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Research Article

FORMULATION AND EVALUATION OF TOPICAL ANTI-INFLAMMATORY HERBAL GEL

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ABSTRACT

Objective: The present investigation aims at development and evaluation of herbal anti-inflammatory gel using of methanolic extract from *Emblica officinalis* fruits (MEEOF) and *Aegle marmelos* fruits (MEAMF).

Methods: The gels were prepared using Carbopol 934, various concentrations of MEEOF, MEAMF, propylene glycol 400, methylparaben, propylparaben, and required amount of distilled water. 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, skin irritation on animal model (rabbit), and 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. ICH guidelines have followed for stability studies.

Results: Results reveal that gel showed good appearance, homogeneity, and spreadability. Viscosity is ranging between 4200 and 4500 centipoises. All formulations have shown no skin irritation to animals. Formulations F4 and F5 significantly inhibited the inflammation to the extent of 56.66%, 61.66% at 3 h and 59.21%, 63.15% at 4 h, respectively, while the reference drug reduced the inflammation by 66.66% at 3 h and 76.31% at 4 h. The preparation was stable under normal storage conditions and did not produce any skin irritation, i.e., erythema and edema when applied over the skin on storage.

Conclusion: It was concluded that anti-inflammatory effect of F4 and F5 was comparable to standard.

Keywords: Herbal anti-inflammatory gel, *Aegle marmelos, Emblica officinalis.*

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INTRODUCTION

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as the increase of vascular permeability, increase of protein denaturation, and membrane alteration. It is defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area. The most common causes of inflammation are infections, burns and trauma, and many types of immune reactions [1].

Aegle marmelos is a slow-growing, medium-sized tree, up to 12–15 m tall with short trunk, thick, soft, flaking bark, and spreading, sometimes spiny branches, the lower ones drooping. This plant is having great potential to cure the diseases such as diabetes, cholesterol, peptic ulcer, inflammation, diarrhea, dysentery, anticancer, cardioprotective, antibacterial, antifungal, radioprotective, antipyretic, analgesic, constipation, respiratory infection, antioxidant, hepatoprotective, and wound healing. Fruit is rich in nutritional value and is a rich source of glucose, sugar, fiber, protein, fat, minerals, fibers, carbohydrates, calcium, phosphate, potassium, iron, Vitamins A, Vitamin B1, nicotinic acid, riboflavin, and Vitamin C. Root and fruits contain coumarins such as scoparone, scopoletin, umbelliferone, marmesin, and skimming. Fruits contain xanthotoxol, imperatorin and alloimperatorin, aegeline, and marmelline [2-10].

Emblica officinalis known as amla belongs to the *Euphorbiaceae* family. *E. officinalis* has been reported to possess potential antioxidant effect. All parts of the plant including fruit, seed, leaf, root, bark, and flower are used in various Ayurvedic/Unani herbal preparations. Amla shows antioxidant, analgesic, antipyretic, adaptogenic, immunomodulatory, and antiulcerogenic activities. *E. officinalis* contains phytoconstituents such as apigenin, gallic acid, ellagic acid, chebulinic acid, quercetin, chebulagic acid, corilagin, isostrictiniin, methyl gallate, luteolin, Emblicanin A, Emblicanin B, phyllaemblicin B, punigluconin, and pedunculagin. Gallic acid, ellagic acid, 1-O-galloyl-beta-D-glucose, 3,6-di-O-galloyl-D-glucose, chebulinic acid, quercetin, chebulagic acid, corilagin, 1,6-di-O-galloyl-beta-D-glucose, 3 methyl gallic acid (3 ethoxy 4,5 dihydroxy benzoic acid), and isostrictinin were isolated [11-22]. In spite of *A. marmelos* and *E. officinalis* has been used for the treatment of inflammation, no report exists on the development of topical dosage forms from extract of these plants. *E. officinalis* has antioxidant potential also hence the present study is aimed at formulating and investigating the effective anti-inflammatory topical gel using methanolic extracts of both plants.

