Original Article

# Protective effect of ginger against the pentylenetetrazole-induced seizure threshold model in streptozocin treated-diabetic mice

Abdolkarim Hosseini[1], Naser Mirazi[2]*, Ali Gomar[3]

1. Department of Physiology, Faculty of Biological Science, Shahid Beheshti University, Tehran, Iran
2. Department of Biology, Faculty of Basic science, Bu-Ali Sina University, Hamedan, Iran
3. Department of Biology, Science & Research Institute, Islamic Azad University, Tehran, Iran

## Abstract

**Introduction:** There is evidence that diabetes affects seizure susceptibility. Ginger (*Zingiber officinale* Roscoe) which is used in traditional medicine has antioxidant activity and neuroprotective effects. The aim of this study was to evaluate the seizure threshold induced by pentylenetetrazole (PTZ) in diabetic mice after induction of diabetes with streptozocin and to examine the possible role of ginger extract in this manner.

**Methods:** The anticonvulsant effect of ginger was investigated using *i.v.* PTZ-induced seizure models in non-diabetic and diabetic mice. Different doses of the hydroethanolic extract of ginger (50 and 100 mg/kg; i.p.) were administered daily for 2 weeks before PTZ challenge. The effect of ginger on the appearance of three separate seizure endpoints e.g. myoclonic, generalized clonic, and tonic extension phase were recorded.

**Results:** The results showed that the ginger extract has anticonvulsant effects in the experimental model of seizure tested as it significantly increased the seizure threshold. Diabetic animal's shows high blood glucose level and lower seizure threshold compared with non-diabetic control animals. Hydroethanolic extract of ginger significantly increased the onset time of myoclonic seizure ($p<0.001$) and significantly prevented generalized clonic ($p<0.001$) and forelimb tonic extension ($p<0.001$) seizure induced by PTZ in both non-diabetic and diabetic animals compared with control group.

**Conclusion:** Based on the results, the hydroethanolic extract of ginger has anticonvulsant effects in diabetic mice, possibly through hypoglycemic effect, antioxidant mechanisms, and oxidative stress inhibition.

# Introduction

Diabetes mellitus (DM) is one of the common metabolic disorders associated with many complications in various organs (Esteghamati et al., 2009). This condition has profound impact on both central and peripheral nervous system and interfere with normal cellular metabolism in many complication prone cell types and is particularly damaging to neurons (Ghasemi et al., 2010). Many previously reported studies also have shown that hyperglycemia in the adult diabetic patients can increase the susceptibility to seizures (Chou et al., 2007;

Raghavendra et al., 2007; Huang et al., 2008). In consistent with human study, some studies reported that alloxan-induced diabetic hyperglycemia was associated with proconvulsant effects in the electroshock seizure model in the adult rats (Koltai and Minker, 1975) and mice (Tutka et al., 1998). Tutka et al. also reported that bicuculline-induced seizure threshold is significantly decreased in streptozocin-induced diabetic mice. Although there is some evidences that hyperglycemia mainly contributes to the alteration in the seizure threshold in diabetic animals and patients (Schwechter et al., 2003; Huang et al., 2008), the underlying mechanism in this phenomenon is remain to be discovered.

Many of the synthetic drugs used for treatment of seizure and epilepsy are not affordable because of their high cost. Some effective and new methods, like vagus nerve stimulation (VNS) or deep brain stimulation (DBS), are very expensive and not achievable for many patients in the developing countries. Also, patients with chronic epileptic disorders require prolonged periods of treatment and this can cause a significant financial burden to the health-care system and increases the chance of side effects (Engel et al., 2007). In recent years, traditional and herbal medicine is gaining popularity due to its widespread availability, moderate efficacy, and no or fewer side effects and low cost as compared with synthetic drugs (Edraki et al., 2014).

*Zingiber officinale* Roscoe (Zingiberaceae), or ginger is widely used as a spice. Ginger has been used for thousands of years for the treatment of numerous ailments, such as colds, arthritis, migraines, and hypertension (Iris and Wachtel-Galor, 2011). It is used in traditional Asian medicine for the treatment of stomachaches (Mascolo et al., 1989), nausea, diarrhea, and joint and muscle pain (Ojewole, 2006). Several research groups have demonstrated that ginger has anti-inflammatory effect, antioxidant activity (Jeena et al., 2013) and neuroprotective effect in diabetic animals (Shanmugam et al., 2011; El-Akabawy and El-Kholy, 2014). Recent reports suggest that ginger can improve insulin sensitivity and glycemic status in type 2 diabetic patients (Arablou et al., 2014), and that administration of ginger possesses potent anticonvulsant effect (Hosseini and Mirazi, 2014).

Laboratory models for epilepsy induction make it possible to analyze the mechanisms and

predisposing factors of epilepsy and to evaluate anticonvulsive drugs and treatment modalities. One of these models is an intravenous pentylenetetrazole (*i.v.* PTZ) infusion with a constant flow rate in animal models which elicits seizure response in a reliable, reproducible, and rapid manner (Mandhane et al., 2007). The objective of the present study was to evaluate the anticonvulsant activity of hydroethanolic extract of *Z. officinale* in the timed *i.v.* PTZ seizure test in diabetic mice.

