Heliyon 10 (2024) e34870

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Solanesol alleviates CFA-induced chronic inflammatory pain via inhibition of proinflammatory cytokines in spinal glial cells

Yuan-yuan Wang [a,1], Yi-fan Li [b,1], Zhen-feng Zhou [c,*]

[a] Department of Endocrinology, Xixi Hospital of Hangzhou, Hangzhou, China
[b] School of Basic Medical Sciences, Hangzhou Normal University, Hangzhou, China
[c] Department of Anesthesiology, Hangzhou Women's Hospital (Hangzhou Maternity and Child Health Care Hospital, Hangzhou First People's Hospital Qianjiang New City Campus, The Affiliated Women's Hospital of Hangzhou Normal University), Hangzhou, China

ARTICLE INFO

Keywords:
Solanesol
Chronic inflammatory pain
Spinal glial cells
Proinflammatory cytokines

ABSTRACT

Solanesol, an aliphatic terpene alcohol predominantly found in solanaceous plants, has gained recognition for its anti-inflammatory, antibacterial, and neuroprotective properties. This study investigates the potential efficacy of solanesol in alleviating chronic inflammatory pain induced by injection of complete Freund's adjuvant (CFA) into the left hind paw. Behavioral assessments revealed a significant reduction in mechanical and thermal hypersensitivity following solanesol administration, accompanied by a partial alleviation of concomitant anxiety-like behaviors. Mechanistically, Western blot analysis demonstrated a substantial decrease in the levels of TNF-α and IL-1β after solanesol administration. Immunohistochemical staining further revealed a notable suppression of microglial and astrocytic activation induced by CFA injection. These findings collectively suggest that solanesol holds promise as a latent therapeutic agent for the treatment of chronic inflammatory pain.

1. Introduction

Pain, a complex and subjective experience, manifests as an uncomfortable sensation and emotional response often associated with substantial or potential tissue damage [1]. Chronic inflammatory pain, a significant subtype encountered in the clinical setting, presents a distressing problem persisting for months to years [2]. This condition affects approximately 100 million adults in the United States, resulting in an annual financial cost exceeding $600 billion [3]. Numerous diseases, including COVID-19, diabetes, anxiety, depression, and tumors, are accompanied by chronic inflammatory pain, significantly impacting patients' quality of life and leading to productivity loss [4]. However, current analgesics, such as opioids and non-steroidal anti-inflammatory drugs (NSAIDs), primarily modulate pain transduction and transmission in neurons, demonstrating limited success in controlling disease progression [5] and often causing serious adverse reactions and toxicities [6]. Therefore, there is an urgent need to explore novel painkillers for chronic inflammatory pain.

Recent evidence highlights the crucial role of activated spinal microglia and astrocytes in the developing and maintenance of chronic pain [7]. Inflammatory stimuli activate these cells, causing changes in morphology and number. Additionally, they synthesize and release specific cytokines that mediate chronic inflammatory pain, heightening the neuroexcitability of nociceptive pathways and

* Corresponding author.
E-mail address: zhenfeng9853@163.com (Z.-f. Zhou).
[1] These authors contribute equally: Yuan-yuan WANG, Yifan Li.

https://doi.org/10.1016/j.heliyon.2024.e34870
Received 13 June 2023; Received in revised form 8 July 2024; Accepted 17 July 2024
Available online 18 July 2024

participating in peripheral and central sensitization [6]. Among these specific cytokines, TNF-α and IL-1β are upregulated in microglia and astrocytes during chronic inflammatory pain [8], subsequently enhancing excitatory synaptic transmission and suppressing inhibitory synaptic transmission in the spinal cord, resulting in thermal and mechanical hypersensitivities [9].

In recent years, the exploration of bioactive compounds, even derived from common crop species, has provided a foundation for disease treatment. One such example is solanesol, a 45-carbon isoprenoid mainly extracted from solanaceous plants such as tobacco leaves, eggplant, and pepper [10]. Widely used in the pharmaceutical industry as a synthetic intermediate of coenzyme Q10 and vitamin K2 [11], solanesol has demonstrated positive effects against bacteria, fungus, viruses, cancer, inflammation, and ulcer [12]. Additionally, various solanesol conjugates play a key role in reversing multidrug resistance and sensitizing tumor cells to traditional anti-cancer therapy [13]. Despite these diverse applications, no study to date has investigated whether solanesol contributes to the regulation of chronic inflammatory pain.

2. Materials and methods

2.1. Animals

Adult C57BL/6 mice, aged 6–8 weeks and weighed 15–25g, were obtained from the laboratory animal center of Hangzhou Normal University. The gender ratio of mice was 1:1 (female to male). They were housed in groups of four or five in a temperature-controlled (25 ± 1 °C), humidity-relative (60 ± 5 %) environment with a 12-h light/dark cycle. Food and water were available *ad libitum*. All experimental protocols had been reviewed and approved by the Animal Ethics and Welfare Committee (AEWC) of Hangzhou Normal University. In adherence to ethical principles, we aimed to maintain consistent effects while minimizing the number of experimental animals used and mitigating their suffering.

