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ORIGINAL RESEARCH

The Centrally-Mediated Mechanisms of Action of Ferulic Acid–Induced Antinociception

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ABSTRACT

This study aimed to investigate the central antinociceptive effects of ferulic acid, a common phenolic compound found in various medicinal plants used for pain relief, and the contribution of cholinergic, serotonergic, opiopidergic and noradrenergic modulation in ferulic acid–induced antinociception. The hot-plate (integrated supraspinal response) and tail-immersion (spinal reflex) tests were used to measure pain thresholds in mice. The involvement of noradrenergic, serotonergic, opioidergic, and cholinergic mechanisms in the antinociception induced by 80 mg/kg (po) ferulic acid were investigated by examining the effects of 1 mg/kg yohimbine as an α_2-adrenoceptor antagonist, 1 mg/kg ketanserin as a serotonin 5-$HT_{2A/2C}$ receptor antagonist, 5 mg/kg naloxone as a nonspecific opioid antagonist, 5 mg/kg atropine as a nonspecific muscarinic antagonist, and 1 mg/kg mecamylamine as a nonspecific nicotinic antagonist pretreatments in mice. Ferulic acid at the doses of 80 mg/kg produced antinociception in hot-plate test and tail-immersion test. Yohimbine and naloxone, but not ketanserin, atropine and mecamylamine, remarkably reversed the antinociceptive effect of ferulic acid in hot-plate test while yohimbine, naloxone, atropine and mecamylamine, but not ketanserin, remarkably reversed the antinociception in tail-immersion test. These results indicated that ferulic acid induces central antinociception through mechanisms involving an interaction with supraspinal/spinal noradrenergic, opioidergic, and spinal cholinergic systems, excluding serotonergic system. All these modulatory systems manage the analgesic effect of ferulic acid with perfect coordination. Therefore, it seems that ferulic acid can be used in pain management as a coadjuvant or monotherapeutic agent.

Keywords: Antinociception; cholinergic pathway; ferulic acid; noradrenergic pathway; opioidergic pathway.

1. Introduction

Pain is an unpleasant sensory and emotional experience that may be acutely or chronically related with various disturbances such as lesions, traumatic injury, tumors, inflammatory diseases, and diabetes (1-2). Although a number of analgesics are available, certain problems such as tolerability, tolerance, abstinence syndrome, insufficiency, possible drug interactions, and side-effects also exist. Hence, the development of analgesics with minimal side effects is still ongoing (3). In this respect, phenolic compounds have gained attention in pain management. *Trans*-ferulic acid [(*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid)] is a caffeic acid derivative and a phenolic compound commonly found in food items, such as artichokes, eggplants and maize bran (4). Ferulic acid exhibits antioxidant, antiallergic, hepatoprotective, anticarcinogenic, anti-inflammatory,

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antimicrobial, antiviral, vasodilatory and antithrombotic effects (5). In recent years, ferulic acid–induced analgesia has been demonstrated in some experimental studies. However, data on its mechanism of action are limited. It has been shown that sodium ferulate, sodium salt of ferulic acid, attenuates the thermal and mechanical hyperalgesia in chronic constriction injury model of neuropathic pain by decreasing the pain transmitted by primary afferent neurons mediated by P2X3 receptor (6). It has also been demonstrated that the treatment with ferulic acid improved the behavioral abnormalities and decreased the levels of noradrenalin, serotonin, dopamine, substance P, NF-κβ p65, and caspase-3 in the hippocampus and frontal cortex in reserpine-induced pain model in mice (7). Ferulic acid is beneficial in mitigating the painful states associated with vincristine-induced painful neuropathy through its anti-inflammatory actions with reduction in oxidative stress (8). However, the available studies are inadequate to demonstrate the underlying mechanisms involved in the antinociceptive effects of ferulic acid *in vivo*. Pain perception and control process is a complex network from the periphery to the central nervous system, spinally and supraspinally. The nociceptors detect noxious stimuli, travel through the spinal cord, and make synaptic connections with second-order neurons in the gray matter column of the dorsal horn. A part of second-order neurons have ascending axons and project to the brainstem or the thalamocortical system (9). The impulses originate from the brain stem nucleus, "descend" to the spinal level, and affect the signal transmission of pain at the dorsal horn (9-10). The perception and control of pain are mediated through these ascending and descending networks that include endogenous opioid-, monoamine-, and acetylcholine-mediated mechanisms (11). This study aimed to investigate the central antinociceptive effects of ferulic acid and the contribution of cholinergic, serotonergic, and noradrenergic modulation in ferulic acid–induced antinociception by using the hot-plate (integrated supraspinal response) and tail-immersion (spinal reflex) tests in mice.

