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Effect of thymoquinone on acetic acid-induced visceral nociception in rats: role of central cannabinoid and α_2-adrenergic receptors

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Abstract

Thymoquinone (TQ) is the main biologically active substance of *Nigella sativa* (black seeds). It has anti-cancer, anti-inflammatory, anti-diabetic, anti-oxidative and anti-nociceptive properties. This study was aimed to explore the effect of TQ on acetic acid-induced visceral nociception. The central mechanisms of the effect of TQ were investigated using cannabinergic (AM251) and α_2-adrenergic (yohimbine [Yoh]) antagonists. The lateral ventricle of the brain was cannulated for intracerebroventricular (ICV) injections. Visceral nociception was induced by intra-peritoneal (IP) injection of acetic acid (1.00% in a volume of 1.00 mL). Measuring the latency time to the first writhing appearance and counting the number of writhing in 5-min intervals for a period of 60 min were performed. Locomotor activity was determined using an open-field test. Oral administration (PO) of 2.50 and 10.00 mg kg^{-1} TQ increased the latency time to the first writhing appearance and decreased the number of writhing. The AM251 (5.00 µg *per* rat; ICV) and Yoh (5.00 µg *per* rat; ICV) partially prevented TQ (10.00 mg kg^{-1}; PO)-induced anti-nociception. Locomotor activity was not altered by these treatments. The results of the present study showed that TQ had the ability to reduce visceral nociception caused by IP injection of acetic acid. The central mechanisms of this action of TQ might be partially mediated by cannabinergic and α_2-adrenegic receptors.

Introduction

Visceral pain, the pain arising from the activation of nociceptors of inflamed, damaged and diseased internal organs, is one of the most common reasons for medical consulting.[1] The information from the stimulated nociceptors enters the spinal cord and is transmitted through the dorsal column pathways to the supra-spinal centers processing visceral pain such as periaqueductal grey, thalamus, amygdala, cingulate cortex and prefrontal cortex.[2] Numbers of neurotransmitter systems such as cannabinoids, glutamate, gamma aminobutyric acid and opioids are employed in the supra-spinal processing of visceral pain.[3] The improving effects of medicinal plants and their active substances in pain management are caused by interference with local, spinal and supra-spinal pain mechanisms. For example, curcumin, the active component of *Curcuma longa*, attenuated ulcerative colitis-induced visceral hyperalgesia by reducing *receptor potential vanilloid 1* expression in colon and dorsal root ganglion.[4]

Cannabinoids are the derivatives of the cannabis plant, and exert their effects through G protein-coupled cannabinoid 1 (CB_1) and 2 (CB_2) receptors.[5] Cannabinoids are involved in the regulation of a number of physiological processes such as appetite, blood pressure, learning and memory, and are effective in the management of pathological conditions such as Parkinson's disease and migraine.[6] These chemical compounds affect inflammatory and neuropathic pains processing at the local, spinal and supra-spinal levels.[7]

The autonomic nervous system and the locus coeruleus of the brain are responsible for the use of noradrenaline to carry out physiological processes such as cardiovascular regulation, cognition and attention through α- and β-adrenergic receptors.[8,9] In addition to the treatment of hypertension and sympathetic hyperactivity, α_2-adrenergic receptors play an essential role in creating sedation and analgesia.[10] Descending pathways originating from brainstem structures such as locus coeruleus noradrenergic neurons are involved in the inhibition of

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spinal cord transmission of visceral pain through α_2-adrenergic receptors activation.[11]

Nigella sativa plant, known as black cumin or black seeds, belongs to the Ranunculaceae family and is widely used in traditional medicine to treat several pathological conditions such as asthma, fever and inflammation.[12] Although *N. sativa* contains many chemical compounds such thymol and carvacrol, the main biologically active component of the plant is thymoquinone (TQ).[13] The anti-inflammatory, anti-cancer, anti-diabetic and digestive and kidney protective effects of TQ have been suggested by pharmacological studies.[14,15] The TQ crosses through blood-brain barrier and exerts beneficial effects in treating some neurological diseases such as epilepsy, depression and anxiety.[16] Although the effect of TQ on visceral nociception has not been reported, pharmacological studies have suggested anti-nociceptive effects of TQ in other pain models such as formalin test.[17]

Pharmacotherapy of visceral pain is typically less efficacious and limited due to multiple adverse side effects.[18] Thus, the use of medicinal plants and their active substances along with drug treatment has raised a new perspective in the medical management of pain.[19] Considering the above-mentioned findings, this study was planned to investigate the effect of oral administration (PO) of TQ on visceral nociception caused by intra-peritoneal (IP) injection of acetic acid. To follow the central mechanism of TQ effect, intracerebroventricular (ICV) injections of N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251; a CB_1 receptor antagonist) and yohimbine (Yoh; an α_2-adrenergic receptor antagonist) were done alone or after TQ treatment.

