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FORMULATION AND EVALUATION OF HERBAL OIL BASED OINTMENT FOR TOPICAL TREATMENT OF INFLAMMATION

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ABSTRACT

Essential oils and fixed oils are established since years in the clinical management of inflammatory disorders. Present study was designed to formulate and evaluate herbal based anti-inflammatory ointment to reduce the inflammation of the skin and muscle. Ointment was formulated with combination of selected of essential and fixed oils which have been reported as potential anti-inflammatory agents. Three combinations; F1, F2 and F3 were prepared and F3 was selected based on the stability and activity study reports. Anti-inflammatory activity was evaluated by carrageenan induced paw edema model in mice. Ointment was applied externally, once in two hours three times a day and once in six hrs. for consecutive day, after the onset of inflammation. The formulation (F3) showed enhanced anti-inflammatory activity by reducing the edema and inflammation by 85% compared to the standard Dual scan Gel which showed 70% reduction (contains diclofenac, diethylamine, oleum lini, methyl salicylate & menthol). From the study, it is concluded that, formulation F3possesses potential anti-inflammatory activity compared to conventional anti-inflammatory gel and can be a promising formulation for external application to treat inflammation.

Keywords: Herbal, Ointment, Inflammatory Disorders, Essential Oil

INTRODUCTION

The clinical symptoms of inflammation were described with four cardinal signs; redness, swelling, heat and pain and later the fifth one as loss of function. In 1793, the Scottish surgeon John Hunter described that inflammation as; it is not a disease, but it is a non-specific reaction which has a curative effect on the host. Inflammation may be acute response damage of the tissue) or chronic (due to pathological consequences like arthritis). Tissue injury causes release of local chemical mediators like histamine, arachidonic acid/metabolites, kinins which produces increased capillary permeability resulting in edema and cell infiltration at the site of tissue injury. In acute inflammation, cellular events helps to restore homeostasis of damaged tissue resulting in subsiding acute inflammation. But chronic inflammation needs treatment in order to overcome the further harmful effects **[1, 2]**.

Generally, nonsteroidal anti-inflammatory drugs like diclofenac, ibuprofen are used to treat inflammation. Diclofenac is a drug of choice to treat pain and swelling of muscle, joints and bones, especially in osteoarthritis and rheumatoid arthritis **[3]**, which needs regular use of anti-inflammatory drugs/analgesics. Diclofenac is used for both internal administration and for external application to treat inflammation and pain **[4]**. Regular use of nonsteroidal anti-inflammatory drugs is reported to cause renal problems, gastrointestinal complications (erosion and ulceration) and cardiac risks like myocardial infarction and stroke **[5]**. Therefore, it has become essential to have safer and effective drugs to combat inflammation especially if the treatment demands long term use of the drugs. In this context, natural remedies which have been used and recognized throughout the human history will come to the rescue. Present study includes designing and development of an ointment containing selected essential oils and fixed oils as therapeutic ingredients which are well documented as antioxidants and anti-inflammatory and because of their remarkable versatility they have find their applications in food, chemical, cosmetic and pharmaceutical industries. The study would be advantageous for industries to manufacture a new effective anti-inflammatory formulation without huge economic sacrifice.

MATERIALS AND METHODS

Materials: All the materialsused were purchased fromvarious vendors. Neem oil (Dagdu teli trading company, Ravivar Peth, Nashik), camphor oil (Arya Vaidya sala, Kottakkal, Kerala),til oil, coconut oil, garlic oil(local purchase from market), ginger oil (Dravida organics-Vcos cosmetics, new Delhi), clove oil, tea tree oil (Sunder Pragati products, Nashik), Patchouli oil, olibanum

oil (RV essential company, new Delhi), Eucalyptus oil (Everest co., Ahmednagar).λ Carrageena-AR was purchased from Sigma chemicals.

Animals: Swiss albino mice of either sex weighing about 20-28g, aged between of 2-3 months were employed in the investigation. The study was approved by institutional animal ethics committee (Reg.no.1858/PO/Re/S/16/CPCSEA).

Animals were acclimatized for 6 days under regular circumstances. Nutrimix standard -1020 feed, was given to the animals along with a regular supply of water. Animals were kept fasting for 24 hrs with free access to drinking water before the commencement of the study.

Methodology-

Preparation of ointment by Fusion Method with modification [6]

Ingredients used:

- Yellow bees wax -12gms
- Cocoa butter- 03gms
- Sesame oil-08ml

Method of preparation-: All the ingredients were taken in a china dish and melted on water bath with thorough stirring (F).

