Original Article

The Improvement of Pain Behavior and Sciatic Nerves Morphology in Mice Model of Painful Diabetic Neuropathy upon Administration of Ginger (*Zingiber officinale* Roscoe.) Extract and Its Pungent Compound, 6-Shogaol

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Abstract

Background: 6-Shogaol is one of the bioactive compounds from ginger (*Zingiber officinale* Roscoe.) that had been widely used in many diseases. This research was aim to investigate the effect of ginger extract and its pungent compound, 6-shogaol on pain behavior and sciatic nerve morphology in mice model of painful diabetic neuropathy. **Materials and Methods:** Seventy-two male BALB/c mice were divided into nine groups. Diabetes was induced by single intraperitoneal injection of streptozotocin (STZ) 110 mg/kg body weight (BW). The mice were considered diabetic when the serum glucose level was at least 200 mg/dL. Thermal and mechanical hyperalgesia was measured using tail-flick latency and Randall–Selitto test once a week. Daily oral administration of 6-shogaol doses 5, 10, and 15 mg/kg BW, ginger extract doses 100, 200, and 400 mg/kg BW, or gabapentin dose 100 mg/kg BW was started at week 4. Diabetic mice without treatment and mice without STZ induction were used as controls. At week 7, the mice were euthanized, and paraffin sections of 1% osmium tetroxide-stained sciatic nerve samples were observed. **Results:** Ginger extract and 6-shogaol, but not gabapentin, produced dose-dependent lowering blood glucose effect. However, the mean of serum glucose level was not <200 mg/dL. After 4 weeks of hyperglycemia, the diabetic groups showed signs of hyperalgesia. The ginger extract, 6-shogaol, and gabapentin administration attenuated the hyperalgesic effect. The microstructure of sciatic nerves in diabetic mice that received ginger extract and 6-shogaol was less damaged compared to the diabetic control group. **Conclusion:** From this research, ginger extract and its pungent compound, 6-shogaol, showed anti-hyperalgesic and neuroprotective effects.

Keywords: 6-Shogaol, axon morphology, ginger extract, painful diabetic neuropathy, sciatic nerves, *Zingiber officinale*

Introduction

Half of the diabetic patients suffer from diabetic neuropathy, and almost 50% of them report painful symptoms,[1,2] such as hyperalgesia and allodynia.[1] Reduced glucose uptake induces cellular damage due to insufficient adenosine triphosphate production. Impairment of neurotrophic support, activation of protein kinase C, activation of poly (ADP-ribose) polymerase, and formation of advanced glycation end products in a diabetic individual[3,4] modulate various biochemical pathways, which lead to structural and functional abnormality of the peripheral nerves including the sciatic nerves. Various cytokines and excitatory neurotransmitters also contribute to downregulation of the pain threshold.[1,5] Therefore, the mechanism of painful diabetic neuropathy (PDN) development is very complex.

Due to its complex mechanism, the treatment of PDN is very challenging. Natural resources are an excellent material of choice because it allows mass production and increases the

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value to local products. One of the natural drug candidates for PDN is ginger. Recent studies reported that ginger showed some biological activities and efficacy to control hyperglycemia *in vitro* and *in vivo* and also showed efficacy in clinical trials for the diabetic patient.[6-8] The 6-shogaol, one of the pungent components in ginger, has been proven as an antioxidant and in an *in vitro* experiment demonstrated as a ligand to the transient receptor potential cation channel subfamily V member 1 (TRPV1), a polymodal receptor that is important in PDN pathophysiology.[9,10] Activation of TRPV1 in diabetic mice induces action potential by increasing calcium influx and releasing neuropeptides including glutamate and causes pain.[11] However, prolonged TRPV1 activation, such as during capsaicin exposure, desensitized TRPV1 and reduced pain.[12,13] There was a report that 6-shogaol activated TRPV1 channels by noncovalent bonding.[14] Based on an *in silico* molecular docking study using AutoDock Vina software (http://vina.scripps.edu/), 6-shogaol had the highest affinity to TRPV1 compared to the other substances, i.e. 8-shogaol, 10-shogaol, 6-gingerol, 8-gingerol, and 10-gingerol.[15] Whether 6-shogaol attenuates hyperalgesia in PDN is yet to be elucidated.

