

THE ANALGESIC PROPERTIES OF THE FLAVONOID GALANGIN IN EXPERIMENTAL ANIMAL MODELS OF NOCICEPTION

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Abstract

This study aimed to investigate the antinociceptive properties of galangin using various nociception models. Galangin's effects were assessed across different doses (50, 100, 150, and 200 µg/kg bw) in mice through multiple pain tests. The results were compared to those of mice treated with acetylsalicylic acid or morphine, with or without naloxone. In addition to standard pain models, capsaicin- and glutamate-induced tests were employed to explore the involvement of the vanilloid and glutamatergic systems in galangin's pain-relief mechanisms. It was found that galangin exhibited a significant, dose-dependent reduction in pain behaviour during the acetic acid-induced writhing test, with a 72.2% inhibition observed at a 200 µg/kg bw dose. In the hot plate test, it increased latency period, with a 72.0% increase at the same dose. Galangin also significantly inhibited both neurogenic and inflammatory phases in the formalin-induced paw-licking test. These effects were reversed by naloxone treatment, indicating opioid system involvement. Galangin further showed inhibitory effects on neurogenic nociception induced by capsaicin and glutamate. Enzyme-linked immunosorbent assay (ELISA) revealed that galangin, at doses of 100 and 200 µg/kg bw, significantly reduced pro-inflammatory cytokine levels (IL-1β, TNF-α, IFN-γ, and NO) in mouse serum. The study concluded that galangin possesses antinociceptive activity mediated through central and peripheral pathways, modulating vanilloid receptors, opioid receptors, and the glutamatergic system. This research highlights galangin's potential as a pain-relieving agent in adult mice.

Rezumat

Studiul a urmărit investigarea proprietăților antinociceptive ale galanginei folosind diferite modele experimentale. Efectele galanginei au fost evaluate în diferite doze (50, 100, 150 și 200 µg/kg greutate corporală) la șoareci prin intermediul mai multor teste de durere. Rezultatele au fost comparate cu cele ale grupurilor tratate cu acid acetilsalicilic sau morfină, cu sau fără naloxonă. În plus față de modelele standard de durere, au fost utilizate testele capsaiciniei și glutamatului pentru a explora implicarea sistemelor vanilloid și glutamatergic în mecanismele de ameliorare a durerii. După administrarea galanginei, rezultatele au arătat o reducere semnificativă, dependentă de doză, a comportamentului dureros în timpul testului de contracție indus de acid acetic, cu o inhibiție de 72,2% observată la o doză de 200 µg/kgc. În testul plăcii fierbinți, aceasta a crescut perioada de latență, 72,0% la aceeași doză. Galangina a inhibat, de asemenea, în mod semnificativ atât faza neurogenă, cât și cea inflamatorie în testul formalinei. Aceste efecte au fost antagonizate de tratamentul cu naloxonă, ceea ce indică implicarea sistemului opioid. Galangina a prezentat efecte inhibitoare asupra nocicepției neurogene induse de capsaicină și glutamat. Testul imunoenzimatic a arătat doze de 100 și 200 µg/kgc de galangină, au redus semnificativ nivelurile serice de citokine proinflamatorii (IL-1β, TNF-α, IFN-γ și NO).

Keywords: galangin, nociception, flavonoids, naloxone, vanilloid, glutamate

Introduction

Pain is an unpleasant sensation caused by damage to body tissues or associated with it. It is widely accepted by scientists that pain, whether resulting from actual or potential tissue damage, helps prevent further harm to the organism [1]. One of the two main types of pain caused by harmful stimuli to bodily tissues is nociceptive pain, which typically

occurs when nociceptors are stimulated by extreme cold, high heat, intense mechanical pressure, or various chemical agents [2]. In recent years, there has been a growing interest in understanding the underlying mechanisms of pain, such as the neurotransmitter systems, ion channels, neuromodulators, and receptors involved in the pain neural pathways [3, 4].

Experiencing pain can have various adverse impacts, including a reduction in overall quality of life, decreased productivity, increased absenteeism from work, disability, and even leading to unemployment in some cases, affecting up to a third of the population. Consequently, the associated annual cost of these outcomes is estimated to be in the billions of dollars [5]. Despite the high cost of treatment, predicting the effectiveness of analgesics for individual patients remains challenging and often fails to meet expectations for pain relief. One possible explanation for this could be the side effects of many medications, which may discourage patients from following their physicians' recommendations.

