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TO FORMULATE AND EVALUATE HERBAL ANTI-INFLAMMATORY GEL OF FRUIT EXTRACT OF *LAGENARIA SICERARIA*

[1] Ms. Neha R. Madavi, [2] Prof. Amit R. Jaiswal

[1]Department of Pharmaceutics, P.R. Pote Patil College of Pharmacy, Amravati

[2]Department of Pharmaceutics, P.R. Pote Patil College of Pharmacy, Amravati

Maharashtra, India

Abstract : The purpose of the current study is formulated and evaluates herbal anti-inflammatory gel of fruit extract of Lagenaria Siceraria for topical application anti-inflammatory activity. Using the methanolic fruit extract lagenaria siceraria. Gelling agent used in this study was 1% w/w concentration of carbopol- 934. The in vivo studies were conducted on Sprague dawley rats of either sex (120-200 gm). Change in edema volume of the rat hind paw was measured. The anti-inflammatory effect produced after topical administration of herbal gel formulation on Carrageenan-induced rat paw edema exhibited a high degree of reproducibility. The initial physicochemical parameters of formulations (F1, F2, F3) i.e. pH, viscosity, spreadability, homogeniety, washability and stability were also examined. The all prepared formulations F1 batch i.e., 5% of Lagenaria Siceraria herbal gel shows anti-inflammatory activity carrageenan-induced rat edema. The preparation remained stable under typical storage conditions and caused no skin irritation.

Keywords **- Herbal Gel, Inflammation, Lagenaria Siceraria Fruit Extract, Cucurbitacea, Carrageenan, Methanolic Extract, Evaluation**

1. INTRODUCTION :

Inflammation is a complicated process that is commonly related painful and includes events examples include enhanced vascular permeability, protein denaturation, and membrane. Alterations, it is a protective in reaction to manifests as redness, discomfort, heat, swelling, and a lack of function in damaged region. Inflammation is typically caused by infections, burns, trauma, and immunological disorders reactions[1].

There are mainly two types of inflammation which are as follows

1.1 Acute inflammation: Acute Inflammation is defined by the exudation of fluids and plasma proteins, as well as the movement of leukocyte, particularly neutrophils, into the wounded region. Acute inflammatory response is a defensive mechanism aimed at destroying germs, viruses, and parasites while also promoting wound repair[2]. An acute inflammation occurs when develops

quickly and becomes severe in a short period of time. The signs and symptoms are: generally only there for a few days, but might linger for a few weeks in others cases[3].

1.2 Chronic Inflammation: Chronic inflammation can develop as a result of acute inflammatory processes that occur over a period of weeks, months, or years. A variety of chemical mediators are generated during both acute and chronic inflammation. Cyclooxygenase enzymes break down arachidonic acid, releasing a vast range of inflammatory mediators, including prostaglandins. However, inflammation is a normal process in the body; it might have serious repercussions for individuals, including the appearance of pain, edema, fever and other symptoms[4].

The use of anti-inflammatory medicines is beneficial in the treatment of various diseases. The existing anti-inflammatory medicines (steroidal and nonsteroidal) have a wide spectrum of negative effects. As a result, several investigations are being conducted to identify anti-inflammatory medicines derived originated from natural sources. In this sense, medicinal herbs are commonly used utilized in traditional medicine throughout many nations to heal a variety of inflammatory disorders, including skin irritation. However, for most plants in use, the true effectiveness and active ingredients remain unclear. Consequently, experimental research targeted at demonstrating the pharmaceutical characteristics of these flora and identifying the applicable active principles are required [5, 6].

