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Preparation and Evaluation of Anti inflammatory Polyherbal Gel

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ABSTRACT

The aim of present study was to assess the anti-inflammatory activity of polyherbal formulation of leaves of *H. aciatica*, *C. gigantean* & *A. aspera* were collected and authenticated. Extractions of dried leaves and rhizome were carried out with ethanol in soxhlet apparatus. The polyherbal formulation showed the significant anti-inflammatory activity comparable to the standard drug Diclofenac sodium against carrageenan induced rat paw edema method. The polyherbal formulation reduced the inflammation induced by carrageenan by 49.3% and 61.73% on oral administration at 100 mg/ kg and 200 mg/kg respectively as compared to the control treated group.

Keywords: Edema, Polyherbal, Carrageenan.

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INTRODUCTION

Topical drug delivery systems are gaining increased in popularity, and several drugs have been successfully delivered by this route for both local and systemic action. Gels have better potential as a vehicle to administer drug topically in comparison to the ointment because they are non-sticky, requires low energy during formulation [1]. Drug delivery through the skin has been a promising concept for a long time because the skin is easy to access, has a large surface area with vast exposure to the circulatory and lymphatic networks and the route is non-invasive. Gel consists of a natural or synthetic polymer forming a three-dimensional matrix throughout a dispersion medium or hydrophilic liquid. After application, the liquid evaporates leaving the drug entrapped in a thin film of the gel-forming matrix physically covering the skin [2]. The presence of a network formed by the interlocking of particles of the gelling agent gives rise to the rigidity of a gel. The nature of the particles and the type of form that is responsible for the linkages determine the structure of the network and the property of the gel [3]. The available anti-inflammatory drugs present a wide range of side effects. Therefore, many studies are being directed to find anti-inflammatory agents from natural sources. *H. aciatica*, *C. gigantean* & *A. aspera* are quite known for its property of anti-inflammatory action. The search for anti-inflammatory drugs in modern time was marked by the introduction of salicin for the treatment of acute-rheumatism.[4] A variety of anti-inflammatory drugs are flooding the world market today but a very few are relatively non-toxic and fit for long term consumption. Moreover, discontinuation of drug therapy in chronic inflammatory conditions often leads to reappearance of symptoms. A large number of synthetic drugs having side-effects are available for promoting anti-inflammatory activity. Even then there is a search for herbal drugs for the same having less side effects.[5]

MATERIALS AND METHOD

The plants were selected on the basis of their antimicrobial activities and their medicinal uses reported in the literatures. The herbs (*H. aciatica*, *C. gigantean* & *A. aspera*) were purchased from plant drug supplier and sent to NISCAIR, Delhi for the identification. All other chemicals were of analytical grade and used without further purification.

Monographic Analysis of Herbs

The individual herbs were evaluated with regard to their standard specifications according to the Herbal Pharmacopoeia of India (1996). The tests carried out were loss on drying, extractive values and ash values.[6]

Preparation of Herbal Extract

The dried powder of *H. aciatica*, *C. gigantean* & *A. aspera* powders were prepared separately by taking 150g drugs and 400ml respective solvents into the round bottom flask for 24hr. Few drops of methyl paraben was added to prevent the fungal growth. Initially round bottom flask was occasionally shaken for 6hr and left aside for 18hr. Next day the extracts obtained by filtering, filtrate was distilled till semisolid mass and spray dried to get the dry uniform extract. Percentage practical yield was calculated on the basis of air dried material.[7]

Identification of Active Constituents in the Herbal Extract

HPTLC analysis of aqueous extract of *H. aciatica*, *C. gigantean* & *A. aspera* for asiaticin, glycyrrhithinic acid and gallic acid respectively was carried out with the markers to check the extracts qualitatively and quantitatively. The HPTLC was carried out using a Hemilton 100 μ l HPTLC syringe, Camag Linomat V automatic spotting device, Camag twin trough chamber, Camag TLC Scanner-3, WINCAT integration software, aluminium sheet precoated with Silica Gel 60 F254, 0.2mm thickness. The most recent automatic device "CAMAG LINOMAT V" was used to apply a band of 6mm width with different concentration of test and standard solutions. Sample application is the most critical step for obtaining good resolution for quantification in HPTLC. The plate was developed in CAMAG glass twin-through chamber (10-10cm) previously saturated with the solvent for 60min (temperature 25.2°C, relative humidity 40%). The development distance was 8cm. Subsequently scanning was done. The mobile phase was selected on the basis of trial and error method. The plates were scanned at UV 366nm and 254nm using CAMAG TLC Scanner-3 and LINOMAT-V. Rf value of separated compounds with peak area were recorded. Mobile Phase selected for *C. longa* (Alcohol) marker Curcumin, (CHCl₃: ethanol: gl. acetic acid (94:5:1)), *G. glabra* (Water) marker Glycyrrhithinic acid; Toluene: ethyl acetate: gl. acetic acid (12.5:7.5:0.5) and *T. chebula* (Water) Gallic acid (CHCl₃: ethyl acetate: formic acid (2.5:2:0.8)).[8,9,10]