2.2. Chronic inflammatory pain model and solanesol treatments

The chronic inflammatory pain model was established based on previous report [14]. Subcutaneous injections of 10 μL of 50 % CFA (MACKUN, CAS: NONE6436) were administered into the left hind paw of all mice. Successful modeling was confirmed by local inflammation and noticeable swollen paw following CFA injection. Solanesol (Fig. 1A), obtained from MCE (Cat. No.: HY-N0576, United States), was dissolved in sterile DMSO and further diluted with sterile saline (0.9 % sodium chloride solution), following the provided instructions. The administered volume of solanesol was 50 mg/kg, determined through preliminary tests to achieve optimal efficacy.

2.3. Experimental procedure

All animals were randomly assigned to one of four groups: (i) Con + Veh; (ii) Con + Sol; (iii) CFA + Veh; (iv) CFA + Sol. The chronic inflammatory pain model was induced in the CFA group as previously described, with control animals receiving a subcutaneous injection of 10 μL sterile saline into the left hind paw at the same time. Following successfully modeling, mice in the Con + Sol group and

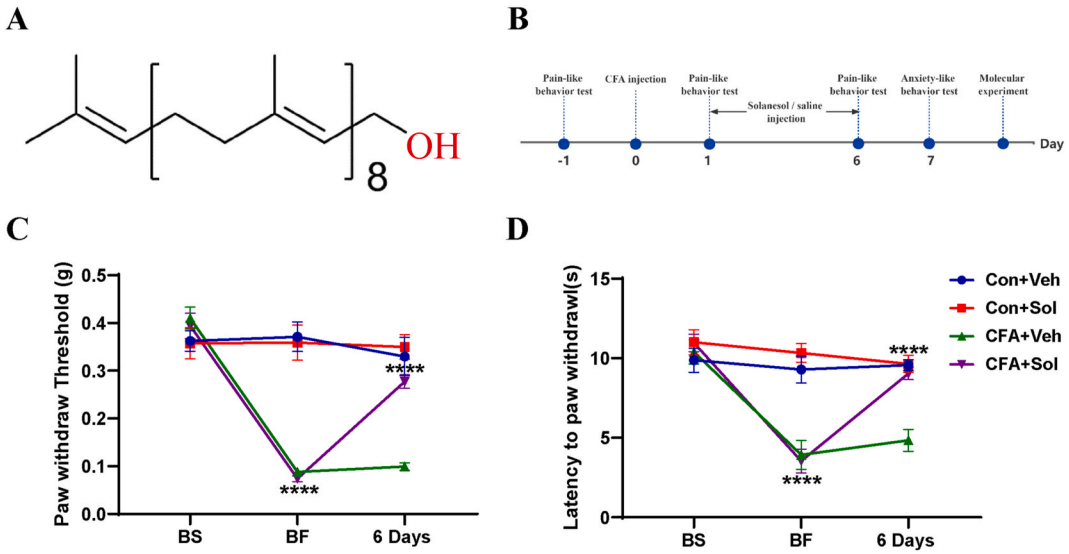

Fig. 1. Solanesol reverses the mechanical allodynia and thermal hypersensitivity caused by CFA (A) solanesol chemical structure. (B) The detailed experimental plan. (C) Mechanical allodynia and (D) Thermal hyperalgesia of mice in the four groups. Results are expressed as the mean ± SEM. (Two-way RM ANOVA followed by Sidak's test, n = 9 per group, ****P < 0.0001) BS: baseline; BF: before solanesol administration.

CFA + Sol groups were intraperitoneally injected with solanesol daily for a week, while mice in other groups received an isodose saline injection under identical conditions. All mice underwent a 10-min adaptation in the experimental settings before each testing session, with all behavioral tests conducted at the same time on the trial days. Paw withdrawal threshold assessments for mechanical and heat stimuli were conducted before CFA injection, before solanesol injection, and 6 days after solanesol injection. Anti-anxiety tests were performed after 6 days after solanesol injection. Following the completion of behavioral tests, animals were promptly sacrificed and the fifth lumbar (L5) spinal cord was collected for Western blot and immunofluorescence staining. A schematic representation of the experimental protocols is shown in Fig. 1B.

2.4. Mechanical allodynia

Mechanical allodynia was accessed using the von Frey filament as described by Chaplan [15]. Animals were individually placed in a separate box on a raised mesh grid, exposing their hind paws. The von Frey filament was vertically applied to the central planter of the left hind paw, kept bent for at least 5 s or until a positive sign of paw withdrawal, accompanied by licking and quivering, was observed. In the case of a negative reaction, progressively stronger filaments were selected. The positive response was the opposite. The paw-withdrawal threshold (PWT) was calculated using Dixon's up-down method [16], with intervals between two consecutive tests no less than 5 min.