Materials and Methods

Animals. Male *Wistar* rats weighing 240 - 260 g were used throughout the study. They were kept in standard breeding conditions (temperature: 22.00 ± 0.50 ˚C; humidity: 60.00 - 70.00% and 12 hr light-dark cycles beginning at 7.00 AM) with free access to food and water. Behavioral recording was done between 10:00 AM – 14:00 PM. Veterinary Ethics Committee of Urmia University, Urmia, Iran (Ethical Code: IR-UU-AEC-972-PD-3) approved the study protocol.

Drugs. Chemical compounds including TQ (Sigma-Aldrich, St. Louis, USA), AM251 (Sigma-Aldrich) and Yoh (Sigma-Aldrich) were used in the present study. Acetic acid, Tween 80 and dimethyl sulfoxide (DMSO) were purchased from Merck Co., Darmstadt, Germany. Ketamine hydrochloride 10.00% and xylaxine hydrochloride 2.00% were purchased from Alfasan (Woerden, The Netherlands). Chemical solutions were prepared 30 min before use. The TQ and Yoh were dissolved in normal saline (NS) *via* adding two drops of Tween 80. The AM251 was dissolved in DMSO 5.00%.

Study protocol. The current study was conducted based on the timeline and administration methods described below. Cannulation of the lateral ventricle of the brain as well as visceral pain and locomotor tests were performed on days 1, 10 and 14, respectively. On test days, PO administration of TQ and its vehicle (V) as well as ICV injections of Yoh and AM251 and their Vs were performed 45 and 5 min before the beginning of the test, respectively. At the end of the experiments (day 15), the brains of the animals were taken out to verify the location of the cannula. Between each test, the used apparatus was carefully cleaned and dried. All examiners were blinded to the study protocol.

Lateral ventricle cannulation. To deliver the test drugs into the brain structures, a guide cannula was implanted in the lateral ventricle of the brain.[20] Under anesthesia induced by IP injection of ketamine (100.00 mg kg^{-1}) and xylazine (10.00 mg kg^{-1}), after exposure of the bregma of the fixed animal in the stereotaxic apparatus, a 23.00-gauge, 13.00-mm stainless-steel guide cannula was placed in the lateral ventricle of the brain according to the following coordinates: 1.40 mm posterior to the bregma, 2.00 mm lateral to the midline and 4.00 mm below the top of the skull.[21] Observing the pulsating clear colorless cerebrospinal fluid flow from the tip of the guide cannula confirmed the correct placement of the cannula in the lateral ventricle of the brain.[20] After anchoring of guide cannula in skull bones, to obturate the cannula, a stylet was placed inside it. After IP injection of penicillin G potassium (50,000 IU kg^{-1}; Jaber-Ebne-Hayyan Pharmaceutical Co., Tehran, Iran), the animals were monitored until they fully regained consciousness.

Animal grouping. In the present study, 72 rats were divided into 12 groups of six as follows: Group 1 (V + NS group) received PO administration of TQ V before ICV injection of NS. Groups 2, 3 and 4 (TQ 0.625 + NS, TQ 2.50 + NS and TQ 10 + NS groups, respectively) received PO administration of TQ at doses of 0.625, 2.50 and 10.00 mg kg^{-1}, before ICV injection of NS, respectively. Group 5 (V, PO + DMSO, ICV group) received PO administration of TQ V before ICV injection of DMSO 5.00%. Group 6 (TQ 10.00, PO + DMSO, ICV group) was treated with 10.00 mg kg^{-1} TQ before ICV injection of DMSO 5.00%. Group 7 (V, PO + AM251 5.00, ICV group) received PO administration of TQ V before ICV injection of 5.00 µg *per* rat AM251. Group 8 (TQ 10.00, PO + AM251 5.00, ICV group) received PO administration of 10.00 mg kg^{-1} TQ before ICV injection of 5.00 µg *per* rat AM251. Group 9 (V, PO + V, ICV group) was treated with PO administration of TQ V before ICV injection of Yoh V. Group 10 (TQ 10.00, PO + V, ICV group) was treated with PO administration of 10.00 mg kg^{-1} TQ before ICV injection of Yoh V. Group 11 (V, PO + Yoh 5.00, ICV group) was treated with PO administration of TQ V before ICV injection of 5.00 µg *per* rat Yoh. Group 12 (TQ 10.00, PO + Yoh 5.00, ICV group) received PO

