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Original Article

Pennogenin Exerts Anti-inflammatory and Anti-nociceptive Effects in Several Models of Inflammation and Nociception in Mice

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ABSTRACT

Background: The nociceptive pain is a fundamental sensory response that works as a crucial protective mechanism for the human body. Chronic pain constitutes a substantial public health issue, impacting millions of individuals globally. Objectives: The present work focused at investigating the beneficial activities of pennogenin against nociceptive and inflammatory models induced by various stimuli. **Materials and Methods:** In the present study, the Swiss mice were utilized and the nociception was produced in the mice using various chemical stimuli and thermal stimuli techniques. The experimental mice were treated with the pennogenin at 5, 10 and 15 mg/kg concentrations before the stimuli induction. The anti-inflammatory activities of the pennogenin were examined using carrageenan-triggered inflammation model in mice. The inflammatory cytokine levels were assessed using commercial kits. **Results:** The results of this work proved that the pennogenin treatment effectively elevated the reaction time on hot plate, enhanced the response time in the tail immersion time in hot water, reduced the writhing numbers and decreased licking responses in the mice. Moreover, pennogenin treatment also reduced the carrageenan-stimulated paw edema, decreased the peritoneal penetrations of leukocytes and pro-inflammatory cytokine levels in the experimental mice, which indicates the anti-inflammatory potentials of the pennogenin. **Conclusion:** In conclusion, the current findings highlighted the anti-inflammatory and antinociceptive properties of pennogenin in several heat- and chemically-produced pain and inflammation models.

Keywords: Capsaicin, Carrageenan, Hot plate method, Inflammation, Pain response, Pennogenin.

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INTRODUCTION

Acute pain, also known as nociceptive pain, is a fundamental sensory response that works as a crucial protective mechanism for the human body. The nociceptive pain develops from the activation of specialized nerve receptors, which respond to various forms of noxious stimuli, including mechanical, thermal and chemical stimuli. Nociception, the physiological process of detecting and transmitting these harmful sensory inputs, is a complex mechanism that has been the subject of broad study in the area of neuroscience.[1] Acute pain is a common and widespread experience, affecting individuals across the globe. Recent epidemiological works have estimated that the global prevalence of acute pain ranges from 10% to 55% in the general population, with higher rates observed in specific patient populations, such as those undergoing surgical procedures or experiencing injuries. The burden of acute pain is further compounded by its potential to transform into chronic pain, a debilitating condition that can significantly impair an individual's quality of life.[2]

The causes of acute pain are diverse and can be accredited to the several factors, both physiological and pathological. Noxious stimuli, such as mechanical pressure, high temperatures, or chemical irritants, can activate nociceptors and trigger the pain response. Additionally, various pathological conditions, including tissue injury, inflammation and nerve damage, can also lead to the perception of acute pain.[3] Nociception involves a complex interplay between peripheral and central nervous system mechanisms. Nociceptors, specialized sensory receptors found throughout the body, identify harmful stimuli and transmit this information to the brain. The activation of nociceptors stimulates several neurochemical and electrophysiological events, including the release of neurotransmitters and the generation of action potentials, which ultimately lead to the conscious perception of pain.[4] The central processing of nociceptive information involves

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the integration of sensory, emotional and cognitive factors, which can modulate the intensity and duration of the pain experience. Pathological changes in the nervous system, such as central sensitization, can lead to the amplification of pain signals and the onset of chronic pain conditions.[5]

The treatment of nociception, or acute pain, remains a significant challenge in the medical field. Current treatments, such as non-steroidal anti-inflammatory drugs, opioid analgesics and anti-epileptics, often have limited effectiveness or undesirable side effects. To address this challenge, researchers have been exploring the potential of plant bioactive compounds as a new therapeutic approach.[6] One of the primary benefits of plant-derived medicines is their capacity to target multiple mechanisms involved in nociception. For instance, certain plant-derived compounds have been shown to possess anti-inflammatory, analgesic and neuroprotective activities, making them potentially more effective than traditional treatments.[7] Additionally, these natural compounds may have a better safety profile compared to synthetic drugs, as they are often associated with fewer side effects.[8] Pennogenin 3-O-β-chacotrioside (Pennogenin) is the principal bioactive ingredient extracted from Paris polyphylla, a plant utilized in traditional folk medicine throughout Asian nations.[9] It has been already well reported that pennogenin has extensive pharmacological properties including, antitumor,[10] gastroprotective,[11] anti-inflammatory,[12] and anticancer[13] properties.

While the anti-inflammatory and other bioactive effects of pennogenin was well documented, its antinociceptive effects remain unexamined. The present work aims to examine the beneficial activities of pennogenin against nociceptive and inflammatory models induced by various stimuli.

MATERIALS AND METHODS

Chemicals

Pennogenin and other major drugs and chemicals including, diclofenac, morphine, naloxone, dexamethasone, capsaicin, acetic acid, formalin and glutamate were purchased from Sigma-Aldrich, USA. The commercial kits for evaluating biochemical parameters were obtained from Abcam, USA.

Experimental animals

Swiss albino mice weighing 23-30 g was employed in this work. The mice were maintained in a controlled, sterile atmosphere with temperature 23±2°C and 12-hr light/dark sequence, with unrestricted contact to a pellet diet and drinking water. All mice were subjected to a 2-hr fasting period prior to commencing the studies. All studies were performed as per the regulations established by the institutional ethics committee. All mice were acclimatized for a week in a laboratory prior to the commencement of the tests.

Hot plate test

The hot plate technique was conducted to evaluate the nociceptive reactions of the mice.[14] Mice were positioned within the heated surface. The interval between the positioning of mice on the platform and the subsequent licking, shaking, or skipping of their hind feet was meticulously noted as a latency of response. The experimental mice received various dosages of pennogenin (5, 10 and 15 mg/kg) 30 min before to the thermal shock on a hot plate. Morphine was provided as standard drug. Naloxone, an opioid antagonist, was delivered concurrently with pennogenin and morphine to assess the reversal properties. All mice were meticulously observed prior to and at 0, 30th, 60th, 90th and 120th min following the appropriate treatments. The cut-off of 30 sec was established and this exposure duration was adequate to observe any responses from the mice without causing tissue damage.

Tail immersion assay

The primary anti-nociceptive effect of pennogenin was investigated according to the methodology outlined previously.[15] The water bath was calibrated to a specified temperature of 55±0.5°C. Briefly, mice were administered the pennogenin and morphine as outlined in the hot plate assay. Approximately 3 cm of the distal portion of the tail was saturated in hot water. The pain response was observed with the rapid withdrawal of the tail. The duration of tail dipping and removal was meticulously recorded at 30 min prior to and following the 30th, 60th, 90th and 120th min intervals. Due to the potential for tissue injury from prolonged immersion in hot water, a maximum immersion duration of 15 sec was established, with extended immersion times indicating the analgesic impact of pennogenin.

