DOI: *http://dx.doi.org/10.18502/cjn.v24i1.???*

Current Journal of Neurology

Original Paper

Curr J Neurol 2025; 24(1): 30-40

Evaluation of the antinociceptive effects of syringic acid in male mice: Using formalin, writhing, and hot plate pain models

Received: 04 Sep.2024
Accepted: 02 Nov. 2024

Yaghoob Farbood[1], Alireza Sarkaki[1], Nima Bakhtiari[2], Samireh Ghafouri[1], Elnaz Harooni[1]

[1] Persian Gulf Physiology Research Center, Medical Basic Sciences Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[2] Pain Research Center, Imam Khomeini Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Keywords
Antioxidants; Mice; Pain; Polyphenols; Syringic Acid

Abstract

Background: Syringic acid (SA) is a natural phenolic compound with antioxidant and anti-inflammatory properties. Due to limited studies on the analgesic effect of SA, we decided to comprehensively investigate this effect. Thus, the analgesic activity of SA was assessed for the first time using the formalin and writhing models, in addition to the hot plate (HP) test, involving its action on opioid, GABAergic, nitric oxide (NO)/cGMP, and ATP-sensitive K^+ channel pathways. Furthermore, we examined exploratory and locomotor behaviors post SA administration.

Methods: A total of 231 mice were randomly assigned to groups of 7. SA was administered at doses of 25, 50, and 100 mg/kg. To investigate the possible pathways, naloxone, flumazenil, L-NAME/methylene blue, and glibenclamide were administered before SA injection. Behavioral tests were performed using the open-field (OF) apparatus. Statistical analysis was performed using one-way (or two-way) analysis of variance (ANOVA) with Tukey, least significant difference (LSD), and Bonferroni post hoc tests. All the results were evaluated under blind conditions.

Results: SA showed significant analgesic effects in the acute ($P < 0.050$) and chronic ($P < 0.001$) phases of formalin ($P < 0.050$) and writhing tests ($P < 0.001$) but not in the HP test. Furthermore, SA decreased exploratory behavior. Opioid receptor blockade reduced the number of writhes ($P < 0.050$). Moreover, using L-NAME increased the pain reaction time in the HP test ($P < 0.010$).

Conclusion: SA exhibited analgesic effects in the formalin and writhing models, but not in the HP test.

How to cite this article: Farbood Y, Sarkaki A, Bakhtiari N, Ghafouri S, Harooni E. Evaluation of the antinociceptive effects of syringic acid in male mice: Using formalin, writhing, and hot plate pain models. Curr J Neurol 2025; 24(1): 30-40.

Corresponding Author: Elnaz Harooni
Email: elnazharooni@gmail.com

Blocking opioid receptors in the writhing test reduced the analgesic effect of SA. Exploratory behavior increased when flumazenil, naloxone, and L-NAME were injected before SA administration.

Introduction

Pain is a distressing sensation commonly associated with various diseases. While acute pain is a natural sensory mechanism necessary to protect an individual from injury, persistent pain can significantly affect a person's life.[1] Inflammatory pain, a type of chronic pain, results from the release of inflammatory mediators from damaged tissue.[2] Both acute and chronic pain are significant health concerns, and current treatment methods, which primarily involve the use of opioid analgesics and non-steroidal anti-inflammatory drugs (NSAIDs), have restrictions related to the risk of abuse and safety.[3,4] The roles of antioxidants in moderate inflammation and pain have been widely investigated.[5] Polyphenols are beneficial antioxidants that have been extensively studied for their ability to prevent and treat several medical conditions. Phenolic acids are the most abundant secondary metabolites in plants and exhibit similar functional and structural features.[6,7] Multiple studies have examined the effects of phenolic compounds on inflammation and pain, and the role of natural products such as phenolic acids in preventing various diseases has been widely demonstrated.[8-10]

Syringic acid (SA) is a phenolic compound found in many plant tissues, including fruits.[11,12] It has been studied for its potential biomedical effects, such as antioxidant, anti-inflammatory, neuroprotective,[13,14] anti-cancer,[15] hepatoprotective,[16] and antidepressant[17] properties in experimental studies. Research has also suggested that SA treatment may increase nitric oxide (NO) availability, decrease lipid peroxides, and lower antioxidant levels in rat blood samples.[18] However, the analgesic effects of this antioxidant have not been comprehensively characterized. Only one study has investigated the effect of SA on pain in thermal models,[19] and its effect on inflammatory and chronic pain and its mechanism of action remain unknown. Given the limited number of available studies on the analgesic effects of SA, this study aimed to investigate whether SA, as a potential antioxidant, has analgesic effects in models of acute and inflammatory pain. Therefore, we comprehensively evaluated the analgesic effects of SA at 3 different doses using standard pain-induction models. In addition, we examined its effect on exploratory and locomotor behavior and investigated the role of opioids, GABAergic, NO/cGMP pathways, and ATP-sensitive K+ channels using pharmacological antagonists.

Materials and Methods

Animals: A total of 231 adult male mice weighing 25-35 g were used in this experiment, with 7 mice placed in each group based on previous similar experimental studies. The mice were assigned to different treatment groups in a completely randomized manner. The mice were housed under controlled laboratory conditions (22 ± 2 °C, 55% humidity) with a 12:12 hour light-dark cycle and had ad libitum access to food and tap water. One week before the beginning of the experiment, the mice were brought to the experimental environment and allowed to acclimatize to the environment to minimize stress. Behavioral experiments and subsequent data analyses were performed by different experimenters who were unaware of group assignments to minimize potential bias. All procedures were approved by the Local Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Iran, (ethics code: IR.AJUMS.ABHC.REC.1398.045). Behavioral tests were conducted during the same period each day under identical environmental conditions, and all animals were tested under the same conditions. In the end, the mice were euthanized through deep anesthesia with ketamine and xylazine.

Chemicals and drugs: Formalin and acetic acid were purchased from Merck (Darmstadt, Germany). SA, l-NAME, methylene blue, and glibenclamide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ketamine and xylazine were obtained from Alfasan Chemical Co. (Woerden, Netherlands) and morphine and aspirin from Darou Pakhsh (Pars Darou, Iran).

Experimental design: This study was conducted in two separate parts, including:

Experiment 1: This experiment included 21 mice divided into 3 subgroups to induce different pain models.

1- The negative control group received an intraperitoneal (i.p) dose of normal saline.

2- Positive control 1 received a dose of morphine (10 ml/kg, i.p.) as an opioid analgesic.

3- The treatment group received a dose of SA (25 mg/kg, i.p.).

4- The treatment group received a dose of SA

(50 mg/kg, i.p.).

