Original Article

Formulation And Evaluation Of Hydrogel Containing Martynia Annua And Ocimum Sanctum Leaves Extract For Anti-Inflammatory Activity

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Abstract

Objective: The purpose of this research was to develop a hydrogel formulation of the methanolic fraction of Martynia annua and Ocimum sanctum and evaluate its potential anti-inflammatory effects in a number of animal models.

Materials and Methods: The leaves of Martynia annua and Ocimum sanctum were used in the preparation of six unique hydrogel formulations, five of which contained extract, and one of which did not contain any extract at all. This was done in accordance with the standard method, and different proportions of Carbopol 940 and sodium carboxymethyl cellulose (CMC) were used. The prepared hydrogel was evaluated for optimization purposes and assessed for anti-inflammatory efficacy in mice with ear edema caused by Xylene and Croton oil, respectively. The effect was seen by the assessment of the percent inhibition of ear edema as well as biochemical indicators such as the level of nitric oxide and the level of myeloperoxidase (MPO).

Results: Results from a phytochemical analysis of M. annua and o. sanctum petroleum ether extract revealed the presence of sterols, terpenoids, and fatty oils, while results from an analysis of ethanol extract of both plant leaves revealed the presence of glycosides, phenolic compounds, flavonoids, and amino acids. Hydrogels produced from the methanol fraction of O. sanctum and M. annua have been shown to have anti-inflammatory properties, according to the current investigation. The findings that croton oil may stimulate neutrophil recruitment to mice ear tissue. Fortunately, Voltaren Emulgel and extract hydrogels were shown to considerably ($P<0.01$) lower MPO levels and NO levels in the ears of mice, suggesting that they might help solve the issue.

Conclusion: Ear edema caused by xylene was inhibited by a methanol fraction of leaves from M. annua and Ocimum sanctum. This may be because the extract blocks the production, release, or activity of xylene, a compound thought to have a role in the inflammation. Inhibiting the production, release, or activity of histamine is how methanol extracts show their anti-inflammatory effects. Flavonoids in the methanol extract fraction of M. annua and Ocimum sanctum leaves may account for their noteworthy activity.

Key words: Anti-inflammatory, Carbopol 940, hydrogel, Martynia annua, Ocimum sanctum ,Voltaren Emulgel

INTRODUCTION

Abnormal inflammation may cause persistent pain, redness, swelling, stiffness, and normal tissue damage, and is often the result of inflammatory illnesses that trigger the immune system to attack the body's cells or tissues. After an infection or injury to a tissue, the host's immune system launches a coordinated reaction that protects and heals the tissue using molecular signals provided by either the host or the disease agent. [1] Inflamed areas have an increased blood flow due to vasodilation. As a result, fluid, big molecules, and white blood cells are allowed to pass through the capillaries from the blood into the tissue. Chemotaxis directs white blood cells (especially neutrophils and monocytes) to the location of an injury. [2,3] The inflammatory response is characterized by redness, heat, swelling, and discomfort. Increased blood flow causes skin to become red and hot to the touch. The accumulation of fluid and white blood cells at the site of inflammation causes swelling. Inflammation causes pain due to the production of chemical substances and the compression of nerves in the area. [4]

Martynia annua Linn is a popular plant used to alleviate a variety of symptoms, including urological discomfort, inflammation, sore throat, scabies, and itchiness. Santal tribal members in India use the whole plant topically to treat a variety of ailments, including high body temperature, thinning hair, scabies, ulcers, and carbuncles. Many people in rural regions also use the paste made from M. annua Linn's leaves to treat wounds on themselves or their pets. Seeds include fatty acids such palmitic, stearic, and arachidic, while the leaves contain chlorogenic acid. Leaf components include [5] P-hydroxy benzoic acid, snapic acid, and fatty acids such palmitic acid and stearic acid. [6]

M. annua was extracted using both water and alcohol, and the results showed that it contains 28 different compounds, the majority of which are composed of oleic acid. Other prominent biological components include pelargonidin- 3-5-diglucoside, cyanidin-3- galactoside, p-hydroxy benzoic acid, gentisic acid, arachidonic acid, linoleic acid, palmitic acid, stearic acid, apigenin, and apigenin-7-oglucuronide. [6,7]

An fragrant perennial herb belonging to the Lamiaceae family, Ocimum sanctum is also known as holy basil, tulsi, simply tulsi. Traditional medicinal applications for the Tulsi plant include expectorant, analgesic, anticancer, antiasthmatic, antiemetic, diaphoretic, antidiabetic, antifertility, hepatoprotective, hypotensive, hypolipidemic, and antistress agent uses for the plant's leaves, blossoms, stem, root, and seeds. A tulsi aqueous decoction has also been used to treat a variety of conditions, including fever, bronchitis, arthritis, and convulsions, amongst others. While the leaf volatile oil[2] contains eugenol (1-hydroxy-2- methoxy-4-allylbenzene), eugenol (also called eugenic acid), ursolic acid, carvacrol (5-isopropyl-2-methylphenol), linalool (3,7-dimethylocta-1,6-dien-3-ol), limatrol, caryophyllene (4,11,11-trimethyl Xylose and polysaccharides are the components that make up the sugars. [3]

In light of the findings of earlier studies and the existing body of published research, the purpose of the current investigation was to assess the anti-inflammatory potential of M. annua Linn and Ocimum sanctum leaves, as well as in the form of a hydrogel formulation, were tested using a variety of animal models. Both of these plant leaves were chosen because of the traditional knowledge and chemical contents that have been documented in the accessible literature as being beneficial for inflammatory conditions.

MATERIALS AND METHODS

Chemicals and Reagents

The following chemicals were obtained from Otto Chemie Pvt. Ltd. in Mumbai, India: carbopol 940; sodium carboxy methyl cellulose; methyl paraben; and propyl paraben. The following chemicals were obtained from Sigma-Aldrich in Mumbai, India: triethanolamine, myeloperoxidase (MPO), nitric oxide (NO), naphthylethylenediamine dihydrochloride, sulfanilamide, and hexadecyltrimethylammonium bromide. Both the xylene and the croton oil were acquired from the HiMedia Lab in Mumbai, India. The analytical grade was used for all general reagents and solvents that were utilised.