# Materials and methods

**Plant material and preparation of the extract**

The fresh rhizomes of *Z. officinale* (herbarium code no. 1483) was purchased from the Institute of Medicinal Plants Tehran, Iran. The extract was prepared based on the previously reported method (Kar et al., 2003). Approximately 200 g of the dried rhizome powder from *Z. officinale* were extracted with 3 L of 80% aqueous ethanol using the percolation method at room temperature. The extracts were filtered through filter paper and evaporated to dryness under reduced pressure at a maximum of 45°C using a rotary evaporator. *Z. officinale* extract was dissolved in normal saline to a stock concentration of 50% (w/v) and then stored at 4°C. The dosage calculations were based on body weight of animals.

**Drugs**

Drugs used were streptozocin (STZ; Sigma, St. Louis, MO, USA), PTZ (purchased from the Sigma, Bristol, UK), and Phenobarbital Na (purchased from the Chemidaru Industrial Company, Iran). STZ was dissolved in 5 mM citrate buffer (pH 4.0) (El-Akabawy and El-Kholy, 2014). PTZ was prepared in saline as 1% w/v solution. Based on previous study on dose response of phenobarbital (Markowitz et al., 2011), the dose of 30 mg/kg (dissolved in physiologic saline solution) was chosen as suitable dose for this investigation. Appropriate vehicle controls were assigned for each experiment.

**Animals**

Adult male Swiss mice that weighed 30 ± 2 g (Pasteur Institute, Tehran, Iran) were used in the study. The animals were housed in standard polycarbonate cages in a temperature-controlled room (22 ± 2°C) on a 12-h light/dark cycle with free

access to food and water and were acclimated at least one week before experiments. The experiments were conducted between 9:00 AM and 3:00 PM. Animals were handled in accordance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health (NIH) publication 86-23; revised 1985). All the protocols were also approved by the institutional ethics committee of Bu-Ali Sina University. Each mouse was used only once.

Animals were made diabetic by intraperitoneal injection of 60 mg/kg STZ (El-Akabawy and El-Kholy, 2014). Control animals only received citrate buffer. After 3 days following STZ administration, blood samples were taken from tail vein and hyperglycemia was confirmed by measuring blood glucose levels, using a glucometer (GlucoDr. Plus, AGM-3000, Korea). Animals showing fasting blood glucose higher than 250 mg/dl were considered as diabetic mice. Normal and STZ-induced diabetic animals were each randomly divided into four groups including control groups treated with vehicle or phenobarbital and treatment groups treated with ginger extract at the dose of 50 and 100 mg/kg for 2 weeks. Each group included eight animals.

### The timed *i.v.* PTZ infusion test in mice

The test was conducted in mice based on the previously reported method (Mandhane et al., 2007) with some modification. After the treatment period, PTZ challenging dose were evaluated. In the time of experiment, the operator was unaware (blinded) of the specific treatment groups to which an animal belonged. A butterfly cannula (needle size 30G, DENTSPLY MPL Technologies, England) attached to an insulin syringe prefilled with PTZ solution was used. For the purpose of infusion, the animal was restrained and needle was inserted into the tail vein. The needle was connected by polyethylene tubing with a plastic syringe that was placed in the syringe pump (GMS Syringe Pump, Singapore). The accuracy of needle placement in the vein was confirmed by appearance of blood in the cannula. The needle was secured to the tail by a special tape. The animal was kept in a transparent Perspex box with holes for ventilation. In this way animal could move freely in the box without strain on the attached cannula with no severe struggling. The syringe contained 1% solution of PTZ in saline, which was administered into the vein of unrestrained animal at a constant rate of 1 ml/min. The time intervals from the start of infusion of PTZ solution to the appearance of three separate endpoints, *i.e.*, first myoclonic twitch, generalized clonus with loss of righting reflex and forelimb tonus, were recorded. The thresholds were calculated separately for each endpoint according to the following formula: threshold dose of PTZ (mg/kg) = (infusion duration (s) × infusion rate (ml/s) × PTZ concentration (mg/ml) × 1000)/body weight kg.

### Statistical analysis

Statistical analysis was performed using SPSS software (version 22; SPSS Inc., Chicago, IL., USA). Kolmogrov-Smirnov test was used to analyze the normality of the data distribution. Seizure thresholds were expressed as the amount of PTZ (in mg/kg) ± SEM (standard error of the mean) needed to produce the first apparent sign of each endpoint, and analyzed with the two-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. $P < 0.05$ was considered statistically significant. The assessment was done using Microsoft EXCEL software.

# Results

### Effect of ginger on the blood glucose (BG) levels and body weight changes

The effect of ginger on blood glucose level of STZ-induced diabetic mice is presented in Table 1. At the beginning, the BG level of STZ-induced diabetic mice was significantly increased compared with normal control (NC) group ($p < 0.001$). By the end of the experiment, the BG level of diabetic control (DC) group was significantly higher than that of NC group. However, after one week of ginger treatment, the BG levels of mice in diabetic ginger groups were decreased significantly. Compared with DC group, the BG levels of mice in diabetic ginger extract (50 mg/kg) (DGE-50) and diabetic ginger extract (100 mg/kg) (DGE-100) groups were significantly reduced ($p < 0.05$), at 7 days ($p < 0.05$) and at 14 days ($p < 0.01$), respectively. There was no significant differences in BG levels among normal phenobarbital and ginger-treated groups compared with NC group.