In this study, the anti-hyperalgesic capacity of ginger extract and 6-shogaol is tested in streptozotocin (STZ)-induced diabetic mice that undergo PDN. Tail-flick latency and paw withdrawal threshold were used to measure the thermal and mechanical pain behavior, respectively. In addition, qualitative assessment of sciatic nerve's morphology will be presented.

Materials and Methods

Materials

Rhizomes of ginger (*Zingiber officinale* var Roscoe.) were obtained from Klaten, Central Java, Indonesia, and identified in the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia. Dried ginger (0.5 kg) was macerated three times (5 × 24 h) using ethanol 96% and evaporated to get the thick extract. The ethanolic extract from this process was obtained with the 5.6% yield.

The 6-shogaol was purchased from Chemfaces, Wuhan, China (CAS No. 555-66-8). Gabapentin was obtained from PT Indofarma, Indonesia. Dimethylsulfoxide, sodium carboxymethylcellulose, and phosphate-buffered saline (PBS) were purchased from Life Technologies Corporation (GIBCO), Singapore. Osmium tetroxide was purchased from Sigma-Aldrich Pte Ltd., Singapore. STZ was purchased from Nacalai Tesque Inc., Japan.

Animals

Seventy-two male BALB/c mice (30–40 g) were obtained from the Experimental Animal Unit, Animal Research and Development Centre, Universitas Gadjah Mada, Yogyakarta. The mice were housed and maintained under the standard conditions of 12-h light/dark cycle, 25°C ± 2°C, and 60%–70% humidity and had free access to food and water. All the animals were acclimatized for 1 week before experimentation. The experiment procedures were approved by the Institutional Animal Ethics Committee of the Integrated Research and Testing Laboratory, Universitas Gadjah Mada (number KE/FK/559/EC/2016).

Streptozotocin-induced painful diabetic neuropathy

Mice were fasted overnight for 14–16 h before the STZ induction. A single high dose of STZ of 110 mg/kg body weight (BW) (dissolved in freshly prepared cold citrate buffer 1.1%, pH 4.5) was administered intraperitoneally. One week after STZ induction, mice with serum glucose levels >200 mg/dl[16] were considered as diabetic and selected for the next experiments. Blood samples were obtained from the retro-orbital vein, and the serum glucose was measured using Glucose GOD FS kit (Diagnostic Systems GmbH, Holzheim, Germany) based on glucose oxidase-peroxidase method. Serum glucose was measured at baseline, 7 days after induction, and once a week at week 4, 5, 6, and 7. Mice BW were measured using an animal scale once a week. The mice were divided into nine groups, eight mice for each group, i.e., normal control (non-STZ induction), diabetic control (received vehicle); ginger extract (100, 200, and 400 mg/kg BW), 6-shogaol (5, 10, and 15 mg/kg BW), and gabapentin (100 mg/kg BW). Oral administration of the test substance was done once daily from week 4 after STZ induction until week 7. Diagram scheme of this research is illustrated in Figure 1.

Pain behavioral testing

Pain behavioral testings were assessed before STZ injection (baseline) and every week until week 7. The tests were done 60 min after the administration of the test substance (ginger extract, 6-shogaol, and gabapentin). All tests were performed in three replications.

Tail-flick test

The thermal hyperalgesia was measured using tail-flick analgesiometer (Columbus Instrument, 1430, Ohio, USA). The tail of each mouse was exposed to a nichrome-radiant heat. The intensity of the heat was adjusted to give a basal latency range of 4–8 s in all mice. The mice that had response out of the range were excluded from the experiment. The tail-flick latency was defined as the time interval that was needed by mice to flick

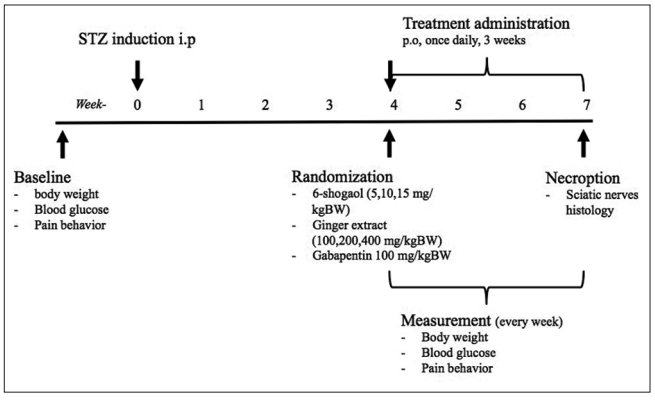


Figure 1: Diagram scheme of the research

its tail after the exposure of the radiant heat. The maximum cutoff time was set to 15 s to prevent tissue damage.[17,18] The percentage of maximum possible effect (MPE) was calculated according to the formula:[19] % MPE = (test latency – predrug latency)/(baseline cutoff latency – predrug latency) ×100.