5- The treatment group received a dose of SA (100 mg/kg, i.p.).

The SA doses were selected in consultation with a pharmacologist and based on previous studies to cover an appropriate range for assessing dose-dependent analgesic effects.[13]

6- Positive control 2: received a dose of aspirin (300 mg/kg, intraperitoneally) as an anti-inflammatory analgesic drug.

All drugs were administered 30 minutes before the induction of the pain model.

Experiment 2: Each group included 3 subgroups for different tests.

1- Negative control: received a normal saline dose as a vehicle.[20]

2- The treatment group received a single dose of SA (the effective dose for each test selected in experiment 1).

3- The treatment group received a dose of flumazenil ($GABA_A$ receptor inhibitor) 1 mg/kg, i.p. + SA.

4- The treatment group received a dose of naloxone (opioid μ receptor inhibitor) 5 ml/kg, i.p. + SA.

5- The treatment group received a dose of methylene blue (cGMP inhibitor) 10 mg/kg, i.p. + SA.

6- The treatment group received a dose of L-NAME (NO synthase inhibitor) 30 mg/kg, i.p. + SA.

7- The treatment group received a dose of glibenclamide (ATP-sensitive K+ ion channel blocker) 10 mg/kg orally + SA.

In experiment 2, the action mechanisms were investigated by pretreating the mice with flumazenil, naloxone, and L-NAME for 30 minutes and glibenclamide and methylene blue for 15 minutes before the injection of SA (Figure 1).

Formalin-induced pain: Briefly, 20 μl of 2.5% formalin solution was administered to the dorsal area of the left hind paw of the mice. The total duration of biting, licking, or elevation of the injected paw was recorded for each animal immediately following the injection. Formalin induces biphasic pain; the initial (neurogenic) stage occurs within the first 5 minutes immediately after injection, and the second (inflammatory) stage occurs 15–30 minutes later. The mice were pretreated with SA, morphine, aspirin, or saline 30 minutes before formalin injection.[21]

Acetic-acid-induced abdominal pain

Writhing test: To induce abdominal pain, a 0.6% (v/v) acetic acid solution was intraperitoneally administered to each mouse. The mice were placed in an apparatus, and the cumulative writhes (contractions of the body, trunk, and hind limb extension) were counted over 30 minutes.[22] The mice were treated with SA, morphine, aspirin, or vehicle 30 minutes before acetic acid injection.

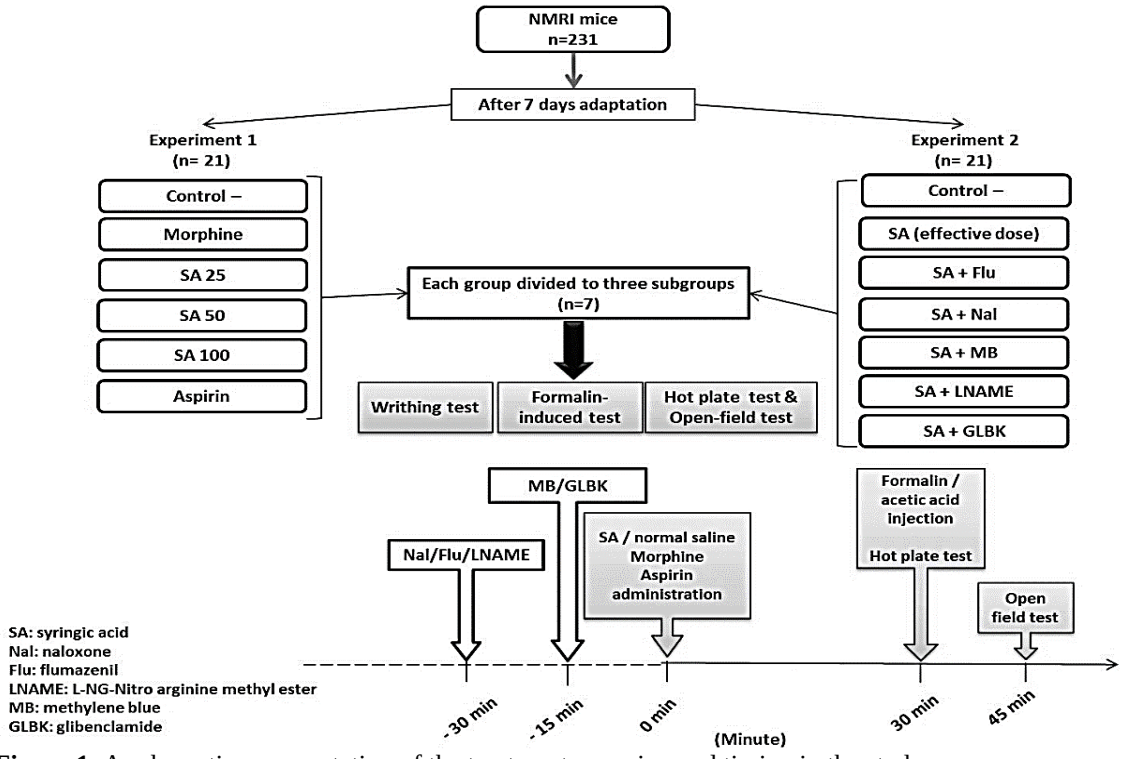

Figure 1. A schematic representation of the treatment grouping and timing in the study

Hot plate (HP) latency test: We used a HP to assess acute thermal pain, according to previous studies.[23] Each animal was placed on a 55 ± 0.5 °C metal surface, and the latency to the first explicit reaction, such as licking, shaking the hind paw, or jumping, was recorded. We considered a maximum cutoff time (= 30) to prevent tissue damage. This test was performed at baseline and 5, 10, 15, 30, and 60 minutes after SA, vehicle, morphine, and aspirin administration. To evaluate the antinociceptive response in the HP test, the percentage of maximal possible effect (%MPE) was calculated, which is a standard approach commonly used in similar studies.

$$\text{MPE} = \frac{(\text{Post drug latency} - \text{predrug latency})}{(\text{Cutoff period} - \text{predrug latency})}\%$$

Open-field (OF) test: Immediately after the HP test, each mouse was transferred to the OF apparatus. This test was performed as previously described.[8] Each animal was placed in a box (40 cm × 40 cm) that was evenly divided into 9 squares. After habituation to the apparatus for 5 minutes, the number of line crossings with all 4 paws (ambulation or crossing) and the number of rearing were recorded within 5 minutes by a blind observer of the treatment. After each exposure, the box was cleaned with 75% ethanol. This procedure was repeated using naloxone (3 mg/kg, i.p.), flumazenil (1 mg/kg, i.p.), and L-NAME (30 mg/kg, i.p.) for 30 minutes, and methylene blue (10 mg/kg, i.p.) and glibenclamide (10 mg/kg, i.p.) for 15 minutes prior to SA administration.

