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# Formulation and Evaluation of an Herbal Anti-Inflammatory Gel Containing *Eupatorium* Leaves Extract

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This study evaluated a noble herbal gel formulation containing extract from the leaves of *Eupatorium adenophorum* for its topical anti-inflammatory activity against carrageenan induced oedema. Gelling agent used in this study was 1% w/w concentration of carbopol- 934. The studies were conducted on Albino Wistar rats of either sex (150-200 gm). Change in oedema volume of the rat hind paw was measured. The anti-inflammatory effect produced after topical administration of herbal gel formulation on Carrageenan-induced hind paw oedema exhibited a high degree of reproducibility. The initial physicochemical parameters of formulations i.e. pH, viscosity, spreadability, extrudability and stability were also examined. The pH of all the formulations was near about 6.8, which lies in the normal pH range of the skin. The preparation was stable under normal storage conditions and did not produce any skin irritation, i.e., erythema and oedema for about a month, when applied over the skin.

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**Keyword:** Herbal Gel, Inflammation, *Eupatorium* Leaves extract, Oedema.

**1. Introduction:** *Eupatorium* (Asteraceae) is a large genus of herbs, shrubs or under shrubs, distributed chiefly in tropical America, a few species occurring in Europe, Africa, Asia and India<sup>[1]</sup>. Different parts of the species *Eupatorium adenophorum* Spreng are used in Ayurveda and other folk medicines for the treatment of cut, wounds, as antifungal<sup>[2]</sup>, antimicrobial<sup>[3]</sup>, and analgesic agent<sup>[4]</sup>.

A recent study on the ethanolic leaf extract of *E. adenophorum* has reported anti-inflammatory activity in dinitrofluorobenzene induced foot paw oedema in mouse for the first time<sup>[5]</sup>. Since, inflammation is a pathophysiological response of living tissues to injuries that leads to the local accumulation of plasmatic fluid and blood cells<sup>1</sup>

and if not controlled, it can lead to development of diseases such as chronic asthma, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and so forth<sup>[6-11]</sup>, the present study was designed to formulate and evaluate the herbal gel formulation containing methanol extract of *E. adenophorum* leaves for its anti-inflammatory potential in carrageenan induced paw oedema in rats.

## 2. Material and Methods

### 2.1 Preparation of methanolic extract

The leaves of *E. adenophorum* Spreng were collected from Rajpur Road, Dehradun (Uttara khand) and authenticated by Botanical Survey of India, Northern Regional Centre, Dehradun with

the Accession number 1127802, 1127803. The leaves were dried under shade and then powdered coarsely with a mechanical grinder. The powder was passed through sieve No. 40 and stored in an airtight container for further use. Hundred grams of powdered leaves were extracted with methanol as a solvent by hot extraction method using soxhlet apparatus. The resulting extract was cooled and filtered. The filtrate was evaporated in vacuum to give a residue.

## 2.2 Formulation of topical preparation

Herbal gel was prepared using carbopol-934 as a gelling agent in 1% w/w concentration with deionized water using mechanical stirrer. The pH of the gel was adjusted to neutral by addition of small quantities of triethanolamine with continuous stirring. 1 % w/w herbal extract of *E. adenophorum* was added to the gel and stirred for sufficient time for homogeneous mixing of extract in gel base. Prepared gel was filled in collapsible tubes and stored at a cool and dry place. Physical parameters such as colour, appearance, and feeling on application were recorded. pH of the gel was recorded using a pH meter.

## 2.3 Viscosity

Viscosity of gel was measured by using Brookfield viscometer with spindle #7.

## 2.4 Extrudability<sup>[12]</sup>

The gel formulations were filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. The weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 500 gm was placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed. The percent of the extruded gel was calculated (>90% extrudability: excellent, >80% extrudability: good, >70% extrudability: fair).

## 2.5 Spreadability<sup>[13]</sup>

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. By this method

spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2 g) under study was placed on the ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with a hook. A 1 kg weight was placed at the top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80 g with the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm was noted. A shorter interval indicated better spreadability. Spreadability was calculated using the following formula:

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

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

## 2.6 Stability study<sup>[14]</sup>

The stability study was performed as per ICH guidelines. The formulated gel was filled in collapsible tubes and stored at different temperatures and humidity conditions, viz. 25±2 °C / 60±5% RH, 30±2 °C / 65±5% RH, 40±2 °C / 75±5% RH for a period of three months and studied for appearance, pH and spreadability.