Acetic acid-induced nociception

The antinociceptive efficacy of pennogenin was evaluated by acetic acid-stimulated writhing technique as outlined previously.[16] Mice were administered with 5, 10 and 15 mg/kg of pennogenin and diclofenac as standard drug 30 min prior to the acetic acid administration. The total occurrences of abdominal writhing during the 25 min following the acetic acid induction was recorded and data were compiled.

Capsaicin and glutamate-induced nociception

The antinociceptive effects of pennogenin were evaluated using capsaicin and glutamate-induced techniques, as suggested previously.[17] The mice were received the pennogenin and diclofenac treatments as outlined in the acetic acid assay 1-hr prior to the capsaicin treatment. Animals were individually placed in see-through observation booths to evaluate nociceptive responses and the instances of paw licking was recorded and data were compiled. In a glutamate-induced nociception, mice were treated as outlined in the glutamate assay 1 hr prior to the intraplantar infusion of glutamate. Subsequent to the treatments,

all mice were located in transparent chambers and the total licking incidences was recorded.

Formalin-induced nociception

The formalin-induced nociceptive test was conducted as previously outlined.[18] Briefly, formalin (1%) in saline (0.9%) was injected into the surface of the hind paw of the mice and then placed in see-through observation chambers. Mice were administered with pennogenin (5, 10 and 15 mg/kg) and morphine as standard drug 30 min prior to formalin injection. To assess the antinociceptive activities, the duration of time that mice spent flinching their formalin-injected paw was recorded and data were compiled.

Carrageenan-triggered paw edema

The anti-inflammatory activity of pennogenin was evaluated using carrageenan-triggered paw edema, as outlined previously.[19] The paw edema in mice was evaluated using a plethysmometer, with the volume of right hind paw analyzed before the treatments. Mice were subsequently administered with pennogenin (5, 10 and 15 mg/kg) and indomethacin orally as standard drug 30 min prior to the intraperitoneal treatment of carrageenan. The paw volume of each mice was evaluated 4 hr post-inflammatory induction.

Analysis of peritoneal leukocyte infiltrations

The anti-inflammatory properties of pennogenin were examined by assessing leukocyte infiltrations into the peritoneal cavity. The mice were administered with pennogenin as described in carrageenan-triggered paw edema test. Following 1 hr, the mice were administered with carrageenan (500 µg). Subsequently, the peritoneal fluid was collected following a 6-hr carrageenan administration and the leukocyte concentration within the peritoneal fluid was assessed to evaluate the inflammatory reactions in the experimental mice.

Analysis of inflammatory cytokines

The anti-inflammatory properties of pennogenin was studied in a carrageenan-induced air pouch technique. Before to the commencement of the assay, the mice were anesthetized and their back side was meticulously shaved using sterile razors. The air pouches were created by injecting sterile air into the dorsal region twice for 3 days. Mice with pouches were subjected to carrageenan (0.5 mL administration) to induce an inflammatory reaction, followed by treatment with pennogenin (5, 10 and 15 mg/kg) and dexamethasone as a standard. Following 1 hr, mice were euthanized and air pouches in the mice were administered with saline (2 mL) to collect the inflammatory cells. The TNF-α, IL-1β and IL-6 concentrations were quantified with commercial diagnostic kits (Abcam, USA).

Analysis of behavioral conditions by open field test

The open field assay was utilized to assess the behavioral changes in the pennogenin-treated experimental mice. The mice were administered treated with pennogenin and morphine as outlined in a formalin assay. After 1 hr of treatment, mice were located in an open field apparatus with 50 cm×50 cm×50 cm, divided into 25 cubes. Behavioral alterations in the mice were observed.[20]

Statistical analysis

Data were denoted as Mean±SD derived from three replicate assessments. A one-way ANOVA and Tukey's *post hoc* assay was performed for the statistical analysis of results using GraphPad Prism. A significance of $p<0.05$ was established for comparisons between control and treatment groups.

RESULTS

Effect of pennogenin on hot plate-induced nociception in the experimental mice

The protective properties of pennogenin on nociception triggered by the hot plate in mice were assessed and the findings are displayed in Table 1. The present findings proved that the response time of control mice was comparatively shorter than that of the pennogenin-treated mice. The mice treated with pennogenin (5, 10 and 15 mg/kg) had an increased reaction time on hot plate compared to control, indicating its antinociceptive effects. The morphine also enhanced the reaction time of mice, comparable to the pennogenin. Furthermore, the pennogenin also enhanced the response time of mice when concurrently treated with naloxone. These findings evidenced the antinociceptive properties of the pennogenin.

Effect of pennogenin on tail immersion-induced nociception in the experimental mice

The antinociceptive effects of pennogenin were evaluated using the tail immersion technique and the outcomes are revealed in Table 2. The response times of pennogenin (5, 10 and 15 mg/kg)-treated mice were considerably enhanced on the hot water compared to the control group. The control mice demonstrated a shorter response time than the pennogenin-administered mice. The standard drug morphine also significantly enhanced the tail immersion duration, which is comparable to the effects of pennogenin treatment. Despite the challenge of naloxone, the treatments with pennogenin and morphine enhanced the response duration in hot water, indicating the antinociceptive efficacy of pennogenin (Table 2).

Effect of pennogenin on carrageenan-stimulated inflammatory response

Anti-inflammatory activities of the pennogenin on a carrageenan-triggered paw edema in experimental mice were examined and data were presented in Table 3. The pennoogenin

administration at 5, 10 and 15 mg/kg dosages remarkably reduced the carrageenan-triggered paw edema in mice. In comparison with 5 and 10 mg/kg of pennogenin, the 15 mg/kg dosage of pennogenin shown superior efficacy and the results were strongly supported by the results of standard drug indomethacin treatment.

Effect of pennogenin on acetic acid-induced nociception in the experimental mice

Figure 1 illustrates the findings of the pennogenin treatment on an acetic acid-stimulated nociception in mice. The findings showed that the acetic acid-treated mice had enhanced writing numbers. Whereas, the treatment of 5, 10 and 15 mg/kg of pennogenin considerably diminished the frequency of writhing in acetic acid-induced mice. The diclofenac treatment also reduced the frequency of writhing in acetic acid-induced mice. The results of pennogenin and standard drug diclofenac administrations were comparable, indicating the antinociceptive activities of pennogenin (Figure 1).

Effect of pennogenin on glutamate-induced nociception in mice

The protective activities of pennogenin on the glutamate-induced nociception in mice were examined and the findings are illustrated in Figure 2. The glutamate-induced mice exhibited an elevated frequency of licking, indicating the presence of pain experience. In contrast, pennogenin treatment at 5, 10 and 15 mg/kg concentrations significantly decreased the licking frequency in the glutamate-challenged mice (Figure 2). Both diclofenac and pennogenin treatments significantly decreased the licking incidences in the glutamate-induced mice, which proves its antinociceptive properties.