Data were checked for normality using the Shapiro–Wilk test. Given the normal distribution of the data and homogeneity of variances, parametric tests, including one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, were used to compare the groups. All statistical analyses were performed using GraphPad Prism (version 8.4.3; GraphPad Software Inc., San Diego, CA, USA) and IBM SPSS (version 26, IBM Corp., Armonk, NY, USA). One-way ANOVA was conducted to compare group means, and post hoc tests, including Tukey's test (Prism) and the least significant difference (LSD) test (SPSS), were used where appropriate. Two-way ANOVA was used for the HP test, along with the Bonferroni post-hoc test. All data are expressed as mean ± SEM, and a significant difference was considered to be at $P < 0.05$. In addition to p-values, 95% confidence intervals (CI) for the differences between the group means were calculated. Effect size (ES) was estimated using Cohen's d to assess the magnitude of group differences.

Results

Antinociceptive response in the formalin test: The results of the acute phase of the formalin test (Figure 2A) showed that 100 mg/kg SA displayed a significant antinociceptive effect compared to the negative control group [$P < 0.050$ LSD post hoc test, ES = 1.48, 95% confidence interval (CI): -16.16-133.3]. Although the 95% CI was –16.16-133.3, the ES was large, indicating a significant difference. As shown in figure 2B, SA resulted in a significant reduction in pain reaction time within the chronic phase of the test at doses of 50 and 100 mg/kg compared to the negative control ($P < 0.001$, ES = 3.29, 95% CI: 42.71-152.1 and ES = 3.40, 95% CI: 53.85-163.3).

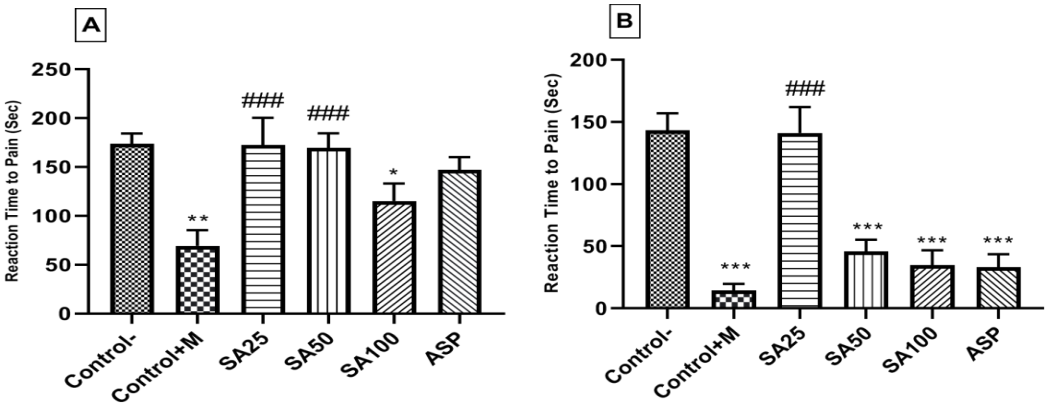

Figure 2. Effect of SA on formalin response in acute (A) and chronic (B) phases
The results are presented as mean ± SEM [n = 7]. Statistical significance was calculated by ANOVA followed by Tukey's or LSD post hoc tests
$^{*}P < 0.05$ and $^{***}P < 0.001$ compared with Control- group; $^{###}P < 0.001$ compared with Control + M group; Control-: Vehicle (normal saline)
Control + M: Control + Morphine; SA: Syringic acid; ASP: Aspirin

As expected, morphine had a significant analgesic effect in this test during both the acute (P < 0.010, ES = 2.91, 95% CI: 29.70 to 179.2) and chronic phases (P < 0.001, ES = 4.34, 95% CI: 74.05-183.5), while aspirin treatment was only effective during the chronic phase compared to the negative control (P < 0.001, ES = 3.46, 95% CI: 55.57-165).

Antinociceptive response in the writhing test: The results of the writhing test, shown in figure 3, indicate that both 25 and 100 mg/kg doses of SA significantly reduced the writhing count compared to the negative control mice (P < 0.010, ES = 2.05, 95% CI: 4.741-43.13; P < 0.050, ES = 1.52, 95% CI: 0.28-38.68, respectively). Moreover, morphine (P < 0.001, ES = 2.87, 95% CI: 14.50-52.89) and aspirin (P < 0.010, ES = 2.50, 95% CI: 9.57-47.96) caused a significant decrease in writhe count compared to the negative control.

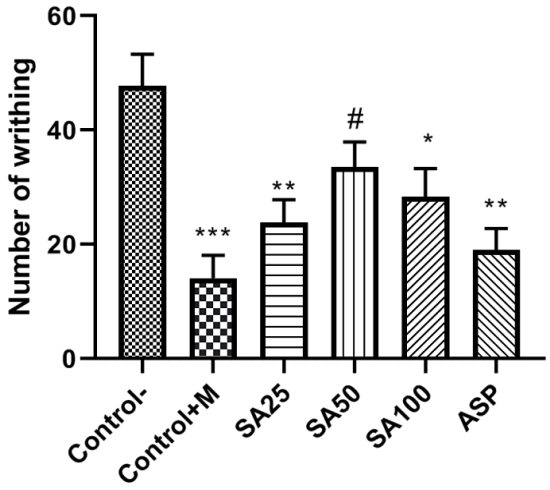

Figure 3. Effect of administration of SA on writhing behavior induced by intraperitoneal administration of 0.1 ml/kg of acetic acid [0.6%] in mice [n = 7]

Data are presented as mean ± SEM. Statistical significance was calculated by ANOVA followed by Tukey's or LSD post hoc tests

$^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with Control- group; $^{\#}P < 0.05$ compared with Control + M group; Control-: Vehicle (normal saline)

Control + M: Control + Morphine; SA: Syringic acid; ASP: Aspirin

Antinociceptive response in the HP test: Figure 4 shows the effect of SA on the HP test. SA did not extend the reaction time to pain at 5-60 minutes post-injection compared to the negative control group. Morphine, as a reference drug, significantly increased the latency time at 15 minutes (P < 0.050 LSD pot hoc, ES = 0.71, 95% CI: -1.74-16.6), 30 minutes (P < 0.010, ES = 1.77, 95% CI: 4.98-23.3), and 60 minutes (P < 0.010, ES = 1.90, 95% CI: 4.98-23.3) after SA injection.

