


Original Article

Zingiber officinale extract pre-treatment ameliorates astrocytes activation and enhances neuroprotection in pentylenetetrazol-induced kindling model of epilepsy in mice

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Abstract

Introduction: Recently, herbal medicine is widely used as an alternative and complementary therapy in several neurological disorders such as epilepsy. The anti-inflammatory and neuroprotective effects of *Zingiber officinale* or ginger have been well-documented. The present study was designed to evaluate the effects of ginger extract pre-treatment on seizures behavior, neuronal density and astrocytes activation in pentylenetetrazol (PTZ)- induced kindling model.

Methods: Kindling model was induced in mice by repetitive administration of PTZ at sub convulsive dose. Hydroalcoholic extract of ginger at doses of 25, 50 or 100 mg/kg were daily injected 10 days before PTZ injections and intraperitoneal administration of extract was continued 1h before each PTZ injection. Immunostaining against NeuN and GFAP as neuronal and astrocyte markers, respectively, was carried out on brain tissue sections.

Results: Our data showed that ginger extract pre-treatment, especially at dose of 100 mg/kg, reduced the seizures behavior in PTZ receiving animals. Immunostaining against NeuN biomarker demonstrated that neuronal death was alleviated in animals under treatment of ginger extract. Furthermore, application of ginger extract attenuated the number of GFAP expressing cells in hippocampus of fully-kindled animals.

Conclusion: Overall, our data suggest that ginger pre-treatment exerts significant neuroprotective effect by attenuation of astrocytes activation in PTZ-induced kindling model. It can be concluded that ginger might be used as effective supplementary agent in epileptic patients.

Keywords:

Kindling;
Pentylenetetrazol;
Zingiber officinale;
Neuroprotection;
Astrocytes activation

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Introduction

Epilepsy is regarded as one the most chronic

neurological disorders affecting 50 million people worldwide (Vezzani et al., 2011). Epilepsy is characterized by spontaneous recurrent seizures and it has been well addressed that neuronal

hyperexcitability possesses major role in epileptogenesis (Devinsky et al., 2013).

Glial cells including astrocytes, microglia and oligodendrocytes are regarded as the most abundant cells in the central nervous system (Pelvig et al., 2008). Astrocytes and microglia are involved in various physiological functions and play important roles in homeostasis of the nervous system (Devinsky et al., 2013). Upon the injury, the proliferation of glial cells increases and activated cells migrate to the damage area. Activated astrocytes and microglia effectively inhibit the spread of injury to the surrounding region of lesion site. Despite the beneficial effect of glial cells, activated astrocytes and microglia ultimately form glial scar which leads to neuronal dysfunction and axonal loss (Guo et al., 2014). Gliosis is considered as common hallmark of brain injuries such as stroke (Barreto et al., 2011) and neurodegenerative disorders including Alzheimer's disease (AD) (Guo et al., 2014) and epilepsy (Lehrmann et al., 2008).

It has been well-understood that changes in morphology, molecular composition and proliferation of astrocytes occur in epileptic foci (Devinsky et al., 2013). Additionally, astrocytes activation has been found in experimental models of epilepsy and brain tissues of epileptic patients (Devinsky et al., 2013). It has been shown that activated glial cells can promote epileptogenesis through enhancement of excitability and inflammation. Activated glial cells induce neuronal hyperexcitability by disruption of ions, water and neurotransmitters regulation (Devinsky et al., 2013). Furthermore, astrocytes and microglia release several inflammatory factors which in turn facilitate the epileptogenesis process (Vezzani and Granata, 2005; Vezzani et al., 2008; Vezzani et al., 2011; Vezzani et al., 2013).

Accumulating body of evidences demonstrated that some anti-inflammatory drugs can effectively reduce the seizures behavior (Ikonomidou-Turski et al., 1988; Marchi et al., 2011). Despite the emergence of numerous anti-epileptic drugs, the current therapeutic approaches are effective only in 40% of epileptic patients (Schmidt, 2011). Therefore, a demand for producing of new types of anti-epileptic drugs continuously exists.

Currently, herbal medicine has been introduced as complementary and alternative strategy in treatment of epilepsy. Ginger is derived from the rhizome of the

Zingiber officinale Roscoe and it is widely being used as spice and food additive in worldwide (Hosseini and Mirazi, 2014). Ginger or its effective compounds such as gingerol or shogaol have been shown to exhibit beneficial effects in treatment of several diseases such as rheumatoid arthritis, neuropathy disorders, gingivitis and stroke (Lantz et al., 2007). Several evidences have demonstrated that *Z. officinale* has antioxidant (Masuda et al., 2004; Mashhadi et al., 2013), anti-inflammatory (Grzanna et al., 2005; Lantz et al., 2007) and neuroprotective effects both *in vitro* and *in vivo* (El-Akabawy and El-Kholy, 2014).

