Verbascum exuberans Hub.-Mor.'ın *in vivo* antinosiseptif ve antienflamatuvar etkileri

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ÖZ

GİRİŞ ve 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. Buna dayanarak, ağrı ve enflamasyon tedavisinde, tedavi edici potansiyeli nedeniyle *Verbascum* L. cinsine yönelik kapsamlı araştırmalar yürütülmektedir. Bunların arasından, *Verbascum exuberans* Hub.-Mor'ın antinosiseptif etkinliğini, bu etkide nitrerjik, serotonerjik ve opioiderjik yolaklar üzerindeki rolünü ve antienflamatuvar aktivitesini araştırdık.

YÖNTEM ve GEREÇLER: Ekstre (250 ve 500 mg/kg)'nin 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 ölçüldü. 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 belirlendi. Karragenan ile oluşturulmuş arka ayak pençe ödem modeli ise ekstre (250 mg/kg)'nin antienflamatuvar aktivitesinin belirlenmesinde kullanıldı.

BULGULAR: Ekstrenin santral spinal düzeyde etkili olduğu fakat santral supraspinal düzeyde etkili olmadığı ve periferal antinosiseptif etkili olduğu görüldü. Ekstrenin antinosiseptif etkinliği büyük ölçüde nitrerjik yolağın üzerinden düzenlenirken, opioiderjik yolağın ise kısmen aracılık ettiği belirlendi. Ek olarak, ekstrenin, zamana bağımlı ödem ilerlemesini ve sitokin (TNF-*α* ve IL-1*β*) birikimlerini önemli ölçüde engellemesi nedeni ile antienflamatuvar etkili olduğu bulundu.

TARTIŞMA ve SONUÇ: *V. exuberans*'ın ağrı ve enflamasyonun giderilmesinde yüksek yararlı potansiyeli ile yeni bir kaynak olduğu düşüncesindeyiz.

ANAHTAR KELİMELER: *Verbascum exuberans* Hub.-Mor., Scrophulariaceae, antinosiseptif aktivite, antienflamatuvar etki, tramadol

The *in vivo* antinociceptive and antiinflammatory effects of *Verbascum exuberans* Hub.-Mor.

ABSTRACT

INTRODUCTION: 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. Among them, we investigated the antinociceptive effect of *Verbascum exuberans* Hub.-Mor, and its contributions to nitrergic, serotonergic and opioidergic pathways as well as its antiinflammatory acitivity.

METHODS: Tail clip, tail flick, and hot plate tests were used to determine the central (spinal and supraspinal), while acetic acid-induced writhing test was measured for the peripheral antinociceptive effect of the extract (250 and 500 mg/kg). Nω-Nitro-L-arginine methyl ester, cyproheptadine, and naloxone were combined with the extract (250 mg/kg) to assess the involvement of nitrergic, serotonergic, or opioidergic pathways, respectively. Carrageenan-induced hind paw edema model was used to assess the antiinflammatory effect of the extract (250 mg/kg).

RESULTS: The extract presents central spinal but not central supraspinal antinociceptive effect and shows 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 (TNF-*α* and IL-1*β*) productions.

DISCUSSION and CONCLUSION: We suggest that *V. exuberans* could be 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