2. Materials and Methods

2.1. Animals

Experimental studies were performed using 3-4 months old Swiss albino female mice. A total of 102 animals were housed in a well-ventilated room with 12-h light/dark cycles at 22 ± 1°C and allowed free access to food and water *ad libitum*. The animals habituated to the laboratory environment for at least 1 week prior to the experiments. Six hours before the experimental procedures, the animals received only water to avoid possible food interaction with ferulic acid. Animal care and research protocols were based on the principles and guidelines adopted by the Guide for the Care and Use of Laboratory Animals (NIH Publication no. 85-23, revised in 1985) and approved by the Local Ethics Committee of Anadolu University, Eskisehir, Turkey.

2.2. Drugs and treatments

The following drugs were used in this study: *trans*-ferulic acid, morphine sulfate, yohimbine, ketanserin, naloxone, atropine, and mecamylamine (Sigma, St. Louis, MO, USA). All drugs were dissolved in saline. All animals were randomly divided into groups with six in each. The control group received only solvent vehicle. Morphine (5 mg/kg), an opioid agonist, was used as a reference drug. Ferulic acid was administered orally (po) by a gavage needle (18 G × 3 inch × 2.25 mm) at the doses of 20, 40, and 80 mg/kg. The other drugs were injected intraperitoneally (ip). For investigating the mechanisms of action, the mice were pretreated with 5 mg/kg muscarinic receptor antagonist atropine 15 min before, 1 mg/kg nicotinic receptor antagonist mecamylamine 20 min before, 1 mg/kg serotonin 5-$HT_{2A/2C}$ receptor antagonist ketanserin 30 min before, 1 mg/kg α_2-adrenoceptor antagonist yohimbine 30 min before, and 5 mg/kg opioid antagonist naloxone 15 min before the administration of 80 mg/kg ferulic acid. The measurements of pain threshold were performed by using hot-plate and tail-immersion tests 45 min after ferulic acid administration. Doses and drug administration schedules were selected based on previous reports (12-14).

2.3. Analgesia test procedures

Analgesia tests were performed between 11:00 and 17:00 h. Intervals of 1–2 min were allowed between each test, and the test protocol sequence was as follows.

2.3.1. Hot-plate test

The pain reflexes in response to a thermal stimulus were measured using a Hot-Plate Analgesia Meter (No. 7280, Ugo Basile Instruments, Comerio, Italy) (15). The mice were gently put on the surface of the hot plate, set to 55 ± 0.5°C. The latency of hind paw licking, hind paw flicking, or jumping was measured as reaction time. The cutoff time was taken as 20 s to minimize hind paw damage.

2.3.2. Tail-immersion test

The painful reactions in the animals were induced by thermal stimuli through dipping the tail tips into a hot water bath (Heto, Allerod, Denmark) at 52.5 ± 0.2°C (16). An area of the

tail of mice was immersed in hot water. The withdrawal time of the tail from the hot water was noted as reaction time. The maximum cutoff time for immersion was 15 s to avoid the injury of tissues of the tail.

The results of the hot-plate and tail-immersion tests were expressed as a percentage of the maximal possible effect (MPE%), which was calculated via response latency against thermal stimulus (17):

MPE% = [(Postdrug latency) – (Predrug latency)] / [(Cutoff time) – (Predrug latency)] × 100

2.3.3. Data analyses

Statistical differences were analyzed non-parametrically by Kruskal–Wallis followed by the post-hoc Dunn test. The statistical analyses were carried out using GraphPad Prism version 5.0. The results were expressed as the mean ± standard error of the mean to show variation in groups. Differences were considered significant when $P \leq 0.05$.