Lagenaria siceraria, sometimes known as bottle guard, is a member of the Cucurbitaceae family and the genus Lagenaria[7]. Lagenaria siceraria is an essential medicinal herb that has to treat a variety of ailments. The bottle gourd's leaf, oil, fruits, and seeds are edible, and the locals used it to cure diabetes, jaundice, colitis, ulcers, piles, hypertension, insanity, congestive heart failure, and skin diseases[8]. Chemically, Lagenaria siceraria includes physiologically active phytoconstituents such as oleanolic acid, ß- Sitosterol, campesterol, kaempferol, flavonoids, alkaloids, and isoquercetin triterpenes, and fragrant compounds. It is possible that be seen as a significant gift from nature to humanity. Scientists and researchers have claimed that Lagenaria siceraria has anthelmintic, antioxidant, antidepressant, anticancer, and antihyperlipidemic properties and cardioprotective, hepatotoxic, antianxiety, antibacterial, diuretic, analgesic, anti-inflammatory, and anti-urolithiatic properties. Global interest in herbal medicine is growing by the day, and scientific experiments the pharmacological characteristics of biologically active plant ingredients have been found to be as effective as synthetic agents in treating a variety of diseases[9].

Gel formulations are used to administer the medicine specifically, they are easier to apply, enhance the contact time and have less adverse effects than other topical preparations and oral administration[10].

In spite of Lagenaria siceraria has being used to cure inflammation; there is no record of the creation of topical dosage formulations from an excerpt one of these plants. Hence the current work aims to formulate topical gels utilizing methanolic extracts of Lagenaria siceraria plant and to investigate the different physicochemical the features of the gel and assess the anti-inflammatory properties against Carrageenan caused edema in rat paws.

Figure 1. Lagenaria siceraria Fruit

2. EXPERIMENTAL METHOD & MATERIAL

2.1 Collection and authentication of fruit material

The fruit was acquired from the vegetable market of Amravati. They were the authenticated at the Taxonomist of Department of Botany Shri Shivaji Science College, Amravati India. Were the voucher specimen was placed on the herbarium sheet.

2.2 Preparation of methanolic extraction of plant material

Lagenaria siceraria the fruits were thoroughly washed, sliced into thin circular slices, and dried. The dried fruits plant material was then ground into a gritty powder. The coarsely powdered dried fruits of Lagenaria siceraria (50 g) were extracted with methanol using the Soxhlet extraction method for 24 hours. After the extraction was completed, the solvent was distilled, concentrated in vaccuo, and stored at freezing temperatures.

Ingredients

Carbopol 934, Methylparaban, Propylparaben, Propylene glycol-400, Triethanolamine, Water.

Animal

Sprague dawley the current study employed rats of any sex that weighs between 120 - 200 g obtained from Smt. Kishoritai Bhoyar College of Pharmacy, Nagpur (New Kamptee). The Animal Ethical Committee accepted the experimental procedure in accordance with the CPCSEA rules. The rats were kept at a regulated temperature (25±2ºC) with a 12-hour the dark-light cycle. They were fed rodent Pellets and water as needed. (Reg. No: 853/PO/Re/S/04/CCSEA)

3. PRELIMINARY PHYTOCHEMICAL SCREENING

The phytoconstituents contained in the methanolic extract of Lagenaria siceraria were identified using conventional techniques.

3.1 Test for carbohydrates (Benedict's test)

In 2ml of extracts, combine 2ml Benedict's reagent is heat. The formation of orange-red precipitate suggests carbohydrates are present[11].

3.2 Test for Flavanoids (alkaline reagent test)

2ml of 2% sodium hydroxide solution was mixed with 2ml in the extract. The Yellow precipitation has appeared suggests in the presence of flavonoids[12].

3.3 Test for Triterpenoids (Salkowski's test)

In 2ml add to the extract 1ml of chloroform, followed then, a few drops of concentrated H_2SO_4 were applied to the edge in the test tube and shake well. The creation the color yellow in the lower layers confirms the existence of triterpenoids[12].

3.4 Test for Saponins (frothing test)

2ml the extract was diluted with 10ml of in a test tube, combine distilled water with agitated for 5 minutes. The presence of stability froth shows the existence of saponins[13].

3.5 Test for tannins (ferric chloride test)

2ml the extract was combined with only a few drops of a 10% Changes in ferric chloride solution in color to dark blue or green suggests the existence of gallic tannins and catechol tannins[13].

