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Experimental Evaluation of Polyherbal Extract of *Ocimum Sanctum, Clitoria Ternatea,* And *Eclipta Prostrata* for Anti-Inflammatory Activity in Rat

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Abstract:
Inflammation is a process that occurs when the body responds to damage or harmful factors like infections or injuries. It often causes redness, swelling, pain, and reduced function in the area. While many medicines are used to treat inflammation, they can sometimes cause side effects. This has led scientists to look for alternatives, such as medicines made from plants. Plants contain various natural compounds that can help reduce inflammation. This opens the door to creating topical treatments, like creams or ointments, using plant extracts.
The main goal of this study was to create a multi-plant ointment using extracts from three plants: *Ocimum sanctum, Clitoria ternatea,* and *Eclipta prostrata*. The researchers aimed to test how the ointment looked and felt, and to see if it might help reduce inflammation in a lab experiment.
To prepare the ointment , the plant materials were gathered, dried in the shade, ground into powder, and then soaked in ethanol for three days. This process helped to extract the active ingredients. The powdered parts-5 grams each of *Ocimum sanctum* leaves, *Clitoria ternatea* flowers, and *Eclipta prostrate* leaves-were mixed with ethanol and left to steep. The liquid extract was then added to a base made of wool fat, cetostearyl alcohol, hard paraffin, and soft paraffin. This base helps the ointment maintain its shape and spread well.
The team tested the ointment in several ways. They checked its color, smell, thickness , pH level, spreadability, stickiness, and how well it dissolved in liquids. They also assessed its safety for skin. To test its ability to reduce inflammation , they used a specific method: they injected carrageenan into the paws of rats to induce swelling and then applied the ointment to some of the animals. The group that did not receive the ointment was used as a control. Diclofenac, a known anti-inflammatory drug, was used as a reference.
The ointment turned out to be yellowish-green in color, had a distinct smell, and felt thick and smooth. Its pH was 6.92, which is close to the skin's pH. It spread well-74 mm in the test-and had a viscosity of 42 cP. The ointment was slightly sticky and could be washed off with water. It didn't dissolve in water but did dissolve in ether and chloroform. When tested on rats, the group that received the ointment had less swelling in their paws compared to the group that did not receive any treatment. The data did not include exact numbers to quantify how much swelling was reduced or whether the difference was statistically significant.
The study shows that it is possible to create an ointment using these three plants. The preliminary results suggest it might help reduce inflammation. However, more work is needed. The researchers need to conduct tests with clear numerical data, perform statistical analyses, improve the consistency of the formula, and verify safety before declaring it ready for use in humans.

Keywords: formulation; anti-inflammatory activity; *Ocimum sanctum*; *Clitoria ternatea; Eclipta prostrata*; herbal ointment; carrageenan-induced paw edema .

I. INTRODUCTION

Inflammation is a way that body tissues react when there is an infection, an injury or something harmful. This reaction includes the teamwork of cells chemicals that cause inflammation and changes in blood vessels. These actions help get rid of things and fix damaged parts. The signs of inflammation are redness, swelling, warmth, pain. Not being able to use the area properly. Even though short-term inflammation is usually good if it lasts long or is too strong it can harm the body and make long-term inflammation worse.

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The body uses chemicals to control the inflammation process. These chemicals include things like prostaglandins, histamine, cytokines, leukotrienes and nitric oxide. These chemicals change how blood vessels work bring in cells and make nerves sensitive to pain. Regular medicines that fight inflammation like -steroidal drugs are often used to help with inflammation.. Some of these medicines can have bad effects especially if used for a long time or by people who are more sensitive. This has led to looking for ways to treat inflammation including special compounds from plants that have healing properties.[1-3]

Plants used in medicine have many different chemicals inside them like flavonoids, phenolic compounds, terpenoids and other less common chemicals. Some of these chemicals have been found to do things that help with inflammation in studies. But the effects of plant extracts depend on what chemicals they have how they are. How they are tested. So careful testing is needed before we can say for sure if they work.[2]