3. Results

3.1. Central analgesic effect of ferulic acid

The antinociception induced by ferulic acid in the hot-plate and tail-immersion tests are shown in Figure 1A and 1B, respectively. It was found that 80 mg/kg (po) ferulic acid and 5 mg/kg (ip) morphine enhanced the response latency against thermal stimulus significantly ($P < 0.05$, $P < 0.001$, respectively) compared with the control group in the hot-plate test. Ferulic acid also succeeded in inducing antinociception at the dose 80 mg/kg as morphine ($P < 0.01$, $P < 0.001$, respectively) by enhancing pain thresholds in the tail-immersion test. The maximum possible effect of ferulic acid was not as high as morphine.

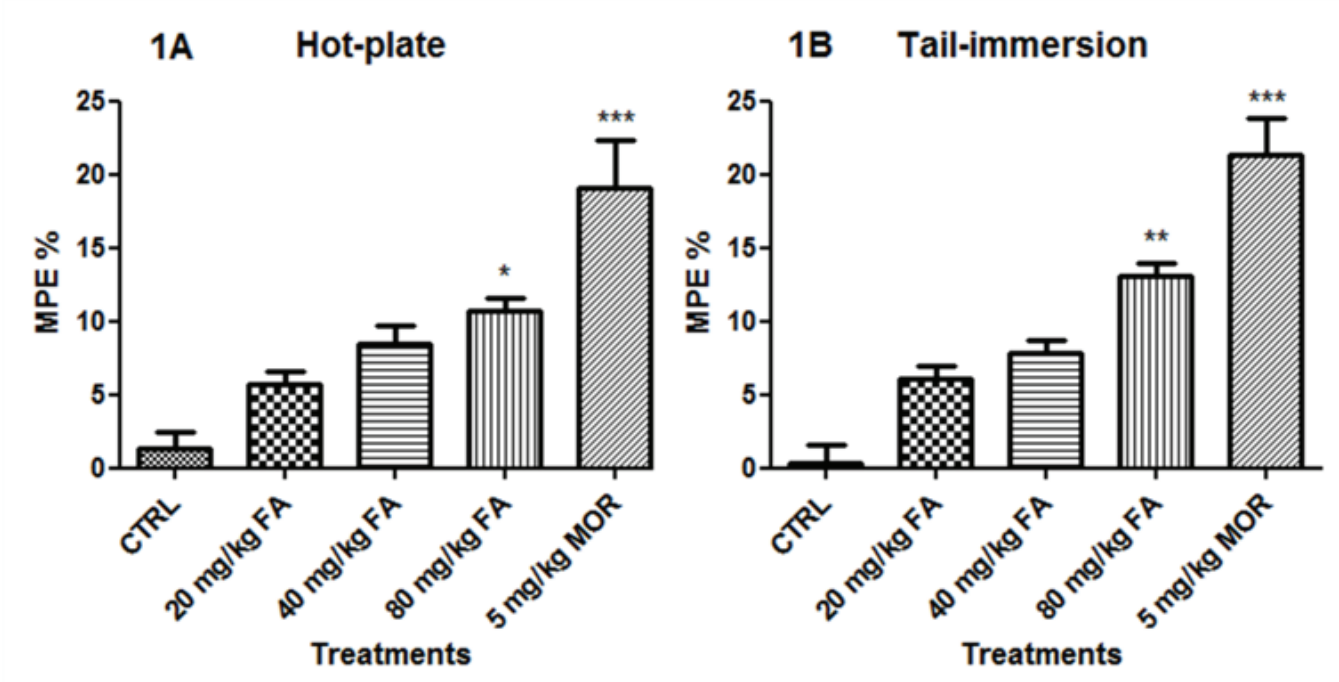

Figure 1. The antinociceptive effect of 20, 40, and 80 mg/kg (po) ferulic acid (FA) in the hot-plate (A) and tail-immersion (B) tests. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$: significant difference from control (CTRL). (n=6 per each group) The statistical analyses were performed using Kruskal–Wallis followed by the post-hoc Dunn test. MOR, Morphine. MPE%: The percentage of the maximal possible antinociceptive effect.

