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[6]-Shogaol and [6]-Gingerol active ingredients may improve neuropathic pain by suppressing cytokine levels in an experimental model

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Background/aim: Neuropathic pain (NP) is a type of chronic pain usually caused by damage to the somatosensory system. Bioactive antioxidant compounds, such as curcumin and ginger, are widely preferred in the treatment of NP. However, the ingredient-based mechanism that underlies their pain-relieving activity remains unknown. The aim of this study was to investigate the therapeutic effects of trans-[6]-Shogaol and [6]-Gingerol active ingredients of the *Zingiber officinale* Roscoe extract on the spinal cord and cortex in the neuroinflammatory pathway in rats with experimental sciatic nerve injury.

Materials and methods: Forty-six volatile phenolic components were identified in ginger samples using gas chromatography–mass spectrometry analysis. Thirty 3-month-old male 250–300 g Wistar Albino rats were divided into three groups as (i) sham, (ii) chronic constriction injury (CCI), and (iii) CCI+ginger. NP was induced using the CCI model. A ginger extract treatment enriched with trans-[6]-shogaol and [6]-gingerol active ingredients was administered by gavage at 200 mg/kg/day for 7 days. On the 14th day of the experiment, locomotor activity was evaluated in open field and hyperalgesia in tail flick tests.

Results: In behavioural experiments, a significant decrease was observed in the CCI group compared to the sham group, while a significant increase was observed in the CCI+ginger group compared to the CCI group ($p < 0.05$). In the spinal cord and cortex tissues, there was a significant increase in the TNF-α, IL-1β, and IL-18 neuroinflammation results of the CCI group compared to the sham group, while there was a significant decrease in the CCI+ginger group compared to the CCI group.

Conclusion: In this study, ginger treatment was shown to have a therapeutic effect on neuroinflammation against sciatic nerve damage.

Key words: [6]-shogaol, [6]-gingerol, ginger, neuroinflammation, neuropathic pain

1. Introduction

Neuropathic pain (NP) is a type of chronic pain that is usually caused by damage to the somatosensory system [1]. Approximately 20% of the adult population worldwide suffers from chronic pain each year [2], and neuroinflammation has an important role in the development of chronic pain. Neuroinflammation is also an underlying cause of many central nervous system (CNS) diseases, including Alzheimer's disease, Parkinson's disease, multiple sclerosis and psychiatric disorders [3]. In neurodegenerative diseases and spinal cord injury, neuroinflammation is a consequence of direct damage to the CNS, causing further neuronal degeneration and cell death (i.e. secondary injury) [3]. In chronic pain (i.e. neuropathic and inflammatory pain), neuroinflammation is usually due to peripheral damage and excessive neuronal activity of primary sensory neurons. Therefore, CNS neuroinflammation after peripheral injury is relatively mild and does not cause marked neuronal loss [4,5]. Cytokines such as tumour necrosis factor (TNF-α) and interleukin-1β (IL-1β) cause neurodegeneration in various regions of the brain associated with brain dysfunction in neurodegenerative disease (hippocampus and dentate gyrus) and impair memory and synaptic plasticity [6,7]. In contrast, TNF-α and IL-1β act as neuromodulators in the spinal cord dorsal horn after peripheral injury and trigger or enhance synaptic plasticity as well as inflammatory and NP [8–10].

Chronic pain, including NP caused by nerve injury and spinal cord injury, inflammatory pain caused by arthritis, cancer pain, and pain caused by drug therapy, are all caused by neuroinflammation in the spinal cord [11]. This neuroinflammation is triggered by activity-dependent release of glial activators (neurotransmitters,

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chemokines, and proteases) from the central terminals of primary afferent neurons and/or disruption of the blood–brain barrier [11]. Furthermore, neuroinflammation produces antiinflammatory cytokines and pro-resolution lipid mediators to normalise neuroinflammation, synaptic plasticity and abnormal chronic pain [11]. TNF-α is one of the most widely studied and potent inflammatory cytokines and has been shown to be expressed by microglia, astrocytes, and primary sensory dorsal root ganglion neurons [12,13]. IL-1β, another important inflammatory cytokine, is expressed by both microglia and astrocytes in the spinal cord [14,15], while IL-18 is induced in microglia after nerve injury and chronic opioid exposure [16,17]. TNF-α increases excitatory currents, IL-6 decreases inhibitory currents, and IL-1β increases excitatory currents and decreases inhibitory currents [18].

Among the different types of chronic pain, NP, caused by damage to the nervous system, including peripheral fibres and central neurons, is very difficult to treat and affects 7%–10% of the general population [19]. Current treatment options for NP are limited and opioid analgesics have serious side effects and can cause opioid use disorder. Recent studies have revealed the role of bioactive compounds in the diet in reducing NP. We evaluated the effects of commonly consumed bioactive compounds (ginger, curcumin, omega-3 polyunsaturated fatty acids, epigallocatechin gallate, and resveratrol) on NP and NP-related neuroinflammation [19]. Cellular studies have shown that these bioactive compounds reduce inflammation through the suppression of NF-κB and MAPK signalling pathways that regulate apoptosis/cell survival, antioxidant, and antiinflammatory responses. Animal studies strongly suggest that, when consumed regularly, these bioactive compounds have a pronounced antiNP effect as demonstrated by reduced mechanical allodynia, mechanical hyperalgesia, thermal hyperalgesia, and cold hyperalgesia [19].