It has been well documented that anti-inflammatory effects of shogaol is partly mediated by inhibiting the production of prostaglandin E2 (PGE2), cyclooxygenase-2 (COX-2), and proinflammatory cytokines such as interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α), and by down-regulating inducible nitric oxide synthases, p38 mitogen-activated protein kinase and nuclear factor kappa B (NF- κ B) expression (Ha et al., 2012; Shim et al., 2011). [6]-shogaol treatment also remarkably increases the level of histone H3 acetylation and heat-shock protein 70 and suppresses the expression of histone deacetylase 1 (Shim et al., 2011). In addition, it has been shown that ginger treatment increases the neuroprotection by augmentation of brain anti-oxidant defense mechanisms (Shanmugam et al., 2011).

Previous studies suggested the beneficial effects of ginger in various neurological disorders such as AD (Grzanna et al., 2004; Oboh et al., 2012). In recent years, the effect of acute and chronic administration of ginger extract on seizure threshold was evaluated in acute model of seizure (Hosseini and Mirazi, 2014; Hosseini and Mirazi, 2015).

To our knowledge, there is no study that examined the anti-convulsant mechanism of ginger extract on kindling model of epilepsy. Pentylentetrazol (PTZ) as GABA_A receptor antagonist is extensively used to evaluate the epileptogenesis process as well as testing of novel antiepileptic drugs (Dhir, 2012). For development of PTZ-induced chemical kindling model of epilepsy, PTZ is repetitively administrated at sub-convulsive dose. The neuronal loss and glial activation in hippocampus of PTZ receiving animals has been well-addressed (Anissian et al., 2018; Gol et al., 2017; Kaur et al., 2015).

The present study was designed to examine the

effect of ginger extract pre-treatment on seizures behavior in PTZ-induced kindling model. Furthermore, the level of astrocytes activation and neuronal density was evaluated in fully-kindled animals which have received the ginger hydroalcoholic extract.

Materials and methods

Drugs

PTZ and sodium valproate (VPA) were obtained from Sigma-Aldrich (St.Louis, Mo USA).

Preparation of ginger hydroalcoholic extract

The rhizomes of *Z. officinale* were purchased from local market and authenticated by a botanist (herbarium number: 94302). Three hundred grams of dried ginger rhizomes were mechanically powdered and mixed with 500 ml of ethanol. The prepared mixture was shaken for 72 hours in 25°C using shaker-incubator (labnet. 311DS. USA). The supernatant solvent was filtered through a whatman filter paper. Solvent evaporation was carried out by rotary evaporator under vacuum condition (Moghadamnia et al., In press). Prepared extract was dissolved in sterile saline solution containing 0.1% Tween-80 and was injected at appropriate dose based on body weight of animals.

Animals

In this study, male NMRI mice (20-30 g) were purchased from the animal house of the Babol University of Medical Sciences (Babol, Iran). Animals were housed in a room with constant temperature (22±2 °C) and humidity percent was 60. Mice were kept with free access to food and water on a 12 h light/dark schedule. All experimental procedures were approved by local ethics committee at the Babol University of Medical Sciences which was in according to international guidelines on the use of laboratory animals.

Experimental design

In the present study, 30 NMRI mice (n=6 for each experimental group) were randomly assigned into 5 experimental groups as saline+PTZ, VPA+PTZ, ginger extract (25 mg/kg)+PTZ, ginger extract (50 mg/kg)+PTZ and ginger extract (100 mg/kg)+PTZ. In control group, animals received the pre-treatment of

saline containing 0.1% Tween-80 at dose of 10 ml/kg as vehicle of ginger extract for 10 days and its intraperitoneally (ip) injection was continued until the end of experiments. In VPA+PTZ as positive control group, animals received the ip injection of VPA at dose of 300 mg/kg and experimental procedure was similar as that described in control group (Bough and Eagles, 2001). In ginger extract experimental groups, mice received the ip injections of ginger hydroalcoholic extract at doses of 25, 50 or 100 mg/kg (Hosseini and Mirazi, 2014) for 10 days and administration of ginger extract was also continued before each PTZ injection. Inclusion criteria for animals were their sex (male) and race (NMRI) and habitation in the lab, one week before the experiments. The exclusion criteria were being used previously for any behavioral tests and having underlying diseases (Ebrahimzadeh et al., 2017). Additionally, mice were excluded from the study if they become kindled after the first five injections of PTZ.

After the last injection of PTZ, animals were sacrificed and brain tissues were removed. To determine the effect of ginger extract on neuronal density and astrocytes activation of hippocampus, immunostaining against NeuN (a neuronal specific nuclear protein) and glial fibrillary acidic protein (GFAP) as astrocyte biomarker was carried out on brain sections.

PTZ-induced kindling model of epilepsy

In order to induce kindling model in animals, PTZ was administrated as we described previously (Gol et al., 2017; Hashemian et al., 2017). Briefly, PTZ at dose of 36.5 mg/kg was injected intraperitoneally every 48 h for 20 consecutive days. After each PTZ injection, mice were monitored with a video camera for 20 min and seizure parameters including maximum seizure stage, latency to the onset of myoclonic jerks (MJ, S2 latency), generalized tonic-clonic seizures (GCTS) latency (S4 or S5 latency) and duration of GCTS were recorded for each animal. The seizure stages were classified as follows: 0, no response; 1, ear and facial twitching; 2, convulsive waves through the body; 3, myoclonic jerks and rearing; 4, clonic-tonic convulsions and turnover into side position and 5, generalized clonic-tonic seizures and loss of postural control (Davoudi et al., 2013). After manifestation of three consecutive stage 4 or 5 seizures, animals

were considered fully kindled.