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to alleviate pain resulting from inflammation. Nonetheless, their extended use has been linked to severe adverse effects, including bleeding, peptic ulcers, and gastrointestinal lesions [6]. While NSAIDs remain a commonly prescribed therapeutic option, these risks underscore the importance of exercising caution and monitoring patients closely when prescribing them for prolonged periods.

Likewise, opioids are a significant category of analgesics that are commonly prescribed to manage short-term post-operative or neurogenic pain. However, opioids have several severe side effects, including constipation, respiratory depression, and tolerance [7]. As a result, alternative therapeutic agents like plant-based pharmaceuticals are gaining more attention as potential treatment options because they have a milder effect and fewer adverse effects.

Several models can be used to evaluate the antinociceptive properties of a compound. The acetic acid-induced writhing test is a model commonly used to evaluate the antinociceptive properties of a compound, the hot plate and the formalin-induced paw licking test [8-12].

The capsaicin-induced paw-licking test is another model commonly employed in pain research, which induces both analgesia and hyperalgesia. This test involves the activation of TRPV1 receptors by capsaicin, a constituent of chili pepper. The activation of these receptors leads to an influx of calcium ions (Ca^{2+}) and sodium ions (Na^+) primarily Ca^{2+} into afferent neurons. This, in turn, stimulates C- or $\text{A}\delta$ -fibers, causing neurogenic pain [13-15].

Moreover, the glutamate-induced paw-licking test is another model commonly utilized in pain research. Glutamate, an essential excitatory amino acid and neurotransmitter abundant in the central nervous system, plays a crucial role in many physiological and pathophysiological processes [16]. Glutamate receptors are present in both the central and peripheral nervous systems and their activation results in nociceptive transmission, making them highly relevant to the study of pain.

Flavonoids have garnered the interest of researchers due to their widespread occurrence in plants and significant pharmacological activity. Research has indicated that flavonoids are involved in both inflammation and antinociception [17]. It has been found that several flavonoids bind to opioid and GABAA receptors, resulting in potent antinociceptive effects in various pain models. This analgesic effect can be induced either directly by inhibiting peripheral afferent pathways or indirectly by enhancing activity in descending pathways [18, 19].

Galangin is a naturally occurring flavonoid that is present in honey and the root of *Alpinia officinarum* Hance (*Zingiberaceae* family) [20]. Galangin has been found to possess antibacterial, antiperoxidative, anti-obesity, anti-tumour, anti-apoptotic and anti-inflammatory properties in terms of pharmacological effects [21-26]. Furthermore, in the neurodegenerative pathologies, galangin is effective in preventing scopolamine-induced dementia [27] and protecting neuronal cells against ischemia/reperfusion (I/R) damage [28].

The current study aimed to assess the antinociceptive potential of galangin in male mice using a range of nociception models: the acetic acid-induced writhing test, hot plate test, formalin-induced paw licking test, capsaicin-induced paw licking test, and glutamate-induced paw licking test. The study also aimed to identify any potential mechanisms of antinociception exhibited by galangin by conducting various nociception behavioural tests.

Materials and Methods

Experimental animals

Male Swiss albino mice, weighing between 25-29g and bred at the Animal House Unit of the Hashemite University, Jordan were used in this study. The Animal Research Ethics Committee at Hashemite University, Jordan approved the animal care and experimental procedures, with IRB number HU 150/2022, and all procedures were conducted in by the National Institutes of Health Guide for the Care and Use of Laboratory Animals [29].

The mice were kept in a regulated environment with a temperature of $20 \pm 2^\circ\text{C}$ and a 12-hour light/dark cycle, with the lights on from 06:00 am to 06:00 pm, and they had unlimited access to food and water. To minimize stress, the mice were allowed to adapt to the laboratory settings by transferring them to the testing area one hour before the experiments. The mice were observed by two trained observers who were not informed about the experimental design to prevent any potential bias. The experiments were conducted in a soundproofed room to minimize any external disturbances.

Acetic acid-induced writhing test

Six groups of mice were treated intraperitoneally according to the previously described method [30]. The first group served as a control and received a solution consisting of 5% and 95% distilled water. The remaining four groups were given galangin in doses of 50, 100, 150 and 200 µg/kg bw, respectively, in the same vehicle solution. The sixth group was given a dosage of 100 mg/kg bw of acetylsalicylic acid (A5376, Sigma-Aldrich, Merck, USA). The doses of galangin were selected after conducting pilot experiments. Following the treatments with either galangin, acetylsalicylic acid (ASA), or the vehicle, each mouse received a dosage of 10 mL/kg bw of body weight of acetic acid 0.6% (A6283, Sigma Aldrich, Merck) after 60 minutes. In this experiment, the mice were administered with acetic acid, and the animals were monitored for 30 minutes to record the frequency of complete writhing events. Complete writhing was characterized as body elongation, abdominal contraction, pelvis twisting, and/or trunk twisting accompanied by limb extension. The percentage of inhibition of writhing (PIW) was calculated using the formula: % Inhibition = [(C - T)/C] × 100, [31]. In this formula, C denotes the total number of observed writhing episodes in the control group, while T represents the total number of observed writhing episodes in the treated group.

