

Antinociceptive and Anti-inflammatory Effects of Hydroalcohol Extract of *Vitex agnus castus* Fruit

M. Ramezani, Gh. Amin, E. Jalili

Abstract—In present study the effects of anti-inflammatory and antinociceptive of vitex hydro-alcoholic extract were evaluated on male mice. In inflammatory test mice were divided into 7 groups: first group was control. The second group, positive control group, received dexamethasone (15 mg/kg) and the other five groups received different doses of hydroalcohol extract of *Vitex* fruit (265, 365, 465, 565, and 665 mg/kg). The inflammation was caused by xylene-induced ear edema. Formalin test was used for evaluation of antinociceptive effect of extract. In this test, mice were divided into 7 groups: control, morphine (10mg/kg) as positive control group, and Vitex extract groups ((265, 365, 465, 565, and 665 mg/kg). All drugs were administered intraperitoneally, 30 min before each test. The data were analyzed using one-way ANOVA followed by Tukey-kramer multiple comparison test. Results have shown significant anti-inflammatory effects of extract at all doses as compared with control ($P < 0.05$). In the first phase of formalin test (0-5 min) none doses of extract could inhibit acute pain, but in the second phase (15-30 min) chronic pain decreased at 265, 365, 465, and 565 mg/kg doses ($P < 0.05$). The results of this research indicated that Vitex extract remarkably inhibited inflammation and second phase of nociception (inflammatory pain) and can be used for treatment of inflammatory diseases.

Keywords—Anti-inflammatory, Antinociceptive, Mice, *Vitex agnus castus*.

I. INTRODUCTION

VITEX AGNUS CASTUS (Verbenaceae) is a deciduous shrub with finger-shaped leaves and slender violet flowers. This plant is native to Middle East and southern Europe. For pepper-like taste, it has been named as monk's pepper [1], [2]. Also it is commonly known as women's Herb because *Vitex* has been traditionally used for a variety of gynecologic conditions, including premenstrual syndrome (PMS), corpus luteum insufficiency, hyperprolactinaemia, and menopause [3]. There are reports that *Vitex* extract may enhance female fertility and suppress tumor growth activity [4], [5]. Essential oils of *vitex* have antibacterial and antifungal effects [6]. Surveys indicate that the side effects following *Vitex* treatment are mild and reversible [3]. But use of it should

be avoided during pregnancy or lactation [7]. Some constituents of *Vitex* genus may have anti-inflammatory, lipxygenase inhibitory, and analgesic properties [8]- [10]. Studies show *Vitex agnus castus* contains following chemical compounds: Essential oils (limonene, pinene and sabinene), Iridoid glycosides (aucubin and agnoside), Flavonoids (casticin, kampferol, quercetagenin, orientin and isovitexin), Diterpenes (vitexilactone, rotundifuran), Essential fatty acids (oleic acid, linolenic acid, palmitic acid and stearic acid) [4], [11]. In Iranian traditional medicine, *Vitex agnus castus* fruit are known for carminative, laxative, appetizer, aphrodisiac, sedative, and anti-inflammatory activity [1], [11]. Therefore the purpose of this study was to investigate the effectiveness of the *Vitex* fruits as anti-inflammatory and antinociceptive agents on mice.

II. MATERIAL AND METHODS

The fruit of *Vitex agnus castus* were collected of 15 Km. NW of Qom (Center of Iran) in Sep 2008, and dried in shadow followed by grinding. The sample was botanically identified by Dr. Gh. Amin Ph.D., from Department of Pharmacognosy and a voucher specimen (THE-6699) was deposited at the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. Then the powder (100g) was extracted exhaustively by percolation method at room temperature with a hydroalcohol solution (ethanol/water 80% v/v). The mixture was filtered and concentrated under reduced pressure at 30-40°C. For injections the extract were freshly dissolved in Tween 20/Normal saline 10% v/v. Selection of doses was on the basis of LD50=1.65g/kg of *Vitex agnus castus* fruit [12].