Randall–Selitto test

The mechanical hyperalgesia was measured using Randall–Selitto paw pressure test analgesiometer (Ugo Basile SRL, Varese, Italy). An increasing mechanical force was directly applied to the dorsal surface of the hind paws of the mice through a conical probe until a withdrawal response, i.e. mouse paw withdrawal, vocalization or struggled, resulted. The point of application was marked with ink to maintain the location over repeated trials. The paw withdrawal threshold was recorded in gram, and the cutoff threshold was set at 250 g.[18,20] The maximum possible effect was calculated according to the following formula:[19] % MPE = (test pressure threshold – pretest pressure threshold/(baseline cutoff pressure threshold – pretest pressure threshold) ×100.

Histological evaluation

On week 7, mice were euthanized, and the segment of the sciatic nerve exposed upon mid-thigh incision was removed and fixed in 10% paraformaldehyde in 0.1 M PBS (pH 7.4) overnight. The sciatic nerves were washed in PBS and then immersed in osmium tetroxide 1% for 3 days in 4°C. At day 4, the sciatic nerves were dehydrated gradually using ethanol, cleared in xylene, and embedded in paraffin. The thin (4 μm) sections were cut using a microtome before examination using a light microscope (Zeiss observer Z1, Massachusetts, USA).[21] The sciatic nerve morphology was compared between the groups.

Statistical analysis

The blood glucose level, BW, tail-flick latency, and paw withdrawal threshold were presented as mean ± standard error of the mean. Statistical differences between groups were analyzed using one-way ANOVA followed by *post-hoc* Tukey test if all values were eligible using normality and homogeneity tests (GraphPad InStat version 3.10; GraphPad Software Inc., La Jolla, CA 92037, USA). The $P < 0.05$ was considered statistically significant.

Results

The effect of ginger extract and 6-shogaol on serum glucose levels and body weight

One week after STZ induction, the range of serum glucose level in diabetic groups was from 209.5 to 599.4 mg/dL with mean at 332.44 ± 39.50 mg/dL. The value was significantly higher compared to the normal control group (102.31 ± 5.74 mg/dL) ($P < 0.001$). The serum glucose level in diabetic groups increased until week 4 at a mean level 414.28 ± 52.72 mg/dl [Table 1] in the range of 205.38 mg/dL–678.4 mg/dL. After 3 weeks receiving ginger extract or 6-shogaol, at week 7, all of the ginger and shogaol groups showed significantly lower serum glucose level compared to the serum glucose level in the diabetic control group (514.22 ± 26.5 mg/dL; $P < 0.001$). It was noted that until week 7, the serum glucose level in all diabetic mice, except the group that received gabapentin, were still >200 mg/dL. The group that received gabapentin did not show a significant difference in the serum glucose level compared to the diabetic control group ($P > 0.05$). The groups received the highest dose of ginger extract 400 mg/kg BW and 6-shogaol 15 mg/kg BW had the lowest mean of serum glucose level at 221.95 ± 23.13 mg/dL and 208.25 ± 49.37 mg/dL [Table 1], respectively. Those two groups started to show significant lower serum glucose level at week 6, sooner than any groups that received 6-shogaol or ginger extract.