METHODS

Preparation of methanolic extracts

Fruits of *Aegle marmelos* and *E. officinalis* were collected from Rahata region (Ahmednagar, Maharashtra). Botanical Survey of India, Pune, with voucher specimen number AMRGA012 and EOMRGA013 authenticates plants.

Fruits of *E. officinalis* were dried under shade after cutting into small pieces and then coarsely powdered with a mechanical grinder. The powder was passed through sieve No. 40 and extracted with methanol as solvent in Soxhlet extractor. Fresh fruit pulp of *A. marmelos* was extracted with methanol in Soxhlet apparatus. The resulting extracts were cooled and filtered. The filtrate was evaporated in vacuum to give a residue.

Formulation of topical gel

Herbal gel was prepared using gelling agent Carbopol 934 in 1% w/w concentration with deionized water using mechanical stirrer. Then, skin pH (6.8–7) was maintained by dropwise addition of tri-ethanolamine

with continuous stirring. Various concentrations 5, 10, 15, 20, and 25% w/w of both extracts were added as shown in Table 1 to the gel and stirred for sufficient time for homogeneous mixing of extract in gel base. Collapsible tubes were used for filling of prepared gel. These formulations were stored at a cool and dry place. Formulation was evaluated for following parameters [23].

Organoleptic evaluation
Physical parameters such as color and appearance were recorded.

Viscosity
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, and >70% extrudability: Fair) [24].

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 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 [25]. Spreadability was calculated using the following formula

$$S = M \times L / T$$

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 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 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 [26-28].

Table 1: Composition of various formulations containing MEEOF and MEAMF

Ingredients	Quantity in %				
	F1	F2	F3	F4	F5
MEEOF	5	10	15	20	25
MEAMF	5	10	15	20	25
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.

MEEOF: Methanolic extract from *Emblica officinalis* fruits, MEAMF: Methanolic extract from *Aegle marmelos* fruits

Homogeneity
All developed gels were packed in containers and then tested for homogeneity by visual inspection. They were tested for their appearance and presence of any aggregates [26-28].

Grittiness
All the formulations were evaluated microscopically for the presence of any appreciable particulate matter which was seen under light microscope. Hence, obviously the gel preparation fulfills the requirement of freedom from particular matter and form grittiness as desired for any topical preparation [26-28].

Stability study
ICH guidelines were followed for stability study. The formulated gel was filled in collapsible tubes and stored at different temperatures and humidity conditions, namely 25±2°C/60±5% RH, 30±2°C/65±5% RH, and 40±2°C/75±5% RH for a period of 3 months and studied for appearance, pH, and spreadability [29,30].

Skin irritation test
The intact skin of Wistar rats of either sex with average weight 150–200 g was used. The hairs were removed from the rat 3 days before the experiment. Prepared gel formulations were used on the test animal and gel base on control group. The animals were treated daily for 7 days, and erythema and edema on the treated skin were examined [31].

Evaluation of anti-inflammatory activity
Animals
Albino Wistar rats of either sex with average weight 150–200 g were used. All animals used in the study were housed in standard environmental conditions and fed with standard rodent diet with water *ad libitum*. All animal procedures were followed in three groups, namely control, test, and standard of six animals each. The Institutional Animal Ethical Committee approved protocol of experiment (CPCSEA/1093), and all the animals used in this work were treated according to the norms established by CPCSEA.

Carrageenan-induced rat paw edema
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 right hind paw of rat 1 h before each experiment. Herbal gel 0.2 g 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. 1% valdecoxib gel 0.2 g was applied in the same way as a standard. 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 plethysmometer [25,32-35]. Percentage inhibition in paw volume is calculated using the formula.

$$\%\text{Inhibition} = [\text{Paw volume}(\text{Control}) - \text{Paw volume}(\text{Test})] \times 100 \div \text{Paw volume}(\text{Control})$$

Statistical analysis
Data were reported as the mean±standard error of the mean. Data analysis was done by one-way analysis of variance followed by Dunnett's test using GraphPad version 7. Probability values of 0.05 ($p<0.05$) or less were considered statistically significant; *$p<0.05$, **$p<0.01$ ***$p<0.001$ versus control.