INTRODUCTION

Pain is a major global health problem and its treatment is challenging.[1] Despite of the present scientific advancement in pain therapies, potent, safe, and effective drugs are still lacking for many painful symptoms.[2] Furthermore, many of the current available therapies for pain are accompanied by severe adverse effects.[3] Therefore, optimization of the currents and identification of new pain relievers is still a major focus of both the pharmaceutical industry and academics.[4] In recent years, increasing interest has been devoted on herbal remedies as potential therapeutic agents in the management of pain and inflammation. Among them, *Verbascum* L. genus (Scrophulariaceae), also commonly known as “mullein”, has a long tradition in classical medicine and it has been used around the globe for diverse of purposes.[5,6] Particularly, the leaves and flowers of *Verbascum densiflorum* Bertol., *V. phlomoides* L. and *V. thapsus* L. have expectorant, mucolytic and demulcent properties which in traditional Turkish folk medicine are used to treat respiratory disorders such as bronchitis, dry coughs, tuberculosis and asthma. These species are also used to treat haemorrhoids, rheumatic pain, superficial fungal infections, wounds and diarrhoea, and have inhibitory activities against the murine lymphocytic leukemia and influenza viruses A2 and B. The oil prepared from the flowers is used to treat earache and is applied externally for eczema and other types of inflammatory skin conditions. These species are reported to be mildly diuretic and to have a soothing and antiinflammatory effect on the urinary tract, as well as acting as a mild sedative. They are traditionally consumed as a tea to relieve abdominal pains.[6,7,8] Additionally, the roots, leaves, flowers, and/or aerial parts provided from the *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 fil. and *V. pyramidatum* M. Bieb. are also used to treat for painful symptoms in a wide range of diseases.[9,10,11]

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 displayed significant roles as safe and efficient pain stillers. Altogether, this highlights the potency of the species from *Verbascum* genus 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), namely defined in Turkish as 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 equal volume of petroleum ether (0.496 g, 2.43% w/w) (Sigma 270709) (to remove chlorophyll and other lipophylic constituents) at least for four times. Finally, the remaining methanolic extract was lyophilized.

Animals and housing

Adult, healthy, 49 male Swiss albino mice (each group, n=7; W, 32±4 g) and adult, healthy, 32 male Spraque Dawley rats (each grup, 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 h in cycle. This study was approved by the Animal Care and Use Committee at Eskisehir Osmangazi University (Protocol No 333-1/2013) and are in accordance with the National Institute 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, and 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 to carrageenan. Carrageenan was given subcutanously (sc). For the experimental antinociceptive study design, the mice were randomly divided into 7 groups, received i.p. injections of (1) sterile physiological saline (0.1 ml/10g) as negative control, (2) low dose *V. exuberans* (250 mg/kg), (3) high dose *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, (7) tramadol (10 mg/kg) as positive control, respectively. The animals of (4)-(6) were accompanied by L-NAME, cyproheptadine, naloxone, respectively, 30 min prior to the extract administration, while (1)-(3) and (7) received empty injections. Additionally, *V. exuberans* or tramadol were administered 60 min before the post-drug experiments. For the experimental antiinflammatory model design, the rats were randomly divided into 4 groups, recieved injections of (1) sterile physiological saline 0.1 ml/100g as negative control, (2) sterile physiological saline 0.1 ml/100g, (3) indomethacin 10 mg/kg as positive control, (4) low dose *V. exuberans* (250 mg/kg), respectively. The animals of (2)-(4) were accompanied by sterile physiological saline, indomethacin, *V. exuberans*, respectively, 30 min prior to the carrageenan (100 µl, 1% w/v in saline) administration, while (1) received solely 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 administers standardized pressure was positioned 2-2.5 cm from the base of the tail. The response time of biting or turning to the clip was recorded. The tail flick test was performed with a beam of high-intensity light focused on the dorsal surface of the tail. The latency time between the onset of the infrared heat stimulus and the movement of the tail out of the light beam of the apparatus (MAY, 9604-A Tail Flick Unit Commat, Ankara, Turkey) was determined. The hot plate test[22] was used to investigate central supraspinal antinociception. The

animals were put on a surface of a plate which was heated and kept at a temperature of 55±0.1ºC using a hot plate unit (UGO BASILE Hot/Cold Plate 35100). The latency time of paw licking or jumping was recorded for the hot plate test.

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

The cut-off time for the tail clip, tail flick, and hot plate tests were set at 30 s, and were performed consecutively and executed twice with the same animal for pre-drug and post-drug latency times. The results were expressed as the percentage of the maximal possible effect (%MPE). The formula is %MPE=[(postdrug latency-predrug latency)/(cut off time-predrug latency]×100. Furthermore, acetic acid-induced writhing test was performed at last.