administration of 10.00 mg kg^{-1} TQ before ICV injection of 5.00 µg *per* rat Yoh. Because the base of Yoh and AM251 Vs was NS, to investigate possible differences caused by the ICV injection effects of NS plus two drops of Tween 80 (V of Yoh) and DMSO 5.00% (V of AM251) with NS, groups 1, 5 and 9 were planned in the animal grouping. Chemical compound doses used here are in accordance with previous studies,[20,22,23] and also our preliminary experiments.

The PO and ICV administrations. The TQ was dissolved in NS *via* adding two drops (100.00 µL) of Tween 80 and administered by gavage at a constant volume of 0.50 mL *per* rat. The ICV injections of AM251 V (DMSO 5.00%), AM251, Yoh V (NS + two drops of Tween 80) and Yoh in a volume of 2.00 µL *per* rat were performed with a 30-guage injection needle connected to a 10.00-µL Hamilton's syringe. Initially, AM251 and Yoh were dissolved at a dose of 5.00 mg in 2.00 mL of their respective V and then, 5.00 µg of each (in 2.00 µL) was injected into the lateral ventricle of the brain.

Visceral nociceptive test. Acetic acid model was used to induce visceral nociception. For this purpose, each animal was placed in a clear Plexiglas box (30.00 × 30.00× 30.00 cm) for a 30-min adaptation period. Following IP injection of 1.00 mL acetic acid (1.00%), latency time to the first abdominal wall contraction (writhing) appearance was recorded and the numbers of writhing were counted in 15-min intervals for a 60-min period. An abdominal constriction has been defined as a contraction wave of the abdominal wall followed by a stretching of the body from the rump to the hind legs.[24]

Locomotor test. On day 14, an electronic activity box (BorjSanat, Tehran, Iran) was used to assess the animal's locomotion in a Plexiglas box (40.00 × 40.00 × 40.00 cm). Following the administration protocol mentioned above, animals were carefully put in the center of the activity box and then, the number of beam breaks caused by animal movement was recorded in a 5-min session as a measure of locomotor activity.[24]

Statistical analysis. Data were statistically analyzed using GraphPad Prism (version 8.2; GraphPad Software Inc., San Diego, USA). Data obtained from 15-min intervals (time-points data) were analyzed using two-way repeated measures ANOVA followed by Bonferroni's *post hoc* test. Latency time to the first writhing appearance, the 60-min period writhing numbers and beam break numbers were analyzed by one-way ANOVA followed by Tukey's *post hoc* test. Data are presented as mean ± SEM. A p value smaller than 0.05 was considered for all results.

Results

Regarding Figures 1 - 3, the latency times to the first writhing appearance, number of writhing at 5-min intervals and total number of writhing at a 60-min period after IP injection of acetic acid showed no significant differences in groups 1, 5 and 4, respectively. The above-mentioned values did not significantly differ among groups 4, 6 and 10. These results showed that ICV injections of NS plus two drops of Tween 80 and DMSO 5.00% did not produce any significant effects compared to the NS. On the other hand, the reducing effects of TQ (10.00 mg kg^{-1}) on visceral nociception were not altered by central administration of Tween 80 and DMSO 5.00%.

Following PO administration of 2.50 and 10.00 mg kg^{-1} TQ, but not the dose of 0.625 mg kg^{-1}, acetic acid-induced decreases of latency time to the first writhing appearance were significantly increased (p < 0.01 and p < 0.001, respectively; Fig. 1A). The ICV injection of AM251 (5.00 µg *per* rat; Fig. 1B) and Yoh (5.00 µg *per* rat; Fig. 1C) alone produced no significant effects. However, the increased latency time to the first writhing appearance induced by 10.00 mg kg^{-1} TQ was reversed by ICV administrations of AM251 (Fig. 1B) and Yoh (Fig. 1C). By comparing the results of the 4th columns of Figures 1B and 1C, a significant difference was observed between the reversing effects of AM251 and Yoh.