2.5. Thermal hyperalgesia (Hargreaves test)

Paw withdrawal latency (PWL) was measured to assess thermal hypersensitivity as previous studies described [17]. The planter hot sting instrument (Ugo Basile, Italy) was used for thermal hypersensitivities, with the intensity adjusted to ensure an average paw withdrawal latency of approximately 8–15 s in normal mice before the test. Mice were separately placed in clear plastic boxes on a raised glass plate. A hot sting instrument under the plate positioned radiant heat stimulus directly to the center of the left hind paw. The heat stimulus was automatically shut off when the animal withdrew its hind paw or the radiant heat lasted for 20 s which we set as maximum for protection. Left paws were measured at least 10-min intervals for a total of three tests. PWL was defined as the time between the initiation of radiant heat and the hind paw withdrawal.

2.6. Elevated plus maze test

The elevated plus maze (EPM) test was carried out according to previous report [18]. The apparatus, elevated 50 cm above the ground, consisted of two open arms (5 cm × 30 cm) and two closed arms (5 cm × 30 cm and 15 cm high walls) on each side. Before each test, the apparatus underwent thorough cleaning with 75 % ethanol and animals were subjected to gentle handling to alleviate any nervousness. Then mice were separately placed in the center area, facing an open arm, and allowed to explore the maze freely for a duration of 5 min. An overhead camera recorded their entries and duration in each arm, and the ANY-maze software was employed for the analysis of animal movements.

2.7. Open field test

The open field test (OFT) was carried out based on a previous study [19]. The apparatus underwent thorough cleaning with 75 % ethanol before each testing session. Then animals were individually placed in a white opaque plastic box featuring a square arena (45 x 45 × 45 cm^3). They were allowed to explore freely for a duration of 15 min, during which a camera positioned overhead traced their motor and exploration behaviors. Behavioral data were analyzed using the ANY-maze software (Stoelting, Wood Dale, IL 60191, USA). The motor activity of mice was accessed through their total distance traveled.

2.8. Western blot analysis

Upon deep anesthesia induced by intraperitoneal injection of pentobarbital, mice were swiftly sacrificed to extract protein from the dorsal horn of spinal cord L4-5 regions. The tissue harvested was homogenized in RIPA buffer with PMSF (CAS No.: 329-98-6, MCE). Following grinding, protein extracts were centrifuged at 12,000 rpm for 15 min at 4 °C. Then BCA assays were used to estimate protein concentrations. Equal sample amounts were loaded onto 12 % separating gels and subsequently transferred onto a poly vinylidene difluoride (PVDF) membrane at 4 °C. The membrane was blocked with Tris-buffered saline (TBST) containing 5 % skimmed milk for 1h at room temperature. Subsequently, it was probed with the primary antibodies against Anti-TNF-α Rabbit mAb (1:400, servicebio Cat. GB11188), Anti-IL-1β Rabbit mAb (1:1500, servicebio Cat.GB11113) and Anti-β-actin Mouse mAb (1:1000, huabio EM21002) overnight at 4 °C. The following day, it underwent incubation with secondary antibodies [1:10,000, Sigma, A0545 (goat anti-rabbit), A9044 (goat anti-mouse)] for 1h at room temperature. The membrane was washed in TBST 4 times for 10 min between each process. The density of the target proteins was detected using the ECL kit (FD8020, FDbio, Hangzhou, China) and the relative content was digitized with ImageJ program (NIH, Bethesda, MD, USA). The final band density was calculated after being standardized to β-actin which we used as a loading control.

2.9. Immunofluorescence staining

Following deep anesthesia of the mice, animals were perfused initially with phosphate-buffered saline (PBS) and subsequently with 4 % paraformaldehyde (PFA). The lumbosacral enlargement segments of the tissue were collected and immersed in PFA overnight for post-fixation. The specimens then underwent dehydration using a gradient of sucrose in PBS (15 % and 30 %, respectively). Subsequently, the tissue was freeze-mounted in OCT and sectioned into 16 μm slices at −20 °C using a Thermo NX50 cryostat.

