

VITEX AGNUS-CASTUS FRUIT EXTRACT RESTRAINS ACQUISITION AND EXPRESSION OF MORPHINE-INDUCED TOLERANCE IN MICE

Saberi, M. ^{1*}; Chavooshi, B.²; Ghorbani, M.¹; Saberi, F.³

¹Department of Pharmacology and Toxicology, Applied Neuroscience Research Center, Faculty of Pharmacy, Baqiyatallah University of Medical Sciences, Tehran, Iran

²Department of psychology, Faculty of Psychology and Education, University of Tehran, Tehran, Iran

³Faculty of Medicine, Iran University of Medical sciences, Tehran, Iran

[*m_s_saber@yahoo.com](mailto:m_s_saber@yahoo.com)

Abstract

Tolerance to the analgesic effect of opioids is the major concern of long-term administration of these compounds. In this study, the effects of hydroalcoholic extract of *Vitex agnus-castus* (VAC) on the acquisition and expression of morphine-induced tolerance were evaluated in mice by tail-flick test. To evaluate the VAC effects on the development or expression of morphine tolerance, animals received VAC (i.p.), 30 min before morphine (50 mg/kg; s.c.) during induction period once daily for 3 days; or 30 min before challenge dose of morphine (5 mg/kg) before (day 1) and after morphine-induced tolerance (day 4), respectively. The analgesic effect of VAC was evaluated every 30 min till 2 h. VAC at the doses of 120 and 180 mg/kg could suppress the development of tolerance. In addition, VAC at the dose of 180 mg/kg attenuated the expression of morphine-induced tolerance. While, the VAC alone had no analgesic effect, its combination with morphine at the dose of 180 mg/kg, could significantly enhance the antinociceptive effect of morphine. VAC can attenuate the expression of morphine's tolerance. Based on the enhancement of the analgesic effect of morphine's challenge dose by VAC, it seems that the expression is more attenuated by additive effect of VAC than reversal of tolerance. The ability of VAC to ameliorate the acquisition and expression of morphine-induced tolerance in mice may be worthwhile for traditional chronic pains treatment in combination with opioids without concern of tolerance and dependence.

Key words: *Vitex agnus-castus*, Morphine, Antinociception, Tolerance, Tail-flick test, Mice

Introduction

The long-term efficacy of opioids, like morphine, is often limited by the development of tolerance to their analgesic effect. As a consequence, opioid monotherapy may result an inadequate analgesia. Regarding to the multiplicities mechanisms which are involved in the pain, [1] the opioids tolerance could be overcome by combination of opioids with one or more non-opioid analgesics to obtain a more favorable balance of analgesia. Recently, herbal medicines have attracted more attention for several advantages including more safety compliance and less expense. *Vitex agnus-castus* (VAC) known as vitagnus or chaste tree is a deciduous shrub and is native to the Mediterranean Europe and central Asia. This plant had been well known in Iranian ancient medical schools and Alkandi used this plant for the treatment of epilepsy and psychosis in 1200 A.D. [2] Traditionally, VAC fruit extract has been widely used for the treatment of many female disorders, such as menstrual irregularity, premenstrual syndrome (PMS) and cyclic mastalgia. [3, 4] In addition, VAC has been used to treat pain, swelling, inflammation, headaches, rheumatism, and sexual dysfunction. Although further rigorous studies are needed to assess the safety of VAC, the available data indicates that VAC is a safe herbal medicine. [2] The major compounds of VAC are Casticin, Luteolin, Rotundifuran and Agnuside. [5] The dopaminergic effects of VAC extract have been proven in animal models and clinical trials. [6, 7] In vitro investigations have elucidated that the lipophilic extract acts as agonist for mu and kappa opioid and D₂ dopaminergic receptors, [8] while aqueous fraction has more tendency to bind with delta opioid receptors. [9] On the other hand, dopamine has been implicated in the acquisition of tolerance to the opioid induced analgesia. [10,11] It has been suggested that activation of opioid receptors located on dopaminergic neurons in the striatum and nucleus accumbens [12] may play an important role in the development of tolerance and sensitization to the opiates. [13] Based on the dopaminergic effects and additional pharmacological actions of VAC extract via opioid receptors, the aim of the study was to evaluate the possible effects of VAC extract on the acquisition and expression of tolerance to the morphine-induced antinociception in tail-flick model of pain in mice.