The BW of normal control group increased along the experiment, but the BW of diabetic mice was decreased after STZ injection. At week 4, the diabetic groups had lower BW compared to the baseline and normal control groups ($P < 0.001$). At week 7, there was no significant

Table 1: Effect of ginger extract (*Zingiber officinale* Roscoe.) and 6-shogaol on serum blood glucose level in normal and diabetic mice

Group	Blood glucose (mg/dL)(mean±SEM)				
	Baseline	Week 4	Week 5 after STZ injection	Week 6 after STZ injection	Week 7 after STZ injection
Normal	100.84±5.91	103.73±4.62	106.62±3.26	105.33±5.10	107.31±4.65
Diabetic	107.92±5.02	414.28±52.72***	479.62±29.91***	481.90±25.97***	514.22±26.50***
Ginger extract (100 mg/kg BW)	89.73±6.47	453.70±60.06***	430.62±39.55***	372.15±36.41***	309.91±36.20**,##
Ginger extract (200 mg/kg BW)	95.78±9.06	448.81±58.61***	380.83±46.87***	296.65±33.35***,##	261.04±34.11***,##
Ginger extract (400 mg/kg BW)	105.16±6.70	435.23±58.59***	290.29±39.76***	231.90±30.85***,##	221.95±23.13***,###
6-Shogaol (5 mg/kg BW)	99.91±7.60	442.83±56.57***	362.65±52.03***,##	329.53±51.89***,#	273.39±49.63***,##
6-Shogaol (10 mg/kg BW)	94.75±9.53	448.62±55.74***	328.54±28.80***,##	292.32±44.70**,#	214.81±24.66***,###
6-Shogaol (15 mg/kg BW)	109.70±5.43	432.87±61.30**	316.20±40.31***,##	254.61±40.80**,##	208.25±49.37##
Gabapentin (100 mg/kg BW)	103.22±7.73	430.80±55.32***	440.71±26.81***	457.44±26.36***	464.79±22.33***

Data are presented as the mean±SEM of eight mice. ***P<0.001, **P<0.01 significantly different versus the normal group, #P<0.05, ##P<0.01, ###P<0.001 significantly different versus the diabetic group. 6-Shogaol and ginger extract were administered for 3 weeks starting week 4 after STZ injection. SEM: Standard error of the mean, STZ: Streptozotocin

difference in BW of the diabetic control group compared to the mice that received 6-shogaol (5, 10, and 15 mg/kg BW), ginger extract (100, 200, and 400 mg/kg BW), and gabapentin 100 mg/kg BW [Table 2].

The effect of ginger extract and 6-shogaol on painful diabetic neuropathy

Before STZ injection, the mean tail-flick latency and paw withdrawal threshold were not significantly different than the normal control ($P > 0.05$). The mean value of tail-flick latency and paw withdrawal threshold was significantly decreased 1 week after STZ injection, indicating the hyperalgesia effect. The thermal and mechanical hyperalgesia persisted to decrease until week 4 ($P < 0.001$). Administration of ginger extract (100, 200, and 400 mg/kg BW) and 6-shogaol (5, 10, and 15 mg/kg BW) attenuated the decrease in tail-flick latency [Table 3] and paw withdrawal threshold [Table 4] in a dose-dependent manner ($P < 0.001$). The highest dose of ginger extract, 400 mg/kg BW and 6-shogaol, 15 mg/kg BW showed the best anti-hyperalgesic effect, better than gabapentin 100 mg/kg BW either in thermal or mechanical-induced hyperalgesia.

Ginger extract and 6-shogaol produced similar maximum possible anti-thermal and mechanical hyperalgesic effect (MPE). However, the MPE of 6-shogaol was slightly higher than the ginger extract. The tail-flick latency showed that the administration of 6-shogaol dose 15 mg/kg BW had significantly higher MPE (111.49% ± 7.23%) than the diabetic group (12.93% ± 5.51%, $P < 0.001$) and was also significantly higher than gabapentin 100 mg/kg BW (73.29% ± 6.18%, $P < 0.05$), [Figure 2]. At week 7, the tail-flick latency in 6-shogaol dose 15 mg/kg BW group was not significantly different compared to the normal control group. However, the paw withdrawal threshold in the 6-shogaol dose 15 mg/kg BW group was still lower than the normal control group ($P < 0.05$).