Preparation of Topical Formulations

The topical gel was prepared by soaking the carbopol 934 in water for 24 h and their compositions are given in Table 1. The herbal extracts were incorporated into prepared gel base in two different concentrations.[11]

Table 1: The Compositions of Gel Formulations

Ingredients	F1	F2
<i>H. aciatica</i>	1 gm	2 gm
<i>C. gigantean</i>	1 gm	2 gm
<i>A. aspera</i>	1 gm	2 gm
Carbopol 934	2 gm	2 gm
Propylene glycol	2ml	2ml
Ethanol	5ml	5ml

Triethanolamine	q. s. to neutralize the gel base	q. s. to neutralize the gel base
Water	5ml	5ml

Antimicrobial Activity

The anti-microbial activity of each formulation was assessed by cup plate method. The zone of inhibition was measured in nutrient agar medium, employing *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* as test organisms.[12,13]

Anti inflammatory Studies

The animal study was carried out with permission from CPCSEA no. IAEC NO: 1161/ac/08/CPCSEA. Healthy inbred Wister albino rats of either sex, (150-180 g) were selected and housed in polypropylene cages at a well-ventilated, temperature-controlled (30±1oC) animal room with food and water ad libitum. Animals were periodically weighed before and after experiments. Animals were divided in four groups of 6 animals each. The control group receives vehicle orally, while other groups receives test drug and standard drug respectively. The animals were treated with drugs by oral route and subsequently one hour after treatment, 0.1ml of 1% suspension of carageenan in normal saline was injected to the sub planter region of left hind paw to induce edema. The paw volume was measured initially at 1, 3 and 5 hours after carageenan injection using plathismometer. The difference between the initial and subsequent reading gave the actual edema volume which was compared with control. The difference of average values between treated animals and control group is calculated for each time interval and evaluated statistically. [14, 15] The percent inhibition is calculated using the formula as follows-

$$\% \text{edema inhibition} = [1 - (V_t / V_c)] * 100.$$

V_t and V_c are edema volume in the drug treated and control groups respectively.

Statistical Analysis

The results of these experiments are expressed as means±SEM of six animals in each group. The data was subjected to one-way ANOVA and the values of $p \leq 0.01$ were considered statistically significant.

RESULTS AND DISCUSSION

Monographic Analysis of Herbs

Table 2 shows the results of monographic analysis of the herbs, performed according to the Indian Herbal Pharmacopoeia and WHO guideline for quality control of herbal raw materials. It was found that the moisture content of all the extract were less than 10%. The extractive values and ash

vales of all the extracts were within the pharmacopoeias limit. It indicates the good quality of raw materials.

Preparation of Herbal Extract

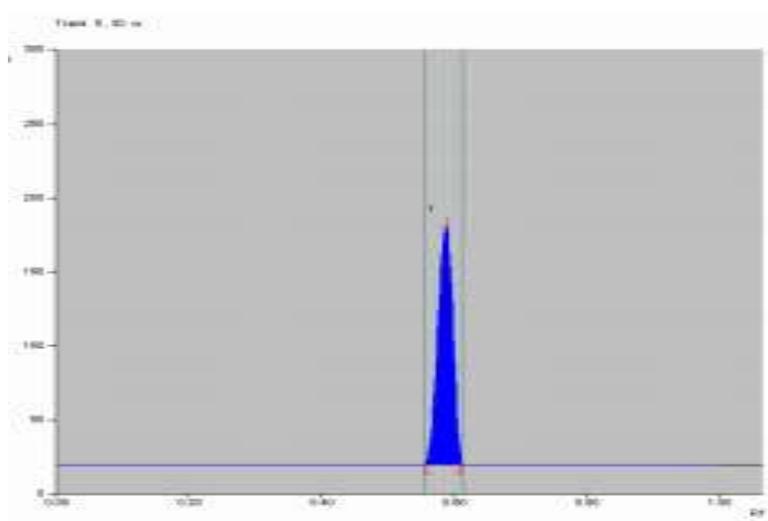
The extract prepared had different color and odor according to raw materials from which they are extracted.