Methods

The adult male NMRI mice (Pasteur Institute, Iran) weighing 20–30 g were maintained on a 12/12-h

light/dark cycle (lights on at 07:00 h) and constant temperature (22±2 °C). Animals were allowed free access to food and water except during the experiments. The animals were acclimatized to the set up for a 45-min prior to the experiments and all experiments were conducted between 14:00 and 17:00 h, during the light portion of the cycle. Animals were allowed free access to food and water except during the experiments. All experiments were executed with the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti University; M.C. Efforts were made to minimize the number and potential suffering of experimental subjects.

The tail-flick test was used for evaluating the antinociception and acquisition of morphine tolerance. The latency to withdraw the tail from a feedback-controlled projector lamp focused on the dorsal surface of tail was used as a measure of nociceptive responsiveness. Tail-flick latency (TFL) more than 8 (s) was considered as a cut-off point to avoid any tissue damage. To determine the baseline latency, two tail-flick tests were done before the pre and post tests for each mouse and the average of them considered as baseline latency. TFL times (s) are expressed as percentage of maximal possible effect (%MPE) using the equation:

$$\%MPE = \frac{\text{Post-drug latency (s)} - \text{Baseline latency (s)}}{\text{Cut-off value (s)} - \text{Baseline latency (s)}} \times 100$$

The animals were rendered tolerant to the morphine using the method by a previous study on the induction of morphine tolerance in mice. [14] Morphine was purchased from Temad Daru Company of Iran. Firstly, the antinociceptive response to a challenge dose of morphine (5 mg/kg; s.c.) was determined by tail-flick test at 30-min intervals (0.5, 1, 1.5 and 2 h) on day 1 (n = 7-8 in each group). Then the tolerance induction was begun on day 1 by administration of morphine (50 mg/kg; s.c.) once daily for 3 days. To evaluate the acquisition of tolerance to the morphine's antinociception, the response to the challenge dose was determined again for each mouse on day 4 as post-test as cited above. The challenge dose of morphine was selected as already described by Chavooshi et al. (2009). [15] The hydro-alcoholic extract of VAC was prepared by Sina Daru Company of Iran. To evaluate the effects of VAC on the induction of morphine's tolerance, animals (n = 7-8) received various doses of VAC (60,

120 or 180 mg/kg; i.p.), 30-min before morphine (50 mg/kg; s.c.) or vehicle once daily for 3 consecutive days. The effect of the challenge dose of morphine (5 mg/kg; s.c.) was tested prior to (day 1) and following the induction period of morphine tolerance (day 4) at different time set intervals as cited above. The doses of VAC were selected as described previously. [16] To assess the effects of VAC on the expression of morphine-induced tolerance, the animals ($n = 7-8$) received different doses of VAC (60, 120 or 180 mg/kg; i.p.) or vehicle 30-min before challenge dose of morphine (5 mg/kg) following morphine-induced tolerance (on day 4). The antinociceptive response to the challenge dose of morphine for each animal was determined by tail-flick test on day 4 (post-tolerance induction) at different time set intervals, as described above.

The antinociceptive effect of various single doses of VAC (60, 120 or 180 mg/kg; i.p.) alone or its vehicle (control) were determined at different time set intervals (0.5, 1, 1.5 and 2 h), 30-min post injection. Also, the analgesic response of the VAC in combination with morphine (5 mg/kg; s.c.) was determined as cited above. The animals ($n = 7-8$) received either VAC or vehicle, 30-min prior to morphine injection. All obtained data were analyzed using the GraphPad Prism software (version 5.0) and presented as mean \pm SEM (standard error of mean). The mean of %MPEs in all groups were subjected to two-way analysis of variance (ANOVA) followed by Bonferroni's test as a post test. P values less than 0.05 were considered to be statistically significant.