Tissue sections were blocked with 5 % bovine serum albumin in 0.3 % Triton X-100 in PBS for 1h at room temperature. The sections were then incubated with primary antibodies as following: ionized calcium-binding adapter molecule 1 (Iba-1) (1:500, OBPGP049-01, Oasis, Hangzhou, China) and S100 calcium binding protein B (S100β) (1:500, OB-PRB050-01, Oasis). Following primary antibody incubation, the slices were then incubated with the secondary antibodies [Alexa647-conjugated goat anti-rabbit (1:1000, A0468, Beyotime, Shanghai, China), Alexa488-conjugated goat anti-guinea pig (1:1000, OB-GP488-50, Oasis)] and 4′,6-diamidino-2-phenylindole (DAPI) (1:1000) for 1h. We washed the section in PBS 3 times for 5 min between each step. Finally, the sections were

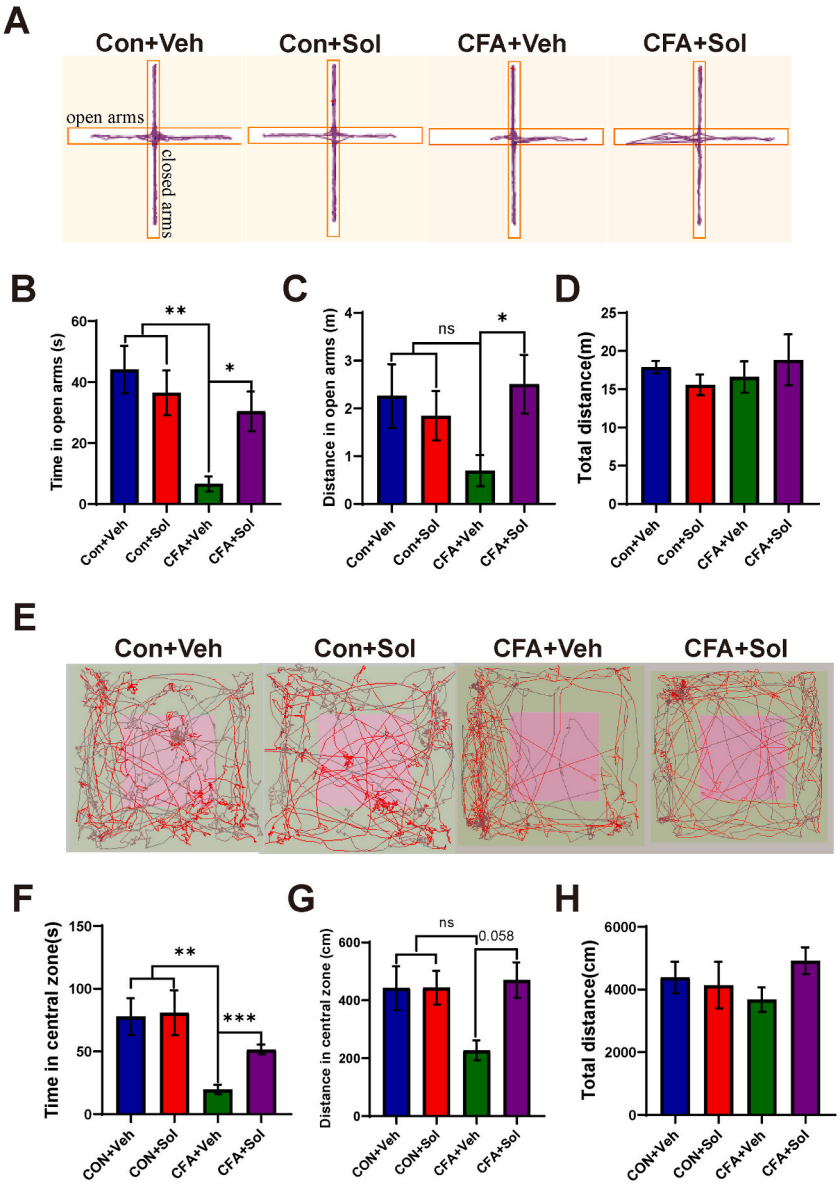

Fig. 2. Solanesol alleviates anxiety-like syndrome concomitant with chronic inflammatory pain (A) Representative tracks in the EPM among the four groups. (B) Summarized data showed the entries into open arms in the EPM. (C) Time spent in open arms in the EPM. (D) Total distance traveled in the EPM. (E) Representative tracks in the OFT among the four groups. (F) Time stayed in the central area in the OFT. (G) Distance moved in the central area in the OFT. (H) Total distance traveled in the OFT. Results are expressed as the mean ± SEM. (*unpaired t*-test, n = 9 per group, *P < 0.05, **P < 0.01).

captured using a fluorescence microscope (Olympus, VS200). For statistical analysis, 3 to 5 consecutive sections per tissue per mouse at 120 μm intervals in at least 3 mice were selected for quantification. The fluorescent density of positive cells was quantified in a defined area of interest in the spinal cord dorsal horn using ImageJ software. The scorer remained blinded to the drug treatment throughout the analysis.

2.10. Data analysis

Statistical analyses were performed using Prism 8.0 (GraphPad Software, La Jolla CA, USA). Results were analyzed by *t*-test one-way or two-way repeat measure ANOVA (Two-way RM ANOVA). Sidak's test or Tukey's test was used for post hoc comparisons. All data were presented as mean and standard errors of the means (mean ± SEM), and statistical significance was considered at $P < 0.05$.

