FORMULATION AND ANTI-INFLAMMATORY EVALUATION OF POLY HERBAL GEL

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Summary

The present investigation was aimed to formulate pathophysiology based herbal gel formulation for the most common chronic joint inflammatory disease rheumatoid arthritis. For treating arthritis, initially inflammation should be reduced. Topical application of drug at the site of application reduces swelling more effectively than internally administered drug. Four medicinal plants were selected which have been traditionally used as anti-inflammatory and anti-arthritic drug and their extracts proven to be more potent than crude drugs. Oleo gum resins of *Boswellia serrata*, fruits of *Capsicum annum*, rhizomes of *Curcuma longa* and roots of *Withania somnifera* were selected and their extracts were prepared. These extracts were combined and gel formulation was made using carbopol 934P. For pharmacological evaluation of gel, carrageenan induced rat paw edema method was followed. After triple application of prepared gel, it showed 61.64% of activity and it was compared with allopathic standard gel and known ayurvedic product showed 67.04% and 54.48% respectively.

Key words: Anti-inflammatory, Gel formulation, Carrageenan, Carbopol 934P

Introduction

Rheumatoid arthritis is the chronic joint disease with an external symptom of inflammation. In modern medicine, non-steroidal anti-inflammatory agents used as first line treatment to relieve the symptoms of arthritis. The modern medicine is limited only upto symptomatic treatment therefore the search for screening and development of drugs for their anti-inflammatory activity is an unending problem. Use of herbal medicine will be the alternative method once joints gets inflammed, drugs through systemic circulation will have difficulty of penetration into inflammed part, so they do not show good healing effect.

Hence for treating arthritis, initially inflammation should be reduced. Internally administered drug although relieves inflammation takes lot of time. Topical application of drug at the site of inflammation reduces swelling in a short period of time. Further maximum amount of drug will directly reach the affected part which gives more action comparing to systemic therapy.

In rheumatoid arthritis earliest change is swelling and congestion of the synovial membrane and the underlying connective tissues, which become infiltered with lymphocytes, plasma cells and macrophages. Effusion of synovial membrane occurs, with the formation of lymphoid follicles resembling immunologically active lymph node inflammatory granulation tissue spreads over and under the articular cartilage, which is progressively eroded and destroyed. Later fibrous or bony ankylosis may occur. Muscles adjacent to inflamed joint and there may be focal infiltration with lymphocytes[1]. Number of drugs reviewed for topical anti-inflammatory and anti-rheumatic activities and from that four drugs were selected, as their extracts act in different stages in the pathophysiology of disease.

Capsaicin from *Capsicum annum* act as counter irritant as well as analgesic. It depletes the neurohumoral substance released from sensory nerve endings which is the cause for chemogenic pain and increased vascular permeability[2]. β-Boswellic acid and other related pentacyclic triterpene acids from *Boswellia serrata* improves blood supply to joints and have inhibitory effect on leukocyte population[3]. Curcumin from *Curcuma longa* scavenge the free oxygen radical produced in inflammation[4]. Withanoloids from *Withania somnifera* inhibit increased level of acute phase reactants[5].

Materials and Methods

Collection of samples:

The fresh fruits of *Capsicum annum*, dried rhizomes of *Curcuma longa*, oleo gum resin of *Boswellia serrata* and dried roots of *Withania somnifera* were collected from Trichirappalli district, Tamilnadu in the month of May. The plants were identified and authenticated by the Taxonomist and voucher specimen was deposited at Department of Botany, Pune University, Pune.

The samples were cleaned, dried under shade and size reduced according to the need for extraction procedure. Carbomer 934 P was supplied from Indus laboratories, Pune.

Extraction of the Crude drugs:

Immature fresh fruits of *Capsicum annum* were digested with acetonitrile for 24 hours. The extract was concentrated to get capsaicin[6].

The coarsely powdered air-dried rhizomes of *Curcuma longa* were extracted with methanol in a soxhlet extractor. The methanolic extract was concentrated and dried on a steam bath[7].

Dried and coarsely powdered oleo gum resin of *Boswellia serrata* was defatted by maceration with petroleum ether at room temperature for seven days. Defatted material was subjected to hot continuous extraction process in soxhlet using 80% methanol as a solvent. The extract was distilled to remove excess of methanol. The concentrated product was dried and stored in a well-closed container[8].

The finely powdered roots of *Withania somnifera* was subjected to cold percolation with methanol and the total percolate concentrated in vacuum to remove all the organic solvent with the addition of some water towards last stages of concentration. This aqueous concentrate was repeatedly shaken with ether and ether layer is separated and concentrated to get extract containing withanolid[9].

Formulation:

Gel formulation was prepared according to the formula [Table-1]. Anti-inflammatory activity of the prepared gel was determined by carrageenan induced rat hind paw edema method and the values were compared with standard marketed formulations Fengel® (Diclofenac) Pure health pharmaceuticals, Pune and Nopane® (Ayurvedic), Dabur Research Ltd New Delhi.