A. Animals

Male NMRI mice weighing 20-25 g were obtained from Pasture institute (Tehran, Iran). The animals were housed in standard laboratory conditions and allowed access to water and food *ad libitum*. They were maintained under constant temperature ($21 \pm 2^\circ\text{C}$) and in a 12h light-dark cycle. The experimental protocol was approved by the animal care review committee of TUMS (Tehran University of Medical Sciences).

B. Xylene-induced ear edema

The method described previously by Atta and Alkofahi [13] was used. Male mice were divided into groups of eight mice each. After 30 min of the i.p. injection of extract at doses of

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265, 365, 465, 565, and 665 mg/kg body wt., 0.03 ml of xylene was applied on the anterior and posterior surfaces of the right ear. The left ear was considered as control. Control animals received (Tween/Normal saline 10% v/v) or dexamethasone (15 mg/kg). Two hours after xylene application, the mice were sacrificed and both ears were removed. Circular sections of both treated and untreated ears were taken using a cork borer with a diameter of 7 mm and weighed. The difference in weight between left untreated ear sections and right treated ear section was calculated.

C. Formalin test

The method described previously by Hunskaar and Hole [14] was used. Pain was induced by injecting 0.02 ml of 2.5% formalin (40% formaldehyde) in distilled water in the sub plantar of the right hind paw. Male mice were divided into groups of eight mice each. Extract was administered intraperitoneally at doses of 265, 365, 465, 565, and 665 mg/kg body wt., 30 min before formalin injection. The control group received the same volume (Tween/Normal saline 10% v/v). Morphine was used as positive control (10 mg/kg i.p.). The animals were observed to evaluate the licking time (an index of nociception) during the first phase, Neurogenic (0-5 min), and the second phase, inflammatory (15-30 min), after formalin injection.

D. Statistical analysis

Results were analyzed using One way ANOVA followed by Tukey- Kramer multiple comparison test. The data were expressed as mean values \pm S.E.M. and difference between the means of treated and control groups was considered significant at $P < 0.05$.

III. RESULTS

A. Xylene-induced ear edema in mice

Topical application of the *Vitex* extract (225-565 mg/kg body wt.) to the mouse ear resulted to potent suppression ($P < 0.05$) of acute edema induced by xylene (Table I). Inhibition percent was equal to that of dexamethasone (15 mg/kg). The most effective doses in inhibition of inflammation was 265mg/kg.

B. Formalin test

In formalin test, all doses of extract showed no effect in blockade of first phase of response (0-5 min) as compared with control (Table II). This phase corresponded to neurogenic pain. In the second phase of response (15-30 min) or inflammatory phase 265, 365, 465, and 565 mg/kg doses of extract showed a significant reduction of licking activity by 59.1, 65.8, 75.8, and 78.1 ($P < 0.05$), respectively as compared with control group.

Morphine was used as positive control and acted throughout the phases (Table II).

TABLE I
EFFECT OF *VITEX AGNUS CASTUS* HYDROALCOHOL EXTRACT (H.E.) ON XYLENE-INDUCED EAR EDEMA IN MICE

Treatment (Dose)	Ear swelling (mg)	Inhibition (%)
Control	11.75 \pm 1.83 ^a	—
Dexamethasone (15mg/kg)	2.1 \pm 0.6 ^c	82.1
h.e. (265mg/kg)	2.9 \pm 2.88 ^{bc}	75.3
h.e. (365mg/kg)	3.42 \pm 2.75 ^{bc}	70.9
h.e. (465mg/kg)	5.31 \pm 3.7 ^{bc}	54.8
h.e. (565mg/kg)	4.73 \pm 46.8 ^{bc}	59.7
h.e. (665mg/kg)	6.72 \pm 16.4 ^b	42.8

Values are the mean \pm S.E.M. Means with different letters have significant difference ($P < 0.05$) compared to control. Differences between groups were statistically analyzed by One-way analysis of variance (ANOVA) followed by Tukey- Kramer multiple comparison test ($n=8$).