Ginger (*Zingiber officinale* Roscoe) consists of a complex combination of biologically active components, among which the compounds ginger, shogaol, and paradol are reported to account for the majority of its antiinflammatory properties. Various ginger compounds and extracts have been tested as antiinflammatory agents, where the lengths of the side chains determine the level of efficacy [19]. β-[6]-gingerol, a combination of gingerols, is more effective than individual compounds in reducing inflammatory mediators. Borgonetti et al. showed that 200 mg/kg ginger once daily by gavage for 7 days starting from the 3rd day of nerve injury improved mechanical and thermal allodynia in mice with sciatic nerve injury [20]. In this study, the chronic constriction injury (CCI) model defined by Bennett and Xie [21] was used to induce NP and ginger treatment was started on the 7th day after surgery.

Chronic pain can be neuropathic or inflammatory. NP results from damage to the peripheral nervous system (PNS) or the CNS. Chronic NP burden has been shown to be associated with the complexity of NP symptoms, including anxiety and depression, and with poor outcomes [19]. Nerve injury often leads to neuroplastic changes in the peripheral and central elements of the pain system, resulting in neuronal hyperexcitability and sensitisation that produce spontaneous and evoked pain, such as mechanical and thermal hypersensitivity. The current symptomatic therapies for the treatment of NP rarely focus on the actual causes and have long-term side effects that limit treatment [20]. *Zingiber officinale* Roscoe (Zingiberaceae), known as ginger, is included in many official pharmacopoeias of different countries and contains nonvolatile components with biological activity, such as gingerols, shogaols, and paradols, along with zingerone in the dried rhizome [20]. Nerve damage in NP causes neuroinflammation and neuroplastic alterations in the peripheral and central neurons associated with sensitization and hyperexcitability [22]. It has been found that NP may result in an imbalance between reactive oxygen species (ROS) and endogenous antioxidants, which can cause neuroinflammation after nerve damage [22]. Therefore, there is an urgent need to develop new effective and safe analgesic and antiinflammatory alternatives without side effects [22].

The antiNP effect of bioactive compounds can be attributed to their ability to interact directly or indirectly with PNS and CNS signalling through their antiinflammatory and antioxidant properties. In this study, the therapeutic effects of ginger extract enriched in phenolic compounds, such as shogaol and gingerol, on neuroinflammation in the PNS and CNS were investigated in rats with experimental sciatic nerve injury.

2. Materials and methods

2.1. Animals and experimental design

Three-month-old male Wistar Albino rats weighing 250–300 g were used. Three experimental groups were formed as (i) sham, (ii) CCI, and (iii) CCI+ginger (Figure 1). NP was induced using the CCI model [21]. Animals in the sham and CCI+ginger groups were treated with ginger extract by gavage at a dose of 200 mg/kg/day for 7 days. Ginger 200 mg/kg, which is an effective dose in the treatment of sciatic nerve injury, was used in this study [20]. On 7th day and the 14th day of the experiment, locomotor activity was measured using an open field test and hyperalgesia was evaluated using the tail flick test. After the behavioural experiments, the rats were sacrificed and the levels of TNF-α, IL-1β, and IL-18 in the spinal cord and cortex tissues were evaluated using the ELISA method.

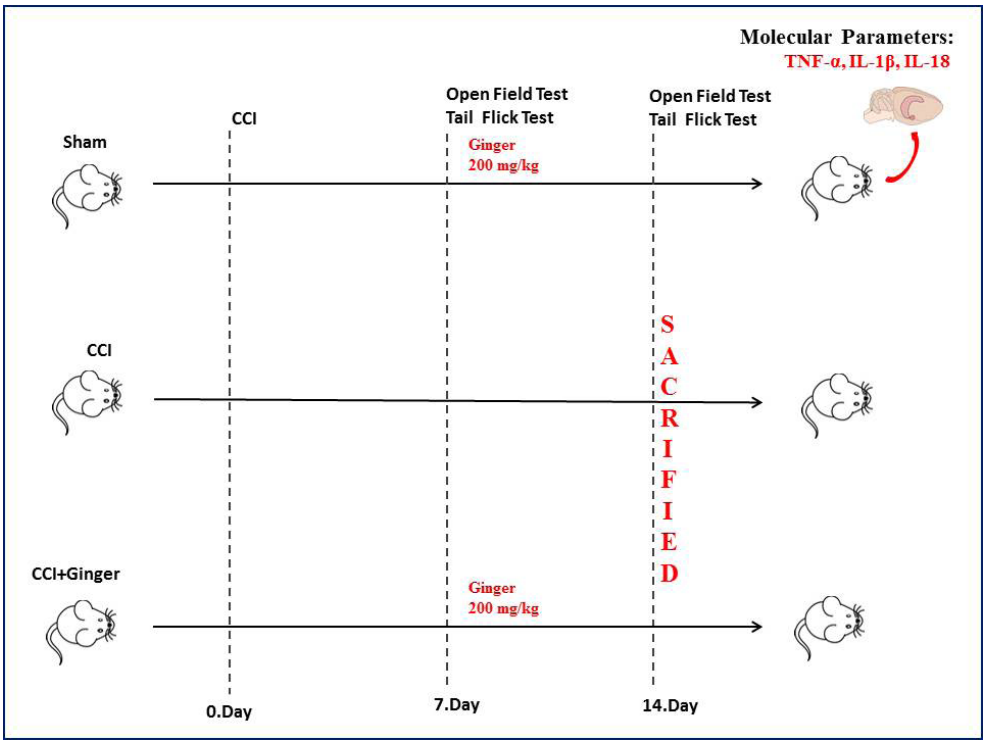

Figure 1. Experimental design.