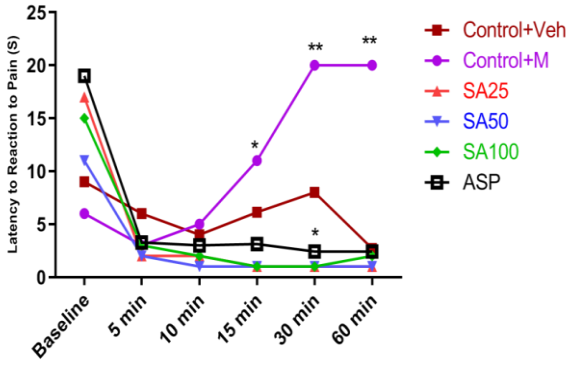

Figure 4. Effect of SA [25, 50, and 100 mg/kg] on the percentage of maximum possible effect (%MPE)/ latency time in the HP test

The results are presented as mean ± SEM [n = 7]

$^{*}P < 0.05$, $^{**}P < 0.01$ compared with control- group; Control-: Vehicle (normal saline)

Control + M: Control + Morphine; SA: Syringic acid; ASP: Aspirin

The effect of SA on motor activity in OF test: The effect of SA on the motor performance of the animals was evaluated 40 minutes post-administration. Locomotion was evaluated by counting ambulation (crossing lines with 4 paws) and the number of rearing that indicated exploratory behavior (the mice stood on their hind legs, raised their forelimbs, and extended their heads). There was no significant change in the number of line crossings in the SA treatment group compared to the negative control group (Figure 5A). SA 25 mg/kg had no significant effect, whereas 50 mg/kg (P < 0.050, ES = 2.02, 95% CI: 2.40-29.9) and 100 mg/kg doses of SA (P < 0.010, ES = 3.06, 95% CI: 6.40-29.9) significantly decreased rearing in mice compared with that in the negative control group (Figure 5B).

The possible mechanisms of the analgesic effect of SA in the formalin test: Compared with the SA 100 group, none of the studied pathways caused a difference in the time of pain response to formalin injection in either the initial or inflammatory phase of the formalin test (Figure 6A, B).

The possible mechanisms of the analgesic effect of SA in writhing test: As shown in figure 7, inhibition of opioid receptors with naloxone significantly increased the number of writhes compared to the SA 25 group [P < 0.050, LSD post hoc test, ES = 1.14, 95% CI: -30.15- (-3.86)]. However, the other pathways did not have a significant effect on this test.

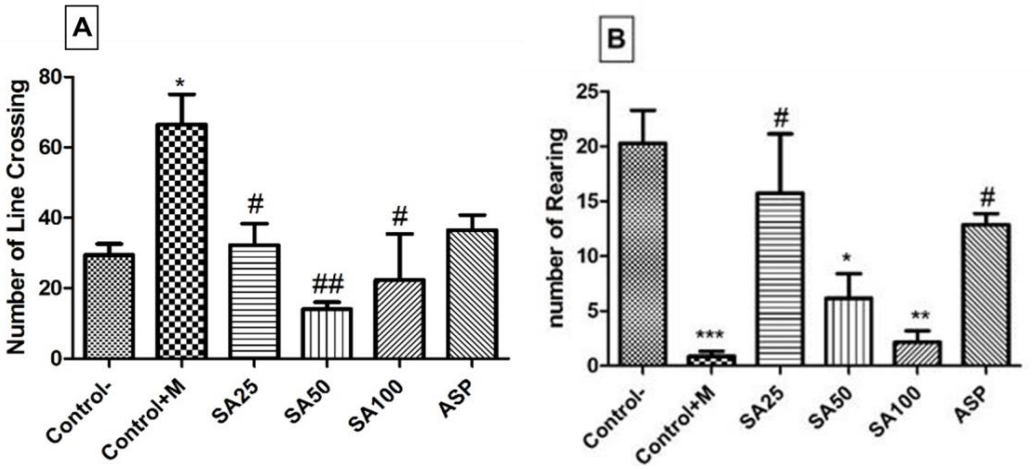

Figure 5. Effect of syringic acid (SA) in the open-field (OF) test, number of line crossing [A] and number of rearing [B]

The results are presented as mean ± SEM [n = 7]. Statistical significance was calculated by ANOVA followed by Tukey or LSD post hoc tests

$^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with Control- group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared with Control + M; Control-: Vehicle (normal saline)

Control + M: Control + Morphine; SA: Syringic acid; ASP: Aspirin

The possible mechanisms of the analgesic effect of SA in HP test: Figure 8 shows the effects of intraperitoneal administration of the selected inhibitors on SA thermal pain latency time. In the SA100 + LNAME group, the pain latency time increased at 15 and 30 minutes after SA injection compared to that in the SA 100 group (ES = 1.95, 95% CI: 1.239-16.19 and ES = 2.83, 95% CI: 0.9529-15.90, respectively; $P < 0.050$). No significant differences were observed between the other groups.

The possible mechanisms of SA in OF test: As shown in figure 9, the administration of flumazenil [ES = 1.24, 95% CI: -19.3-(-1.85)], naloxone [ES = 0.94, 95% CI: -20.3-(-2.85)], and L-NAME (ES = 0.92, 95% CI: -19.9-(2.42)] before SA injection significantly elevated the number of rearing behaviors in the animals compared to the SA 100 treatment ($P < 0.05$). However, no significant differences were observed in the number of line crossings (Figure 9).

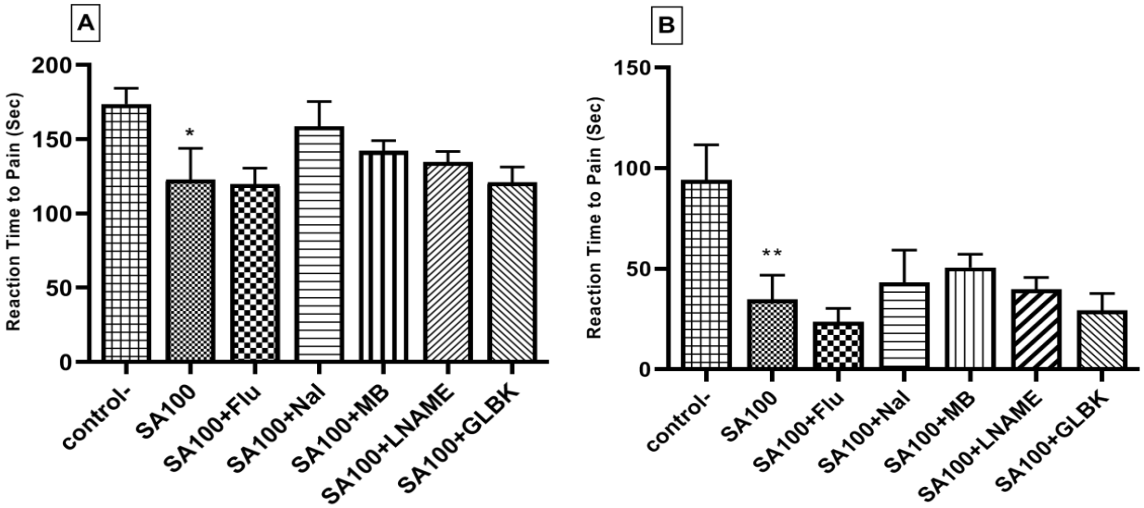

Figure 6. Involvement of opioidergic, GABAergic, and NO/cGMP systems, and ATP-sensitive K+ channels in the formalin test in acute (A) and chronic (B) phases