Hot plate test

For this test, we used eight groups of mice. The first group, which served as the vehicle control group, was given a solution made up of 5% DMSO in distilled water. Groups two to five were treated with galangin at different doses of 50, 100, 150, and 200 µg/kg bw, respectively. Group seven received morphine (M8777, Sigma Aldrich, Merck) that was dissolved in sterile saline and given intraperitoneally at a dosage of 5 mg/kg bw. To investigate the role of the opioidergic system, groups six and eight were given naloxone hydrochloride, a non-selective opioid receptor antagonist, at a dosage of 5 mg/kg bw intraperitoneally, 15 minutes before being treated with galangin (200 µg/kg bw) and morphine (5 mg/kg bw i.p.), respectively. All treatments were administered intraperitoneally, and they were given 60 minutes before the mice were subjected to testing on the hot plate analgesiometer at a temperature of $55 \pm 5^\circ\text{C}$. To determine the reaction time of the mice, we measured the time interval between placing the animal on the hot plate and when it started to paw lick. The reaction time was measured twice: first before treatment and then again 60 minutes after treatment. We expressed the results as the percentage increase in baseline, which was calculated using the following formula: Percentage increase in baseline = ((A-B)/B) × 100. Here, A represents the reaction time after treatment, and B represents the reaction time before treatment [32].

Formalin-induced paw licking test

The experiment involved the use of nine groups of mice. The first group was designated as the vehicle control and received distilled water containing 5% DMSO. The second to fifth groups were given galangin at doses of 50, 100, 150 and 200 µg/kg bw, respectively. The sixth group was administered morphine (5 mg/kg bw), while the seventh group received ASA (100 mg/kg bw). Before administering galangin (200 µg/kg bw) or morphine, group eight was given an opioid receptor antagonist called naloxone hydrochloride (5 mg/kg, i.p. bw), while group nine was given morphine (5 mg/kg bw) to study the function of the opioidergic system. The treatments were given through an intraperitoneal injection, 15 minutes before the administration of the experimental substances. After 60 minutes, we induced pain by injecting 20 µL of 2.5% formalin into the subplantar region of the right hind paw. We assessed the nociceptive response by measuring the time spent by each mouse licking the site where formalin was injected. We recorded the licking times during two phases: the early phase (neurogenic), which was 0 - 5 minutes after formalin injection, and the late phase (inflammatory), which was 15 - 30 minutes after formalin injection. To calculate the percentage of licking inhibition, we used the following formula: PIL = [(Licking time (control) - Licking time (treatment))/Licking time (control)] × 100, [33].

Capsaicin-induced paw licking test

The objective of this experiment was to evaluate the impact of galangin on the vanilloid receptor (TRPV1) and its antinociceptive properties. The experiment consisted of six groups of mice (n= 6 mice/group) that were administered either distilled water with 5% DMSO (group 1 - vehicle control), galangin at various doses (groups 2 - 5 at 50, 100, 150 and 200 µg/kg bw, respectively), or the TRPV1 receptor antagonist, capsazepine (group 6 at a dose of 0.17 mmol/kg bw) through intraperitoneal injection. After one hour, each mouse's right hind paw was injected with 20 µL (1.6 µmol/paw) of capsaicin (211275, Sigma Aldrich, Merck) using the intraplantar route, and the nociceptive response was recorded by measuring the duration of time each mouse spent biting or licking the injection site for a period of 0 to 5 minutes [32].

Glutamate-induced paw licking test

To evaluate the antinociceptive effects of galangin on glutamatergic receptors, an experiment was performed on five groups of mice, each group consisting of six mice. The first group received an intraperitoneal injection of 5% DMSO as a vehicle control, while the remaining four groups were given various doses of galangin (50, 100, 150 and 200 µg/kg bw i.p.). After 60 minutes, 20 µL of glutamate (1446600, Sigma-Aldrich, Merck) was injected into the ventral surface of the right hind paw of each animal. The

mice were observed for 15 minutes, and the amount of time each mouse spent licking and/or biting the glutamate injection site was recorded [34].