The effect of ginger extract and 6-shogaol on axon morphology of sciatic nerves in painful diabetic neuropathy

Osmium tetroxide staining in sciatic nerve paraffin sections indicated that many myelinated axons were damaged at 7 weeks after STZ induction in diabetic mice compared to normal control. Figure 3a shows the morphology of the normal sciatic nerves, characterized with structured axons that are

Table 2: Effect of 6-shogaol and ginger extract (*Zingiber officinale* Roscoe.) on body weight change in normal and diabetic mice

Group	Body weight (g) (mean±SEM)				
	Baseline	Week 4	Week 5	Week 6	Week 7
Normal	36.33±0.89	40.13±1.13	40.20±0.80	40.73±0.95	40.93±0.85
Diabetic	35.66±1.00	30.51±1.61***	30.98±1.40***	31.14±1.31***	31.56±1.30***
Ginger extract (100 mg/kg BW)	38.15±1.01	33.96±1.33	34.25±1.24	34.15±1.24*	34.06±0.97*
Ginger extract (200 mg/kg BW)	37.10±1.53	32.09±1.45**	32.78±1.44*	32.54±1.39**	31.85±1.29***
Ginger extract (400 mg/kg BW)	36.46±0.40	34.09±1.19	34.59±1.29	35.60±1.24	35.65±1.20
6-Shogaol (5 mg/kg BW)	37.81±0.67	34.94±1.26	35.41±1.15	36.53±1.05	37.03±1.08
6-Shogaol (10 mg/kg BW)	36.89±1.16	33.20±1.51*	32.88±1.26**	34.24±1.23*	35.38±1.45
6-Shogaol (15 mg/kg BW)	35.88±0.77	33.14±0.84*	33.90±0.93*	34.79±1.07*	34.78±0.84*
Gabapentin (100 mg/kg BW)	37.40±1.65	34.70±2.36	35.18±2.40	35.88±2.23	35.73±2.32

6-Shogaol and ginger extract were administered for 3 weeks starting week 4 after STZ induction. Data are presented as the mean±SEM of eight mice. ***P<0.001, **P<0.01, *P<0.05 significantly different versus the normal group. SEM: Standard error of the mean, STZ: Streptozotocin

Table 3: The effect of ginger extract (*Zingiber officinale* Roscoe.) and 6-shogaol on thermal-induced hyperalgesia under painful diabetic neuropathy in mice