The results are presented as mean ± SEM [n = 7]. Statistical significance was calculated by ANOVA followed by Tukey or LSD post hoc tests

$^{*}P < 0.5$ and $^{**}P < 0.01$ compared with Control- group; Control-: Vehicle (normal saline)

SA: Syringic acid; Flu: Flumazenil; Nal: Naloxone; MB: Methylene Blue; GLBK: Glibenclamide

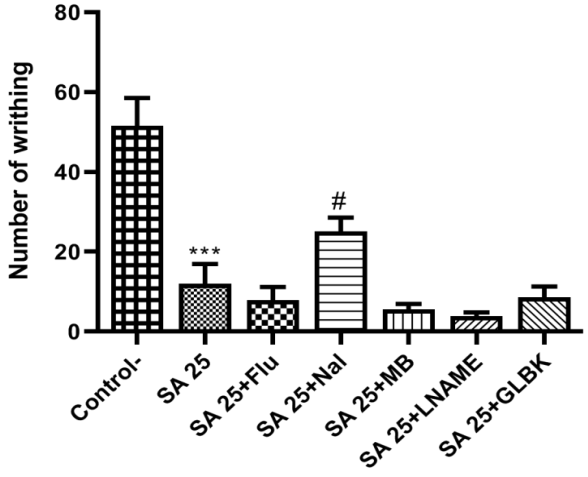

Figure 7. Involvement of opioidergic, GABAergic, and NO/cGMP systems, and ATP-sensitive K+ channels in writhing abdominal test

The results are presented as mean ± SEM [n = 7]. Statistical significance was calculated by ANOVA followed by Tukey's or LSD post hoc tests

***P < 0.05 compared with Control- group; Control-: Vehicle (normal saline)

SA: Syringic acid; Flu: Flumazenil; Nal: Naloxone; MB: Methylene Blue; GLBK: Glibenclamide

Discussion

This study investigated the effects of SA pretreatment on 3 pain models. The findings showed that SA's antinociceptive effects were partly dose-dependent and varied across models. SA reduced nociceptive responses in formalin- and acetic acid-induced pain tests, which are models of neurogenic and inflammatory pain. In the chronic formalin phase, 50 and 100 mg/kg doses were effective, whereas 25 mg/kg was not. In the acute phase, only 100 mg/kg showed significant activity.

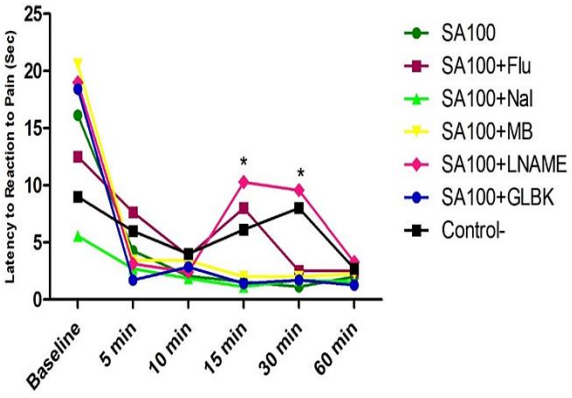

Figure 8. Involvement of opioidergic, GABAergic, and NO/cGMP systems, and ATP-sensitive K+ channels in the percentage of maximum possible effect (%MPE)/ latency time in the HP test

The results are presented as mean ± SEM [n = 7]

**P < 0.01 compared with Control- group; Control-: Vehicle (normal saline)

SA: Syringic acid; Flu: Flumazenil; Nal: Naloxone; MB: Methylene Blue; GLBK: Glibenclamide

In the writhing test, 25 and 100 mg/kg doses were effective. However, no tested dose produced considerable analgesia in the HP test, suggesting limited efficacy in central thermal pain pathways. These results indicate that SA's antinociceptive action is more prominent in peripheral inflammatory models at higher doses. Additionally, SA reduced exploratory behavior during the OF test, but there were no changes in locomotion.

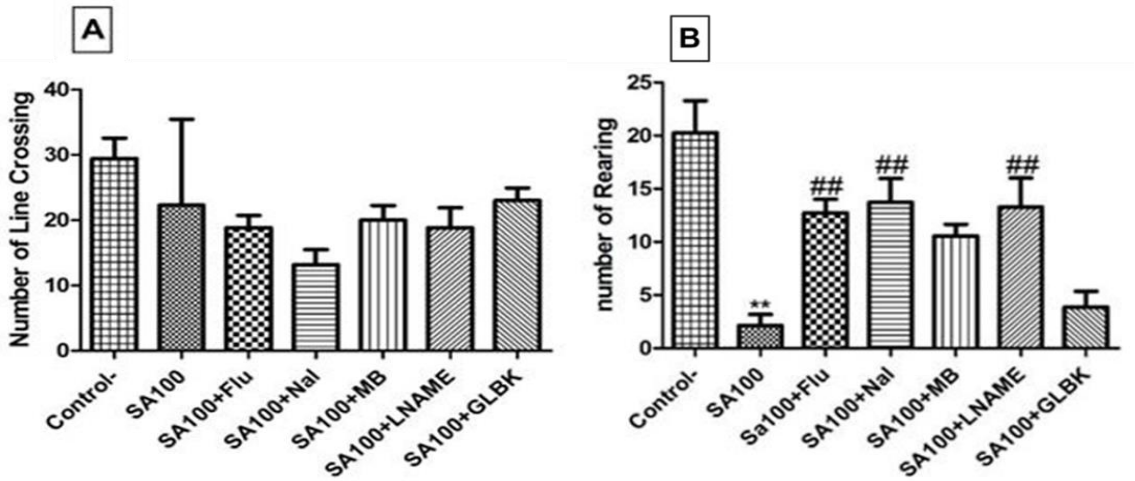

Figure 9. Number of line crossing (A) and number of rearing (B) in the open-field (OF) test