Immunostaining

Immunostaining procedure was performed according to our previous study (Anissian et al., 2018). Briefly, animals were deeply anesthetized and transcardially perfused with phosphate buffer saline (PBS) and 4% paraformaldehyde (PFA). Brain tissues were removed and post-fixed in PFA for 12-16 h. Coronal brain sections (6 μ m) from dorsal part of hippocampus were prepared by cryostat instrument (MICROM HM 525, Thermo Scientific). For immunostaining, tissue sections were washed with PBS and non-specific bindings were blocked using 10% normal goat serum + 0.3% Triton X100 for 1 h. Then, sections were incubated with primary antibodies including rabbit anti-GFAP (1:400, Z0334, Dako) or rabbit anti-NeuN (1:500, ab177487, Abcam inc.) overnight at 4 °C. After washing with PBS, secondary antibody (Goat anti-rabbit Alexa Fluor®594, 1:1000, ab150116, Abcam inc.) was added for 1 h. Washing step was done with PBS and DAPI (4',6-diamidino-2-phenylindole) was used for nuclear staining. Tissue sections were evaluated under fluorescence microscope (Olympus IX71, Japan) and images were captured by a DP-72 camera. Quantification of immunostaining results was performed based on our previous reports (Gol et al., 2017; Naeimi et al., 2018). In brief, the number of GFAP and NeuN positive cells were manually counted using Image J software (version 1.42 V, NIH, USA). To assess the number of cells per mm², cell counts were divided by the area of micrograph. Three sections from each slide, three slides from each animal and three rats were used for each experimental group (27 sections for each group).

Statistical analysis

Normality of data was measured by Shapiro–Wilk tests and equal variance using Levene's test. Maximum seizures stage data were assessed by Kruskal-Wallis non-parametric test, followed by Dunn's post-test. Latency to the onset of MJ, GCTS latency and GCTS duration were analyzed using two way-analysis of variance, followed by Bonferroni's post hoc test. Analysis of histological results was carried out by One-way ANOVA, followed by Tukey post -test. *P* value <0.05 was considered statistically significant. Statistical analyses and drawing of figures

were carried out using GraphPad Prism 6.01 (GraphPad Software, Inc., San Diego, CA).

Results

Ginger extract pre-treatment decreased seizures behavior in PTZ- induced kindling model

In order to evaluate the effect of ginger extract pre-treatment on seizures behavior, maximum seizures stage, latency to the onset of MJ, GCTS latency and duration of GCTS were assessed in PTZ-induced kindling model of epilepsy in mice. Administration of VPA caused a statistically significant decrease in the seizures stage compared with control group ($P<0.05$). Interestingly, behavioral data analysis also showed that ginger extract pre-treatment at dose of 100 mg/kg markedly decreased the maximum seizures stage compared to the control group ($P<0.05$, (Fig. 1A). Furthermore, administration of ginger extract at high dose could significantly increase the latency to onset of MJ at injections 8, 9 and 10 of PTZ ($P<0.05$). We could not find any significant difference in latency of MJ between VPA receiving animals and control group at injections 6, 7, 8, 9 and 10 of PTZ ($P=0.9403$, $P=0.9792$, $P=0.3191$, $P=0.2413$ and $P=0.0776$ respectively; Fig. 1B).

Statistical analysis demonstrated that application of VPA considerably increased the latency of GCTS compared to the control group ($P<0.001$). A significant increase in latency of GCTS was observed in fully kindled animals which were pre-treated with high dose of extract (100 mg/kg) compared to the control group. Ginger extract administration at the dose of 25 mg/kg significantly increased the latency of GCTS compared to the control group at injections 6 and 8 of PTZ ($P<0.05$ and $P<0.01$, respectively). Our data also showed a significant difference in latency of GCTS between VPA and ginger extract group at dose of 50 mg/kg ($P<0.05$, Fig. 2A). Furthermore, behavioral data indicated that duration of GCTS significantly reduced in fully kindled animals which were under treatment of VPA and high dose of ginger extract compared to the control group. A significant reduction in duration of GCTS was also found in animals which were treated with low dose of ginger extract (25 mg/kg) compared with control group, but ginger at dose of 50 mg/kg had no impact on decreasing of GCTS duration (Fig. 2B).

