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ORIGINAL ARTICLE

The *In Vivo* Antinociceptive and Antiinflammatory Effects of *Verbascum exuberans* Hub.-Mor.

Verbascum exuberans Hub.-Mor.'ın *İn Vivo* Antinosiseptif ve Antiinflamatuvar Etkileri

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ABSTRACT

Objectives: Safe and effective drugs are still lacking for many pain therapies. In recent years, growing interest has been devoted thus on herbal drugs as an option to identify new pain killers. Based on this, extensive researches are carried out on *Verbascum* L. genus due to its therapeutic potency on pain and inflammation therapy. In this study, among *Verbascum* species, the antinociceptive effect of *Verbascum exuberans* Hub.-Mor., and its contributions to nitrergic, serotonergic, or opioidergic pathways as well as its antiinflammatory acitivity were investigated.

Materials and Methods: Tail clip, tail flick, and hot plate tests were used to determine the central (spinal and supraspinal) antinociceptive effect, while an acetic acid-induced writhing test was used to measure the peripheral antinociceptive effect of the extract (250 and 500 mg/kg). The extract (250 mg/kg) was then combined with nω-nitro-L-arginine methyl ester, cyproheptadine, and naloxone to evaluate its involvement in nitrergic, serotonergic, or opioidergic pathways, respectively. Carrageenan-induced hind paw edema model was used to determine the antiinflammatory effect of the extract (250 mg/kg).

Results: The extract shows central spinal but not central supraspinal antinociceptive effect, and presents peripheral antinociceptive effect. The antinociceptive actions of the extract is largely regulated via targeting the nitrergic pathway, while the opioidergic pathway is partly involved. Further, the extract shows antiinflammatory effect due to the significant inhibitions on the time dependent edema progression and the cytokine (tumor necrosis factor-alfa and interleukin-1beta) productions.

Conclusion: *V. exuberans* could be stated as a new source with a high beneficial potential in alleviating pain and inflammation.

Key words: *Verbascum exuberans* Hub.-Mor., *Scrophulariaceae*, antinociceptive effect, antiinflammatory effect, tramadol

ÖZ

Amaç: Birçok ağrı tedavisi için güvenli ve etkili ilaçların arayışı hala devam etmektedir. Nitekim, son yıllarda, yeni ağrı kesicilerin keşfine bir seçenek olarak bitkisel ilaçlara ilginin arttığı görülmektedir. Bu bilgiye dayanarak, ağrı ve enflamasyon tedavisinde, tedavi edici potansiyeli nedeniyle *Verbascum* L. cinsine yönelik kapsamlı araştırmalar yürütülmektedir. Bu çalışmada, *Verbascum* türleri arasından, *Verbascum exuberans* Hub.-Mor.'ın antinosiseptif etkinliğini, bu etkide nitrerjik, serotonerjik ve opioiderjik yolaklar üzerindeki rolünü ve antiinflamatuvar aktivitesini araştırılmıştır.

Gereç ve Yöntemler: Ekstrenin (250 ve 500 mg/kg) santral (spinal ve supraspinal) antinosiseptif aktivitesi tail clip, tail flick ve hot plate testleri ile, periferal antinosiseptif etkisi ise asetik asit ile oluşturulmuş kıvranma testi ile ölçülmüştür. Daha sonra, ekstre (250 mg/kg) nω-nitro-L-arginin metil ester, siproheptadin ve nalokson ile kombine edilerek, sırasıyla, ekstrenin nitrerjik, serotonerjik ve opioiderjik yolaklardaki rolü belirlenmiştir. Karragenan ile oluşturulmuş arka ayak pençe ödem modeli ise ekstrenin (250 mg/kg) antiinflamatuvar aktivitesinin belirlenmesinde kullanılmıştır.

Bulgular: Ekstrenin santral spinal düzeyde etkili olduğu; ancak santral supraspinal düzeyde etkili olmadığı ve periferal antinosiseptif etkili olduğu görülmüştür. Ekstrenin antinosiseptif etkinliği büyük ölçüde nitrerjik yolağın üzerinden düzenlenirken, opioiderjik yolağın ise kısmen aracılık ettiği belirlenmiştir. Ayrıca, ekstrenin, zamana bağımlı ödem ilerlemesini ve sitokin (tümör nekroz faktörü alfa ve interlökin 1beta) birikimlerini önemli ölçüde engellemesi nedeni ile antiinflamatuvar etkili olduğu bulunmuştur.

