ANTI-INFLAMMATORY ACTIVITY OF HYDROGEL FORMULATIONS OF *CURCULIGO ORCHIOIDES* (GAERTN) RHIZOMES.

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Summary

Curculigo orchioides Gaertn. (Family: Amaryllidaceae) commonly known as 'kali-musali' widely used in ayurvedic and traditional system of medicine for the treatment of inflammation. The objective of the present investigation was to study the anti-inflammatory activity of gel formulations of *Curculigo orchioides* (G.) rhizomes against carrageenan induced rat paw edema. The gels were formulated using the different concentration of gelling agent i.e. carbomer 940 and sodium CMC polymer. FC_1 and FS_2 showed significant ($P<0.01$) anti-inflammatory activity as compared with control. So FC_1 and FS_2 gel formulations were taken as optimized formulation for further study of physical evaluation.

Keywords: *Curculigo orchioides*, carrageenan, anti-inflammatory, diclofenac gel

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Introduction

In Indian system of medicine, a large number of drugs of either herbal or mineral origin have been advocated for various types of diseases and other different unwanted conditions in humans [1]. Ayurvedic medicines are largely based upon herbal and herbomineral preparations and have specific diagnostic and therapeutic principles [2]. Inflammation is a disorder involving localized increases in the number of leukocytes and a variety of complex mediator molecules [3]. Prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation. Their biosynthesis has also been implicated in the pathophysiology of cardiovascular diseases, cancer, colonic adenomas and Alzheimer's diseases [4, 5].

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects [6, 7]. The research into plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs. Non steroidal anti-inflammatory drugs (NSAIDs) have been increasingly introduced as topical preparation for local treatment of musculoskeletal soft tissue rheumatic conditions. This concept may be extended to the selective COX-2 inhibitors, so as to minimize their side effect. Topical administration requires lower doses as compared to oral dosage forms; hence it is hypothesized that systemic absorption may be negligible [8]. *Curculigo orchioides* (G.) shows potent anti-inflammatory activity, so it was thought to that the formulations and pharmacological evaluation of C*urculigo orchioides* (G.) rhizomes was carried out [9, 10, 11].

Material and Methods

Plant Material

The rhizomes of *Curculigo orchioides* (G.) (Family: *Amaryllidaceae*) were purchased from local market of Jalgaon. Maharashtra, India. Rhizomes of *Curculigo orchioides* Gaertn. were authenticated by Botanical Survey of India, Pune. India. The Voucher no. CUOPDPL1.

Preparation of Extract

The rhizomes of *Curculigo orchioides* (G.) were shade dried and pulverized. The air-dried powder was subjected to exhaustive extraction with ethanol in a soxhlet extractor and concentrated under vacuum and dried in a dessicator. The percentage yield of ethanol extract was 11.66 % w/w.

Preliminary Phytochemical Analysis of Extract

Ethanol extract was subjected for preliminary phytochemical investigation for the identification of the various phytoconstituents employing standard screening test [12]. Conventional protocol for detecting the presence of alkaloids, tannins, carbohydrates, gums, mucilage, steroids, glycosides, flavonoids etc. was used.

Drugs and Chemicals

Carrageenan was purchased from Sigma, USA. Diclofenac sodium gel was purchased from medical store. Carbomer 940, sodium carboxy methyl cellulose, methyl paraben and propyl paraben were obtained as a gift sample from Ajanta Pharmaceutical, Jalgaon. India. Propylene glycol, glycerin, methanol and ethanol were purchased from Jinendra Scientific, Jalgaon.

Formulation of herbal gel

Carbomer 940 gel [FC]

Carbomer 940 was soaked in 50 ml of water in different concentration. of carbomer i.e.1.0 % and 1.25 %. On the next day they stirred uniformly to form the mucilage. In each of the mucilage, drug extract was added (previously dissolved in methanol) with constant stirring. Preservative methyl paraben and propyl paraben were added. It was neutralize with triethanolamine solution with constant stirring and glycerin was added to form a clear gel. The final gels were sparkling and light brown in colour

Sodium CMC gel [FS]

Sodium CMC was weighed in different concentration 3.5 % and 4.0 %; and distilled water was added with constant stirring. The stirring was done slowly to avoid the entrapment of air bubble. Preservative methyl paraben and propyl paraben were added and then drug extract in methanol (previously dissolved in propylene glycol and glycerin) was added. The mixture was stirred to homogenize for about 10 min [13, 14, 15]. The composition of herbal gel formulations is shown in Table 1.