The results are presented as mean ± SEM [n = 7]. Statistical significance was calculated by ANOVA followed by Tukey or LSD post hoc tests

**P < 0.01 compared with Control- group; ##P < 0.01 compared with SA 100 group; Control-: Vehicle (normal saline)

SA: Syringic acid; Flu: Flumazenil; Nal: Naloxone; MB: Methylene Blue; GLBK: Glibenclamide

The effects of SA were also evaluated in comparison to standard analgesics, such as aspirin and morphine. However, there are limitations to the use of these 2 drug groups. Long-term use of NSAIDs can result in peptic ulceration and cardiovascular effects, making it challenging.[4] Moreover, the use of opioids, such as morphine, is limited owing to the risk of dependence, which can lead to abuse and addiction.[3]

Herbal medicines have recently gained popularity due to having fewer adverse effects than synthetic drugs. Polyphenols, including SA, often have antinociceptive and anti-inflammatory properties, primarily through the inhibition of lipoxygenase, cyclooxygenase, and NF-κB signaling pathways.[24,25] This study examined the potential effects of SA, a natural polyphenol antioxidant, on pain modulation in mouse models.

We used the formalin test to assess both acute and chronic pain,[26] as it allows a distinction between the central and peripheral mechanisms of analgesia.[27] In our study, 100 mg/kg SA significantly reduced nociceptive behaviors during the acute (neurogenic) phase, whereas both 50 and 100 mg/kg doses attenuated the chronic (inflammatory) phase. The inhibition observed during the chronic phase was comparable to that observed with aspirin, indicating a peripheral anti-inflammatory effect. Consequently, a dose of 100 mg/kg was determined to be optimal for subsequent experiments.

The writhing test is a widely used model for examining visceral pain induced by chemical stimuli. In this method, intraperitoneal injection of acetic acid triggers the activation of peripheral nociceptors and promotes the release of inflammatory mediators.[28] These mediators subsequently sensitize nociceptive neurons, making this model suitable for evaluating the effects of anti-inflammatory and analgesic agents, such as NSAIDs and opioids.[29] In our study, SA significantly reduced the number of abdominal writhes following acetic acid administration, indicating an apparent antinociceptive effect in this inflammatory pain model.[30] This supports the potential of SA to modulate peripheral pain pathways associated with chemical and inflammatory stimuli.

The HP test (thermal threshold test) was used to evaluate the central analgesic activity of SA. In this model, behavioral responses such as hind paw licking and jumping are commonly interpreted as indicators of spinal and supraspinal pain processing.[31] Our findings showed that SA did not significantly alter reaction latency time compared to the control group, suggesting limited or no effect on thermal nociception. This finding is inconsistent with that of Okur and Şakul, who reported the analgesic effects of SA at 50 and 100 mg/kg doses in the HP model.[19] The observed discrepancy may be due to variations in experimental conditions, such as differences in mouse strains, treatment protocols, or timing of assessments between the two studies.[19]

In the present study, SA demonstrated a significant analgesic effect during the acute phase of the formalin test; however, this effect was not observed in the HP test. This discrepancy can be attributed to the distinct mechanisms underlying these 2 pain models. While the formalin test mainly reflects the activity of peripheral nociceptors in response to chemical stimulation, the HP test is more reliant on the central processing pathways activated by thermal pain. These observations indicate that the antinociceptive effect of SA in our study was primarily mediated by peripheral mechanisms. Its limited efficacy in the HP model may suggest a reduced impact on central thermal nociception or necessitate alternative dosing strategies or timing to reveal such effects.

The effects of SA were examined for the first time using formalin and writhing tests, revealing greater efficacy in mitigating chronic or inflammatory pain than neurogenic pain. This may be attributed to SA's anti-inflammatory and antioxidant properties, which modulate various genes and signaling pathways involved in inflammatory responses.[32] These effects likely occur through suppression of pro-inflammatory cytokines such as interleukin-13 (IL-13), IL-4, IL-5, and tumor necrosis factor (TNF-α).[13] As inflammation is a pivotal factor in the onset and persistence of pain,[33] the ability of SA to downregulate these mediators highlights its analgesic potential. Our findings align with those of previous studies that demonstrated the antinociceptive properties of polyphenolic compounds.[9,25]

We investigated the possible impact of SA on motor activity in mice using OFT. Our findings showed a dose-dependent reduction in rearing behavior at higher doses of SA. Since SA did not significantly affect ambulation, this selective reduction suggests that SA may reduce exploratory behavior without inducing general sedation. Interestingly, Dalmagro et al. showed

that injection of a lower dose of SA (1 mg/kg) for 1 week had no impact on locomotion, suggesting dose- and duration-dependent effects.[34] In our study, 25 mg/kg had no significant effect on movement compared to the control group. For comparison, morphine reduced rearing behavior similarly to SA but increased locomotor activity, which is consistent with the findings of Zhang and Kong, who reported that morphine enhanced movement in the OFT.[35]

To better understand the potential mechanisms of the analgesic effects of SA, several pharmacological inhibitors targeting specific pathways were administered prior to SA treatment. Inhibition of $GABA_A$ receptors by flumazenil 30 minutes before SA injection did not interfere with the analgesic effects of SA, suggesting that GABAergic mechanisms may not be directly involved in SA-induced analgesia. Behavioral studies suggest that low concentrations of $GABA_A$ receptor agonists reduce formalin-induced pain behaviors, whereas high concentrations increase pain.[36] In addition, based on previous literature and findings, we know that these types of receptors are not the only regulators of pain. Many inhibitory and stimulatory neurotransmitters are involved, and simply blocking these receptors may not affect the pain.

Naloxone, an opioid receptor antagonist, was injected 30 minutes before SA administration. In the writhing test, naloxone significantly increased the number of abdominal contractions compared to SA alone, suggesting that opioid receptors may mediate, at least partially, the antinociceptive effects of SA. Previous studies investigating the role of opioid pathways in plant extracts containing SA as the main phenolic acid and opioid receptor inhibitor did not report a reduction in the analgesic effect of SA.[37] In the study by Okur and Şakul, the injection of naloxone reversed the antinociceptive effect of SA in the HP test, which is consistent with our results.[19]

The NO/cGMP signaling pathway was examined in 2 separate groups by intraperitoneal administration of L-NAME and methylene blue 30 and 15 minutes before the SA injection, respectively. L-NAME enhanced the analgesic effect of SA in the HP test at 15 and 30 minutes post-injection, which may suggest that inhibition of NO synthesis may potentiate SA's action. Pain signal transmission in the spinal cord is known to involve NO/cGMP-dependent signaling pathways. NO plays a complex role in pain regulation. Evidence shows that inhibition of the NO/cGMP signaling pathway significantly reduces pain in animals and humans. Blocking NO or cGMP synthesis in the spinal cord alleviates nociception.[38] However, some conflicting findings suggest that NO may possess pain-relieving properties in the spinal cord.[39] Therefore, although our findings are consistent with a potential regulatory role of NO in nociception, further mechanistic studies are necessary to confirm this and its involvement in the antinociceptive pathway of SA.