Sonuç: *V. exuberans*'ın ağrı ve enflamasyonun giderilmesinde yüksek yararlı potansiyeli ile yeni bir kaynak olduğu ifade edilebilir.

Anahtar kelimeler: *Verbascum exuberans* Hub.-Mor., *Scrophulariaceae*, antinosiseptif aktivite, antiinflamatuvar etki, tramadol

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INTRODUCTION

Pain is a major global health problem and its treatment is challenging.[1] Despite the present scientific advancements in pain therapies, potent, safe, and effective drugs are still lacking for many pain conditions.[2] Furthermore, many of the currently available treatments for pain are accompanied by adverse effects.[3] Therefore, optimization of the current pain relievers and identification of new ones are still a major focus of both the pharmaceutical industry and academics.[4] In recent years, increasing interest has been devoted to herbal remedies as potential therapeutic agents in the management of pain and inflammation. Among them, the genus *Verbascum* L. (Scrophulariaceae), also commonly known as mullein, has a long tradition in classical medicine and it has been used around the globe for diverse purposes.[5,6] In particular, the leaves and flowers of *Verbascum densiflorum* Bertol., *V. phlomoides* L., and *V. thapsus* L. have expectorant, mucolytic, and sedative properties that in Turkish folk medicine are used to treat respiratory disorders such as bronchitis, dry coughs, tuberculosis, and asthma. These species are also applied for the treatment of hemorrhoids, rheumatic pain, superficial fungal infections, wounds, and diarrhea. The oil prepared from the flowers is used to treat otitis media and is applied externally for eczema and other types of inflammatory skin conditions. These species are reported to be mildly diuretic and are applied for pruritic conditions of the urinary tract. Furthermore, they are traditionally consumed as a tea to relieve abdominal pain.[6-8] Additionally, the roots, leaves, flowers, and/or aerial parts of *Verbascum* species including *V. pumilum* Boiss. and Heldr., *V. orientale* (L). All., *V. cheiranthifolium* Boiss. var. *cheiranthifolium* Boiss., *V. chrysochaete* Stapff, *V. lasianthum* Boiss. ex Bentham, *V. symes* Murb. et Rech.f., and *V. pyramidatum* M. Bieb. are also used to treat painful symptoms in a wide range of diseases.[9-11]

Besides the folkloric uses, in general, pharmacological studies have shown that *Verbascum* species possess unique biological properties that can be beneficial for medical purposes. More importantly, *V. chionophyllum* Hub.-Mor., *V. pycnostachyum* Boiss. and Heldr., *V. latisepalum* Hub.-Mor., *V. salviifolium* Boiss.,[12] *V. lasianthum* Boiss. ex Benth., *V. pterocalycinum* var. *mutense* Hub.-Mor.,[13,14] *V. mucronatum* Lam.,[15] *V. mallophorum* Boiss. and Heldr.,[16] *V. xanthophoeniceum* Griseb.,[17] and *V. phlomoides* L.[18] as well as their isolated active compounds played significant roles as safe and efficient pain-killers. Altogether, this highlights the potency of the species from the genus *Verbascum* in pain and inflammation therapy. Considering thus the biological potential and the limited scientific information of this plant, in the present study, we for the first time investigated the antinociceptive and the antiinflammatory effects of the methanol extract prepared from *V. exuberans* Hub.-Mor. aerial parts, in experimental animal models.

MATERIALS AND METHODS

Plant material and extraction

V. exuberans (Scrophulariaceae), which in Turkish is named zibil sığırkuyruğu,[19] was collected from Manisa, Turkey. The endemic voucher specimen (KA 1243) is deposited at the Herbarium of the Faculty of Science and Arts of Celal Bayar University in Manisa, Turkey. The air-dried and powdered aerial parts of the plant material (20.354 g) was extracted with methanol (Sigma 34860) using a Soxhlet apparatus for 48 hours at 55°C. The obtained methanolic extract was filtered and evaporated in a rotator evaporator to give crude extract (2.534 g, 12.45% w/w). Subsequently, the crude methanolic extract was dissolved in distilled water and partitioned with an equal volume of petroleum ether (0.496 g, 2.43% w/w) (Sigma 270709) (to remove chlorophyll and other lipophilic constituents) at least four times. Finally, the remaining methanolic extract was lyophilized.