Proinflammatory cytokines measurements

3 hours after formalin test, serum was collected and the levels of IL-1 β , TNF- α , IFN- γ , and NO were measured using a two-site sandwich enzyme-linked immunosorbent assay (ELISA) in accordance with the instructions provided by the manufacturer (CUSABIO, Wuhan, China).

Statistical analysis

The Prism 5 software (GraphPad Software, USA) was used to perform data analysis. The results are presented as the mean \pm standard error of the mean (SEM), and any variations between groups were evaluated using one-way ANOVA, and then verified with Tukey's post-hoc test. Any statistical significance was established at $P < 0.05$.

Results and Discussion

Galangin reduced writhing induced by acetic acid

Intraperitoneal administration of galangin at dosages of 100, 150 and 200 $\mu\text{g}/\text{kg}$ bw caused a significant decrease ($P < 0.001$) in the number of acetic acid-induced writhing episodes in mice who received treatment, as compared to the control group (Figure 1). Furthermore, the impact of galangin was observed to be dose-dependent ($P < 0.05$). The group that was administered the highest dose of galangin exhibited a reduction of approximately 72.2% in the percentage of writhing inhibition when compared to the control group ($P < 0.001$). In contrast, the reduction in nociceptive behaviour produced by the reference drug, ASA (100 mg/kg), was around 78.4% when compared to the control group (Figure 1).

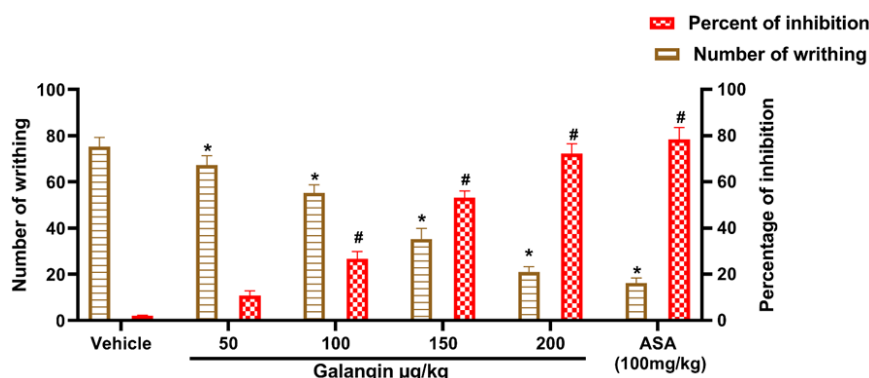


Figure 1:

The effect of galangin on writhing induced by 0.6% acetic acid in mice. The experiment involved six mice that received injections of either 5% DMSO (vehicle), galangin at various doses (50, 100, 150, 200 $\mu\text{g}/\text{kg}$ bw i.p.), or acetylsalicylic acid (ASA) at a dose of 100 mg/kg bw

Mean values \pm SEM were calculated, and statistical analysis demonstrated that galangin led to a significant reduction in the number of writhing compared to the vehicle (* $P < 0.001$). Moreover, the percentage of inhibition caused by galangin was significantly different from the vehicle (# $P < 0.001$)

Galangin increased latency time in the hot plate test

The administration of galangin at various doses (50, 100, 150 and 200 $\mu\text{g}/\text{kg}$ bw) resulted in a significant ($P < 0.001$) increase in the time taken by the animals to lick their posterior paw when placed on a hot plate (Figure 2). The effect of galangin was observed to be dependent on the dose ($P < 0.05$), with the highest dose (200 $\mu\text{g}/\text{kg}$ bw) showing the most significant reduction in pain compared to the control group. The galangin-treated group (200 $\mu\text{g}/\text{kg}$ bw) displayed a 72% increase in baseline latency time, which was statistically significant ($P < 0.001$). However, the reference drug morphine caused a greater increase in latency time (94.4%). In order to investigate how galangin reduces pain, we gave mice naloxone 15 minutes before giving them either 200 $\mu\text{g}/\text{kg}$ bw of galangin or 5 mg/kg bw of morphine. We then tested the mice's response to a hot plate. The results showed that naloxone significantly reduced

the pain-relieving effect of morphine ($P < 0.001$) compared to morphine alone. Similarly, naloxone also significantly reduced the pain-relieving effect of galangin (200 $\mu\text{g}/\text{kg}$ bw) ($P < 0.001$) compared to galangin alone (Figure 2). However, some of the pain-relieving effects of galangin remained even after the administration of naloxone, indicating that galangin may have another mechanism for reducing pain in addition to the opioid system.