As shown in Table 2, there was no significant differences in initial body weight of mice among groups. The body weight of mice in normal group increased regularly during the experiment. Compared

**Table 1:** Blood glucose (mg/dl) of separate groups of control and diabetic mice 0 and 3 days, 1 and 2 weeks after injection of either citrate buffer (normal control) or streptozocine (diabetic)

| Treatment | Blood Glucose (mg/dl) | | | |
|---|---|---|---|---|
| | 0-day | 3-day | 1-week | 2-week |
| Normal | | | | |
| Vehicle control | 107.85 ± 4.63 | 102.57 ± 3.25 | 105.42 ± 4.99 | 109.14 ± 2.99 |
| Phenobarbital | 112.87 ± 4.82 | 111.25 ± 2.79 | 107.75 ± 2.96 | 114.87 ± 2.77 |
| Ginger extract-50 | 107.62 ± 3.82 | 109.75 ± 6.30 | 108 ± 4.97 | 108.37 ± 4.35 |
| Ginger extract-100 | 107 ± 1.90 | 105.37 ± 4.96 | 109.37 ± 2.99 | 107.62 ± 4.21 |
| Diabetic | | | | |
| Vehicle control | 105.85 ± 4.41 | 462 ± 11.32[a] | 515.57 ± 9.77[a] | 501.28 ± 13.4[a] |
| Phenobarbital | 112.25 ± 3.84 | 484.87 ± 9.94[a] | 508.62 ± 15.42[a] | 476.75 ± 14.05[a] |
| Ginger extract-50 | 105.28 ± 1.76 | 488.85 ± 6.20[a] | 468.85 ± 14.22[a,b] | 455.42 ± 12.89[a,b] |
| Ginger extract-100 | 112.5 ± 5.99 | 491.62 ± 5.74[a] | 467.87 ± 12.45[a,b] | 440.25 ± 12.39[a,c] |

Results are presented as mean ± SEM (n=8).
[a] Significantly different vs. normal control at $p < 0.001$.
[b] Significantly different vs. diabetic control at $p < 0.05$.
[c] Significantly different vs. diabetic control at $p < 0.01$.

**Table 2:** Body weight (g) of separate groups of control and diabetic mice 0 and 3 days, 1 and 2 weeks after injection of either citrate buffer (normal control) or streptozocine (diabetic)

| Treatment | Body Weight (g) | | | |
|---|---|---|---|---|
| | 0-day | 3-day | 1-week | 2-week |
| Normal | | | | |
| Vehicle control | 30.48 ± 0.65 | 31.41 ± 0.72 | 36.42 ± 1.01 | 41.87 ± 1.30 |
| Phenobarbital | 30.12 ± 0.63 | 30.93 ± 0.58 | 35.92 ± 1.08 | 40.46 ± 1.61 |
| Ginger extract-50 | 31.18 ± 0.66 | 32.10 ± 0.70 | 37.56 ± 1.10 | 42.60 ± 1.47 |
| Ginger extract-100 | 29.90 ± 0.46 | 31.05 ± 0.50 | 38.03 ± 1.03 | 41.07 ± 1.33 |
| Diabetic | | | | |
| Vehicle control | 30.70 ± 0.61 | 31.82 ± 0.64 | 26.66 ± 1.17[b] | 27.80 ± 1.29[b] |
| Phenobarbital | 29.60 ± 0.72 | 30.71 ± 0.75 | 26.02 ± 1.15[b] | 29.88 ± 1.72[b] |
| Ginger extract-50 | 30.37 ± 0.73 | 31.48 ± 0.74 | 27.55 ± 1.06[b] | 32.30 ± 1.22[b] |
| Ginger extract-100 | 31.02 ± 0.59 | 32.02 ± 0.62 | 27.75 ± 0.95[b] | 34.38 ± 1.08[a,c] |

Results are presented as mean ± SEM (n=8).
[a] Significantly different vs. normal control at $p < 0.01$.
[b] Significantly different vs. normal control at $p < 0.001$.
[c] Significantly different vs. diabetic control at $p < 0.05$.

to normal control mice, DC group mice exhibited a significant loss in body weight ($p<0.001$). At the end of the experiment, the body weight of the mice in diabetic phenobarbital (DP) and DGE-50 groups showed no significant increase compared with DC group. However, DGE-100 group mice that administered by ginger extract of 100 mg/kg showed a significant increase in body weight compared with DC group ($p<0.05$).