Animals and housing

Forty-nine adult, healthy, male Swiss albino mice (each group, n=7; *W*, 32±4 g) and 32 adult, healthy, male Sprague Dawley rats (each group, n=8; *W*, 240±20 g) were purchased from the animal breeding laboratories of Eskisehir Osmangazi University, Medical and Surgical Experimental Animals Implementation and Research Center. The animals were left for a week to acclimatize to animal room conditions and maintained on a standard pellet diet and water (*ad libitum*). All animals were kept at 22±2°C, with 45-50% relative humidity, a light/dark cycle of 12 h, and 10-15 changes of fresh air per hour in each cycle. The study was approved by the Animal Care and Use Committee at Eskisehir Osmangazi University (protocol no: 333-1/2013) and is in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Study designs and experimental groups

Nω-nitro-L-arginine methyl ester (L-NAME) hydrochloride (N5751), cyproheptadine hydrochloride (C6022), tramadol hydrochloride (42965), λ-carrageenan (C1013), and indomethacin (I7378) were purchased from Sigma, while naloxone hydrochloride was purchased from Inresa. All of the drugs including *V. exuberans* and carrageenan were dissolved in sterile physiological saline. The drugs were administered intraperitoneally (ip) except for carrageenan. Carrageenan was given subcutanously (sc). For the experimental antinociceptive study design, the mice were randomly divided into 7 groups and received ip injections of (1) sterile physiological saline (0.1 mL/10 g) as a negative control, (2) a low dose of *V. exuberans* (250 mg/kg), (3) a high dose of *V. exuberans* (500 mg/kg), (4) *V. exuberans* 250 mg/kg+L-NAME 100 mg/kg, (5) *V. exuberans* 250 mg/kg + cyproheptadine 50 µg/kg, (6) *V. exuberans* 250 mg/kg + naloxone 1 mg/kg, and (7) tramadol (10 mg/kg) as a positive control, respectively. The animals of groups (4)-(6) were also given L-NAME, cyproheptadine, and naloxone, respectively, 30 min prior to the extract administration, while groups (1)-(3) and (7) received empty injections. Additionally, *V. exuberans* or tramadol was administered 60 min before the postdrug experiments. For the experimental antiinflammatory model design, the rats were randomly divided into 4 groups, which received injections of (1) sterile physiological saline 0.1 mL/100 g as a negative control, (2) sterile physiological saline 0.1 mL/100 g, (3) indomethacin 10 mg/kg as a positive control, and (4) a low

dose of *V. exuberans* (250 mg/kg), respectively. The animals of groups (2)-(4) were also given sterile physiological saline, indomethacin, and *V. exuberans*, respectively, 30 min prior to the carrageenan (100 μL, 1% w/v in saline) administration, while group (1) received only sterile physiological saline.

Experimental antinociceptive activity tests

Tail clip[20] and tail flick[21] tests were used to investigate central spinal antinociception. For the tail clip test an artery clip that exerts standardized pressure on the tail was positioned 2-2.5 cm from the base of the tail. The biting and turning response to the tail clip was recorded. The tail flick test was applied using a focused beam of high intensity light to the tail. The latency time to "flick" or withdraw the tail from the heat stimulus apparatus was noted (MAY, 9604-A Tail Flick Unit Commat, Ankara, Turkey). The hot plate test[22] was used to investigate central supraspinal antinociception. The animals were put on a hot plate surface unit (Ugo Basile Hot/Cold Plate 35100) that was stabilized at 55±0.1°C. The latency time of paw licking or jumping was recorded for the hot plate test.

The acetic acid-induced writhing test[23] was used to assess peripheral antinociception. After 5 min of 0.6% acetic acid (60 mg/kg, i.p.) administration, stretching movements of the animals (arching of back, development of tension in abdominal muscles, elongation of body, or extension of forelimbs) were counted for 10 min.