TABLE II
EFFECT OF *VITEX AGNUS CASTUS* HYDROALCOHOL EXTRACT (H.E.) ON FORMALIN-INDUCED PAIN TEST IN MICE
Licking Times (s)

Treatment (Dose)	0-5 min	Inhibition (%)	15-30 min	Inhibition (%)
Control	80.6 \pm 31.5 ^{ab}	—	60 \pm 44.1 ^a	—
Morphine (10mg/kg)	15.7 \pm 14.1 ^c	80.5	4.2 \pm 4 ^b	93
h.e.(265mg/kg)	65 \pm 57.7 ^{abc}	19.35	24.5 \pm 22.2 ^b	59.1
h.e.(365mg/kg)	97.7 \pm 36.7 ^a	21.2	20.5 \pm 19.2 ^b	65.8
h.e.(465mg/kg)	84.7 \pm 40.9 ^{ab}	-5	14.5 \pm 12 ^b	75.8
h.e.(565mg/kg)	35.1 \pm 9.2 ^{bc}	56.4	13.1 \pm 10.9 ^b	78.1
h.e.(665mg/kg)	52.5 \pm 24 ^{abc}	34.8	25.8 \pm 20.3 ^{ab}	57

Values are the mean \pm S.E.M., Means with different letters have significant difference ($P < 0.05$) compared to control. Differences between groups were statistically analyzed by One-way analysis of variance (ANOVA) followed by Tukey- Kramer multiple comparison test ($n=8$).

IV. DISCUSSION

Data of this experiments indicated that the hydroalcohol extract of *Vitex agnus castus* fruits have reverse dose dependent activity (with increasing dose of extract the activity decreased) against inflammation as revealed in the xylene-induced ear edema. Xylene causes instant irritation of the mouse ear, which leads to fluid accumulation and edema characteristic of the acute inflammatory response [13]. The inhibition percent offered by 256 mg/kg of extract was nearly same as to that of dexamethasone (75.3% versus 82.1%).

In the formalin test, morphine as a centrally acting analgesic drug, produced an inhibitory effect on both phases whereas the hydroalcohol extract showed antinociceptive activity only on the second phase (15-30 min). Thus the extract may not act via central mechanisms. In the formalin test, the pain in the early phase is caused due to the direct stimulation of the sensory nerve fibers by formalin while the pain in the late phase is due to inflammatory mediators, like prostaglandin, histamine, serotonin, and bradykinin [15], [16] that confirm anti-inflammatory effect of *Vitex agnus castus* fruits.

Phytochemical studies have revealed the existence of essential oils such as α -pinen, sabinen, limonen, and cineol as secondary metabolites in *Vitex agnus castus* [17].

α -pinen as a terpenoids is known to possess anti-inflammatory activity [18]. It was reported that limonen decrease licking time in the second phase of the formalin test and this activity was not inhibited by naloxone. Thus limonen did not act via opioid receptors and may exert his activity via suppression of inflammatory mediators [19]. Investigations have shown that cineol also act by this mechanism [20]. Therefore anti-inflammatory effect of *vitex* may be due to the existence of these constituents.

Casticin, a major flavonoid that was isolated from *Vitex rotundifolia* L. (native to sea coast of china) had significant anti-nociceptive using acetic acid writhing test and anti-inflammatory effect on acute inflammation by xylene-induced ear edema [21], [22]. Recently several secondary metabolites were isolated from *Vitex agnus castus* plant and screened for anti-inflammatory activity. P-hydroxybenzoic acid, methyl 3,4-dihydroxybenzoate, and 3,4-dihydroxybenzoic were found to have significant anti-inflammatory activity in a cell-based contemporary assay [23]. However, there is a need fo further studies to understand the action mechanisms of *Vitex agnus castus* ingredients.

V. CONCLUSION

In conclusion, the results of the present study provide evidence for the anti-inflammatory and analgesic activity of *Vitex agnus castus* fruits which can affirm the traditional usage of this plant.

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