3.6 Test for glycosides (Keller-Kilani test)

4ml using one drop of glacial acetic acid 2.0% $FeCl_3$ were added to 10ml of extract, this is followed by 1ml of concentrated H_2SO_4. The Brown color formation rings in between the layers suggests the existence in cardiac glycosides[14].

Table1: Phytochemical analysis of methanol extract of lagenaria siceraria.

Sr.no.	Phytoconstituent	Test Result
1.	Carbohydrates	Absent
2.	Flavonoids	Present
3.	Triterpenoids	Present
4.	Saponins	Present
5.	Tannins	Present
6.	Glycosides	Present

4. PREFORMULATION OF DOSAGE FORM

Characterizations of Lagenaria siceraria Fruit Extract

Color: Brown

Odour: Characteristic

State: Powder Form

5. FORMULATION OF DOSAGE FORM

The dispersion technique was employed to prepare topical gels because of carbopol polymers are stirred in water at room temperature to readily disseminate it. All of the components they were weighed accurately.

Carbopol 934 was then distributed in 50 ml distilled water. While stirring constantly, methyl paraben and propyl paraben were individually diluted in 5 ml of distilled water and heated in a water bath. The solution was chilled before adding propylene glycol 400. After adding the fruit extract, the solution was combined with the carbopol 934 solution, and the volume was increased to 100 ml with distilled water. Finally, a triethanolamine (TEA) was added in suitable quantities to the mixture. While stirring continuously to adjust the appropriate strength of gel. Then the weight and pH of the gel determined[15].

Table 2: Gel composition for 25gm of lagenaria siceraria fruit extract.

		Quantity (in gm)		
Sr.no	**Ingredients**	**F1**	**F2**	**F3**
1	**MELSF**	1.25	2.5	3.75
2	**Carbopol 934**	0.25	0.25	0.25
3	**Methylparaben**	0.05	0.05	0.05
4	**Propylparaben**	0.025	0.025	0.025
5	**Propylene glycol 400**	1.25	1.25	1.25
6	**Triethanolamine**	q.s	q.s	q.s
7	**Distilled Water**	Up to 25ml	Up to 25ml	Up to 25ml

MELSF: Methanolic Extract from Lagenaria Siceraria Fruits

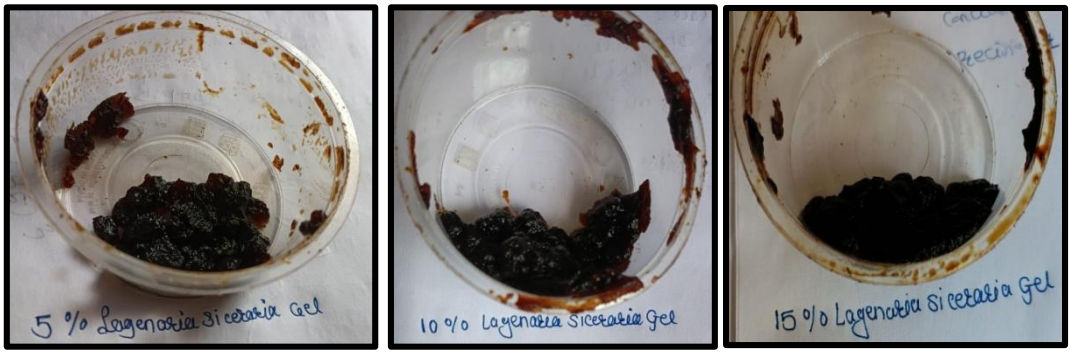


Figure 2.Various formulation of Lagenaria siceraria fruit extract herbal gel

6. PHYSICOCHEMICAL EVALUATIONS OF DOSAGE FORM

6.1 Pharmaceutical Evaluation

6.1.1 Physical appearance[16]

The colour herbal gel composition was found to be brown Appearance was determined to be smooth upon application.

6.1.2 pH

A digital pH meter was used to measure the pH of different gel compositions. One gram of gel was dissolved 50 milliliters of distilled water and kept for two hours. The pH in each formulation was measured in three times and the average results were computed.

6.1.3 Homogeneity

Following the gels had been established in the container, they were visually inspected for uniformity.