Experimental antiinflammatory activity test

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 rats. The increase in the 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 in every 60 min during 6 h after carrageenan injection. Meanwhile, the blood samples were drawn from each rat via cardiac puncture under anaesthesia pre- and post-carrageenan (solely at 6 h) injection. Within the blood collection, the blood samples were precipitated by centrifugation of 10.000 rpm for 3 min at 4°C. The extracted sera samples were aliquoted, and were kept at -20°C till use. The tumor necrosis factor (TNF)-*α* (eBioscience BMS630) and the interleukin (IL)-1*β* (Invitrogen KRC3011) assays were measured using an enzyme-linked immune sorbent assay (ELISA) commercial kit according to the manufacturer's instructions. The proinflammatory cytokine productions were calculated after plotting the standard curves, and are expressed as pg/ml.

Statistical analysis

Statistical significance was assessed using the One-way or the Two-way Analysis (one factor repeated) of Variance followed by the Tamhane or the Tukey tests for multiple comparisons, respectively. *P* values indicating statistically significant between the mean values are defined as $p<0.05$ or $p<0.001$.

RESULTS AND DISCUSSION

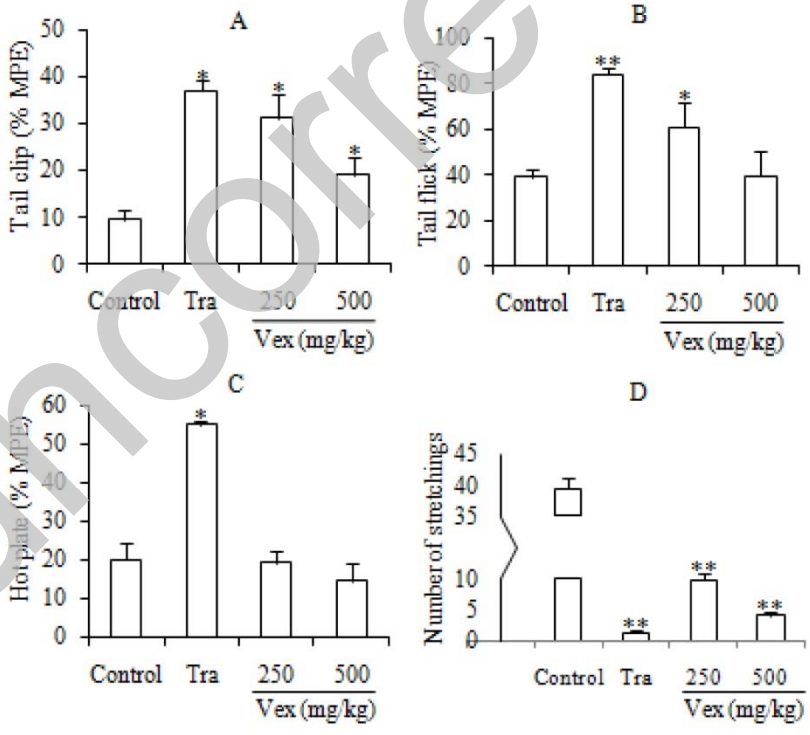
V. exuberans has a profound central antinociceptive effect via the spinal system

