Exploring the analgesic potential of isorhamnetin: insights from formalin-induced pain and diabetic neuropathy models

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ABSTRACT. – OBJECTIVE: Isorhamnetin, a naturally occurring flavonoid compound, holds paramount importance as a primary constituent within several medicinal plants, exhibiting profound pharmacological significance. The aim of this study is to investigate the pain-relieving attributes of isorhamnetin in murine models through both formalin-induced pain and diabetic neuropathy scenarios.

MATERIALS AND METHODS: To achieve our objective, isorhamnetin was orally administered to mice at varying dosage levels (10 to 100 mg/kg). Pain-related behaviors were assessed using the formalin test during its secondary phase. Additionally, the potential pain-alleviating effect of isorhamnetin was evaluated in a diabetic neuropathy model induced by streptozotocin. Additionally, we carried out advanced interventions using naloxone, which is a well-known antagonist of opioid receptors, yohimbine, which blocks α2-adrenergic receptors, and methysergide, which inhibits serotonergic receptors, during the formalin test.

RESULTS: The oral intake of isorhamnetin showed a decrease in behaviors associated with pain that was proportional to the dose observed during the second phase of the formalin test when induced by formalin. In the diabetic neuropathy model, isorhamnetin administration effectively reversed the reduced pain threshold observed. Notably, naloxone, the opioid receptor antagonist, effectively counteracted the pain-relieving effect produced by isorhamnetin in the formalin test, whereas yohimbine and methysergide did not yield similar outcomes. Isorhamnetin also led to a reduction in elevated spinal cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) levels triggered by formalin, with this effect reversed by pre-treatment with naloxone. The compound also suppressed heightened spinal phosphorylated CREB (p-CREB) levels caused by diabetic neuropathy.

CONCLUSIONS: This research determined that isorhamnetin has notable abilities to relieve pain in models of formalin-induced pain and diabetic neuropathy. The pain-relieving mechanism of isorhamnetin in the formalin-induced pain model seems to be connected to the activation of spinal opioid receptors and the adjustment of CREB protein amounts. This insight improves our knowledge of how isorhamnetin could be used therapeutically to treat pain conditions stemming from formalin-induced pain and diabetic neuropathy.

Key Words:
 Nociception, Diabetic neuropathy, Naloxone, Flavonoid, p-CREB.

Introduction

Isorhamnetin, a flavonoid compound that occurs naturally, is the main component identified in several medicinal plants. This compound exhibits a broad spectrum of pharmacological activities beneficial for cardiovascular diseases[1] and a variety of tumors[2]. Moreover, it possesses the potential to prevent neurodegenerative diseases such as Alzheimer's disease[3]. It also has pharmacodynamic properties against hyperuricemia[4] and

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pulmonary fibrosis[5]. Furthermore, it has anti-inflammatory[6], antidiabetic[7], immunoregulatory[8], antioxidant[9], and antiviral[10] roles. The therapeutic effects of isorhamnetin are associated with its modulation of signaling pathways, including nuclear factor kappa B (NF-κB)[11], phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)[12], mitogen-activated protein kinase (MAPK)[13] along with their subsequent factors. Consequently, exploring the pharmacological actions and underlying mechanisms of isorhamnetin represents a key area of current scientific investigation.

The cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) is important in pain transmission modulation. For instance, expressions of CREB protein are changed in the dorsal root ganglia (DRG) and spinal cord in response to nociceptive pain. Crown et al[14] suggested that CREB proteins are involved in allodynia development in models of spinal cord injury. Moreover, CREB protein's expression is seen to be significant in several pain models, including those for neuropathic pain, diabetic neuropathy, and pain treated with capsaicin[15-18]. Nevertheless, the specific impact of isorhamnetin on spinal CREB protein in controlling induced anti-nociception remains unclear.

Multiple pieces of research[19,20] have shown that isorhamnetin has anti-inflammatory properties. The pain from the formalin test is often considered to stem from inflammation. Furthermore, a significant number of studies[20-23] have identified inflammation as a key pathological factor in diabetic neuropathy. Although isorhamnetin is recognized for its anti-inflammatory and anti-diabetic effects, its potential anti-nociceptive (pain-relieving) capabilities have yet to be explored. Therefore, this study was initiated to investigate the anti-nociceptive effects of isorhamnetin, focusing particularly on pain models induced by inflammation. Moreover, this research aimed to uncover the molecular mechanisms behind the anti-nociceptive effects of isorhamnetin.

Materials and Methods

Experimental Animals

In this study, 30 adult male Swiss albino mice, with body weights ranging from 24 to 28 grams and bred at the Hashemite University's Animal House Unit, were used. The Institutional Review Board at the Hashemite University in Zarqa, Jordan, approved all experimental procedures (IRB: 8/5/2019/2020). The mice were kept in an environment regulated at a temperature of $21 \pm 1°C$, under a 12-hour light/dark cycle, with lights on from 06:00 to 18:00 hours. They had constant access to sufficient food and water. To reduce stress, the mice were brought to the testing area one hour before the experiments to acclimate to the lab settings. Two experienced observers (M.O. and R.A.D.), who were not informed about the specifics of the experimental design, meticulously monitored the mice during the experiments to eliminate any observational bias.