2.2. CCI surgery

The CCI model described by Bennett and Xie (1988) was used for the induction of NP [21]. In brief, rats were anaesthetized with isoflurane (5% for induction, 2.5% for maintenance), and a 1-cm incision was made along the longitudinal axis of the right hind leg distal to the hip, 3–4 mm below the femur. Then, 4 loose ligatures (4/0 chromic catgut) were tied proximal to the sciatic trifurcation approximately 1 mm apart. The ligations were loosened to minimize nerve constriction and allow epineural blood flow. After the procedure, the surgical incision was immediately sutured, and a povidone–iodine solution was applied externally. The rats were housed in separate cages for 4 h after the CCI surgery and were allowed to recover for 1 week before treatments. For the sham group, the sciatic nerve was exposed, similar to the CCI model, but no ligatures were placed. On the 14th day, the pain tests were completed and the cortex and spinal cord tissues of the subjects were taken.

2.3. Ginger extraction

The ginger powder was obtained from Bağdat Co. Ltd. in Türkiye and authenticated by Dr Sevil Özkınalı. A series of voucher specimens were stored at the Hitit University Department of Chemistry [23]. Extraction was carried out at 100 °C in a Soxlet device by adding 300 mL of ethyl alcohol to 4.0 g of powdered ginger [24,25]. Ethyl alcohol, a polar protic solvent, is preferred for the extraction of polar phenolic compounds such as shogaols and gingerols. At the end of the experiment, the ethyl alcohol is evaporated with the help of an evaporator and the remaining viscous mixture is weighed. A similar process is repeated with water and methyl alcohol to release the phenolic compounds in ginger.

2.4. Behavioural tests

2.4.1 Open field test

Locomotor activity was carried out in a setup with a base of 80 × 80 cm and a wall height of 40 cm. For rats to explore the apparatus, they were placed in the centre of the field and monitored and recorded by the video surveillance system (Noldus Ethovision XT System, Netherlands) for 5 min. The total distance (cm) and frequency of movement were calculated to evaluate locomotor activity [26].

2.4.2 Tail flick test

Thermal hyperalgesia was also evaluated by the tail flick test, in which the animal's tail is exposed to a heat source [27]. When the animal feels uncomfortable, it automatically raises its tail. Briefly, the 2 cm portion of the distal tail was immersed into a 52.5 ± 0.2 °C water bath. The time the rats took to flick their tail was recorded as tail flick latency; the cutoff latency was 15 s to avoid injury of the tissues of the tail [28].

2.5. Tissue collection

The animals were killed by decapitation on day 14 after the behavioural experiments. The whole cerebral cortex

and ipsilateral or contralateral spinal cord (T7/8-L5) were dissected from the brain and stored frozen at –80 °C. The tissues were homogenized in phosphate buffered saline (pH 7.4), centrifuged at 12,000 rpm for 20 min at 4 °C, and the supernatants were used for biochemical analyses.

2.6. Biochemical analysis

2.6.1. Enzyme-linked immunosorbent assay (ELISA)

The levels of TNFα, IL-1β, and IL-18 were quantified using commercially available ELISA kits (R&D Systems, MN, USA) for rat TNF-α (EK710127), IL-1β (EK710260), and IL-18 (EK710281) according to the manufacturer's instructions. The concentrations of TNF-α, IL-1β, and IL-18 in the samples were calculated from their corresponding absorbance values via the standard curve. The data were normalized to total tissue protein and expressed as pg mg^{-1} tissue protein.

2.6.2 Protein measurements

Protein concentrations were measured in the sample tissues at 595 nm by a modified Bradford assay using a Coomassie Plus reagent with a bovine serum albumin standard (Pierce Chemical Company, Rockford, IL, USA).

2.6.3 Gas chromatography–mass spectrometry (GC–MS) analysis

A Shimadzu GCMS QP 2010 ULTRA with a high-performance quadrupole mass filter was used for the detection of volatile phenolic compounds in *Zingiber officinale*. An RXI-5MS capillary column (30 m; 0.25 mm; 0.25 μm) was used. For the GC–MS analysis, the temperature of the injection port was set to 250 °C, the split ratio was adjusted to 25:1, and the carrier gas was helium (99.999 % pure) with a flow rate of 1.0 mL/min. The oven temperature program started at 40 °C for 3 min, and was then raised to 240 °C at a rate of 4°C/min, where it remained for 10 min. The analysis was completed in 63 min. The working solutions of *Zingiber officinale* were prepared at 1 mg/mL in ethanol. For the MS (Shimadzu with a high-performance quadrupole mass filter) analysis, the ion source temperature was set to 200 °C, and the transfer line temperature was set to 250 °C (Figure 2).

2.6.4 Qualitative and quantitative analysis of Zingiber officinale by GC–MS

We detected 46 volatile phenolic compounds in the ginger samples (Bağdat Co. Ltd., Türkiye). The total ion chromatograms and detailed information on various compounds are shown in the Table. In the GC–MS analysis, a broad array of masses was obtained within a scan time of 5.0 to 60.3 minutes (scan range 20–450 m/z) and the presence of metabolites was determined with various retention times. The types of metabolites were verified by comparing the generated spectral model with those of the built-in spectral library developed by the National Institute of Standards and Technology (NIST, Washington, DC, USA), data version W9N11. As seen in the Table, most of the identified metabolites belong to the 5 main classes of sesquiterpenes (38.26%), phenolic compounds (29.95%), terpenes (8.08%) and their derivatives (14.3%), and fatty acids (6.4%). The superior performance of ginger in the aforementioned antioxidant assay can be attributed to the presence of several metabolites in the sesquiterpene, phenolic, and terpenoid classes, as profiled in the Table. The main aroma components of ginger are 11 phenolics and 7 sesquiterpenes, and it has been determined that there are 13 terpenes, 4 fatty acids, 4 terpenoids, 5 sesquiterpene

