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Research Article

TOPICAL ANTIINFLAMMATORY ACTIVITY OF *TRIPODANTHUS ACUTIFOLIUS* FLOWERS GEL FORMULATION

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ABSTRACT

This study evaluated a new herbal preparation containing extract from flower of *Thripodanthus acutifolius* "corpo" for its antiinflammatory activity against carrageenan induced edema. Gelling agent used was gel base (Carbopol 940®). The gel was evaluated for various parameters such as stability, pH, spreadability, skin irritancy and organoleptic characters.

The physicochemical appearance of the formulation was consistent, brilliant, dark amber, due to the color of the extract, without apparent loss of his structure during 60 days in conditions of rest.

Topical administration of corpo gel showed significant antiedematogenic activity. The maximal activity was 20 % at 2.5 h after carrageenan injection and maintained for 4.5 h of assay.

The results concluded that topical preparation containing 4% of corpo gel possesses antiiflammatory effect which can be useful for the treatment of local inflammation.

Keywords: *Tripodanthus acutifolius,* Topical gel formulation, Antiinflammatory activity

INTRODUCTION

The plants belonging to Loranthaceae family are found to be a rich source of substances of phytoclinical interest[1]. Many plants from this family are used in traditional system of medicine[2].

Tripodanthus acutifolius, previously named *Phrygilanthus acutifolius*, is commonly referred to as "corpo". It is a hemi parasite found on the "Calchaquí Valley". The flowers are used for ornamental purposes and in folk medicine for throat pain, respiratory infections and hypertensive diseases. Recently, our research group demonstrated antibacterial[3], antiinflammatory, antinociceptive, antipyretic[4], diuretic[5] and antioxidant[6] activities and no toxic effects in the flower extract.

Inflammation is a physiophathological response of living tissues to injuries that leads to the local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases. Therefore, the uses of non-steroidal antiinflammatory drugs are helpful in the therapeutic treatment for regional inflammatory disorders such us muscle pain, osteoarthritis and rheumatoid arthritis. In this context, medicinal plants are widely used in folk medicine of many countries to treat different inflammatory process[7,8,9,10,11,12].

It is well known that transdermal gels are more popular among all topical preparations due to ease of applications and better percutaneous absorption than other semisolid forms. However, few studies on topical gel formulations reported the therapeutic effects of drugs in a variety of experimental inflammation and inflammatory pain condition[13,14].

The present research has been undertaken with the aim to develop a transdermal gel formulation with aqueous flowers extract, prepared using carbopol 940. During the development step of formulation it was evaluated stability, spreadability and organoleptic characters. Further, the in vivo antiinflammatory activity of the gel formulation was studied using a rat model.

MATERIALS AND METHODS

Plant material

The plant materials used in this study consisted of the flowers of *Tripodanthus acutifolius* (Ruiz & Pav.) Eichler (Corpo) collected during the flowering season (May–June) in Amaicha del Valle, in the province of Tucumán, Argentina. The voucher herbarium (no. 511125) specimen was identified by using morphological, anatomical and histochemical techniques and by comparison with an identified specimen at the herbarium "Miguel Lillo" of San Miguel de Tucumán, Tucumán, Argentina.

Preparation of extract

Aqueous extract was prepared from the flowers of the plant under study. The dried materials were milled into particles of about 5-10 mm in diameter. For the aqueous extracts, samples were soaked for 2 days in 100 mL of distilled water. The extract was filtered through Whatman Paper No. 1 and evaporated by a lyophilizer to dryness.

Preparation of topical extract gel preformulation

The formulation was development used a gel base prepared with anionic hydrophilic colloid (carboxypolymethylene (Carbopol 940®) added with glycerine (emollient), methylparabene (preservative) and triethanolamine (for neutralized pH). Carbopol is commonly used in cosmetic and pharmaceutical products because of its high stability, compatibility and low toxicity.