Production of Streptozotocin-Induced Diabetic Neuropathy Model

An animal model for diabetic neuropathy was created by administering 0.1 mg/kg streptozotocin *via* intraperitoneal injection as a single dose. The study took place five weeks following the streptozotocin injection.

Von-Frey Test

Anti-nociception and mechanical allodynia were measured using the Von-Frey test, following the methodology described by Bonin et al[24]. For this test, each mouse was placed inside a transparent glass chamber with a wire mesh bottom. The mice were given a 30-minute period to acclimate to the test environment before the test began. After this adaptation period, Von-Frey filaments (sourced from North Coast Medical, Inc., Gilroy, CA, USA) were applied to the plantar surface of the mice's feet, employing an ascending and descending force method.

Intraplantar Formalin Test

Following the formalin test guidelines set forth by Hunskaar et al[25], a 10 μl injection of 5% formalin was administered subcutaneously beneath the plantar surface of the left hind paw. Immediately following this injection, the animals were transferred to an acrylic observation chamber. The time spent by the animals engaging in behaviors such as licking, shaking, and biting the injected paw was precisely recorded with a stopwatch, which served as an indicator of nociceptive response. The initial phase of this response usually peaked at 0 to 5 minutes, and the later phase occurred at 20 to 40 minutes after the formalin injection. These intervals reflect the immediate effects on nociceptors and the responses to inflammatory pain, respectively, as detailed by Hunskaar and Hole[19]. Before undergoing the formalin test, the animals were given oral doses of isorhamnetin,

ranging from 10 to 100 mg/kg, 30 minutes before the test.

Pretreatment of Antagonists

Initially, the mice received an intraperitoneal pretreatment with one of the following: saline, naloxone (5 mg/kg), methysergide (5 mg/kg), or yohimbine (5 mg/kg), administered 10 minutes before the oral intake of either a control substance or a uniform dose of isorhamnetin (100 mg/kg). Following this, the behavior indicative of nociception caused by formalin was observed and evaluated.

Protein Extraction and Western Blot

The lumbar section of the mice's spinal cord was extracted and washed twice with cold Tris-buffered saline (made of 20 mmol/L Trizma base and 137 mmol/L NaCl, with a pH of 7.5). Directly after washing, the tissue was homogenized in sodium dodecyl sulfate lysis buffer (containing 62.5 mmol/L Trizma base, 2% w/v sodium dodecyl sulfate, and 10% glycerol), with 0.1 mmol/L Na_3VO_4, 3 mg/mL aprotinin, and 20 mmol/L sodium fluoride (NaF). The mixture was then sonicated briefly, and protein levels were determined using a detergent-compatible protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA), using bovine serum albumin as a reference. After adding 0.1% w/v bromophenol blue, the proteins were boiled and then separated by electrophoresis on polyacrylamide gels ranging from 6% to 10%. The proteins were subsequently transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA). The membranes were then incubated overnight with antibodies targeting p-CREB (Abcam, Discovery Drive, Cambridge, UK, 1:1000) and β-actin (Cell Signaling Technology, Danvers, Boston, MA, USA, 1:1000) in a blocking buffer. After four 20-minute washes in Tris-buffered saline with 20% Tween-20 (TBST) (consisting of 10 mM Trizma base, pH 8.0, 150 mM NaCl, and 20% Tween 20), the membranes were incubated with an anti-rabbit IgG-horseradish peroxidase conjugate (1:4000) in blocking buffer at room temperature for an hour. Following four more 20-minute washes with TBST, the membranes were treated with an enhanced chemiluminescence (ECL)-plus solution (Millipore, Billerica, MA, USA) and analyzed with a Luminescent Image Analyzer (LAS-4000, Fuji Film Co., Tokyo, Japan) to detect the emitted light. Signal intensities were quantified using Multi-Gauge Version 3.1 (Fuji Film, Tokyo, Japan) and expressed as percentages relative to the control.

Drugs

Isorhamnetin, streptozotocin, naloxone, yohimbine, and methysergide were acquired from Sigma-Aldrich (St. Louis, MO, USA) and were freshly prepared prior to utilization.

Statistical Analysis

Statistical evaluation was performed with a one-way ANOVA, succeeded by Bonferroni's post-hoc test, using GraphPad Prism Version 8.0.1 for Windows (GraphPad Software, San Diego, CA, USA). A significance level was established with p-values below 0.05. The results were displayed as the mean ± standard error of the mean (S.E.M.).

Results

Effect of Isorhamnetin on Pain Response in the Formalin Test

The primary objective of this experiment was to evaluate the analgesic effect of isorhamnetin on pain responses induced by formalin injection in mice. Figure 1 demonstrates that, initially, mice in the control group, which received a vehicle treatment, exhibited immediate pain behaviors upon receiving a 5% formalin injection through the intraplantar route. These responses peaked within the first 5 minutes, characterizing the initial phase of nociception. This acute phase was succeeded by a prolonged inflammatory response, observable between 20 to 40 minutes post-formalin administration, defined as the secondary phase of pain.