3.2. Mechanism of action studies

The pretreatment of mice, which were given 80 mg/kg ferulic acid, with 1 mg/kg yohimbine decreased the pain thresholds remarkably, but not statistically significant, in both the hot-plate and tail-immersion tests, as shown in Figure 2A and 2B, respectively. The pretreatment with yohimbine prevented the visible effect of ferulic acid. Figure 3 shows that the pretreatment with ketanserin did not significantly alter the pain thresholds in both the hot-plate (Fig. 3A) and tail-immersion (Fig. 3B) tests. On the contrary, naloxone

remarkably reversed the enhancement of pain response latency in the hot-plate and tail-immersion tests and, the pretreatment with naloxone prevented the visible effect of ferulic acid (Fig. 4A and 4B, respectively). Figure 5 shows the reversal effect of atropine on antinociception induced by 80 mg/kg ferulic acid. Atropine did not significantly reverse the MPE% of 80 mg/kg ferulic acid in the hot-plate and tail-immersion tests (Fig. 5A and Fig. 5B). The pretreatment with atropine prevented the visible effect of ferulic acid in tail immersion test although the ferulic acid was still effective in hot plate test ($P < 0.05$). Similar to atropine pretreatment results, Figure 6 shows that the pretreatment with mecamylamine did not change the MPE% of 80 mg/kg ferulic acid in the hot-plate test and ferulic acid remained effective ($P < 0.05$) (Fig. 6A). Conversely, the pretreatment with mecamylamine partially antagonized the effect of ferulic acid in the tail-immersion test (Fig. 6B) and the visible effect of ferulic acid was disappeared.

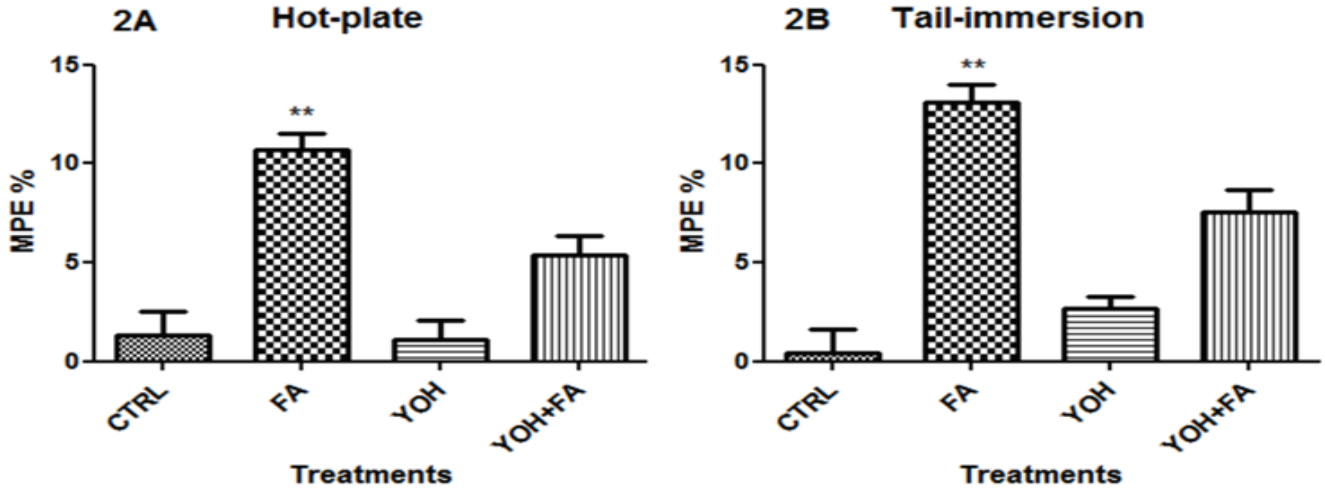

Figure 2. The reversal effect of 1 mg/kg yohimbine (YOH) (ip) on 80 mg/kg (po) ferulic acid (FA)-induced antinociception in the hot-plate (A) and tail-immersion (B) tests. $^{**}P < 0.01$: significant difference from control (CTRL). (n=6 per each group) The statistical analyses were performed using Kruskal–Wallis followed by the post-hoc Dunn test. MPE%: The percentage of the maximal possible antinociceptive effect.