Ocimum sanctum, called holy basil or Tulsi is a plant from the Lamiaceae family. Its leaves have chemicals, including eugenol, flavonoids, phenolic compounds and terpenoids. Scientists have looked at its ability to fight radicals and reduce inflammation. These chemicals give a reason to look at *Ocimum sanctum* as part of a plant-based treatment for inflammation.[3,4]

Clitoria ternatea, also called butterfly pea is a plant from the *Fabaceae* family. Its flowers have chemicals like anthocyanins, ternatins along with flavonoids and other plant chemicals. These chemicals have been studied for their ability to fight radicals and possibly fight inflammation. Adding *Clitoria ternatea* to a mix of plants might give helpful chemicals but we still need experiments to prove its role in a mixed treatment.[5-7]

Eclipta prostrata, also called daisy is a plant from the Asteraceae family. It has been used in medicine and studied for its properties. It has chemicals like wedelolactone that have drawn attention in research on inflammation. These findings show that *Eclipta prostrata* is worth looking at as part of a plant-based treatment.[8,9]

Putting plants together in one medicine might help study how they work together.. Just having multiple plants doesn't mean they work better together. We need to find out how each one helps and if they mix well with each other. This needs experiments to check.[3,5,8]

Ointments are a type of medicine that is put on the skin. They need to have the texture, thickness and other features to work well. Checking these features helps make sure the medicine is good and ready for testing.[13]

The carrageenan-induced rat paw oedema model is used in labs to study inflammation. When a rats paw swells after being treated it is compared to a group that is not treated and a group that gets a known medicine. To get results we need to measure things right use enough rats and do proper statistics to see if the effect is real.[11,12]

Based on this this study made an ointment with extracts from *Ocimum sanctum, Clitoria ternatea* and *Eclipta prostrata*. The ointment was checked for some of its chemical features and its early ability to fight inflammation was tested using the rat paw model. This study gives a starting point for research, on this mix of plants.

II. Materials and Methods

2.1. Materials

The study used leaves from *Ocimum sanctum* and flowers from *Clitoria ternatea.* Leaves from *Eclipta prostrata* were also included as plant materials. Ethanol was used as the extraction solvent. Wool fat, cetostearyl alcohol, hard paraffin, and soft paraffin were used in the preparation of the ointment base. Diclofenac was used as the reference anti-inflammatory treatment in the animal experiment.
The chemicals used in the formulation and experimental procedures were reported to be of analytical grade. The dissertation identifies a meter, heating mantle, and Brookfield viscometer among the instruments used for formulation evaluation.

2.2. Collection and Preparation of Plant Materials

The selected plant materials were prepared for extraction. The leaves and flowers were shade-dried for two to three days and reduced to a medium-coarse powder. The dried powders were used for the extraction procedure.

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The botanical identity of each plant should be documented before publication. The collection location, collection date, authentication procedure, and voucher specimen numbers should be reported if available.[8,9]

2.3. Preparation of Ethanolic Extract

The extraction procedure involved 5 g *of O. sanctum* leaves, 5 g of powdered *C. ternatea* flowers, and 5 g of powdered E. *prostrata* leaves. The combined plant material was macerated in 100 mL of ethanol for 72 hours.

Following maceration , the extract was separated by filtration. The resulting liquid extract was stored under refrigerated conditions until processing. A portion of the extract was reserved for screening, while the remaining portion was used for formulation.

The extract was subsequently concentrated by heating at 70°C to facilitate solvent evaporation , as described in the original experimental procedure.

For reproducibility , the final manuscript should specify whether the three plant materials were extracted together or separately before mixing. The extraction yield, final extract mass, residual solvent assessment, and storage duration should also be reported if these measurements are available.[13]

2.4. Preparation of Polyherbal Ointment

The ointment base was prepared using wool fat, cetostearyl alcohol, hard paraffin , and soft paraffin. The hard paraffin was melted using a heating method, after which the remaining base ingredients were added and mixed until a uniform preparation was obtained.