Sensory neurons transduce mechanical, thermal, and chemical stimuli into nerve impulses which then travel to higher centres to initiate painful sensations, a process referred to as nociception.[25] In our study, we used tail clip, tail flick, and hot plate tests to evaluate central nociception. Tail clip is mechanical test, while tail flick and hot plate are thermal nociceptive tests.[26] Furthermore, the nociceptive threshold response is supraspinally organised in the hot plate test, while the tail clip and the tail flick tests are spinally mediated.[27] In our experiments, we used a high (500 mg/kg) and a low (250 mg/kg) doses of *V. exuberans* (Fig. 1). Treatment with the high dose of the extract importantly decreased the behavioral nociceptive responses of mice to the mechanical noxious stimuli compared to the control in the tail clip test ($p<0.05$) (Fig. 1A), but did not affect the behavioral nociceptive responses to the thermal noxious stimuli in both the tail flick and the hot plate tests ($p>0.05$) (Fig. 1B and 1C). Interestingly, the low dose of the extract showed even 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 the tail flick tests ($p<0.05$) (Fig. 1A and 1B), rather than in the hot plate test did ($p>0.05$) (Fig. 1C). Moreover, in the tail clip test, the antinociception of the extract showed similar effect compared to tramadol ($p>0.05$). Finally, the significant alterations on both the mechanical and the 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 effect of the extract is due to its action on the spinal but not on the supraspinal system.

V. exuberans shows 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 the sensitization of the nociceptive receptor to the peripherally released endogenous prostaglandins (PG)s, in particular $PGE_{2\alpha}$ and $PGF_{2\alpha}$ as well as lipoxygenase products and cytokines.[28] In this study, the behavioral nociceptive response of mice to the chemical noxious stimuli was exceedingly prohibited by *V. exuberans* at both the doses compared to the control ($p<0.001$), thus clearly indicating a peripheral antinociceptive effect. The effect was stronger at the higher dose of the extract when compared to the lower dose ($p<0.05$) (Fig. 1D). In our experiments, we used the well characterized drug tramadol as positive control.[29,30]. As expected 10 mg/kg of tramadol showed central spinal, central supraspinal, and peripheral antinociceptive effects compared to the control in all the experimental nociceptive tests ($p<0.001$) (Fig. 1). Strikingly, when compared to tramadol, the inhibition of peripheral pain of the extract has greater benefit than the inhibition of central pain, suggesting that *V. exuberans* might be a new alternative for the treatment of pain.