Table 1.Components of various gel formulations:

Formulations				
Ingredients	FC_1	FC_2	FS_1	FS_2
Curculigo orchioides extract (%)	10.0	10.0	10.0	10.0
Carbomer 940 (%)	1.0	1.25	----	----
Na CMC (%)	----	----	3.5	4.0
Triethanolamine (%)	0.7	0.7	----	----
Propylene glycol (%)	----	----	18.0	18.0
Glycerin (%)	10.0	10.0	10.0	10.0
Methyl Paraben (%)	0.18	0.18	0.18	0.18
Propyl Paraben (%)	0.02	0.02	0.02	0.02
Alcohol (%)	10.0	10.0	10.0	10.0
Water	q. s	q. s	q. s	q. s

Experimental Animals

Wistar albino rats (150-180 g) of either sex were procured from Calcutta Fish Aquarium, Indore, India and used throughout the study. They were kept in polypropylene cages and maintained at a temperature of 25 ± 2 ^{0}C and relative humidity of 45% to 55% under 12-h light: 12-h dark cycle. The animals had free access to food pellets (Amrut Feeds, Pune, India), and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Hon'ble Loksevak Madhukarrao Chaudhari, College of Pharmacy, Faizpur, India.

Pharmacological Screening of Formulations

Anti-inflammatory activity of Curculigo orchioides alcoholic extract gels by carragenan induced rat paws oedema

The rats were divided into six groups (n = 6). The different groups were treated topically with FC_1, FC_2, FS_1 and FS_2 alcoholic extract of *Curculigo orchioides* (G.), Diclofenac gel (1%) and vehicle control (gel base). The administration of formulations and drug was 1 hr prior to injection of 0.1 ml of 1% freshly prepared suspension of carrageenan in normal saline in the right hind paw sub plantar of each rat.

The paw volume was measured initially and then at 1, 3, 5 and 7 h after the carrageenan injection by using plethysmometer. The anti-inflammatory effect of alcoholic extract of *Curculigo orchioides* (G.) was calculated by the following equation: -

$$\text{Anti-inflammatory activity (\%)} = (1\text{-}Vt/Vc) \times 100$$

Where Vt represents the paw volume in drug treated animals and Vc represents the paw volume of control group animals [16].

Evaluation

Homogeneity test

A small quantity of gel was pressed between the thumb and the index finger and the consistency of the gel was noticed (whether homogeneous or not) and if there was any coarse particles appeared or detached on finger.

Organoleptic characteristics

Gel was tested for color, odor, texture, phase separation or bleeding as well as the feel upon application (stiffness, grittiness, greasiness and tackiness) ones the preparation was on the skin and also after two minutes of application.

pH

The pH of the gel was measured using pH meter. One gram of gel was dissolved in 100 ml distilled water and stored for 2 hours. The measurement of pH of each formulation was done in triplicate and average values are calculated.

Viscosity

The viscosity of gel was studied by using Brookfield Viscometer YR-1 by using spindle no. 7 at 10 rpm. The rheology of the gel was studied using Brookfield Viscometer For Carbomer 940 and sodium CMC using spindle no 7 were found to be appropriate respectively and speeds were selected at 10, 20, 30, 40, 50 and 70 rpm. Different viscosities at respective spindle speeds were obtained for an ascending and descending curve. Rate of shear and shearing stress were calculated [17].

Spredability
An excess of gel was placed between two glass slides and a 1000 gm weight was placed on the slide for 5 minutes to compress the sample to a uniform thickness. The bottom slide was anchored to the apparatus and weights of 100 gm placed in the pan. A time in second needed to separates to slide was taken as a measure of spreadability [18].

Skin irritation study
10 Wister rats of either sex weighing between 150-200 g were used. Animals were divided in to 2 groups of 5 animals each. Hairs were depleted from the back of wistar rats with the help of depilatories and area 4 cm^2 was marked on both the sides. One side served as control while the other as test and animals were used after 24 hrs. After hair depletion, gel was applied (1 g) once a day for 7 days and sight was covered with cotton bandage and observed for any sensitivity and the reaction if any was graded as, [13].

Stability studies
The gel was subjected to 37˚C± 2˚C at 65 ± 5% RH temperature and relative humidity during stability studies for 1, 2 and 3 month. Gel was evaluated for various parameters after every 3 months.

Statistical Analysis
All values were expressed as mean ± SEM. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Dunnett test. *P* values < 0.05 were considered to be statistically significant when compared to control.

Results

Preliminary Phytochemical Screening
Preliminary phytochemical screening of the ethanol extract of *Curculigo orchioides* revealed the presence of alkaloids, tannins, carbohydrates, steroids, triterpenoides, flavonoides and glycosides.

Carrageenan induced rat paw edema
The result of gel formulations of *Curculigo orchioides* alcoholic extract against carrageenan-induced paw edema is shown in Table 2.