Effect of pennogenin on capsaicin-induced nociception in mice

Figure 3 illustrates that the capsaicin administration significantly increased the licking frequency in the experimental mice. Whereas, the treatment of pennogenin at dosages of 5, 10 and 15 mg/kg respectively, remarkably diminished the capsaicin-induced licking responses in the experimental mice, indicating its antinociceptive properties. The standard drug diclofenac also reduced the licking frequency in the experimental mice that supports the effects observed with 15 mg/kg pennogenin treatment (Figure 3).

Effect of pennogenin on formalin-induced nociception in mice

The protective properties of pennogenin on a formalin-induced biphasic nociceptive response in the experimental mice were presented in Figure 4. The mice treated with formalin exhibited an elevated frequency of licking in both phases, indicating the onset of nociception. Whereas, the treatment of 5, 10 and 15 mg/

Table 1: Effect of pennogenin on hot plate-induced nociception in the experimental mice.

Treatment (mg/kg)	Pre-treatment	Response time (s) (% MPE)			
		30 min	60 min	90 min	120 min
Control	11.06±0.32	12.30±0.14	12.14±1.81	11.16±0.45	16.41±0.19
Pennogenin (5 mg)	12.73±0.35	14.11±0.46 (15.32)	17.22±0.25 (22.55) #	18.12±0.50 (23.32) *	18.48±0.27 (36.26) *
Pennogenin (10 mg)	10.68±0.33	14.02±0.44 (19.36)	17.62±0.45 (32.66) #	19.52±0.19 (56.48) *	18.76±0.25 (26.56) *
Pennogenin (15 mg)	11.33±0.20	15.33±0.10 (23.66)	16.51±0.26 (45.26) #	20.81±0.50 (56.32) *	20.90±0.62 (86.44) *
Morphine (5 mg)	10.96±0.74	18.45±0.25 (22.33)	19.99±0.64 (65.44) #	22.04±0.48 (26.56) *	23.36±0.50 (31.96)*
NLX (2 mg)+Control	11.86±0.36	13.65±0.40	14.64±0.33	15.48±0.22	14.34±0.24
NLX (2 mg)+Pennogenin (5 mg)	11.59±0.33	15.45±0.26 (33.48)	13.32±0.31 (16.25) #	16.35±0.11 (19.45) *	18.40±0.17 (45.65) *
NLX (2 mg)+Pennogenin (10 mg)	11.52±0.25	13.40±0.32 (32.39)	13.95±0.24 (17.32) #	16.69±0.26 (55.96) *	18.52±0.15 (65.48) *
NLX (2 mg)+Pennogenin (15 mg)	12.46±0.22	13.36±0.15 (21.22)	12.91±0.52 (23.33) #	17.40±0.10 (45.15) *	19.48±0.18 (24.15) *
NLX (2 mg)+Morphine (5 mg)	11.66±0.29	13.42±0.12 (15.96)	15.87±0.36 (20.32) #	17.56±0.34 (19.89) *	21.44±0.29 (55.54) *

Data were denoted as mean±SD derived from three replicate assessments. A one-way ANOVA and Tukey's *post hoc* assay was performed using GraphPad Prism to scrutinize the data. Note: '#' and '*' represents the statistical significance level at $p<0.01$ and $p<0.05$, respectively when compared between treatment groups.

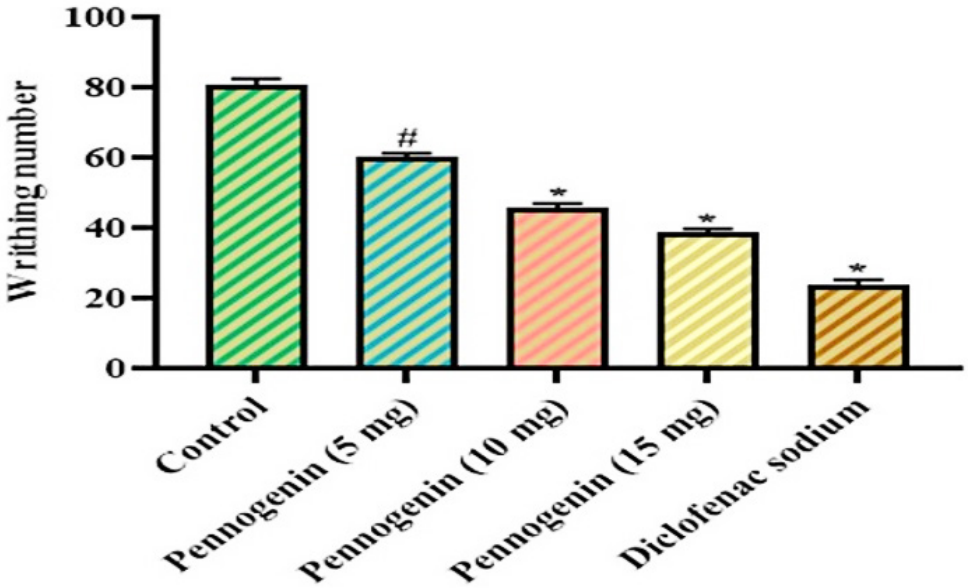

Figure 1: Effect of pennogenin on the acetic acid-induced nociception in the experimental mice. Data were denoted as mean±SD derived from three replicate assessments. A one-way ANOVA and Tukey's *post hoc* assay was performed using GraphPad Prism to scrutinize the data. Note: '#' and '*' represents the statistical significance level at $p<0.01$ and $p<0.05$, respectively when compared between treatment groups.

