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RESEARCH ARTICLE

Formulation, Characterization, and Evaluation of Topical Anti-inflammatory Herbal Gel

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ABSTRACT

The aim of the present investigation was to prepare and evaluate topical gel containing capsaicinoid and/or aqueous extract of *Tridax procumbens* L. (AETP) leaves and/or aqueous extract of *Ocimum sanctum* leaves. First gel base was prepared using various different concentrations of Carbopol-934, propylene glycol 400, methylparaben, and propylparaben and required amount of distilled water. The optimized base was selected for the incorporation of capsaicinoid and AETP. Then, skin pH (6.8–7) was maintained by dropwise addition of triethanolamine. Prepared formulations were evaluated for physical appearance, pH, spreadability, viscosity, and homogeneity. Prepared formulations have proceeded for skin irritation on the animal model (rabbit). All gels were evaluated for anti-inflammatory activity using carrageenan-induced rat paw edema model on Albino Wistar rats of either sex (150–200 g). Change in edema volume of the rat hind paw was measured, and percent inhibition was calculated. Stability studies have meted out as per the ICH guidelines for 3 months at different temperatures and humidity. Results reveal that all formulations have shown good appearance, homogeneity, and spreadability. The viscosity of all formulations is ranging between 3500 and 5000 centipoises. All formulations have shown no skin irritation, i.e., erythema and edema to animals. Formulations F1, F2, and F3 have shown significant (P<0.001) anti-inflammatory activity and shown significant inhibition of the inflammation to the extent of 42.37%, 55.93%, and 45.76% at 3 h and 68%, 69.33%, and 54.67% at 4 h, respectively, while the reference drug; diclofenac sodium reduced the inflammation by 59.32% at 3 h and 74.67% at 4 h.

Keywords: Capsaicinoids, herbal anti-inflammatory gel, *Ocimum sanctum* carrageenan-induced rat paw edema, *Tridax procumbens*

INTRODUCTION

Inflammation is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area.[1,2] Capsaicinoids contain capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin. Capsaicin is a topical analgesic used in the treatment of chronic pain in many diseases, including osteoarthritis, rheumatoid arthritis, diabetic neuropathy, and post-herpetic neuralgia.[3-10] *Tridax procumbens* reported anti-inflammatory. The phytochemical investigation reports the isolation of lipid constituents, sterols, flavonoids, polysaccharide, and bergenin derivatives from *T. procumbens*.[11-13] *Ocimum sanctum* L. contains eugenol and cardinene. *O. sanctum* has been shown medicinal properties such as analgesic activity and anti-inflammatory properties.[14-22]

The present study was designed for preparation of anti-inflammatory topical gel. Capsaicinoid has

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long been used as a traditional medicine to treat pain, and to enhanced anti-inflammatory activity aqueous extract of *T. procumbens* (AETP) and aqueous extract of *O. sanctum* (AEOS) was used as these herbs have anti-inflammatory potential.

MATERIALS AND METHODS

Leaves of *T. procumbens* and *O. sanctum* were collected from Nasik district of Maharashtra State (INDIA) in August 2018 and authenticated at Botanical Survey of India, Pune, where a sample (voucher number – RDBTP11, RDBOS16) has been deposited. Shade-dried and powdered leaves were extracted with water using Soxhlet extractor. The solvent was evaporated under reduced pressure. Aqueous extract obtained was used for the preparation of herbal gel.

Isolation of capsaicinoids

20 g of finely ground chilies were placed in the extraction shell of a Soxhlet extractor and methanol was percolated through the sample until no more green color appeared in the percolated methanol. The extracts were cooled and adjusted to a volume of 200 ml with methanol. 5 g of charcoal was added to the colored extract. The slurry was heated to boiling for the duration of 3 min, filtered and the charcoal residue washed several times with methanol. The clear extract was then evaporated to dryness in the flash evaporator. The oil residue was dissolved in petroleum ether and transferred to a separating funnel and washed with distilled water. After discarding the aqueous layer, the ether layer was evaporated to dryness in the flash-evaporator. The purified oily residue remaining was dissolved in methanol, collected in a volumetric flask.[23-29]

Formulation of topical gel

The herbal gel was prepared using Carbopol-934 as a gelling agent in 1% w/w concentration with deionized water using mechanical stirrer. Then, skin pH (6.8–7) was maintained by dropwise addition of triethanolamine with continuous stirring. Different ingredients such as capsaicinoids, and/or AETP, and/or AEOS were added to the gel and stirred for sufficient time for homogeneous mixing. Prepared gel was filled in collapsible tubes and stored at a cool and dry place. The formulation is evaluated for the following parameters.[30]

Organoleptic evaluation

Physical parameters such as color and appearance were recorded.

Viscosity

The viscosity of gel was measured using Brookfield viscometer (Brookfield viscometer RVT) with spindle number 7.

