulations using methanolic extracts of different plant materials in different concentrations shown in Table 1. Formulations A to D were prepared, first 1 g of carbopol 934 was dispersed in 50 ml of distilled water kept the beaker aside to swell the carbopol 934 for ½ h and then stirred to mix the carbopol 934 to form gel. 5 ml of distilled water was taken and required quantity of methylparaben and propylparaben were dissolved by heating on water bath. Solution was cooled, and propylene glycol 400 was added. Further required quantity of methanolic extracts of different plant materials were mixed to the above mixture and volume made up to 100 ml by adding remaining distilled water. Finally, all ingredients were mixed properly to the carbopol 934 gel with continuous stirring and triethanolamine was added dropwise to the formulation for adjustment of required skin pH (5.8-6.2) and to obtain the gel at required consistency.

Preparation of cream formulation (Formulation E, F, G, and H)[16]

Ingredients were weighed as per the details are given in Table 2. The oily phase (Part A) that consisted of emulsifier (steric acid) and other oil soluble component was heated to 75°C over a water bath.

Water soluble component was added to water (Part B) and heated to the same temperature followed by addition of ginger extract, turmeric extract, *Allium* extract, *Capsicum* extract in a % proportions as given in Table 2 and with constant stirring added methylparaben

Table 1: Composition of gel formulation

Components	Composition (% w/w)			
	Formulation I (A)	Formulation II (B)	Formulation III (C)	Formulation IV (D)
Ginger extract (%)	5	10	15	20
Turmeric extract (%)	0.05	0.1	0.2	0.4
Garlic extract (%)	1	2	5	10
Capsicum extract (%)	0.1	0.5	0.75	1
Carbopol 934	1 g			
Methylparaben (0.5%)	0.2 ml			
Propylparaben (0.2%)	0.1 m			
Propylene glycol 400 (5%)	5 ml			
Triethanolamine (q.s)	1.2 ml			
Distilled water	Up to 100 ml			

Table 2: Formulation ingredients of polyherbal cream

Part A (oily phase)		Part B (aqueous phase)	
Ingredients	% w/w	Ingredient	% w/w
Light liquid paraffin	27.85	Triethanolamine	1.71
Steric acid	8.57	Glycerin	10.71
Glycerol monostearate	7.50	Methylparaben	0.21
Cetostearyl alcohol	4.28	Propylparaben	0.21
Microcrystalline wax	0.21	Ginger extract, turmeric extract, garlic extract, capsicum extract	Quantities in proportion as mentioned in Table 3
Hard paraffin wax	1.00	Distilled water	q.s. 100%

Table 3: Concentration of different extracts in formulations cream

Extract	E (%)	F (%)	G (%)	H (%)
Ginger extract	5	10	15	20
Turmeric extract	0.05	0.1	0.15	0.2
Garlic extract	1	2	5	10
Capsicum extract	0.1	0.5	0.75	1.0

Table 4: Concentration of different extracts in formulations of ointment

Extract	I (%)	J (%)	K (%)	L (%)
Ginger extract	5	10	15	20
Turmeric extract	0.05	0.1	0.15	0.2
Garlic extract	1	2	5	10
Capsicum extract	0.1	0.5	0.75	1.0

and propylparaben. To the heated aqueous, phase aqueous mixture oily phase was incorporated on magnetic stirrer with constant stirring.

Preparation of ointment formulation[17] (Formulation I, J, K, and L)

Ointment containing polyherbal extract was prepared by fusion method. Specified concentration of poly(ethylene glycol) (PEG) 4000 was melted in porcelain dish on boiling water bath. PEG 600 was heated and added to melt PEG 4000. Mixture was removed from heat and stirred. Then, extracts were dissolved in 20 % propylene glycol and then added to PEG mixture and stirred until congealing. Excipients were taken according to the weight of various concentrations of polyherbal extracts in Tables 3 and 4.

Physical evaluation of formulations

pH

The pH was measured using a pH meter, which was calibrated before each use with standard buffer solutions at pH 4, 7, and 9. The electrode was inserted into the sample 10 min before taking the reading at room temperature.

Viscosity

The viscosity of the formulations was checked using a Brookfield Viscometer (DV-I PRIME, USA). The gels were rotated at 10, 20, 50, and 100 rotations/min. The viscosity of the gel was obtained by multiplying the corresponding dial reading with the factor given in the Brookfield Viscometer catalog.

Spreadability

Spreadability is expressed in terms of time in seconds taken by two slides to slip off from the gel when placed in between the slides under the direction of a certain load. The excess amount of sample was placed between the two glass slides, and a definite amount of weight was placed on these glass slides to compress the glass slides of uniform thickness. A weight of 70 g was added and the time required to separate the two slides was noted. Spreadability was calculated using the formula:

$S = ML/T.$

Where, M = Weight tied to upper slide, L = Length of glass slides, T = Time taken to separate the slides.

Stability

The stability studies were conducted for all the formulations. The formulations were kept at two different temperatures 4 ± 2°C and 30 ± 2°C, 65 RH, for 3 months. The pH and the viscosity of the formulations, which were determined after 3 months, were compared with the initial pH and viscosity.

Acute toxicity studies

Acute toxicity study was performed in accordance with Organization for Economic Cooperation and Development (OECD) guidelines 425.[3] Acute toxicity refers to the effects on the whole body of a single dose of a chemical or several doses within a 24-h period. Acute toxicity data are used mainly to: (i) Identify lethal/toxic doses of chemicals for humans (primarily for the regulatory purposes of classification and labeling), (ii) indicate the mode of toxicity in humans, including the susceptibility of key target organs, and (iii) provide a rough guide for dose selection in repeat dose tests in animals. ATS were carried out according to guidelines by the OECD 2001. In this study, we have performed acute toxicity studies and calculated lethal dose 50%. No adverse effect or mortality was detected in animal's polyherbal formulations.

Pharmacological evaluation

Evaluation of anti-inflammatory activity

Paw edema method[18]

The rats were challenged by subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the sub plantar side of the left hind paw. The paw was marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume was measured plethysmographically immediately after carrageenan injection at 30 min, 01 h, 02 h, and 03 h. All the groups of animals received treatment as given in Table 5:

Cotton-pellet-induced granuloma in rats[19]

All the animals were anesthetized with ether. The axillary skin was shaved and disinfected with 70% ethanol. An incision was made and by a blunt forcep subcutaneous tunnels were formed, and a sterilized cotton pellet (20 ± 1 mg) was placed in both axillas. The controls, test drug, standard drug, were applied 0.5 g each for 7 consecutive days starting from day of cotton implantation. At the 8th day, rats were anesthetized again, and the cotton pellet (along with granular tissue formed around) were removed surgically and freed from extraneous tissue. The pellets were weighed immediately for wet weight. Then, pellets were dried in an incubator at 60°C until a constant weight was obtained.

Ultraviolet (UV) erythema in guinea pigs method

18 h prior testing, the animals were shaved on both flanks and the back, and then they were chemically depilated by a suspension of barium sulfide. 20 min later, the depilation paste and fur were rinsed off in running warm water. On the next day, the test compounds were applied topically 30 min before UV exposure. Control animals were treated with the base alone. Five animals were used for each treatment and control group. Treated groups received single application 0.5 g of respective formulations. The guinea pig was placed in a leather cuff with a hole of 1.5 cm × 2.5 cm size punched in it, allowing UV radiation to reach only this area. An original Hanau UV burner Q600 was warmed up for about 30 min before use and placed at a constant distance (20 cm) above the animal. The investigator had to protect him/her by gloves and UV glasses. The erythema was scored at 30 min, 1 h, 2 h, and 3 h after exposure. A group of animals was also given standard drug (diclofenac sodium gel).