## 2.7 Primary Dermal Irritation Index (PDII)

Dermal irritation is the production of reversible damage to the skin following the application of a test substance for up to 4 hours. Primary dermal irritation index (PDII) is a method for classifying topical formulations into various categories based on acute toxic reactions observed upon single application of a formulation on skin. Based on the PDII score, the formulation can be graded as irritating or non-irritating.

## 2.8 Selection and maintenance of animals

Healthy young male albino rabbits, weighing 1.5 – 2 kg at the start of the experiment, were used as

the experimental animals in the present study (Clinical Ethical clearance No: 1145/a/07/CPCSEA/2011/6). The animals were housed together in a clean tank which was spacious enough for free movement of the animals and accommodation to hold drinking water and feed. Room temperature was  $25\pm 30$  °C, humidity was 45-55% with a light period of 12 h (06.00 to 18.00). The animals were fed with commercially available standard pellet chow and filtered tap water.

### 2.9 Preparation of animals

Approximately 24 hours before the test, fur was removed by closely clipping the dorsal area of the trunk of the animals. Care was taken to avoid abrading the skin and only animals with healthy, intact skin were used for the study.

### 2.10 Application of the herbal gel

Half a gram of the herbal gel, as the test substance, was applied to an area of approximately 6 cm<sup>2</sup> of skin and covered with a gauze patch. The patch was loosely held in contact with the skin by means of a suitable semi-occlusive dressing for 4 hours and was then removed. At the end of the exposure period, i.e. 4 hours, residual test substance was removed without altering the existing response or the integrity of the epidermis. Observations were recorded an hour after the removal of the patch. Control animals were prepared in the same manner and 0.5 gram of the gel base, i.e. gel formulated using all the ingredients except the herbal mixture, was applied to the control animals and observations were made similar to the test animals. Both the control and the test animals were observed every day for any occurrence of skin irritation or toxic reactions such as oedema or erythema. Per observation of skin, a value between 0 and 4 was recorded where 0 meant no skin erythema and eschar formation and 1, 2, 3 and 4 stood for very slight, well defined, moderate and severe erythema to eschar formation, respectively. It also scored from 0–4, where 0 stood for no oedema and 4 stood for severe oedema.

Primary Dermal Irritation Index (PDII) =

$$\frac{\text{PDII observed on 12 + 24 + 48 + 72 hrs}}{4}$$

### 2.11 Classification system based on PDII

< 0.5: non-irritating, 0.5-2.0: slightly irritating, 2.1-5.0: moderately irritating and >5.0: severely irritating.

### 2.12. 28 days repeated dose dermal toxicity of the developed herbal gel formulation

28 days repeated dose dermal toxicity of the herbal gel formulation was conducted on the rabbit skin to evaluate the cumulative toxicity of the herbal gel upon repeated application along with the study of behavioral, hematological and biochemical parameters. Both the control and the test animals were observed every day for any occurrence of toxic or irritation reactions such as oedema or erythema.

### 2.13 Hematological analysis

Blood samples of all the test and control rabbits were collected by vein puncture on the 14<sup>th</sup> and on the 28<sup>th</sup> day of the study. Estimation of haemoglobin percentage was done using haemocytometer.

### 2.14 Biochemical analysis

For determining blood sugar, total cholesterol, creatinine, urea, total and direct bilirubin, protein, SGOT, SGPT, alkaline phosphatase and acid phosphatase, blood samples were collected separately from each control and experimental animal by retro orbital puncture on the 14<sup>th</sup> and 28<sup>th</sup> day of the study.

### 2.15 Evaluation of anti-inflammatory activity Animals

Albino Wistar rats of either sex, weighing 150–200 g were used. They were housed in standard environmental conditions and fed with standard rodent diet with water *ad libitum*. All animal procedures were followed in three groups (Control, Test and Standard) of six animals each.