Galangin reduced licking time in both the early and late phases after formalin

In this experiment, mice were given varying doses of galangin (100, 150 and 200 $\mu\text{g}/\text{kg}$ bw) and then monitored for decreases in licking times following formalin injection. The results demonstrated that all doses of galangin produced substantial reductions in both the early and late phases of licking times (Figure 3) in a comparable manner. A reference drug, morphine, was also used and resulted in a significant

reduction in both phases. However, acetylsalicylic acid did not display any significant effect on the early phase of licking time (as shown in Figure 3A), but it did lead to a significant reduction in the late phase (P < 0.001, Figure 3B). In order to examine the role of the opioidergic system in the pain-relieving effects of galangin and morphine, mice were administered naloxone 15 minutes before receiving either galangin (200 µg/kg bw) or morphine (5 mg/kg bw) and then subjected to the formalin-induced paw-licking test. The results showed that

when naloxone was given before morphine, it significantly increased the licking time in both the early and late phases in comparison to the group that only received morphine (P < 0.001). On the other hand, when naloxone was given before galangin, the licking times were significantly reduced in both the early and late phases to the same extent (P < 0.001). This suggests that the pain-relieving effects of galangin are achieved through a different mechanism of action than the opioidergic system.

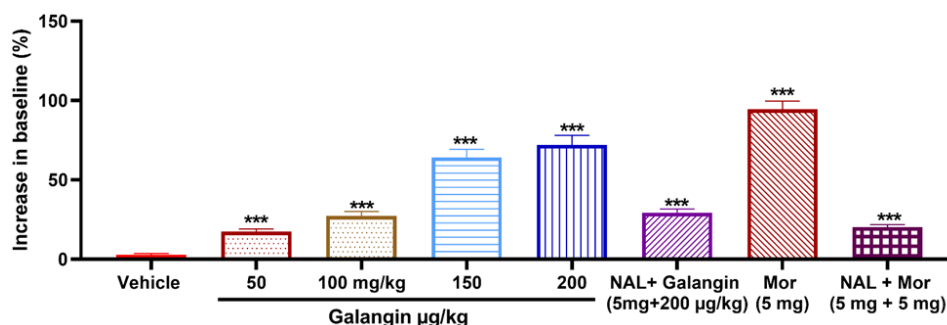


Figure 2.

Six mice were tested using the hot plate method to determine the impact of galangin. Mean values were calculated, along with the standard error of the mean (S.E.M.)

A significant difference was found when comparing to the control (5% DMSO), as indicated by ***P < 0.001. In addition, a significant difference was noted when compared to both 200 µg/kg bw galangin and 5 mg morphine, as denoted by # # #P < 0.001. NAL – naloxone, Mor – morphine

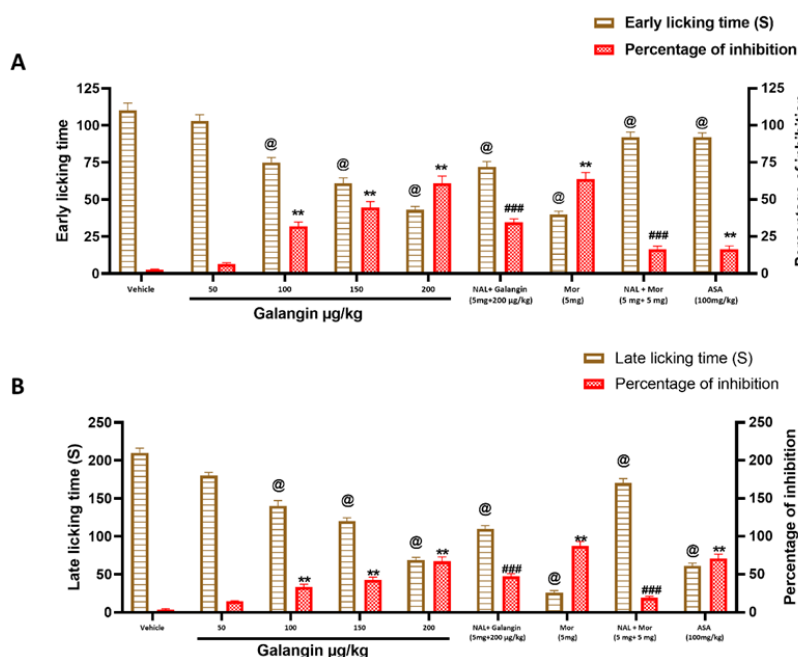


Figure 3.