Evaluation of analgesic activity

Tail flick method

In which the recording of basal reaction time to radial heat by placing the tip (last 1-2 cm) of the tail on radial heat source. The tail withdrawal from the heat (flicking response) was recorded as the end point. Normally a mouse withdraws its tail in 3-5 s. A cut of period 15 s was observed to prevent damage to the tail. Any animal failing to withdraw its tail in 3-5 s was rejected from the study. Nearly about 3-5 basal reaction time for each mouse was taken at a gap of 5 min to confirm normal behavior of animal. The test and standard were applied to divided group and response time was noted at 30 min, 1 h, 2 h, and 3 h after the drug application. As the reaction time reached 15 s, it was considered maximum analgesia and the tail was removed from the source of the heat to avoid tissue damage. A group of animals was also applied standard drug formulation (diclofenac). Cut off time of 15 s.

Table 5: For gel formulation

Groups gel formulation	Treatment	Groups cream formulation	Treatment ointment formulation	Groups	Treatment	Quantity applied topically all groups (I-XVIII)
I	Control base	VII	Control base	XIII	Control base	0.5 g
II	Formulation A	VIII	Formulation E	XIV	Formulation I	0.5 g
III	Formulation B	IX	Formulation F	XV	Formulation J	0.5 g
IV	Formulation C	X	Formulation G	XVI	Formulation K	0.5 g
V	Formulation D	XI	Formulation H	XVII	Formulation L	0.5 g
VI	Diclofenac gel	XII	Diclofenac cream	XVIII	Diclofenac ointment	0.5 g

Hot plate method

The temperature of hot plate was controlled at 55-56°C. This was a copper plate or a heated glass surface. The animal was placed on the hot plate and time until either licking or jumping occurs was recorded in stopwatch. The latency was recorded before and after 30, 60 min 2 h and 3 h following topical applications of standard and test compound. A group of animals was also given a standard drug (diclofenac sodium formulation).

Tail immersion method

Procedure

The lower 5 cm portion of the tail was marked. This part of tail was immersed in cup of freshly filled water of 55°C within a few seconds the rat reacts by withdrawing the tail. The reaction time was recorded in sec unit by a stopwatch. After each determination, the tail was carefully dried. Control group received an application of 0.5 g of the base. The test group received application of 0.5 g of gel (A-D), cream (E-H), and ointment (I-L) formulations. A group of animals was also given standard drug (diclofenac sodium formulation).

Evaluation

The reaction time was determined before and periodically after topical application of the formulations and standard drug (diclofenac sod. formulation) after 30 and 60 min, 2 h and 3 h. The cut off time for immersion was 15 s.

Statistical analysis

Data were presented as mean ± standard deviation wherever applicable. For continuous variables, Student's *t*-test was used to differentiate mean difference. For comparison between more than 2 groups, one-way analysis of variance was used followed by *post hoc* analysis. $P < 0.05$ was considered significant. Statistical analysis was performed using SPSS version 21.

Results

Physical evaluation of prepared topical formulations

Viscosity

It was found that the viscosity decreases shearing and the lower viscosity favor easy spreadability (Tables 6 and 7).

Spreadability

The lower value of spreadability indicates the lesser work required to spread the formulation over the skin, which means formulation was easily spreadable by applying a small amount of shear (Table 8).

Stability

There was no change in the color of formulations at the end of observation periods suggesting physical stability and apparently no chemical reaction between the ingredients even after storage at different storage conditions.

Table 6: Composition of prepared ointment formulation

Name of ingredient	I (%)	J (%)	K (%)	L (%)
PEG 4000	20	20	20	20
PEG 600	10	10	10	10
Methanol	2	2	2	2
Propylene glycol	20	20	20	20
Methylparaben	0.2	0.2	0.2	0.2
Propylparaben	0.2	0.2	0.2	0.2
Ginger extract	5	10	15	20
Turmeric extract	0.05	0.1	0.15	0.2
Garlic extract	1	2	5	10
Capsicum extract	0.1	0.5	0.75	1.0
Water	q.s. to 100%	q.s. to 100%	q.s. to 100%	q.s. to 100%

PEG: Polyethylene glycol

Table 7: Viscosity (cps) of prepared formulations

	Viscosity (cps)			
RPM	100	50	20	10
Gel formulation q				
A	11,000	19,600	41,000	64,000
B	10,000	19,500	41,100	64,200
C	10,000	19,400	42,000	65,000
D	12,000	18,400	42,300	60,000
Cream formulation				
E	13,000	21,000	40,000	60,000
F	12,000	20,000	39,000	58,000
G	11,500	19,000	38,000	61,000
H	13,000	21,500	40,000	60,500
Ointment formulation				
I	14,000	19,800	43,000	64,000
J	13,500	19,000	43,500	64,500
K	13,800	19,500	43,700	64,200
L	13,000	19,800	42,800	64,600

pH

In this study, the pH of freshly prepared formulation of gel, cream, and ointment was determined as given in Table 9 and which is within the range of skin pH (Table 9).

Acute toxicity study

The acute toxicity studies of the different plant extracts were studied, and all the animals tolerated the maximum test doses of the extract, as there were no clinical signs of toxicity or mortality of the animals at a dose from 5 to 2000 mg/kg body weight. The animals could tolerate a dose >2000 mg/kg body weight.

Table 8: Spreadability of prepared formulations

Gel formulation	Spreadability (g.cm/s)	Cream formulation	Spreadability (g.cm/s)	Ointment formulation	Spreadability (g.cm/s)
A	18.89±1.11	E	17.09±0.12	I	18.0±0.1
B	19.12±1.01	F	18.01±0.32	J	17.9±0.12
C	20.11±0.1	G	16.98±0.12	K	16.90±0.38
D	18.01±0.21	H	20.10±0.1	L	19.01±0.25

Table 9:The pH of each prepared formulation

Gel formulation	pH	Cream formulation	pH	Ointment formulation	pH
A	5.89	E	5.92	I	5.93
B	5.90	F	5.85	J	5.85
C	5.85	G	5.80	K	5.88
D	5.70	H	5.84	L	5.90

Evaluation of anti-inflammatory activity

Effect of gel formulations on paw edema volume

Group I received single application of 0.5 g of gel base. Group II-V received single application of 0.5 g of gel Formulation A to D, respectively. Group VI was used for the study of diclofenac sodium gel (Table 10). Mean paw edema (±SD) in control group animals (Group I) was found at 30 min, 1 h, 2 h, and 3 h (Figure 13).

Group II animals showed percent edema inhibition was found to be 8, 29, 45, and 45 at 30 min, 1 h, 2 h, and 3 h, respectively, Formulation B (Group III) at 13, 33, 47, and 49 at 30 min, 1 h, 2 h, and 3 h, respectively, Formulation C (Group IV) at 22, 41, 49 and 50 at 30 min, 1 h, 2 h, and 3 h, respectively, Formulation D (Group V) at 22, 75, 80, and 81 at 30 min, 1 h, 2 h, and 3 h, respectively, and diclofenac gel (Group VI) at 92, 100, 100, and 100 at 30 min, 1 h, 2 h, and 3 h, respectively.

Effect of cream formulations on paw edema volume

Group VII received single application of 0.5 g of cream base. Group VIII and XI received single application of 0.5 g of cream Formulation E, F, G, and H, respectively. Group XII was used for the study of diclofenac sodium cream (Table 11) (Figure 14).

Mean paw edema (±SD) in control group animals (Group VII) was at 30 min, 1 h, 2 h, and 3 h, respectively. Group VIII animals showed percent edema inhibition was found to be 8, 31, 35, and 44 at 30 min, 1 h, 2 h, and 3 h, respectively, Formulation F (Group IX) at 12, 34, 42, and 47 at 30 min, 1 h, 2 h, and 3 h, respectively, Group X at 26, 45, 49, and 53 at 30 min, 1 h, 2 h, and 3 h, respectively, Group XI at 69, 77, 80, and 83 at 30 min, 1 h, 2 h and 3 h, respectively, and diclofenac cream (Group XII) at 98, 100, 100, and 100 at 30 min, 1 h, 2 h, and 3 h, respectively.

Effect of ointment formulations on paw edema volume

Group XIII received single application of 0.5 g of ointment base. Group XIV to XVII received single application of 0.5 g of ointment Formulation I, J, K, and L, respectively. Group XVIII was used for the study of diclofenac sodium ointment (Table 12).