Identification and Collection of Plant Materials

During the months of August and September in 2018, plant material consisting of M. annua leaves and Ocimum sanctum leaves was gathered from the area around SRK University Campus in Bhopal, Madhya Pradesh, India. The Jawaharlal Nehru Krishi Vishwavidyalaya in Jabalpur was responsible for the

identification and verification of the plant specimens. The plant leaves that were collected should be dried in the shade to produce a powder, and then they should be stored for further processing, such as extraction and other investigations.

Extraction and Phytochemical Screening

For the extraction process, dried powdered M. annua leaves as well as Ocimum sanctum leaves were used (200 g each). Following the first extraction of plant components with petroleum ether for defatting, the mark was allowed to dry before undergoing a second round of extraction with ethanol using the Soxhlet apparatus technique.

Following the completion of the extraction process, the ethanol was removed in its entirety using a vacuum evaporator while the pressure was lowered. As a result, a totally dry extract was produced for further applications. The leaves of M. annua and Ocimum sanctum were used to create petroleum ether and ethanol extracts, which were then put through qualitative analytical testing to determine whether or not certain chemical ingredients were present. [8-10]

Fractionation of Ethanol Extract of M. annua and Ocimum sanctum Leaves

Both a chloroform soluble fraction and a chloroform insoluble fraction were produced by repeatedly treating the filtrate ethanol extract with chloroform after first concentrating it and then thoroughly drying it out. After repeatedly dissolving the chloroform insoluble fraction in methanol, we were able to get the methanol insoluble fraction as well as the methanol soluble fraction. A higher yield may be obtained from the methanol soluble fraction as opposed to the methanol insoluble fraction. The methanol-soluble fraction was subjected to concentration and drying before being used as a topic for subsequent studies and formulation production.

Formulation Preparation and Characterization

The modified procedure of Chirayath et al. was used to generate six distinct hydrogel formulations, five of which included extract, one of which did not contain extract, and one of which served as a control. [11] To summarize, 100 milliliters of distilled water was stirred continuously while several concentrations of carbopol 940 and sodium carboxymethyl cellulose (CMC) were added to the mixture. We started with around 10 milliliters of distilled water, then dissolved the necessary amount of methylparaben and propylparaben in it using heating on a water bath, and finally allowed it to cool. This solution was subsequently supplemented with glycerin. To obtain the optimal formulation, an extract concentration of precisely 1% w/w was measured out and added to the previously swollen polymer while it was being continuously stirred at 650 rpm in a close vessel and the temperature was kept at 29 degrees Celsius. This process was repeated until a homogeneous gel was obtained, and the volume was brought up to 200 ml by adding any remaining distilled water. After all was said and done, the formulation was given the necessary quantity of 97% triethanolamine, which was added drop by drop in order to achieve the desired pH range for the skin (between 6.8 and 7), and it was mixed gently in order to achieve a homogenous mixture [Table 1 and 2]. For the manufacture of the control formulation, a process very identical to the one described above was followed, but no plant extract was used.

Table 1: Formulations of hydrogel containing methanol extract of Martynia annua leaves (MAMFH)

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**
Carbopol 940 (gm)	0.5	1	1.5	2	3
Sodium CMC	3	2	1	1	0.5
Extract (%w/w)	1	1	1	1	1
Propylene glycol 400 (5%)	5	5	5	5	5
Methyl Paraben (0.5%) (ml)	0.2	0.2	0.2	0.2	0.2
Propyl Paraben (0.2%) (ml)	5	5	5	5	5
Triethanolamine (ml)	q. s.	q. s.	q. s.	q. s.	q. s.

Distilled water (ml) q.s.	100	100	100	100	100

Table 2: Formulations of hydrogel containing methanol fraction of Ocimum sanctum leaves (OCMFH)

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**
Carbopol 940 (gm)	0.5	1	1.5	2	3
Sodium CMC	3	2	1	1	0.5
Extract (%w/w)	1	1	1	1	1
Propylene glycol 400 (5%)	5	5	5	5	5
Methyl Paraben (0.5%) (ml)	0.2	0.2	0.2	0.2	0.2
Propyl Paraben (0.2%) (ml)	5	5	5	5	5
Triethanolamine (ml)	q. s.	q. s.	q. s.	q. s.	q. s.
Distilled water (ml) q.s.	100	100	100	100	100

Characterization of Prepared Hydrogel Containing Methanol Extract of M. annua Leaves (MAMFH)

Physical characters

Visual observations were made of the herbal gel's color and look, both of which are examples of physical criteria. After placing the gels in the container, all of the generated gels were visually inspected to determine whether or not they met the criteria for homogeneity. They were examined to determine their appearance and whether or not any aggregates were present.

Measurement of pH

A digital pH meter was used in order to ascertain the pH of the different gel compositions. Two grams of gel were dissolved in two hundred milliliters of distilled water, and the mixture was let to sit for two and a half hours. Three separate readings of the pH of each formulation were taken, and an average was then determined from those readings.

Spreadability

The instrument used to measure spreadability was a wooden block with a pulley attached to one end. This block was placed at the center of the table. The slip and drag properties of gels were used as the measurement foundation for this approach, which examined the spreadability of gels. On the ground slide, an excess of the gel (approximately 3 grams' worth) that was being studied was deposited. After that, the gel was placed between this glass slide and another glass slide that had the dimensions of a permanent ground slide and was equipped with a hook. A weight of one kilogram was put on top of the two slides for a period of six minutes in order to remove air from the space between the slides and to generate a uniform layer of the gel. The borders of the gel were cleaned of any excess that had accumulated there. After that, a pull of 90 g weight was applied to the top plate with the assistance of a string that was linked to the hook, and the amount of time that it took for the top slide to travel a distance of 8.0 cm was timed and recorded in seconds. [12] When compared to longer intervals, higher spreadability is shown by shorter ones.Spreadability was calculated using the formula given below:

$S = M \times L/T$

Where, S = Spreadability, M = Weight in the pan (tied to the upper slide), L = Length moved by the glass slide and T = Time (in sec.) taken to separate the slide completely each other.