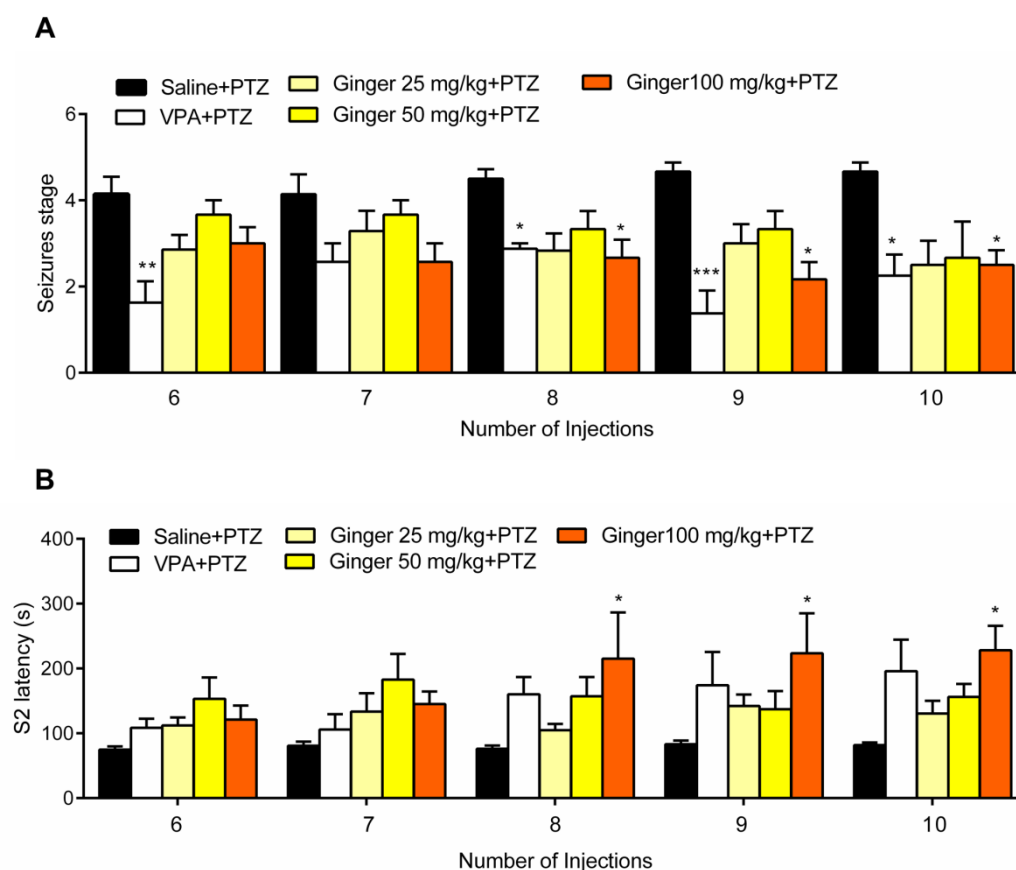


Fig.1. Effect of ginger extract on seizures behavior in PTZ receiving animals. (A) Ginger extract pre-treatment decreased the seizures stage. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$ compared to the control group, $n=6$. (B) Ginger extract increased the latency of myoclonic jerks in PTZ-induced kindling model. * $P<0.05$ compared to the control group, $n=6$.

Ginger extract pre-treatment enhances the neuronal density of hippocampus in PTZ-induced kindling model of epilepsy

In order to assess the neuroprotective effect of ginger extract, immunostaining against NeuN as mature neuronal marker was carried out on brain sections obtained from the dorsal part of hippocampus (Fig. 3A). NeuN immunostaining and quantification of the data showed the number of NeuN positive cells was dramatically reduced in CA3 region of hippocampus of fully kindled animals which were treated by saline or ginger extract at doses of 25 and 50 mg/kg compared to intact animals ($P<0.001$). A significant increase in NeuN expressing cells was found in animals group which received VPA compared to the control group ($P<0.001$). Administration of ginger extract at doses of 25 ($P<0.001$), 50 ($P<0.01$) and 100 mg/kg ($P<0.001$) considerably increased the neuronal density compared to the control group. There was also a statistical significant in number of NeuN positive cells in VPA receiving animals compared to the ginger extract groups which received doses of 25 ($P<0.01$) or 50 mg/kg ($P<0.001$). In

addition, application of ginger at its high dose (100 mg/kg) could markedly alleviate the neuronal loss following PTZ injections when compared with doses of 25 ($P<0.01$) and 50 mg/kg ($P<0.001$, Fig. 3B).

Ginger extract administration ameliorates astrocytes activation in PTZ-induced kindling model of epilepsy

To evaluate the effect of ginger extract pre-treatment on astrocytes activation, immunostaining against GFAP, as astrocyte marker was performed on brain sections (Fig. 4A). GFAP immunostaining and its quantification indicated the number of GFAP expressing cells considerably increased in fully kindled animals which have received saline compared with intact animals ($P<0.001$). Our data also showed that the number of GFAP immunoreactive cells significantly increased in animals under treatment of ginger extract at doses of 25 and 50 mg/kg compared to the intact ($P<0.05$) and control groups ($P<0.05$). The level of astrocytes activation was remarkably reduced in animals which were treated with VPA compared with control group