### 2.16 Carrageenan-induced rat paw oedema<sup>[15,16]</sup>

Animals were fasted for 24 hrs before the experiment with water *ad libitum*. Approximately 50µl of a 1% suspension of carrageenan in saline was prepared 1 h before each experiment and was injected into the plantar side of right hind paw of rat. 0.2 g of herbal gel containing 1% *E. adenophorum* extract was applied to the plantar surface of the hind paw by gentle rubbing 50 times with the index finger. Rats of the control groups received the plain gel base and 0.2 g 1% Valdecoxib gel applied in the same way was used as a standard. Drugs or placebo were applied 1h before the carrageenan injection. Paw volume was measured immediately after carrageenan injection and at 1, 2, 3 and 4 hrs intervals after the administration of the noxious agent by using a plethysmometer.

### 2.17 Statistical Analysis

Data are reported as the mean±SEM (Standard Error Mean) and were analyzed statistically by means of analysis of variance (ANOVA) followed by Student's t-test. Values of  $p < 0.05$  are regarded as significant.

## 3. Results

The herbal gel was prepared and subjected to evaluation of various parameters. The gel was greenish in colour with a translucent appearance and cooling sensation throughout the evaluation period. The pH was constant throughout the study to about 6.8 and the gel did not produce any irritation upon application to the skin. Extrudability was excellent while spreadability was less variant after performing stability studies from that of the initially prepared gel. The initial viscosities were recorded at 25 °C. Furthermore, the stability study's results revealed the preparation was stable under normal storage conditions.

The Primary Dermal Irritation Index (PDII) of the formulation was 0.00; hence, according to OECD

guidelines the formulation can be classified as non-irritant to the rabbit skin. No clinical signs of dermal toxicity were observed in any of the animals treated with the test substance upon repeated application of the herbal gel for up to 28 days.

The control and the experimental rabbits showed no signs of convulsions. Hematological profiles of the experimental rabbits were studied after the repeated application of herbal gel daily for 28 days. Hematological parameters such as total counts of RBC and WBC, differential count of WBC and hemoglobin percentage were normal before treatment and after 14 and 28 days of application of the herbal gel. Biochemical parameters of blood such as blood sugar, total cholesterol, creatinine, urea, total and direct bilirubin, total protein, SGOT, SGPT, alkaline phosphatase and acid phosphatase of both test and the control rabbits were evaluated for any change in these parameters due to the application of the herbal gel with respect to the control rabbits. The changes were statistically insignificant.

These results indicated that the herbal gel had no adverse effects on the biochemical parameters of the blood. The anti-inflammatory effect produced after topical administration of herbal gel formulation on Carrageenan-induced hind paw oedema exhibited a high degree of reproducibility.

**Table 1:** Extrudability of the herbal gel at the time of preparation (Mean ± SEM)

Extrudability	Mean of three tubes (Initial month)
Net wt of formulation in tube (g)	12.34±0.011
Wt. of gel extruded (g)	11.32±0.014
Extrudability amount percentage	91.73±0.005

**Table 2:** Spreadability of the herbal gel during the evaluation period (Mean  $\pm$  SEM)

Evaluation Condition	Spreadability (g.cm/sec) Mean of three readings
Initial month	30.03 $\pm$ 0.015
After 3 months at 25 $\pm$ 2 °C/ 60 $\pm$ 5% RH	30.01 $\pm$ 0.012
After 3 months at 30 $\pm$ 2 °C/ 65 $\pm$ 5% RH	29.98 $\pm$ 0.012
After 3 months at 40 $\pm$ 2 °C/ 75 $\pm$ 5% RH	29.92 $\pm$ 0.014

**Table 3:** Viscosity of the herbal gel at the time of preparation

RPM	Viscosity (cps) – Initial month
50	22910
75	18790
100	15260
150	13089

**Table 4:** Effect of topical administration of Herbal gel on carrageenan-induced rat paw oedema

	Early Phase	Percentage Inhibition	Late Phase	Percentage Inhibition
Control	53.9 $\pm$ 2.051	-	94.2 $\pm$ 1.933	-
Standard	39.2 $\pm$ 2.342	27.27	61.5 $\pm$ 0.987	34.71
Herbal gel	49.4 $\pm$ 1.120	8.35	75.7 $\pm$ 0.851	19.64

#### 4. Conclusion

Carrageenan induced oedema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins whereas the second phase is related to the release of prostaglandin and slow reacting substances<sup>[15]</sup>. The data presented in this study demonstrate that *E. adenophorum* leaves extract in the form of gel possess significant topical anti-inflammatory properties, supporting their traditional use for the treatment.

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