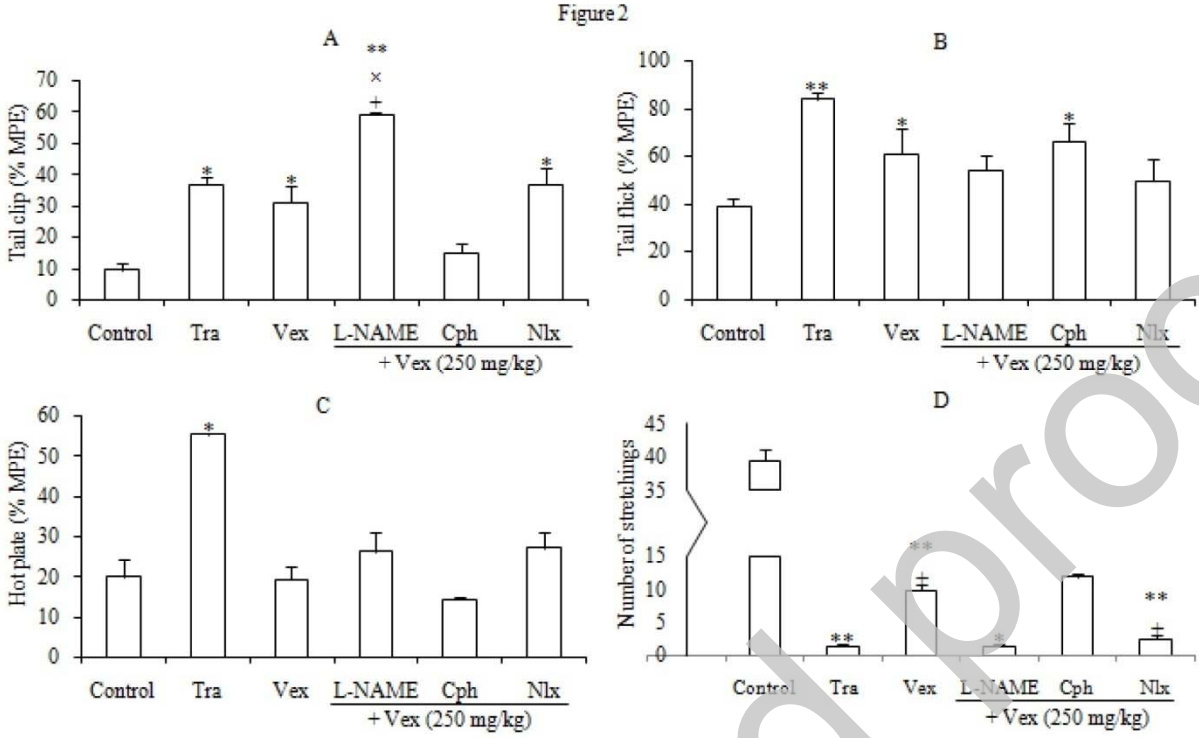
Figure 1



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

Activation of the L-arginine (arg)-nitric oxide (NO)-cGMP-K_{ATP} channel pathway results in antinociception. NO contribute to antinociceptive effect via opening the K^+ channel and activation of guanylate cyclase-cGMP system.[30] 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 non-selective nitric oxide synthase (NOS) inhibitor (Fig. 2). Addition of L-NAME to 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 the extract 250 mg/kg alone (p<0.05) in both the tail clip and the writhing tests (Fig. 2A and 2D). Moreover, the enhanced antinociceptive responses of the extract presented a higher effect than tramadol in the tail clip test (p<0.05) (Fig 2A), while in the writhing test showed a similar potential (p>0.05) (Fig. 2D). Besides, the extract did insignificantly affect the behavioral nociceptive latencies of mice to the thermal noxious stimuli compared to the control and the extract 250 mg/kg alone in both the tail flick and the hot plate tests (p>0.05) (Fig. 2B and 2C). However, the observation that in the presence of L-NAME the extract remains its antinociception properties rather than showing increased activity in the tail flick test. This could be related to the concurrent effect of NO that depends on dosage levels, the rate, and timing of its release.[31,32] As a 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.

Figure 2



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 (Fig. 2). Addition of cyproheptadine to mice pretreated with the plant extract did not significantly change the mechanical and the thermal nociceptive threshold latencies compared to the control in both the tail clip and the hot plate tests (p>0.05) (Fig. 2A and 2C). The supplement of cyproheptadine let the extract to decrease the behavioral nociceptive responses of mice compared to the control in both the tail flick and the writhing tests (p<0.05), while the nociceptive latencies showed insignificant alterations compared to the extract 250 mg/kg alone (p>0.05) (Fig. 2B and 2D). Together these results indicate that cyproheptadine does not evoke the obvious antinociceptive properties of *V. exuberans*. The data from animal studies suggested that 5-HT and 5-HT receptors play a role in modulating nociceptive reflexes in a complex manner. They can inhibit or activate nociceptive responses depending on the type of nociceptive stimuli, subtype of the receptor, and the dose of agonists and antagonists. Especially 5-HT_2 and 5-HT_3 receptors were reported to modulate nociceptive transmission.[33] Since cyproheptadine is a high-affinity 5-$HT_{1C,2}$ receptor antagonist, our data suggest that at least these receptors do not participate in the antinociceptive effect of *V. exuberans*.