The cut-off time for the tail clip, tail flick, and hot plate tests was set at 30 seconds and they were performed consecutively and executed twice with the same animal for predrug and postdrug latency times. The results were calculated via the formula of maximal possible effect %=[(postdrug latency-predrug latency)/(cut-off time-predrug latency] x100. Furthermore, the acetic acid-induced writhing test was performed last.

Experimental antiinflammatory activity test

The carrageenan-induced hind paw edema model[24] was used to investigate the antiinflammatory potential. The inflammation was induced by a sc injection of 100 μL of 1% freshly prepared solution of carrageenan into the right hind paws of the rats. The increases in paw thicknesses were considered to be edema and were measured by a micrometric compass (Ozaki, Co, Tokyo, Japan). The measurements of the rat paws were performed just before the carrageenan injection, that is, at "0 h (time 0)" and then every 60 min over 6 h after the carrageenan injection. Meanwhile, blood samples were drawn from each rat via cardiac puncture under anesthesia pre- and postcarrageenan (solely at 6 h) injection. Within the blood collection, the blood samples were precipitated by centrifugation at 10,000 rpm for 3 min at 4˚C. The extracted serum samples were aliquoted and were kept at -20˚C until use. The tumor necrosis factor (TNF)-α (eBioscience BMS630) and the interleukin (IL)-1β (invitrogen KRC3011) assays were measured using ELISA. The proinflammatory cytokine production was calculated after plotting the standard curves and is expressed as pg/mL.

Statistical analysis

Statistical significance was assessed using One-Way or Two-Way analysis (one factor repeated) of variance followed by the Tamhane or Tukey test for multiple comparisons, respectively. Significance between the mean values is defined as $p<0.05$ or $p<0.001$.

RESULTS AND DISCUSSION

V. exuberans has a profound central antinociceptive effect via the spinal system

Sensory neurons encode mechanical, thermal (heat or cold), and chemical stimuli into nerve fibers that travel via the spinal cord to the brain to stimulate painful sensations, a process known as nociception.[25] In the present study, we used tail clip, tail flick, and hot plate tests to assess central nociception. The tail flick and hot plate tests are thermal nociceptive tests, whereas the tail clip test is mechanical.[26] Furthermore, the nociceptive threshold response is supraspinally organized in the hot plate test, while the tail clip and tail flick tests are spinally mediated.[27] In our experiments, we used a high (500 mg/kg) dose and a low (250 mg/kg) dose of *V. exuberans* (Figure 1). Treatment with the high dose of the extract significantly decreased the behavioral nociceptive responses of the mice to the mechanical noxious stimuli compared to the control in the tail clip test ($p<0.05$) (Figure 1A), but did not affect the behavioral nociceptive responses to the thermal noxious stimuli in the tail flick test or the hot plate test ($p>0.05$) (Figure 1B and 1C). Interestingly, the low dose of the extract showed a higher potency to relieve pain. *V. exuberans* at 250 mg/kg dose alone decreased the behavioral nociceptive responses compared to the control in the tail clip and tail flick tests ($p<0.05$) (Figure 1A and 1B), but not in the hot plate test ($p>0.05$) (Figure 1C). Moreover, in the tail clip test, the antinociception of the extract showed an effect similar to that of tramadol ($p>0.05$). Finally, the significant alterations in both the mechanical and thermal nociceptive threshold latencies of mice at 250 mg/kg dose indicate that *V. exuberans* has a profound central antinociceptive effect. Additionally, the central antinociceptive action of the extract affects the spinal but not the supraspinal system.

V. exuberans shows a peripheral antinociceptive effect

The abdominal constriction response induced by acetic acid was used to evaluate the potential of *V. exuberans* as a peripherally acting pain reliever. Acetic acid stimulates the pain nerve endings and induces contraction of abdominal muscles *via* sensitization of the nociceptive receptor to the peripherally released endogenous prostaglandins (PGs), in particular $PGE_{2\alpha}$ and $PGF_{2\alpha}$ as well as lipoxygenase products and cytokines.[28] In the present study, the behavioral nociceptive response of mice to the chemical noxious stimuli was greatly inhibited by *V. exuberans* at both doses compared to the control ($p<0.001$), thus clearly indicating a peripheral antinociceptive effect. Furthermore, the effect was stronger at the higher dose of the extract than at the lower dose ($p<0.05$) (Figure 1D). In our experiments, we used the well characterized drug tramadol as a positive control.[29,30] As expected, 10 mg/kg tramadol showed both central (spinal and supraspinal) and peripheral antinociceptive effects compared to the control in all the experimental nociceptive tests ($p<0.001$) (Figure 1). Strikingly,

when compared to tramadol, the inhibition of peripheral pain by the extract has greater benefit than the inhibition of central pain, suggesting that *V. exuberans* might be a new alternative for pain therapy.