The results of the experiment that investigated the effects of galangin on early and late phases of paw licking behaviour in mice that were induced with 2.5% formalin. The mice (n = 6) were given various injections, including a control injection of 5% DMSO (vehicle), different doses of galangin, morphine, or acetyl salicylic acid (ASA).

Additionally, a group of mice were given naloxone prior to being injected with either galangin or morphine. The data are presented as mean values with standard error of the mean (SEM). Statistical significance is denoted by the symbols **P < 0.001 and @ P < 0.001, which indicate significant differences in percentage of inhibition and licking time, respectively, compared to the vehicle group. The symbol # # #P < 0.001 indicates significant differences compared to the groups treated with galangin (200 µg/kg bw) or morphine (5 mg/kg bw, i.p.)

Licking time was reduced after the galangin treatment following the capsaicin injection

The study aimed to investigate the potential involvement of the vanilloid system in the antinociceptive effects of galangin. The experiment involved administering different doses of galangin (50, 100, 150 and 200 µg/kg bw) to mice and measuring the licking time. The results showed that all doses of galangin significantly reduced the licking time in mice, with

a dose-dependent effect observed. Additionally, when capsazepine, a capsaicin antagonist, was administered along with galangin, the licking time in mice was significantly reduced by 76.7%. This suggests that galangin may exert antinociceptive effect, at least in part, through the vanilloid system. The statistical significance of the results was determined using appropriate tests, and the mean values with SEM were reported (Figure 4).

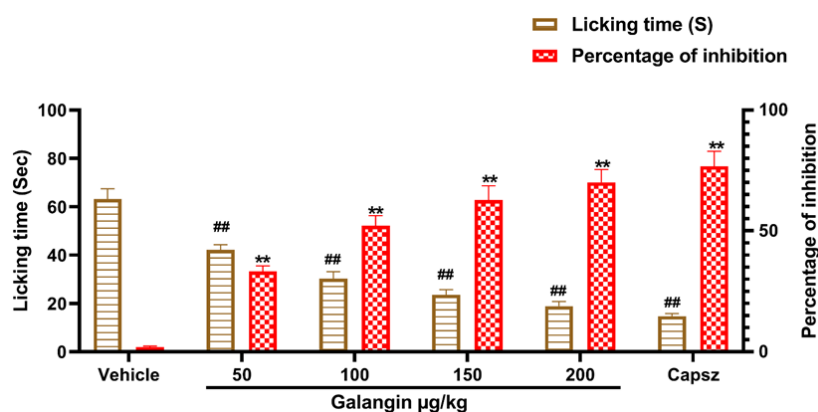


Figure 4.

The impact of galangin on paw licking induced by capsaicin in mice. The study involved six male mice divided into five groups, with each group receiving either 5% DMSO (vehicle), galangin at doses of 50, 100, 150 and 200 µg/kg bw (i.p.), or capsazepine (Capsz, 0.17 mmol/kg bw, i.p.). After 60 minutes, capsaicin was administered to the mice, and the duration of paw licking was noted

The findings revealed that galangin significantly decreased the percentage of inhibition when compared to the control group (indicated by **P < 0.01) and resulted in a substantial difference in licking time compared to the control group (denoted by ##P < 0.01)

Galangin reduced paw licking time after glutamate injection

When mice were administered glutamate and then galangin at doses of 100, 150 and 200 µg/kg bw,

there was a significant reduction (P < 0.001) in the paw licking time, as shown in Figure 5. Furthermore, the effectiveness of galangin was found to be dose-dependent (P < 0.05).

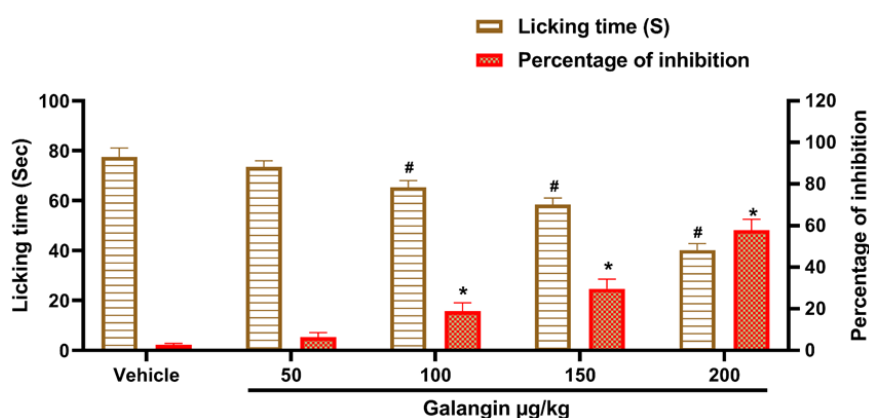


Figure 5.