3. Results

3.1. Solanesol reverses the mechanical allodynia and thermal hypersensitivity caused by CFA

To assess the analgesic role of solanesol, we established a mouse model of chronic inflammatory pain through hindpaw injection of CFA. Successful induction of chronic inflammatory pain was confirmed by evident paw swelling and reduced paw withdrawal threshold (PWT) and paw withdrawal latency (PWL) in the CFA group (Fig. 1C and D). However, mice in the CFA + Sol group exhibited restored PWT and PWL compared to those receiving saline injection. No significant differences in sex were observed during the experiment. These findings indicated that solanesol administration effectively reverses CFA-induced chronic inflammatory pain.

3.2. Solanesol alleviates anxiety-like syndrome concomitant with chronic inflammatory pain

We investigated the potential anti-anxiety effects of solanesol in CFA-induced chronic inflammatory pain using the EPM and OFT. The motor and exploration behaviors of mice were traced as shown in Fig. 2A and E. Results revealed a decrease in time spent in the open arms of the EPM (Fig. 2B) and in the center area of the OFT (Fig. 2F) in CFA-injected mice, suggesting anxiety-like behavior. Although no statistically significant differences were observed in the distance traveled in the open arms (Fig. 2C) and center area (Fig. 2G), a clear trend was apparent. However, mice treated with solanesol exhibited increased time (Fig. 2B) and distance traveled in the open arms of the EPM (Fig. 2C) and OFT (Fig. 2F and G), indicating anxiolytic effects. Total distance traveled showed no significant differences among groups in the EPM and OFT (Fig. 2D and H). These results collectively suggest that solanesol possesses anxiolytic effects in CFA-injected mice.

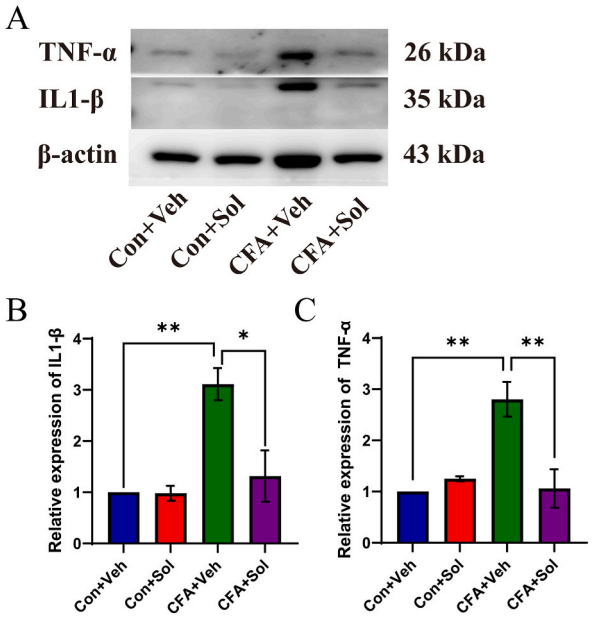

Fig. 3. Spinal TNF-α and IL-1β downregulated after solanesol administration in CFA-induced model mice (A) Typical imaging of proinflammatory cytokines TNF-α and IL-1β. (B) Statistical results of IL-1β in the four groups. (C) Statistical results of TNF-α in the four groups. Results are expressed as the mean ± SEM. (*unpaired t*-test under, n = 3 per group, **P < 0.01).

3.3. Spinal TNF-α and IL-1β downregulated after solanesol administration in CFA-induced model mice

Under conditions of injury, proinflammatory cytokines, including TNF-α and IL-1β are released to sensitize nociceptive sensory neurons, contributing to pain formation [20].To investigate whether solanesol inhibits the inflammatory signaling pathway could be inhibited by solanesol injection, we assessed the expression levels of TNF-α and IL-1β in the L5 spinal cord using Western blot analysis. As depicted in Fig. 3, the expression of proinflammatory cytokines TNF-α (Fig. 3A and B) and IL-1β (Fig. 3A and C) in the spinal cord significantly increased in response to the CFA injection. However, a notable decrease in these cytokines was observed one week after solanesol injection, supporting our hypothesis. These findings indicate that solanesol administration exerts analgesic effects by downregulation of spinal TNF-α and IL-1β.

3.4. Solanesol administration can suppress the activation of microglia and astrocytes in the spinal cord following CFA injection

Under normal conditions, microglia and astrocytes remain relatively quiescent but become activated and play a role in the etiopathogenesis of neurological disease after injury [21]. Activated microglia exhibit hypertrophied soma, thickened processes, and increased ionized calcium-binding adapter molecule-1 (Iba1) [22], while activated astrocytes are characterized by S100β expression [23]. In this study, we conducted immunostaining against Iba1 and S100β, specific markers of microglia and astrocytes, respectively.