V. exuberans mediates its central spinal and peripheral antinociception by targeting the nitrergic pathway

Activation of the L-arginine (arg)-nitric oxide (NO)- cyclic guanosine monophosphate (cGMP)-ATP sensitive K^+ channel pathway results in antinociception. NO mediates the antinociceptive effect via phosphorylation of the K_{ATP} channel and thereby activation of the guanylate cyclase-cGMP system.[31] To explore the role of the nitrergic pathway in the antinociceptive effect of *V. exuberans*, we combined the plant extract with L-NAME, a competitive L-arg-based nonselective NO synthase inhibitor (Figure 2). Addition of L-NAME to the mice pretreated with the extract ameliorated the behavioral nociceptive responses to the mechanical and the chemical noxious stimuli compared to the control ($p<0.001$) and 250 mg/kg extract alone ($p<0.05$) in both the tail clip and writhing tests (Figure 2A and 2D). Moreover, the enhanced antinociceptive responses of the extract exhibited a higher effect than tramadol in the tail clip test ($p<0.05$) (Figure 2A), while in the writhing test they showed similar potential ($p>0.05$) (Figure 2D). In addition, the extract nonsignificantly affected the behavioral nociceptive latencies of mice to the thermal noxious stimuli compared to the control and 250 mg/kg extract alone in both the tail flick and hot plate tests ($p>0.05$) (Figure 2B and 2C). However, in the presence of L-NAME the extract retained its antinociception properties rather than showing increased activity in the tail

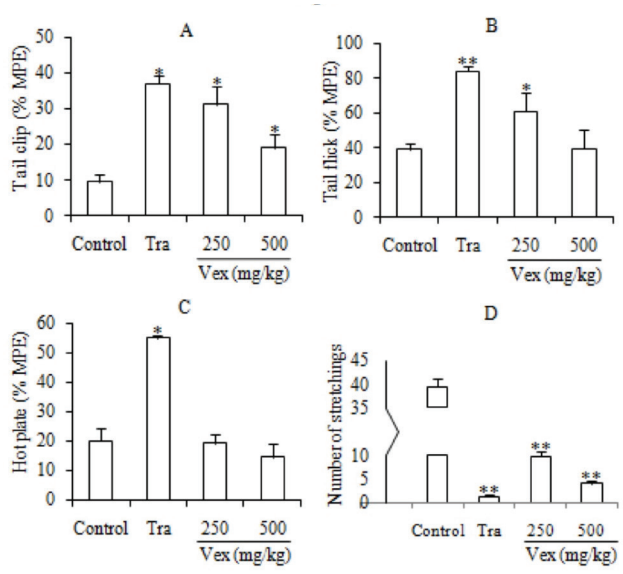

Figure 1. The effects of *V. exuberans* on central and peripheral nociception (Swiss albino mice; each group, n=7; *W*, 32±4 g). The central spinal antinociceptive effect was determined by the tail clip (A) and the tail flick (B) tests, while the central supraspinal antinociceptive activity was assessed by the hot plate test (C). The peripheral antinociceptive activity was determined by the acetic acid-induced writhing test (D). The latency time responses were defined as % MPE for the central antinociceptive tests, while for the peripheral antinociceptive test the movement responses were defined as the number of stretchings. All the test results were expressed as mean ± SEM. *$p<0.05$ compared to control, **$p<0.001$ compared to control, as determined by one way analysis of variance followed by the Tamhane test.