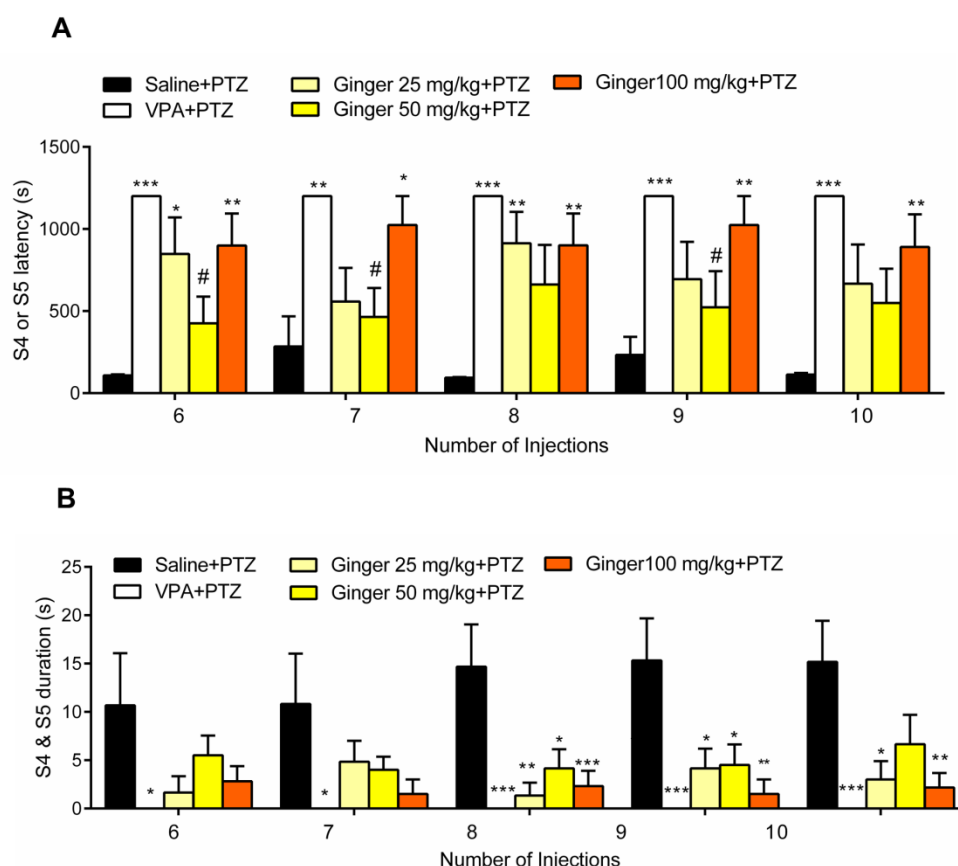


Fig.2. Illustration of the experimental protocols. Rats were administered by increasing doses of morphine (0.1, 0.2 and 0.3 mg/ml; each dose was administered for 48 hours) then a dose of 4 mg/ml was given per day for 21 consecutive days. After the last oral administration of morphine on day 21, rats treated by daily oral *Syzygium aromaticum* (4 mg/kg) for 14 days. SA=treatment with *Syzygium aromaticum*

($P < 0.001$). Additionally, a significant difference was observed in VPA receiving animals and ginger extract groups at doses of 25 and 50 mg/kg ($P < 0.05$). Interestingly, ginger extract pre-treatment at the dose of 100 mg/kg significantly attenuated the level of astrocytes activation in CA3 region of hippocampus in comparison with PTZ treated animals ($P < 0.001$, Fig. 4B).

Discussion

Herbal medicine has been emerged as alternative therapeutic strategy in treatment of several neurological disorders such as epilepsy (Liu et al., 2017). In this study, the effect of ginger extract pre-treatment on seizures behavior, neuronal density and glial activation were examined in PTZ-induced kindling model of epilepsy in mice. Our results indicate that administration of ginger extract effectively reduces seizures in fully kindled animals. In contrast to previous reports (Hosseini and Mirazi, 2014; Hosseini and Mirazi, 2015), we could not

observe any remarkable antiepileptic effect of ginger extract at doses of 25 and 50 mg/kg and its application at high dose (100 mg/kg) could considerably reduce the seizures behavior in PTZ-induced kindling model of epilepsy. Interestingly, treatment with ginger extract at all applied doses ameliorates the level of hippocampal neuronal loss and astrocytes activation following PTZ administration.

Previous report showed that single dose injection of ginger reduced the seizures behavior in acute model of seizure (Hosseini and Mirazi, 2014). Additionally, another study by this group also showed that chronic administration of ginger for 1 week decreased the seizure parameters in PTZ-induced seizure model (Hosseini and Mirazi, 2015). Further study by Hosseini and colleagues demonstrated that hydroethanolic extract of ginger exerts protective effects against PTZ-induced seizure threshold model in diabetic mice (Hosseini et al., 2016). In consistent with aforementioned studies, our data also indicated that ginger extract possesses anticonvulsant effect in