6.1.4 Washability

The composition was put to the skin, and the ease of washing with water was checked.

6.1.5 Viscosity

The viscosity of the herbal gel was evaluated use a Brookfield viscometer at 5 rpm and spindle no.6. Each reading was collected once the sample reached equilibrium after two minutes.

6.1.6 Spreadability

Spreadability relates in order to amount in which gel spreads when applied. There was spreadability determined using the slip and drag properties of gels. Two collections of glass slides with standard dimensions were taken. The herbal gel mixture was used on one of the slides. The other gel was sandwiched between two slides for a span of 7.5 cm. An excess of the gel below examination (approximately 2 g) was put on the ground slide. Finally, the gel was placed between this slide and another glass slide that has same dimensions of the fixed ground slide. A weight of one kilogram had been placed over 5 minutes at the top of the slide to remove air and create a consistent coating gel between the slides. There was too much gel scraped off the edges. The upper plate was then pulled, and the time (in seconds) taken from the top slide to travel a length of 7.5 cm was recorded. A shorter gap suggests more spreadability.

The spreadability was determined using the following formula:

$S = M \times L / T$

In this equation,

S = represents spreadability

M = represents weight in the pan (to the top slide)

L = represents glass slide length

T= represents time (in seconds) necessary to completely separate the slides

6.2 In Vivo Evaluation of Anti-inflammatory Activity Animals

Sprague dawley rats we utilized either have sex an average weight of 120-200 g. All of the animals utilized in this investigation were housed in regular circumstances and fed with a conventional rodent diet food has free access to water ad libitum. All animal treatments were implemented among three groups: control, standard, and treatment, each with six animals. The Protocol of experiment was approved by the Institutional Animal Ethical Committee. (IAECAE/853), and all every animal utilized in this study were handled in accordance with CPCSEA guidelines.

6.2.1 Determinations of anti-inflammatory activity of 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 formulation (5, 10, 15, %) will be 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% herbal gels 0.2 g were applied in the same way as a standard. Drugs or placebo were 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[17].

Percentage inhibition in paw volume is calculated using the formula.

%Inhibition = [Paw volume (Control) − Paw volume (Test)] × 100 ÷ Paw volume (Control)

Statistical analysis

Data were reported as the mean ± SEM (Standard Error Mean) and Data analysis was done by one-way analysis of variance (ANOVA) followed by Tukey's test using. Probability values of 0.05 ($p<0.05$) or less were considered statically significant:*$p<0.05$, **$p<0.01$***$p<0.001$ versus control.

7. OBSERVATIONS

7.1 Physicochemical Evaluations of Gel

The herbal gel was made with Carbopol 934, varying amounts of methanolic extraction from Lagenaria siceraria fruit (MELSF), propylene glycol 400, methylparaben, propylparaben, purified water, and triethanolamine. The prepared gels tested for appearance, viscosity, spreadability, pH, and homogeneity, and the findings are presented in Table 3. The gel compositions have a brown due with a transparent the appearance and a smooth feel when applied, which remained consistent throughout the stability testing period. All of these formulas demonstrated excellent viscosity. The pH levels of each created formulations varied from 6 to 7, which is regarded appropriate to avoid irritation when applied onto the skin. All formulas remain homogenous and free of gritty particles after three months.

Table 3: Physicochemical evaluation of various gel formulations

		Observation		
Sr.no	**Evaluation Parameter**	**F1**	**F2**	**F3**
1	Gel appearance	Brown	Brown	Brown
2	pH	6.8 -7	6.7-7	6.8-7
3	Homogeneity	Good	Good	Good
4	Washability	Good	Good	Good
5	Viscosity	4560 cps	4561cps	4562cps
7	Spradability	19.30cm	19.30cm	19.31cm