Significant increases in fluorescence intensity of Iba-1 (Fig. 4A and B) and S100β (Fig. 4A and C) were observed in the L5 spinal cord of CFA-injected mice, indicative of activated microglia and astrocytes. However, mice injected with solanesol exhibited a marked reduction in fluorescence intensity for both Iba-1 (Fig. 4B) and S100β (Fig. 4C). These results suggest that solanesol administration may attenuate the activation of glial cells triggered by chronic inflammatory pain, thereby alleviating chronic pain.

4. Discussion

Previous reports have highlighted the efficacy of pro-inflammatory cytokine antagonists in profoundly alleviating hyperalgesia, indicating the potential of anti-inflammatory drugs to partially attenuate chronic inflammatory pain [24]. In this study, we investigated the potential analgesic and anti-inflammatory properties of solanesol through a series of experiments. Behavioral tests revealed that intraperitoneally injection of 50 mg/kg solanesol reduced the sensitivity of mice to thermal and mechanical stimuli in chronic pain model mice, concurrently alleviating anxiety-like behaviors to a certain extent. Western blot analysis demonstrated a decline in the release of proinflammatory cytokines after solanesol administration, such as IL-1β and TNF-α. Additionally, solanesol administration

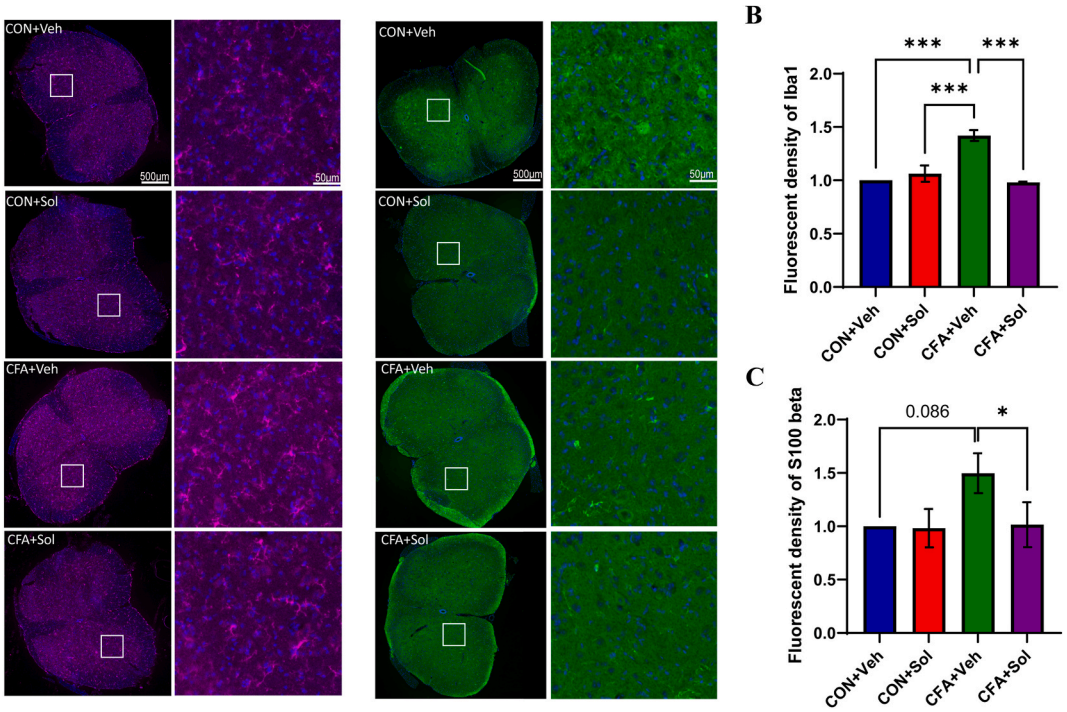

Fig. 4. Solanesol administration can suppress the activation of microglia and astrocytes in the spinal cord following CFA injection (A) Representative images for Iba-1 and S100βin the spinal cord after CFA injection, and Sol (50 mg/kg) administration suppressed the enhanced activation of Iba-1 and S100β. Mean immunofluorescence intensity of Iba-1 (B) and S100β (C). Results are expressed as the mean ± SEM. (*unpaired t*-test under, n = 4 per group, **P < 0.01, ****P < 0.0001).

suppressed spinal microglia and astrocyte activation. These results implied that solanesol has the potential to alleviate CFA-induced chronic inflammatory pain and concomitant anxiety-like behaviors via the inhibition of spinal glial cell-mediated proinflammatory cytokines.

In clinical settings, sex differences in pain responses are well-documented, with increased pain sensitivity observed among women [25]. While our experiment did not find any sex differences, and the specific mechanisms were not explored in this paper, it is essential to acknowledge the existing literature suggesting different immune cells may mediate mechanical pain hypersensitivity in males and females [26,27].

