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Evaluation of anti-inflammatory activity of herbal gel formulation

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Abstract

This study evaluated a new herbal gel preparation containing extract from leaves of Ocimum Sanctum (OS) for its topical anti-inflammatory activity against carrageenan induced edema and anti-nociceptive effect. Gelling agent used in this study was 1% w/w concentration of carbopol-940. The studies were conducted on wistar rats of either sex (160-180 g). Change in oedema volume of the rat hind paw was measured. From the study we concluded that combination of Ocimum Sanctum (OS) potentiated the anti-inflammatory and anti-nociceptive effect topically.

Key Words: Anti-inflammatory effect; Herbal formulation.

INTRODUCTION

Inflammation or phlogosis is a pathophysiological response of living tissues to injuries that leads to the local accumulation of plasmatic fluid and blood cells[1]. During the development of inflammation, the concerted actions of molecular signaling determine whether inflammatory cells undergo migration, activation, proliferation, differentiation, or clearance. Many inflammatory processes are self-limiting and self-resolving, which suggests the existence of endogenous anti-inflammatory and/or proresolution mediators during the course of inflammation.

Ocimum Sanctum has been investigated extensively for its antioxidant and neuroprotective effect[1]. Radio protective effect[2]. Antioxidant and cyclooxygenase inhibitory action[3]. Antibacterial activity[4]. Immunotherapeutic Potential[5], anti-inflammatory, analgesic and antipyretic activity[6]. Hepatoprotective activity[7]. Protective effect of *Ocimum sanctum* in

experimental liver injury[8]. Antispermatogenic effect[9]. Hypoglycemic effect[10], gastric antiulcer activitry[11]. Antistress activity[12]. Alterations in glycogen content and carbohydrate metabolism in rats[13], evaluation of antidiabetogenic properties[14], cardiac changes in rats[15]. Cardiaprotective potential[16], Antifertility effect[17]. Chemopreventive activity[18], regulation of thyroid function[19]. Antinociceptive action[20]. Antidiabetic, antihypercholesterolaemic and antioxidant effect[21], wound healing[22].

In order to verify their topical anti-inflammatory potential, in present study herbal drug was extracted using methanol as a solvent from leaves of *Ocimum sanctum.* Extract obtained, was then formulated in gels. Then, prepared gel was evaluated for its anti-inflammatory activity using carrageenan-induced paw edema, and anti-nociceptive effect using hot plate in albino wistar rats were studied.

MATERIALS AND METHODS

Preparation of methanolic extract

The leaves of *Ocimum Sanctum* was collected, and cut in to small pieces dried in hot air oven at 55^0C for 20 min. The leaves were grinded mechanically to make powder. 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.

Formulation of topical preparation

Herbal gel prepared using carbopol-940 as a gelling agent in 1% w/w concentration with deionized water using mechanical stirrer. The pH of gel was adjusted to neutral by addition of small quantities of triethanolamine with continuous string. 1 % w/w herbal extract of *Ocimum Sanctum* was added to the gel and stir for sufficient time homogeneous mixing of extracts in gel base. Prepare gel were filled in collapsible tubes and stored at cool and dry place.

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 three groups (Control, Test and Standard) of six animals in each group were used for experiment.

Carrageenan-induced rat paw edema[23, 24]

Animals were fasted for 24 hrs before the experiment with water *ad libitum*. Approximately 50 µl 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% *Ocimum Sanctum* 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 1 h 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.

Hot-plate test

The method used was a modification of previously reported method. Mice were placed into a 10 cm wide glass cylinder on a hot plate maintained at 55 ºC. Control latency was determined for each mouse. The normal latency (reaction time) was 3-5 second. The responses were calculated. The reaction time was recorded when animals jumped or licked their paws. Seven mice per group, dose were injected i.p. with saline (10 ml/kg, as control), the Valdecoxib is used as a standard before extract administration) and tested at various times (0, 30, 60, 90, 120,150, 180 and 240 Min) thereafter to establish a time course.

Statistical analysis

Data are reported as the mean ± SEM. 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.

RESULTS

Table 1: Effect of topical administration of Herbal gel on carrageenan-induced paw edema in rats

	EARLY PHASE	PERCENTAGE INHIBITION	LATE PHASE	PERCENTAGE INHIBITION
CONTROL	52.8 ± 2.853	-	95.2 ± 1.393	-
STD.	37.8 ± 2.354	28.40	62.6 ± 0.927	34.24
O.S. 1%	48.4 ± 1.030	8.33	75.4 ± 0.748	20.79

Table 2: Hot-plate results for Herbal gel in albino rats

	0 MIN	30 MIN	60 MIN	90 MIN	120 MIN	150 MIN	180 MIN	210 MIN	240 MIN
CONTROL	5.60±0.003	5.3±0.022	5.1±0.057	5.4±0.070	5.5 ±0.070	6.0 ±0.230	5.4 ±0.149	5.9±0.13	5.7 ±0.145
STD	5.1±0.016	6.4±0.014*	7.6±0.013	7.8±0.013**	7.7±0.011**	8.2±0.046 **	8.9±0.036 **	8.6±0.033**	8.5±0.050 **
O.S. 1%	5.0 ±0.017	6.1±0.038**	6.8±0.063	6.4±0.080	7.1±0.076**	7.5±0.020 *	7.3±0.012 **	7.0±0.009**	7.2±0.025 **

DISCUSSION

The anti-inflammatory activity after topical administration of herbal gel formulations were studied, Carrageenan-induced hind paw edema is the standard experimental model of acute inflammation. Moreover, the experimental model exhibits a high degree of reproducibility. Carrageenan 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 to the release of prostaglandin and slow reacting substances[23]. The results of anti-inflammatory activity after topical administration of herbal gel reported in Table 1. Statistical analysis showed that the edema inhibition by preparation containing extract is significantly differing from control group at all the concentrations tested which is compared with the formulation containing both plant extract. The results showed that the anti-inflammatory effect of the formulation containing 1% of the herbal gel and another formulation containing both extract was equivalent to the effect of standard gel formulation.

From these overall results, we can conclude that topical preparation containing at least 1 % of herbal gel and another formulation containing both extract possesses both anti-inflammatory and antinociceptive effect which can be useful for the treatment of local inflammation[24].

The data presented in this study demonstrate that the reported herbal drugs possess significant topical anti-inflammatory properties, supporting their traditional use for the treatment. Indeed, their extracts were able to inhibit the inflammatory activity on topical application.

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