Upon administering oral isorhamnetin at various doses ranging from 10 to 100 mg/kg, significant mitigation of pain behaviors was observed, particularly during the secondary inflammatory phase of the response. As depicted in Figure 1, the reduction in pain behaviors was dose-dependent, with higher doses of isorhamnetin demonstrating a more pronounced effect.

In summary, the findings from this experiment suggest that isorhamnetin effectively reduces pain responses in mice subjected to the formalin test, especially during the inflammatory phase of pain. This supports the potential of isorhamnetin as a therapeutic agent in managing pain associated with inflammation.

Impact of Isorhamnetin on Pain Behavior in a Model of Diabetic-Induced Neuropathy

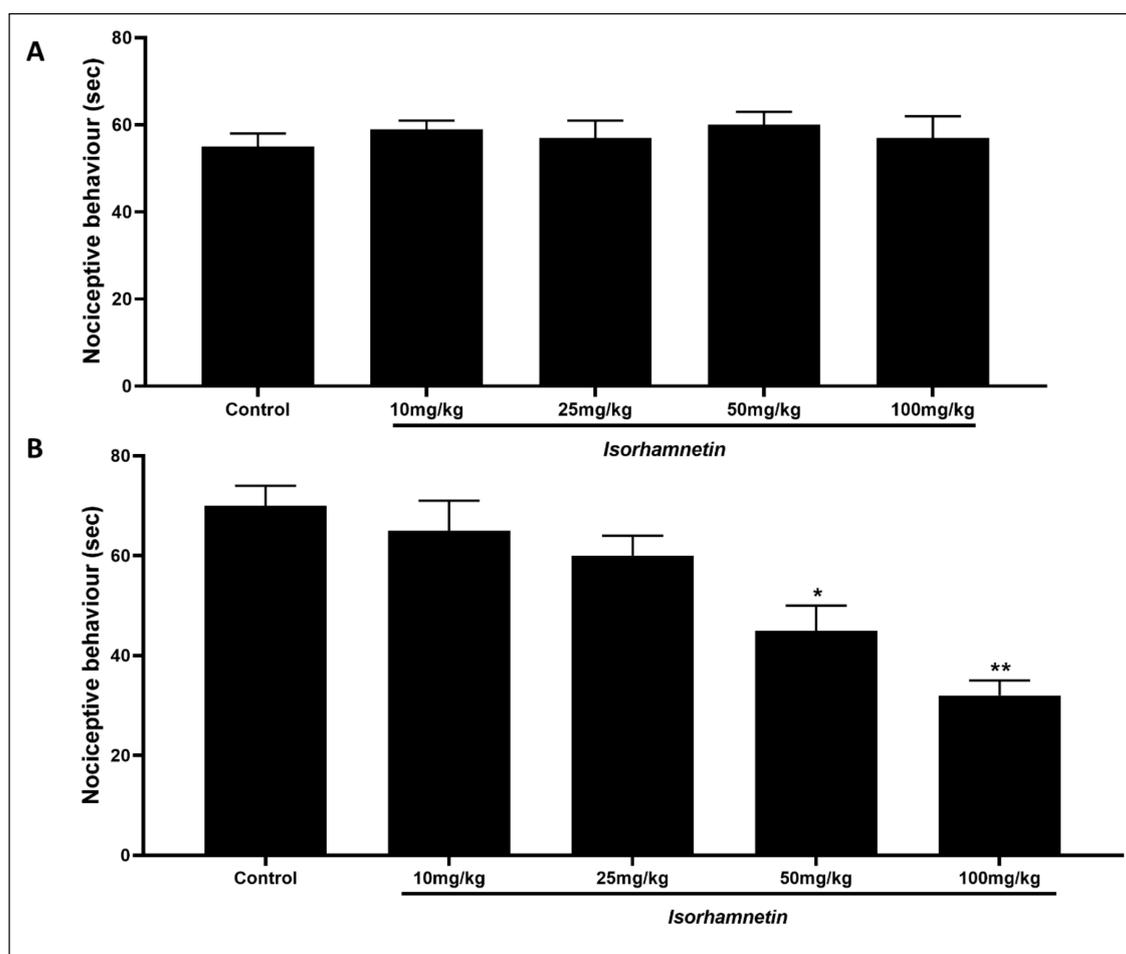The aim of this portion of the study was to assess the efficacy of isorhamnetin in modulating pain