The base was allowed to cool before incorporating the concentrated extract. The extract was incorporated by levigation , initially mixing it with a quantity of the base to produce a smooth paste. Additional base was then gradually incorporated to obtain an ointment.

According to the dissertation , the extract-containing formulation was prepared using 1 mL of extract and a sufficient amount of ointment base to make 10 g.

The formulation composition and final extract concentration should be verified before publication. The exact quantity of each excipient in the batch , the final extract content expressed as mass per mass or volume per mass, and the preparation temperature should be stated wherever the original records permit.[14]

2.5. Physicochemical Evaluation

The prepared ointment was subjected to physicochemical evaluation.

2.5.1. Organoleptic properties: The formulation was visually examined for color , odor, and consistency. These characteristics were recorded to describe the appearance of the prepared ointment.

2.5.2. pH determination: One gram of the formulation was dispersed in 100 mL of diluted Tween 80 solution, as described in the dissertation. The pH was determined using a meter. The electrode calibration procedure and replicate measurements should be documented if available.

2.5.3. Spreadability: A quantity of ointment was placed between two glass slides , compressed under a 100 g weight for five minutes. The time required to separate the slides under the specified conditions was recorded. Spreadability was intended to be calculated using the following relationship:

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

Where:

S = Spreadability

M = Applied weight

L = Length or distance specified by the experimental setup

T = Time required to separate the slides

The apparatus dimensions, distance traveled , units, and calculation should be checked before reporting the final numerical value.

2.5.4. Viscosity: Viscosity was determined using a Brookfield viscometer with spindle number 4 at 6 rpm , as reported in the dissertation. The measured dial reading and the applicable instrument conversion factor were used to calculate the viscosity. Instrument calibration and measurement temperature should be reported if documented.

2.5.5. Washability: A small quantity of the formulation was applied to the skin , and its ease of removal was examined. The observation was recorded qualitatively.

2.5.6. Solubility: The formulation was examined for its reported solubility characteristics in selected solvents. Because the preparation is an ointment containing a paraffin-based vehicle, the test conditions and whether the assessment concerned the formulation or the extracted constituents should be clarified.

2.5.7. Skin irritation assessment: The dissertation reports an irritation assessment following topical application. The final manuscript should specify the test model, amount applied, observation period, scoring method, control , and ethical approval applicable to this procedure. A single observation

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after ten minutes should not be presented as evidence of comprehensive dermal safety.[13,`14]

2.6. Experimental Evaluation of Anti-inflammatory Activity

The anti-inflammatory activity of the polyherbal formulation was investigated using the carrageenan-induced rat paw edema model.

According to the dissertation , female albino rats were maintained under laboratory conditions and provided with standard laboratory feed and water. The animals were assigned to three groups, with three animals per group, as described in the section.

The groups were designated as follows:

Group I: Carrageenan-treated inflammatory control.

Group II: Reference treatment with diclofenac.

Group III: Treatment with the formulation.[11,12,15]

Acute inflammation was induced by administering 0.1 mL of 1% (w/v) carrageenan into the subplantar region of the right hind paw , as reported in the dissertation. Paw measurements were recorded using Vernier calipers at the observation intervals.

The dissertation describes observations at 0, 1 , 2, 3, and 4 hours. The final manuscript should confirm the measurement schedule, treatment timing, administration route, dose of diclofenac, amount of ointment applied, and method used to measure paw edema .

The animal study was reported as having Institutional Animal Ethics Committee approval. The approval number, approval date, approved animal number , and applicable institutional and CPCSEA requirements should be reported accurately.

2.7. Calculation of Percentage Inhibition of Paw Edema

I was calculated the percentage inhibition from the recorded oedema measurements using the prespecified calculation method.