Results

The antinociceptive response to the challenge dose of morphine (5 mg/kg; s.c.) for both vehicle and pre-tolerant groups on day 1 had no difference at all time set of intervals (Fig. 1A). To assess the morphine's tolerance, the animals received morphine (50 mg/kg) or vehicle (10 ml/kg) once daily for 3 days. Two-way ANOVA indicated that the antinociceptive response (the mean of %MPEs values) to the challenge dose of morphine on day 4 decreased significantly in morphine-treated animals that received morphine during the induction period in comparison with the vehicle-treated group [$F(1,48)=54.2$, $P<0.0001$; Fig. 1B] at all time set intervals. These findings are clearly indicative of the acquisition of tolerance to the morphine-induced antinociception following 3 days of morphine treatment. To evaluate the effects of VAC on the induction of morphine's tolerance, the animals

received different doses of VAC (60, 120 or 180 mg/kg; i.p.), 30-min before morphine (50 mg/kg; s.c.) administration once daily for 3 days during the induction period. The obtained data revealed that pretreatment of animals with VAC significantly reduced the acquisition of tolerance to morphine antinociceptive effect in a dose-dependent manner, as indicated by increment of %MPE in VAC treatment groups. However, the lowest dose of VAC (60 mg/kg) had no effect on the acquisition of morphine tolerance. Two-way ANOVA followed by Bonferroni's test indicated that in morphine-treated animals which received either 120 or 180 mg/kg of VAC prior to the morphine injection during the induction period, the antinociceptive responses to the challenge dose of morphine (5 mg/kg; s.c.) on day 4 were increased significantly in comparison to control group [$F(3,96)=11.37$, $P<0.0001$; Figure 2].

We evaluated then whether a single dose of VAC prior to the challenge dose of morphine (tolerance expression) was sufficient or daily administration of VAC was necessary to inhibit the morphine tolerance. Thus, the animals received VAC (60, 120 or 180 mg/kg; i.p.) or vehicle (10 ml/kg; i.p.), 30-min before challenge dose of morphine (5 mg/kg) prior to and following morphine-induced tolerance. Data analysis indicated that VAC at the dose of 180 mg/kg attenuated the expression of morphine tolerance [$F(3,96)=62.33$, $P<0.0001$; Figure 3] at all-time set intervals. To determine the antinociceptive effect of VAC alone, the tail-flick test were done at 0.5, 1, 1.5 and 2 h after single injection of different doses of the VAC (60, 120 or 180 mg/kg; i.p.). As shown in Fig. 4, two-way ANOVA indicated that there was no significant difference between analgesic effects (MPEs) of various doses of VAC alone when compared to those of the vehicle group [$F(3,96)=0.116$, $P=0.9513$; Figure 4]. In addition, to evaluate the effects of VAC on morphine-induced antinociception, the animals received various doses of VAC (60, 120 or 180 mg/kg; i.p.), 30-min before morphine (5 mg/kg; s.c.) injection. Data analysis showed that the co-administration of VAC at the dose of 180 mg/kg with morphine (5 mg/kg) significantly increased the %MPE in comparison to the morphine alone treated group [$F(3,96)=10.58$, $P<0.0001$; Figure 5].

Discussion

The present study showed that while VAC alone has no antinociceptive effect in the tail-flick test, as an acute pain model, it could suppress the acquisition of morphine tolerance. In addition, VAC enhanced the antinociceptive effect of morphine and attenuates

the expression of tolerance to the antinociceptive effect of morphine. Morphine and related opioid drugs produce potent analgesia by activating specific receptors associated with spinal and brain neurons involved in nociceptive signaling. [17] Chronic administration of these drugs, however, produces a state of tolerance, indicated by the loss of drug potency. In clinical situations, the consequence of the opioid induced tolerance is escalation of drug dose and limitation of the opioids efficacy in the management of severe pain syndromes. [18]