Table 2: The evaluation of herbal extracts

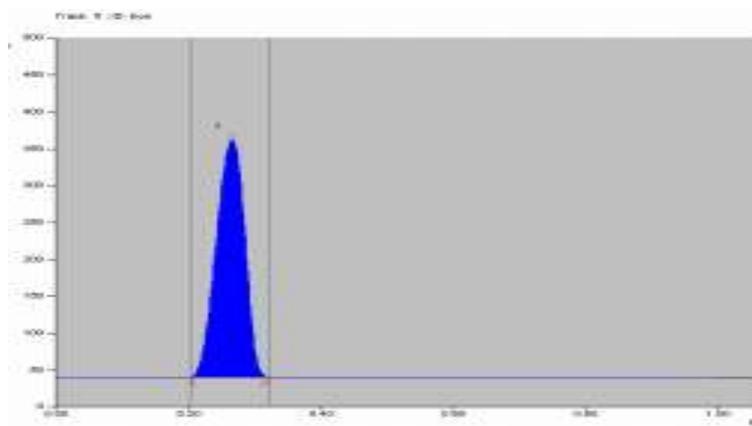
Parameters	<i>H. asiatica</i>		<i>C. gigantean</i>		<i>A. aspera</i>	
	Obtained Value	Pharmacopoeial Limit	Obtained Value	Pharmacopoeial Limit	Obtained Value	Pharmacopoeial Limit
Loss on drying	7.98±0.52	NMT 12	7.66±0.38	NMT 10	6.75±0.25	NMT 12
Alcohol Soluble Extractive	10.15±2.22	NLT 8	10.37±0.33	NLT 9.5	16.21±0.21	NLT 16
Water Soluble Extractive	13.29±0.25	NLT 9	15.38±0.39	NLT 6	20.25±0.43	NLT 17
Total Ash	7.07± 0.062	NMT 9	22.32±0.32	NMT 19	9.10±0.10	NMT 27
Acid Insoluble Ash	0.51±0.028	NMT 1	6.19±0.23	NMT 6	1.40±0.08	NMT 2

Identification of Active Constituents in the Herbal Extracts

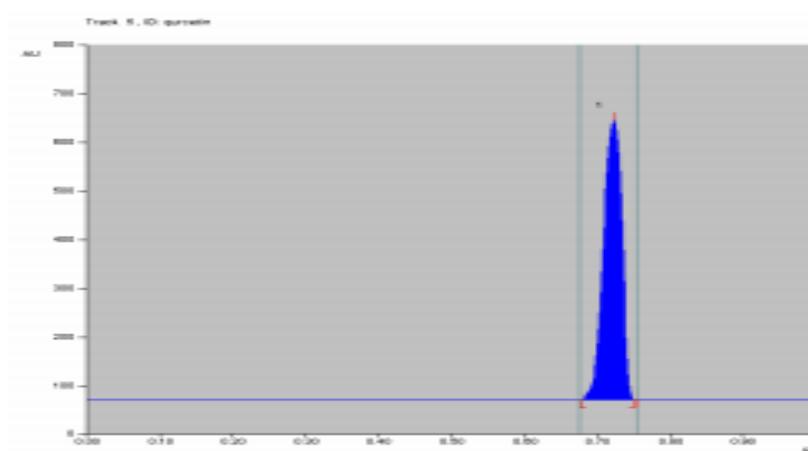
HPTLC analysis of the herbal extracts was performed and the chromatograms of active constituents and extracts are shown in Figure 1. It was found that the *R_f* values of the active constituents in the chromatogram of extract were match with chromatogram of active constituents. It indicates the presence of active constituent in extracts. The heights of peaks also indicate the presence of active constituents in significant amount in extract.



HPTLC of *H. asiatica*



HPTLC of C. gigantean



HPTLC of A. aspera

Preparation of Topical Formulations

The gel base without herbal extract was transparent and had good viscosity. The color was changed to brown after adding the extract and viscosity also slightly decreased due to addition of extract

Anti-Microbial Activity of the Formulations

The results of the antimicrobial activity of formulations are illustrated in Table 3. The gel base without the herbal extracts did not show any zone of inhibition while in case of formulation F1 F2, the zone of inhibitions were found to increase on increasing the concentration of extracts both the formulations were shown the better activity against Yeast and mold and *Staphylococcus aureus* than *Escherichia coli*.

Table 3: The Anti microbial activity of extracts

Sr. No.	Microbial analysis	Limit	Aqueous extract		
			<i>H. asiatica</i>	<i>C. gigantean</i>	<i>A. aspera</i>
1.	Total aerobic microbial count	10^5 /gm	NG	10cfu/g	10cfu/g
2.	Total yeast and mold count	10^5 /gm	NG	10cfu/g	NG
3.	<i>E. coli</i>	10^1 /gm	AB	AB	AB
4.	<i>S. aureus</i>	Absent	AB	AB	AB

Table 4 Anti-inflammatory Activity of Polyherbal formulation:

Sr. No.	Groups	Dose (mg/kg, p.o.)	0 hrs	1 hrs	2hrs	3 hrs	4 hrs	% Inhibition
1	Control	-	9.0±0.089	9.4±0.085	10.2±0.12	11.25±0.09	11.3±0.10	-
2	Std.Gel	150	8.36±0.09	9.7±0.11	7.53±0.07	7.0±0.05	61±0.07	44.60
3	Formulation	300	7.37±0.06	8.4±0.07	6.14±0.07	5.5±0.08	4.27±0.11	62.43

Values are mean \pm sem $p < 0.001$, $p < 0.01$ when compared with the control values of corresponding hours, n = 6.

CONCLUSION

Polyherbal formulation possesses potent anti-inflammatory activity as it inhibits maximum edema at 5 hrs, which was comparable to that of standard Diclofenac sodium. Since, serotonin, histamine and prostaglandins are the major mediators of inflammation, anti inflammatory effect of polyherbal formulation could be due to inhibition of either their synthesis or release possibly due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis at third stage of inflammation. Based on the results of the present study, it can be concluded that polyherbal formulation showed significant anti inflammatory activity.

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