When paw oedema is expressed as the increase in paw volume or another validated measurement relative to baseline , the used calculation is:

Percentage inhibition = $[(C - T) / C] \times 100$

Where:

C = Mean oedema response in the inflammatory control group at the selected time point.

T = Mean oedema response in the treated group at the time point.

I must use measurements obtained at the same time point for the calculation. I must specify the definition of the paw oedema response in the final manuscript.

I need the original numerical observations for the control group, diclofenac group , and polyherbal treatment group to compute this value. I was not reported any percentage inhibition without those data[11,12]

III. RESULTS

3.1. Physicochemical Evaluation of the Polyherbal Ointment

The prepared polyherbal ointment was evaluated for selected pharmaceutical characteristics. The polyherbal ointment exhibited a yellowish - green color , an odor, and a viscous consistency. The polyherbal ointment had a pH of 6.92, a spreadability of 74 mm, and a viscosity of 42 cP. The polyherbal ointment was described as slightly washable and sticky. Solubility observations indicated that the polyherbal ointment was insoluble in water but soluble in ether and chloroform. The preliminary irritation assessment recorded the ointment as non - irritant under the test conditions.

Table 1. Physicochemical characteristics of the ointment

Evaluation parameter	Reported observation
Colour	Yellowish green
Odour	Aromatic
Consistency	Viscous
pH	6.92
Spreadability	74 mm
Solubility	Soluble in ether and chloroform; insoluble in water
Washability	Slightly washable and sticky
Skin irritation	Reported as non-irritant
Viscosity	42 cP

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These observations provide a description of the polyherbal ointment. However , replicate measurements, measurement variability, instrument details, and confirmatory testing are needed to establish reproducibility and formulation quality.

3.2. Preliminary Anti - Inflammatory Evaluation

The anti - inflammatory activity of the ointment was investigated using the carrageenan - induced rat paw edema model. Three experimental groups were described: an inflammatory control group, a diclofenac - treated reference group, and an ointment - treated group. The dissertation reports that paw edema increased in the inflammatory control group and that reduced paw swelling was observed in the diclofenac - treated group and the ointment - treated group. The polyherbal ointment treatment was described as producing a reduction in paw edema compared with the inflammatory control.

These findings provide evidence that the polyherbal ointment may possess anti - inflammatory activity under the reported experimental conditions. Nevertheless, the available descriptive observations do not establish the magnitude of the effect compared to diclofenac.

3.3. Quantitative Analysis and Interpretation

A numerical comparison of the three groups requires the recorded paw measurements at each observation time. The mean edema response, variability, percentage inhibition, and statistical significance must be calculated from the data. The percentage inhibition of paw edema cannot be established reliably from descriptions alone. Similarly, the available information is insufficient to conclude that the polyherbal ointment is equivalent to or comparable with diclofenac.

The physicochemical observations indicate that the polyherbal ointment containing the selected plant extracts was successfully prepared and subjected to evaluation. Further work is required to establish batch - to - batch reproducibility, stability, dermal safety, and quantitative pharmacological activity.

IV. ANIMAL STUDIES

4.1. Experimental Animals

The anti - inflammatory activity of the polyherbal ointment was tested on albino rats in a laboratory setting.

According to the protocol described in the dissertation , female albino rats were obtained from the animal house at Nandha College of Pharmacy in Perundurai. The rats were kept under laboratory conditions and were given laboratory feed and water freely.

The dissertation describes three groups, each containing three rats. The rats were used to study the effect of the formulation on carrageenan - induced paw edema .

The age, strain, individual body weights, acclimatization period, housing conditions, and allocation procedure should be reported from the original laboratory records.[15]

4.2. Experimental Design

The rats were divided into three groups to compare the response after the administration of carrageenan, a reference anti - inflammatory treatment, and the prepared polyherbal formulation.

Group I - Inflammatory control: Rats received carrageenan to produce paw edema .