Finally, the involvement of ATP-sensitive potassium channels was examined using glibenclamide, an inhibitor of these channels. Research has demonstrated that compounds or pathways that stimulate these channels can alleviate both long- and short-term pain induced by formalin in rodent models.[40] Administration of glibenclamide (10 mg/kg) did not significantly affect the antinociceptive response of SA, suggesting that this pathway may not be crucial for the mechanism of action of SA in the tested models.

A wide variety of nociceptors and channels in the body are responsible for detecting noxious stimuli, including glutamate, N-methyl-D-aspartate (NMDA), and toll-like receptors. In addition, channels include transient receptor potential (TRP) and voltage-gated calcium channels.[41,42] Therefore, in addition to the mechanisms investigated in this study, other nociceptors are involved in the pain mechanism through which SA exerts analgesic effects.

Limitations: First, molecular and biochemical analyses are needed to elucidate the exact pathways. Second, the study focused on short-term behavioral assessments after single-dose SA administration. The long-term efficacy and safety of repeated administration were not evaluated. Third, the mechanisms of the antinociceptive effects of SA have only been partially explored using pharmacological inhibitors. Finally, experiments were conducted using male mice, not accounting for sex-based differences in pain perception and drug responses. Since hormones in women can affect pain threshold and drug efficacy, future studies should include both sexes to reflect clinical variation.

Conclusion

In conclusion, SA pre-treatment exhibited notable antinociceptive effects in models of inflammatory and visceral pain, particularly during the chronic

phase of the formalin and acetic acid-induced writhing tests. These findings suggest that SA may be more effective in alleviating inflammatory pain, likely through peripheral mechanisms of action. No significant effect was observed in the HP test, indicating less effectiveness for centrally mediated thermal pain relief. Further investigation is necessary in this area, particularly regarding the impact of SA on chronic pain. The findings of this study indicate the potential of SA as a natural compound with analgesic effects in inflammatory pain. However, further studies on the effective dose, long-term safety, and molecular mechanisms, particularly in chronic pain models, are necessary to assess the transferability of these results to humans.

Conflict of Interests

The authors declare no conflict of interest in this study.

Acknowledgments

The data used in this article were extracted from the master's thesis of Elnaz Harooni. thesis. This work was supported by the deputy of research affairs of Ahvaz Jundishapur University of Medical Sciences (grant No. APRC-9806) and was performed in the Medical Basic Sciences Research Institute, Persian Gulf Physiology Research Center, Iran.

References

1. Lee GI, Neumeister MW. Pain: Pathways and Physiology. Clin Plast Surg 2020; 47(2): 173-80.
2. Onasanwo SA, Rotu RA. Antinociceptive and anti-inflammatory potentials of kolaviron: mechanisms of action. J Basic Clin Physiol Pharmacol 2016; 27(4): 363-70.
3. Vearrier D, Grundmann O. Clinical Pharmacology, Toxicity, and Abuse Potential of Opioids. J Clin Pharmacol 2021; 61 Suppl 2: S70-S88.
4. Solomon DH, Husni ME, Libby PA, Yeomans ND, Lincoff AM, Lüscher TF, et al. The Risk of Major NSAID Toxicity with Celecoxib, Ibuprofen, or Naproxen: A Secondary Analysis of the PRECISION Trial. Am J Med 2017; 130(12): 1415-22.e4.
5. Ilari S, Giancotti LA, Lauro F, Gliozzi M, Malafoglia V, Palma E, et al. Natural Antioxidant Control of Neuropathic Pain-Exploring the Role of Mitochondrial SIRT3 Pathway. Antioxidants (Basel) 2020; 9(11): 1103.
6. Robbins RJ. Phenolic acids in foods: an overview of analytical methodology. J Agric Food Chem 2003; 51(10): 2866-87.
7. Rathee P, Chaudhary H, Rathee S, Rathee D, Kumar V, Kohli K. Mechanism of action of flavonoids as anti-inflammatory agents: a review. Inflamm Allergy Drug Targets 2009; 8(3): 229-35.
8. Falade T, Ishola IO, Akinleye MO, Oladimeji-Salami JA, Adeyemi OO. Antinociceptive and anti-arthritic effects of aqueous whole plant extract of Trianthema portulacastrum in rodents: Possible mechanisms of action. J Ethnopharmacol 2019; 238: 111831.
9. Shahidi S, Komaki A, Raoufi S, Salehi I, Zarei M, Mahdian M. The Anti-nociceptive Effect of Ellagic Acid in Streptozotocin-induced Hyperglycemic Rats by Oxidative Stress Involvement. Basic Clin Neurosci 2021; 12(6): 861-72.
10. Kamothi DJ, CL M, PK P, Kumar D, AG T. Synthesis, Characterization and Ameliorating Effect of Quercetin Nanoparticles against Ethion-Induced Oxidative Stress in Male Rats. Explor Anim Med Res 2023; 13(2): 231-42.
11. Pacheco-Palencia LA, Mertens-Talcott S, Talcott ST. Chemical composition, antioxidant properties, and thermal stability of a phytochemical enriched oil from Acai (Euterpe oleracea Mart.). J Agric Food Chem 2008; 56(12): 4631-6.
12. Srinivasulu C, Ramgopal M, Ramanjaneyulu G, Anuradha CM, Suresh Kumar C. Syringic acid (SA) – A Review of Its Occurrence, Biosynthesis, Pharmacological and Industrial Importance. Biomed Pharmacother 2018; 108: 547-57.
13. Li Y, Zhang L, Wang X, Wu W, Qin R. Effect of Syringic acid on antioxidant biomarkers and associated inflammatory markers in mice model of asthma. Drug Dev Res 2019; 80(2): 253-61.
14. Güzelad Ö, Özkan A, Parlak H, Sinen O, Afşar E, Öğüt E, et al. Protective mechanism of Syringic acid in an experimental model of Parkinson's disease. Metab Brain Dis 2021; 36(5): 1003-14.
15. Periyannan V, Veerasamy V. Syringic acid may attenuate the oral mucosal carcinogenesis via improving cell surface glycoconjugation and modifying cytokeratin expression. Toxicol Rep 2018; 5: 1098-106.
16. Adeyi OE, Somade OT, Ajayi BO, James AS, Adeyi AO, Olayemi ZM, et al. Syringic acid demonstrates better anti-apoptotic, anti-inflammatory and antioxidative effects than ascorbic acid via maintenance of the endogenous antioxidants and downregulation of pro-inflammatory and apoptotic markers in DMN-induced hepatotoxicity in rats. Biochem Biophys Rep 2023; 33: 101428.
17. Dalmagro AP, Camargo A, Pedron NB, Garcia SAM, Zeni ALB. Morus nigra leaves extract revokes the depressive-like behavior, oxidative stress, and hippocampal damage induced by corticosterone: a pivotal role of the phenolic syringic acid. Behav Pharmacol 2020; 31(4): 397-406.
18. Kumar S, Prahalathan P, Raja B. Syringic acid ameliorates (L)-NAME-induced hypertension by reducing oxidative stress. Naunyn Schmiedebergs Arch Pharmacol 2012; 385(12): 1175-84.
19. Okur ME, Şakul AA. Mechanism of antinociceptive action of syringic acid. J Res Pharm 2021; 25(3): 277-86.
20. Hajipour S, Farbood Y, Dianat M, Nesari A, Sarkaki A. Effect of Berberine against Cognitive Deficits in Rat Model of Thioacetamide-Induced Liver Cirrhosis and Hepatic Encephalopathy (Behavioral, Biochemical, Molecular and Histological Evaluations). Brain Sci 2023; 13(6): 944.
21. Gomes NM, Rezende CM, Fontes SP, Matheus ME, Fernandes PD. Antinociceptive activity of Amazonian Copaiba oils. J Ethnopharmacol 2007; 109(3): 486-92.
22. Niemegeers CJ, Van Bruggen JA, Janssen PA. Suprofen, a potent antagonist of acetic acid-induced writhing in rats. Arzneimittelforschung 1975; 25(10): 1505-9.
23. Aykan DA, Kesim M, Ayan B, Kurt A. Anti-inflammatory and antinociceptive activities of glucagon-like peptides: evaluation of their actions on serotonergic, nitrergic, and opioidergic systems. Psychopharmacology (Berl) 2019; 236(6): 1717-28.
24. Morteza-Semnani K, Mahmoudi M, Heidar MR. Analgesic activity of the methanolic extract and total alkaloids of Glaucium paucilobum. Methods Find Exp Clin Pharmacol 2006; 28(3): 151-5.
25. Fatemi R, Moghaddam HF, Farbod Y, Beygtashkhani R. Effects of Crocin on brain neurotrophins, cognition, balance and pain in toxic-induced demyelination model. Acta Neurol Taiwan 2024; 33(2): 48-59.
26. Trongsakul S, Panthong A, Kanjanapothi