RESULTS

Physical evaluations of ointment formulation
The herbal gel was prepared using Carbopol 934, various concentrations of methanolic extract from *E. officinalis* fruits (MEEOF), methanolic extract from *A. marmelos* fruits (MEAMF), propylene glycol 400, methylparaben, propylparaben, distilled water, and triethanolamine. Prepared gels were subjected for appearance, viscosity, spreadability, pH, and homogeneity, and results are shown in Table 2. All gel formulations have pale green color with a translucent appearance and have smooth

feel on application which was remain same on stability testing period. All these formulations have shown optimum viscosity. The pH values of all prepared formulations ranged from 6 to 7 which is considered acceptable to avoid the risk of irritation on application to the skin. All formulations when prepared and after 3 months remain homogeneous without any gritty particle. Furthermore, the stability study's results revealed that the preparation was stable under normal storage conditions.

Extrusion of the gel

The extrusion of the gel from the tube is an important during its application and in patient 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.

Acute skin irritation study

Results of skin irritation test indicate that prepared gels were not produce irritation, redness, or edema on application and free from dermatological reaction.

Investigation of anti-inflammatory activity of various gel formulations

Anti-inflammatory activity of various gel formulations was investigated by carrageenan-induced paw edema method, and results obtained are shown in Table 4. Edema inhibition in carrageenan-induced rat paw edema by various formulations and standard 1% valdecoxib is represented in Fig. 1. Formulations with 5% and 10% extract did not show significant percent inhibition of rat paw edema, whereas formulations containing 15%, 20%, and 25% have shown significant percent inhibition. Formulations F4 and F5 significantly inhibited the inflammation to the extent of 56.66%, 61.66% at 3 h and 59.21%, 63.15% 4 h, respectively, while the reference drug reduced the inflammation by 66.66% at 3 h and 76.31% at 4 h. The anti-inflammatory effect of F4 and F5 was comparable to that of valdecoxib at respective time point.

DISCUSSION

Five different concentrations of MEEOF and MEAMF were used for preparation of topical gel formulation, and they were stable during the period of stability testing.

All formulations were subjected for investigations of anti-inflammatory activity using carrageenan-induced rat paw edema. Carrageenan-induced paw edema in rat has known as a sensitive method for studying of non-steroidal anti-inflammatory agents and shows a biphasic event which is attributed to the different mediators. At the first phase means at about 2 h after carrageenan injection, hyperemia mainly induces because of the release of histamine and serotonin, whereas prostaglandins and bradykinin potentiate the second phase of edema by mobilization of leukocytes. The edema was reached its highest thickness 4 h after the application of the stimulus [28]. Investigation anti-inflammatory efficacy of the topical gel preparations of *A. marmelos* and *E. officinalis* was best

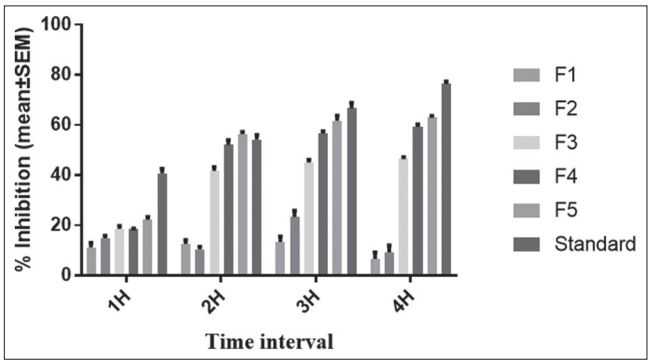

Fig. 1: Percent edema inhibition in carrageenan-induced rat paw edema by various formulations and standard 1% valdecoxib.