Viscosity

The Brookfield viscometer with spindle No. 7 at 50 revolutions per minute and room temperature was used to test the gel's viscosity. The reading on the matching dial was taken down. The dial reading was

multiplied by a factor that was provided in the user manual for the Brookfield Viscometer in order to determine the viscosity of the gel.

Drug content determination

Two grams of the produced gel were combined with two hundred milliliters of the appropriate solvent, which was ethyl alcohol. After filtering the stock solution, aliquots of various concentrations were generated by appropriate dilutions, and the drug content was measured by measuring the absorbance at 253 nm using a UV/Vis spectrophotometer (Shimadzu UV 1700).

In vitro drug release study of hydrogel

For the in vitro release investigations, a Franz diffusion cell of 3.7 centimeters in diameter was used. As a permeation cell, we used a glass tube that measured 10 centimeters in height and had an exterior diameter of 3.7 centimeters. A sample of exactly 1 gram was weighed, and then it was positioned on a semipermeable cellophane membrane such that it occupied a circle with a diameter of 3.7 centimeters. Rubber bands were used to create a watertight seal around the laden membrane as it was stretched over the bottom open end of a glass tube with a diameter of 3.7 centimeters. The tube, which served as the donor compartment, was submerged in a beaker that had 100 milliliters of phosphate buffer with a pH value of 6.8. (receptor compartment). After being submerged to a depth of one centimeter below the surface of the buffer, the cell was removed. Throughout the duration of the experiment, a magnetic stirrer was used to keep the temperature of the system at 37 1 degrees Celsius and the speed at 30 revolutions per minute. After taking samples of 3 milliliters each at 15, 30, 45, 60, 90, 120, 180, and 240 minute intervals, the volume of each sample was replaced with the same amount of freshly prepared buffer in order to keep the volume constant. Spectrophotometric analysis was performed at 253 nm on the samples without dilution or filtration in order to determine the amount of luteolin present. [13,14] In order to quantify the quantity of medication that was released, a calibration curve of luteolin was developed using known concentrations that were within the required range.

Dermal irritation study of hydrogel formulation

In order to assess whether or not a single topical application of hydrogel has the ability to irritate the skin of rabbits, a basic skin irritation test was carried out on the animals. We gave three young adult albino rabbits of either sex unrestricted access to the lab and fed them a commercial pellet meal with water available on demand. All of the rabbits were in good health. Before beginning the dosing process, the animals spent a total of nine days becoming used to the environment of the laboratory. The temperature in the animal room was maintained at a stable range of 19–24 degrees Celsius. A tiny animal clipper was used the day before the treatment to remove hairs from the dorsal and trunk areas of rabbits.

On the day when the dose was administered, but before it was applied, the animals' health was assessed, and an examination of the skin was done to look for any abnormalities. There was no evidence of any prior skin inflammation. After caging the animals, we applied 2–3 grams of the hydrogel to a 6 cm2 intact region on each animal. After being exposed to hydrogel for a period of four hours, the test locations were meticulously cleansed to remove any trace of the chemical that had been used. Following the removal of the hydrogel, roughly 1, 24, 48, and 72 hours later, each individual assessment of the test dosage was graded using the Draize Scoring System. [15] The main dermal irritation index was used to determine the degree of irritancy that a substance exhibited.

$$\text{PDII} = \frac{(\text{PDI for } 1, 24, 48 \text{ and } 72 \text{ h})}{4}$$

Stability studies of hydrogel formulation

The ICH standards require that all of the manufactured hydrogel formulations be tested for stability for a period of six months at a temperature and relative humidity (RH) of 40°C 2°C and 75% RH 5% RH, respectively. The acceptability and stability of the formulations were taken into consideration when evaluating the hydrogel's long-term stability in terms of the physical changes that may occur. In order to

assess hydrogel formulations, physical changes such as phase separation and color changes, odor, consistency of the formulations, which in turn affected their stability, and other desirable formulation features were taken into consideration.

For a period of 29 days, test samples of the hydrogel formulation were maintained in a variety of temperature settings, including at 39 degrees Celsius and at room temperature. The samples were checked at regular intervals to look for any signs of physical deterioration, such as changes in consistency or the appearance of an unpleasant color or smell. A centrifuge was used to assess the stability of the formed hydrogel during centrifugation in graded cylinders of 10 milliliters capacity for a period of 12 minutes at a speed of 10,000 revolutions per minute (Remi). The formulation that demonstrated resistance to the centrifugation process was chosen for further testing and assessment.

Anti-inflammatory Activity

Animal protocol

The mice used in the anti-inflammatory research were Swiss albino mice of either sex that weighed between 95 and 100 g and were in good health. They will spend one week prior to the experiment being conducted in the animal home, which will have a regulated room temperature of 25.2 degrees Celsius, relative humidity ranging from 44–56%, and light and dark cycles of 10 and 14 hours, respectively. The procedures for the care of the animals and the experiments themselves were carried out in compliance with CPCSEA and IAEC. Animals were chosen at random, tagged to enable for individual identification, and then housed in their cages for at least seven days prior to receiving their doses so that they could get used to the circumstances of the laboratory. For the purpose of the experiment, the animals will be divided into groups and kept in polyacrylic cages. The animals have unrestricted access to food and water at all times. However, before the surgical treatment, they had to abstain from food and drink for a period of forty-eight hours. The animals were separated into the following groups, each of which included five of the respective animal species.