**Effect of ginger on the threshold for the myoclonic seizures in normal and diabetic mice**

The effect of ginger on seizure thresholds for the first myoclonic twitch in the *i.v.* PTZ seizure threshold test

in non-diabetic and diabetic mice is shown in Figure 1 ($F_{7,63} = 52.428$, $p<0.001$). As shown in this figure, diabetes effect in lowering the seizure threshold in DP, DGE-50 and DGE-100 groups was compared with corresponding normal groups ($p<0.001$, $p<0.01$ and $p<0.05$, respectively). Results show that ginger at doses of 50 to 100 mg/kg increased thresholds for myoclonic seizures in the timed *i.v.* PTZ infusion test in both normal and diabetic mice after 2-weeks treatment ($p<0.001$). There was no significant differences in seizure threshold among normal and diabetic phenobarbital groups compared with corresponding ginger-treated group.
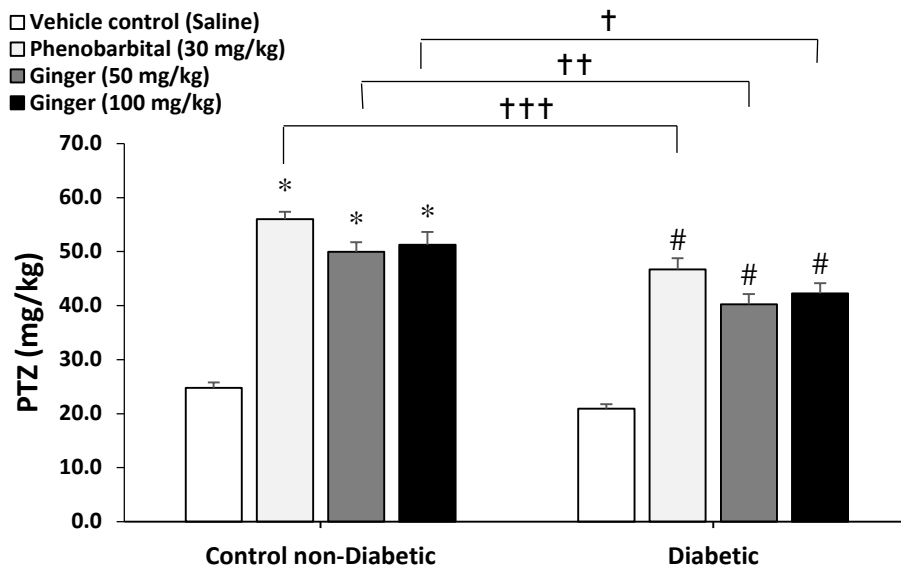
**Fig.1.** The effect of ginger on the threshold for the myoclonic seizures in the *i.v.* PTZ seizure threshold test in mice. Data are presented as mean ± SEM (n=8).

\* *p*<0.001 significantly different vs. corresponding normal vehicle control.

\# *p*<0.001 significantly different vs. corresponding diabetic vehicle control.

† *p*<0.05, †† *p*<0.01 and ††† *p*<0.001 significantly different vs. corresponding groups.
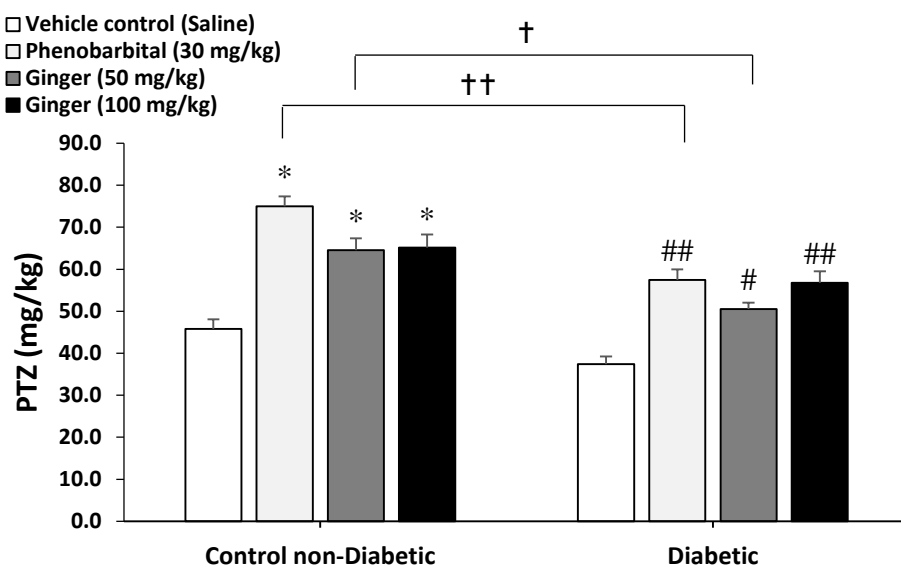


**Fig.2.** The effect of ginger on the threshold for the generalized clonic seizures in the *i.v.* PTZ seizure threshold test in mice. Data are presented as mean ± SEM (n=8).

\* *p*<0.001 significantly different vs. corresponding normal vehicle control.

\# *p*<0.01 and ## *p*<0.001 vs. corresponding diabetic vehicle control.

† *p*<0.01 and †† *p*<0.001 significantly different vs. corresponding groups.