Table 2: Physical evaluation of various gel formulations

Formulation	Appearance	Viscosity	Spreadability	pH	Homogeneity
F1	Pale green	4520	24.36	6.3	Homogeneous
F2	Pale green	4260	22.35	6.5	Homogeneous
F3	Pale green	4300	24.83	6.8	Homogeneous
F4	Pale green	4500	19.32	7	Homogeneous
F5	Pale green	4580	19.14	6.7	Homogeneous

Table 3: Extrudability study of various gel formulations

Formulation	Weight of formulation	Weight of gel extruded	Extradibility amount (%)	Grade
F1	15.2	13.1	86.18	Good
F2	15.64	12.9	82.48	Good
F3	15.95	13.42	84.13	Good
F4	15.26	13.15	86.17	Good
F5	15.23	12.7	83.38	Good

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

Treatment	Paw volume (ml) at various time intervals after carrageenan administration							
	1 h		2 h		3 h		4h	
	Mean±SEM	%Inhibition	Mean±SEM	%Inhibition	Mean±SEM	%Inhibition	Mean±SEM	%Inhibition
Control	0.27±0.0109	-	0.48±0.095	-	0.6±0.012	-	0.76±0.062	-
F1	0.24±0.0195	11.11	0.42±0.0138	12.5	0.52±0.043	13.33	0.71±0.084	6.57
F2	0.23±0.042	14.81	0.43±0.0011	10.41	0.46±0.0028	23.33	0.69±0.0081	9.21
F3	0.22±0.0091*	18.51	0.28±0.0046	41.66	0.33±0.0027**	45	0.39±0.013**	48.68
F4	0.22±0.0099***	18.51	0.23±0.015***	52.08	0.26±0.061***	56.66	0.31±0.0083**	59.21
F5	0.21±0.048**	22.22	0.21±0.0074	56.25	0.23±0.0077**	61.66	0.28±0.0038	63.15
Standard	0.16±0.0092**	40.74	0.22±0.0085***	54.16	0.2±0.0096	66.66	0.18±0.0086***	76.31

SEM: Standard error of the mean; ***p<0.001, **p<0.01, *p<0.05 compared to the vehicle treated group. One way ANOVA followed by Dunnett's Test

demonstrated when concentrations of methanolic extract used were above 15%, and F4 and F5 formulation shown same results means that concentration range of extracts required for effective use was 15–25%. Phytochemical analysis of MEAMF showed the presence of alkaloids, terpenoids, coumarins, tannins, polysaccharides, and flavonoids [36]. *E. officinalis* contains tannins, flavonoids, phenolic compounds, saponins, terpenoids, ascorbic acids, carbohydrates, and many other compounds [20]. Flavonoids have been shown to inhibit cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, and NADPH-oxidase, all involved in reactive oxygen species generation. Another anti-inflammatory property of flavonoids is their suggested ability to inhibit neutrophil degranulation. Modulation of the activity of pro-inflammatory enzymes is one of the most important mechanisms of action for flavonoids. Pro-inflammatory enzymes, such as cytosolic phospholipase A2, cyclooxygenases, lipoxygenases, and inducible NO synthase, produce very potent inflammatory mediators, and therefore, their inhibition contributes to the overall anti-inflammatory potential of flavonoids [37,38]. *A. marmelos* and *E. officinalis* were reported to have anti-inflammatory activity, and rational behind incorporation of *E. officinalis* is its potent antioxidant effect thus potentiation of anti-inflammatory activity of prepared topical gel.

CONCLUSION

Results shown that gel formulations are good in appearance, homogeneity, extrudability, and spreadability. Formulation containing 25% methanolic extract from MEEOF and MEAMF has shown significant anti-inflammatory activity in carrageenan-induced rat paw edema model.

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AUTHORS' CONTRIBUTIONS

Mahendra A. Giri and Rasika D. Bhalke have equally contributed for thematic preparation and editing of the manuscript.

CONFLICTS OF INTEREST

The authors declared that there are no conflicts of interest.

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