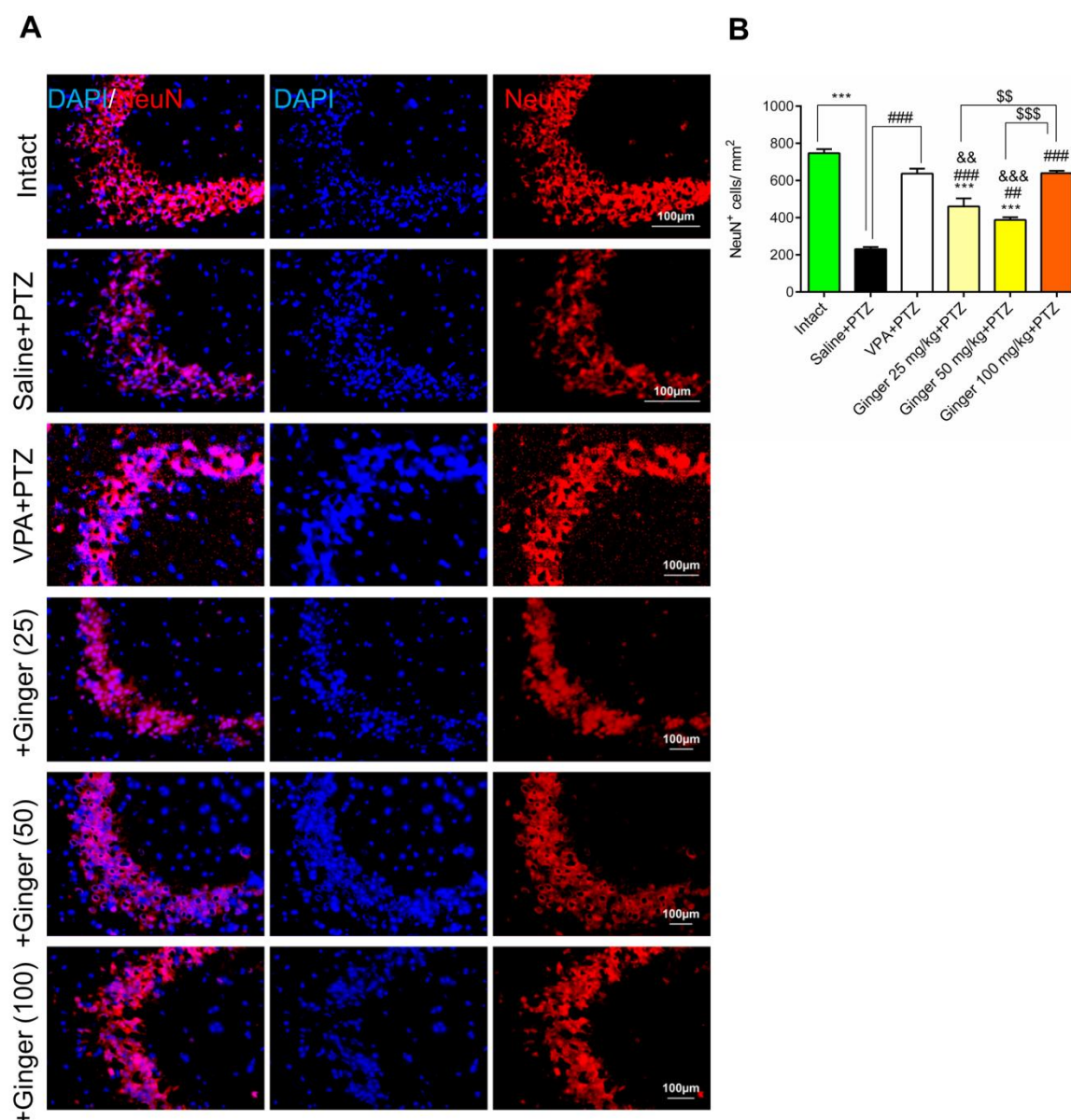


Fig.3. Effect of ginger extract administration on hippocampal cell density following PTZ injection. (A) Immunostaining against NeuN as mature neuronal marker in CA3 region of hippocampus. (B) Quantification of immunostaining data showed that ginger extract at dose of 100 mg/kg attenuated the adverse effect of PTZ on neuronal survival. *** $P < 0.001$ compared to the intact; ## $P < 0.01$ and ### $P < 0.001$ compared to the control group; && $P < 0.01$ and &&& $P < 0.001$ compared to the VPA+PTZ; \$\$ $P < 0.01$ and \$\$\$ $P < 0.001$ compared to the ginger extract at doses of 25 or 50 mg/kg. Scale bar: 100 μ m, DAPI: nuclei stain, NeuN: neuronal marker, $n = 3$.

PTZ-induced kindling model. However, Hosseini et al., used single intravenous infusion of PTZ to develop the acute model of epilepsy, while in our study, PTZ was repetitively administrated at sub convulsive dose to induce the chemical kindling model of epilepsy. In contrast to acute model, PTZ-induced kindling model is regarded as most widely accepted animal model for studying the process of epileptogenesis and evaluation of new antiepileptic drugs (Dhir, 2012). Although, previous studies revealed the anticonvulsant effects of ginger, the precise mechanism underlying such effect is not still

investigated. It has been hypothesized that the antiepileptic effect of ginger may be mediated by antioxidant mechanisms and inhibition of oxidative stress. Additionally, it has been postulated that ginger can increase the seizure threshold via its modulatory effect on both excitatory and inhibitory neurotransmitters and inhibitory impacts on various types of calcium channels (Hosseini and Mirazi, 2014).