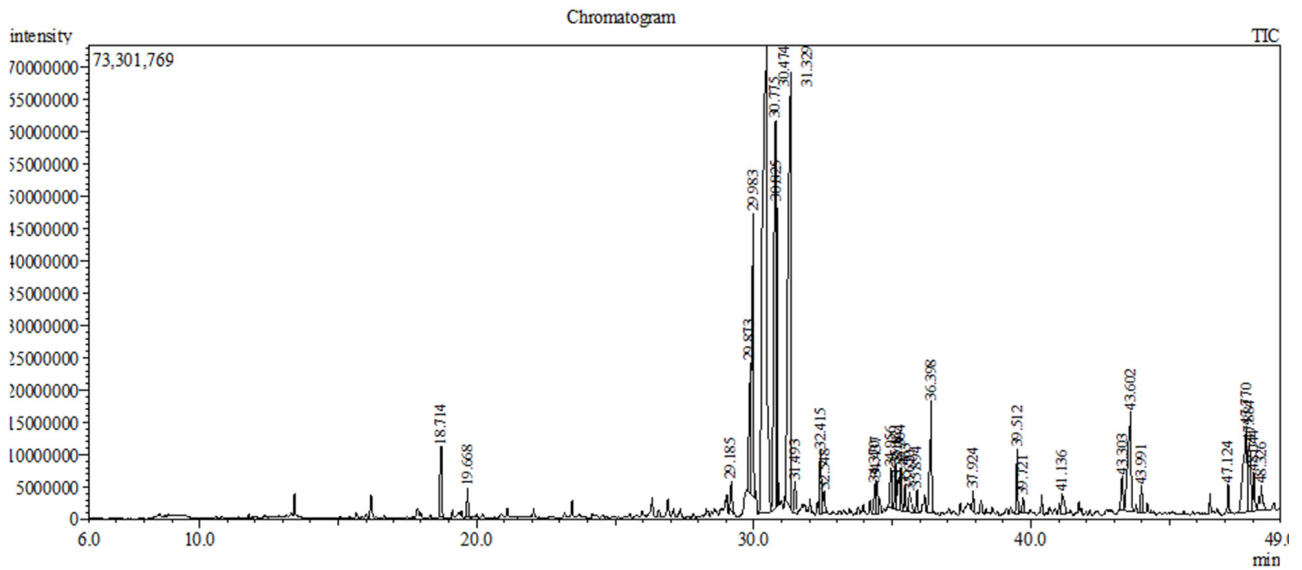

Figure 2. The total ion chromatograms of ginger extract as determined via GC–MS.

Table. Identified components of the ginger extract

No	Compound	Formula	Retention time (min)	% area	Retention index	Classes
1	1,3-butanendiol	$C_4H_{10}O_2$	5.192	0.68	0	alcohols
2	endo-borneol	$C_{10}H_{18}O$	18.714	0.87	1138	terpenes
3	α-terpineol	$C_{10}H_{18}O$	19.668	0.33	1143	terpenes
4	(–)-β-chamigrene	$C_{15}H_{24}$	29.185	0.37	1507	sesquiterpenes
5	R-α-curcumene	$C_{15}H_{22}$	29.873	2.05	1480	aromatic monoterpenoids
6	S-α-curcumene	$C_{15}H_{22}$	29.983	4.22	1524	aromatic monoterpenoids
7	zingiberene	$C_{15}H_{24}$	30.474	18.18	1496	monocyclic sesquiterpene
8	(E,E)-α-farnesene	$C_{15}H_{24}$	30.775	6.05	1458	sesquiterpenes
9	β-bisabolene	$C_{15}H_{24}$	30.825	2.40	1500	sesquiterpenes
10	β-sesquiphellandrene	$C_{15}H_{24}$	31.329	10.26	1523	sesquiterpenes
11	α-patchoulene	$C_{15}H_{24}$	31.493	0.41	1459	triterpene
12	*trans*-nerolidol	$C_{15}H_{26}O$	32.415	0.74	1564	sesquiterpenes
13	dodecanoic acid	$C_{12}H_{24}O_2$	32.548	0.41	1570	saturated fatty acids
14	guaiol	$C_{15}H_{26}O$	34.370	0.43	1614	sesquiterpenoid alcohols
15	caryophyllene oxide	$C_{15}H_{24}O$	34.437	0.58	1507	terpenes
16	zingerone	$C_{11}H_{14}O_3$	34.956	0.49	1638	phenolics
17	β-eudesmol	$C_{15}H_{26}O$	35.121	0.57	1656	terpenes
18	α-eudesmol	$C_{15}H_{26}O$	35.210	0.49	1598	terpenes
19	α-bisabolol	$C_{15}H_{26}O$	35.304	0.59	1688	sesquiterpene alcohols
20	(R,R)-α-bisabolol	$C_{15}H_{26}O$	35.463	0.27	1625	sesquiterpene alcohols
21	β-bisabolol	$C_{15}H_{26}O$	35.620	0.40	1619	sesquiterpene alcohols
22	bicyclo[4.3.0] nonane, 2,2,6,7-tetramethyl-7-hydroxy-	$C_{13}H_{24}O$	35.894	0.26	0	sesquiterpenoids
23	*E*-nerolidol	$C_{15}H_{24}O$	36.398	1.56	1572	sesquiterpene alcohols
24	2-cuparenol	$C_{15}H_{22}O$	37.924	0.24	1776	phenolics
25	campherenone	$C_{15}H_{24}O$	39.512	0.79	0	terpenes
26	2-methyl-5-(2,6,6-trimethyl-cyclohex-1-enyl)-pentane-2,3-diol	$C_{15}H_{24}O$	39.721	0.26	1776	terpenes
27	campherenone	$C_{15}H_{24}O$	41.136	0.46	0	terpenes
28	geranyl-*p*-cymene	$C_{20}H_{30}$	43.303	0.42	2006	terpenes
29	*n*-hexadecanoic acid	$C_{16}H_{32}O_2$	43.602	2.61	1968	saturated fatty acids
30	geranyl-α-terpinene	$C_{20}H_{32}$	43.991	0.32	1962	terpenes
31	(–)-nortrachelogenin	$C_{20}H_{22}O_7$	47.124	0.35	1328	phenolics
32	9,12-octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	47.770	2.72	2183	fatty acids
33	7-tetradecenal, (Z)-	$C_{14}H_{26}O$	47.884	1.50	1609	fatty aldehydes
34	geranyllinalool	$C_{20}H_{34}O$	48.044	0.59	2046	terpene alcohols
35	octadecanoic acid	$C_{18}H_{36}O_2$	48.326	0.61	2167	fatty acids
36	(4-methoxy-phenyl)-(2-nitrocyclohexyl)-methanol	$C_{14}H_{19}NO_4$	49.701	1.63	2148	aromatic terpenes
37	zingerone	$C_{11}H_{14}O_3$	49.896	1.01	1638	phenolics
38	*trans*-6-Shogaol	$C_{17}H_{24}O_3$	51.468	13.34	0	phenolics
39	ZO-3-(6)-gingerdione	$C_{17}H_{24}O_4$	52.121	1.03	0	phenolics