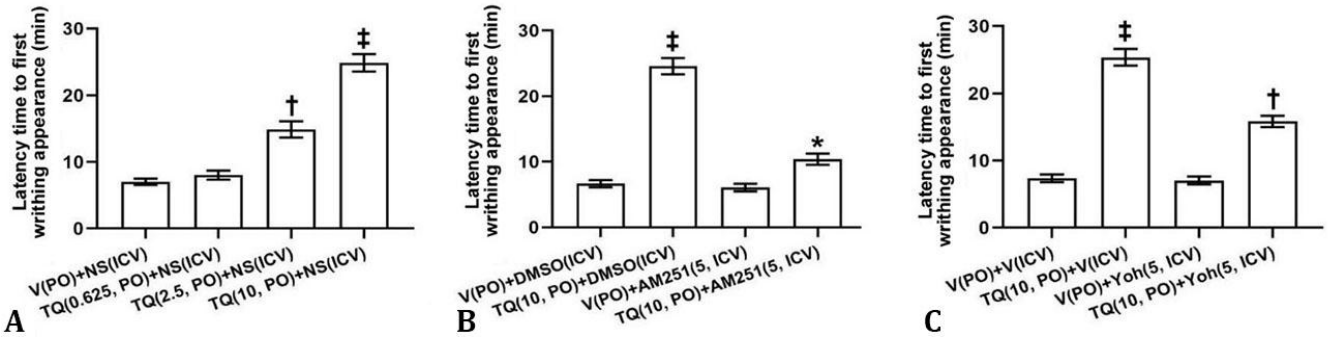

Fig. 1. Latency time to the first writhing appearance after oral administration (PO) of thymoquinone (TQ; **A**), intracerebroventricular (ICV) injection of N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251; **B**) and yohimbine (Yoh; **C**) alone and after TQ administration in acetic acid model of visceral nociception. The TQ was administered orally 45 min and AM251 and Yoh were injected ICV 5 min before intra-peritoneal injection of acetic acid. * p < 0.05, † p < 0.01 and ‡ p < 0.001 in comparison with V (PO) + NS (ICV). V: Vehicle; NS: Normal saline; DMSO: Dimethyl sulfoxide.

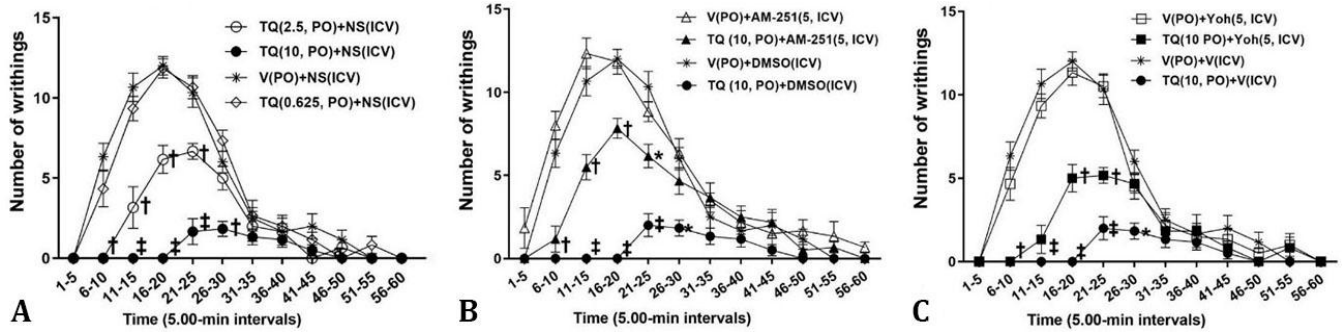

Fig. 2. The number of writhing at 5-min intervals after oral administration (PO) of thymoquinone (TQ; **A**), intracerebroventricular (ICV) injection of N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251; **B**) and yohimbine (Yoh; **C**) alone and after TQ administration in acetic acid model of visceral nociception. The TQ was administered orally 45 min and AM251 and Yoh were injected ICV 5 min before intra-peritoneal injection of acetic acid. * $p < 0.05$, † $p < 0.01$ and ‡ $p < 0.001$ in comparison with V (PO) + NS (ICV). V: Vehicle; NS: Normal saline; DMSO: Dimethyl sulfoxide.

