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### Topical anti-inflammatory activity of herbal gel formulation

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#### Abstract

The management and treatment of pain is probably one of the most common and yet the most difficult aspects of medicinal practice. This study evaluated a new herbal preparation containing extract from leaves of *Vitex negundo* (VN) for its topical anti-inflammatory activity against carrageenan induced edema, formalin test, anti-nociceptive effect. Gelling agent used in this study was 1% w/w concentration of carbopol-940 in the formulation. The studies were conducted on wistar rats of either sex (160-180 g). The change in oedema volume of the rat hind paw was measured. From the study we observed that the 1 % herbal formulation also potentiated the anti-inflammatory and anti-nociceptive effect topically.

**Key Words:** Anti-inflammatory effect; Topical gel, Herbal formulation, Anti-inflammatory Herbal gel.

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#### 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. 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 anti-inflammatory agents are helpful in the therapeutic treatment of these pathologies. In this context, medicinal plants are widely used in folk medicine of many countries to treat different inflammatory conditions and, in particular, skin inflammations. However, for many of the plants in use the real efficacy and/or the relevant active principles are unknown. Consequently, experimental studies aimed to demonstrate the pharmacological properties of these plants and to identify the relevant active principles are needed<sup>1</sup>.

*Vitex negundo* Linn. (VN) has been investigated extensively for its anti-inflammatory and analgesics activities but it was only Telang *et al* (1999) who noticed the inhibitory activity of the extract on prostaglandin biosynthesis and confirmed NSAID-like activity<sup>2, 3</sup>, anti-inflammatory, antipyretic and febrifuge properties<sup>4</sup>, anti-inflammatory activity<sup>5, 6</sup>, CNS activity<sup>7</sup>, Hepatoprotective effect<sup>8</sup>, anticonvulsant<sup>9</sup> and bronchial relaxant actions<sup>10</sup> are claimed in literature.

In order to verify their topical anti-inflammatory potential, herbal drug was extracted using methanol as a solvent from leaves of *Vitex negundo*, dry powder was obtained and utilized for gel preparation, then evaluated for anti-inflammatory activity using carrageenan-induced paw edema, formalin test and anti-nociceptive in Albino Wistar rats.

## MATERIAL AND METHODS

### Preparation of methanolic extract:

The leaves of *Vitex negundo* was collected, and cut in to small pieces dried in hot air oven at 55 °C 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 stirring. 1 % w/w herbal extract 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

Animals were fasted for 24 hrs. before the experiment with free access to water. 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% VN extract was applied to the plantar surface of the hind paw by gently 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<sup>11</sup>.

**Formalin test**

The antinociceptive activity was determined using the formalin test described in literature<sup>12</sup>. 0.2 g of herbal gel containing 1% of VN extract was applied to the dorsal surface of the left hind paw by gently rubbing 50 times with the index finger. Rats of the control groups received only the plain gel base. 1% Valdecoxib gel was applied in the same way as a standard. Fifteen minutes later, the antinociceptive activity was determined using the formalin test. 50µl. of 2.5% formalin was injected to the dorsal surface of the left hind paw. The rat was observed for 60 min after the injection of formalin, and the time spent licking the injected hind paw was recorded. The first 5 min post formalin injection is known as the early phase and the period between 15 and 60 min as the late phase.