Table. (Continued).

40	butanoic acid, 3,7-dimethyl-2,6-octadienyl ester, (E)-	$C_{14}H_{24}O_2$	52.350	0.33	1550	terpenes
41	6-gingerol	$C_{17}H_{26}O_4$	53.518	6.37	2396	phenolics
42	carinol	$C_{20}H_{26}O_6$	55.599	0.79	3296	phenolics
43	*cis*-8-shogaol	$C_{19}H_{28}O_3$	56.163	4.43	0	phenolics
44	(E)-4-(2',6',6'-trimethyl-1',2'-epoxy-cyclohexyl)-3-penten-2-one	$C_{14}H_{22}O_2$	57.175	0.62	0	terpenes
45	gingerol	$C_{17}H_{26}O_4$	59.208	0.68	2396	phenolics
46	1-(2,4-dihydroxyphenyl)-2-(4-methoxy-3-nitrophenyl)ethanone	$C_{15}H_{13}NO_6$	60.299	1.22	2728	phenolics

alcohols, 1 aldehyde, and 1 alcohol derivative, among other components.

2.7. Statistical analysis

SPSS version 20.0 was used for all analyses. The results are given as mean ± standard error of the mean (SEM), and p values less than 0.05 were considered significant. One-way analysis of variance (ANOVA) was used for the data analysis with normality conditions checked using the Shapiro–Wilk test. The Tukey test was used for posthoc analysis.

3. Results

3.1. Effects of ginger on locomotor activity and thermal hyperalgesia

The total distance (cm) and frequency values, the determinants of the locomotor scores from the open field test, were significantly decreased in the CCI group on the 7th day compared to the sham group ($p < 0.05$) (Figures 3a and 3b). Similarly, the tail flick latency of the CCI rats was significantly reduced in the tail flick test on the 7th day, as compared to the sham group ($p < 0.05$) (Figure 3c). On the 14th day, a significant decrease was observed in the total distance ($p < 0.05$), frequency ($p < 0.05$), and latency ($p < 0.05$) values of the CCI group compared to the sham group, while a significant increase was observed in the total distance, frequency and latency values of the CCI+ginger group compared to the CCI group (Figure 4).

3.2. TNFα, IL-1β, IL-6 and IL-18 levels in cerebral cortex and spinal cord tissues

The levels of TNF-α ($p < 0.05$), IL-1β ($p < 0.05$), and IL-18 ($p < 0.05$) in the cerebral cortex and spinal cord are shown in Figures 5 and 6. The levels of TNF-α, IL-1β, and IL-18 in the cerebral cortex of the CCI group were significantly increased compared to the sham group ($p < 0.05$). The ginger treatment significantly decreased all the proinflammatory cytokine levels in the cerebral cortex tissues of the CCI rats on the 14th day ($p < 0.05$ for all). The levels of TNF-α ($p < 0.05$), IL-1β ($p < 0.05$), and IL-18 ($p < 0.05$) in the spinal cord of the CCI group were also significantly increased compared to the sham group. The ginger treatment significantly decreased all the proinflammatory cytokine levels in the spinal cord tissues of the CCI on the 14th day ($p < 0.05$ for all).

Zingiberene (18.18%), trans-[6]-shogaol (13.34%), β-sesquiphellandrene (10.26%), [6]-gingerol (6.37%), and (E,E)-α-farnesene (6.05%) compounds (Figure 7) were determined by the GC–MS analyses to be the most abundant compounds in the ginger extract. Among these main components, zingiberene, β-sesquiphellandrene and (E,E)-α-farnesene are in the sesquiterpene family, while trans-[6]-shogaol and [6]-gingerol are phenolic compounds. In the literature, terpene derivatives have been found to reduce neuroinflammation through HDAC-1 inhibition in a mouse neuropathy model [29]. Also, zingiberene, a sesquiterpene derivative, was found to be the most promising HDAC1 inhibitor [29]. Zingiberene has various pharmacological properties such as anticancer [30], antioxidant [31], antiulcer [32], antiviral [33], and antibacterial [34] effects. Among the other main components, phenolic compounds such as shogaol, gingerol, gingerdione, 2-cuparenol, carinol, nortrachelogenin, and zingerone were detected in the ginger extract. These compounds are known to have antioxidant and antiinflammatory activities [35]. Terpenoids, like phenolic compounds, have been extensively studied in the literature, and the evidence of their antioxidant potential is well recorded [36].