Our data demonstrated that ginger extract reduced the cell death and increased the neuronal density in hippocampus of fully kindled animals. Previous

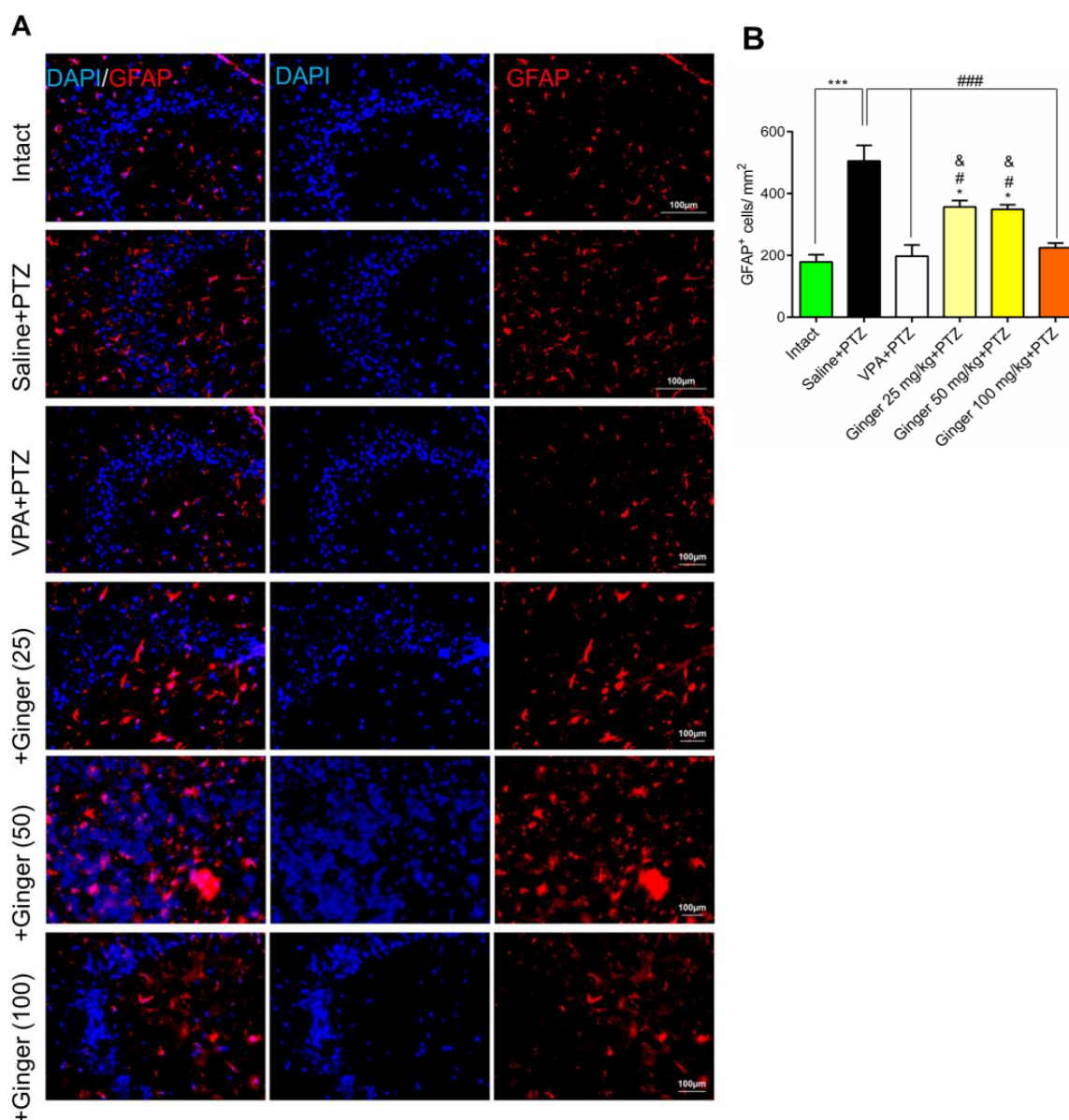


Fig.4. Effect of ginger administration on astrocytes activation. (A) Immunostaining against GFAP as astrocyte marker. (B) Quantification of GFAP immunostaining data indicated that high dose of ginger extract reduced the number of activated astrocytes in CA3 region of fully kindled animals. *** $P < 0.001$ compared to the intact; # $P < 0.05$ and ### $P < 0.001$ compared to the control group; & $P < 0.05$ compared to the VPA+PTZ. Scale bar: 20µm, DAPI: nuclei stain, GFAP: astrocyte marker, $n = 3$.

reports also indicated that ginger or its effective compound 6-shogaol possesses neuroprotective function both *in vitro* and *in vivo* (El-Akabawy and El-Kholy, 2014; Ha et al., 2012; Shanmugam et al., 2011). Furthermore, Hussein et al. (2017) study showed that ginger exerts neuroprotective effects against monosodium glutamate-induced neurotoxicity through alleviation of DNA oxidative marker 8-OHdG, accumulation of β -amyloid as well as alteration of neurotransmitter levels. Ha et al. (2012) report indicated that 6-shogaol exhibits remarkable neuroprotective effects in transient global ischemia model through inhibition of microglia activation. 6-Dehydrogingerdione (6-DG), one of the major

components of dietary ginger, effectively scavenges various free radicals and enhances neuroprotection *in vitro*. Mechanistic study demonstrated that the neuroprotective effect of 6-DG is mediated via activation of the Keap1-Nrf2-ARE pathway (Yao et al., 2014). Moreover, ginger extract administration remarkably enhances the neuroprotection in animal model of diabetes disease (El-Akabawy and El-Kholy, 2014; Shanmugam et al., 2011). The results of these studies suggested that the neuroprotective function of ginger extract is partly mediated via acceleration of anti-oxidant defense mechanisms and attenuation of oxidative stress and inflammation (El-Akabawy and El-Kholy, 2014; Shanmugam et al., 2011).