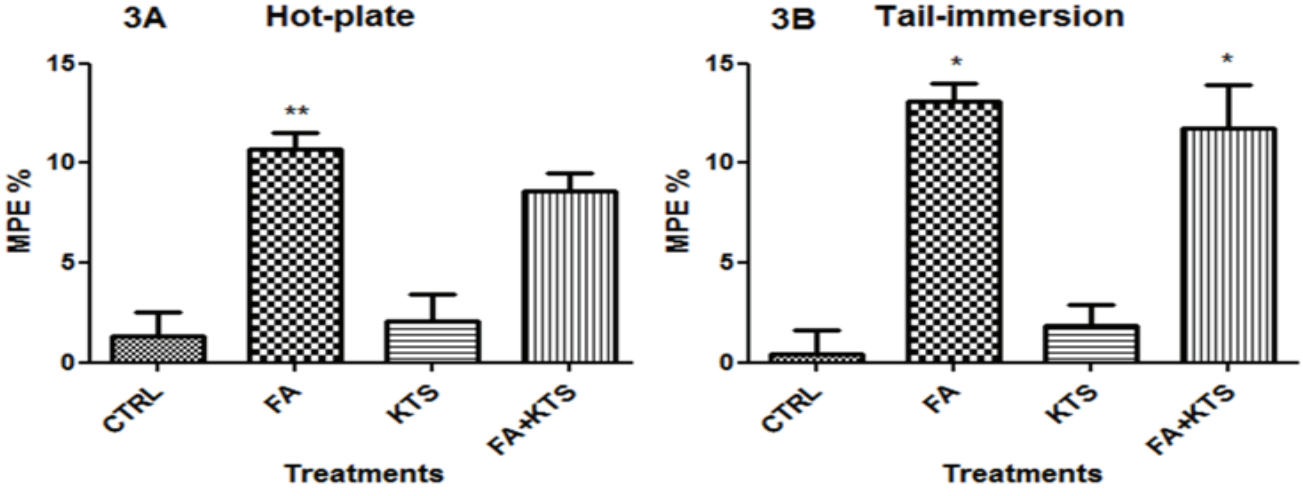

Figure 3. The reversal effect of 1 mg/kg ketanserin (KTS) (ip) on 80 mg/kg (po) ferulic acid (FA)-induced antinociception in the hot-plate (A) and tail-immersion (B) tests. $^{*}P < 0.05$, $^{**}P < 0.01$: significant difference from control (CTRL). (n=6 per each group) The statistical analyses were performed using Kruskal–Wallis followed by the post-hoc Dunn test. MPE%: The percentage of the maximal possible antinociceptive effect.