Volume of mean paw edema (±SD) in control group animals (Group XIII) was measured at 30 min, 1 h, 2 h, and 3 h, respectively.

Group XIV animals showed percent edema inhibition 10, 40, 47, and 51 at 30 min, 1 h, 2 h, and 3 h, respectively, and Group XV at 15, 40, 48, and 53 at 30 min, 1 h, 2 h, and 3 h, respectively, Group XVI at 25, 49, 55, and 58 at 30 min, 1 h, 2 h, and 3 h, respectively, Group XVII at 65, 80, 83, and 83 at 30 min, 1 h, 2 h, and 3 h, respectively, and Group XVIII at 85, 100, 100, and 100, percent edema inhibition at 30 min, 1 h, 2 h, and 3 h, respectively.

Anti-inflammatory effect of gel formulations using cotton-pellet-induced granuloma method

Table 13 summarizes the effect of gel formulations on cotton pellet granuloma. Group I was treated with application of 0.5 g of gel base for 7 consecutive days. Group II to V received application of 0.5 g of Formulation A, B, C, and D, respectively, and Group VI was treated with application of 0.5 g of diclofenac gel for 7 consecutive days. Group II to V showed percent granuloma inhibition 48, 53, 61, and 68, respectively, Group VI showed 70% granuloma inhibition (Figure 16).

Anti-inflammatory effect of cream formulations using cotton-pellet-induced granuloma method

Table 14 summarizes the effect of cream formulations on cotton pellet granuloma. Group VII was treated with application of 0.5 g of cream base for 7 consecutive days. Group VIII to XI received an application of 0.5 g of Formulation E, F, G, and H, respectively, and Group XII was treated with application of 0.5 g of diclofenac cream for 7 consecutive days. Group VIII to XI showed percent granuloma inhibition 47, 56, 62, and 68, respectively. Group XII showed 70% granuloma inhibition (Figure 17).

Anti-inflammatory effect of ointment formulations using cotton-pellet-induced granuloma method

Group XIII was treated with application of 0.5 g of ointment base for 7 consecutive days. Group XIV to XVII received an application of 0.5 g of Formulation I, J, K, and L, respectively, and Group XVIII was treated with application of 0.5 g of diclofenac cream for 7 consecutive days. Group XIV to XVII showed percent granuloma inhibition 47, 58, 65, and 70, respectively. Group XVIII showed 74% granuloma inhibition (Table 15) (Figure 18).

Anti-inflammatory effect of topical formulations on erythema development

Following scoring system was used to calculate erythema formation.

Table 10: Effect of gel formulations on paw volume (% inhibition)

Groups	Formulation	Edema paw volume (mean±SD)				% Inhibition of paw edema			
		30 min	1 h	2 h	3 h	30 min	1 h	2 h	3 h
I	Control (base)	0.41±0.08	0.52±0.09	0.58±0.11	0.58±0.07	-	-	-	-
II	A	0.38±0.07*	0.37±0.08*	0.32±0.06*	0.32±0.05*	8	29	45	45
III	B	0.36±0.06*	0.35±0.07*	0.31±0.06*	0.30±0.07*	13	33	47	49
IV	C	0.32±0.08*	0.31±0.06*	0.30±0.05*	0.29±0.04*	22	41	49	50
V	D	0.14±0.07*	0.13±0.02*	0.12±0.02*	0.11±0.02*	22	75	80	81
VI	Standard group (diclofenac gel)	0.05±0.00	0.00±0.00	0.00±0.00	0.00±0.00	92	100	100	100

*$P<0.05$, P values compared with control (value express as mean of 6 animals). SD: Standard deviation

Table 11: Effect of cream formulations on paw volume (% inhibition)

Groups	Formulation	Edema paw volume (mean±SD)				% Inhibition of paw edema			
		30 min	1 h	2 h	3 h	30 min	1 h	2 h	3 h
VII	Control (base)	0.42±0.07	0.53±0.06	0.55±0.12	0.58±0.09	-	-	-	-
VIII	E	0.39±0.06*	0.37±0.06*	0.36±0.07*	0.33±0.06*	8	31	35	44
IX	F	0.37±0.05*	0.35±0.05*	0.32±0.06*	0.31±0.08*	12	34	42	47
X	G	0.31±0.07*	0.29±0.06*	0.28±0.04*	0.27±0.04*	26	45	49	53
XI	H	0.13±0.04	0.12±0.01*	0.11±0.03*	0.10±0.03*	69	77	80	83
XII	Standard group (diclofenac cream)	0.01±0.00	00±0.00	00±0.00	00±0.00	98	100	100	100

*$P<0.05$, P values compared with control (value express as mean of six animals). SD: Standard deviation

Table 12: Effect of ointment formulations on paw volume (% inhibition)

Groups	Formulations	Edema paw volume (mean±SD)				% Inhibition of paw edema			
		30 min	1 h	2 h	3 h	30 min	1 h	2 h	3 h
XIII	Control (base)	0.40±0.08	0.55±0.10	0.58±0.05	0.59±0.03	-	-	-	-
XIV	I	0.36±0.09*	0.33±0.06*	0.31±0.07*	0.29±0.04*	10	40	47	51
XV	J	0.34±0.06*	0.33±0.07*	0.30±0.07*	0.28±0.07*	15	40	48	53
XVI	K	0.30±0.09*	0.28±0.03*	0.26±0.04*	0.25±0.02*	25	49	55	58
XVII	L	0.14±0.04*	0.11±0.02*	0.10±0.02*	0.10±0.02*	65	80	83	83
XVIII	Standard group (diclofenac ointment)	0.06±0.00	00±0.00	00±0.00	00±0.00	85	100	100	100

*$P<0.05$, P values compared with control (value express as mean of 6 animals). I-L ointment formulation

Table 13: Effect of gel formulations on cotton-pellet-induced granuloma

Groups	Formulations	Wet weight (mg)	Dry weight (mg)	Percentage of inhibition
I	Control (base)	184±17.25	106±5.81	-
II	A	132±12.52*	55±11.23*	48*
III	B	128±16.51*	50±6.42*	53*
IV	C	121±18.41*	41±7.47*	61*
V	D	119±14.56*	34±7.52*	68*
VI	Standard (diclofenac gel)	114±14.23	32±6.50	70

$P<0.05$. P values compared with control A-D gel formulation

Erythema score: 0 = No erythema observed, 1 = Slight erythema, 2 = Mild erythema, 3 = Moderate erythema, and 4 = Severe erythema.

After application of all formulations, it was noted that gel base application showed no erythema inhibition up to 3 h while application of gel Formulation A showed slightly erythema inhibition and the onset of action was observed from 1 h and sustain up to 3 h. Further, gel Formulation D showed significant erythema inhibition and the onset of action was observed from 1 h and sustains up to 3 h. Results are tabulated in Table 16 and Figure 1.

After application of all formulations, it was noted that cream base application showed no erythema inhibition up to 3 h while application of gel Formulation A showed slightly erythema inhibition and the onset of action was observed from 1 h and sustain up to 3 h. Further, gel Formulation D showed significant erythema inhibition and the onset of action was observed from 1h and sustains up to 3 h. Results are tabulated in Table 17. After application of all formulations, it was noted that ointment base application showed no erythema inhibition up to 3 h while application of gel Formulation A showed slightly erythema inhibition and the onset of action was observed from 1 h and sustain up to 3 h. Further, gel Formulation D showed significant erythema inhibition and the onset of action was observed from 1 h and sustains up to 3 h. Table 12 summarizes that % erythema score for the formulations including gel, cream, and ointment and marketed standard diclofenac gel at 30 min, 1 h, 2 h, and 3 h. Results are tabulated in (Table 18) (Figures 2 and 3).