The major finding of this study suggests that the VAC pretreatment can inhibit the acquisition of morphine tolerance, dose-dependently. One common possible mechanism of tolerance development suggests opioid receptors involvement during chronic opioid administration. [19] Consistent with this mechanism, VAC may affect the opiate systems including μ , δ , and κ opiate receptors and endogenous opiate peptides such as β -endorphin. [7] Meier et al. (2000) have reported that an ethanolic and several subfractions of a methanolic extracts of VAC show affinity for opiate receptors. [6] On the other hand, the opioid system has close functional links to the dopaminergic system in several areas, including the substantia nigra, mesocortex and mesolimbic projections. [20] Previous studies have suggested that D_2 dopamine agonists could attenuate opioid tolerance. [11] Moreover, opioids are known to modulate dopamine release in a variety of brain areas. [20] Some properties of opioids, including hyperlocomotion and reward, are at least partly mediated through dopamine receptors. [21] Also, morphine has increased the metabolism of dopamine in the septum and nucleus accumbence. [22] In addition, several studies have indicated that VAC acts on dopamine D_2 receptors. [6, 8]

Moreover, previous studies showed that steroid hormones influence female rat's response to noxious stimuli. [23, 24] Nociception varies across the estrous cycle. [25] Proestrous rats, with high concentration of estradiol and progesterone, have shown more latency to tail-flick and paw flick in response to radiant heat, compared to diestrous rats that have low hormone levels. [26] Also, it had been shown that administration of estradiol compared to vehicle, increased latencies in response to the heat stimuli. [27] It has also been suggested that some elements in the VAC extract may act on the estrogen (ER- β) receptors. [28] The estrogenic effects of VAC extract maybe related to the presence of linoleic acid as an estrogenic

compound. [5] Liu et al. (2004) [28] have suggested that linoleic acid from the fruits of VAC can bind to estrogen receptors and induce certain estrogen inducible genes. So, this mechanism also may be involved in the VAC extract effects on morphine tolerance but more investigations are needed to elucidate the precise mechanism. We showed that VAC alone has no antinociceptive effect, Our results are in consistent with the report of Ravishankar et al. (1985) who failed to observe the analgesia from ethanolic extract of *Vitex-negundo* (another species of *Vitex*) in tail-flick method. [29] In contrast, Gupta and Tandon (2005) had shown that *Vitex-negundo* produced a significant antinociception in the experimental models such as tail-flick test and acetic acid induced writhing. [30] This discrepancy might be attributed to the different experimental situations as they used rats for tail-flick test, low dose of acetic acid (0.6%) in writhing test and applied higher doses of *Vitex-negundo* (100-500 mg/kg) in their study.

In the other set of our experiments, we showed that VAC enhances morphine's antinociceptive effects in tail-flick test. As cited above, the interaction of VAC with opioid receptors and dopaminergic system may contribute to the latter effect. As a result, co-administration of drugs such as VAC with morphine which can produce therapeutic analgesia with lower dose of morphine would be of great clinical implication. It has been previously reported that the acquisition and expression of morphine-induced tolerance are distinct phases. [31] Our results indicate that VAC can attenuate the expression of morphine's tolerance. Based on the enhancement of the analgesic effect of morphine's challenge dose by VAC, it seems that the expression is more attenuated by additive effect of VAC than reversal of tolerance. In conclusion, based on the present study, while VAC can suppress both the acquisition and expression of morphine-induced tolerance, VAC has no analgesic effect alone. To elucidate the mechanisms involved in these observed effects further investigation are warranted.