Figure 1. Isorhamnetin's impact on formalin-induced nociceptive response. Mice were orally administered with varying doses of isorhamnetin (ranging from 10 to 100 mg/kg) for a duration of 30 minutes prior to subcutaneous injection of formalin (5%, 10 μl) into the plantar region of the left hind paw. The combined duration of licking, biting, and shaking of the injected paw was assessed in two time intervals: 0-5 minutes (1st phase) (**A**) and 20-40 minutes (2nd phase) (**B**). The vertical bars on the graph represent the standard error of the mean. Each experimental group consisted of 8-10 animals. The data were analyzed using one-way ANOVA, which showed a significant effect of isorhamnetin on formalin-induced nociceptive response, $F_{(3, 36)} = 5.67$, $p < 0.05$ for the 1st phase and $F_{(3, 36)} = 8.22$, $p < 0.01$ for the 2nd phase, indicating a dose-dependent reduction in pain behaviors (*$p < 0.05$; **$p < 0.01$, compared to the control group).

sensitivity in a diabetic neuropathy model. This was achieved by measuring the mechanical pain threshold following the induction of diabetes in mice. According to Figure 2, a significant reduction in the threshold for mechanical stimulation was observed five weeks after administering a single intraperitoneal injection of streptozotocin; this decrease in pain threshold is indicative of heightened pain sensitivity, a hallmark of diabetic neuropathy.

Pre-treatment with oral doses of isorhamnetin, ranging from 10 to 100 mg/kg approximately 30 minutes prior to the evaluation of mechanical pain, was found to counteract the lowered pain threshold. Notably, the reversal of pain sensitivity was dose-dependent, with higher doses of isorhamnetin showing greater efficacy, as detailed in Figure 2. The dose-dependent amelioration suggests a significant role of isorhamnetin in modulating pain perception under conditions of diabetic neuropathy.

In conclusion, the data from this experiment underscore isorhamnetin's potential as an analgesic agent in diabetic neuropathy models, specifically its capacity to improve mechanical pain thresholds. These outcomes highlight isorhamnetin's therapeutic promise in addressing the pain associated with diabetic-induced neuropathic conditions.

Modulation of Phosphorylated CREB Protein in the Spinal Cord by Isorhamnetin in Models of Formalin-Induced Pain and Diabetic-Induced Neuropathy

The primary objective of this investigation was to explore how isorhamnetin affects the expression of phosphorylated CREB (p-CREB) protein in the spinal cord, specifically in the context of formalin-induced pain and diabetic-induced neuropathy. This inquiry is rooted in understanding the molecular mechanisms underlying isorhamnetin's potential analgesic and anti-inflammatory effects.

This part of the study focused on evaluating the modulation of p-CREB protein expression in the spinal cord following exposure to pain stimuli from formalin injection and diabetic-induced neuropathy. Lumbar spinal cord protein extracts analyzed 30 minutes after formalin injection showed an increase in p-CREB levels, as depicted in Figure 3A. Notably, this increase was mitigated by the oral administration of 100 mg/kg isorhamnetin. Furthermore, in a model of diabetic neuropathy induced by streptozotocin, diabetic mice displayed a significant rise in CREB phosphorylation compared to their non-diabetic coun-

terparts. Intriguingly, this enhanced phosphorylation was diminished when treated with 100 mg/kg isorhamnetin, administered either as a single dose or over three separate doses, as illustrated in Figure 3B.

Effect of Naloxone, Yohimbine, or Methysergide on Pain Response in the Formalin Test

The aim of this experiment was to determine how isorhamnetin mediates its analgesic effects, specifically investigating its interactions with opioidergic, serotonergic, and adrenergic pathways. This analysis was prompted by the hypothesis that isorhamnetin's pain-relieving properties could be partly due to its influence on these neurological systems.

To explore this, we conducted experiments as shown in Figure 4, where isorhamnetin's anti-nociceptive effect was tested in the presence of naloxone (an opioid receptor antagonist), methysergide (a serotonergic antagonist), and yohimbine (an α-2 adrenergic antagonist). Each substance was administered to assess its ability to reverse the analgesic effects of isorhamnetin in a pain model.
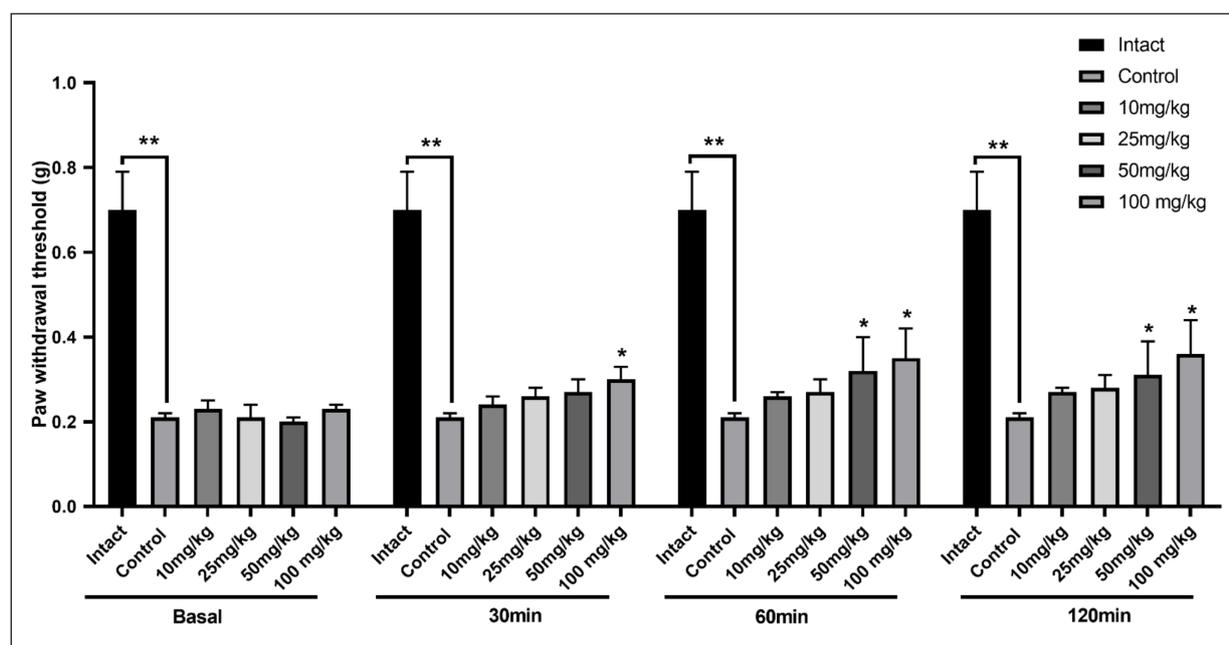