In carrageenan induced rat paw edema, FC_1 and FS_2 showed significant ($P<0.01$) reduction of rat paw edema. The FC_1 and FS_2 showed maximum inhibition 34.61 % and 46.15% after 1 h of drug treatment in carrageenan induced paw edema whereas the standard drug showed 57.69% of inhibition when compared to control group. Whereas FC_2 and FS_1 showed 30.76 % and 34.61% inhibition which may be due its high viscosity to resists release of drug extract. Hence FC_1 and FS_2 gel formulations were taken as optimized formulation for further study of physical evaluation.

Table 2.Effect of *Curculigo orchioides* gel on carrageenan induced paw edema in rats.

Treatment	Differences in Paw edema volume in ml (% inhibition).			
	1 h	3 h	5 h	7 h
Control	0.26±0.0084	0.58±0.0070	0.86±0.011	1.03±0.010
Diclofenac gel (1 %)	0.11±0.0071** (57.69 %)	0.29±0.0095** (50 %)	0.44±0.0079** (48.83 %)	0.51±0.014** (50.48 %)
FC_1	0.17±0.011** (34.16 %)	0.41±0.019** (29.31 %)	0.64±0.030** (25.58 %)	0.77±0.026** (25.24 %)
FC_2	0.18±0.0060** (30.76 %)	0.43±0.015** (25.86 %)	0.64±0.021** (25.58 %)	0.78±0.022** (24.27 %)
FS_1	0.17±0.015** (34.61 %)	0.45±0.022** (22.41 %)	0.69±0.019** (19.76 %)	0.86±0.013** (16.50 %)
FS_2	0.14±0.011** (46.15 %)	0.33±0.0071** (43.10 %)	0.50±0.0084** (41.86 %)	0.60±0.0088** (41.74 %)

Data was expressed as mean ± S.E.M (n=6) and statistical analysis was carried out by One Way ANOVA followed by Dunnett test. **P<0.01 Vs control.

Evaluation of Formulations

Selected gel formulations were evaluated for there organoleptic characters, homogeneity, pH, viscosity, spredability, safety evaluation and stability study. It was found that all formulation gels were smooth, elegant, with pleasant odor and were not gritty which indicated good texture of formulation. The results are shown in Table 3.

Table 3.Result for pH, Viscosity and Spredability:

Formulation	pH	Viscosity (cps)	Spredability (g.cm/ sec)
FC_1	6.4	39700	26.65 ± 0.507
FS_2	5.8	42700	20.68 ± 0.123

Mean of three reading ± S. D

Rheology study

The rheology of test sample determines at 10, 20, 30, 40, 50 and 70 rpm and it showed a change in yield value. The results are shown in Table 4 and 5. The effect of storage time on the rheology of the gel was studied by storing the gel for variable periods of 0, 1, 2 and 3 month at room temperature and calculate yield value at 10, 20, 30, 40, 50 and 70 rpm.

Skin irritation study

Gels were subjected to skin irritation test and it was found that no erythema, pruritis or allergic reaction had occurred when applied to the skin in all cases.

Stability studies

The effect of storage time on the pH, Viscosity and Spredability of the gel was studied by storing the gel for variable periods of 0, 1, 2 and 3 month at room temperature. The results are shown in Table 6

Table 4.Rheological studies of FC_1 at 37^0c ± 2 at 65 ± 5% RH.

	FC_1								
		Month 0		Month 1		Month 2		Month3	
	RPM	Shear rate (sec)	Shear stress (dynes /cm^2)	Shear rate (sec)	Shear stress (dynes /cm^2)	Shear rate (sec)	Shear stress (dynes /cm^2)	Shear rate (sec)	Shear stress (dynes /cm^2)
ACENDING	10	0.0416	106.23	0.0416	103.23	0.0416	98.86	0.0416	96.5
	20	0.0827	128.81	0.0827	116.01	0.0827	120.7	0.0827	115.89
	30	0.118	142.44	0.118	127	0.118	127.2	0.118	125.51
	40	0.168	152.63	0.168	138.84	0.168	135.29	0.168	135.29
	50	0.2067	169.59	0.2067	147.62	0.2067	145.26	0.2067	142.63
	70	0.2889	176.11	0.2889	158.11	0.2889	148.15	0.2889	146.15
DECENDING	50	0.2067	158.78	0.2067	143.44	0.2067	137.8	0.2067	137.1
	40	0.168	146.7	0.168	125.63	0.168	122.6	0.168	128.22
	30	0.126	135.81	0.126	112.47	0.126	117.24	0.126	118.1
	20	0.0827	116.81	0.0827	99.5	0.0827	102.5	0.0827	100.6
	10	0.0416	91.6	0.0416	88.7	0.0416	86.6	0.0416	84.2

Table 5. Rheological studies of FS_2 at 37^0c ± 2at 65 ± 5% RH.