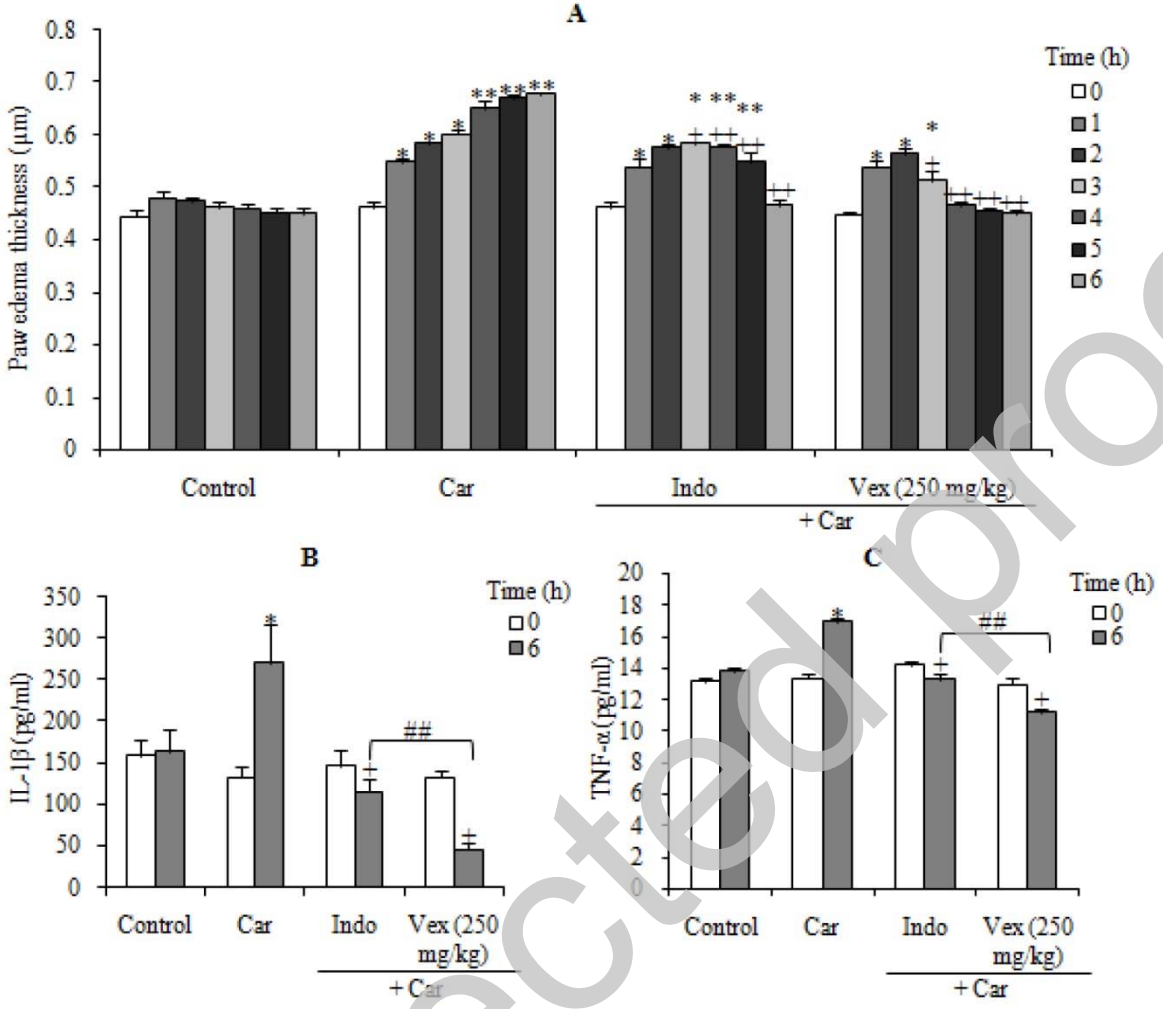
Naloxone partly inhibits V. exuberans-induced antinociception