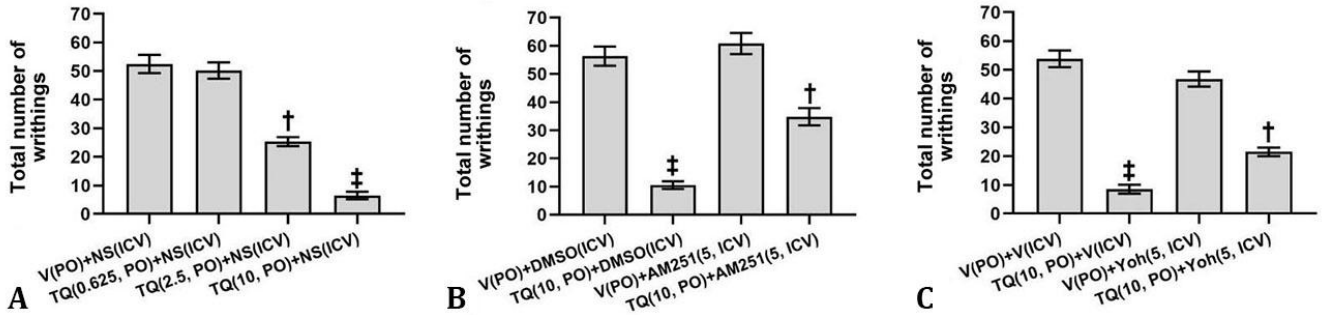

Fig. 3. Total number of writhings over a 60-min period after oral administration (PO) of thymoquinone (TQ; **A**), intracerebroventricular (ICV) injection of N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251; **B**) and yohimbine (Yoh; **C**) alone and after TQ administration in acetic acid model of visceral nociception. The TQ was administered orally 45 min and AM251 and Yoh were injected ICV 5 min before intra-peritoneal injection of acetic acid. † $p < 0.01$ and ‡ $p < 0.001$ in comparison with V (PO) + NS (ICV). V: Vehicle; NS: Normal saline; DMSO: Dimethyl sulfoxide.

Figure 2 shows the number of writhing in consecutive 5-min intervals. In (TQ 10, PO + NS, ICV), (TQ 10, PO + DMSO, ICV) and (TQ 10, PO + V, ICV) groups, the maximum numbers of writhing were determined at the 2nd - 6th 5-min intervals. The TQ at a dose of 0.625 mg kg^{-1} had no effect; whereas, at doses of 2.50 and 10.00 mg kg^{-1}, significantly reduced the 2nd - 5th and 2nd - 6th 5-min intervals of the number of writhing ($p < 0.01$ and $p < 0.001$, respectively; Fig. 2A). The ICV injection of AM251 (5.00 µg *per* rat; Fig. 2B) and Yoh (5.00 µg *per* rat; Fig. 2C) alone did not alter 5-min intervals of the number of writhing. The ICV injection of AM251 (5.00 µg *per* rat) after PO administration of TQ (10.00 mg kg^{-1}) reversed the decreased number of writhing at 3rd - 6th 5-min intervals (Fig. 2B). The decreased number of writhing at 4th - 6th 5-min intervals induced by PO administration of 10.00 mg kg^{-1} TQ was reversed by ICV injection of 5.00 µg *per* rat Yoh (Fig. 2C). Significant differences were observed between the reducing effects of AM251 and Yoh regarding 4th and 5th 5-min intervals.

The increased number of writhing at the 60-min period induced by acetic acid, significantly decreased by PO administration of 2.50 ($p < 0.01$) and 10.00 ($p < 0.001$) mg kg^{-1} TQ (Fig. 3A). The TQ at a dose of 0.625 mg kg^{-1} was without effect (Fig. 3A). The decreased 60-min period number of writhing induced by 10.00 mg kg^{-1} TQ did not change after alone ICV injection of AM251 (5.00 µg *per* rat; Fig. 3B) and Yoh (5.00 µg *per* rat; Fig. 3C). The TQ-induced reduction of the 60-min period number of writhing at a dose of 10.00 mg kg^{-1} was significantly reversed by ICV injection of AM251 (Fig. 3B) and Yoh (Fig. 3C). A significant difference was indicated between reversing effects of AM251 and Yoh (4th columns of Figs. 3B and 3C).