Table 14: Effect of cream formulations on cotton-pellet-induced granuloma

Groups	Formulations	Wet weight (mg)	Dry weight (mg)	Percentage of inhibition
VII	Control (Base)	175±16.25	109±5.98	-
VIII	E	134±12.92*	58±4.25*	47*
IX	F	129±16.33*	48±4.96*	56*
X	G	123±18.74*	41±4.36*	62*
XI	H	117±12.35*	35±3.54*	68*
XII	Standard group (diclofenac cream)	115±10.25	33±6.50	70

P<0.05, *P* values compared with control E-H cream formulation

Table 15: Effect of ointment formulations on cotton-pellet-induced granuloma

Groups	Formulations	Wet weight (mg)	Dry weight (mg)	Percentage of inhibition
XIII	Control (base)	172±12.25	111±5.22	-
XIV	I	140±12.82*	59±4.98*	47*
XV	J	132±16.83*	47±4.10*	58*
XVI	K	126±13.74*	39±4.58*	65*
XVII	L	118±12.35*	33±4.51*	70*
XVIII	Standard group (diclofenac ointment)	116±11.25	29±6.54	74

P<0.05, *P* values compared with control I-L ointment formulation

Table 16: Effect of gel formulations on erythema development

Groups	Gel formulation	Erythema score				% Erythema inhibition			
		30 min	1 h	2 h	3 h	30 min	1 h	2 h	3 h
I	Gel base	4	4	4	4	-	-	-	-
II	A	4	3	3	3	0	25	25	25
III	B	4	3	2	2	0	25	50	50
IV	C	3	2	2	1	25	50	50	75
V	D	1*	0*	0*	0*	75*	100*	100*	100*
VI	Standard group (diclofenac gel)	1	0	0	0	75	100	100	100

P<0.05, *P* values compared with control

Analgesic effect

Analgesic effect of gel formulations using tail flick method

Tail flicking response in second in Group I was almost same at all observed times as compared to predrug reaction time. Tail flicking response of Group II to V in second was found significantly increased as compared with control Group I. Group VI showed increased tail flicking response as compared with control Group I. It was observed that the analgesic effect of Formulation D was almost equivalent to diclofenac sodium gel after 3 h (Table 19 and Figure 4).

Table 17: Effect of cream formulations on erythema development

Groups	Cream formulation	Erythema score				% Erythema inhibition			
		30 min	1 h	2 h	3 h	30 min	1 h	2 h	3 h
VII	Cream base	4	4	4	4	-	-	-	-
VIII	E	4	3	3	3	0	25	25	25
IX	F	4	3	2	2	0	25	50	50
X	G	3	2	2	2	25	50	50	50
XI	H	2	1	0	0	50	75	100	100
XII	Standard group (diclofenac cream)	1	0	0	0	75	100	100	100

P<0.05, *P* values compared with control

Table 18: Effect of ointment formulations on erythema development

Groups	Ointment formulation	Erythema score				% Erythema inhibition			
		30 min	1 h	2 h	3 h	30 min	1 h	2 h	3 h
XIII	Ointment base	4	4	4	4	0	0	0	0
XIV	I	4	4	3	3	0	0	25	25
XV	J	4	3	3	2	0	25	25	50
XVI	K	3*	2*	2*	2*	25*	50*	50*	50*
XVII	L	2*	1*	1*	0*	50*	75*	75*	100*
XVIII	Standard group (diclofenac ointment)	1	0	0	0	75	100	100	100

P<0.05, *P* values compared with control

Analgesic effect of cream formulations using tail flick method

Tail flicking response in second in Group VII was almost same at all observed times as compared to predrug reaction time. Tail flicking response of Group VIII to XI in second was found significantly increased as compared with control Group VII. Group XII showed increased tail flicking response as compared with control Group VII. It was observed that the analgesic effect of Formulation H was almost equivalent to diclofenac sodium cream after 3 h (Table 20 and Figure 5).

Analgesic effect of ointment formulations using tail flick method

Tail flicking response in second in Group XIII was almost same at all observed times as compared to predrug reaction time. Tail flicking response of Group XIV to XVII in second was found significantly increased as compared with control Group XIII. Group XVIII showed increased tail flicking response in second as compared with control group XIII. It was observed that the analgesic effect of Formulation L was almost equivalent to diclofenac sodium ointment after 3 h (Table 21, Figure 6).

Evaluation of analgesic activity of gel formulations by hot plate method

Group I received single application of 0.5 g of gel base. Group VI was treated with single application 0.5 g of diclofenac gel. Paw licking/jumping response in second was found to be significantly increased in

Table 19: Analgesic effect of gel formulations using tail flick method

Groups	Formulations	Tail flick response in seconds (±SD)				
		Predrug reaction time	After 30 min	After 1 h	After 2 h	After 3 h
I	Control (base)	3.3±0.21	3.25±0.38	3.16±0.27*	3.21±0.33*	3.20±0.54
II	A	3.5±0.22*	6.0±0.79*	6.60±0.71*	6.62±0.64*	6.63±0.38*
III	B	3.9±0.32*	7.6±0.82*	8.20±0.76*	8.36±0.65*	8.38±0.47*
IV	C	3.1±0.29*	8.0±0.67*	9.10±0.91*	9.22±0.78*	9.32±0.48*
V	D	3.5±0.32*	9.0±0.77*	9.60±0.85*	9.82±0.84*	9.84±0.69*
VI	Standard group (diclofenac gel)	3.8±0.17	9.2±0.54	9.83±0.27	10.10±0.81	10.16±0.34

**$P<0.01$. P values compared with control A-D gel formulation. Series 1-30 min, Series 2 – 1 h, Series 3 – 2 h, Series 4 – 3 h. SD: Standard deviation

Table 20: Analgesic effect of cream formulations using tail flick method

Groups	Formulations	Tail flick response in seconds (±SD)				
		Predrug reaction time	After 30 min	After 1 h	After 2 h	After3 h
VII	Control (base)	3.32±0.29	3.35±0.39	3.56±0.41	3.21±0.31	3.22±0.42
VIII	E	3.55±0.33*	6.52±0.55*	6.60±0.84*	6.42±0.55*	6.73±0.54*
IX	F	3.91±0.41*	6.61±0.79*	8.20±0.86*	8.36±0.63*	9.15±0.52*
X	G	3.19±0.25*	8.09±0.45*	9.10±0.74*	9.22±0.75*	9.85±0.58*
XI	H	3.51±0.16*	9.2±0.87*	9.60±0.54*	9.82±0.84*	10.05±0.95*
XII	Standard group (diclofenac cream)	3.82±0.19	9.3±0.54	9.83±0.94	10.10±0.98	10.16±0.61

**$P<0.01$. P values compared with control E-H cream formulation. Series 1 – 30 min, Series 2 – 1 h, Series 3 – 2 h, Series 4 – 3 h. SD: Standard deviation

Table 21: Analgesic effect of ointment formulations using tail flick method

Group	Formulation	Tail flick response in seconds (±SD)				
		Predrug reaction time	After 30 min	After 1 h	After 2 h	After 3 h
XIII	Control (base)	4.10±0.23	4.25±0.40	3.56±0.47	3.98±0.33	3.99±0.33
XIV	I	3.96±0.45*	6.52±0.51*	6.60±0.51*	6.72±0.55*	6.79±0.54*
XV	J	3.91±0.49*	7.78±0.72*	8.30±0.71*	8.66±0.71*	9.25±0.50*
XVI	K	3.39±0.25*	8.89±0.44*	9.35±0.46*	9.62±0.81*	9.85±0.59*
XVII	L	3.51±0.54*	9.18±0.91*	9.57±0.47*	10.33±0.87*	10.35±0.58*
XVIII	Standard group (diclofenac ointment)	3.82±0.13	9.32±0.54	10.83±0.99	10.90±0.81	10.96±0.61

**$P<0.01$. P values compared with control I-L ointment formulation. Series 1 – 30 min, Series 2 – 1 h, Series 3 – 2 h, Series 4 – 3 h. SD: Standard deviation

Group II to V as compared with control Group I. It was also observed that Group VI treated animals showed significantly increased paw licking/jumping response as compared with control Group I. The response of Formulation D was almost equivalent to Group VI treated animals (diclofenac gel) (Table 22 and Figure 7).