Incorporation oils: All the below mentioned ingredients were incorporated individually in the ointment base (F)maintained at 55^0C, with continuous stirring until the formation of perfect ointment consistency. Three formulations with different combination of oils were prepared and screened for anti-inflammatory activity.

Table 1: List of therapeutic oils used for formulations with composition

S. No.	Name of ingredient	Quantity		
		F1	F2	F3
1.	Almond oil	1ml	2ml	4ml
2	Sesame oil	2ml	4ml	8ml
3	Clove oil	1ml	2ml	4ml
4	Ginger oil	1ml	2ml	4ml
5	Garlic oil	1ml	2ml	4ml
6	Eucalyptus oil	1ml	2ml	4ml
7	Neem oil	1ml	2ml	4ml
8	Tea tree oil	1ml	2ml	4ml
9	Wintergreen oil	1ml	2ml	4ml
10	Coconut oil	1ml	2ml	4ml
11	Patchaouli oil	1ml	2ml	4ml
12	Olibanum oil	1ml	2ml	4ml
13	Camphor oil	1ml	2ml	4ml
14	Cocoa butter	2gm	3gm	3gm
15	Yellow beeswax	2gm	6gm	12gm

(A) Evaluation of ointment [7-9]:

a) **Organoleptic evaluation-:** Organoleptic evaluation of the ointment was carried visually and by touch. Physical evaluation for colour, odour, phase separation and

skin feel test for texture and homogeneity was carried out to test the presence of gritty particles if any.

b) **pH Determination**- The pH is determined with the help of pH meter.2.5 g of test formulations were taken in a dry beaker separately and added 50 ml of water heated on water bath until it melts. pH of all the three test samples were determined using a pH meter. The testwas carried out in triplicates and the average of three readings was taken.

c) **Spreadability test:** Parallel glass plate method was used. The device consists of fixed glass slideon a wooden block with a pulley at one end of it. Around 3g of ointment was placed and sandwiched between this plate and the other glass plate provided with a hook having the dimension of fixed glass plate. Initially, 1 kg weight was kept on the two plates for 3 min to drive out air and to get a consistent and uniform film of the ointment in between the plates. Excess of ointment was removed off from the ends of the glass plates. A pull of 240g was placed on the top plate and subjected pull down with the assistance of the spring attached to the hook. Ointment spreadability was calculated using the below formula

S = M×L/T

Where, S = Spreadability

M = Weight tied to the upper slide)

L = Length moved by the glass slide

T = Time taken to separate the slide completely each other (in seconds)

d) **Accelerated stability study:** Freeze-Thaw Cycle: Samples were subjected to various temperatures and the test was conducted for twelve days with six cycles each at temperature 2^0C, 35^0C and 50^0C for 24 hours.

e) **Skin irritation study:** Test formulations were screened on mice topically by applying 500mg of ointment on a shaved area of mice and observed regularly upto 48 hrs for any redness, swelling, flush or any kind of allergic reactions. Zero scoring was given for no signs of irritation.

f) **Microbial growth**: The ointment was tested for microbial contamination or growth by streaking the sample onto the agar media plates and incubating the plates for 24 hours at 37°C. Agar contrast without sample was used for comparison.

(B) Screening for anti-inflammatory activity [10]: Carrageenan induced paw edema model (with modification): Test animals (mice) were divided into five groups

of six each. All the test animals were fasted overnight with free access to drinking water. The sub-planter area of a mouse's hind paw was injected with 1% carrageenan, subcutaneously under diethyl ether anaesthesia.

Group A: Negative control (Without any treatment)

Group B: Treated with Test Formulation (F1)

Group C: Treated with Test Formulation (F2)

Group D: Treated with Test Formulation (F2)

Group E: Positive control **(**Treated with standard formulation Dual scan Gel)

Group A (negative control) treated topically with vehicle (distilled water). Animals of group B, C, D and E were treated topically with formulations F1, F2, F3 and standard respectively (around 500mg), once in two hours three times during screening followed by once in six hours for the consecutive day. Edema was induced by administering 0.1ml of 1% carrageenan in normal saline into plantar region of right hind paw, beneath plantar of aponeurosis. Ointment was applied after 30 minutes of carrageenan injection. Paw volume was measured before inducing edema with the help of plethysmograph followed by 1st, 3rd, and 5th hour after carrageenan injection and also measured after 24hrs. Difference of initial and respective subsequent paw volume was considered as actual edema volume. Percentage of inhibition of inflammation was calculated using below formula **[11]**

% inhibition=100(1-Vt/Vc)

Where by

Vc= Mean Paw Edema volume in control

Vt =Mean Paw Edema volume in the treated group

Statistical analysis: Data was analyzed by means of Analysis of variance (ANOVA) followed by Student's t-test. Values, $p<0.05$ are considered as significant.