Herbal gel was prepared with gel base (85 g), corpo extract (4 g) was resuspended in propilenglicol (1 mL) and distilled water (9 mL). The preservative used was a mixture of propylparabene and methylparabene (1 mL) (Solbac ®)

Analysis of the stability

The formulation was packaged in impermeable polypropylene containers for 60 days at 25±3ºC/AH (ambient humidity). Samples from formulation container were evaluated every 7 days at room temperature.

For the semisolid forms transferred aliquots of 2 g in pipes of test, they were closed and were controlled visually in regular form.

Evaluation of formulated gels

The organoleptic features of the samples were examined at the same temperature, lighting and packaging conditions to assess variations in appearance, phase separation, color, sheen and smell.

The pH values of prepared gels were checked by using a calibrated digital pH meter at constant temperature.

Gel spreadability

The spreadability evaluates the consistency of a semisolid form in order to assure that his extension on the skin. It is a term expressed to denote the extent of area to which gel readily spreads on application to skin or affected part. The therapeutic efficacy of a formulation also depends upon its spreading value.

A sample of 20 mg of each formulation was pressed between two slides on which weights of 2g, 4g, 7g and 10g were placed at intervals of one minute. The diameters during each interval were given as the area (mm^2), according to the National Formulary[15]. The variation of the area as a function of weight was then analyzed. The results obtained are an average of three determinations.

Animals

The Wistar rats (190-240 g) of either sex were selected for this study and were obtained from the Bioterio of Facultad de Bioquímica, Química y Farmacia, Instituto de Biología (INSIBIO).

All animals were kept under normal laboratory conditions of humidity, temperature (25±1°C) with photoperiod (12 h light/12 h dark cicle), and allowed free access to food and water ad libitum. The studies were conducted in accordance with the internationally accepted principles for laboratory animal use and care (EEC Directive of 1986; 86/609/EEC).

Antiinflammatory activity

The topic antiinflammatory activity was evaluated by the method of the induction of the edema according to Winter et al. (1962)[16]. In this study there were used 3 groups of six rats Wistar each one.

Group 1: gel base (negative control)

Group 2: gel of corpo water extract (4 %)

Group 3: gel of diclofenac dietilamina 1.16% (positive control) (Curinflam ®)

Paw edema was induced by injecting 0.1 mL of a 1.5% (w/v) suspension of carrageenan in physiological solution into the sub plantar side of right leg of the rat. After injection (1.5 h, maxima inflammation), 0.05 g of corpo gel (4 %) was applied to the plantar surface with circular massage, during 1 minute up to the penetration of the gel. Rats of control groups received 0.05 g of gel base and 0.05 g of diclofenac (1.16%) gel respectively. Paw volume (mL) was measured at 2.5, 3.5 and 4.5 h intervals after injection of the noxious agent. The % decrease in edema at each time point for the gel formulation was also calculated in comparison to control group. The antiinflammatory activity was expressed as percent inhibition of paw edema and was calculated by taking the values in the control group (gel base) as 100% inflammation.

Statistical analysis

Data are reported as mean ± S.D. Student´s t-test was used for statistical evaluation.

RESULTS AND DISCUSSION

Macroscopic aspects and analysis of stability

The physical and organoleptics characters evaluated for gels are represented in table1.The physicochemical appearance of the formulation was consistent, brilliant, dark amber, due to the color of the extract, without apparent loss of his structure during 60 days in conditions of rest, which shows that degradations of the formulations do not take place.

Table 1: **Physical and organoleptics characters of the preformulation**

Physical and organoleptics characters	Recent preparation of the gel of corpo	Corpo gel preparation at 60 days	Gel base
Color	Dark amber	Dark amber	Colorless
Brightness	Shiny	Shiny	Shiny
Smell	Characteristic of flowers used	Characteristic of flowers used	Odorless
Transparency	Media	Media	High
Tactile sensation	Soft	Soft	Soft
Aspect	Homogeneous, unctuous	Homogeneous, unctuous	Homogeneous, unctuous touch
pH	touch, free of lumps	touch, free of lumps	5.5±0.1
	6 ± 0.5	6 ± 0.3	

The results expressed in the table 1 indicated that the characters organoleptics obtained were acceptable for the natural product. The color was dark amber, typical for the extract used for this preparation. The greasiness to the tact is good and presence of grooms is not observed.