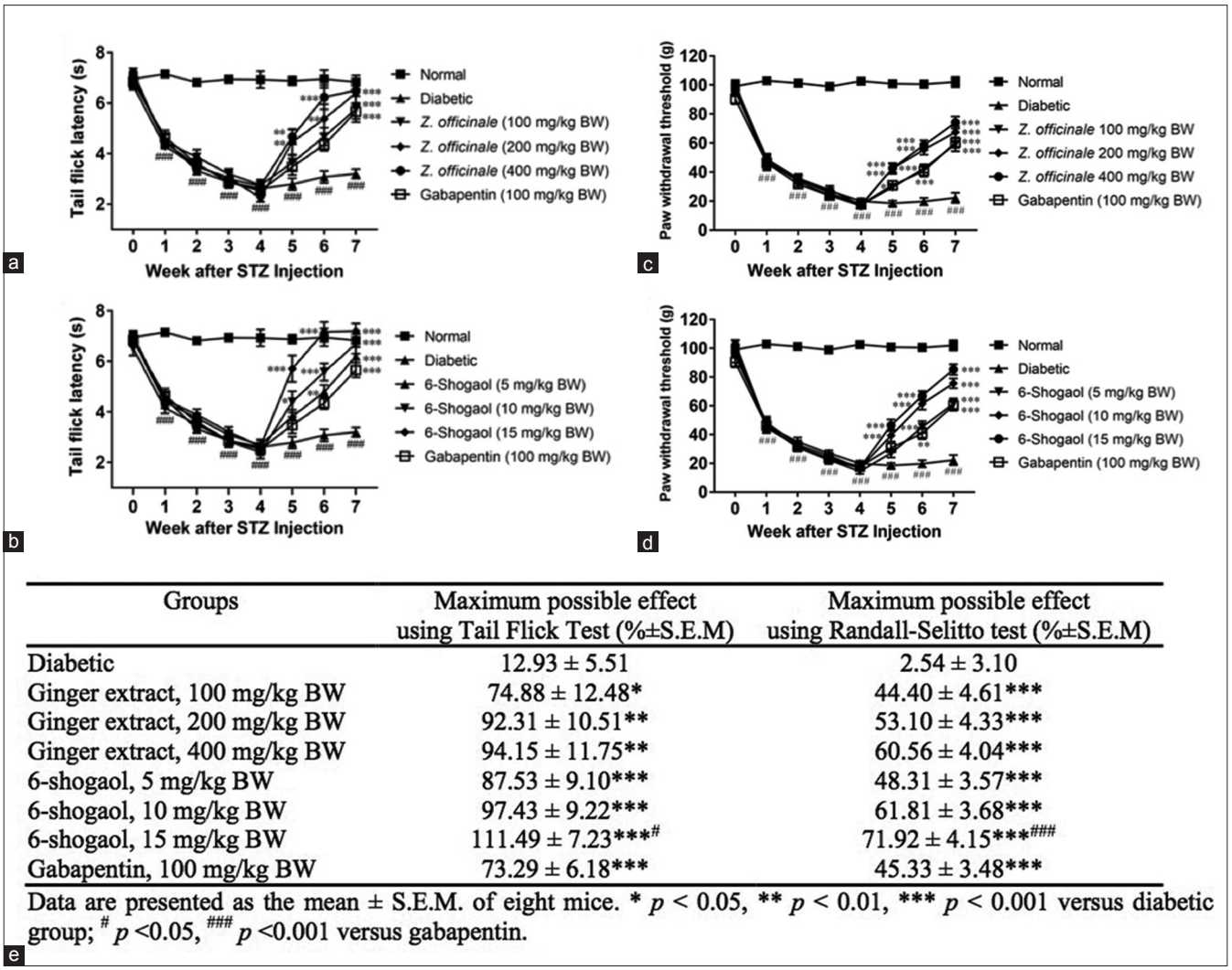
Group	Tail flick latency (s) (mean±SEM)				
	Baseline	Week 4 after STZ injection	Week 5 after STZ injection	Week 6 after STZ injection	Week 7 after STZ injection
Normal	6.96±0.20	6.93±0.33***	6.88±0.17***	6.95±0.35***	6.83±0.27***
Diabetic	6.97±0.24	2.62±0.17$^{###}$	2.79±0.23$^{###}$	3.08±0.24$^{###}$	3.20±0.19$^{###}$
Ginger extract (100 mg/kg BW)	6.97±0.15	2.75±0.22$^{###}$	3.64±0.29$^{###}$,*	4.67±0.35$^{##}$,**	5.76±0.51**
Ginger extract (200 mg/kg BW)	6.68±0.16	2.25±0.15$^{###}$	4.46±0.50$^{###}$,**	5.37±0.59**	6.40±0.48***
Ginger extract (400 mg/kg BW)	7.19±0.19	2.72±0.18$^{###}$	4.67±0.30$^{###}$,**	6.23±0.48***	6.50±0.49***
6-shogaol (5 mg/kg BW)	6.86±0.25	2.70±0.21$^{###}$	3.82±0.27$^{###}$*	4.79±0.27$^{##}$,**	6.20±0.38***
6-shogaol (10 mg/kg BW)	6.51±0.28	2.41±0.26$^{###}$	4.40±0.42$^{###}$,**	5.58±0.34$^{#}$,**	6.69±0.39***
6-shogaol (15 mg/kg BW)	6.88±0.25	2.63±0.26$^{###}$	5.71±0.52***	7.17±0.39***	7.20±0.30***
Gabapentin (100 mg/kg BW)	6.84±0.24	2.53±0.25$^{###}$	3.48±0.33$^{###}$	4.35±0.24$^{###}$,**	5.66±0.29$^{#}$,***

Data are presented as the mean±SEM of eight mice. $^{##}P$<0.01, $^{###}P$<0.001 significantly different versus the normal group. ***P<0.001, **P<0.01, *P<0.05 significantly different versus the diabetic group. SEM: Standard error of the mean, STZ: Streptozotocin

Table 4: The effect of ethanolic extract (*Zingiber officinale* Roscoe.) and 6-shogaol on mechanical-induced hyperalgesia under painful diabetic neuropathy in mice