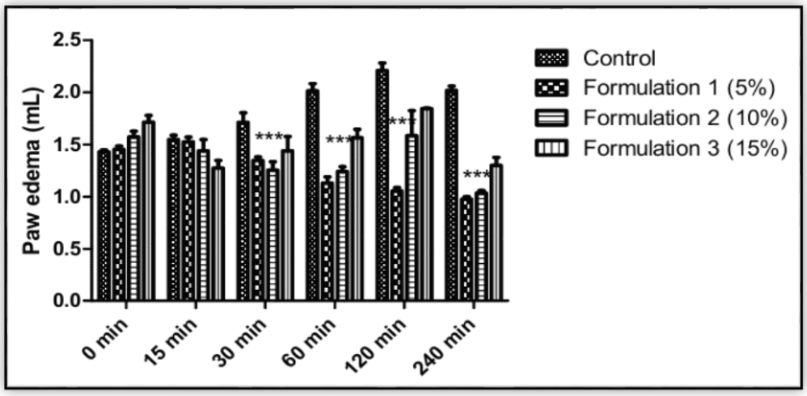
7.2 Investigation of anti-inflammatory activity of various gel formulations

The anti-inflammatory effects of different gel compositions were examined using the carrageenan-induced rat paw edema technique and the findings are presented in Table 4. & Graph no.1 depicts how various formulations reduce carrageenan-induced rat paw edema. Formulations with 5%, 10%, and 15% extract did, whereas formulations with 5%, 10%, and 15% showed substantial percent inhibition. Formulations F1 strongly decreased inflammation to the amount of -5.59% at 2 h and -3.001% at 3 h, respectively, whereas the reference medicine reduced inflammation. The anti-inflammatory impact of F1 was equivalent in comparison herbal gel at the respective time points.

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

Treatment	**Paw volume (ml) at various time intervals after carrageenan administration**					
Time	**0min**		**15 min**		**30min**	
	Mean±SEM	**%Inhibition**	**Mean±SEM**	**%Inhibition**	**Mean±SEM**	**%Inhibition**
Control	1.43±0.016667	-	1.54833±0.037872	-	1.7133±0.08348	-
F1	1.455±0.02826	-	1.525±0.043509	-17.8013	1.3483±0.030131	-23.3361
F2	1.5733±0.0508	-	1.44±0.099972	-21.7151	1.255±0.074377	1.647564
F3	1.715±0.05919	-	1.275±0.067361	36.73962	1.44±0.126557	7.085466
Time	**60min**		**120 min**		**240mim**	
	Mean ± SEM	**%Inhibition**	**Mean ±SEM**	**%Inhibition**	**Mean ±SEM**	**% Inhibition**
Control	2.01666±0.061854	-	2.21±0.066416		2.02±0.037859	-
F1	1.13±0.054671	-7.11573	1.055±0.30162	-5.59814	0.97833±0.20872	-3.00199
F2	1.241667±0.04251	7.801469	1.58666±0.21797	0.075473	1.03667±0.0213	-2.0265
F3	1.565±0.075526	-2.83093	1.845±0.006124	-1253.77	1.30166±0.07021	-6.03504

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 Taukey Test.



Time Intervals (in min)

Graph1: The percentages of edema inhibition in carrageenan-induced rat paw edema by different formulations.

Figure 3.Evaluation of anti-inflammatory activity of formulated herbal gel

8. DISCUSSION

Three different concentrations of MELSF were used for preparation of herbal gel formulation.

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 15min 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 3h after the application of the stimulus. Investigation anti-inflammatory efficacy of the herbal gel preparations of Lagnaria siceraria was best demonstrated when concentrations of methanolic extract used were 5 %(F1) formulation shown same results means that concentration range of extracts required for effective use was 5%. Phytochemical analysis of MELSF showed the presence of flavonoids. Lagnaria siceraria contains tannins, flavonoids, , saponins, terpenoids, ascorbic acids, carbohydrates, and many other compounds . 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.Lagenaria siceraria were reported to have anti-inflammatory activity, effect thus potentiation of anti-inflammatory activity of prepared herbal gel.

9. CONCLUSION

The results show that herbal gel formulations have acceptable appearance, homogeneity, washability, viscosity, and spreadability. A formulation containing 5% methanolic extract of MELSF demonstrated considerable Anti-inflammatory activity in a carrageenan-induced rat paw edema method.

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