The impact of galangin on glutamate-induced paw licking in mice. The experiment involved five groups of six male mice, which received either 5% DMSO or galangin at various doses ranging from 50 to 200 µg/kg bw. After an hour, the mice were given 20 µL of glutamate through intraplantar administration into the right hind paw, and the duration of paw licking was observed

Results indicated that galangin caused a significant reduction in the percentage of inhibition compared to the control group (denoted by *P < 0.001), and there was also a significant difference in the licking time (#P < 0.001)

Proinflammatory cytokines levels

In order to investigate the anti-inflammatory and antinociceptive mechanism of galangin, we measured the levels of four pro-inflammatory cytokines (IL-1 β , TNF- α , IFN- γ and NO). As shown in Figure 6, galangin treatment at doses of 100 $\mu\text{g}/\text{kg}$ bw and

200 $\mu\text{g}/\text{kg}$ bw significantly inhibited the levels of IL-1 β and NO. Furthermore, galangin treatment also inhibited the levels of pro-inflammatory cytokines TNF- α and IFN- γ in serum, and this inhibition showed a dose-dependent relationship with galangin.

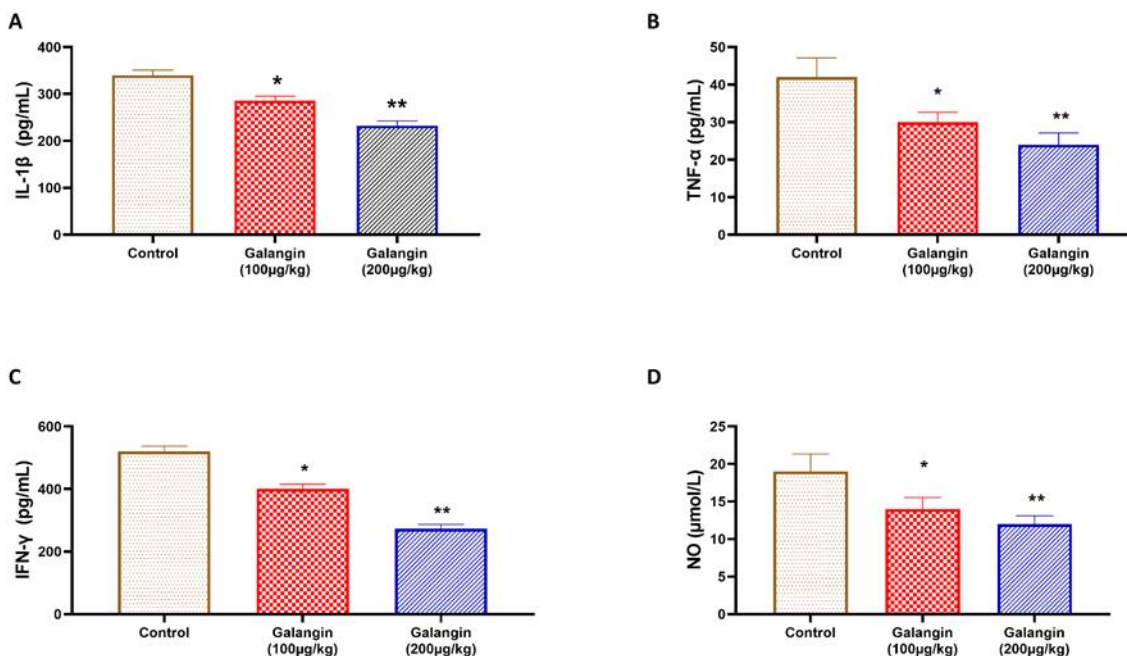


Figure 6.

The effect of galangin on cytokines level

The results indicated that galangin caused a significant decrease in cytokines levels, as indicated by * $P < 0.001$

The study's results demonstrate, for the first time, the antinociceptive effects of galangin. The obtained data suggest that galangin can inhibit nociceptive effects induced by acetic acid, which is a well-known pain inducer. The nociceptive effects of acetic acid are thought to be caused by the release of inflammatory cytokines such as TNF- α , IL-1 β and IL-8, as well as nitric oxide (NO), from peritoneal mast cells and macrophages. These substances activate peripheral nociception receptors in the peritoneum, leading to the sensation of nociceptive pain [35, 36]. Le Bars *et al.* (2001) reported that intraperitoneal (i.p.) administration of acetic acid quickly resulted in nociceptive pain [37-41]. The present study suggests that galangin may have inhibited nociception induced by acetic acid through the suppression of peripheral COX and LOX levels. This, in turn, would have indirectly reduced the production of pain mediators, including prostaglandins, indicating that galangin has a peripheral antinociceptive effect. However, caution must be exercised as other non-analgesic agents such as antihistamines, anticholinergic agents, and muscle relaxants can lead to false-positive results in the acetic acid-induced writhing test [41]. Therefore, we completed our study with additional methods such as formalin and hot plate tests to

determine whether the antinociceptive effects of galangin are mediated centrally or peripherally.