## Effect of ginger on the threshold for the generalized clonic seizures in normal and diabetic mice

As shown in Figure 2, ginger affected thresholds of generalized clonic seizures ($F_{7,63}$ = 23.732, *p*<0.001) provoked by *i.v.* PTZ infusion in non-diabetic and diabetic mice. In this phase, DP and DGE-50 groups

showed lower seizure threshold compared with corresponding normal animals (*p*<0.001 and *p*<0.01, respectively). Statistically significant raise in the threshold for clonic seizures was observed in normal groups treated with ginger at the doses of 50 and 100 mg/kg compared with NC group (45.8 ± 2.3 mg/kg vs. 64.52 ± 2.83 mg/kg; *p*<0.001 and 45.8 ± 2.3 vs.
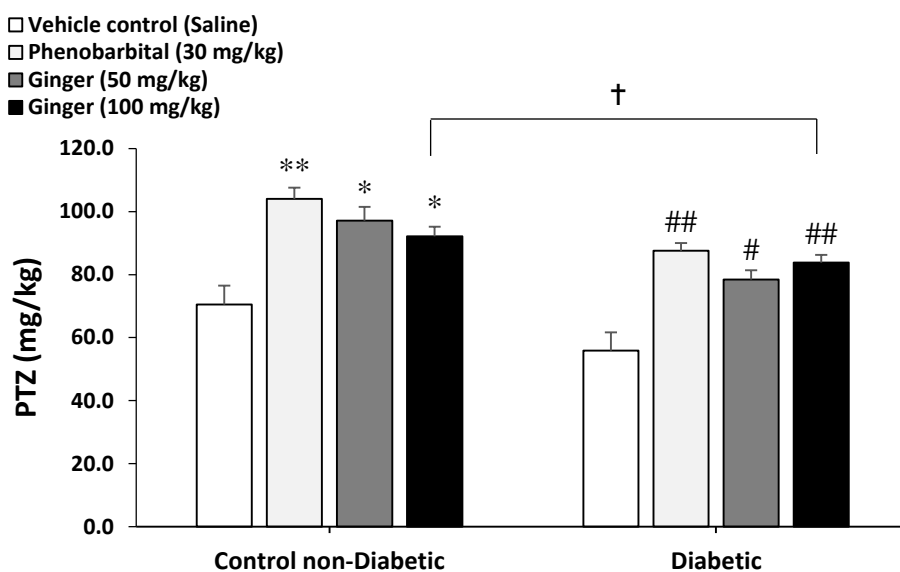
**Fig.3.** The effect of ginger on the threshold for the forelimb tonic extension in the *i.v.* PTZ seizure threshold test in mice. Data are presented as mean ± SEM (n=8).

* *p*<0.01 and ** *p*<0.001 significantly different vs. corresponding normal vehicle control.

# *p*<0.01 and ## *p*<0.001 significantly different vs. corresponding diabetic vehicle control.

† *p*<0.05 significantly different vs. corresponding groups.

65.13 ± 3.13 mg/kg; *p*<0.001, respectively) and diabetic groups treated with ginger at the doses of 50 and 100 mg/kg compared with DC group (37.42 ± 1.8 vs. 50.54 ± 1.53 mg/kg; *p*<0.01 and 37.42 ± 1.8 vs. 56.81 ± 2.68 mg/kg; *p*<0.001, respectively). There was no significant differences in seizure threshold among normal and diabetic phenobarbital groups compared with corresponding ginger-treated group.

**Effect of ginger on the threshold for the forelimb tonic extension in normal and diabetic mice**

As shown in Figure 3, ginger treatment significantly increased the threshold for forelimb tonic extension in the timed *i.v.* PTZ infusion test, compared with control group ($F_{7,63}$ = 14.455, *p*<0.001). At this phase also, diabetic ginger-treated group at the dose of 100 mg/kg showed lower seizure threshold compared with corresponding normal group (*p* < 0.05). One-way ANOVA revealed that ginger significantly increased the threshold for tonic seizures and *post hoc* analysis showed significant anticonvulsant effect for the dose of 50 and 100 mg/kg in normal animals compared with NC group (70.52 ± 6.03 vs. 92.19 ± 2.97 mg/kg; *p*<0.01 and 70.52 ± 6.03 vs. 97.13 ± 4.39 mg/kg; *p*<0.01, respectively). Diabetic animals treated with ginger at the doses of 50 and 100 mg/kg also showed increased threshold of tonic seizures compared with DC group (55.83 ± 5.87 vs. 78.48 ± 2.85 mg/kg;

*p* < 0.01 and 55.83 ± 5.87 vs. 83.87 ± 2.37 mg/kg; *p* < 0.001, respectively). There was no significant differences in seizure threshold of mice among normal and diabetic phenobarbital groups compared with corresponding ginger-treated group.

# Discussion

The present study investigated the anticonvulsant activity of *Z. officinale* in the timed PTZ infusion test in non-diabetic and diabetic mice. This test is considered as a very sensitive model of acute seizures which enables screening of various agents with different mechanisms of action, both pro- and anticonvulsant. Moreover, it makes possible to assess influence of the investigated compounds on separate components of seizure behavior. In the present study, we found that in the STZ-induced diabetic mice, the threshold of PTZ-induced clonic seizures is decreased on the second week after induction of diabetes with STZ injection. We also demonstrated that the PTZ-induced seizure threshold was significantly decreased in diabetic mice compared with 2-week non-diabetic animals.