Tra: Tramadol, Vex: *V. exuberans*, MEP: Maximal possible effect, SEM: Standard error mean

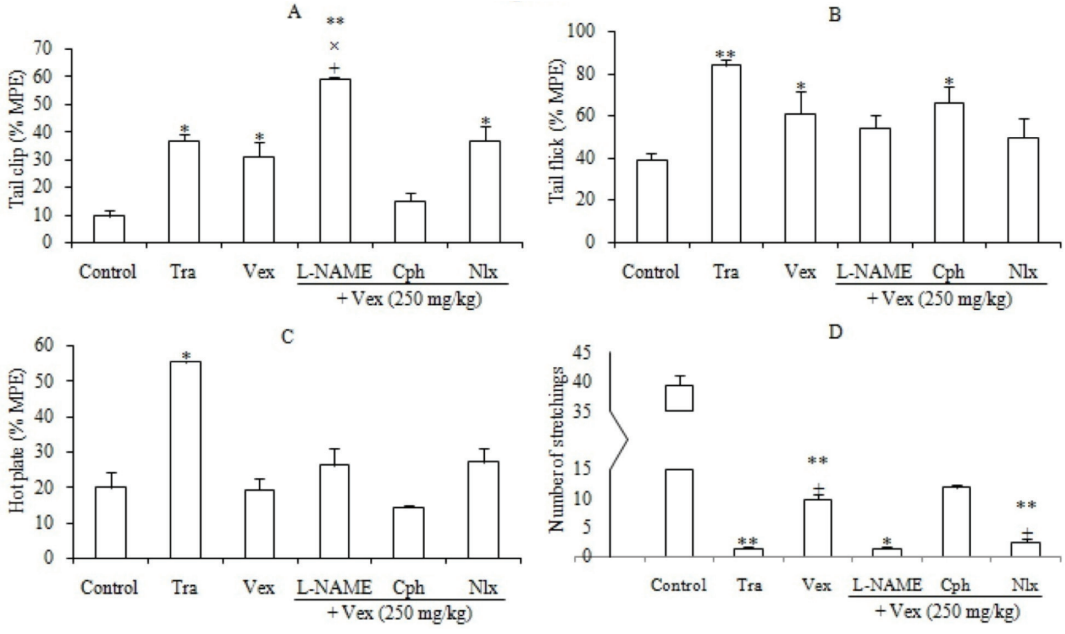

Figure 2. The effects of *V. exuberans* and its combinations on central and peripheral nociception (Swiss albino mice; each group, n=7; *W*, 32±4 g). The central spinal antinociceptive effect was determined by the tail clip (A) and the tail flick tests (B), while the central supraspinal antinociceptive activity was assessed by the hot plate test (C). The peripheral antinociceptive activity was determined by the acetic acid-induced writhing test (D). The latency time responses were defined as % MPE for the central antinociceptive tests, while for the peripheral antinociceptive test the movement responses were defined as the number of stretchings. All the test results were expressed as mean ± SEM. *$p<0.05$ compared to control; **$p<0.001$ compared to control, ×$p<0.05$ compared to 10 mg/kg tramadol, +$p<0.05$ compared to the single dose of 250 mg/kg *V. exuberans*, as determined by One-Way analysis of variance followed by the Tamhane test.

Tra: Tramadol, Vex: *V. exuberans* 250 mg/kg, Cph: Cyproheptadine, Nlx: Naloxone, MEP: Maximal possible effect, SEM: Standard error mean

flick test. This could be related to the concurrent effect of NO, which depends on dosage levels and the rate and timing of its release.[32,33] In conclusion, our results indicate that *V. exuberans* showed its antinociceptive effect in a L-NAME reversible manner, suggesting a central spinal and peripheral nitrergic mechanism. The results imply that the composition of the plant extract might have a specific effect on the nitrergic pathway.