The pH of the gel formulation was in the range of 6 ± 0.3 to 6 ± 0.5, compatible with the normal pH range of the skin and would not produce any skin irritation, similar results was obtained by Das and Ahmed[11]. There was no significant change in pH values as a function of time after 60 days of preparation. The measurement of pH of the formulations is necessary to detect alterations during time storage, ensuring that the pH value is compatible with the components of the formulation and with the application place, avoiding irritation.

It was found in this study that the formulations showed functional and physical stability in the period of assay. None of the gels presented detectable alteration during 60 days to temperature set.

Gel Spreadability

Spreadability is the parameter which will help in the uniform application of gel to the skin. The results of the test of spreadability showed (table 2) that the formulated gels presented major spreadability compared with the gel base and the gel of diclofenac (Curinflam®) (figure 1).

The expressed results indicated that the average spreadability with a weight of 14, 97 g were 359 mm^2, inside the allowed limits (500 mm^2).

Table 2: Values of the spreadability with regard to the weight of the gels

Weight (g)	Spreadibility	Average (mm^2)	Gel of diclofenac

	Gel of corpo	Gel base	
4.97	153 ± 0.5	78± 0.7	133± 0.6
6.97	201± 0.3	95± 0.7	176± 0.5
8.97	254± 0.2	132± 0.6	201± 0.3
11.97	307± 0.1	153± 0.5	227± 0.2
14.97	359± 0.1	176± 0.5	254± 0.2

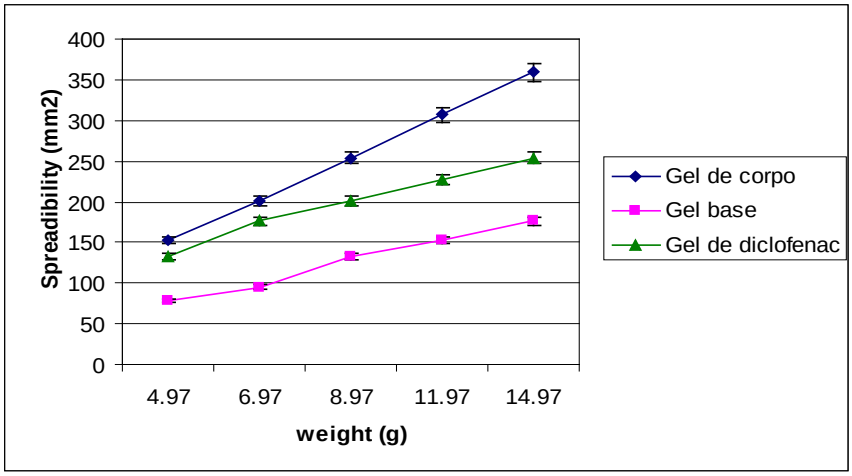

Fig. 1: The spreadibility was expressed in terms of the variation of the area (mm²) as a function of weight in one minute. Results are means±SD of three experiments

It is clearly evident that the gel formulation showed good homogeneity and spreadability.

With regard to the greasiness it does not leave residues, does not stain and allows to rub with a soft massage the skin where it was applied. After applying the gel of corpo is not observed in the rate any cutaneous deleterious manifestation.

The results evidenced that the formulation could be considered stable since the rheological parameters were constant during the period of study.

Evaluation of the antiinflammatory topical activity

The antiinflammatory activity after topical administration of corpo gel was studied. Carrageenan induced paw edema is the standard experimental model of acute inflammation. Moreover, the experimental model exhibits a high degree of reproducibility. The induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins whereas the second phase is related with the release of prostaglandins and slow reacting substances.