Evaluation of analgesic activity of cream formulations by hot plate method

Group VII received single application of 0.5 g of cream base. Group VIII to XI received single application of 0.5 g of Formulation E to H, respectively. Group XII was treated with single application 0.5 g of diclofenac cream. Paw licking/jumping response in second was found to be significantly increased in Group VIII to XI as compared with control Group VII. It was also observed that Group XII treated animals showed significantly increased paw licking/jumping response in second as compared with control Group VII (Table 23 and Figure 8).

The response of Formulation H was almost equivalent to Group XII treated animals (diclofenac cream) after 3 h.

Evaluation of analgesic activity of ointment formulations by hot plate method

Group XIII received single application of 0.5 g of ointment base. Group XIV to XVII received single application of 0.5 g of Formulation I to L, respectively. Group XVIII was treated with single application 0.5 g of diclofenac ointment. Paw licking/jumping response in second was found to be significantly increased in Group XIV to XVII as compared with control group XIII. It was also observed that Group XVIII treated animals showed significantly increased paw licking/jumping response as compared with control Group XIII (Table 24 and Figure 9).

The response of Formulation L in second was almost equivalent to Group XVIII treated animals (diclofenac ointment) after 3 h.

Evaluation of analgesic activity of gel formulations by tail immersion method

Group I received single application of 0.5 g of gel base. Group II to V received single application of 0.5 g of Formulation A to D, respectively.

Table 22: Analgesic effect of gel formulations using hot plate method

Groups	Formulation	Paw licking/jumping response in seconds (±SD)				
		Predrug reaction time	After 30 min	After 1 h	After 2 h	After 3 h
I	Control (base)	3.19±0.32	3.25±0.24	3.28±0.41	3.42±0.31	3.33±0.32
II	A	3.66±0.37*	5.55±0.41*	5.98±0.64*	6.25±0.51*	6.84±0.54*
III	B	3.67±0.15*	5.78±0.41*	6.30±0.78*	6.66±0.41*	6.85±0.49*
IV	C	3.39±0.21*	6.89±0.37*	6.95±0.41*	7.62±0.48*	8.81±0.84*
V	D	3.45±0.27*	8.25±0.57*	9.57±0.27*	10.13±0.97*	10.45±0.41*
VI	Standard group (diclofenac gel)	3.42±0.27	7.32±0.28	8.83±0.87	10.50±0.57	10.56±0.81

**$P<0.01$. P values compared with control A-D gel formulation. Series 1 – 30 min, Series 2 – 1 h, Series 3 – 2 h, Series 4 – 3 h. SD: Standard deviation

Table 23: Analgesic effect of cream formulations using hot plate method

Groups	Formulation	Paw licking/jumping response in seconds (±SD)				
		Predrug reaction time	After 30 min	After 1 h	After 2 h	After 3 h
VII	Control (base)	3.66±0.65	3.68±0.14	3.74±0.18	3.82±0.17	3.83±0.31
VIII	E	3.81±0.56*	5.51±0.22*	5.88±0.33*	6.05±0.54*	6.14±0.41*
IX	F	3.77±0.58*	5.78±0.49*	6.40±0.54*	6.47±0.47*	6.99±0.74*
X	G	4.01±0.40*	5.98±0.37*	6.85±0.71*	7.52±0.57*	8.91±0.62*
XI	H	3.95±0.32*	7.25±0.67*	9.47±0.99*	10.23±0.91*	10.95±0.92*
XII	Standard group (diclofenac cream)	3.42±0.12	7.32±0.61	9.83±0.87	10.81±0.84	10.96±0.29

**$P<0.01$. E-H cream formulation. Series 1 – 30 min, Series 2 – 1 h, Series 3 – 2 h, Series 4 – 3 h. SD: Standard deviation

Table 24: Analgesic effect of ointment formulations using hot plate method

Groups	Formulations	Paw licking/jumping response in seconds (±SD)				
		Predrug reaction time	After 30 min	After 1 h	After 2 h	After 3 h
XIII	Control (base)	3.33±0.32	3.78±0.24	3.84±0.41	3.99±0.40	4.01±0.39
XIV	I	3.65±0.41*	5.64±0.21*	5.98±0.32*	6.15±0.45*	6.33±0.67*
XV	J	3.98±0.16*	5.68±0.54*	6.70±0.57*	6.77±0.60*	6.93±0.52*
XVI	K	3.69±0.19*	6.02±0.41*	6.85±0.51*	7.32±0.61*	8.41±0.74*
XVII	L	3.95±0.24*	7.28±0.56*	9.27±0.86*	10.13±0.81*	10.81±0.77*
XVIII	Standard group (diclofenac ointment)	3.78±0.51	7.32±0.71	9.98±0.91	10.84±0.93	10.91±0.84

**$P<0.01$. P values compared with control I-L ointment formulation. Series 1 – 30 min, Series 2 – 1 h, Series 3 – 2 h, Series 4 – 3 h. SD: Standard deviation

Table 25: Analgesic effect of gel formulations using tail immersion method

Groups	Formulations	Tail flicking response in seconds (±SD)				
		Predrug reaction time	After 30 min	After 1 h	After 2 h	After 3 h
I	Control (base)	4.10±0.42	4.11±0.34	4.18±0.41	4.19±0.31	4.19±0.25
II	A	3.94±0.31*	4.55±0.45*	4.98±0.64*	5.25±0.51*	5.84±0.30*
III	B	3.77±0.37*	5.78±0.51*	6.40±0.78*	6.96±0.41*	6.98±0.47*
IV	C	3.99±0.40*	6.49±0.61*	6.95±0.41*	7.42±0.48*	8.71±0.66*
V	D	3.90±0.40*	8.15±0.74*	9.91±0.27*	10.93±0.87*	11.45±0.89*
VI	Standard group (diclofenac gel)	3.42±0.33	8.32±0.28	9.83±0.87	11.50±0.57	11.56±0.74

**$P<0.01$. P values compared with control A-D gel formulation. Series 1 – 30 min, Series 2 – 1 h, Series 3 – 2 h, Series 4 – 3 h. SD: Standard deviation

Group VI was treated with single application 0.5 g of diclofenac gel. Tail flicking response in second was found to be significantly increased in Group II to V as compared with control Group I. It was also observed that Group VI treated animals showed significantly increased tail flicking response as compared with control Group I (Table 25 and Figure 10).

The response of Formulation D was almost equivalent to Group VI treated animals (diclofenac gel).

Evaluation of analgesic activity of cream formulations by tail immersion method

Group VII received single application of 0.5 g of cream base. Group VIII to XI received single application of 0.5 g of Formulation E to H, respectively. Group XII was treated with single application 0.5 g of diclofenac cream. Tail flicking response in second was found to be significantly increased in Group VIII to XI as compared with control Group VII. It was also observed that Group XII treated animals showed significantly increased tail flicking response as compared with control Group VII (Table 26 and Figure 11).

The response of Formulation H was almost equivalent to Group XII treated animals (diclofenac cream).

Evaluation of analgesic activity of ointment formulations by tail immersion method

Group XIII received single application of 0.5 g of ointment base. Group XIV to XVII received single application of 0.5 g of Formulation I to L, respectively. Group XVIII was treated with single application 0.5 g of diclofenac gel. Tail flicking response in second was found to be significantly increased in Group XIV to XVII as compared with control Group XIII. It was also observed that Group XVIII treated animals showed significantly increased tail flicking response as compared with control Group XIII (Table 27 and Figure 12).

The response of Formulation L was almost equivalent to Group XVIII treated animals (diclofenac ointment).

Discussion

Ginger suppresses prostaglandin synthesis through inhibition of COX-1 and COX-2. The characterization of the pharmacological properties of ginger entered a new phase with the discovery that a ginger extract (EV.EXT.77) derived from *Z. officinale* (family Zingiberaceae) and *Alpina galanga* (family Zingiberaceae) inhibits the induction of several genes involved in the inflammatory response. This discovery provided the first evidence that ginger modulates biochemical pathways activated in chronic inflammation.[15]

Deorukhakar *et al.* investigated anti-inflammatory activity of the polyherbal formulation Entox® containing turmeric in rats for acute and subacute models of inflammation using carrageenan-induced rat paw edema and cotton pellet granuloma methods. The formulation showed a significant anti-inflammatory activity.[20] The present results of the study were also comparable to this study.