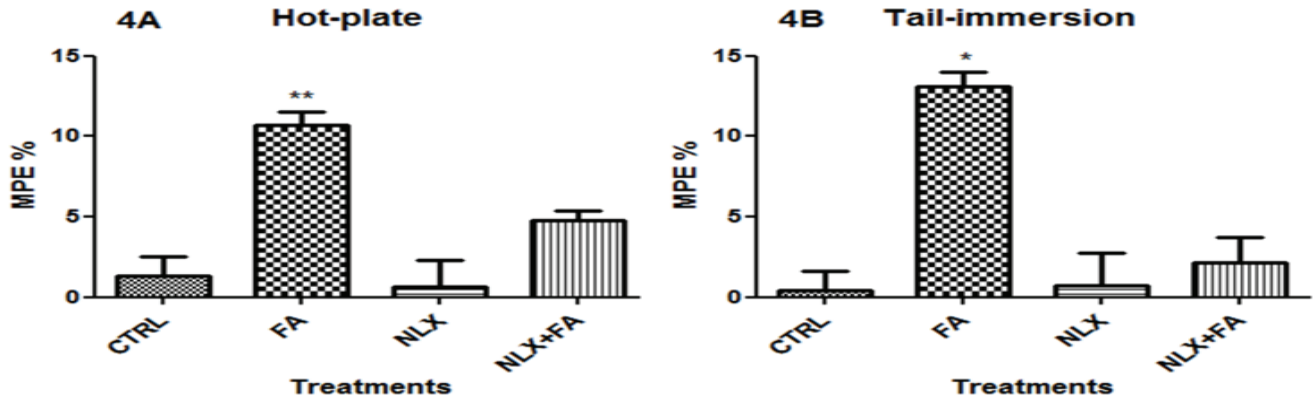

Figure 4. The reversal effect of 5 mg/kg naloxone (NLX) (ip) on 80 mg/kg (po) ferulic acid (FA)-induced antinociception in the hot-plate (A) and tail-immersion (B) tests. $^{*}P < 0.05$, $^{**}P < 0.01$: significant difference from control (CTRL). (n=6 per each group) The statistical analyses were performed using Kruskal–Wallis followed by the post-hoc Dunn test. MPE%: The percentage of the maximal possible antinociceptive effect.

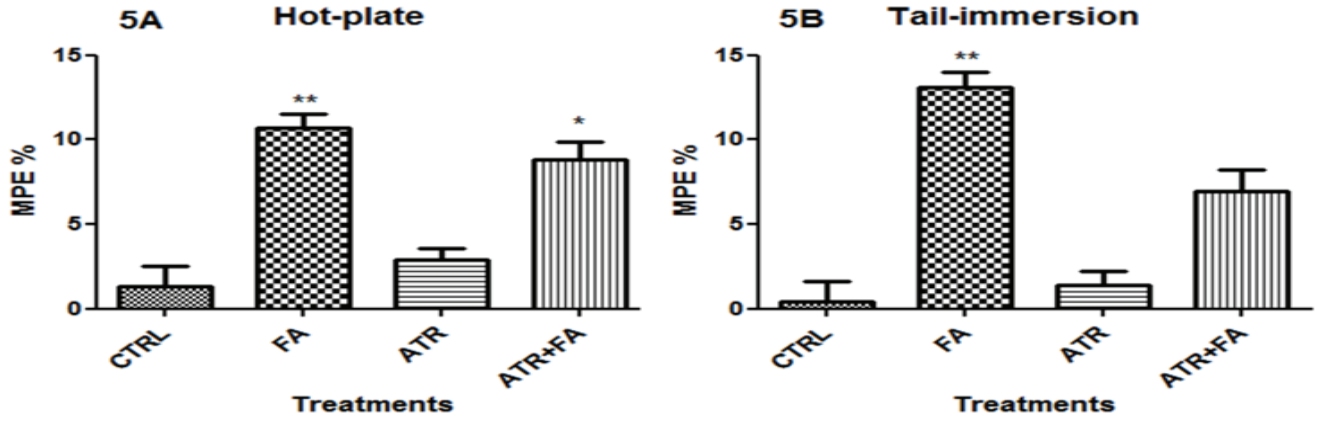

Figure 5. The reversal effect of 5 mg/k atropine (ATR) (ip) on 80 mg/kg (po) ferulic acid (FA)-induced antinociception in the hot-plate (A) and tail-immersion (B) tests. $^{*}P < 0.05$, $^{**}P < 0.01$: significant difference from control (CTRL). (n=6 per each group) The statistical analyses were performed using Kruskal–Wallis followed by the post-hoc Dunn test. MPE%: The percentage of the maximal possible antinociceptive effect.