Group I was control and given hydrogel base without extract, topically to each animal in the group. **Groups II** was given topically prepared hydrogel of methanol fraction of Martynia annua (MAMFH) to each rat of group. **Groups III,** was given prepared hydrogel of methanol fraction of Ocimum sanctum (OSMFH) topically to each animal of group. **Group IV** was referred as standard and given Voltaren Emulgel (1%, Diclofenac Sodium; Novartis India Ltd) topically to each animal of group

Xylene-induced ear edema

On the anterior surface of the right ear of Swiss albino mice, a topical application of extract hydrogel formulation (20 mg/ear) was administered, and at the same time, xylene (0.05 ml) was put on the posterior surface of the same ear. On the front surface of the animal's anterior ear, the control animals were given an identical amount of plain vehicle gel. The treatment for the left ear was never completed. Three hours after applying xylene to both ears, the ear lobes on both ears were cut off and weighed. As a measurement of edema, the difference in weight between the right ear, which had been treated, and the left ear, which had not been treated, was computed. [16] The level of inhibition (%) of edema was calculated using following equation:

$$\%\mathbf{edema} = \frac{\mathbf{wt\ of\ right\ ear} - \mathbf{wt\ of\ left\ ear}}{\mathbf{wt\ of\ left\ ear}}$$

Edema was expressed as the percentage difference between the weight of the inflamed right ear and the non-inflamed left ear of each mouse.

Croton oil-induced ear edema

The acute anti-inflammatory potential test was carried out in accordance with the technique that had been previously described, but with some minor adjustments. [17] On the inner surface of the right ear of each

mouse, 20 L of a fresh solution of 2.5% croton oil was topically administered, while an equivalent volume of gel base was placed as a control on the surface of the left ear. The animals were slaughtered six hours after the inflammation was induced, and ear punch biopsies with a diameter of six millimeters were taken from each of them. Ear punch biopsies were individually weighed using an analytical balance immediately after collection for the purpose of edema measurement and to quantify % edema inhibition in a manner that was comparable to the procedure that had been used before. Following this step, mouse ears were saved for further analysis in order to quantify inflammatory factors.

Ear biopsies that were collected were minced and homogenized in a volume of 500 L of phosphate buffered saline with a pH of 7.4. (137 mMNaCl, 3 mMKCl, 1.5 mM KH2 PO4 , and 10 mM Na2 HPO4). After that, the samples were centrifuged for ten minutes at a speed of 10,000 g and 4 degrees Celsius. The supernatants were used for the purpose of quantifying nitrite, which served as an indirect marker for the formation of nitric oxide (NO). Following that, the pellets were used for the determination of the myeloperoxidase (MPO) enzyme, which served as an indirect marker of neutrophil migration.

Determination of NO

The inflammatory response causes an increase in the synthesis and release of inflammatory mediators, such as cytokines, reactive oxygen species (ROS), and arachidonic acid derivatives, including prostaglandins E2. This damage to tissue is caused by the combination of the release of reactive oxygen species (ROS) and reactive nitrogen oxides (NO), which occurs during the inflammatory process. In addition to this, NO is an effective vasodilator, which plays a role in the process of inflammation and contributes to the development of edema. [18-20]

The levels of NO in the ears of mice were measured in an indirect manner using the Griess reaction. This reaction measures nitrite, which is produced when NO reacts with oxygen. [21] In a nutshell, 50 L of the supernatant from each ear was combined with 50 L of 1% sulfanilamide in 5% phosphoric acid, and the mixture was then left to incubate in the dark at a temperature of 22 degrees Celsius for five minutes. The absorbance at 540 nm was then measured using a microplate reader after 50 L of 0.1% naphthylethylenediamine dihydrochloride had been introduced in the previous step (ELISA, Micro Lab, Ahmedabad, India). The quantity of nitrite was determined using a sodium nitrite standard curve, and the results were presented as the number of nmol of nitrite that was present in each ear.

MPO enzyme estimation

According to the procedure outlined by Krawisz et al., the activity of tissue MPO was analyzed 24 hours after croton oil was applied to the mouse ear. [22] In a nutshell, 6 mm of ear tissue was punched, then chopped, and then homogenized in 10 mL of ice cold, 50 mM potassium phosphate buffer (pH 6) containing 0.5% hexadecyltrimethylammonium bromide. The buffer was mixed at a pH of 6. Sonication and centrifugation at 4 degrees Celsius for twenty minutes and twelve thousand grams were performed on the homogenate (Remi Centrifuge, India). In order to evaluate the MPO activity spectrophotometrically, 0.1 mL of the supernatant was mixed with 2.9 mL of a 50 mM phosphate buffer that was dissolved in 0.0005% hydrogen peroxide. At a wavelength of 460 nm, the enzyme activity was measured. Change in absorbance measured over the course of one minute at room temperature was used to define one unit of MPO activity. The activity was reported as a percentage of MPO in comparison to rats that had been treated with vehicle.

Histological study

Biopsies of the ears were collected from subjects in the control group, the test group, and the standard group. These samples were then fixed in a 10% buffered formaldehyde solution, dried, and embedded in paraffin. In order to evaluate the extent of the edema and the leukocyte infiltration, sections with a thickness of 5 micrometers were prepared, stained with hematoxylin and eosin, and seen by light microscopy at a magnification of 100. [23,24]

Statistical Analysis

All statistical data were given with the values as a mean along with a standard deviation. There were a total of six animals included in the study, and results were deemed statistically significant if P was less than 0.05. The difference in mean values that were determined by doing an analysis of variance (ANOVA) on the data in one direction and then using Tukey's test for multiple comparisons. GraphPad Instat Software was used to do the statistical analysis on the data.

RESULTS

In order to get the ethanol extracts, the dried powder of M. annua and O. sanctum was first defatted with petroleum ether, and then extracted with ethanol. The yields of the petroleum ether extract and the ethanol extract of M. annua were found to be 2.6% and 3.4% w/w, respectively. The powder of the leaves of O. sanctum was subjected to extraction with petroleum ether and ethanol, and the yield of both extracts was found to be 2.1 and 2.7%w/w, respectively. The ethanol extract was then concentrated using a vacuum, and it was stored away for later use. Petroleum ether extract of M. annua showed the presence of sterols, terpenoids, and fatty oils. Ethanol extract of M. annua leaves showed the presence of glycosides, phenolic compounds, flavonoids, and amino acids. On the other hand, ethanol extract of O. sanctum showed the presence of alkaloids, glycosides, and flavonoids. Petroleum ether extract of O. sanctum showed the presence of steroids and fatty acid.