Extrudability

The gel formulations were filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. The weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 500 g was placed over the slides, and then the cap was removed. The amount of the extruded gel was collected and weighed. The percent of the extruded gel was calculated (>90% extrudability: excellent, >80% extrudability: good, >70% extrudability: fair).[31]

Spreadability

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. By this method, spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2 g) under study was placed on the ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of the fixed ground slide and provided with a hook. A 1 kg weight was placed at the top of the two slides for 5 min to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80 g with the help of string attached to the hook and the time (in seconds)

required by the top slide to cover a distance of 7.5 cm was noted. A shorter interval indicated better spreadability. Spreadability was calculated using the following formula:[32]

$S = M \times L/T$

Where,

S = Spreadability

M = Weight in the pan (tied to the upper slide)

L = Length moved by the glass slide

T = Time (in sec.) taken to separate the upper slide from the ground slide.

Measurement of pH

The pH of developed gel formulations was determined using a digital pH meter. The measurement was performed at 1, 30, 60, and 90 days after preparation to detect any change with time. 1 g of the gel was dissolved in 100 ml distilled water and kept aside for 2 h. The measurement of pH of formulation was done in triplicate and average values are calculated.[33-34]

Homogeneity

After the gels have been set in a container, all developed gels were tested for homogeneity by visual inspection. They were tested for their appearance and presence of any aggregates.[33-34]

Grittiness

All the formulations were evaluated microscopically for the presence of any appreciable particulate matter which was seen under a light microscope. Hence, obviously the gel preparation fulfills the requirement of freedom from particular matter and form grittiness as desired for any topical preparation.[35]

Stability study

The stability study was performed as per the ICH guidelines. The formulated gel was filled in collapsible tubes and stored at different temperatures and humidity conditions, namely0, 25±2°C/60±5% RH, 30±2°C/65±5% RH, 40±2°C/75±5% RH for a period of 3 months and studied for appearance, pH, and spreadability.[36,37]

Skin irritation test

The Wistar rats of either sex weighing 150–200 g were used for this test. The intact skin was used. The hair was removed from the rat 3 days before the experiment. The herbal extract containing gel was used on the test animal and gel base on the control group. The animals were treated daily up to 7 days, and finally, the treated skin was examined for erythema and edema.[38]

Evaluation of anti-inflammatory activity

Carrageenan-induced rat paw edema

Albino Wistar rats of either sex, weighing 150–200 g were used. They were housed in standard environmental conditions and fed with standard rodent diet with water *ad libitum*. All animal procedures were followed in seven groups (control, F1, F2, F3, F4, F5, and standard) of six animals each. Animals were fasted for 24 h before the experiment with water *ad libitum*. Edema was induced by injecting 0.1 ml of 1% w/v carrageenan in saline into the plantar side of the right hind paw of rat 1 h before each experiment. 0.2 g of the herbal gel was applied to the plantar surface of the hind paw by gentle rubbing 50 times with the index finger. Rats of the control groups received the plain gel base, and rats of the standard group received 0.2 g 1% diclofenac sodium gel. Drugs or placebo was applied 1 h before the carrageenan injection. Paw volume was measured immediately after carrageenan injection and at 1, 2, 3, and 4 h intervals after the administration of the noxious agent using a Ugo Basile Plethysmometer. The percentage of anti-inflammatory activity was calculated using the formula given below.[39-40]

$\%\ \text{inhibition} = (\text{control group mean} - \text{test group mean})/\text{control group mean} \times 100$

RESULTS AND DISCUSSION

The herbal gel was prepared using combinations of ingredients, as shown in Table 1 and subjected

to the evaluation of various parameters. All gel formulations have different colors as shown in Table 2 and have a smooth feel on the application, which was remain same on stability testing period. The measurement of viscosity of the prepared gel was done with brookfield viscometer. In all these formulations, the optimum viscosity was found and results are deputed in Table 2. The extrusion of the gel from the tube is an important during its application and inpatient acceptance. Gels with high consistency may not extrude from tube whereas, low viscous gels may flow quickly and hence suitable consistency is required to extrude the gel from the tube. Extrudability of all gel formulations was found to be good and results are deputed in Table 3. The pH values of all prepared formulation ranged from 6–7 which are considered acceptable to avoid the risk of irritation on application to the skin. All formulations when prepared were without any gritty particle and after 3 months that is during stability testing period remain homogeneous. Furthermore, the stability study's results revealed that the preparation was stable under normal storage conditions. Results of skin irritation test indicates that prepared gels were not produce irritation, redness or edema on application, and free from dermatological reaction. Percent inhibition in carrageenan-induced rat paw edema by various formulations and standard diclofenac sodium gel is represented in Figure 1. Formulation F1, F2, and F3 have shown significant (P<0.01) anti-inflammatory activity. Formulations F1, F2, and F3 significantly inhibited the inflammation to the extent of 42.37%, 55.93%, and 45.76% at 3 h and 68%, 69.33%, and

Table 1: Composition of formulations containing capsaicinoids, AETP, and AEOS

Ingredients	Quantity in percent				
	F1	F2	F3	F4	F5
Capsaicinoid	0.25	0.25	0.25	–	–
AETP	–	1	–	1	–
AEOS	–	–	1	–	1
Carbopol-934	1	1	1	1	1
Methylparaben (0.5%)	0.2	0.2	0.2	0.2	0.2
Propylparaben (0.2%)	0.1	0.1	0.1	0.1	0.1
Propylene glycol 400 (5%)	5	5	5	5	5
Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.