Figure 2. The analgesic effect of isorhamnetin in a diabetic neuropathy model. A model of diabetic neuropathy was established through a single intraperitoneal injection of 0.1 mg/kg streptozotocin. Observations were made five weeks after the streptozotocin injection. The vertical lines on the graph denote the standard error of the mean. Each group included 8-10 animals. Statistical analysis by one-way ANOVA revealed a significant reduction in pain behaviors in the isorhamnetin-treated groups, $F_{(3, 36)} = 7.03$, $p < 0.01$, compared to the intact group, and $F_{(3, 36)} = 4.56$, $p < 0.05$, compared to the control group, indicating isorhamnetin's analgesic effect in diabetic neuropathy (**$p < 0.01$, compared to the intact group; *$p < 0.05$, compared to the control group).
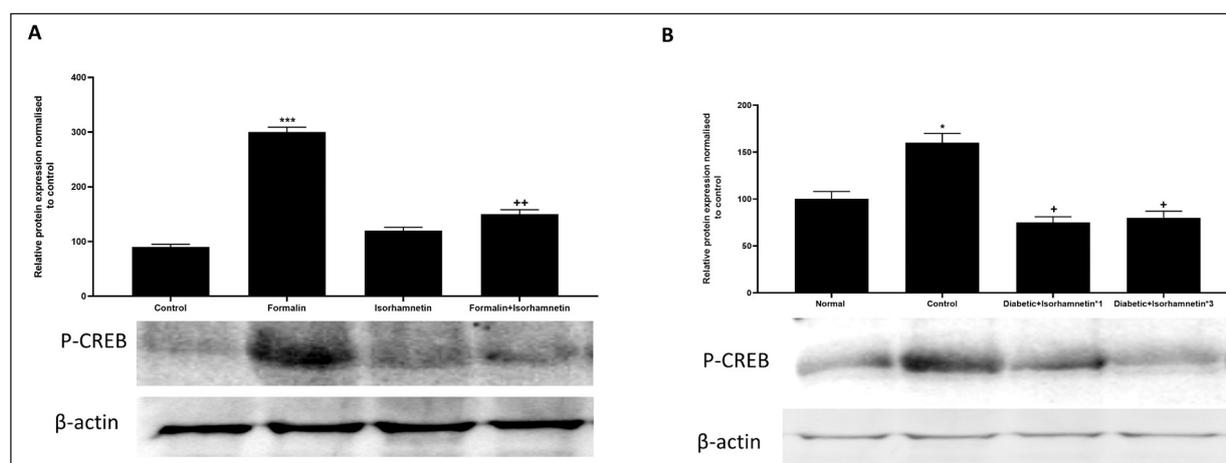
Figure 3. Alterations in phosphorylated CREB protein levels in the spinal cord following isorhamnetin treatment in formalin-induced and diabetic neuropathy pain models. **A**, Western blot analysis was performed on proteins extracted from lumbar spinal cord tissue 30 minutes post formalin injection to assess changes in phosphorylated CREB (p-CREB) levels, involving six animals per experimental group. **B**, Following the induction of diabetic neuropathy *via* streptozotocin injection (conducted five weeks prior), mice received isorhamnetin treatment either once or three times, with the latter administered twice daily (at 10 AM and 4 PM). Protein extraction for Western blot analysis was done 1 hour after the last dose of isorhamnetin. β-Actin served as an internal loading control (diluted 1:1000). Signal intensities were quantified using laser scanning densitometry and expressed as percentages relative to the control group. Data are shown as mean ± SEM. Statistical analysis by one-way ANOVA indicated significant alterations in p-CREB levels, with F (2, 15) = 10.34, $p < 0.001$ for comparisons with the control group; F (2, 15) = 7.88, $p < 0.01$ for comparisons with the formalin-treated group; and F (2, 15) = 4.76, $p < 0.05$ for comparisons with the diabetic group, demonstrating isorhamnetin's regulatory effect on p-CREB expression in the context of formalin-induced pain and diabetic neuropathy (***$p < 0.001$ compared to the control group; ++$p < 0.01$ compared to the formalin-treated group; *$p < 0.05$ compared to the normal group; +$p < 0.05$ compared to the diabetic group).