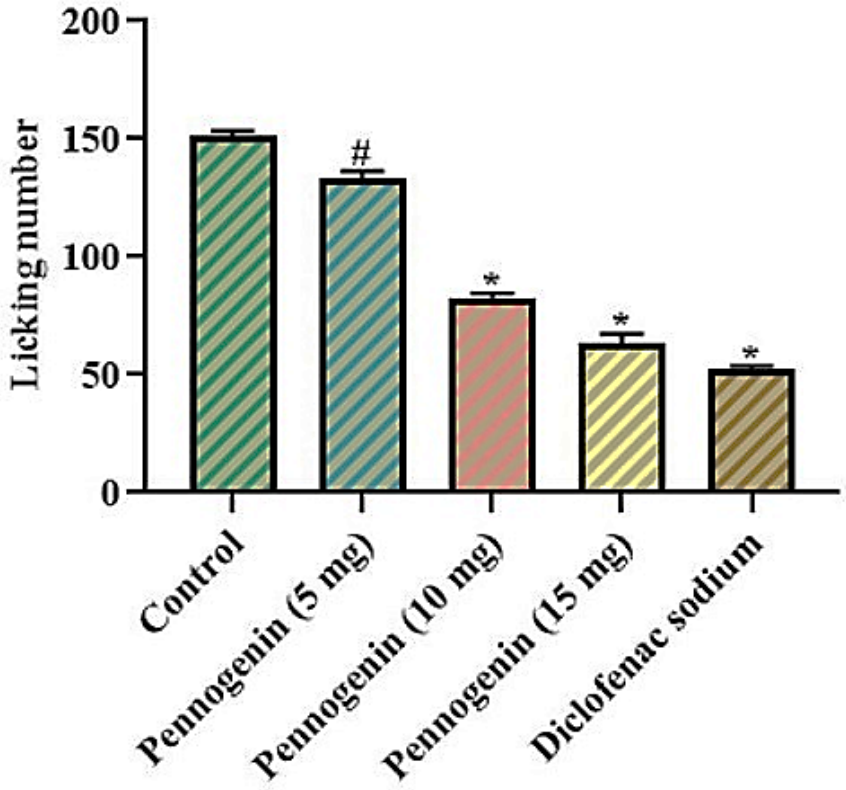

Figure 2: Effect of pennogenin on the glutamate-induced nociception in mice. Data were denoted as mean±SD derived from three replicate assessments. A one-way ANOVA and Tukey's *post hoc* assay was performed using GraphPad Prism to scrutinize the data. Note: '#' and '*' represents the statistical significance level at $p<0.01$ and $p<0.05$, respectively when compared between treatment groups.

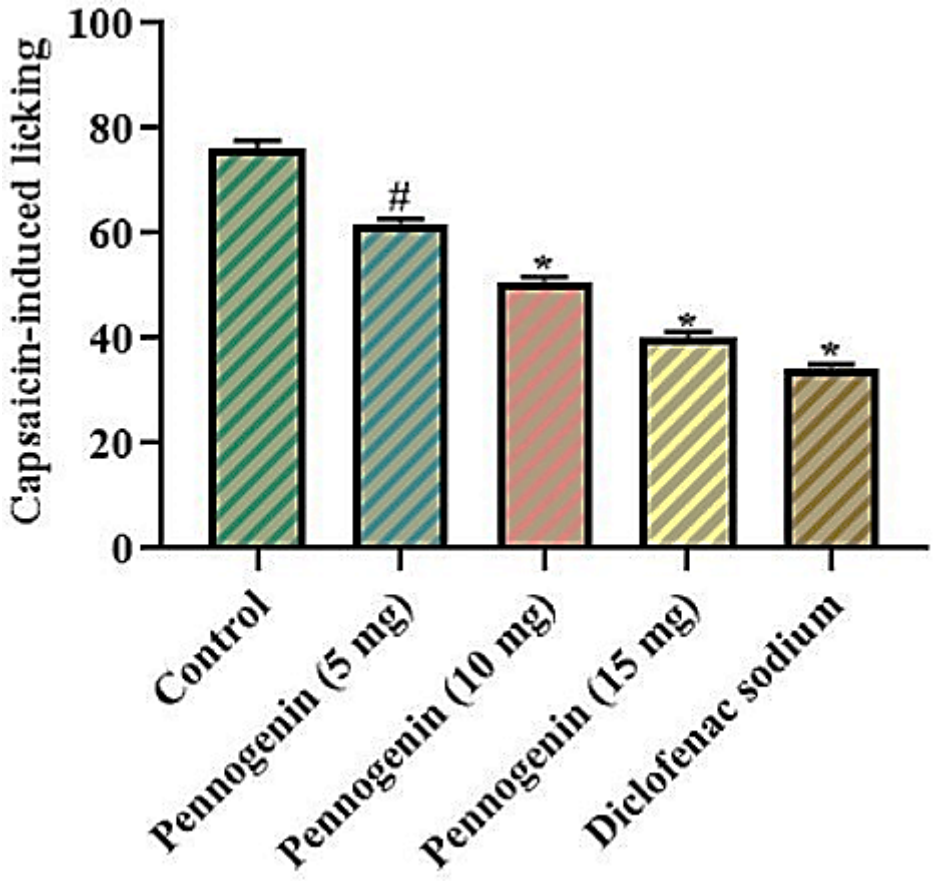

Figure 3: Effect of pennogenin on the capsaicin-induced nociception in mice. Data were denoted as mean±SD derived from three replicate assessments. A one-way ANOVA and Tukey's *post hoc* assay was performed using GraphPad Prism to scrutinize the data. Note: '#' and '*' represents the statistical significance level at $p<0.01$ and $p<0.05$, respectively when compared between treatment groups.

kg of pennogenin considerably diminished the formalin-induced licking incidences. Similarly, the morphine also diminished the frequency of licking induced by formalin (Figure 4).

Effect of pennogenin on carrageenan-initiated leukocyte penetrations in the experimental mice

The protective effects of pennogenin on the carrageenan-initiated leukocyte penetrations in the experimental mice were evaluated and the data were given in Figure 5. The present findings indicated that the carrageenan-induced mice exhibited elevated leukocytes, mononuclear cells and polymorphonuclear cell infiltrations, which confirm the onset of inflammation. However, the treatment pennogenin at 5, 10 and 15 mg/kg concentrations significantly inhibited the peritoneal leukocyte, polymorphonuclear and mononuclear cell penetrations (Figure 5). The administration of standard drug morphine also reduced the carrageenan-induced leukocyte infiltrations, which highlights the anti-inflammatory properties of the pennogenin.

Effect of pennogenin on the inflammatory cytokine levels

Figure 6 illustrates a remarkable elevation in the IL-1β, IL-6 and TNF-α concentration in a carrageenan-induced air pouch model. In contrast, these augmentations were significantly diminished by the pennogenin treatment. The pennogenin at 5, 10 and 15 mg/kg concentrations remarkably reduced the IL-1β, IL-6 and TNF-α concentration in the carrageenan-induced mice. In similar manner, standard drug dexamethasone also effectively reduced the levels of these cytokines, which is comparable to the results of 15 mg/kg of pennogenin.

Effect of pennogenin on the behavioral changes in the experimental mice

The effects of pennogenin on the behavioral alterations in experimental mice were investigated by open field assay and the outcomes are displayed in Figure 7. The mice administered with 5, 10 and 15 mg/kg of pennogenin exhibited a relatively reduced walking square numbers compared with control group, indicating mild sedative activities. The standard drug morphine also considerably diminished the number of walked squares

because of its sedative activities, which supports the activity of pennogenin (Figure 7).