**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 °C. 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.

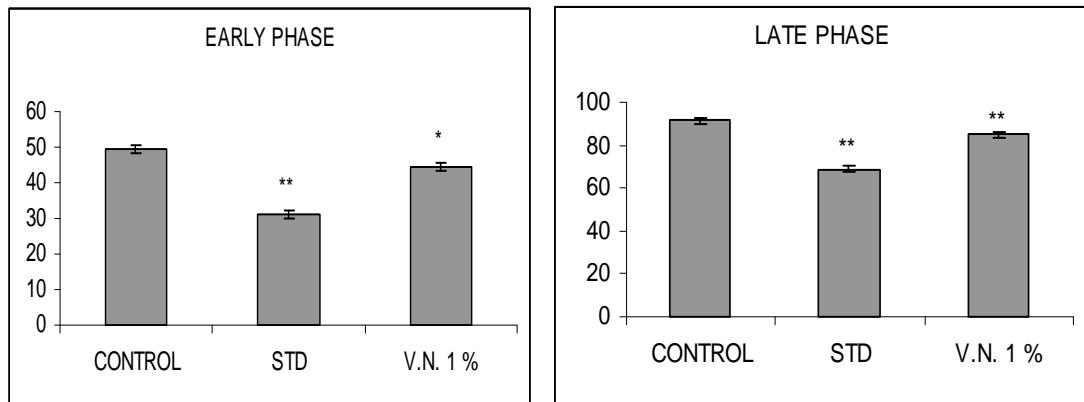
**Statistical analysis:**

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 AND DISCUSSION**

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

MEAN	0 MIN	30 MIN	60 MIN	90 MIN	120 MIN	150 MIN	180 MIN	210 MIN	240 MIN
CONTR OL	0.20 ± 0.003	0.43 ± 0.022	0.41 ± 0.011	0.54 ± 0.012	0.65 ± 0.001	0.80 ± 0.01	0.74 ± 0.014	0.79 ± 0.001	0.77 ± 0.002
STD	0.19 ± 0.016	0.21* ± 0.014	0.26* ± 0.013	0.31** ± 0.013	0.37** ± 0.011	0.42** ± 0.012	0.39** ± 0.016	0.36** ± 0.014	0.35** ± 0.022
V.N. 1%	0.17 ± 0.007	0.23* ± 0.016	0.29* ± 0.015	0.32** ± 0.017	0.35** ± 0.011	0.45** ± 0.012	0.41** ± 0.012	0.40** ± 0.015	0.37** ± 0.014

**Fig.1. Antinociceptive effect of Herbal gel in albino rats in early and late phase:****Table 2: Hot-plate results for Herbal gel in albino rats**

Groups	0 sec	30 sec	60 sec	90 sec	120 sec	150 sec	180 sec	210 sec	240 sec
CONTR OL	3.8 ±0.073	4.4 ± 0.070	3.2 ± 0.066	3.3 ± .096	3.4 ± 0.102	3.8 ±0.10	3.5 ± 0.083	3.7 ± 0.104	3.6 ± 0.050
STD	3.4 ±0.070	4.9 ± 0.050 **	4.5 ± 0.070 **	4.1 ± .086 *	4.9 ±0.050 **	4.6 ±0.107 **	4.5 ± 0.070 **	4.7 ±0.09 **	4.5 ± 0.086 **
V.N. 1%	3.1 ±0.148	5.2 ± 0.158	4.7 ± 0.083**	4.2 ± 0.09	4.1 ± 0.158 **	4.2 ±0.192 **	3.8 ± 0.050 **	4.2 ± 0.158	4.1 ± 0.130

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.

The anti-inflammatory activity after topical administration of herbal gel was 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<sup>13</sup>. 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. The results showed that the anti-inflammatory effect of the formulation containing 1% of the herbal gel was equivalent to the effect of standard gel formulation.

The formalin test is a valid and reliable model of nociception and it is sensitive for various classes of analgesic drugs. Formalin test produced a distinct biphasic response and different analgesics may act differently in the early and late phases of this test. Therefore, the test can be used to clarify the possible mechanism of antinociceptive effect of a proposed analgesic. Centrally acting drugs such as opioids inhibit both phases equally but peripherally acting drugs

such as aspirin, indomethacin and dexamethasone only inhibit the late phase. The late phase seems to be an inflammatory response with inflammatory pain that can be inhibited by anti-inflammatory drugs. The effects of herbal gel on formalin test have been shown in Fig 1. The results for groups, which receive gel containing 1 % of extract, were significantly different from control group on the early phase and late phase. The effect of topical preparation containing extract on the first and second phases of formalin test suggests that its activity may be resulted from its central action. Herbal formulation suppressed hyperalgesia associated with inflammation and may have a beneficial role in the treatment of inflammatory pain.

### CONCLUSION

From these overall results, we can conclude that topical preparation containing at least 1 % of herbal gel possesses both anti-inflammatory and antinociceptive effect which can be useful for the treatment of local inflammation<sup>14</sup>.

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