Acknowledgements

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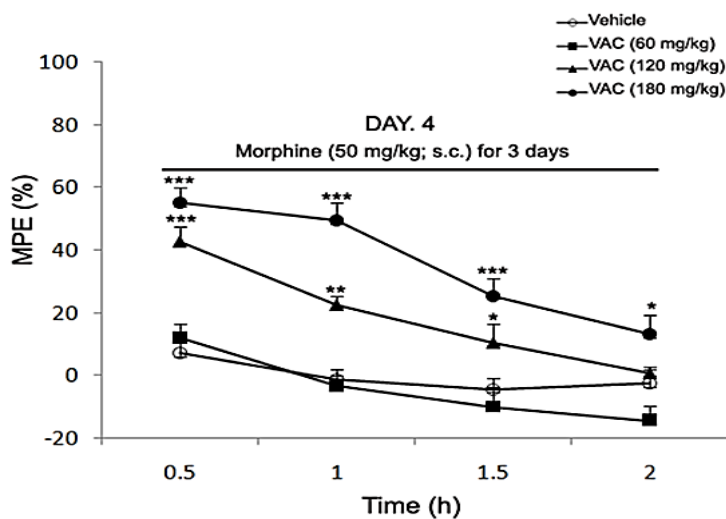
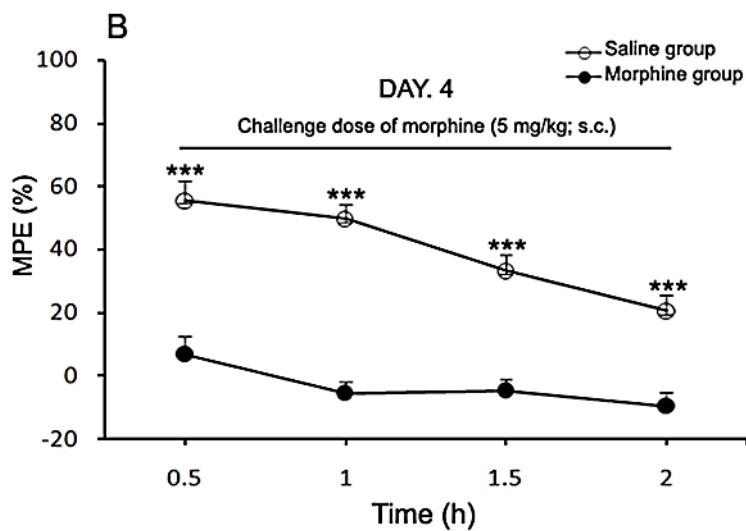
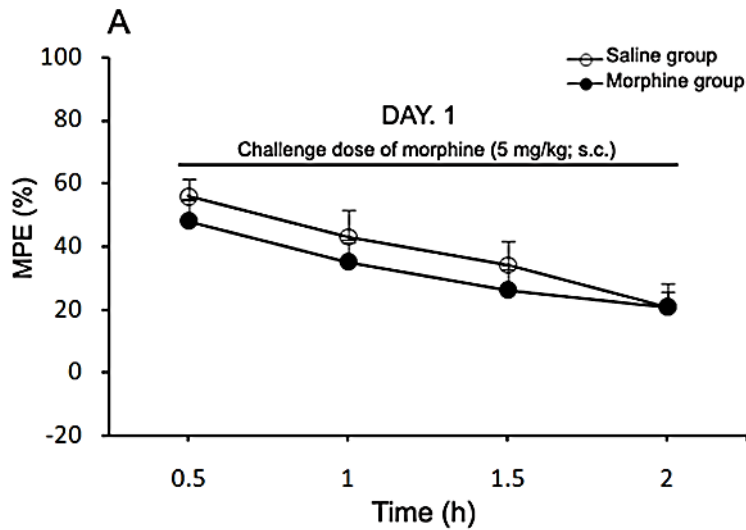


Figure 1. Acquisition of tolerance to morphine-induced antinociception; The effect of challenge dose (5 mg/kg; s.c.) of morphine, (A) before tolerance induction on day 1 and, (B) following 3 consecutive days administration of vehicle 10 ml/kg (○) or morphine 50 mg/kg (●) as an induction period on day 4. Each point represents the mean \pm SEM of percent of maximal possible effect (%MPE) for 7-8 mice.

***P<0.001 in comparison to the vehicle-treated group

Figure 2. The effect of different doses of Vitex agnus-castus (VAC) on the development of morphine tolerance. Animals received VAC (60, 120 or 180 mg/kg; i.p.) or vehicle (10 ml/kg; i.p.), 30-min before morphine (50 mg/kg; s.c.) once daily for 3 days during the induction period. In the day 4, the tail-flick latencies were determined after injection of challenge dose of morphine (5 mg/kg; s.c.). Each point represents the mean \pm SEM for 7-8 mice.