DISCUSSION

The underlying pathways and mechanisms involved in nociception are crucial to understanding the various causes and manifestations of pain, which can range from acute, localized discomfort to debilitating chronic conditions.[21] Chronic pain constitutes a substantial public health issue, impacting millions of individuals globally.[22] Animal models have played a crucial role in advancing our knowledge on the underlying mechanisms of pain perception and the advancement of novel therapies.[23] One such model, the hot plate-induced nociception mice model, has gained considerable attention for its applications and importance in pain research.[24] The hot plate-induced nociception mouse model is a well-established experimental paradigm used to study acute thermal nociception, a type of pain response evoked by exposure to high temperatures. In this model, animals are located on a hot plate and their behavioral responses, such as paw licking, jumping, or withdrawal, are monitored as an indicator of their pain threshold. This model has been widely employed to examine the physiological and pharmacological mechanisms participated in pain processing, as well as to evaluate the efficacy of analgesic compounds in alleviating thermal-induced pain[25] One of the primary applications of the hot plate-induced nociception mice model is in the screening and development of new pain-relieving drugs. By assessing the effects of various pharmacological agents on the pain responses of mice, researchers can identify potential therapeutic candidates and gain insights into their mechanisms of action.[26] In this work, the protective activities of pennogenin on nociception triggered by the hot plate in mice were assessed. The findings clearly proved that the response time of experimental mice on the hot plate was significantly increased by the pennogenin treatment when compared to the control, indicating its antinociceptive effects.

The tail immersion-triggered nociception model in mice is a widely used experimental approach for studying pain perception and the underlying mechanisms. This model involves immersing the mouse's tail in warm water, which triggers a nociceptive reaction and measuring the latency to removal of the tail. This method has been extensively utilized to investigate various aspects of pain, including the evaluation of analgesic drugs, the study of cold pain pathways and the assessment of sensitization processes.[27] One of the key applications of the tail immersion-induced nociception model is the evaluation of analgesic drugs. The nociceptive withdrawal reflex, which is the spinal reflex evoked by painful stimuli, can be quantified using this model, providing a reliable measure of the excitability of the pain system. By administering potential analgesic compounds and measuring changes in the withdrawal latency, researchers can assess the efficacy of these drugs in reducing pain perception.[28] In this work, the antinociceptive properties of pennogenin was studied using tail immersion technique. The reaction times of mice treated with pennogenin were significantly enhanced in the tail immersion assay in hot water than the control, indicating the antinociceptive efficacy of pennogenin.

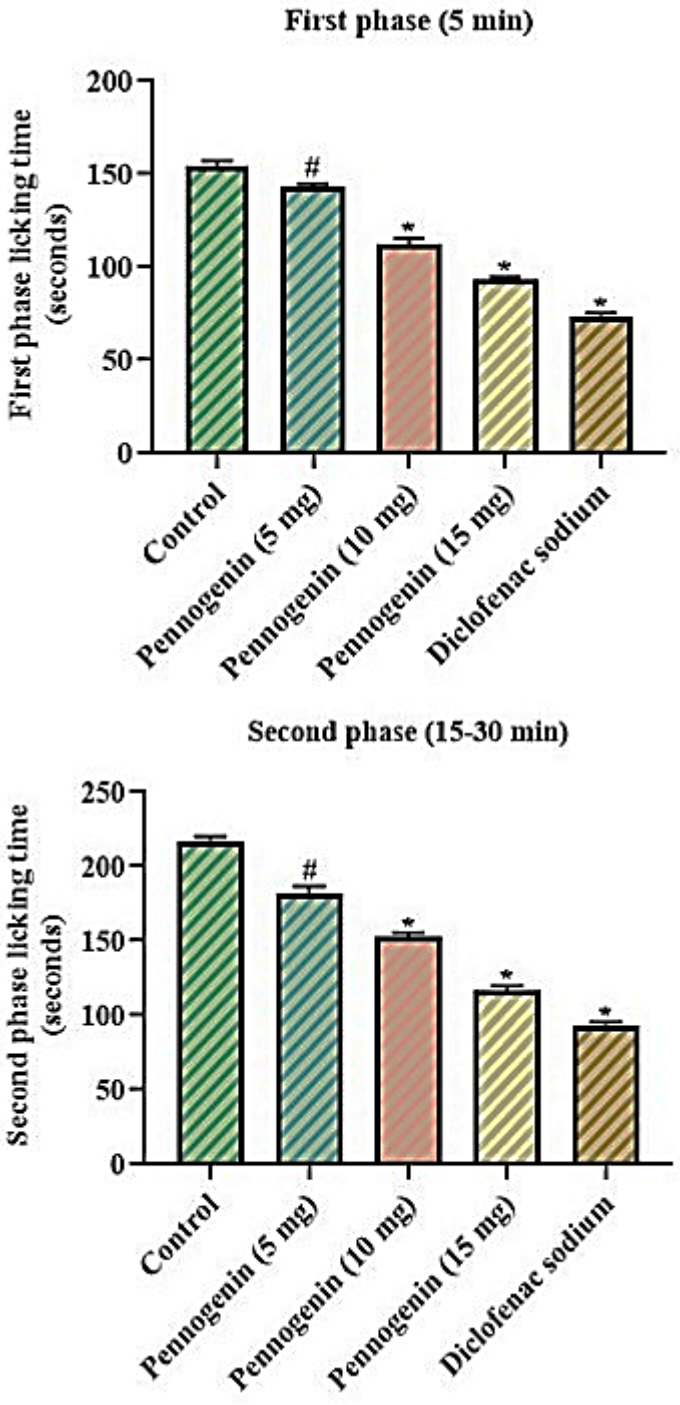

Figure 4: Effect of pennogenin on the formalin-induced nociception in mice. Data were denoted as mean±SD derived from three replicate assessments. A one-way ANOVA and Tukey's *post hoc* assay was performed using GraphPad Prism to scrutinize the data. Note: '#' and '*' represents the statistical significance level at $p<0.01$ and $p<0.05$, respectively when compared between treatment groups.