Cyproheptadine does not affect the antinociceptive properties of V. exuberans

Involvement of the serotonergic pathway mediated antinociceptive effect of *V. exuberans* was tested using cyproheptadine, a serotonin (5-HT) receptor antagonist (Figure 2). Addition of cyproheptadine to mice pretreated with the plant extract did not significantly change the mechanical or thermal nociceptive threshold latencies compared to the controls in the tail clip test or the hot plate test ($p>0.05$) (Figure 2A and 2C). The supplementation of cyproheptadine let the extract decrease the behavioral nociceptive responses of mice compared to the controls in both the tail flick and writhing tests ($p<0.05$), while the nociceptive latencies showed nonsignificant alterations compared to 250 mg/kg extract alone ($p>0.05$) (Figure 2B and 2D). Together these results indicate that cyproheptadine does not evoke the obvious antinociceptive properties of *V. exuberans*. Animal studies report that 5-HT and 5-HT receptors have a complex role in modulating nociceptive reflexes. The complexity of effects produced by the 5-HT receptor in nociceptive transmission is due to the type of nociceptive stimuli, subtype of receptor, and dose of agonists or antagonists.[34] Since cyproheptadine is a high-affinity $5\text{-HT}_{1C,2}$ receptor antagonist, our data suggest that at least these receptors do not directly activate the antinociceptive effect of *V. exuberans*.

Naloxone partly inhibits V. exuberans-induced antinociception

The opioid system is very important in regulating pain. This system participates in both the perception and modulation of the pain process via central and peripheral mechanisms.[35] To explore the contribution of the opioidergic pathway to the antinociceptive effect of *V. exuberans* we used naloxone, a relatively nonselective opioid receptor antagonist (Figure 2). Supplementation of naloxone to mice pretreated with the plant extract caused nonsignificant behavioral nociceptive responses compared to the control and 250 mg/kg extract alone in both the tail flick and hot plate tests ($p>0.05$) (Figure 2B and 2C). The addition of naloxone enabled the extract to enhance the nociceptive latencies compared to the control in the tail clip test ($p<0.05$), whereas the latencies showed non-significant changes compared to both tramadol and 250 mg/kg extract alone ($p>0.05$) (Figure 2A). Our results indicate that the antinociceptive effect of the extract on both the mechanical and thermal nociceptive thresholds of mice was unaltered by naloxone, indicating that the spinally mediated actions of the extract are independent of the central opioidergic system. The therapeutic utility of opioids in pain therapy is limited due to their specific affinity to centrally mediated opioid receptors.[36] Therefore, targeting of peripheral opioid receptors may provide pain relief, while reducing many of the adverse effects.[37] In fact, addition of naloxone allowed the extract to suppress the acetic acid nociceptive stimuli by decreasing the stretching responses compared to both the control ($p<0.001$) and 250 mg/kg extract alone ($p<0.05$) in the writhing test (Figure 2D). Naloxone has a high affinity to μ-opioid receptors and a lower affinity to κ- and δ-opioid receptors. Importantly, our results extend this observation by showing that the antinociception produced by the extract activates these opioid receptors in the periphery. Reinforcing this, most of the opioid antinociceptive effects are mediated via activation of opioid receptors,[38] and these receptors have been identified on the peripheral terminals of afferent nerves, which can be the sites of intrinsic modulation of nociception.[39] In conclusion, *V. exuberans* might be safe with high potency as a pain reliever since the extract acts as a peripheral opioid agonist by decreasing the excitability of sensory nerves and/or inhibiting proinflammatory neuropeptides based on the chemogenic pain model.

V. exuberans inhibits edema progression via reduced proinflammatory cytokines

The antiinflammatory role of *V. exuberans* was tested using the carrageenan-induced model of acute peripheral inflammation and hyperalgesia. The mechanism of carrageenan induces biphasic inflammation. The initial phase (0-2 h) is primarily mediated via the release of histamine, serotonin, and bradykinin, while the late phase (2.5-6 h) is sustained by infiltration of leukocytes and is mainly attributed to the overproduction of PGs.[40] In our study, the paw edema size showed a rapid increase over the first hour of carrageenan injection ($p<0.05$), presented a small peak at 3 h ($p<0.05$), and progressively persisted until at least 6 h compared to the saline controls (2 h, $p<0.05$; 4-6 h, $p<0.001$) (Figure 3A). Following the carrageenan-induced inflammation, at 6 h, IL-1β and TNF-α, which are important peripheral and spinal hyperalgesic proinflammatory mediators,[41] were significantly increased compared to both the precarrageenan (time 0) ($p<0.05$) and the saline controls ($p<0.05$) (Figure 3B and 3C). In contrast, the low dose of *V. exuberans* did not affect edema size during 0-2 h ($p>0.05$), but significantly weakened the peaked edema at 3 h ($p<0.05$) and showed inhibition in the inflamed paw swellings up to 6 h ($p<0.001$) when compared to those of the rats that received carrageenan (Figure 3A). Furthermore, the extract showed a similar potency in both paw size and cytokine production at 6 h compared to the well-described drug indomethacin at 10 mg/kg[42] ($p>0.05$) (Figure 3B and 3C), whereas the extract had a much stronger effect on paw edema size during 3-5 h ($p<0.05$) (Figure 3A). Finally, at 6 h, *V. exuberans* accelerated recovery in the rat paw size as well as the cytokine productions to near normal levels compared to those of the saline and the precarrageenan controls ($p>0.05$), suggesting thus an antiinflammatory effect.