The results revealed a notable interaction between isorhamnetin and the opioid system, as the administration of naloxone reversed isorhamnetin's anti-nociceptive effects, suggesting a significant opioidergic involvement. In contrast, neither methysergide nor yohimbine affected the pain-relieving effects of isorhamnetin, indicating that the serotonergic and adrenergic pathways might not play a central role in isorhamnetin's mechanism of action. Additionally, the administration of naloxone, methysergide, or yohimbine alone did not alter pain responses, further supporting the specificity of isorhamnetin's interaction with these pathways.

Impact of Naloxone on Isorhamnetin-Induced Modulation of Spinal p-CREB Expression in the Formalin Test

The research sought to investigate whether naloxone could counteract the isorhamnetin-induced reduction in spinal phosphorylated CREB (p-CREB) expression, building on findings that naloxone reverses isorhamnetin's anti-nociceptive effects in the formalin test. This was conducted to elucidate the interaction between isorhamnetin and the opioid pathway, specifically regarding the modulation of p-CREB expression within the spinal cord. To this end, Figure 5 displays the results of experiments where mice were pre-treated with naloxone before the administration of isorhamnetin. This setup allowed for the assessment of naloxone's impact on the baseline and isorhamnetin-altered levels of spinal p-CREB expression.

The data revealed that while naloxone alone did not influence the baseline levels of p-CREB, it effectively reversed the reduction in p-CREB expression triggered by isorhamnetin. This outcome indicates a significant interaction between isorhamnetin's analgesic effects and the opioid signaling pathway, specifically in the modulation of p-CREB expression in the spinal cord. It underscores the potential complexity of isorhamnetin's mechanism of action, suggesting that its analgesic properties may, in part, be mediated through an influence on opioid pathway-linked molecular targets.

Discussion

In this study, we observed that isorhamnetin effectively reduces pain behaviors in a formalin-induced model, particularly during the inflamma-

tory phase. This aligns with the biphasic nature of formalin-induced pain, where the initial phase is attributed to direct nociceptor stimulation and the second to inflammation and central sensitization[24]. Interestingly, isorhamnetin's efficacy was more pronounced in mitigating inflammation-related pain.

To investigate whether isorhamnetin can alleviate pain induced mechanically, we created a model of diabetic neuropathy. Five weeks following the establishment of the streptozotocin-induced diabetic neuropathy model, we assessed isorhamnetin's potential anti-nociceptive effects and discovered that it indeed mitigates pain associated with diabetic neuropathy. Furthermore, our observations revealed that isorhamnetin treatment did not affect the blood glucose levels in diabetic mice, suggesting that its pain-relief effects are independent of any anti-diabetic action.

The CREB protein plays a crucial role in pain transmission, with increased expression of phosphorylated CREB (p-CREB) observed in the spinal cord or dorsal root ganglia across various chronic pain models, including neuropathic pain and neuropathy[15,16]. Additionally, p-CREB levels are known to rise in the spinal cord or brain regions in response to acute inflammatory pain, as seen in the formalin pain model[26-30]. In our study, we noted a significant elevation in p-CREB expression in the spinal cord following the induction of pain by formalin. Moreover, isorhamnetin effectively reduced the formalin-induced increase in spinal p-CREB expression. We also found that isorhamnetin treatment decreased the up-regulation of spinal p-CREB expression in a model of diabetic neuropathy. These findings suggest that isorhamnetin's ability to diminish pain may be partially due to its effect on lowering p-CREB levels in the spinal cord in both formalin-induced pain and diabetic neuropathy models.