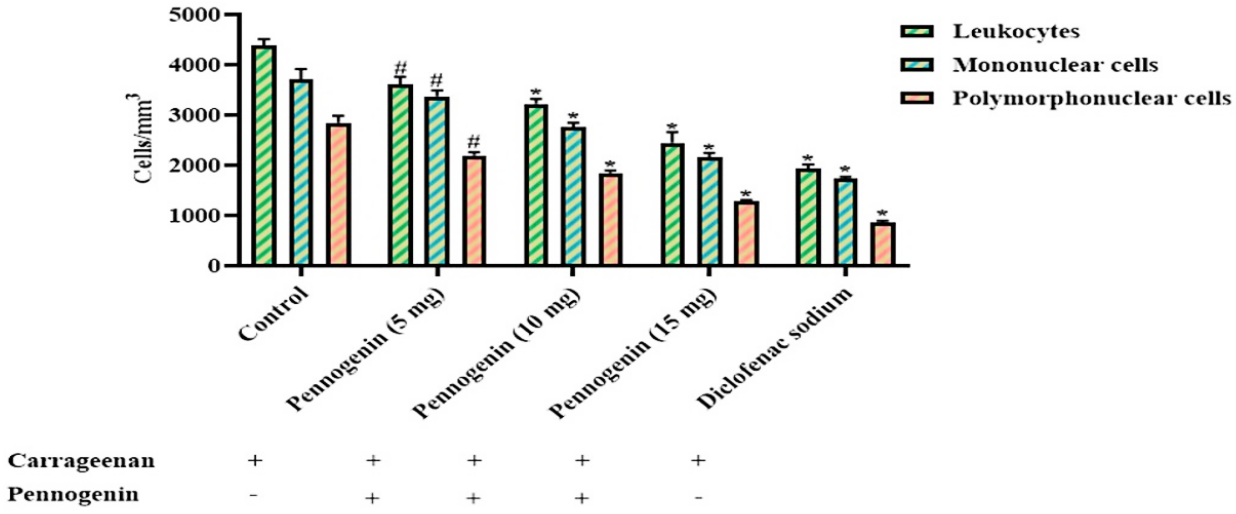

Figure 5: Effect of pennogenin on the carrageenan-induced peritoneal leukocyte infiltration in the experimental mice. Data were denoted as mean±SD derived from three replicate assessments. A one-way ANOVA and Tukey's *post hoc* assay was performed using GraphPad Prism to scrutinize the data. Note: '#' and '*' represents the statistical significance level at $p<0.01$ and $p<0.05$, respectively when compared between treatment groups.

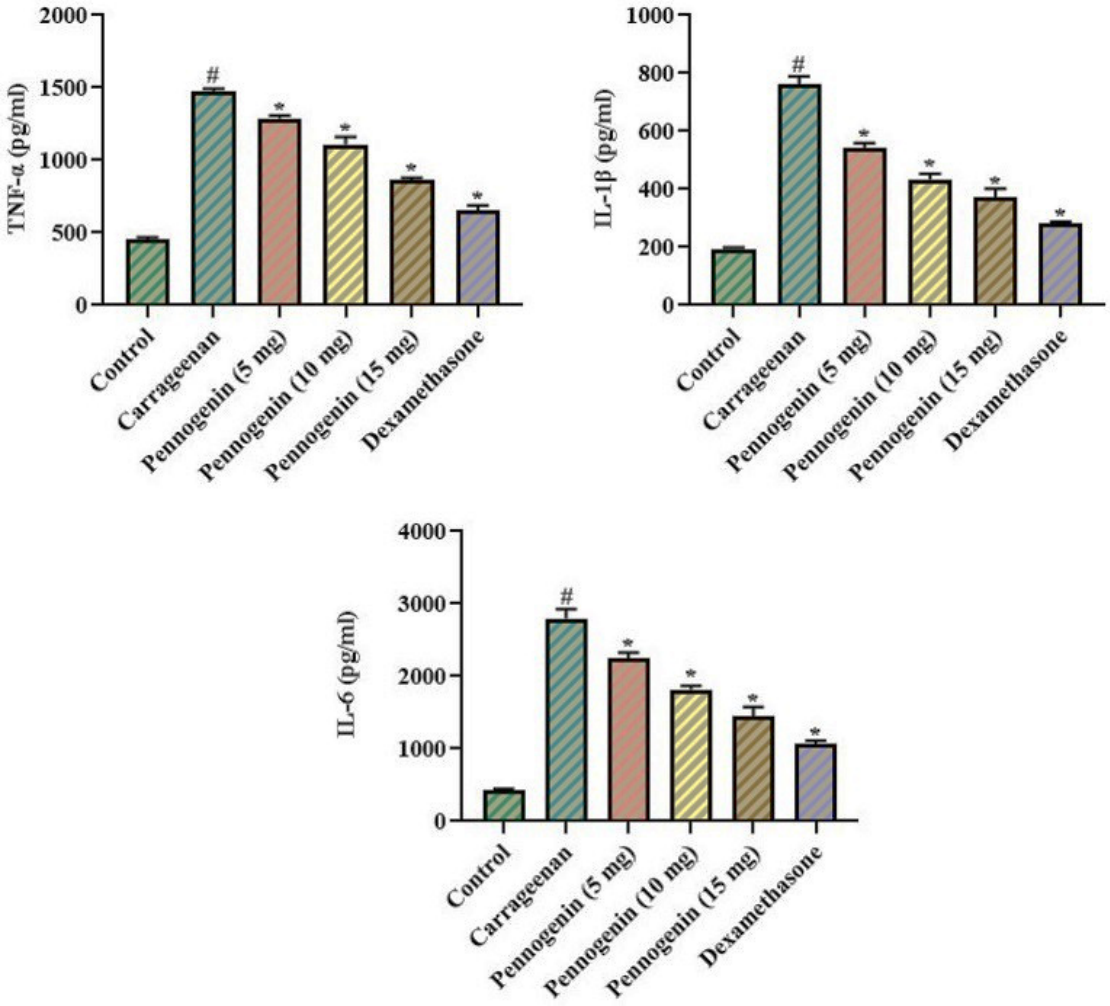

Figure 6: Effect of pennogenin on the pro-inflammatory cytokine levels in the carrageenan-induced air pouch mice model. Data were denoted as mean±SD derived from three replicate assessments. A one-way ANOVA and Tukey's *post hoc* assay was performed using GraphPad Prism to scrutinize the data. Note: '#' and '*' represents the statistical significance level at $p<0.01$ and $p<0.05$, respectively when compared between treatment groups.

The carrageenan-triggered paw edema model is a widely utilized experimental approach to study the anti-inflammatory and analgesic activities of various samples. This model has recognized as be a valuable tool for studying the processes underlying inflammatory processes and evaluating the therapeutic potential of new drug candidates.[29] One of the key applications of the carrageenan-triggered paw edema model is in the assessment of the anti-inflammatory property of drugs and natural compounds. This model allows researchers to measure the degree of swelling and inflammation in the mice paw following the carrageenan administration.[30] Carrageenan is known to induce a biphasic inflammatory response, which mimics the pathological processes observed in various inflammatory disorders. By monitoring the changes in paw volume or weight over time, researchers can quantify the anti-inflammatory effects of their test compounds, giving valuable understandings into their mode of action the novel therapies.[31,32] The anti-inflammatory activities of pennogenin was analyzed in this work by using the carrageenan-triggered paw edema in experimental mice. The treatment of pennogenin significantly reduced the carrageenan-triggered paw edema in mice, which indicates the anti-inflammatory properties of the pennogenin.