D, Taesotikul T. The analgesic, antipyretic and anti-inflammatory activity of Diospyros variegata Kruz. J Ethnopharmacol 2003; 85(2-3): 221-5.
27. Melo AS, Monteiro MC, da Silva JB, de Oliveira FR, Vieira JL, de Andrade MA, et al. Antinociceptive, neurobehavioral and antioxidant effects of Eupatorium triplinerve Vahl on rats. J Ethnopharmacol 2013; 147(2): 293-301.
28. Trevisan G, Rossato MF, Tonello R, Hoffmeister C, Klafke JZ, Rosa F, et al. Gallic acid functions as a TRPA1 antagonist with relevant antinociceptive and antiedematogenic effects in mice. Naunyn Schmiedebergs Arch Pharmacol 2014; 387(7): 679-89.
29. Collier HO, Dinneen LC, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br J Pharmacol Chemother 1968; 32(2): 295-310.
30. Tjølsen A, Hole K. Animal Models of Analgesia. In: Dickenson A, Besson JM, editors. The Pharmacology of Pain. Berlin, Heidelberg: Springer Berlin Heidelberg; 1997. p. 1-20.
31. Ishola IO, Akindele AJ, Adeyemi OO. Analgesic and anti-inflammatory activities of Cnestis ferruginea Vahl ex DC (Connaraceae) methanolic root extract. J Ethnopharmacol 2011; 135(1): 55-62.
32. Srinivasan S, Muthukumaran J, Muruganathan U, Venkatesan RS, Jalaludeen AM. Antihyperglycemic effect of syringic acid on attenuating the key enzymes of carbohydrate metabolism in experimental diabetic rats. Biomed Prev Nutr 2014; 4(4): 595-602.
33. Sommer C, Kress M. Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. Neurosci Lett 2004; 361(1-3): 184-7.
34. Dalmagro AP, Camargo A, Severo Rodrigues AL, Zeni ALB. Involvement of PI3K/Akt/GSK-3β signaling pathway in the antidepressant-like and neuroprotective effects of Morus nigra and its major phenolic, syringic acid. Chem Biol Interact 2019; 314: 108843.
35. Zhang JJ, Kong Q. Locomotor activity: A distinctive index in morphine self-administration in rats. PLoS One 2017; 12(4): e0174272.
36. Towers S, Princivalle A, Billinton A, Edmunds M, Bettler B, Urban L, et al. GABAB receptor protein and mRNA distribution in rat spinal cord and dorsal root ganglia. Eur J Neurosci 2000; 12(9): 3201-10.
37. Arana-Argáez VE, Domínguez F, Moreno DA, Isiordia-Espinoza MA, Lara-Riegos JC, Ceballos-Góngora E, et al. Anti-inflammatory and antinociceptive effects of an ethanol extract from Senna septemtrionalis. Inflammopharmacology 2020; 28(2): 541-9.
38. Schmidtko A, Tegeder I, Geisslinger G. No NO, no pain? The role of nitric oxide and cGMP in spinal pain processing. Trends Neurosci 2009; 32(6): 339-46.
39. Luo ZD, Cizkova D. The role of nitric oxide in nociception. Curr Rev Pain 2000; 4(6): 459-66.
40. Barreras-Espinoza I, Soto-Zambrano JA, Serafín-Higuera N, Zapata-Morales R, Alonso-Castro Á, Bologna-Molina R, et al. The Antinociceptive Effect of a Tapentadol-Ketorolac Combination in a Mouse Model of Trigeminal Pain is Mediated by Opioid Receptors and ATP-Sensitive K(+) Channels. Drug Dev Res 2017; 78(1): 63-70.
41. Wang M, Thyagarajan B. Pain pathways and potential new targets for pain relief. Biotechnol Appl Biochem 2022; 69(1): 110-23.
42. Nazıroğlu M, Braidy N. Thermo-Sensitive TRP Channels: Novel Targets for Treating Chemotherapy-Induced Peripheral Pain. Front Physiol 2017; 8: 1040.