CONCLUSION

Importantly, our data show for the first time the potential of methanol extract from the aerial parts of *V. exuberans* to relieve pain and inflammation in experimental animals. The plant extract showed a central spinal and a peripheral antinociceptive

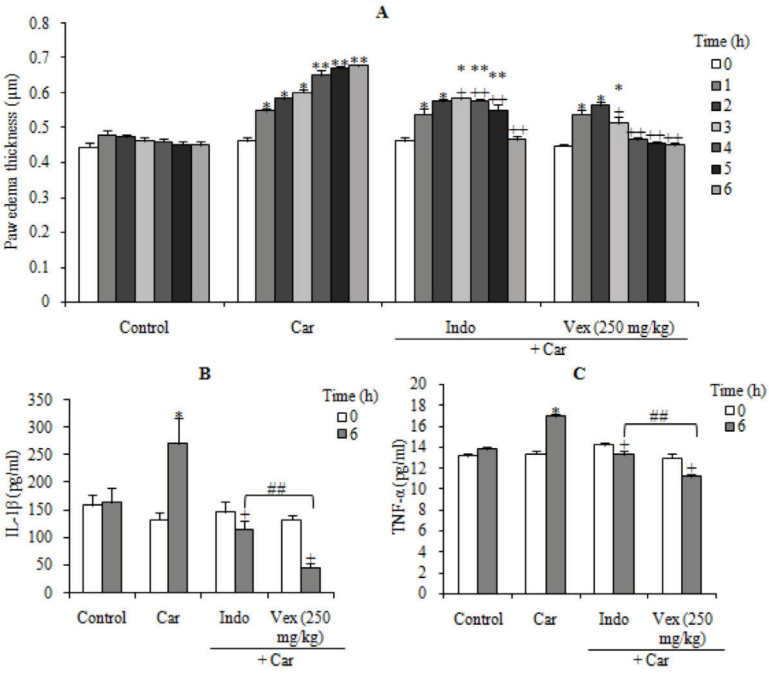

Figure 3. The effects of *V. exuberans* on inflammation (Sprague Dawley rats; each group, n=8; *W*, 240±20 g). The inflammation was induced by carrageenan (100 µL, 1% w/v in saline) into the subplantar surface of right hind paws. The increases in paw thicknesses were considered to be edema and were measured at different time intervals (0-6 h) (A). The proinflammatory cytokine production including IL-1β (B) and TNF-α (C) was measured immediately before the carrageenan injection (0 h) and then after the carrageenan injection solely at 6 h. The values were given as mean ± SEM. *p<0.05 compared to control, **p<0.001 compared to control, +p<0.05 compared to carrageenan, ++p<0.001 compared to carrageenan, ##p<0.001 compared to indomethacin, as determined by two way analysis of variance (one factor repeated) followed by the Tukey test

Car: Carrageenan, Vex: V. exuberans 250 mg/kg, Indo: Indomethacin 10 mg/kg, IL: Interleukin, TNF: Tumor necrosis factor, SEM: Standard error mean

effect as well as antiinflammatory activity. The antinociception induced by the extract is mainly organized via targeting of the nitrergic pathway, while the opioidergic pathway is only peripherally involved. Additionally, the bioactive compounds present in the extract might have a specific effect on the nitrergic pathway. To further understand the mechanism by which *V. exuberans* relieves pain and inflammation, it will be key to isolate and characterize the active agents responsible for the observed pharmacological activities.

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