Group	Paw withdrawal threshold (g) (mean±SEM)				
	Baseline	Week 4 after STZ injection	Week 5 after STZ injection	Week 6 after STZ injection	Week 7 after STZ injection
Normal	99.17±3.27	102.67±1.90***	100.83±2.38***	100.58±2.57***	102.08±3.59***
Diabetic	97.71±3.25	19.99±1.91$^{###}$	18.54±1.71$^{###}$	19.79±2.39$^{###}$	22.29±3.49$^{###}$
Ginger extract (100 mg/kg BW)	95.83±2.29	17.49±1.61$^{###}$	29.58±1.72$^{###}$.**	42.50±2.85$^{###}$.***	59.17±4.78$^{###}$.***
Ginger extract (200 mg/kg BW)	99.99±3.50	17.30±1.94$^{###}$	42.50±3.78$^{###}$.**	55.42±3.42$^{###}$.***	67.50±3.97$^{###}$.***
Ginger extract (400 mg/kg BW)	95.83±3.62	16.87±1.39$^{###}$	42.71±3.00$^{###}$.***	58.54±3.37$^{###}$.***	74.17±4.09$^{###}$.***
6-Shogaol (5 mg/kg BW)	103.34±2.47	14.58±1.86$^{###}$.*	27.08±3.07$^{###}$.*	45.21±4.11$^{###}$.***	61.46±3.67$^{###}$.***
6-Shogaol (10 mg/kg BW)	94.80±4.76	16.67±1.97$^{###}$	39.38±4.09$^{###}$.**	61.46±4.15$^{###}$.***	75.63±3.38$^{###}$.***
6-Shogaol (15 mg/kg BW)	102.09±3.42	16.88±1.16$^{###}$	46.46±4.05$^{###}$.***	67.08±3.18$^{###}$.***	85.21±3.68$^{###}$.***
Gabapentin (100 mg/kg BW)	90.42±3.54	17.71±2.46$^{###}$	30.83±3.09$^{###}$.**	40.83±3.96$^{###}$.***	60.21±3.60$^{###}$.***

Data are presented as the mean±SEM of eight mice. $^{###}P$<0.001 significantly different versus the normal group, ***P<0.001, **P<0.01, *P<0.05 significantly different versus the diabetic group. SEM: Standard error of the mean, STZ: Streptozotocin



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Groups	Maximum possible effect using Tail Flick Test (%±S.E.M)	Maximum possible effect using Randall-Selitto test (%±S.E.M)
Diabetic	12.93 ± 5.51	2.54 ± 3.10
Ginger extract, 100 mg/kg BW	74.88 ± 12.48*	44.40 ± 4.61***
Ginger extract, 200 mg/kg BW	92.31 ± 10.51**	53.10 ± 4.33***
Ginger extract, 400 mg/kg BW	94.15 ± 11.75**	60.56 ± 4.04***
6-shogaol, 5 mg/kg BW	87.53 ± 9.10***	48.31 ± 3.57***
6-shogaol, 10 mg/kg BW	97.43 ± 9.22***	61.81 ± 3.68***
6-shogaol, 15 mg/kg BW	111.49 ± 7.23***$^{\#}$	71.92 ± 4.15***$^{###}$
Gabapentin, 100 mg/kg BW	73.29 ± 6.18***	45.33 ± 3.48***

Data are presented as the mean ± S.E.M. of eight mice. * p < 0.05, ** p < 0.01, *** p < 0.001 versus diabetic group; $^{\#}$ p <0.05, $^{###}$ p <0.001 versus gabapentin.

Figure 2: Tail-flick latency of ginger extract (a) and 6-shogaol (b) versus control group. Paw withdrawal threshold of ginger extract (c) and 6-shogaol (d) versus control group. (e) Maximum possible effect of anti-hyperalgesia after administration of 6-shogaol and ginger extract (*Zingiber officinale* Roscoe.) under painful diabetic neuropathy in mice (week 7)

surrounded by the proportional thickness of myelin sheath to its diameter with many appearances of lipid droplets. The sciatic nerve of the diabetic control group [Figure 3b] showed thin myelin sheath and myelin debris. The group received