Figure 4 shows the number of beam breaks in the current study groups. The number of beam breaks in control group was 94.83 ± 3.11. This value did not change with none of the test chemical compounds mentioned above after PO and ICV administrations.

Discussion

The results of this study showed that PO administration of TQ suppressed acetic acid-induced visceral nociception through increasing the latency time to the first writhing appearance and decreasing the number of writhing. As far as we know, there is no report describing the effect of TQ itself on acetic acid-induced visceral pain in rats. However, oral administration of black cumin seed essential oil produced reducing effects in

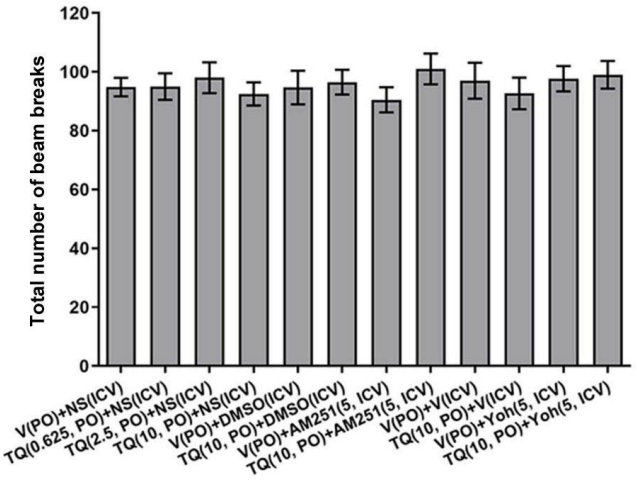

Fig. 4. Total number of beam breaks in an open-field test after oral administration (PO) of thymoquinone (TQ), intracerebroventricular (ICV) injection of N-(piperidin-1-yl)-5-(4-iodophenyl) -1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251) and yohimbine (Yoh) alone and after TQ administration in acetic acid model of visceral nociception. The TQ was administered orally 45 min and AM251 and Yoh were injected ICV 5 min before intra-peritoneal injection of acetic acid. There were no significant differences among treated groups. V: Vehicle; NS: Normal saline; DMSO: Dimethyl sulfoxide.

acetic acid-induced visceral nociceptive responses in mice.[25] In this regard, oral administration of *N. sativa* seed extract reduced the number of writhing induced by acetic acid in albino mice.[26] In other pain models such as chemotherapy (vincristine)- and chronic constriction injury-induced neuropathic pains, the use of TQ alleviated pain symptoms such as mechanical, thermal and cold allodynia *via* oxidative stress and apoptosis suppression in the spinal cord and blood.[27,28] It has been reported that mechanical pain caused by carrageenan injection into the Achilles tendon, to induce tendinopathy, is reduced by peri-tendon injection of TQ and hyaluronic acid-coated TQ liposomes.[29] The PO (2.50 - 10.00 mg kg^{-1}), IP (1.00 - 6.00 mg kg^{-1}) and ICV (1.00 - 4.00 µg *per* mouse) administrations of TQ attenuated the nociceptive response in not only the early phase but also the late phase of the formalin test through a naloxone-sensitive mechanism.[30] Along with the findings mentioned above, it could be raised that TQ, in addition to somatic pain, may also relieve the symptoms of visceral nociception.

In the present study, ICV injection of AM251 prevented visceral anti-nociceptive effect of TQ. In other words, CB_1 receptor of the brain cannabinoid system plays a role in the visceral pain reducing effect of TQ. The expression of the components of endogenous cannabinoid system in the pain pathways resulting from their exogenous manipulation has provided a research platform for the role of cannabinoid system in pain regulation at the peripheral, spinal and supra-spinal levels.[31] In knock-out mice lacking CB_1 receptors in cortical regions, nociceptive responses induced by IP injection of acetic acid were not altered, suggesting the involvement of CB_1 receptors in supra-spinal processing of visceral pain.[32] The CB_1 agonists including delta-9-tetrahydrocannabinol and cannabinol (CBN) produced anti-nociceptive effects in an acetic acid-induced visceral nociception and the anti-nociceptive effects of both compounds were blocked by CB_1 receptor antagonist, rimonabant, but not the CB_2 receptor antagonist, SR144528.[33] The cannabinoid system is used to evaluate the mechanism of action of other substances effective in pain processing. For example, selective antagonists of CB_1 and CB_2 cannabinoid receptors, AM251 and AM630, respectively reversed the anti-nociceptive effect induced by curcumin in the mouse model of intra-plantar injection of carrageenan.[34] In addition, enhancement of pain reduction in hot-plate and acetic acid-writhing tests has been reported after co-administration of CBN and flavonoid composition.[35] There are no reports showing the interaction between TQ and cannabinoid system in processing of pain; therefore, the results included here can be considered as the first report in this field.