Group II - Standard treatment: Rats received diclofenac as the reference anti - inflammatory drug.

Group III - Test treatment: Rats received the polyherbal ointment containing extracts of *Ocimum sanctum*, *Clitoria ternatea*, and *Eclipta prostrata.*

The experimental design aimed to determine whether the test formulation reduced paw edema compared with the control and to compare the response with the reference treatment.

The dose administration route, treatment timing, and amount of ointment applied must be confirmed from the original experimental records before publication.[11,12,15]

4.3. Induction of Acute Inflammation

Acute inflammation was created using the carrageenan - induced rat paw edema model. According to the dissertation, 0.1 mL of a 1% carrageenan suspension was injected into the subplantar region of the right hind paw.

Carrageenan - induced paw edema is a well - known model for evaluating acute inflammatory responses and for screening potential anti - inflammatory agents. The inflammatory response involves changes in blood vessel permeability and the activity of inflammatory mediators.

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The resulting paw swelling was measured using the procedure described in the protocol.[11,12]

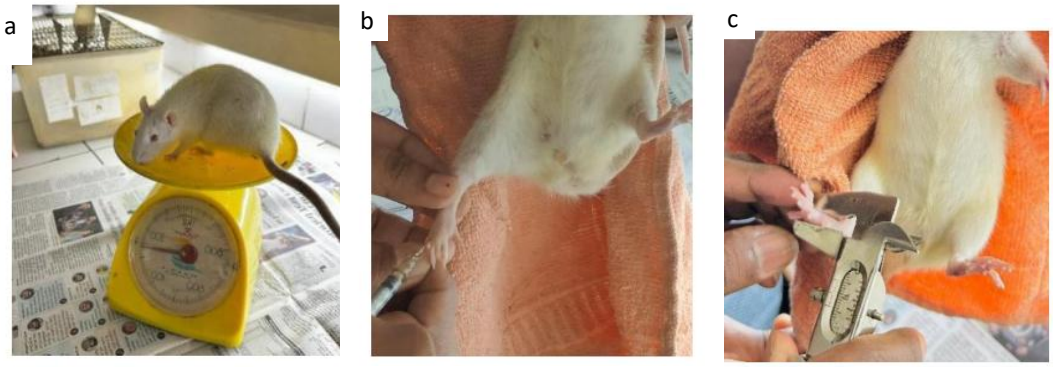

Figure 1(a). Animal weighing procedure (as labelled in the dissertation),**Figure 1(b).** Carrageenan-induced paw oedema procedure (as labelled in the dissertation),**Figure 1(c).** Paw measurement using Vernier 1207abeled1207 (as 1207abeled in the dissertation).

4.4. Measurement of Paw Edema

Paw measurements were taken with Vernier calipers at the observation times. The dissertation describes measurements at baseline and at 1 , 2, 3, and 4 hours after the carrageenan injection.

The paw measurements were intended to track the progression of inflammation in the control group and the reduction in swelling with the reference and test treatments.

For publication , the original records should clarify whether the measured outcome was paw thickness, circumference, or another linear measurement. The same validated measurement method and units must be used consistently across all groups.[14,15]

Table.2 Measurement of Paw Edema

Group	Initial paw value	0 min	1 hour	2 hour	3 hour	4 hour
Negative control	0.43±0.0726	0.75±0.0577	0.84±0.0441	0.88±0.0441	0.93±0.0441	0.93±0.0441
Standard (diclofenac)	0.48±0.1014	0.68±0.0441	0.87±0.0601	0.92±0.0601	0.93±0.0441	0.87±0.0601
Test (polyherbal ointment)	0.38±0.0726	0.70±0.0764	0.80±0.0764	0.90±0.0667	0.92±0.0601	0.85±0.0577