V. Nithya, evaluated the anti-inflammatory activity, analgesic activity of *A. sativum* Linn., on carrageenan induced paw edema in Wistar male rats and aqueous, ethanolic and methanolic extracts, and compared to a positive control drug, Voveran.[26] In this study, the significant anti-

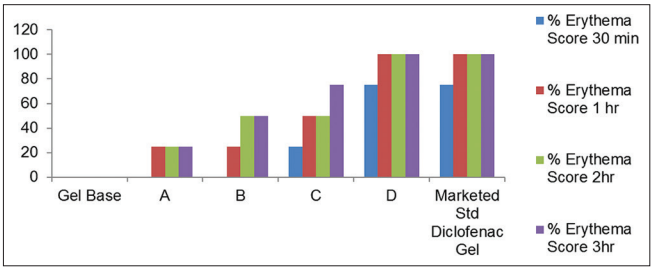

Figure 1: Effect of gel formulations on erythema development

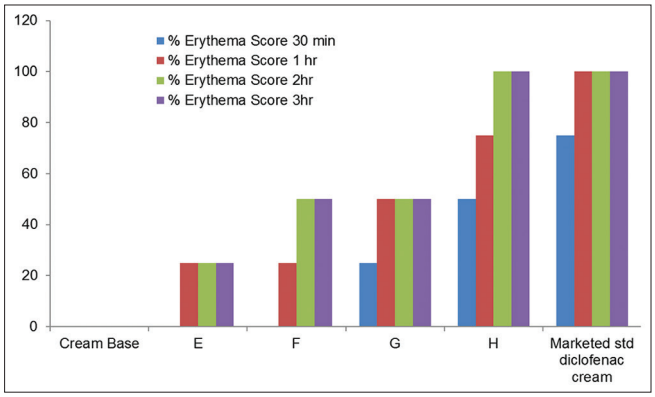

Figure 2: Effect of Cream formulations on erythema development

Table 26: Analgesic effect of cream formulations using tail immersion method

Groups	Formulations	Tail flicking response in seconds (±SD)				
		Predrug reaction time	After 30 min	After 1 h	After 2 h	After 3 h
VII	Control (base)	4.12±0.45	4.18±0.24	4.16±0.22	4.44±0.25	4.52±0.31
VIII	E	3.98±0.66	6.14±0.22	6.68±0.33	6.75±0.54	6.84±0.41*
IX	F	3.85±0.58	6.78±0.49	6.90±0.54	7.47±0.47	7.99±0.74
X	G	3.93±0.40	6.87±0.37	7.99±0.71	9.55±0.57	10.12±0.62
XI	H	3.95±0.32	6.99±0.67	9.49±0.99	9.34±0.91	10.65±0.92*
XII	Standard group (diclofenac cream)	3.65±0.12	7.62±0.61	9.99±0.87	9.99±0.84	10.96±0.29

**$P<0.01$. P values compared with control A-D gel formulation E-H cream formulation. Series 1 – 30 min, Series 2 – 1 h, Series 3 – 2 h, Series 4 – 3 h. SD: Standard deviation

Table 27: Analgesic effect of ointment formulations using tail immersion method

Groups	Formulations	Tail flicking response in seconds (±SD)				
		Predrug reaction time	After 30 min	After 1 h	After 2 h	After3 h
XIII	Control (base)	4.12±0.40	4.21±0.39	4.34±0.33	4.38±0.24	4.39±0.36
XIV	I	3.98±0.16	5.94±0.41	5.99±0.32	6.25±0.45	6.73±0.67*
XV	J	3.98±0.16	5.88±0.54	6.72±0.57	6.92±0.21	7.01±0.68
XVI	K	3.69±0.19	6.08±0.41	6.95±0.55	7.82±0.61	8.61±0.74
XVII	L	3.95±0.24	7.68±0.56	10.17±0.86	11.43±0.81	11.81±0.77*
XVIII	Standard group (diclofenac ointment)	3.68±0.51	7.52±0.71	10.28±0.91	11.84±0.93	11.91±0.84

**$P<0.01$. P values compared with control A-D gel formulation I-L ointment formulation. Series 1 – 30 min, Series 2 – 1 h, Series 3 – 2 h, Series 4 – 3 h. SD: Standard deviation

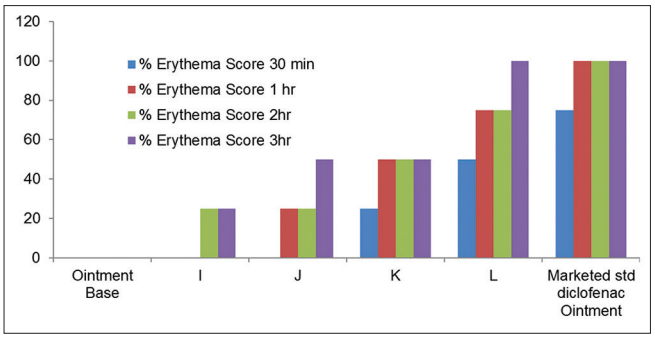

Figure 3: Effect of Ointment formulations on erythema development

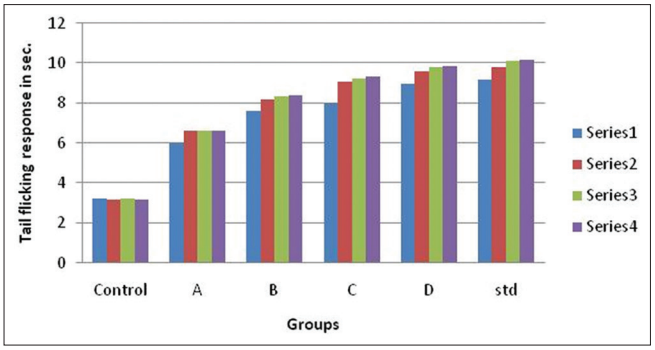

Figure 4: Effect of gel formulations on tail flicking response, Series1-30min, Series2-1hr, Series3-2hr, Series4-3hr

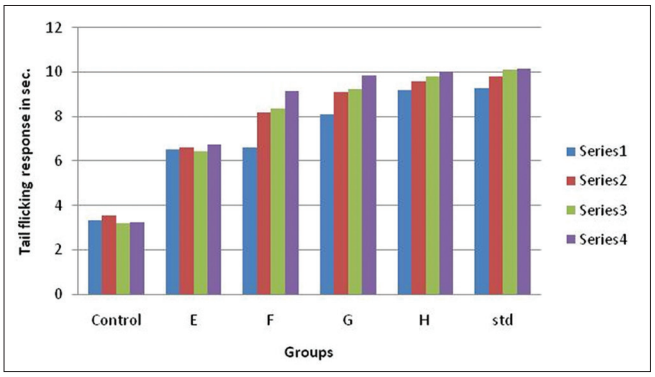

Figure 5: Effect of cream formulations on tail flicking response, Series1-30 min, Series2-1hr, Series3-2hr, Series4-3hr

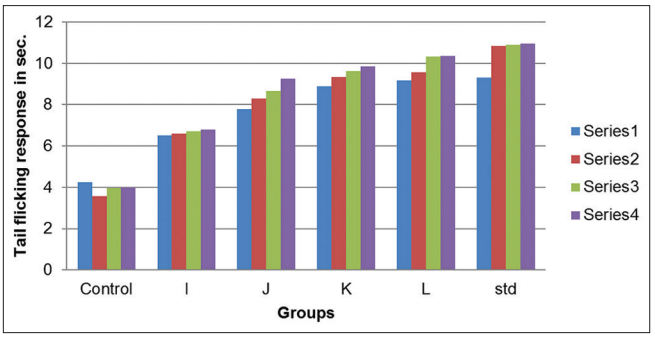

Figure 6: Effect of ointment formulations on tail flicking response, Series1-30min, Series2-1hr, Series3-2hr, Series4-3hr