4. Discussion

NP is chronic pain caused by somatosensory damage. Approximately 20% of the adult population worldwide suffers from chronic pain each year. ROS generation and inflammation play an important role in the NP mechanism. Although anticonvulsants, antidepressants, opioids, and nonopioids are used in the treatment of NP, there is no drug with proven efficacy. Antioxidant bioactive compounds, such as curcumin and ginger,

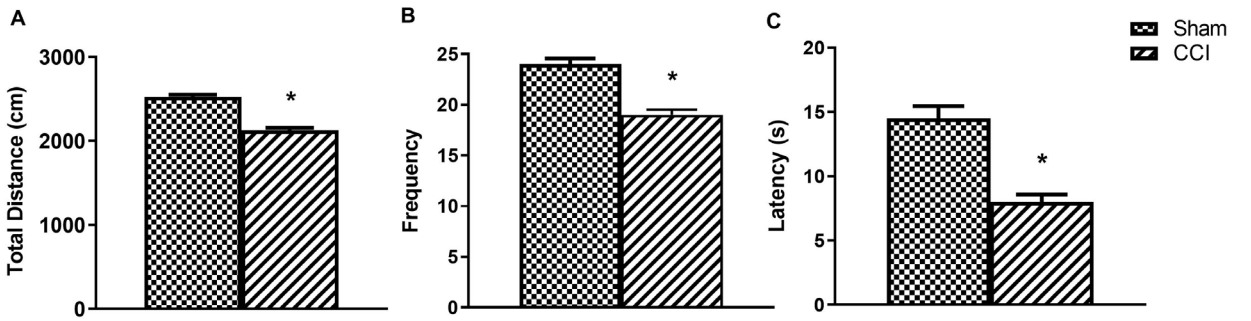

Figure 3. Day 7 baseline open field (OF) test and tail flick (TF) test results. (a) Total distance (cm) from OF, (b) frequency in OF, (c) latency (s) in TF. The * indicates significant difference ($p < 0.05$) compared to the sham group. All data are presented as mean ± SEM.

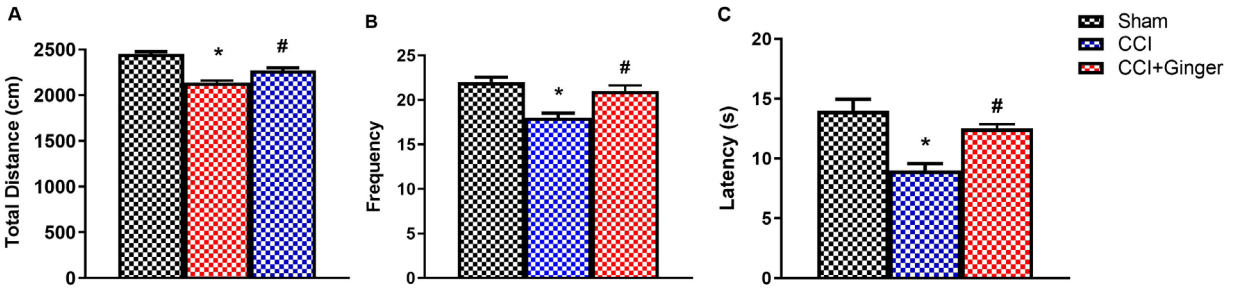

Figure 4. Day 14 baseline OF test and TF test results (n = 10 for each group). (a) Total distance (cm) from OF, (b) frequency in OF, (c) latency (s) in TF. The * indicates a significant difference ($p < 0.05$) from the sham group, and the # indicates a significant difference ($p < 0.05$) from the CCI group, based on one-way ANOVA followed by a Tukey posthoc test. All data are presented as mean ± SEM.

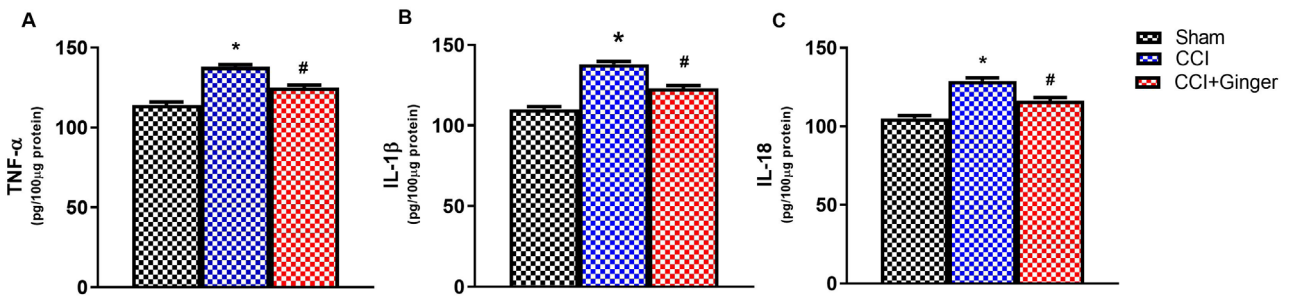

Figure 5. Neuroinflammation results in the cerebral cortex for (a) TNF-α levels, (b) IL-1β levels, and (c) IL-18 levels (n=10, for each group). The * indicates a significant difference ($p < 0.05$) from the sham group, and the # indicates a significant difference ($p < 0.05$) compared to the CCI group, based on one-way ANOVA followed by a Tukey posthoc test. All data are presented as mean ± SEM.