Previous studies have indicated that CFA injection can induce anxiety-like behaviors [28], a common occurrence in patients with chronic pain in clinical practice [20]. Microglial activation has been linked to anxiety-like behavior in adult rats [25], and cytokines, particularly IL-1β, have been considered as potential therapeutic targets for anxiety [29]. Our study aligns with these findings, demonstrating that solanesol partially alleviates anxiety-like symptoms in the context of chronic inflammatory pain. One potential mechanism identified is the reduction of IL-1β and TNF-α production by solanesol. However, further research is needed to elucidate the specific pathways involved.

Accumulating evidence suggests that cytokines are related to inflammatory pain conditions [24], with some produced in spinal glial to facilitate neuropathic pain, including TNF-α, IL-1β and IL18 [30]. Additionally, glial cells produce anti-inflammatory cytokines, such as IL-4, IL-10, transforming growth factor-β (TGFβ) and interferon-α (IFNα), to counteract pain through various regulatory pathways [5]. They regulate chronic pain in many ways, such as activating the mitogen-activated protein kinase (MAPK) pathway, potentiating glutamatergic synaptic transmission, and increasing the frequency and amplitude of spontaneous excitatory postsynaptic currents (sEPSCs) [1]. Consistent with these reports, our study demonstrated that solanesol (50 mg/kg) suppressed the production of TNF-α and IL-1β in the spinal cord, suggesting that solanesol alleviated chronic inflammatory pain by downregulating these cytokines at the spinal cord level.

Chronic pain is characterized by maladaptive changes in the central nervous system activated by noxious or innoxious stimuli, leading to hyperalgesia and allodynia [31]. Recent findings suggested that glia in the spinal cord may serve as new therapeutic targets for treating chronic pain [32]. Microglia regulate their structures and functions through interaction with synapses under normal conditions and release numerous molecules after painful injuries [7]. Astrocytes, one of the most abundant glial cells in the spinal cord, are highly engaged in modulating pain signal pathways under various health and disease situations [33]. Activated by noxious stimuli and inflammation, these glial manifested in their morphological changes, the increased expression of their specific markers S100β or Iba-1, and the activation of glia-specific proinflammatory pathways [25].

In our study, CFA injection induced increased fluorescence intensity of Iba1 labeling in spinal microglial cells, associated with hypertrophic cell bodies and thickened processes. Similarly, immunofluorescence of S100β was increased. However, solanesol treatment declined the spinal expression of Iba-1 and S100β. These results suggested that solanesol reduces the proliferation activity of microglia and astrocytes, providing relief from CFA-induced chronic inflammatory pain. While the current investigation is limited to mice and may not entirely mirror the clinical pain profile in humans, it offers valuable insights into the ability of solanesol to alleviate chronic inflammatory pain by modulating nociceptive transmission in the spinal cord.

5. Conclusion

In summary, this study provides evidence that solanesol possesses the capacity to alleviate CFA-induced chronic inflammatory pain via the inhibition of proinflammatory cytokines in spinal glial cells. These findings contribute to our understanding of the possible mechanism underlying the antinociceptive properties of solanesol. Consequently, solanesol emerges as a promising candidate for the treatment of chronic inflammatory pain, yet its efficacy and safety warrant further exploration in clinical experiments.

Data availability statement

The data presented in the study are available at https://pan.baidu.com/s/1B2J-IytuDZc38vNKQdFfXQ?pwd=0619.

CRediT authorship contribution statement

Yuan-yuan Wang: Writing – review & editing, Writing – original draft, Software, Resources, Formal analysis, Data curation. **Yi-fan Li:** Methodology, Investigation, Data curation, Conceptualization. **Zhen-feng Zhou:** Funding acquisition, Zhenfeng Zhou, Visualization, Validation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was supported by the Zhejiang Provincial Natural Science Foundation of China under Grant No. LGF22H310002.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34870.