The acetic acid-triggered writhing assay is a widely used model of visceral pain, where the administration of acetic acid induces a characteristic abdominal constriction response in mice. This model is particularly useful for evaluating the analgesic properties of various compounds and investigating the underlying mechanisms of visceral pain.[33] Glutamate, a key neurotransmitter in the nociceptive pathway, has been extensively studied using mouse models. Intrathecal or intraplantar injection of glutamate can induce nociceptive behaviors, such as paw licking and lifting, which serve as a proxy for pain perception. These models provide insights into the role of glutamatergic signaling in pain processing and the potential therapeutic targeting of this system.[34] Capsaicin, the active component in chili peppers, is a potent agonist of the transient receptor potential vanilloid 1 channel, a key player in nociception. Topical or systemic administration of capsaicin in mice can elicit nociceptive behaviors, leading to the characterization of the underlying mechanisms and the evaluation of novel analgesic strategies.[35] The formalin-induced nociception model is another widely used tool in pain research. Formalin injection into the hind paw of mice leads to a biphasic nociceptive response. This model allows for the assessment of the differential activities of pharmacological agents on acute and inflammatory pain.[36] These mouse models of nociception have been instrumental in advancing our knowledge on pain pathways and the advancement of novel analgesic strategies.[37] In the current work, the antinociceptive activities of the pennogenin

Table 2: Effect of pennogenin on the tail immersion-induced nociception in the experimental mice.

Treatment (mg/kg)	Pre-treatment	Response time (s) (%MPE)			
		30 min	60 min	90 min	120 min
Control	7.33±0.76	9.09±0.04	9.76±0.25	8.62±0.32	8.21±0.16
Pennogenin (5 mg)	7.59±0.27	9.43±0.20 (12.32)	9.31±0.24 (15.28)	8.73±0.43 (10.23)	10.50±0.30 (16.65)
Pennogenin (10 mg)	7.55±0.28	9.48±0.30 (15.25)#	10.56±0.18 (11.24)*	10.57±0.26 (12.33) *	10.68±0.18 (14.17)*
Pennogenin (15 mg)	8.33±0.25	7.23±0.13 (9.25) #	10.81±0.12 (23.22) *	11.71±0.46 (15.36)*	11.98±0.44 (12.09)*
Morphine (5 mg)	8.28±0.19	10.34±0.28 (20.32)#	10.64±0.26 (28.25) *	12.04±0.76 (65.89) *	8.54±0.07 (24.36) *
NLX (2 mg)+Control	8.42± 0.05	8.37±0.19	8.39±0.05	9.25±0.13	9.45±0.23
NLX (2 mg)+Pennogenin (5 mg)	8.23±0.04	9.47±0.20 (16.39) #	9.41±0.24 (12.15) *	9.56±0.24 (12.23) *	9.55±0.21 (11.23) *
NLX (2 mg)+Pennogenin (10 mg)	8.06±0.03	11.65±0.26 (19.58) #	11.58±0.25 (14.65) *	11.53±0.22 (13.96) *	13.41±0.59 (17.89) **
NLX (2 mg)+Pennogenin (15 mg)	8.15±0.01	9.38±0.24 (13.26) #	9.26±0.12 (11.28) *	9.28±0.55 (15.66) *	10.60±0.29 (16.46) *
NLX (2 mg)+Morphine (5 mg)	8.05±0.80	8.31±0.16 (10.99) #	8.40±0.16 (10.65) *	8.23±0.14 (10.88) *	9.33±0.13 (12.56) *

Data were denoted as mean±SD derived from three replicate assessments. A one-way ANOVA and Tukey's *post hoc* assay was performed using GraphPad Prism to scrutinize the data. Note: '#' and '*' represents the statistical significance level at $p<0.01$ and $p<0.05$, respectively when compared between treatment groups.

was assessed using several chemicals-triggered nociceptive models in the experimental mice. The results of this work demonstrated that the pennogenin significantly reduced the acetic acid-triggered writhing numbers, glutamate-triggered licking incidences, capsaicin-triggered licking responses and formalin-triggered licking numbers in the mice. These findings evidenced the antiniciceptive properties of the pennogenin.

The analysis of carrageenan-induced leukocyte infiltrations in mice model is a valuable tool in understanding the underlying mechanisms of inflammation and immune responses. Carrageenan is a widely used phlogistic agent that induces a robust inflammatory reaction when administered into the peritoneal cavity of mice. This model allows for the study of both mononuclear cells, such as monocytes and lymphocytes, as well as polymorphonuclear cells, primarily neutrophils, that infiltrate the site of inflammation.[38] The infiltration of these leukocyte subsets is a hallmark of the inflammatory process and plays an essential role in the body's defense mechanism against infection, trauma and injury. Understanding the dynamics and regulation of this cellular response is essential for developing novel therapeutic interventions for a wide range of inflammatory conditions.[39] The present findings indicated that the carrageenan-induced mice exhibited elevated peritoneal leukocyte penetrations, which confirms the onset of inflammation. Interestingly, pennogenin treatment significantly inhibited the peritoneal leukocyte, polymorphonuclear and mononuclear cell penetrations in the experimental mice, which highlights the anti-inflammatory properties of the pennogenin.

Emerging evidence suggests that inflammatory responses and specific cytokines, such as TNF-α, IL-1β and IL-6 play an essential role in the pathophysiology of pain.[40] The biochemical origin of pain can be traced back to inflammation and the inflammatory response. Inflammation is a crucial biological reaction triggered by harmful stimuli, such as damaged cells or pathogens, with the goal of eliminating the source of the injury and initiating the healing process. In the context of pain, this inflammatory response can result in the activation of pain receptors, the regulation and transmission of pain signals and ultimately, the