AETP: Aqueous extract of *Tridax procumbens*, AEOS: Aqueous extract *Ocimum sanctum*

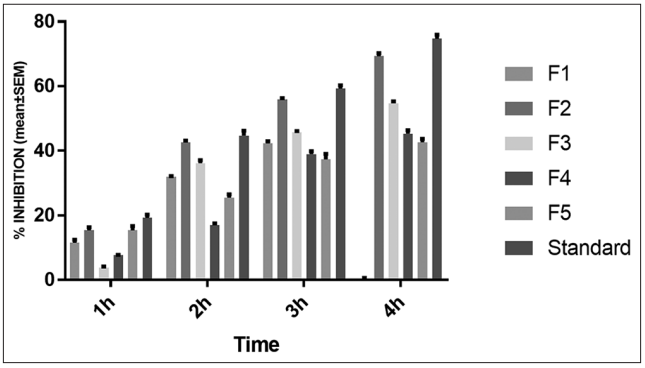

Figure 1: Percent inhibition of inflammation in carrageenan-induced rat paw edema model. Each column represents a mean±standard error of the mean

Table 2: Evaluation parameters of formulations F1–F5

Formulation	Appearance	Viscosity	Spreadability	pH	Homogeneity	SIT
F1	Faint	3651	26.62	6.2	Homogeneous	No reaction
F2	Green	4325	24.82	6.8	Homogeneous	No reaction
F3	Greenish	4287	22.34	6.7	Homogeneous	No reaction
F4	Yellowish green	4652	26.84	7	Homogeneous	No reaction
F5	Green	4103	21.65	6.9	Homogeneous	No reaction

Table 3: Extrudability of F1–F5 herbal gel

Formulation	Weight of formulation	Weight of gel extruded	Extrudability amount (%)	Grade
F1	15.22	13.6	89.35	Good
F2	15.95	13.56	85.01	Good
F3	14.62	12.08	82.62	Good
F4	14.22	13.24	93.10	Good
F5	15.49	13.72	88.57	Good

Table 4: Effect of various formulations on carrageenan-induced paw edema in rats

Treatment	Paw edema volume after (Mean ± SEM)							
	1hr		2hr		3hr		4hr	
	Mean ± S.E.M.	% inhibition	Mean ± S.E.M.	% inhibition	Mean ± S.E.M.	% inhibition	Mean ± S.E.M.	% inhibition
Control	0.26±0.0062		0.47±0.03		0.59±0.06		0.75±0.0074	
F1	0.23±0.025***	11.53	0.32±0.0054***	31.91	0.34±0.0064***	42.37	0.24±0.0095***	68
F2	0.22±0.03***	15.38	0.27±0.0091**	42.55	0.26±0.097***	55.93	0.23±0.01**	69.33
F3	0.25±0.06*	3.84	0.3±0.0051	36.17	0.32±0.0091**	45.76	0.34±0.0073**	54.66
F4	0.24±0.02***	7.69	0.39±0.0064***	17.02	0.36±0.0082***	38.98	0.41±0.006***	45.33
F5	0.22±0.05	15.38	0.35±0.0084	25.53	0.37±0.006	37.28	0.43±0.0054	42.66
Standard	0.21±0.012	19.23	0.26±0.062	44.68	0.24±0.095	59.32	0.19±0.062	74.66

Values are mean ± S.E.M.(Standard Error of the Mean), n = 6, ***P<0.001, **P<0.01, *P<0.05 compared to the vehicle treated group. One way ANOVA followed by Dunnett's Test.

54.67% at 4 h, respectively, while the reference drug; diclofenac sodium reduced the inflammation by 59.32% at 3 h and 74.67% at 4 h, as shown in Table 4. Formulation F2 with capsaicinoids and AETP has shown comparable and significant percentage inhibition as that of standard diclofenac gel and percentage inhibition is also high as compared to formulation F1, which indicates a synergistic effect of capsaicinoids and AETP. F3 with capsaicinoids and AEOS has shown the same effect as that of formulation F1, which indicates AEOS does not have any synergistic effect along with capsaicinoid.

CONCLUSION

Results have shown that gel formulations are good in appearance, homogeneity, extrudability, and spread ability. Synergistic effect between capsaicinoid and AETP as an anti-inflammatory has been observed.

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