References

[1] H.J. Lu, Y.J. Gao, Astrocytes in chronic pain: cellular and molecular mechanisms, Neurosci. Bull. 39 (3) (2023) 425–439.
[2] A. Fayaz, et al., Prevalence of chronic pain in the UK: a systematic review and meta-analysis of population studies, BMJ Open 6 (6) (2016) e010364.
[3] J. Lee, et al., Negative impact of chronic pain: the role of locus of control and perceived family validation of chronic pain, Health Psychol. Open 9 (2) (2022) 20551029221125170.
[4] Y.C. Lee, Effect and treatment of chronic pain in inflammatory arthritis, Curr. Rheumatol. Rep. 15 (1) (2013) 300.
[5] R.R. Ji, Z.Z. Xu, Y.J. Gao, Emerging targets in neuroinflammation-driven chronic pain, Nat. Rev. Drug Discov. 13 (7) (2014) 533–548.
[6] S.L. Whittle, B.L. Richards, R. Buchbinder, Opioid analgesics for rheumatoid arthritis pain, JAMA 309 (5) (2013) 485–486.
[7] R.R. Ji, T. Berta, M. Nedergaard, Glia and pain: is chronic pain a gliopathy? Pain 154 (0 1) (2013) S10–s28. Suppl 1.
[8] H. Cao, Y.Q. Zhang, Spinal glial activation contributes to pathological pain states, Neurosci. Biobehav. Rev. 32 (5) (2008) 972–983.
[9] R.R. Ji, A. Chamessian, Y.Q. Zhang, Pain regulation by non-neuronal cells and inflammation, Science 354 (6312) (2016) 572–577.
[10] K.P. Kotipalli, N.C.V. Rao, K. Raj, Estimation of solanesol in tobacco and non-tobacco plants from Solanaceae family, J. Med. Aromat. Plant Sci. (1 Pt.B) (2008) 30.
[11] X. Xiao, et al., Supercritical CO_2 conditions of CoQ_(10) biotransformation from solanesol by Schizosaccharomyces prombe, J. Food Sci. Biotechnol. 29 (2) (2010) 302–306.
[12] N. Yan, et al., Solanesol: a review of its resources, derivatives, bioactivities, medicinal applications, and biosynthesis, Phytochemistry Rev. 14 (3) (2015) 403–417.
[13] Y. Xiong, et al., Solanesol derived therapeutic carriers for anticancer drug delivery, Int. J. Pharm. 572 (2019) 118823.
[14] D.S. Wang, et al., Anxiolytic-like effects of translocator protein (TSPO) ligand ZBD-2 in an animal model of chronic pain, Mol. Pain 11 (2015) 16.
[15] S.R. Chaplan, et al., Quantitative assessment of tactile allodynia in the rat paw, J. Neurosci. Methods 53 (1) (1994) 55–63.
[16] W.J. Dixon, Efficient analysis of experimental observations, Annu. Rev. Pharmacol. Toxicol. 20 (1980) 441–462.
[17] K. Hargreaves, et al., A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia, Pain 32 (1) (1988) 77–88.
[18] A.A. Walf, C.A. Frye, The use of the elevated plus maze as an assay of anxiety-related behavior in rodents, Nat. Protoc. 2 (2) (2007) 322–328.
[19] Y.J. Wang, et al., Restoration of cingulate long-term depression by enhancing non-apoptotic caspase 3 alleviates peripheral pain hypersensitivity, Cell Rep. 33 (6) (2020) 108369.
[20] M.C. Bushnell, M. Ceko, L.A. Low, Cognitive and emotional control of pain and its disruption in chronic pain, Nat. Rev. Neurosci. 14 (7) (2013) 502–511.
[21] J. Scholz, C.J. Woolf, The neuropathic pain triad: neurons, immune cells and glia, Nat. Neurosci. 10 (11) (2007) 1361–1368.
[22] M. Tsuda, et al., IFN-gamma receptor signaling mediates spinal microglia activation driving neuropathic pain, Proc Natl Acad Sci U S A 106 (19) (2009) 8032–8037.
[23] S.M. Yuan, S100 and S100β: biomarkers of cerebral damage in cardiac surgery with or without the use of cardiopulmonary bypass, Rev. Bras. Cir. Cardiovasc. 29 (4) (2014) 630–641.
[24] W.A. Verri Jr., et al., Hypernociceptive role of cytokines and chemokines: targets for analgesic drug development? Pharmacol. Ther. 112 (1) (2006) 116–138.
[25] Y.L. Wang, et al., Microglial activation mediates chronic mild stress-induced depressive- and anxiety-like behavior in adult rats, J. Neuroinflammation 15 (1) (2018) 21.
[26] R.E. Sorge, et al., Different immune cells mediate mechanical pain hypersensitivity in male and female mice, Nat. Neurosci. 18 (8) (2015) 1081–1083.
[27] Y. Tu, et al., Microglia-independent peripheral neuropathic pain in male and female mice, Pain 163 (11) (2022) e1129–e1144.
[28] M. Narita, et al., Chronic pain induces anxiety with concomitant changes in opioidergic function in the amygdala, Neuropsychopharmacology 31 (4) (2006) 739–750.
[29] M. Li, et al., Lentivirus-mediated interleukin-1β (IL-1β) knock-down in the hippocampus alleviates lipopolysaccharide (LPS)-induced memory deficits and anxiety- and depression-like behaviors in mice, J. Neuroinflammation 14 (1) (2017) 190.
[30] K. Ren, R. Dubner, Neuron-glia crosstalk gets serious: role in pain hypersensitivity, Curr. Opin. Anaesthesiol. 21 (5) (2008) 570–579.
[31] J. Sandkühler, J. Lee, How to erase memory traces of pain and fear, Trends Neurosci. 36 (6) (2013) 343–352.
[32] J.P. O'Callaghan, D.B. Miller, Spinal glia and chronic pain, Metabolism 59 (Suppl 1) (2010) S21–S26.
[33] Y.L. Chen, et al., Mode of action of astrocytes in pain: from the spinal cord to the brain, Prog Neurobiol 219 (2022) 102365.