		FS_2							
		Month 0		Month 1		Month 2		Month3	
	RPM	Shear rate (sec)	Shear stress (dynes /cm^2)	Shear rate (sec)	Shear stress (dynes /cm^2)	Shear rate (sec)	Shear stress (dynes /cm^2)	Shear rate (sec)	Shear stress (dynes /cm^2)
ACENDING	10	0.032	129.3	0.032	122.43	0.032	117.54	0.032	104.58
	20	0.064	142.8	0.064	139.25	0.064	135.14	0.064	123.33
	30	0.097	154.2	0.097	152	0.097	151.25	0.097	145.25
	40	0.128	164.8	0.128	163	0.128	160.48	0.128	151.82
	50	0.1601	182.1	0.1601	175.56	0.1601	170.2	0.1601	165.29
	70	0.224	185	0.224	181.78	0.224	176.68	0.224	171.77
DECENDING	50	0.1601	172.3	0.1601	171.3	0.1601	164.32	0.1601	160.67
	40	0.128	153.7	0.128	153.7	0.128	148.65	0.128	143.48
	30	0.097	142.4	0.097	142.4	0.097	136.17	0.097	132.82
	20	0.064	130.8	0.064	130.81	0.064	126.74	0.064	120.17
	10	0.032	102.6	0.032	104.45	0.032	103.85	0.032	90.26

Table 6. Stability studies of FC_1 and FS_2 at $37^0c \pm 2$ and at 65±5% RH.

	FC_1				FS_2			
	Month				Month			
Parameters	0	1	2	3	0	1	2	3
Viscosity (cps)	39700	38910	39300	39230	42700	42300	42360	42250
pH	6.4	5.8	5.4	6.2	5.8	5.7	6.0	6.1
Spredability (g.cm/sec)	26.65± 0.507	27.27± 0.802	29.13± 0.911	29.94± 0.854	20.68± 0.123	24.70± 0.175	25.10± 0.546	24.23± 0.123

Discussion

The most widely used primary test for screening of anti-inflammatory agents is carrageenan induced edema in the rat paw hind paw [16]. The development of edema in the paw of the rat after injection of Carrageenan is believed to be biphasic event. The initial phase observed during the first hour is attributed to the release of histamine and serotonin; the second phase is due to the release of prostaglandin-like substances [19]. Based on this, it could be argued that the suppression of the first phase may be due to inhibition of the release of early mediators, such as histamine and serotonin, and the action in the second phase may be explained by an inhibition of cyclooxygenase [20].

Ueno et al., [21] found that the injection of carrageenan into the rat paw induces the liberation of bradykinin, which later induces the biosynthesis of prostaglandins and other autocoids, which are responsible for the formation of the inflammatory exudates. Besides, in the carrageenan induced rat paw edema model, the production of prostanoids has been through the serum expression of COX-2 by a positive feedback mechanism [22]. There fore, it is suggested that then mechanism of action of *Curculigo orchioides* alcoholic extract may be related to prostaglandin synthesis inhibition.

Preliminary phytochemical screening indicated the presence of flavonoids in *Curculigo orchioides* alcoholic extract. The rhizomes of *Curculigo orchioides* (G.) two phenolic glucosides named orchiosides A and B were isolated [23].

Selected phenolic compounds and flavonoids were shown to inhibit both the cyclooxygenase and 5-lipxygenase pathways [24-26]. This inhibition reduces the release of arachidonic acid. The exact mechanism by which flavonoids inhibit these enzymes is not clear. Quercetin, in particular, inhibits both cyclooxygenase and lipoxigenase activities, thus diminishing the formation of these anti-inflammatory metabolites [27, 28].

Another anti-inflammatory feature is the ability of flavonoids to inhibit eicosanoid biosynthesis [29, 30]. Eicosanoids, such as prostaglandins, are involved in various immunological responses and are the end products of the cyclooxygenase and lipoxigenase pathways. Flavonoids also inhibit both cytosolic and membranal tyrosine kinases which play key roles in the signal transduction pathway that regulates cell proliferation [31]. Another anti-inflammatory property of flavonoids is their suggested ability to inhibit neutrophils degranulation. This is the direct way to diminish the release of arachidonic acid by neutrophils and other immune cells [32, 33].

Conclusion

The FS_2 gel formulation of rhizomes of *Curculigo orchioides* (G.) showed significant anti-inflammatory activity in carrageenan induced rat paw edema. Hence to put into a nutshell, the active principle/s of rhizomes of *Curculigo orchioides* Gaertn. like tannins, flavonoids and steroids were reported to inhibit PG synthesis [34]. So this phytoconstituents may be responsible for anti-inflammatory activity. However, it needs isolation, structural elucidation and screening of any of the above mentioned active principle/s to pin point activity of drug.

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