Opioid, serotonergic, and adrenergic receptors play significant roles in the modulation of nociceptive processing. For instance, opioid receptors are known to contribute to anti-nociception[31]. Furthermore, the inhibition of spinal serotonergic receptors through the spinal administration of methysergide and spinal noradrenergic receptors through yohimbine counteract the anti-nocicep-
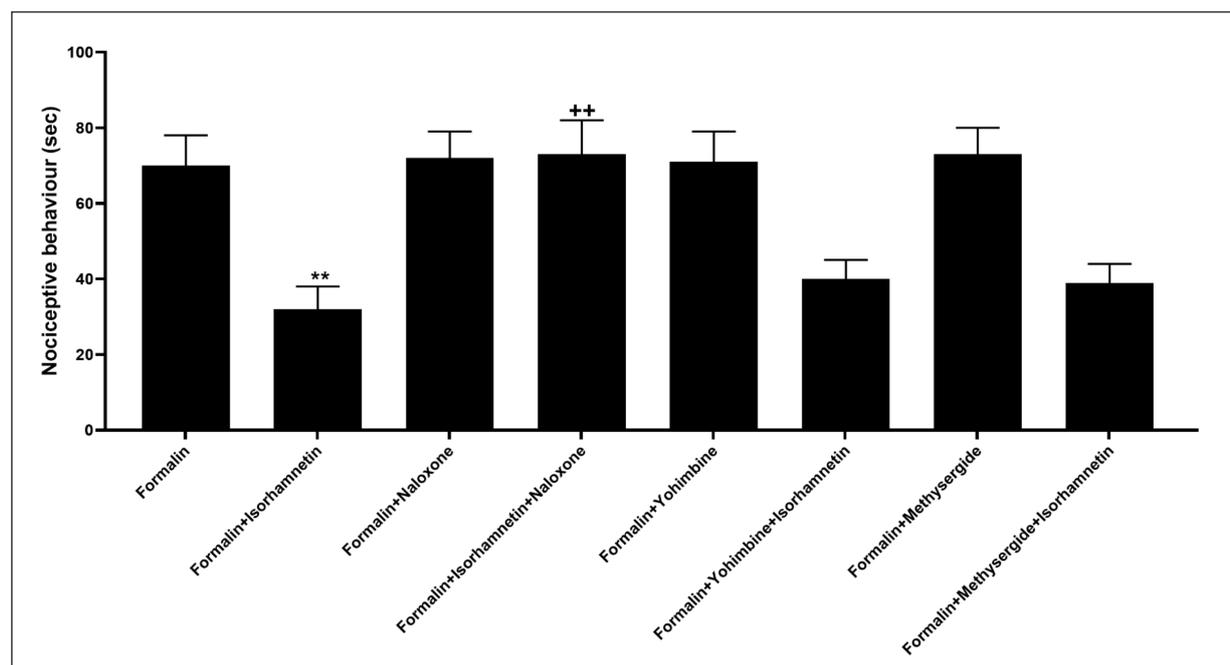

Figure 4. The effect of naloxone, yohimbine, or methysergide on isorhamnetin-induced analgesia in the formalin test. Mice received intraperitoneal pretreatment with naloxone, yohimbine, or methysergide (5 mg/kg) 10 minutes before oral administration of isorhamnetin (100 mg/kg) and 30 minutes prior to the subcutaneous injection of formalin (5%, 10 μl) into the plantar region of the left hind paw. The study included six animals per experimental group. The vertical bars on the graph indicate the standard error of the mean. Statistical analysis revealed significant differences: $F_{(3, 20)} = 8.76$, $p < 0.01$ for comparisons with the formalin-treated group, indicating naloxone reverses isorhamnetin's analgesic effect; and $F_{(3, 20)} = 6.42$, $p < 0.01$ for comparisons with the formalin + isorhamnetin group, showing yohimbine and methysergide do not affect isorhamnetin's action (**$p < 0.01$, compared to the formalin-treated group; ++$p < 0.01$, compared to the formalin + isorhamnetin group).
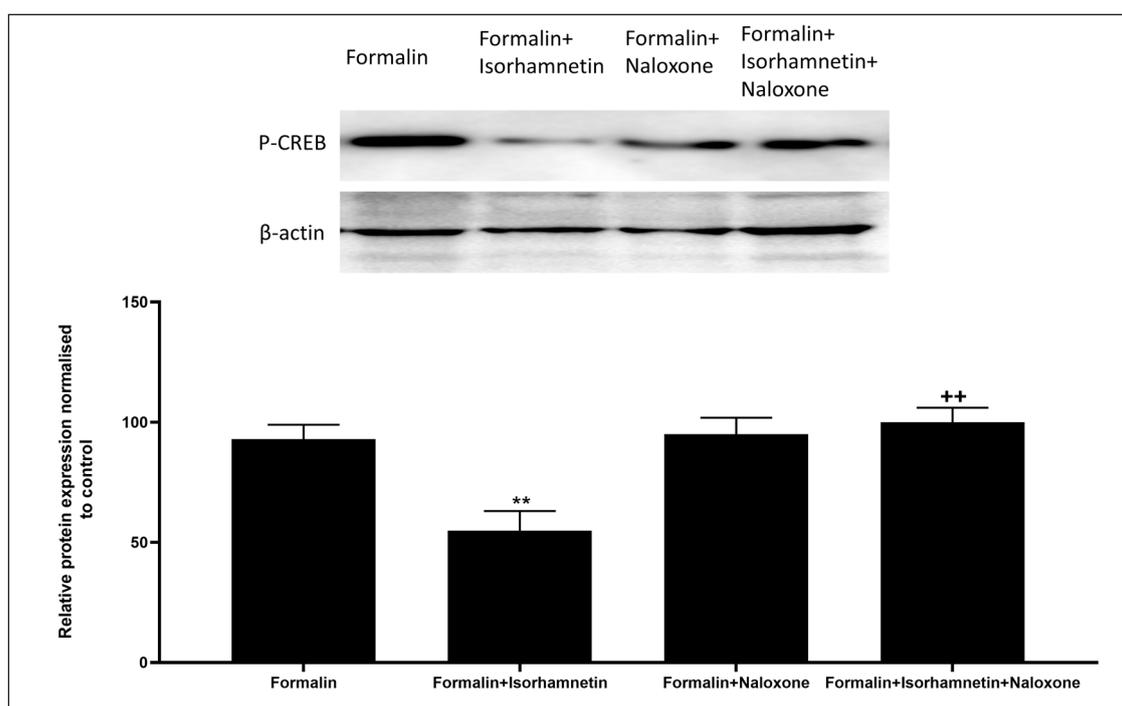
Figure 5. Naloxone's effect on isorhamnetin-induced antinociception and spinal p-CREB expression in the formalin test. Mice underwent a 10-minute intraperitoneal pretreatment with naloxone (5 mg/kg) before receiving oral isorhamnetin (100 mg/kg) 30 minutes before subcutaneous formalin injection (5%, 10 μl) into the plantar region of the left hind paw. Proteins for Western blot analysis were extracted from lumbar spinal cord tissue 30 minutes post formalin administration. The study featured 6 animals per group. β-actin served as an internal loading control (diluted 1:1000). The graph's vertical bars depict the standard error of the mean. Statistical analysis via one-way ANOVA demonstrated significant changes: $F_{(3, 20)} = 5.33$, $p < 0.05$ for comparisons to the formalin-only group, indicating naloxone reverses the antinociceptive effects of isorhamnetin; and $F_{(3, 20)} = 4.89$, $p < 0.05$ for comparisons to the formalin + isorhamnetin group, suggesting naloxone affects the modulation of p-CREB expression by isorhamnetin (**$p < 0.05$, compared to the formalin group; ++$p < 0.05$, compared to the formalin + isorhamnetin group).