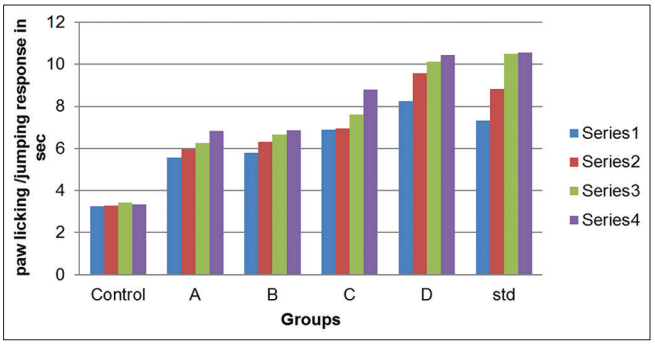

Figure 7: Effect of gel formulations on paw licking/jumping response, Series1-30min, Series2-1hr, Series3-2hr, Series4-3hr

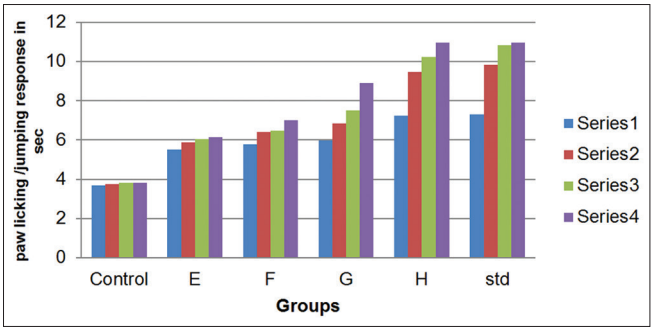

Figure 8: Effect of cream formulations on paw licking/jumping response, Series1-30min, Series2-1hr, Series3-2hr, Series4-3hr

inflammatory activity of all three formulations was observed against carrageenan induced rat paw edema method. In this study, the order of potency of gel formulation (according to mean paw edema volume) was observed as diclofenac gel ≥ Formulation D> Formulation C > Formulation B > Formulation A.

The order of potency was observed as diclofenac cream ≥ Formulation H > Formulation G > Formulation F > Formulation E. Further, the order of potency was observed as diclofenac ointment ≥ Formulation L > Formulation K > Formulation J > Formulation I.

Mishra *et al*. showed that polyherbal formulation of alcoholic extract of C. *longa* and *Boswellia* serrata has significantly decrease in wet weight and dry weight of cotton pellets and suppressed the development of ear edema induced by xylene in mice as compared to the vehicle control group.[21] Further, the results found in the present study are similar to this study.

Karthikeyan studied cotton pellet granuloma in Wistar albino rats was used, anesthetized with ether and the control, test drug (gel, ointment, and cream), and standard drug (diclofenac sod) were used.[27]

In this investigation, all gel formulations showed anti-inflammatory activities in a cotton-pellet-induced granuloma. Among the formulations, erythema induced by UV light was completely abolished at 1 h, 2 h, and 3 h when animals treated with Formulation D and diclofenac gel as compared with control group. It showed Formulation D and diclofenac gel has a maximum activity that other formulations and control group. For cream formulation, the order of potency

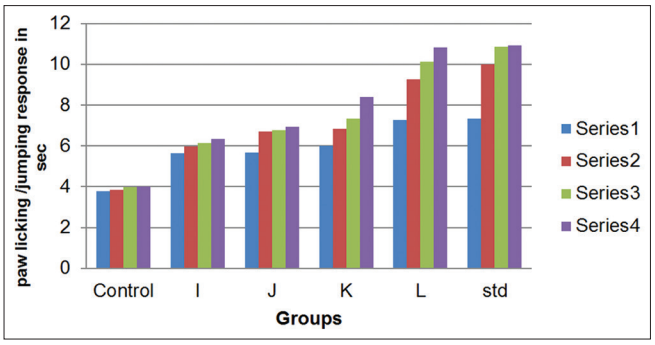

Figure 9: Effect of ointment formulations on paw licking/jumping response, Series1-30min, Series2-1hr ,Series3-2hr, Series4-3hr

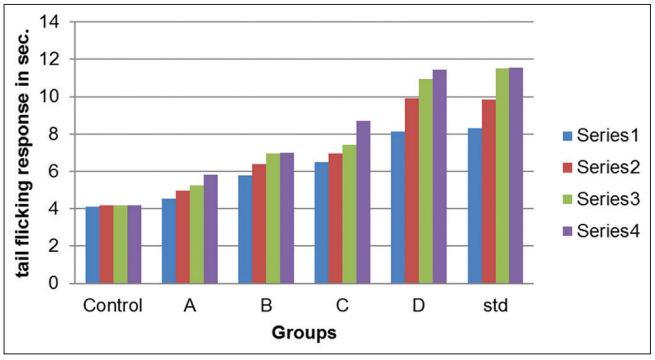

Figure 10: Effect of gel formulations on tail flicking response in sec, Series 1-30 min, Series 2-1hr, Series3-2hr, Series4-3hr

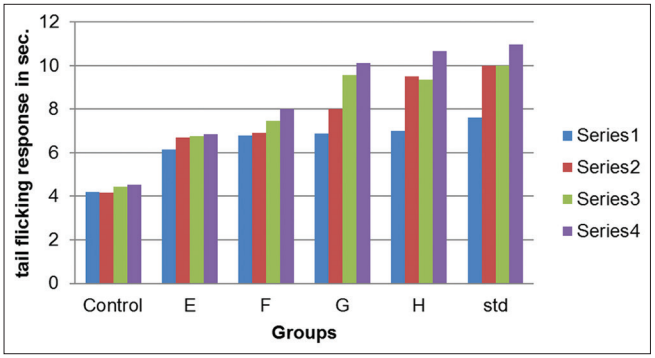

Figure 11: Effect of cream formulations on tail flicking response in sec, Series1-30 min, Series2-1hr, Series3-2hr, Series4-3hr

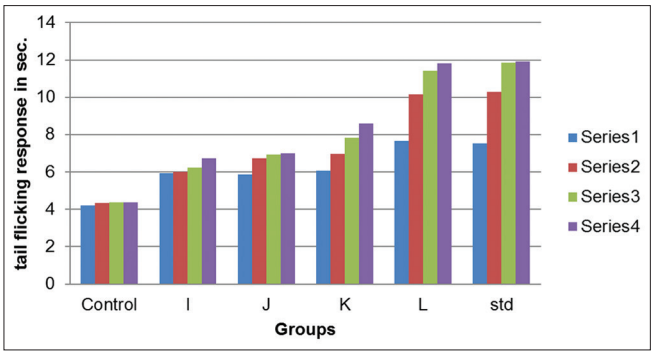

Figure 12: Effect of ointment for mulations on tail flicking response in sec, Series1-30min,Series2-1hr ,Series3-2hr, Series4-3hr

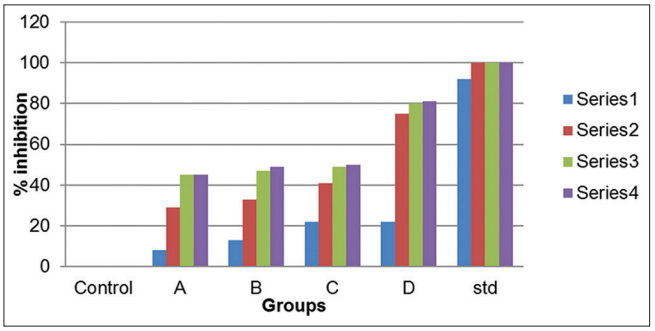

Fig: 13: Effect of gel formulation on paw edema, Series1-30min, Series2-1hr, Series3-2hr, Series4-3hr

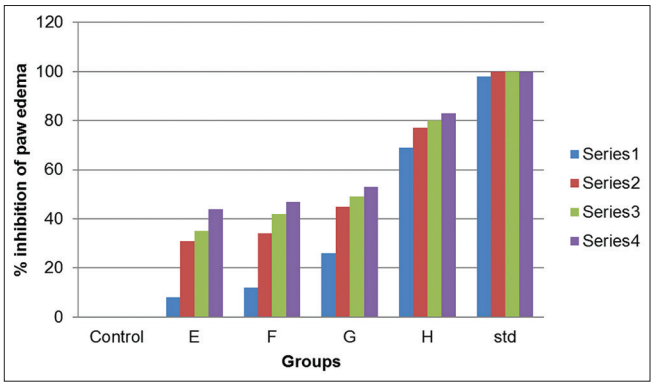

Figure 14: Effect of Cream formulation on paw edema, Series1-30min, Series2-1hr, Series3-2hr, Series4-3hr

was observed as diclofenac cream ≥ Formulation H > Formulation G > Formulation F > Formulation E. The result also showed that the percent granuloma inhibition of Formulation L and diclofenac ointment was better than the percent granuloma inhibition of other ointment formulations.