Previous studies have shown that the seizure susceptibility is increased in diabetic animals (Koltai and Minker, 1975; Tutka et al., 1998; Schwechter et al., 2003). The deterioration in the seizure threshold

could be discussed by the profound effects of diabetic condition on the central nervous system. Accordingly, several studies have shown that in the adult diabetic patients hyperglycemia can precipitate seizures (Chou et al., 2007; Raghavendra et al., 2007; Huang et al., 2008). Hyperglycemia is also known to aggravate ischemic brain damage (Huang et al., 2013; Clark et al., 2014), whereas fasting-induced hypoglycemia has protective effects in ischemia and neurotoxic neuronal damage (Go et al., 1988; Yager et al., 1992). Animal studies also demonstrated that alloxan-induced diabetic hyperglycemia was associated with proconvulsant effects in the electroshock seizure model in the adult rats (Koltai and Minker, 1975) and mice (Tutka et al., 1998). These observations could be explained by the adverse effects of long-term hyperglycemia on the seizure threshold in diabetic patients (Schwechter et al., 2003; Huang et al., 2008).

The results of this study showed that the blood glucose in diabetic animals was significantly increased 3 days after STZ-induced diabetic mice and consistent afterward compared with non-diabetic animals. It was also demonstrated that during the treatment period, glucose levels in ginger-treated diabetic mice was significantly reduced compared with the control diabetic mice. However, it should be noted that serum glucose levels in ginger-treated diabetic rats did not reach normal levels at the dosages used in the present study. Similar results was reported by Akhani et al. (2004) in their study on the effects of ginger juice in STZ-induced diabetic rats. Results of this study are consistent with those clinical findings showing that increased glucose concentrations in adult diabetic patients may provoke seizures (Berkovic et al., 1982; Harden et al., 1991; Bush and Steward, 1995). In the streptozocin-induced diabetic, hyperglycemia had proconvulsant effects in the electroshock and bicuculline seizure models in the adult mice (Tutka et al., 1998). Our data confirm these studies and suggest that hyperglycemia by itself may have proconvulsant effects, as shown for myoclonic, clonic or tonic seizures in our experiment. However, note here that fasting led to slightly decreased glucose concentrations associated with anticonvulsant effects. Caloric restriction diet associated with decreased blood glucose concentrations have anticonvulsant effects in the epileptic EL mice (Greene et al., 2001)

as well as neuroprotective effects in rats after kainic acid (Yager et al., 1992). Our in vivo experiments support findings of proconvulsant effects of high glucose concentrations and increase seizure susceptibility in diabetic animals. In vitro study demonstrated that the hippocampal slices from streptozocin-induced diabetic rats are more prone to produce epileptiform response to a stimulation than control slices (Margineanu et al., 1998). Possible mechanisms of seizures from diabetic hyperglycemia include localized cerebral ischemia with or without a previous underlying structural abnormality, cortical deformation from brain dehydration, and increased metabolism of GABA via the succinic semialdehyde pathway as a result of depression of the Krebs cycle (Messing et al., 1986; Brick et al., 1989). Depressed brain GABA levels presumably reduces the seizure threshold by decreasing cortical inhibition. These evidence seem to suggest that anticonvulsant effect of ginger extract may have been mediated by hypoglycemic effect and thereby elevation of the seizure threshold in diabetic mice.

Several lines of studies indicate that hyperglycemia promote oxidative stress (King and Loeken, 2004; Giacco and Brownlee, 2010) and much evidence that oxidative stress is involved in the etiology of several diabetic complications (Rochette et al., 2014; Saisho, 2014). In addition, in vascular tissue, inflammation can stimulate inducible nitric oxide synthase (iNOS) expression by macrophages and smooth muscle cells, leading to the production of free radical, nitric oxide ($NO^-$) (Griendling and FitzGerald, 2003). Reactive nitrogen species, such as NO, influence signal transduction and cause DNA damage, which contributes to disease processes such as seizure. Nitric oxide is produced by iNOS, which is stimulated in response to various stresses. In addition to the above findings, NO also increases the level of cyclic guanosine monophosphate (cGMP) through the activation of soluble guanylyl cyclase (sGC), which influences a wide range of physiological functions including regulation of seizure threshold (Nidhi et al., 1999). Numerous experimental studies and clinical observations indicate that ginger influences some central nervous system effects (Felipe et al., 2008), but accurate mechanisms of its action are not precisely known. It is highly possible that the observed effects are resulted from the elevated intracellular cGMP level. It was noted that cGMP and

its downstream targets, including PDEs, cGMP-dependent channels and PKG, regulate neurotransmission, long-term potentiation, gene expression, neurotoxicity, and neurodegenerative processes (Wang and Robinson et al., 1997). Components of the NO/cGMP pathway modulate release of both excitatory and inhibitory amino acids in the central nervous system (Prast and Philippu, 2001; Yu and Eldred, 2005). Because imbalance between the excitatory and inhibitory neurotransmission is the main reason of epileptic discharges, cGMP may affect convulsant activity in brain (Garthwaite and Boulton, 1995). High antioxidant activity of ginger and its compounds have been demonstrated in numerous reports (Ghasemzadeh et al., 2010; Jeena et al., 2013). [6]-gingerol, a bioactive component of ginger, was reported to dose-dependently inhibit nitric oxide (NO) production and reduce inducible nitric oxide synthase (iNOS) in lipopolysaccharide (LPS)-stimulated mouse macrophages (Ippoushi et al., 2003). Therefore, it seems that antioxidant property of ginger and its nitric oxide synthase inhibitory effect can be, at least in part, responsible for such inhibiting response in this study.