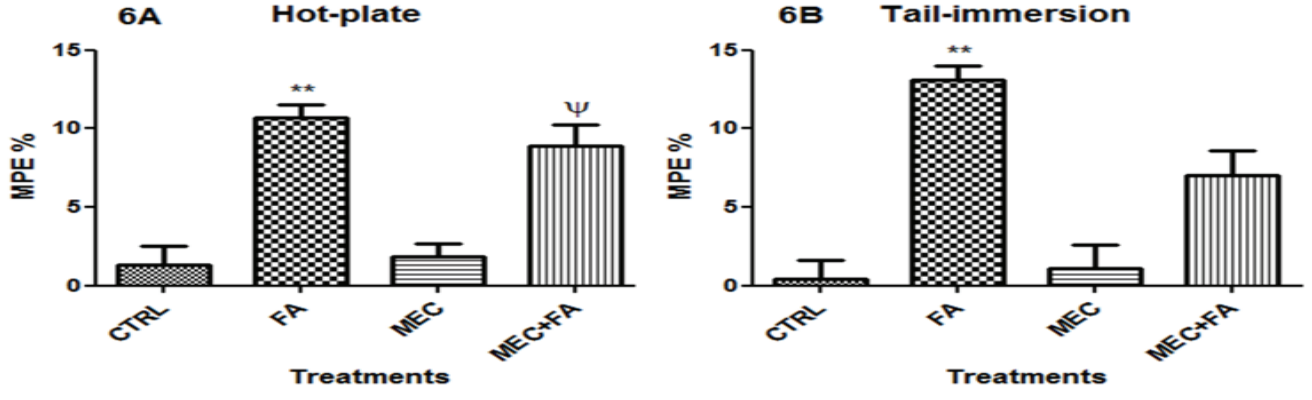

Figure 6. The reversal effect of 1mg/kg mecamylamine (MEC) (ip) on 80 mg/kg (po) ferulic acid (FA)-induced antinociception in the hot-plate (A) and tail-immersion (B) tests. $^{**}P < 0.01$: significant difference from control (CTRL). $^{\Psi}P < 0.05$: significant difference from 1 mg/kg MEC alone. (n=6 per each group) The statistical analyses were performed using Kruskal–Wallis followed by the post-hoc Dunn test. MPE%: The percentage of the maximal possible antinociceptive effect.

4. Discussion

This study demonstrated that orally administered ferulic acid elicited a significant antinociceptive action in mice in the hot-plate and tail-immersion tests. Its antinociceptive effect observed in the hot-plate test was mostly mediated by noradrenergic and opioidergic systems, while the effect in the tail-immersion test was mediated by noradrenergic, opioidergic, and also cholinergic systems.

The tail-immersion response is a simple spinal reflex; however, the hot-plate test is a behavioral model of nociception where more organized behaviors such as hindpaw licking and jumping are elicited, reactions that are controlled by supraspinal mechanisms (18). Since ferulic acid possessed antinociception in the hot-plate and tail-immersion tests, the possible roles of various spinally and supraspinally organized analgesic mechanisms on ferulic acid–induced central antinociception were evaluated in this study. Descending pain pathways are one of the most important pain control systems. The mechanism of descending inhibition and supraspinal/spinal signal integration includes the release of a number of neurotransmitters, particularly endogenous opioids, noradrenaline, serotonin, and acetylcholine, and their activity (19). Thus, the involvement of these modulatory systems in ferulic acid–induced analgesia was investigated.

In this study, yohimbine, α_2-adrenoceptor antagonist, was used, since both presynaptic and postsynaptic α_2-adrenoceptors mediate the antinociceptive effects of noradrenaline (20). It partially reversed the analgesic effect of ferulic acid in both the hot-plate and tail-immersion tests. Previously, Xu et al. (7) showed that 40 and 80 mg/kg ferulic acid increased the noradrenaline levels in the frontal cortex and hippocampus, and claimed that this effect is one of the mechanisms of action of ferulic acid. It is possible that ferulic acid–induced analgesia may be mediated by increased levels of noradrenaline and activation of the spinal/supraspinal α_2-adrenoceptors. However, the analgesic effect of ferulic acid was not completely reversed by yohimbine. Hence, alternative mechanisms of action need to be considered.

The other point of investigation was the connection of opioidergic modulation with ferulic acid-induced antinociception. Non-selective opioid antagonist naloxone was used for investigation, since almost all receptor subtypes, opioid Mu (μ), kappa (κ), and delta (δ), mediate the antinociceptive effect of opioids (21). The results showed that the opioidergic system, especially spinally organized, plays an important role in ferulic acid–induced analgesia, since the effect was remarkably antagonized by naloxone. Unlike other antagonists, the most powerful reversible effect was observed with naloxone pretreatment. Thus, it was thought that ferulic acid may possess analgesic effect mainly via interaction with the opioidergic system. The analgesia induced by selective μ-opioid receptor agonists is closely related with the descending noradrenergic system but not related with the serotonergic system (22-23). In this study, the serotonergic system was not involved in ferulic acid-induced analgesia as in selective μ-opioid receptor agonist-mediated analgesia. However, further detailed studies are needed to prove this speculation.