Table-1: Formula for gel Formulation

Extract of *Capsicum annum*	0.02%
Extract of *Curcuma longa*	0.5%
Extract of *Boswellia serrata*	1.00%
Extract of *Withania somnifera*	0.05%
Propylene glycol	10%
Sodium EDTA	0.15%
Tween 80	0.01%
Carbopol 934 P	2.5%
Water q.s to	100%

Experimental Animals:

Sprague dawely albino rats of either sex (180-240 g) were kept under standard conditions: temperature (24 ± 1^0 C), relative humidity (45-55%) and 12:12 light: dark cycle. The animals were fed with standard rat pellet and water ad libitum. The animals were allowed to acclimatize to laboratory conditions 48 h before the start of the experiment. Groups of 6 rats (18-24g) were used in all sets of experiments. All the experiments were conducted after obtaining permission from the Institutional Animal and Ethical Committee (IAEC)

Carrageenan induced hind paw edema:

Edema induced by the sub plantar injection of Carrageenan 0.1 ml (1% W/V) in the hind paws of the rats and edema was measured by plethysmometer (UGO BASILE, Italy, Cat. 7140). The gel formulation was applied to the inflammed paw of each animal immediately after carrageenan injection [10]. The edema was measured at 1, 2 and 3 hours after application of gel formulation (Table-3). Incase of single application the prepared formulation was applied, once to the inflamed paw immediately after carrageenan injection. Incase of double application, second application was made one hour after first application. Incase of triple application, third application was made one hour after second application.

The same method was followed for Standard marketed diclofenac formulation (Table-4) and Ayurvedic formulation (Table-5) for comparisons.

Table-2. Effect of Gel base (0.5 g) on Carrageenan induced rat paw edema (Control):

No. of Application	Mean edema volume ± S.D. after		
	1 hr	2 hr	3 hr
Single	0.29 ±0.02	0.41 ±0.03	0.56 ±0.02
Double	0.24 ±0.01	0.44 ±0.04	0.57 ±0.04
Triple	0.29 ±0.24	0.38 ±0.31	0.42 ±0.27

Mean edema volume ± S.D. observed after single, double and triple application of gel base.

Table-3. Effect of prepared gel (0.5g) formulation on Carrageenan induced rat paw edema

No. of Application	Mean edema volume ± S.D. after			Percentage inhibition of edema after		
	1 hr	2 hr	3 hr	1 hr	2hr	3 hr
Single	0.23 ± 0.01	0.30 ±0.03	0.33±0.32	20.69	27.91	41.07
Double	0.18 ±0.02	0.29 ±0.03	0.28±0.02	25.94	34.39*	50.53*
Triple	0.22 ±0.12	0.23 ±0.02	0.16±0.03	23.16	39.02*	61.64*

Values are expressed as ± S.D; *P < 0.05 vs control

Table-4. Effect of Diclofenac gel (0.5g) on Carrageenan induced rat paw edema

No. of Application	Mean edema volume ± S.D. after			Percentage inhibition of edema after		
	1 hr	2 hr	3 hr	1 hr	2hr	3 hr
Single	0.21 ±0.02	0.28 ±0.03	0.29±0.02	27.59	31.41	48.21
Double	0.17 ±0.03	0.25 ±0.02	0.24±0.03	30.04	43.60*	56.08***
Triple	0.19 ±0.1	0.21 ±0.02	0.14±0.03	34.16	44.74**	67.04***

Values are expressed as ± S.D; * p< 0.05, ** p< 0.01 *** p< 0.001

Table-5. Effect of Ayurvedic gel formulation (0.5g) on Carrageenan induced rat paw edema

No. of Application	Mean edema volume ± S.D. after			Percentage inhibition of edema after		
	1 hr	2 hr	3 hr	1 hr	2hr	3 hr
Single	0.24 ±0.02	0.31 ±0.02	0.35±0.01	17.24	25.84	37.50
Double	0.19 ±0.02	0.31 ±0.03	0.34±0.02	23.60	29.86*	39.93*
Triple	0.21 ±0.1	0.23 ±0.02	0.18±0.03	23.24	36.95**	54.48**

Values are expressed as ± S.D; * $p< 0.05$, ** $p< 0.01$

Statistical Analysis:

The average paw edema volume in all the groups was compared with that of control group. The percentage inhibition of edema was calculated and the statistical significance was calculated by using students't' test.

Results

Triple application of gel formulation showed significant and maximum anti-inflammatory activity. The acute anti-inflammatory effect by carrageenan induced rat hind paw showed 61.64% for the prepared herbal gel after triple application. The activity was compared with the standard diclofenac gel showed 67.04% and that of known ayurvedic formulation in the market showed 54.48%.

Discussion

The sum total anti-inflammatory effect of the gel formulation is more than the effects of the individual herbal drugs. This may be appropriately put here as the synergistic or potentiated activity exerted by the gel formulation.

A number of synthetic drugs have been used as anti-inflammatory drugs. The major side effects of NSAIDs tablets are gastric irritation and ulceration. Therefore in addition to tablets, the usage of topical preparation is recommended [11].

In the same way, using topical herbal gel formulation will not be sufficient to treat arthritis. A pathophysiolgy based internal herbal medicine should be recommended for better therapeutic effect.

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