Formulations Preparation and Characterization

A number of the hydrogel's physical qualities, including its color, appearance, homogeneity, consistency, phase separation, and odor, were evaluated, and the results are shown in Table 3 and 4.

Table 3 Physical properties of prepared hydrogel formulation containing methanol fraction of Martynia annua leaves (MAMFH)

Parameters	F1	F2	F3	F4	F5
Appearance	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Color	Brownish	Brownish	Brownish	Brownish	Brownish
Odor	Odorless	Odorless	Odorless	Odorless	Odorless
Consistency	Good	Good	Good	Good	Good
Phase separation	No Phase separation	No Phase separation	No Phase separation	No Phase separation	No Phase separation

Table 4 Physical properties of prepared hydrogel formulation containing methanol fraction of Ocimum sanctum leaves (OCMFH)

Parameters	F1	F2	F3	F4	F5
Appearance	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Color	Brownish	Brownish	Brownish	Brownish	Brownish
Odor	Odorless	Odorless	Odorless	Odorless	Odorless
Consistency	Good	Good	Good	Good	Good
Phase separation	No Phase separation	No Phase separation	No Phase separation	No Phase separation	No Phase separation

At room temperature, the hydrogels of both plant leaves extract that had been created were analyzed for their pH value, viscosity, and their ability to be spread out [Table 5 and 6].

Table 5 Evaluation of prepared hydrogel formulations containing methanol extract of Martynia annua leaves (MAMFH)

Parameters	F1	F2	F3	F4	F5
pH	6.98	6.97	7.01	6.98	6.99
Viscosity (cps)	195200	194400	197500	193600	197200

Spreadability (g.cm/s)	14.27±0.51 *	17.64±0.75*	15.82±0.62*	14.37±0.84*	16.82±028*

*n=5

Table 6 Evaluation of prepared hydrogel formulations containing methanol fraction of Ocimum sanctum leaves (OCMFH)

Parameters	**F1**	**F2**	**F3**	**F4**	**F5**
pH	**7.02**	**6.96**	**7.01**	**7.02**	**6.95**
Viscosity	193200	194600	196100	194100	195100
Spreadability (g.cm/s)	15.75±0.47*	16.85±0.76*	16.21±0.28*	15.67±0.58*	16.62±0.77*

*n=5

At the beginning of the process, the pH of the manufactured hydrogel was tested using a pH meter (Systronics, India), and the results showed a range of 6.97–7.01 at room temperature. Therefore, it was preferable for the skin that the pH of the gel have a neutral value since these ingredients did not disrupt the physiology of the skin. At the beginning of the hydrogel formation process, the gel had a viscosity of between 193600 and 197500 cps, and this was measured for formulations that included methanol extract of M. annua leaves (MAMFH). Spreadability of hydrogel formulations was measured to be in the range of 14.27–17.64 g.cm/s, whereas cps were measured for hydrogel formulations that included the methanol fraction of O. sanctum leaves (OCMFH).

The drug content, measured as the luteolin concentration, was determined to be 0.084 g/ml for MAMFH and 0.053 g/ml for OCMFH when the spectrophotometric technique was used to analyze the samples. This information served as the foundation for a research on the drug release from hydrogel formulations. In the beginning, it was noted that the percentage of drug release from the hydrogel that included methanol extract of M. annua leaves (MAMFH) was 8.68% (at 15 minutes) and 52.62% (after 240 minutes), respectively, for the F1 formulation. By first, it was noted that the percentage of drug release from the hydrogel containing the methanol fraction of O. sanctum leaves (OCMFH) was 52.62%, and at 240 minutes, it dropped to 50.83% for the F1 formulation.

When compared to the other formulations, the extract in the F3 formulation showed the greatest percentage of drug release after 240 minutes (66.07), as shown in Figure 1 and 2. According to these findings, the hydrogel formulations that included 1.5% Carbopol 940 and 1% Sodium CMC had a larger proportion of luteolin released after 240 minutes. Up to 240 minutes on the clock, every other combination of hydrogel showed a lower proportion of luteolin being released.

The skin irritation investigation was carried out on Swiss albino mice with the purpose of determining how mild the produced formulations were in comparison to the skin irritation [Table 7 and 8]. The improved formulation F3 did not seem to have any unfavorable effects on the skin of rabbits. The hydrogels that were created all underwent a stability test in accordance with the ICH standard (2013), which consisted of storing them at 27 1 degrees Celsius for about 30 days while monitoring their physical characteristics.

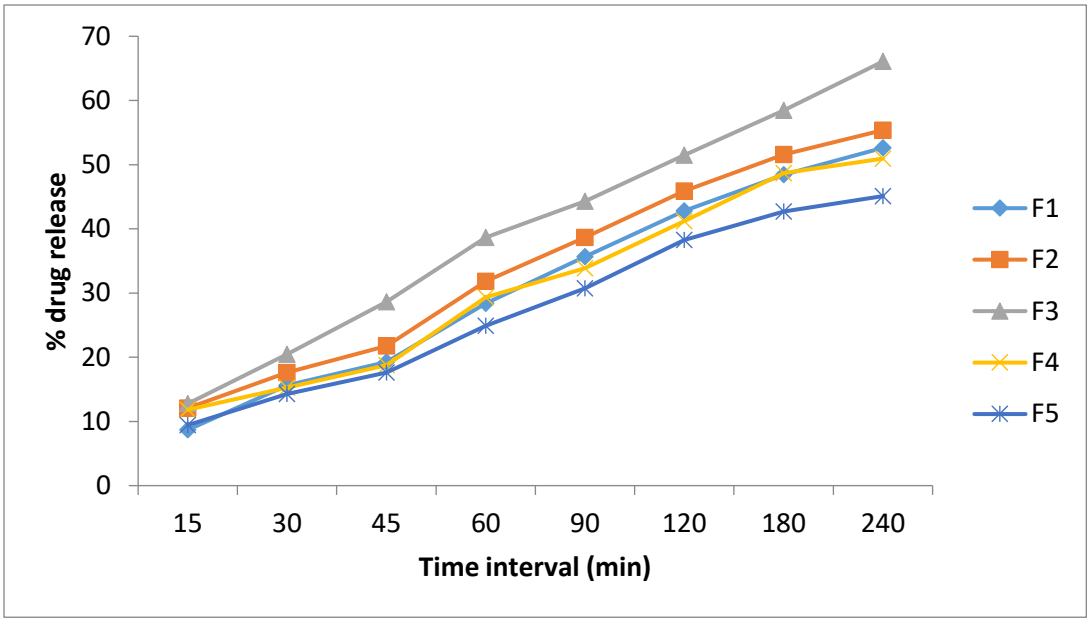

Figure 5.2: Percentage Drug release of different formulations containing methanol extract of Martynia annua leaves