# Conclusion

In conclusion, in the present study it was demonstrated that ginger extract has anticonvulsant effect in the timed PTZ infusion test in both non-diabetic and diabetic mice. Summing up, it can be hypothesized that the anticonvulsant effect of ginger may have been mediated by hypoglycemic effect, antioxidant mechanisms and oxidative stress inhibition. The precise molecular mechanism of anticonvulsant effect of ginger in diabetic seizure should be investigated in further experiments. The results of this study might support new treatment methods using ginger in diabetic patients with epileptic seizure. Due to favorable pharmacodynamic characteristics and lack of acute side effects, the tested interactions might be beneficial and worthy of consideration for testing in clinical trials.

## Acknowledgments

## Conflict of interest

# References

Akhani SP, Vishwakarma SL, Goyal RK. Anti-diabetic activity of *Zingiber officinale* in streptozotocin-induced type I diabetic rats. J Pharm Pharmacol 2004; 56: 101-105.

Arablou T, Aryaeian N, Valizadeh M, Sharifi F, Hosseini A, Djalali M. The effect of ginger consumption on glycemic status, lipid profile and some inflammatory markers in patients with type 2 diabetes mellitus. Int J Food Sci Nutr 2014; 65: 515-20.

Berkovic SF, Johns JA, Bladin PF. Focal seizures and systemic metabolic disorders. Aust N Z J Med 1982; 12: 620-3.

Brick JF, Gutrecht JA, Ringel RA. Reflex epilepsy and nonketotic hyperglycemia in the elderly: a specific neuroendocrine syndrome. Neurology 1989; 39: 394-399.

Bush GH, Steward DJ. Can persistent cerebral damage be caused by hyperglycaemia? Paediatr Anaesth 1995; 5: 385-387.

Chou CH, Lin JC, Peng GS. Status epilepticus associated with initiation of theophylline in an elderly patient with diabetic ketoacidosis. Neurol India 2007; 55: 154-6.

Clark ME, Payton JE, Pittiglio LI. Acute ischemic stroke and hyperglycemia. Crit Care Nurs Q 2014; 37: 182-7.

Edraki M, Akbarzadeh A, Hosseinzadeh M, Tanideh N, Salehi A, Koohi-Hosseinabadi O. Healing effect of sea buckthorn, olive oil, and their mixture on full-thickness burn wounds. Adv Skin Wound Care 2014; 27: 317-23.

El-Akabawy G, El-Kholy W. Neuroprotective effect of ginger in the brain of streptozotocin-induced diabetic rats. Ann Anat 2014; 196: 119-28.

Engel J, Pedley TA, Aicardi J, Dichter MA, Moshé S, Perucca E, et al. Epilepsy: A comprehensive textbook. 2nd ed. Philadelphia: Lippincott Williams & Wilkins, 2007.

Esteghamati A, Khalilzadeh O, Anvari M, Meysamie A, Abbasi M, Forouzanfar M, et al. The economic costs of diabetes: A population-based study in Tehran, Iran. Diabetologia 2009; 52: 1520-7.

Felipe CFB, Fonsêca KS, dos Reis Barbosa AL, Bezerra JNS, Neto MA, de Franc a Fonteles MM, et al. Alterations in behavior and memory induced by the essential oil of *Zingiber officinale* Roscoe (ginger) in mice are cholinergic-dependent. J Med Plants Res 2008; 2: 163-170.

Garthwaite J, Boulton CL. Nitric oxide signaling in the central nervous system. Annu Rev Physiol 1995; 57: 683-706.

Ghasemi M, Shafaroodi H, Karimollah AR, Gholipour T, Nezami BG, Ebrahimi F, et al. ATP-sensitive potassium channels contribute to the time-dependent alteration in

the pentylenetetrazole-induced seizure threshold in diabetic mice. Seizure 2010; 19: 53-8.

Ghasemzadeh A, Jaafar HZ, Rahmat A. Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). Molecules 2010; 15: 4324-4333.

Giacco F, Brownlee M. Oxidative Stress and Diabetic Complications. Circ Res 2010; 107: 1058-70.

Go KG, Prenen GH, Korf J. Protective effect of fasting upon cerebral hypoxic-ischemic injury. Metab Brain Dis 1988; 3: 257-263.

Greene AE, Todorova MT, McGowan R, Seyfried TN. Caloric restriction inhibits seizure susceptibility in epileptic EL mice by reducing blood glucose. Epilepsia 2001; 42: 1371-1378.

Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury. I. basic mechanisms and in vivo monitoring of ROS. Circulation 2003; 108: 1912-1916.

Harden CL, Rosenbaum DH, Daras M. Hyperglycemia presenting with occipital seizures. Epilepsia 1991; 32: 215-220.