The hot plate test is utilized as a means to evaluate the biological properties of new drugs on the supra-spinal and spinal levels, with the exclusion of inputs from peripheral nociception neurons [42]. When a drug or substance increases the latency of mice in discomfort on the hot plate, it suggests centrally mediated activity, similar to opioids [43, 44]. In the current study, the results demonstrated that galangin caused an increase in the latency of mice in discomfort on the hot plate, indicating the centrally mediated antinociceptive activity of galangin. Based on the outcomes of both the acetic acid-induced writhing test and the hot plate test, it is proposed that galangin may exert both central and peripheral antinociceptive activities.

By performing the formalin induced paw-licking test, galangin effectively suppresses nociceptive responses in both phases, demonstrating its potential as a centrally-acting analgesic agent [45]. This is a significant information because opioids, which act centrally, are capable of inhibiting both phases, whereas drugs that act peripherally only inhibit the second phase (*e.g.*, NSAIDs). Additionally, our study demonstrates that the pre-treatment with the non-

selective opioid antagonist, naloxone, considerably reduced the antinociceptive effect of morphine but only partially inhibited the antinociceptive effect of galangin in the hot plate test and the early phase of formalin-induced nociception. These findings suggest that, apart from the opioidergic system, galangin may engage another mechanism to exert its antinociceptive action.

In order to explore how galangin may affect the perception of pain, we conducted paw-licking tests induced by capsaicin and glutamate. Capsaicin is known to trigger the release of inflammatory mediators from the peripheral nervous system, including neuropeptides, neurokinins, nitric oxide, and excitatory amino acids like glutamate and aspartate. Additionally, capsaicin can transmit nociceptive pain signals from vanilloid receptors to the spinal cord [46]. Inflammatory mediators can stimulate and sensitize vanilloid receptors, creating a cycle that increases the levels of inflammatory mediators and intensifies nociception [47].

Based on our results, galangin demonstrated a significant inhibitory effect on capsaicin-induced paw licking that was comparable to the effect produced by capsazepine, a well-known TRPV antagonist, as illustrated in Figure 5. These results suggest that galangin may have the potential to interfere with pain transmission through vanilloid receptors and block the release or activity of inflammatory agents caused by capsaicin. Furthermore, our observation that galangin can alleviate the writhing and inflammatory responses in the second phase of the formalin test supports the notion that it can interfere with the activity of inflammatory mediators. The reduction in licking time observed with capsazepine in our experiments confirms the validity of the capsaicin-induced pain model and validates the effectiveness of our galangin treatment.

We conducted a glutamate-induced paw-licking test to investigate whether galangin can interfere with the glutamate-mediated pain transmission. Mice displayed licking behaviour after the glutamate administration, but this behaviour was considerably reduced by varying concentrations of galangin. This test is similar to the late phase of the formalin test, which is typically inhibited by glutamate receptor antagonists such as MPEP or CPCO [48]. It is worth mentioning that the impact of galangin was limited to the late phase of the formalin test, and there was no observed effect on the neurogenic early phase.

Our research has provided proof that galangin can hinder pain responses induced by glutamate, which suggests that it can regulate pain transmission through the glutamatergic system. This effect may be due to galangin's interaction with glutamate receptors or its ability to impede the release of NO, which is known to play a role in pain signalling. Nonetheless, further investigations are necessary to fully comprehend the role of NO and its downstream signalling in

galangin's antinociceptive activity. Galangin's mechanism of anti-inflammatory and antinociceptive effects is related to various processes, including cytokine and NO. Galangin was found to inhibit the levels of IL-1 β , TNF- α , IFN- γ , and NO in serum. Therefore, we concluded that galangin is a multi-target drug with anti-inflammatory and antinociceptive properties.

Conclusions

The results of our study demonstrate that galangin possesses antinociceptive effects through various physiological pathways within both the peripheral and central nervous systems. More specifically, galangin functions as an opioid receptor agonist and can inhibit the vanilloid and glutamatergic receptor systems.

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Conflict of interest

The authors declare no conflict of interest.

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