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.[34] To explore the contribution of the opioidergic pathway to the antinociceptive effect of *V. exuberans* we used naloxone, a relatively non-selective opioid receptor antagonist (Fig. 2). Supplement of naloxone to mice pretreated with the plant extract displayed insignificant behavioral nociceptive responses compared to the control and the extract 250 mg/kg alone in both the tail flick and the hot plate tests (p>0.05) (Fig. 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 insignificant changes compared to both tramadol and the extract 250 mg/kg alone (p>0.05) (Fig. 2A). Our results indicate that the antinociceptive effect of the extract on both the mechanical and the thermal nociceptive thresholds of mice were unaltered by naloxone, indicating that the spinally mediated actions of the extract is independent of the central opioidergic system. The therapeutic utility of the opioids in the pain therapy are limited due to their specific affinity to centrally mediated opioid receptors.[35] Therefore, targeting of peripheral opioid receptors may provide pain relief, while reducing many of the adverse effects.[36] 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 the extract 250 mg/kg alone (p<0.05) in the writhing test (Fig. 2D). Naloxone has a high-affinity to μ-opioid receptors and though with a lower affinity, at κ- and δ-opioid receptors. Importantly, our results extend this observation by showing that the antinociception produced by the extract activate these opioid receptors in the periphery. Reinforcing this, most of opioid antinociceptive effects are mediated via activation of opioid receptors,[38] and these receptors have been identified on peripheral terminals of afferent nerves, which can be the sites of the intrinsic modulation of nociception.[39] In conclusion, *V. exuberans* might be safe with a 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 of proinflammatory neuropeptides based on the chemogenic pain model.

V. exuberans inhibits the edema progression via reduced proinflammatory cytokines

The antiinflammatory role of *V. exuberans* was tested by 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 the infiltration of leukocytes, and is mainly attributed to the overproduction of PGs.[37] In our study, the paw edema size showed a rapid increase over the first h of carrageenan injection ($p<0.05$), presented a small peak at 3 h ($p<0.05$), and progressively persisted for at least 6-h time point compared to the saline controls (2 h, $p<0.05$; 4-6 h, $p<0.001$) (Fig. 3A). Following the carrageenan-induced inflammation, at 6 h, IL-1β and TNF-α, which are important peripheral and spinal hyperalgesic proinflammatory mediators,[38] were significantly increased compared to both the pre-carrageenan (time 0) ($p<0.05$) and the saline controls ($p<0.05$) (Fig. 3B and 3C). In contrast, the low dose of *V. exuberans* did not affect the edema size during 0-2 h ($p>0.05$), but importantly weakened the peaked edema at 3 h ($p<0.05$), and showed inhibitons in the inflamed paw swellings till the last 6-h time point ($p<0.001$) when compared to those of the carrageenan received rats (Fig. 3A). Further, the extract showed a similar potency in both the paw size and the cytokine productions at the 6-h time point compared to the well-described drug indomethacin 10 mg/kg[39] ($p>0.05$) (Fig. 3B and 3C), whereas the extract displayed a much stronger effect in the paw edema size during 3-5 h ($p<0.05$) (Fig. 3A). Finally, at 6-h time point, *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 pre-carrageenan controls ($p>0.05$), suggesting thus an antiinflammatory effect.

Figure 3



CONCLUSIONS

Importantly, our data shows for the first time the potential of methanol extract from *V. exuberans* aerial parts to relieve pain and inflammation in experimental animals. The plant extract showed a central spinal and a peripheral antinociceptive effect as well as an antiinflammatory activity. The antinociception induced by the extract is mainly organised via targeting 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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Figure Legends

Fig. 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 performed 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 test followed by the Tamhane Test. Abbreviations: Tra: Tramadol; Vex: *V. exuberans*.

Fig. 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 performed 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 tramadol 10 mg/kg; +p<0.05 compared to the single dose of *V. exuberans* 250 mg/kg, as determined by One Way Analysis of Variance test followed by the Tamhane Test. Abbreviations: Tra: Tramadol; Vex: *V. exuberans* 250 mg/kg; Cph: Cyproheptadine; Nlx: Naloxone.

Fig 3. The effects of *V. exuberans* on inflammation (Spraque 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 increase of the paw thicknesses were considered to be edema, and were measured at different time intervals (0-6 hrs) (A). The proinflammatory cytokine productions including IL-1β (B) and TNF-α (C) were measured immediately before carrageenan injection ("0 h") and then after carrageenan injection solely at the 6th 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) test followed by the Tukey test. Car: Carrageenan, Vex: *V. exuberans* 250 mg/kg, Indo: Indomethacin 10 mg/kg.