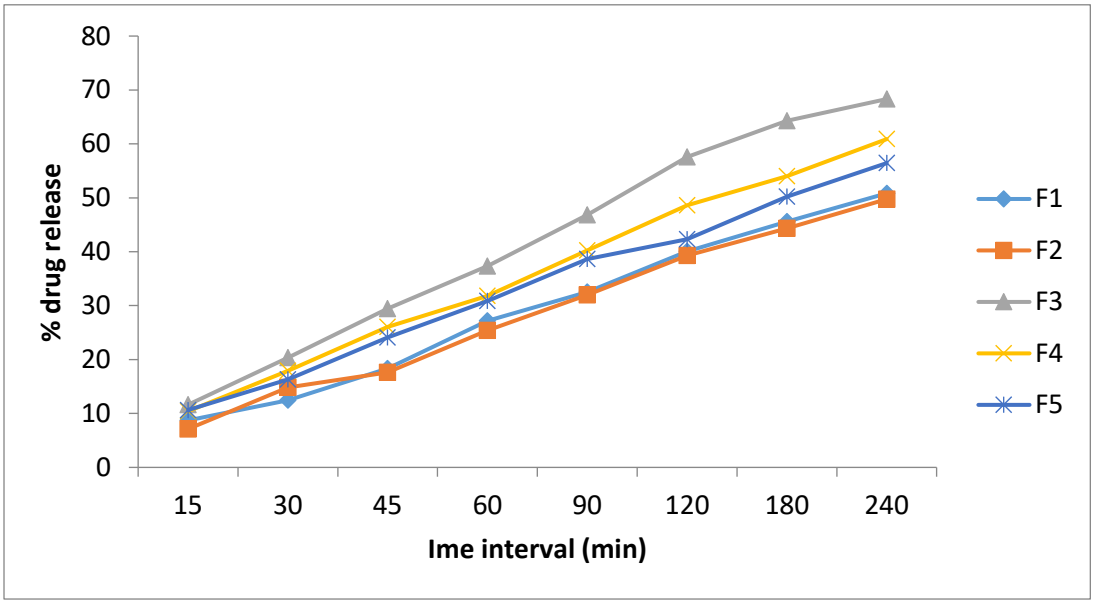

Figure 5.3: Percentage Drug release of different formulations containing methanol fraction of Ocimum sanctum leaves (OCMFH)

Table 7. Primary dermal irritation scores in albino rabbits after exposure to Hydrogels containing methanol extract of Martynia annua leaves

Formulations	Time post instillation (hr)	Incidence of dermal irritation		Total PDIα (Mean score)	Primary Dermal Irritation Index(PDII)
		Erythema	Edema		
F1	1	0	0	0	0.5
	24	1	0	1	
	48	1	0	1	
	72	0	0	0	
F2	1	0	0	0	0.25
	24	1	0	1	

	48	0	0	0	
	72	0	0	0	
F3	1	0	0	0	0.25
	24	1	0	1	
	48	0	0	0	
	72	0	0	0	
F4	1	0	0	0	0.25
	24	1	0	1	
	48	0	0	0	
	72	0	0	0	
F5	1	0	0	0	0.25
	24	1	0	1	
	48	0	0	0	
	72	0	0	0	
	1	0	0	0	0.25
	24	1	0	1	
	48	0	0	0	
	72	0	0	0	

n = 3 albino rabbits; α = Primary dermal irritation = Average erythema + Average edema

Table 8 Primary dermal irritation scores in albino rabbits after exposure to Hydrogels containing methanol fraction of Ocimum sanctum leaves (OCMFH)

Formulations	Time post instillation (hr)	Incidence of dermal irritation		Total PDIα (Mean score)	Primary Dermal Irritation Index (PDII)
		Erythema	Edema		
F1	1	0	0	0	0.5
	24	1	0	1	
	48	1	0	1	
	72	0	0	0	
F2	1	0	0	0	0.25
	24	1	0	1	
	48	0	0	0	
	72	0	0	0	
F3	1	0	0	0	0.25
	24	1	0	1	
	48	0	0	0	
	72	0	0	0	
F4	1	0	0	0	0.5
	24	1	0	1	
	48	1	0	1	
	72	0	0	0	
F5	1	0	0	0	0.25
	24	1	0	1	
	48	0	0	0	
	72	0	0	0	
	1	0	0	0	0.5
	24	1	0	1	
	48	1	0	1	
	72	0	0	0	

n = 3 albino rabbits; α = Primary dermal irritation = Average erythema + Average edema

After a period of 30 days, it was discovered that all attributes, with the exception of color, had remained unchanged [Table 9 and 10]. The hue of the gel was a very light blue grayish shade. During the course of thirty days, the gel did not undergo liquefaction or phase separation at any point. After a period of thirty days, other parameters were analyzed. After a period of 30 days, only small shifts in pH within the range of 6.97–7.01 were observed.