Peters *et al*. have studied the effects of different classes of pharmacological agents applied topically to the skin of guinea pigs and have found indomethacin and pirprofen, both prostaglandin synthetase inhibitors, to be highly effective suppressors of UV erythema. Results appear to further substantiate that prostaglandin synthesis and release may be the primary mechanistic process in the production of erythema and that the model itself can be predictive of both therapeutic and prophylactic effects of agents against sunburn.[22]

After application of all formulations, it was noted that gel base application showed no erythema inhibition up to 3 h while application of gel Formulation D, cream formulation (H), and ointment formulation (L) showed significant erythema inhibition and the onset of action was observed from 30minutes and sustained up to 3 h.

Jayanti and Jyoti estimated the analgesic and antinociceptive effects of *A. sativum* powder, and compared the effects between central and peripheral nociceptive models with that of other established analgesic drugs. Albino rats and mice were used for studying analgesic and antinociceptive activity using various models, viz., acetic acid induced writhing model, Eddy's hot plate for analgesic study, and formalin-induced paw licking model were used for antinociceptive study.[24]

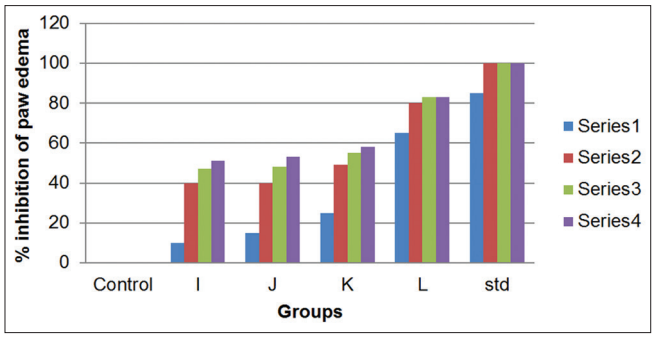

Figure 15: ect of Ointment formulation on paw edema, Series1-30 min, Series2-1hr, Series3-2hr, Series4-3hr

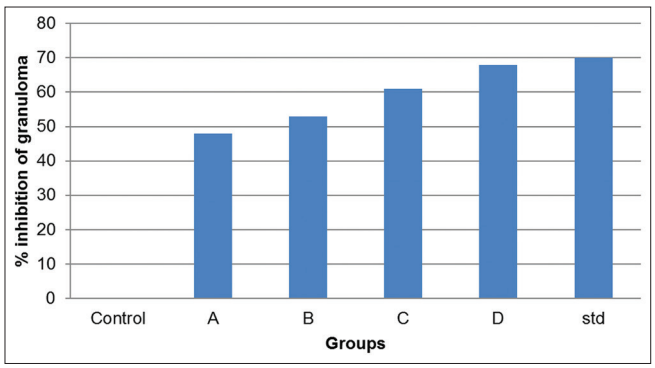

Figure 16: Effect of Gel formulation on cotton pellete induced granuloma

Mathew *et al*. evaluated the analgesic efficacy of garlic extract by employing different pain models such as hot plate and tail flick tests for central analgesia. Further, the 4% sodium chloride induced writhing as a peripheral analgesic model.[25] In this investigation, the order of potency was observed as diclofenac gel ≥ Formulation D > Formulation C > Formulation B > Formulation A. The order of potency was observed as diclofenac cream ≥ Formulation H > Formulation G > Formulation F > Formulation E. The result also showed that the analgesic effect of Formulation L was better than the effect of other ointment formulations.

The paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The significant increase in pain threshold produced by tests and standard in these models suggests the involvement of central pain pathway (Figures 13-18).

In this study, the order of potency was observed as diclofenac gel ≥ Formulation D > Formulation C > Formulation B > Formulation A. The order of potency was observed as diclofenac cream ≥ Formulation H > Formulation G > Formulation F > Formulation E.

The response of Formulation L was almost equivalent to Group XVIII treated animals (diclofenac gel) after 3 h. The results are tabulated in Table 24.

From the study, it was found that potency of diclofenac ointment is almost equivalent to Formulation L ointment.

Mishra *et al*. investigated the analgesic activity of etoricoxib for individual drug therapy and etoricoxib for combination therapy with

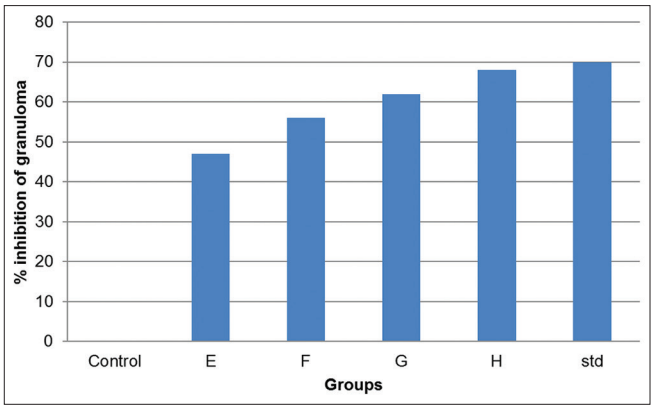

Figure 17: Effect of cream formulation on cotton pellete induced granuloma

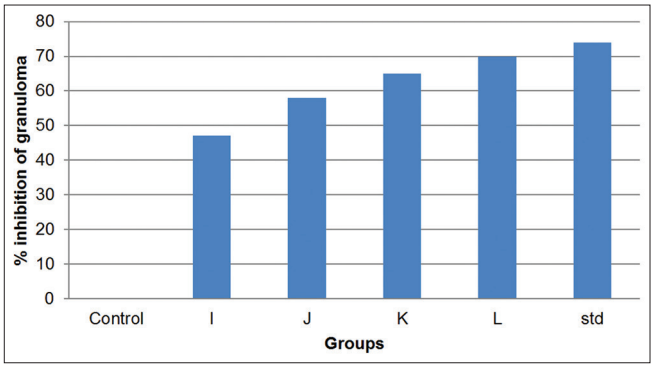

Figure 18: Effect of ointment formulation on cotton pellete induced granuloma

diclofenac potassium using acetic acid induced writhing, hot plate, and tail immersion methods. The results of pharmacological test performed in the present studies suggest the combination of etoricoxib and diclofenac potassium possess potent analgesic activity.[23]

In this study, the response of Formulation D was almost equivalent to Group VI treated animals (diclofenac gel). The order of potency was observed as diclofenac cream ≥ Formulation H > Formulation G > Formulation F > Formulation E. The order of potency was observed as diclofenac ointment ≥ Formulation L > Formulation K > Formulation J > Formulation I.

Conclusion

Gel formulations among prepared topical formulations showed the highest activity and also exhibited nonstaining, good spreadability, and patient compliance. Topical formulations containing spices can be used in chronic inflammatory and pain conditions and devoid of side effects. Thus, topical formulations containing India spices can be a better alternative to conventional nonsteroidal anti-inflammatory drugs topical preparations.

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