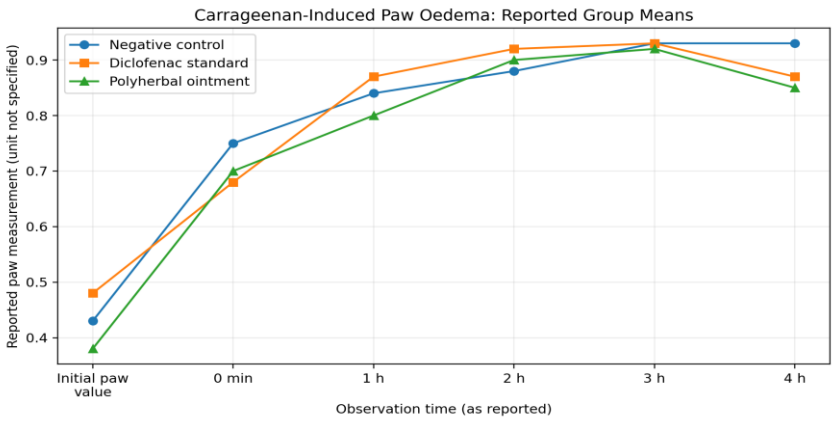

Figure.2 Measurement of Paw Edema

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4.5. Assessment of Anti - Inflammatory Activity

The anti - inflammatory activity of the ointment was assessed by comparing the paw edema response in the test group with that of the inflammatory control group.

The dissertation reports progressive paw swelling in the inflammatory control group and a reduction in swelling in the diclofenac - treated group. A noticeable reduction in paw edema was also reported in the treatment group.

These observations suggest anti - inflammatory potential for the prepared formulation. However, the magnitude of the treatment effect cannot be determined from the observations alone.

The percentage inhibition of paw edema should be calculated from the numerical measurements at each time point. The resulting values should be presented with the group means and appropriate measures of variability.

4.6. Ethical Considerations

The dissertation reports that the animal experiments received Institutional Animal Ethics Committee approval and were conducted in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA) , Government of India.

Before submission , the authors should verify the name of the institutional committee, the approval number, the approval date, and the number of animals authorized under the approved protocol.

The manuscript should also describe the procedures used to minimize pain and distress, the monitoring performed during the study, and any adverse events or humane endpoints specified in the approved protocol.

4.7. Animal Study Findings

The qualitative findings reported in the dissertation indicated differences in paw edema among the three groups. The inflammatory control group exhibited increasing paw swelling, whereas the standard treatment group showed a reduction in inflammation. The polyherbal formulation - treated group also demonstrated a reported reduction in paw edema compared with the control.

These findings provide observations supporting further investigation of the polyherbal formulation - treated group's anti - inflammatory activity. However, without the numerical measurements and appropriate statistical analysis, it is not possible to determine the percentage inhibition, statistical significance, or equivalence of the polyherbal formulation - treated group to diclofenac.

V.DECLARATION

The authors state that this research was conducted to create and test a mixture of herbs called an ointment. The ointment contains Ocimum sanctum, Clitoria ternatea, and Eclipta prostrata. The authors claim this ointment may help reduce swelling. The authors state that the paper they wrote is original . We assert it has not been published anywhere before. We confirm it is not being submitted elsewhere for publication . We affirm that all the information they used is properly credited .

VI.CONCLUSION

This study was conducted to create and test an ointment that contains ethanolic extracts of *Ocimum sanctum, Clitoria ternatea,* and *Eclipta prostrata* to determine its potential for alleviating inflammation. The ointment produced exhibited specific physical characteristics . The pH was 6.92. The spreadability measured 74 mm. The viscosity was 42 cP. No skin irritation was observed during testing. In the rat model, where inflammation was induced by carrageenan, the group that received the ointment exhibited more swelling in the paw than the group that did not receive treatment. This suggests that there may be some anti-inflammatory effect. More work is needed. Additional experiments with various groups, improved statistical methods to assess the stability of the ointment, and evaluations of its safety are all necessary. The results indicate that this research provides a preliminary foundation for investigating this ointment as a potential treatment for skin inflammation

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