Hosseini A, Mirazi N. Acute administration of ginger (*Zingiber officinale* rhizomes) extract on timed intravenous pentylenetetrazol infusion seizure model in mice. Epilepsy Res 2014; 108: 411-419.

Huang CW, Tsai JJ, Ou HY, Wang ST, Cheng JT, Wu SN, et al. Diabetic hyperglycemia is associated with the severity of epileptic seizures in adults. Epilepsy Res 2008; 79: 71-7.

Huang J, Liu B, Yang C, Chen H, Eunice D, Yuan Z. Acute hyperglycemia worsens ischemic stroke-induced brain damage via high mobility group box-1 in rats. Brain Res 2013; 1535: 148-55.

Ippoushi K, Azuma K, Ito H, Horie H, Higashio H. [6]-gingerol inhibits nitric oxide synthesis in activated J774.1 mouse macrophages and prevents peroxynitrite-induced oxidation and nitration reactions. Life Sci 2003; 73: 3427-37.

Iris F, Wachtel-Galor S. Herbal Medicine Biomolecular Clinical Aspects. 2nd ed. New York: CRC Press, 2011.

Jeena K, Liju VB, Kuttan R. Antioxidant, anti-inflammatory and antinociceptive activities of essential oil from ginger. Indian J Physiol Pharmacol 2013; 57: 51-62.

Kar A, Choudhary BK, Bandyopadhyay NG. Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. J Ethnopharmacol 2003; 84: 105-108.

King GL, Loeken MR. Hyperglycemia-induced oxidative stress in diabetic complications. Histochem Cell Biol 2004; 122: 333-338.

Koltai M, Minker E. Changes of electro-shock seizure threshold in alloxan diabetic rats. Experientia 1975; 31: 1369.

Mandhane SN, Aavula K, Rajamannar T. Timed pentylenetetrazol infusion test: a comparative analysis with s.c. PTZ and MES models of anticonvulsant screening in mice. Seizure 2007; 16: 636-44.

Margineanu DG, Niespodziany I, Wulfert E. Hippocampal slices from long-term streptozotocin-injected rats are prone to epileptiform responses. Neurosci Lett 1998; 252: 183-186.

Markowitz GJ, Kadam SD, Smith DR, Johnston MV, Comi AM. Different effects of high- and low-dose phenobarbital on post-stroke seizure suppression and recovery in immature CD1 mice. Epilepsy Res 2011; 94: 138-148.

Mascolo N, Jain R, Jain SC, Capasso F. Ethnopharmacologic investigation of ginger (*Zingiber officinale*). J Ethnopharmacol 1989; 27: 129-140.

Messing RO, Simon RP. Seizures as a manifestation of systemic disease. Neurol Clin 1986; 4: 563-584.

Nidhi G, Balakrishnan S, Pandhi P. Role of nitric oxide in electroshock and pentylenetetrazole seizure threshold in rats. Methods Find Exp Clin Pharmacol 1999; 21: 609-612.

Ojewole JA. Analgesic, anti-inflammatory and hypoglycaemic effects of ethanol extract of *Zingiber officinale* (Roscoe) rhizomes (Zingiberaceae) in mice and rats. Phytother Res 2006; 20: 764-772.

Prast H, Philippu A. Nitric oxide as modulator of neuronal function. Prog Neurobiol 2001; 64: 51-68.

Raghavendra S, Ashalatha R, Thomas SV, Kesavadas C. Focal neuronal loss, reversible subcortical focal T2 hypointensity in seizures with a nonketotic hyperglycemic hyperosmolar state. Neuroradiology 2007; 49: 299-305.

Rochette L, Zeller M, Cottin Y, Vergely C. Diabetes, oxidative stress and therapeutic strategies. Biochim Biophys Acta 2014; 1840: 2709-29.

Saisho Y. Glycemic variability and oxidative stress: A link between diabetes and cardiovascular disease? Int J Mol Sci 2014; 15: 18381-406.

Schwechter EM, Veliskova J, Velisek L. Correlation between extracellular glucose and seizure susceptibility in adult rats. Ann Neurol 2003; 53: 91-101.

Shanmugam KR, Mallikarjuna K, Kesireddy N, Sathyavelu Reddy K. Neuroprotective effect of ginger on anti-oxidant enzymes in streptozotocin-induced diabetic rats. Food Chem Toxicol 2011; 49: 893-897.

Tutka P, Sawiniec J, Kleinrok Z. Experimental diabetes sensitizes mice to electrical- and bicuculline-induced convulsions. Pol J Pharmacol 1998; 50: 92-3.

Wang X, Robinson PJ. Cyclic GMP-dependent protein kinase and cellular signaling in the nervous system. J Neurochem 1997; 68: 443-56.

Yager JY, Heitjan DF, Towfighi J, Vannucci RC. Effect of insulin-induced and fasting hypoglycemia on perinatal hypoxic-ischemic brain damage. Pediatr Res 1992; 31: 138-42.

Yu D, Eldred WD. Nitric oxide stimulates gamma-aminobutyric acid release and inhibits glycine release in retina. J Comp Neurol 2005; 483: 278-91.