The results showed that ICV injection of Yoh prevented the suppressive effect of TQ in acetic acid-induced nociceptive responses. The α_2-adrenergic receptors are involved in local, spinal and supra-spinal mechanisms of pain processing.[36] Visceral nociception induced by IP injection of acetic acid was reduced by clonidine, an α_2-adrenergic receptor agonist.[37] The α_2-adrenergic system, like other neurotransmitter systems, is widely used to investigate the mechanism of action of synthetic analgesics and medicinal plant extracts and their active ingredients.[38] Pre-treatment with Yoh prevented the anti-nociceptive activity of *Alafia barteri* in the acetic acid-induced writhing response.[39] Moreover, ICV injections of famotidine (a histamine H_2 receptor antagonist) and Yoh produced inhibitory effects on centrally-administered crocetin (an active substance of saffron)-induced hypoalgesia in the formalin-induced orofacial nociception.[40]

The results mentioned in this study stated that the inhibitory effects of AM251 and Yoh on the reduction of visceral pain responses caused by TQ were relative and also the inhibitory effect of AM251 was stronger than Yoh. These findings show that in addition to cannabinoid and α_2-adrenergic systems, TQ may also recruit other systems to produce a pain-reducing effect. For example, it has been reported that TQ uses both peripheral and central L-arginine/nitric oxide/cyclic guanosine monophosphate/ K_{ATP} channel signaling pathways to produce an analgesic effect in the formalin model of nociception.[41] Furthermore, ICV injection of naloxone (a general opioid receptors antagonist), naloxonazine (a mu-opioid receptor antagonist) and nor-binaltorphimine (a kappa-opioid receptor antagonist) but not naltrindole (a delta-opioid receptor antagonist) prevented *N. sativa* oil- and TQ-induced anti-nociception in the early phase of the formalin test.[30] In the

orofacial formalin pain test, the reduction of pain following ICV injection of crocetin was partially but equally inhibited by famotidine and Yoh, while naloxone could not.[40] Based on these findings, it can be suggested that natural products or synthetic analgesics use endogenous pain processing systems to different degrees to exert their effects.

Our results indicated that TQ, AM251 and Yoh alone or in combination treatments did not alter the number of beam breaks in an open-field test. This result confirms the findings of Parvardeh *et al*.[41] indicated that ICV injection of TQ did not alter squares crossing number in an open-field test. Also, ICV injections of Yoh and AM251 did not change locomotor activities.[40,42] Hypoactivity and hyperactivity may influence the analgesic effect of anti-nociceptive drugs; therefore, special care needs to be taken to ensure that the recorded anti-nociceptive effect is solely associated with a decrease in pain perception.[43]

Medicinal plants and their active substances are widely used in the treatment of several gastrointestinal disorders through motility disturbance, visceral hypersensitivity, mucosal and immune function, gut microbiota and central nervous system processing alteration.[44] In this regard, the beneficial effects of plants such as *Perilla frutescens, Camelia sinesis, C. longa* and *Scutellaria baicalensis Georgi* in visceral pain treatment have been attributed to their biologically active substances including luteolin, catechins, curcuminoids and baicalin, respectively.[45] *Nigella sativa* and its active ingredient TQ have also been found to have beneficial effects on gastrointestinal disorders.[46,47] At least in this study, TQ reduced visceral nociception by recruiting central CB_1 and α_2-adrenergic receptors, which can be included in the wide range of information related to the effects of TQ.

In conclusion, according to the results of the present study, PO administration of TQ produced anti-nociceptive effects in acetic acid-induced visceral pain without changing locomotor activity. This effect was partially antagonized by ICV injection of AM251 and Yoh, indicating the involvement of central CB_1 and α_2-adrenergic receptors in the function of TQ. In addition to the above-mentioned systems, TQ may also recruit other pain processing mechanisms. Investigating the role of central systems involved in the mechanism of action of natural products may be useful to optimize pain management.

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Conflict of interest

No financial or other conflicts of interest are declared by the authors.

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