RESULTS:

Results obtained are tabulated below

Table 2: Table showing results of physical evaluation of ointment formulation

Ointment formulation	F1	F2	F3
Color	Whitish yellow	Slightly yellowish	Yellowish white
Texture	Moderately soft	Moderately soft	Soft
Gritty particles	Absent	Absent	Absent
Homogeneity	Homogenous	Homogenous	Homogenous
Odour	Pleasant	Pleasant	Pleasant
pH	6±2	6.1±2	6.2±1
Spreadability	10.2±1	14.2±1	18.8±2
Skin Irritation	0	0	0
Accelerated stability studies	No phase separation	No phase separation	No phase separation
Microbial growth	Nil	Nil	Nil

Table 3: Table showing anti-inflammatory activity of ointment formulationin carrageenan induced paw edema in mice (% inhibition)

Samples	Percentage inhibition of edema taken at different time intervals			
	1 hrs.	3 hrs.	5 hrs.	24 hrs.
Negative Control (Without treatment)	0	0	0	0
Positive control (Dual scan Gel)	53±24*	64.24±46**	70.48±22*	74.2±40*
F1	50±22*	62.22±22*	72.46±32*	80.24±22*
F2	50.2±14*	64.4±22**	75.38±22**	86.22±14**
F3	58.8±42***	65.2±40***	85.34±62***	92.2±22***

Mean ±SEM, p< 0.05 was considered as significant, *=Less significant, **=Relatively significant, *=More significant**

DISCUSSION

In the present study, topical ointment based formulation was prepared for the treatment of inflammation. Ointment was preferred, because of its effective penetration which aids the absorption of active ingredients more efficiently, provides sustained release of active ingredients, and forms a protective layer on the skin which prevents further damage to the skin. In this study the active ingredients used are fixed oils and volatile oils which are better compatible with the base used. Base itself acts as an emollient which gives additional healing properties to the formulation. In chronic inflammation like rheumatoid arthritis, inflammatory cells and inflammatory substances attack joints and muscles resulting in severe pain, tenderness, swelling, heat which can results in severe damage to muscles and joints. In such cases external treatment is very much required to reduce the inflammation and pain, since long term use of oral anti-inflammatory drugs (NSAID's have shown numerous adverse effect. In this study, we have tried preparing a topical formulation using combination of fixed oils and volatile oils to treat inflammation. Ingredients used here are volatile oils like Clove oil, Ginger oil, Eucalyptus oil, Tea tree oil, Patchaouli oil, Garlic oil, Wintergreen oil, Olibanum oil and Camphor oil which are well-known anti-inflammatory and anti-oxidants **[12-14]**. Fixed oils like Almond oil, Sesame oil, Coconut oil, Neem oil are used as emollients and to reduce irritation **[15-16]**. The carrier base Cocoa butter and yellow bees wax provides additional affect as emollient which is also one of the essential properties to treat inflammation.

From the present study, it is observed that the formulation F3 was found to be more effective and passes all the valid physical tests as per the standards. Formulations did not show any irritation, phase separation, change in physical properties and found to be stable. pH was found to be around 6.2 which is one of essential property of an ointment. Spreadability of F3 was found to be superior compared to F2 and F1. Anti-inflammatory activity was found to be dose dependant and activity of F3 was found to be significant and better than the standard formulation.

Since carrageenan shows Maximum effect after 24 hrs; reading was also taken at 24th hour and we could observe around 92% inhibition after 24 hrs. The study data demonstrates that the formulation F3 possesses excellent significant topical anti-inflammatory activity. The possible mechanism may be attributed to inhibition of release of histamine, serotonin, kinins and prostaglandins after topical absorption. The enhanced activity may be attributed to the additional effects of combination of oils when compared with standard.

CONCLUSION

From the overall data, it can be concluded that the ointment formulation F3 will be beneficial in the topical treatment of inflammation and hope will be helpful in reducing inflammatory pain in arthritis and local inflammation due to insect bite, allergy itching or related inflammations and will be also of commercial importance as anti-inflammatory formulation.

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