tive effects of morphine given above the spinal level[32-34]. In our current study, we found that opioid receptors, but not α-2 adrenergic or serotonergic receptors, seem to be involved in the anti-nociceptive effects induced by orally administered isorhamnetin.

Furthermore, our research revealed that pre-treating with spinal naloxone reverses the anti-nociception caused by isorhamnetin in the formalin test. This indicates that isorhamnetin's ability to reduce pain in the formalin pain model is, to some extent, mediated by opioid receptors and the p-CREB protein within the spinal cord. Although direct evidence was not obtained in this study, the findings open up the possibility that isorhamnetin may facilitate the release of endogenous opioids in the spinal cord. This supposition is derived from the naloxone-reversible anti-nociceptive effects of isorhamnetin, suggesting an opioid-dependent mechanism that potentially blocks formalin-induced nociceptive signaling at the spinal level. Further direct experimental research is re-

quired to verify this suggested mechanism. It is also hypothesized that the release of endogenous opioids into the spinal cord by isorhamnetin might activate opioid receptors, leading to the observed reduction in p-CREB levels in the spinal cord during the formalin test.

Our research underscores the promising analgesic properties of isorhamnetin. Yet, it is crucial to acknowledge that the safety profile of isorhamnetin remains largely unverified in clinical settings. Despite the encouraging outcomes of our study, thorough safety evaluations, encompassing both long-term toxicity studies and clinical trials, are imperative to comprehensively ascertain the implications of using isorhamnetin for pain relief.

Conclusions

Our findings indicate that isorhamnetin exhibits an anti-nociceptive effect in both formalin-induced pain and diabetic neuropathy models. This effect

appears to be mediated primarily through opioid receptors, rather than α-2 adrenergic or serotonergic receptors. Additionally, the CREB protein, located within the spinal cord, may also play a role in the anti-nociceptive action induced by isorhamnetin.

This study underscores the significant analgesic potential of isorhamnetin, positioning it as a promising natural pain management option. Our results highlight the compound's capacity to modulate pain pathways, suggesting an alternative to traditional painkillers. Although our research marks a significant step forward, it calls for more detailed exploration into the specific mechanisms and long-term impacts of isorhamnetin use. We advocate for further studies on natural substances, opening new avenues for treatment in persistent pain conditions such as diabetic neuropathy. Our contributions to the understanding of natural analgesics pave the way for innovative, more patient-centric approaches to pain management.

Conflict of Interest
The authors declare that they have no conflict of interest.

Authors' Contributions
Conceptualization, A.A., and E.Q.; methodology, Y.B.; software, O.G.; validation M.O. and A.A.; formal analysis, O.G.; investigation, B.O.; resources, A.A.; data curation, M.W.; writing-original draft preparation, R.A.; writing-review and editing, A.A.; visualization, A.A.; supervision, E.Q.; project administration, A.A.; funding acquisition, B.O. All authors have read and agreed to the published version of the manuscript.

Ethics Approval
The necessary approvals for animal care and experimental procedures were obtained from the Animal Research Ethics Committee at the Hashemite University (IRB number: HU 8/5/2019/2020), and all procedures were performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Data Availability
Data is available upon reasonable request.

Acknowledgments

Informed Consent
Not applicable.

AI Disclosure
The authors declare no AI tools were used to write this article.

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