Table 9 Visual observation of prepared hydrogels containing methanol extract of Martynia annua leaves at various temperatures

Formulations	Room temperature(RT)		40±2˚C	
	15 days	30 days	15 days	30 days
F1	No change in colour, odour	No change in colour, odour	No change in colour, odour	No change in colour, odour but slight separation of oil phase
F2	No change in colour, odour	No change in colour, odour	No change in colour, odour	No change in colour, odour
F3	No change in colour, odour	No change in colour, odour	No change in colour, odour	No change in colour, odour
F4	No change in colour, odour	No change in colour, odour	No change in colour, odour	slight separation of oil phase
F5	No change in colour, odour	No change in colour, odour	No change in colour, odour	No change in colour, odour
Hydrogel base	No change in colour, odour	No change in colour, odour	slight separation of oil phase	slight separation of oil phase

Table 10 Visual observation of prepared hydrogels containing methanol fraction of Ocimum sanctum leaves (OCMFH) at various temperatures

Formulations	Room temperature(RT)		40±2˚C	
	15 days	30 days	15 days	30 days
F1	No change in colour, odour	No change in colour, odour	No change in colour, odour	No change in colour, odour but slight separation of oil phase
F2	No change in colour, odour	No change in colour, odour	No change in colour, odour	No change in colour, odour

F3	No change in colour, odour	No change in colour, odour	No change in colour, odour	No change in colour, odour
F4	No change in colour, odour	No change in colour, odour	No change in colour, odour	slight separation of oil phase
F5	No change in colour, odour	No change in colour, odour	No change in colour, odour	No change in colour, odour
Hydrogel base	No change in colour, odour	No change in colour, odour	slight separation of oil phase	slight separation of oil phase

The formulation's level of viscosity determines how easily it may be dispersed. It was determined that the spreadability of formulations for optimal gels was 15.82± 0.62 for MAMFH and 16.21± 0.28 for OCMFH.

It was discovered that the pH of the finished formulations fell within the range of 6.9–7.01, which was discovered to be approximately identical to the pH of skin. Even at ambient temperature, the prepared hydrogel was found to be stable, and even at an enhanced temperature of40°C±2°C, no separation of the oil phase could be seen. There was no indication of phase separation, formation of an offensive odor, or any other signs of physical instability, and the impact of storing the hydrogel at different temperatures was not documented.

According to the findings, they were quite similar to one another in terms of their capability for application or dissemination. The produced hydrogel does not suffer any degradation during storage, not even under circumstances that increase hydrogel stability. As a result, it is possible to draw the conclusion that the formulations were sufficient and acceptable with regard to the physical parameters.

According to the findings of the experiments, the hydrogel formulation F3 that contains methanol extract of M. annua and O. sanctum leaves is superior to those of other formulation combinations and base. When applied topically to rabbit skin, none of the formulations were determined to be irritating, and none of them displayed any signs of skin toxicity.

Anti-inflammatory Activity

Effect on xylene-induced ear edema

In the current investigation, hydrogel formulations of MAMFH (1%) and OSMFH (1%) were administered topically to mice in order to determine the percentage of suppression of xylene-induced ear edema [Table 11]. The effect of MAMFH and OSMFH were found to have a bigger percentage of edema inhibition (66.67%) and (66.28%) respectively, and this inhibition was equivalent to that of the standard group of therapy (65.59%). According to the findings of the current research, both produced hydrogel MAMFH and OSMFH have strong inhibitory effects against acute inflammation.

Table 11 Effect of prepared hydrogel formulations on xylene induced ear edema in mice

Animal groups	**Weight of ear lobe (in gm) Mean ± SEM**	**% inhibition of ear edema**
Control (hydrogel base)	0.93±0.06	-
MAMFH (20 mg/day)	0.31±0.11	66.67*

OSMFH (20 mg/day)	0.34±0.07	63.44*
Standard (Voltaren Emulgel)	0.32±0.12	65.59*

Each value is the mean ± S.E.M. (n = 5), *P < 0.05 compared with control and standard. MAMFH: Hydrogel containing methanol fraction of Martynia annua OSMFH: Hydrogel containing methanol fraction of Ocimum sanctum

Effect on croton oil induced ear edema

In the current investigation, hydrogel formulations of OSMFH (1%) and MAMFH (1%) were administered topically to mice in order to determine the percentage of suppression of croton oil-induced ear edema [Table 12]. The effect of OSMFH and MAMFH was shown to limit edema by a bigger proportion, respectively 49.53 and 52.33 percent. According to the findings of the current research, the produced hydrogel OSMFH has anti-inflammatory properties that may reduce acute inflammation.

Table 12 Effect of extract hydrogel formulations on Croton oil induced ear edema

Animal groups	Weight of ear lobes (in gm) Mean ± SEM	% inhibition of ear edema
Control (hydrogel base)	1.07±0.27	-
MAMFH (20 mg/day)	0.51± 0.06	52.33
OSMFH (20 mg/day)	0.54±0.38	49.53
Standard (Voltaren Emulgel)	0.52±.37	51.40

Each value is the mean ± S.E.M. (n = 5), *P < 0.05 compared with control and standard; MAMFH: Hydrogel containing methanol fraction of Martynia annua OSMFH: Hydrogel containing methanol fraction of Ocimum sanctum

The MPO levels in mice ears were dramatically decreased after treatment with extract hydrogels and Voltaren Emulgel, which was statistically significant (P 0.05). This may be lowered by these methods. When compared to the untreated control group of animals, histological examinations revealed that inflammation and leukocyte infiltration were much less prevalent in the MAMFH and OSMFH treated groups than they were in the group that served as the control. The rats in the control group had an increase of inflammatory cells as well as fibroblast cells.

DISCUSSION

Inflammation is the body's protective reaction to injury and infection; it is a complicated process that involves many distinct cell types as well as many components of blood. Inflammation is the body's natural defense against both damage and infection. Chronic discomfort, redness, swelling, stiffness, and damage to normal tissues may be the outcomes of aberrant inflammation, which can be caused by inflammatory illnesses that result in the immune system attacking the body's own cells or tissues. These conditions can induce abnormal inflammation. The inflammatory response moves fast to locate diseased or wounded tissues, cut them off from the rest of the body, and kill and eradicate any foreign cells or cells that have been harmed. [25] When inflammation cannot be brought under control, it may lead to the death of healthy tissue, which can then result in an inflammatory illness.