reduce inhibitory system activation and are used in pain treatment. These antioxidant compounds are frequently used as therapeutic agents affecting antiinflammatory pathways. The side effects of long-term use of current symptomatic therapies for the treatment of NP limit treatment and rarely focus on the actual causes [20]. *Zingiber officinale* Roscoe (Zingiberaceae), known as ginger, is included in many official pharmacopoeias of different countries and contains nonvolatile components with biological activity, such as gingerols, shogaols, paradols, and zingerone in the dried rhizome [20]. Ginger is a promising bioactive compound used in the treatment of NP due to its antiinflammatory properties [22]. As expected, the GC–MS analysis identified [6]-gingerol and

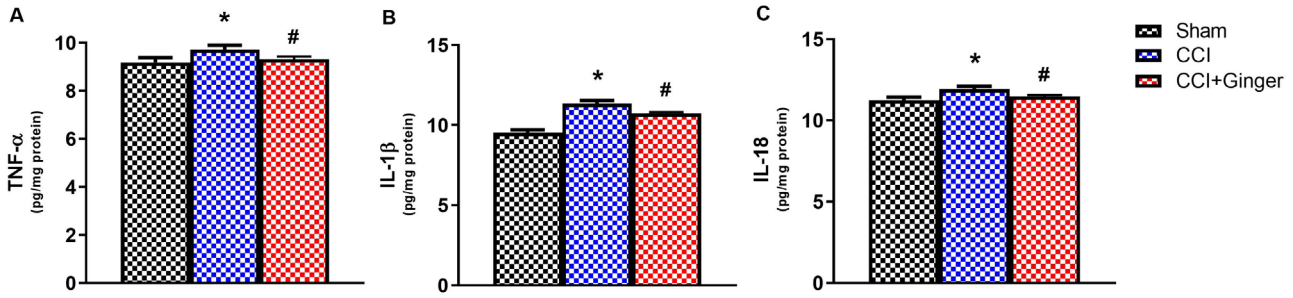

Figure 6. Neuroinflammation results in spinal cord. A) TNF-α levels, B) IL-1β levels, C) IL-18 levels. (n=10, for each group; * p<0.05, shows the difference compared to the Sham group, # p<0.05 shows the difference compared to the CCI group, one-way ANOVA test, followed by Tukey post hoc test). All data are presented as means ± SEM.

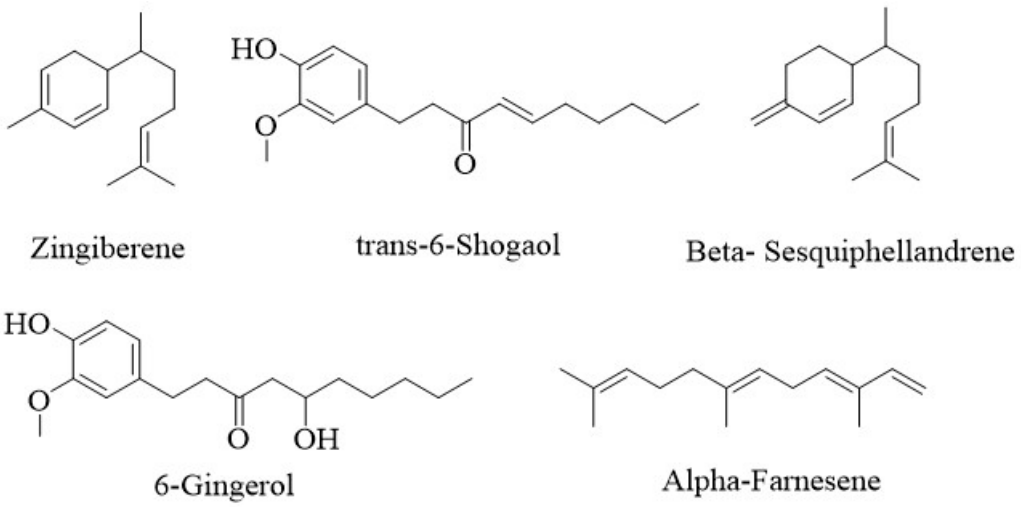

Figure 7. The 5 main components of ginger extract.

[6]-shogaol as major components, as well as zingiberene, β-sesquiphellandrene, and (E,E)-α-farnesene [37]. Gingerols are the main pungent compounds found in the rhizomes of ginger (*Zingiber officinale* Roscoe), and gingerol analogues are thermally unstable and readily undergo dehydration reactions to form the corresponding shogaols, which give dried ginger its characteristic pungent taste. Both gingerols and shogaols exhibit a range of biological activities ranging from anticancer, antioxidant, antimicrobial, antiinflammatory and antiallergic to various CNS activities [37]. Gingerols and shogaols have been thoroughly studied for their antiinflammatory properties, especially concerning the reduction of NF-κBp65 activation and proinflammatory cytokines released from glial cells [20]. The injection of 10 μg [6]-gingerol into the rat spinal cord was found to be effective in relieving NP. [6]-Gingerol was also found to block prion peptide-mediated neurotoxicity associated with hypoxia-inducible factor 1a, while preserving mitochondrial function [37]. Specific primary sensory neurons include a functional vanilloid receptor accountable for the transmission of a pain or itch stimulus to the CNS. This receptor is activated by vanilloids such as capsaicin and high temperatures. It has been determined that [6]-gingerol inhibits capsaicin-induced contraction at a certain dose [37]. Excessive oxidative stress has been linked to the advance of chronic NP [22]. It has been demonstrated that both gingerols and shogaols significantly decreased ccf-mtDNA levels in NP animals treated with spinal nerve ligation [22]. In addition, it has been shown that ginger root extract increased antioxidant capacity and improved mitochondrial function by reducing ROS production in rats [22].