Original Article

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Abstract

Background: 6-Shogaol is one of the bioactive compounds from ginger (*Zingiber officinale* Roscoe.) that had been widely used in many diseases. This research was aim to investigate the effect of ginger extract and its pungent compound, 6-shogaol on pain behavior and sciatic nerve morphology in mice model of painful diabetic neuropathy. **Materials and Methods:** Seventy-two male BALB/c mice were divided into nine groups. Diabetes was induced by single intraperitoneal injection of streptozotocin (STZ) 110 mg/kg body weight (BW). The mice were considered diabetic when the serum glucose level was at least 200 mg/dL. Thermal and mechanical hyperalgesia was measured using tail-flick latency and Randall–Selitto test once a week. Daily oral administration of 6-shogaol doses 5, 10, and 15 mg/kg BW, ginger extract doses 100, 200, and 400 mg/kg BW, or gabapentin dose 100 mg/kg BW was started at week 4. Diabetic mice without treatment and mice without STZ induction were used as controls. At week 7, the mice were euthanized, and paraffin sections of 1% osmium tetroxide-stained sciatic nerve samples were observed. **Results:** Ginger extract and 6-shogaol, but not gabapentin, produced dose-dependent lowering blood glucose effect. However, the mean of serum glucose level was not <200 mg/dL. After 4 weeks of hyperglycemia, the diabetic groups showed signs of hyperalgesia. The ginger extract, 6-shogaol, and gabapentin administration attenuated the hyperalgesic effect. The microstructure of sciatic nerves in diabetic mice that received ginger extract and 6-shogaol was less damaged compared to the diabetic control group. **Conclusion:** From this research, ginger extract and its pungent compound, 6-shogaol, showed anti-hyperalgesic and neuroprotective effects.

Keywords: 6-Shogaol, axon morphology, ginger extract, painful diabetic neuropathy, sciatic nerves, *Zingiber officinale*

Introduction

Half of the diabetic patients suffer from diabetic neuropathy, and almost 50% of them report painful symptoms,[1,2] such as hyperalgesia and allodynia.[1] Reduced glucose uptake induces cellular damage due to insufficient adenosine triphosphate production. Impairment of neurotrophic support, activation of protein kinase C, activation of poly (ADP-ribose) polymerase, and formation of advanced glycation end products in a diabetic individual[3,4] modulate various biochemical pathways, which lead to structural and functional abnormality of the peripheral nerves including the sciatic nerves. Various cytokines and excitatory neurotransmitters also contribute to downregulation of the pain threshold.[1,5] Therefore, the mechanism of painful diabetic neuropathy (PDN) development is very complex.

Due to its complex mechanism, the treatment of PDN is very challenging. Natural resources are an excellent material of choice because it allows mass production and increases the

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that was more related to pain and thermal hyperalgesia. Nevertheless, there was an effect of ginger extract and shogaol on mechanical hyperalgesia. It might be caused by the mild glucose-lowering effect and increase utilization of glucose that prevented axonal damage and resulted in better peripheral nerve morphology. Whether there are other molecular targets of ginger extract and 6-shogaol related to its neuroprotection effect still needs to be elucidated.

The maximum possible effect of 6-shogaol was higher than ginger extract both on mechanical and thermal hyperalgesia. This effect may be due to higher amounts of 6-shogaol administered in 6-shogaol groups compared to the ginger extract group. One limitation of this study is the lack 6-shogaol concentration in the ginger extract. However, based on Ok and Jeong,[28] a total amount of 6-shogaol in ethanol extract of ginger was 3–5 mg/g. If this observation was integrated into our doses, there was 1.2–2 mg of 6-shogaol in the 100–400 mg ginger extract. This amount of 6-shogaol in the ginger extract was much lower than our 6-shogaol's single doses, i.e., 5–15 mg. To obtain the same doses of 6-shogaol, we needed 7–8-fold doses of ginger extract as much as 2.8–3.2 g. The dose volume will be >2.5 ml, the maximum volume of oral administration in mice. The maximum possible effect of 6-shogaol on mechanical and thermal hyperalgesia was also higher than gabapentin. Gabapentin did not affect glucose utilization; hence, it had slightly minimized nerve damage in the diabetic mice, and their effect was solely based on activating $A_2 \partial_1$ site in calcium channel, which induced the release of Gamma-Aminobutyric Acid (GABA) and minimized pain.[29]

Conclusion

From our study, we conclude that 6-shogaol and ginger extract alleviate thermal and mechanical pain behavior in mice model of PDN due to its effect in blood glucose stabilization and neuroprotection. There were many reports related to ginger supplementation in diabetic patients. Ginger extract has been tested in clinical trials for its glucose-lowering effect. Administration of dried ginger for a long period did not induce any adverse effect in diabetic patients. Therefore, clinical trials of 6-shogaol for diabetic neuropathy should be considered. Further study is also needed to elucidate the activity of 6-shogaol and ginger extract in TRPV1 and NR2B to explain their exact mechanism in PDN.

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Conflicts of interest

There are no conflicts of interest.

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