A gelling agent is often included as one of the primary components in hydrogel compositions. If there is not enough of the viscosity enhancer in the hydrogel preparation, you will end up with a simple solution or lotion that has a very poor consistency. On the other hand, if there is too much of the viscosity enhancer, you may end up with gels that have a very high viscosity, which can cause problems with the distribution of drugs and the handling of the gel. [26] We attempted utilizing a gel that included 1-3 percent carbopol-

940 and 1.3 milliliters of triethanolamine, but we discovered that using carbopol-940 to produce the gel resulted in a consistent and smooth gel that did not liquefy after being stored for a significant amount of time at room temperature.

According to the results of the physical examination, the color of the manufactured gels was brownish, and the appearance of the hydrogel was homogenous. Additionally, it was smooth when it was applied. It was discovered that the hydrogel had a consistent and homogeneous structure, and there was no evidence of any phase separation. In the F3 hydrogel formulation combination, the results of all of the other assessment criteria, such as pH, viscosity, spreadability, and consistency, were determined to be satisfactory. Therefore, in order to achieve the optimal drug release profile, the optimal composition of the F3 formulation was determined to be 1.5 grams of carbopol 940 and one percent sodium CMC.

According to the findings, the gel responded quickly to the light pressure that was applied, suggesting that it may be easily disseminated. These guaranteed that the formulation would be able to maintain a sufficient wet contact duration when it was applied at the desired location. [26]

The purpose of the irritation research was to ascertain whether or not the formulation caused any undesirable irritation to the skin of rabbits after a single application of the formulation topically. The findings of this test revealed that one hour after the treatment, all three of the treated locations exhibited a very little erythema. The total occurrence of discomfort, as well as its intensity, reduced as time progressed. After a period of 48 hours, no animal showed any signs of cutaneous discomfort. The PDII for both extract hydrogels was found to be 0.25 when subjected to the circumstances of this investigation. With the exception of the cutaneous irritation that was seen, all of the animals looked to be healthy and active, and there were no other indications of unpleasant toxicity or strange behavior.

In the current investigation, the models that were used provided a wide range for the assessment of the anti-inflammatory activity. In a variety of models, various inducers have been responsible for producing inflammation via the release of inflammatory mediators. Each one of them causes inflammation by a unique set of mechanisms, including an increase in vascular permeability, the influx of leukocytes from the blood into the tissue, granuloma formation, and tissue repair.

One of the many techniques that are utilized in the screening process for anti-inflammatory drugs is one that is based on the capability of such agents to inhibit the edema that is produced in the ear of the animal as a result of the injection of a phlogistic agent. This technique is one of the most frequently used methods. Histamine, xylene, arachidonic acid, phorbol myristate acetate, oxazolone, croton oil, and formalin are the ingredients that are often used in the process of edema production. As a result, the methanol fraction of M. annua and O. sanctum was chosen for hydrogel production and studied for its potential anti-inflammatory effects using ear edema models generated by xylene and croton.

Mice with xylene-induced ear edema are an easy animal model for assessing the efficacy of possible anti-inflammatory medicines, particularly in the event of fluid buildup and edema, which are typical of an acute inflammatory response. In this particular model, the use of xylene results in the formation of neurogenic swelling. There is a correlation between it and the drug P. Substance P is an undecapeptide that plays a role as a neurotransmitter or neuromodulator in a broad range of physiological processes. It is extensively dispersed throughout the nervous system, both in the central nervous system and in the peripheral nervous system. When exposed to painful stimuli, sensory neurons in the spinal cord produce substance P, which, in turn, activates dorsal neurons. Substance P is released by neurons in the midbrain in response to stress, where it helps dopaminergic neurotransmission.

It has been hypothesized that the peripheral release of substance P from sensory neurons has a role in neurogenic inflammation because it promotes vasodilatation and plasma extravasations. Therefore, it has the potential to produce an enlargement of the ear in mice. Results suggest that the acute anti-inflammatory activity of herbal gels tested may be related, at least partially, with their ability to interfere in the pathways involved in the synthesis of NO. This is due to the fact that the activation of intracellular signaling pathways dependent on the enzyme inducible NO synthase by croton oil plays a fundamental role in the control of the inflammatory response in the skin, where the NO produced favors vasodilation that contributes directly to the fomentation of the inflammation [27]

Based on these findings, it seems that the acute anti-inflammatory effect of the produced herbal hydrogels that were evaluated may include interfering with the migration of cells at the site of the inflammation. In

general, we are able to draw the conclusion that the methanolic fraction of M. annua and O. sanctum containing hydrogels slows the migration of neutrophils in areas of inflammation.

Natural goods have lately piqued the interest of many industrial uses, not only as a source for pharmaceutical products, but also for the beneficial benefits that they have on human health. It was discovered that the methanol fraction of M. annua and O. sanctum were able to suppress xylene's ability to cause ear edema. According to the findings of the research, the efficiency of the extract in reducing edema may be related to its capacity to prevent the synthesis, release, or activity of xylene, which is implicated in the inflammation. This is a plausible explanation for the observed results.

MPO is a further enzyme molecule that may be found in neutrophils, as well as monocytes and macrophages, but at considerably lesser concentrations. It is well knowledge that the degree of MPO activity is directly related to the concentration of neutrophils on the tissue that is undergoing inflammation. [28] The drug's ability to inhibit MPO activity, hence reducing the creation of oxidants such hypochlorous acid, is directly tied to the anti-inflammatory capacity of the herbal hydrogel that has been created. When the body's initial response is not adequate to clear the body of the chemicals that cause inflammation, a condition known as chronic inflammation may develop. Chronic inflammation is characterized by the proliferation of fibroblasts in addition to the infiltration of neutrophils and the release of fluid. The growth of proliferative cells, which may either form granuloma or disseminate throughout the body, is the cause of this condition. [29]

CONCLUSION

The results showed that the methanol fraction of the leaves of M. annua and O. sanctum effectively decreased the ear edema during the early phase of inflammation. This indicates that the extracts hydrogel may be blocking the release of histamine and serotonin during the early phase. This led researchers to believe that the anti-inflammatory effects of the methanol extracts were achieved by preventing the production, release, or activity of the histamine molecule. Based on the findings, it is possible to draw the conclusion that the noteworthy activity may be caused by the presence of flavonoids in the methanol extract fraction of the leaves of M. annua and O. sanctum.

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