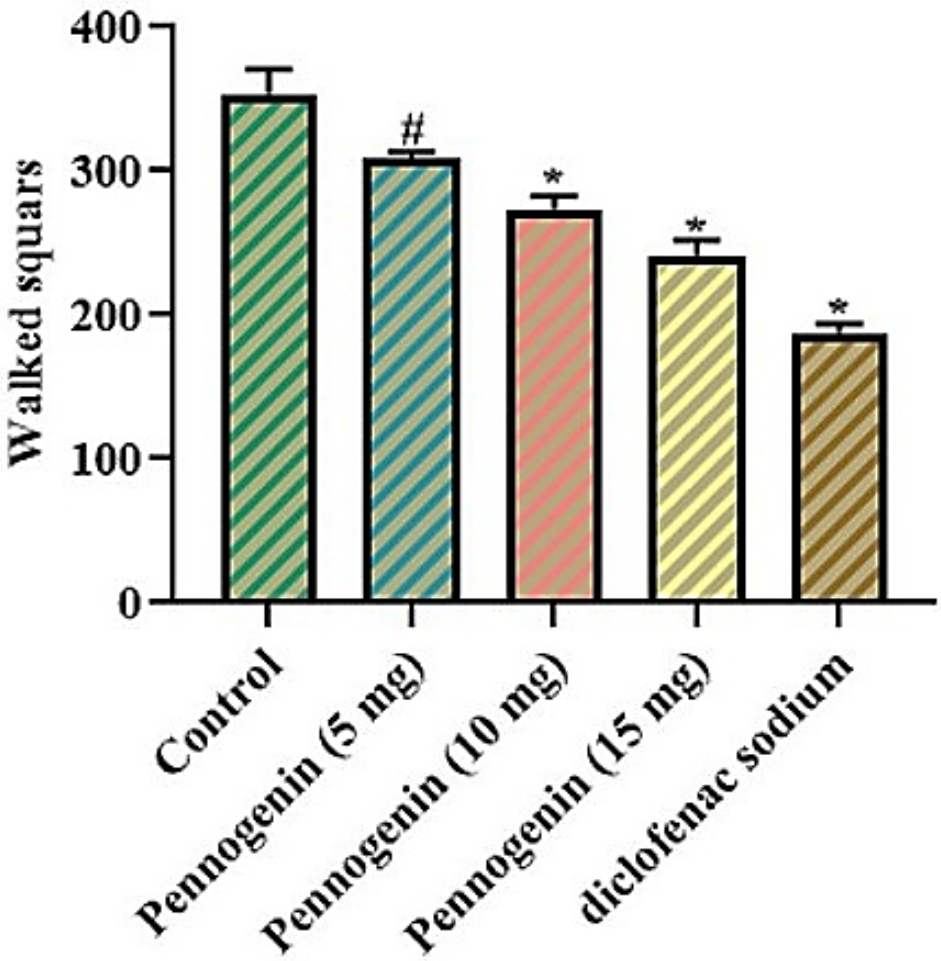

Figure 7: Effect of pennogenin on the behavioral changes in the experimental mice assessed by the open field test. Data were denoted as mean±SD derived from three replicate assessments. A one-way ANOVA and Tukey's *post hoc* assay was performed using GraphPad Prism to scrutinize the data. Note: '#' and '*' represents the statistical significance level at $p<0.01$ and $p<0.05$, respectively when compared between treatment groups.

Table 3: Effect of pennogenin on the carrageenan-induced inflammatory response in the experimental mice.

Treatment (mg/kg)	Response time (s) (%MPE)				
	Basal	1st hr	2nd hr	3rd hr	4th hr
Control	53.34±2.20	178.49±1.95	164.95±1.76	154.49±2.07	127.09±2.13
Pennogenin (5 mg)	59.27±0.60	115.75±2.45 (15.32%)	113.65±2.70 (52.32%)#	108.08±2.85 (91.32%)*	101.74±1.28 (39.96%)*
Pennogenin (10 mg)	51.16±1.36	108.18±2.22 (31.56%)	108.07±1.88 (89.65%)#	94.52±0.85 (41.25%)*	91.08±0.58 (50.26%)*
Pennogenin (15 mg)	46.80±1.28	113.88±2.86 (71.62%)	97.86±1.22 (28.52%)#	89.90±0.44 (35.26%)*	84.05±1.65 (79.89%)*
Indomethacin (10 mg)	56.49±1.61	94.76±0.59 (25.36%)	88.04±3.15 (69.36%)#	87.36±1.39 (26.48%) *	80.40±0.99 (49.25%)*

Data were denoted as mean±SD derived from three replicate assessments. A one-way ANOVA and Tukey's *post hoc* assay was performed using GraphPad Prism to scrutinize the data. Note: '#' and '*' represents the statistical significance level at $p<0.01$ and $p<0.05$, respectively when compared between treatment groups.

development of peripheral and central sensitization.[41] IL-6 has been identified as an emerging regulator of pathological pain, which is characterized by a heightened sensitivity and increased reaction to toxic stimuli. Furthermore, TNF-α and IL-1β have been shown to directly sensitize and activate nociceptors, contributing to the development of hyperalgesia and allodynia.[42,43] The carrageenan-triggered air pouch in mice is a well-established method to study the inflammatory response and the involvement of these cytokines.[44] In this model, an air pouch is formed by administering air under the skin of the dorsal portion of mice, which is then challenged with an inflammatory stimulus, such as carrageenan. This leads to the onset of a localized inflammatory response, characterized by fluid accumulation, inflammatory cells and the generation of various mediators, including cytokines.[45] In this study, the considerable increase in the IL-1β, IL-6 and TNF-α concentrations was noted in a carrageenan-challenged mice. Captivatingly, the pennogenin substantially reduced the TNF-α, IL-1β and IL-6 concentrations, which highlights the anti-inflammatory activities of the pennogenin.

The open field test is a widely utilized technique in the field of neuroscience and behavioral research to assess various aspects of animal behavior, particularly in mice. This technique involves placing a mouse in a novel, enclosed arena and observing its spontaneous activity and exploration patterns, which can provide valuable insights into the animal's emotional state, locomotor abilities and response to novelty.[46] One of the primary applications of the open field test is its capacity to detect subtle changes in mouse behavior that may be indicative of underlying neurological or physiological conditions. For example, the test can be used to assess the impact of various genetic manipulations, pharmacological interventions, or environmental factors on the animal's exploratory behavior, anxiety-like behaviors and overall locomotor activity. By analyzing parameters such as the traveled distance and the number of rearings or grooming episodes, researchers can gain a better understanding of how these factors influence the mouse's behavioral repertoire.[47] In the current study, the activity of pennogenin on the behavioral alterations in the experimental mice were studied by open field assay. The mice treated with pennogenin exhibited a relatively reduced number of walking squares, suggesting mild sedative properties. Overall, the findings of the various assays conducted in the present study clearly proved that the pennogenin has the potent anti-inflammatory and antinociceptive activities.

CONCLUSION

In conclusion, the current findings highlighted the anti-inflammatory and antinociceptive properties of pennogenin in several heat- and chemically-produced pain and inflammation models. Pennogenin treatment significantly inhibited nociceptive responses induced by various chemical and thermal stimuli and inflammation caused by carrageenan in mice. These findings further warrant the more research in the future to enhance the understanding of therapeutic effects of pennogenin.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

TNF-α: Tumor necrosis factor-α; **IL-1β:** Interleukin-1beta; **IL-6:** Interleuikin-6; **ANOVA:** Analysis of variance.

SUMMARY

Acute pain, also known as nociceptive pain, is a fundamental sensory response that works as a crucial protective mechanism for the human body. Noxious stimuli, such as mechanical pressure, high temperatures, or chemical irritants, can activate nociceptors and trigger the pain response. The result of the

present research work has proved the anti-inflammatory and antinociceptive properties of pennogenin in several heat- and chemically-produced pain and inflammation models.

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