The roles of muscarinic and nicotinic cholinergic receptors in ferulic acid–induced analgesia were investigated by using atropine and mecamylamine, respectively, since cholinergic receptors are also involved in descending inhibition (24). Both antagonists relatively antagonized the ferulic acid–induced analgesia only in the tail-immersion test. Hence, it is thought that the cholinergic modulation of ferulic acid–induced analgesia is spinally mediated.

It is well known that the release of opioids, noradrenaline and acetylcholine, is linked as follows. The activation of opioidergic receptors causes noradrenaline release, and spinally released noradrenaline directly stimulates acetylcholine release in the spinal dorsal horn by acting on the α_2-adrenoceptors (20, 25-27). Based on these data and the results, it can be presumed that orally administered ferulic acid–induced antinociception may be induced by stimulating the release of norepinephrine, especially as μ-agonists do, in the spinal cord, which acts on the excitatory α_2-adrenoceptors on cholinergic neurons. However, exhaustive studies performed with specific antagonists and by biomolecular techniques are needed to support this speculation.

In conclusion, ferulic acid possesses a central analgesic effect via spinally/supraspinally mediated noradrenergic and opioidergic, and spinally mediated cholinergic modulation. All these modulatory systems manage the analgesic effect of ferulic acid with perfect coordination. Therefore, it seems that ferulic acid can be used in pain management as a coadjuvant or monotherapeutic agent.

Conflict of interest statement

The authors declare no conflicts of interest.

Acknowledgment

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Ferulik Asitin Santral Antinosiseptif Etkisine Aracılık Eden Etki Mekanizmaları

ÖZ

Bu çalışmada, ağrıyı hafifletmek için kullanılan çeşitli tıbbi bitkilerde bulunan bir fenolik bileşik olan ferulik asitin santral antinosiseptif etkisinin ve bu etkide kolinerjik, opioderjik, serotonerjik ve noradrenerjik yolakların rolünün araştırılması amaçlanmıştır. Farelerde ağrı eşiklerini ölçmek için **s**ıcak plaka (supraspinal aracılı etki) ve kuyruk daldırma (spinal refleks) testleri kullanılmıştır. 80 mg/kg (po) ferulik asidin antinosiseptif etki mekanizmasında noradrenerjik, serotonerjik, opioderjik ve kolinerjik mekanizmaların rolünü araştırmak için sırasıyla; 1 mg/kg α_2-adrenerjik reseptör antagonisti yohimbin, 1 mg/kg 5-$HT_{2A/2C}$ reseptör antagonisti ketanserin, 5 mg/kg nonspesifik opioid antagonisti nalokson, 5 mg/kg nonspesifik muskarinik reseptör antagonisti atropin ve 1 mg/kg nonspesifik nikotinik reseptör antagonisti mekamilamin kullanılmıştır. Sıcak plaka testinde ve kuyruk daldırma testinde 80 mg/kg ferulik asit antinosiseptif etki göstermiştir. Sıcak plaka testinde gözlenen etki belirgin şekilde sadece yohimbin ve nalokson ile geri çevrilirken, kuyruk daldırma testinde ise yohimbin, nalokson, atropin ve mekamilamin ön uygulaması ile geri dönmüştür. Ferulik asit supraspinal/spinal noradrenerjik, opioderjik ve spinal kolinerjik sistemin yer aldığı mekanizmalarla santral antinosiseptif etki göstermektedir. Serotonerjik sistemin ise etkiye katkı sağlamadığı görülmektedir. Özetle, ferulik asidin santral analjezik etkisini etkili bulunan tüm bu modülatör sistemler mükemmel bir uyumla organize etmektedir. Ferulik asidin ağrı tedavisinde tek başına ya da yardımcı ilaç olarak kullanılabileceği düşünülmektedir.

Anahtar kelimeler: Antinosisepsiyon; kolinerjik yolak; ferulik asid; noradrenerjik yolak; opioderjik yolak.

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