The relationship between ginger, which is known to have a therapeutic effect on locomotor activity and thermal hyperalgesia after NP, and neuroinflammation has not been fully elucidated. In the present study, the

therapeutic effects of ginger on the spinal cord and cortex in the neuroinflammatory pathway were investigated in rats with experimental sciatic nerve injury.

We found that ginger treatment could alleviate pain behaviours in CCI rats by reducing proinflammatory cytokine production in cortex and spinal cord tissues. The experimental NP was induced using the CCI model, which is an easily reproducible and reliable method. CCI mimics traumatic mechanical injury in humans very well and demonstrates many of the pathophysiological features of chronic NP [38]. After the CCI surgery, the rats showed abnormal posture and licking of injured hindlimbs, resembling clinical neuropathic symptoms resulting from nerve injury in chronic pain patients [39]. Pain behaviours peaked on the 7th day after CCI [39], and distinctly nociceptive behaviours were apparent until day 14. [40]. Therefore, we evaluated the nociceptive pain behaviours on the 7th and 14th days after CCI surgery. We determined that locomotor activity (open field test) and thermal hyperalgesia (tail flick test) were affected at day 7 in the CCI rats. These results are consistent with previous studies demonstrating increased nociceptive behaviours due to sciatic nerve injury in rats following CCI [39,41,42]. We also determined that 200 mg/kg of ginger treatment provided improvements in the nociceptive behaviours of CCI rats. Borgonetti et al. reported in their study on mice with sciatic nerve damage that 200 mg/kg ginger treatment for 7 days, starting from the 3rd day of nerve damage, improved mechanical and thermal allodynia but did not change locomotor activity [20]. In addition, the characteristic of persistent NP mode is decreased locomotor activity [43]. In contrast, we observed that 200 mg/kg ginger treatment improved both locomotor activity and thermal allodynia. NP begins to occur on the 7th day after surgery in animals with nerve damage [2]. Borgonetti et al. started treatment on day 3 after surgery, whereas we started the ginger treatment on the 7th day after surgery and applied it for 7 days; this might explain the different results for improvement in locomotor activity.

Proinflammatory cytokines are important in the development and maintenance of NP [44]. NP is characterised by glial cell activation and proinflammatory cytokine secretion in the spinal dorsal horn [2]. TNF-α is one of the most potent proinflammatory cytokines expressed by microglia, astrocytes, and primary sensory dorsal root ganglion neurons [45]. IL-1β is another important inflammatory cytokine expressed by both microglia and astrocytes in the spinal cord [45]. Experimental studies have shown that TNF-α and IL-1β induce NP and that anticytokine therapy may be promising in the treatment of NP. It is also known that inflammatory changes in macrophages lead to the secretion of IL-18 as well as IL-1β in both the CNS and the PNS following CCI [46,47]. Cheng et al. reported that CCI injury increased IL-1β and IL-18 in the spinal cord and also decreased claw withdrawal latency and claw withdrawal threshold [39]. They also showed that treatment with loganin, an iridoid glycoside, decreased IL-1β and IL-18 in the spinal cord [39]. Likewise, Wen et al. found that CCI injury significantly decreased paw withdrawal latency and paw withdrawal threshold on days 7 and 14, while TNF-α, IL-1β, and IL-6 levels in the spinal cord were significantly increased [42]. Borgonetti et al. reported that 200 mg/kg ginger treatment inhibited NF-κB signalling activation and reduced the release of IL-1β, TNF-α, and IL-6 in CCI rats [20]. Consistent with these reports, our study showed that CCI injury significantly increased levels of TNF-α, IL-1β, and IL-18 in the cerebral cortex and spinal cord. Treatment with 200 mg/kg ginger for 7 days decreased the levels of TNF-α, IL-1β, IL-6, and IL-18 in the cerebral cortex and spinal cord tissue of CCI rats. Our results show that the ginger treatment regulates locomotor activity and thermal hyperalgesia in CCI rats by its antiinflammatory effects in the cerebral cortex and spinal cord.

Our study has some limitations. We only used male rats, not females. It is known that sex differences are important contributors to pain sensitivity and the analgesic efficacy of treatments. Another important limitation is that the long-term effects of phenolic compounds such as [6]-shogaol and [6]-gingerol obtained from ginger extract have not been investigated; understanding these is important for the application of treatments in clinical settings. In addition, investigating the free radical levels and apoptosis pathways that lead to neuroinflammation would have made a great contribution.

5. Conclusion

As a result of GC–MS quantitative analysis, trans-[6]-shogaol (13.34%) and [6]-gingerol (6.37%) were found to be the main phenolic components in ginger. This supports previous research that found the same results for [6]-gingerol and [6]-shogaol as a result of GC–MS analysis [37]. These components have been investigated for their antiinflammatory properties [20], and our study concurred, concluding that trans-[6]-shogaol and [6]-gingerol, as active ingredients in total ginger extract, may provide a treatment effect on weakened nociceptive behaviour and reduced thermal hyperalgesia caused by sciatic nerve damage. The findings showed that 200 mg/kg ginger extract treatment attenuated nociceptive behaviour and reduced thermal hyperalgesia caused by sciatic nerve injury. Ginger extract treatment, which is rich in phenolic components such as shogaols and gingerols, showed a therapeutic effect on NP by regulating cytokine levels. There is a need for antioxidant treatment strategies to be used alone or in combination with other effective therapies to alleviate NP in the future.

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Ethical approval
The authors declare no competing financial interests. All animal use and experimental protocols were approved and implemented by the Animal Care and Ethics Committee of Erciyes University (01.06.2022/Approval no: 22/132).

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