Topical administration of corpo gel showed significant antiedematogenic activity (table 3, figure 2). The maximal activity was 20 % at 2.5 h after carrageenan injection and maintained for 4.5 h of assay. In early study[4] demonstrated significant corpo antiedematogenic action of 100% for dose of 100 mg/kg orally administrated in Wistar rats before the injection of carrageenan.

The diclofenac produces a decrease of the inflammation of 15 % at 2.5 h, increasing to a percentage of 20 % at 3.5 h and it is kept up to the end.

Similar results were observed for a topical gel formulation of carbopol and *Achillea* and *Ruscus*.extracts extracts[8], for lapachol[9] and for leaves of *Vitex negundo*[17].

Table 3: Effect of topical administration of corpo gel on carrageenan induced paw edema in rats

Time*(h)	Treatment*	Control	Gel of corpo	Gel of diclofenac
T0	Carrageenan 1.5% (0,1 mL)	1.13± 0.02	1.26 ± 0.01	1.23 ± 0.03
T1 (1.5)	Gel base-Gel of corpo - Gel of diclofenac	1.49± 0.03	1.61 ± 0.03	1.58 ± 0.03
T2 (2.5)	_	1.52± 0,02	1.43 ± 0.02	1.51 ± 0.02
T3 (3.5)	_	1.47± 0.02	1.41 ± 0.03	1.45 ± 0.02
T4 (4.5)	_	1.45± 0.03	1.40 ± 0.02	1.41 ± 0.03

T0: the paw volume measured before the carrageenan injection, T1: the paw volume before the treatment with different gels formulations

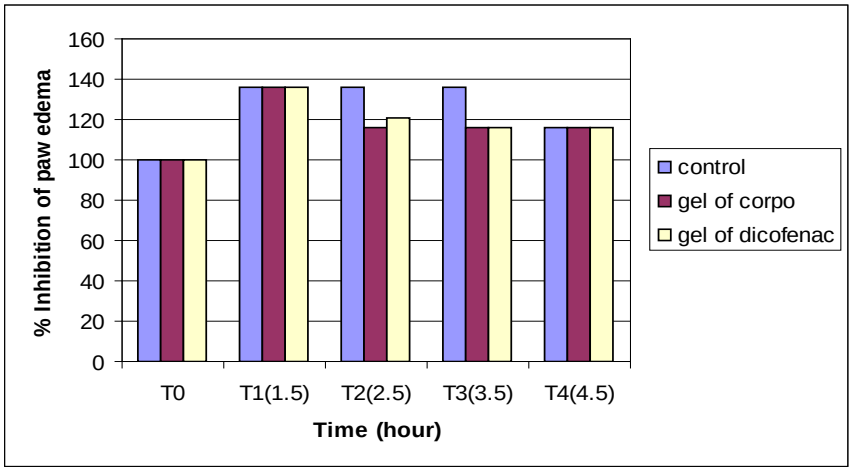

Fig. 2: Antiinflammatory effect of topical administration of corpo gel after carrageenan injection. Results are means ± SD of three experiments

CONCLUSION

The results concluded that topical preparation containing 4% of corpo gel possesses antiiflammatory effect which can be useful for the treatment of local inflammation.

There was elaborated a gel of hackneyed use based on water extract of flowers of corpo, who was compared with a gel of commercial origin (registered trademark) that contains diclofenac 1,16 % as reference to prove the antiinflammatory effect. The prepared formulation was it tipples give plastic containers, do not be transparent with lid to pressure. The gel does not present grooms, is homogeneous with good spreadability of 359 mm^2 and supporting his parameters organoleptics after 60 days of test.

For the checking of the efficiency of the gel in rats Wistar, there was used the method of induction of the edema to plant with carrageenan. There is verified that the gel diminishes the inflammation of a way similar to the obtained one for the diclofenac.

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