*P<0.05, ** P<0.01, ***P<0.001 in comparison to the vehicle group

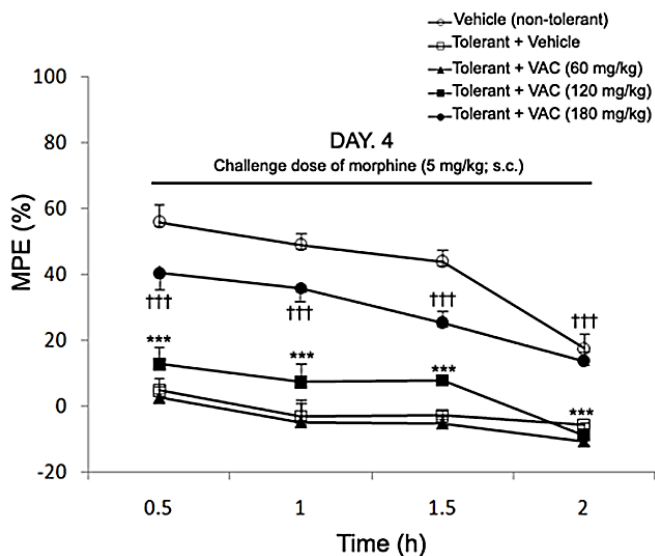


Figure 3. The effect of different doses of Vitex agnus-castus (VAC) on the expression of morphine-induced tolerance. Following 3 days of morphine tolerance induction (50 mg/kg; once daily). Various doses of VAC or vehicle were administered 30-min prior to injection of challenge dose of morphine (5 mg/kg; s.c.) at day 4. Each point is the mean \pm SEM of percent of maximal possible effect (%MPE) for 7-8 mice.

†, * indicates significant in comparison to the morphine-treated (tolerant) and vehicle treated (non-tolerant) groups, respectively, $P < 0.05$, ††† or *** $P < 0.001$

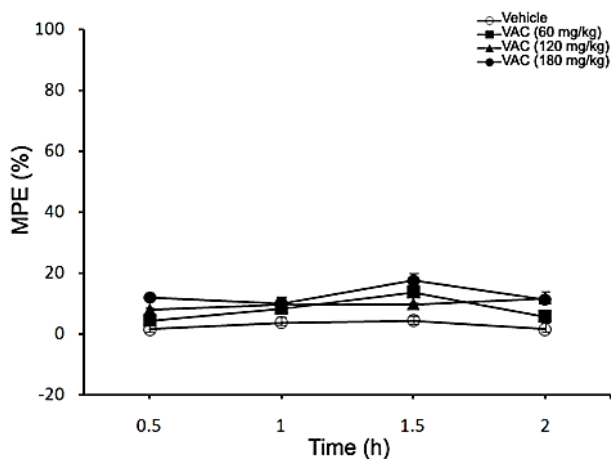


Figure 4. The antinociceptive effects of different doses of Vitex agnus-castus (VAC) alone presented as the percent of maximal possible effect (%MPE). The animals received VAC (60, 120 or 180 mg/kg), 30-min before morphine administration. Each point is the mean \pm SEM for 7-8 mice.

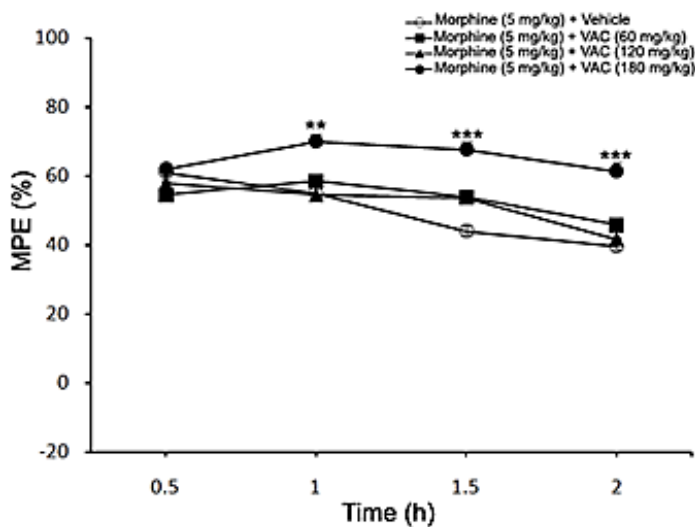


Figure 5. The antinociceptive effects of different doses of Vitex agnus-castus (VAC) in combination with morphine present as the percent of maximal possible effect (%MPE). The animals received VAC (60, 120 or 180 mg/kg), 30-min before the morphine injection (i.p.). Each point is the mean \pm SEM for 7-8 mice.

** $P < 0.01$; *** $P < 0.001$ in comparison to the morphine-treated group