In agreement with previous studies (Arisi et al., 2011; Anissian et al., 2018; Hashemian et al., 2017; Zhu et al., 2015), we also observed a significant increase in the number of astrocytes in PTZ receiving animals. Activated glial cells secrete several inflammatory factors which play an important role in the pathology of neurological disorders (Ben Haim et al., 2015; Liu and Hong, 2003; Maragakis and Rothstein, 2006). The results of this study indicated that ginger administration can attenuate the gliosis following PTZ application. This finding is consistent with previous reports which showed the beneficial anti-inflammatory effects of ginger (Ha et al., 2012; Lantz et al., 2007; Penna et al., 2003). It has been shown that ginger extract or its major components, gingerol or shogaol, efficiently inhibit the production of PGE₂, pro-inflammatory cytokines such as IL-1 β , TNF- α , COX-2 as well as NF- κ B and ameliorate glial activation (Lantz et al., 2007). 6-shogaol significantly inhibits the release of numerous inflammatory factors and attenuates microglia activation both *in vitro* and *in vivo* (Ha et al., 2012). In addition, ginger extract reduces the expression of inflammatory factors and astrocyte activation in streptozotocin-induced diabetic rats (El-Akabawy and El-Kholy, 2014). Therefore, it seems that ginger by its inhibitory effects on oxidative stress, glial activation and release of inflammatory mediators may exert the anti-seizure activity.

Conclusion

In conclusion, results of this study indicate that ginger hydroalcoholic extract reduces the seizure behavior in PTZ receiving animals. The beneficial anticonvulsant activity of ginger is partly mediated via its inhibitory effect on astrocyte activation which in turn leads to enhancement of neuroprotection in fully kindled animals. Based on these findings, ginger might be regarded as a useful supplementary compound in epileptic patients; however, further studies are needed to justify the exact molecular mechanism of ginger in animal model of epilepsy.

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Conflict of interest

No conflict of interest is declared.

References

- Anissian D, Ghasemi-Kasman M, Khalili-Fomeshi M, Akbari A, Hashemian M, Kazemi S, et al. Piperine-loaded chitosan-stpp nanoparticles reduce neuronal loss and astrocyte activation in chemical kindling model of epilepsy. *Int J Biol Macromol* 2018; 107: 973-83.
- Arisi GM, Ruch M, Foresti ML, Mukherjee S, Ribak CE, Shapiro LA. Astrocyte alterations in the hippocampus following pilocarpine-induced seizures in aged rats. *Aging Dis* 2011; 2: 294-300.
- Barreto G, White RE, Ouyang Y, Xu L, Giffard RG. Astrocytes: targets for neuroprotection in stroke. *Cent Nerv Syst Agents Med Chem* 2011; 11: 164-73.
- Ben Haim L, Carrillo-de Sauvage MA, Ceyzeriat K, Escartin C. Elusive roles for reactive astrocytes in neurodegenerative diseases. *Front Cell Neurosci* 2015; 9: 278.
- Bough KJ, Eagles DA. Comparison of the anticonvulsant efficacies and neurotoxic effects of valproic acid, phenytoin, and the ketogenic diet. *Epilepsia* 2001; 42: 1345-53.
- Davoudi M, Shojaei A, Palizvan MR, Javan M, Mirnajafi-Zadeh J. Comparison between standard protocol and a novel window protocol for induction of pentylenetetrazol kindled seizures in the rat. *Epilepsy Res* 2013; 106: 54-63.
- Devinsky O, Vezzani A, Najjar S, De Lanerolle NC, Rogawski MA. Glia and epilepsy: excitability and inflammation. *Trends Neurosci* 2013; 36: 174-84.
- Dhir A. Pentylenetetrazol (ptz) kindling model of epilepsy. *Curr Protoc Neurosci* 2012; Chapter 9: Unite9.37.
- Ebrahimzadeh MA, Chitsaz Z, Shokrzadeh M, Ataie A, Ataee R. Evaluation of anticonvulsant activities of *Eryngium caucasicum* with maximal electroshock and kindling model of seizure in mice. *Iran J Psychiatry Behav Sci* 2017; 11: e3571.
- El-Akabawy G, El-Kholy W. Neuroprotective effect of ginger in the brain of streptozotocin-induced diabetic rats. *Ann Anat* 2014; 196: 119-28.
- Gol M, Ghorbanian D, Hassanzadeh S, Javan M, Mirnajafi-Zadeh J, Ghasemi-Kasman M. Fingolimod enhances myelin repair of hippocampus in pentylenetetrazol-induced kindling model. *Eur J Pharm Sci* 2017; 96: 72-83.
- Grzanna R, Lindmark L, Frondoza CG. Ginger--an herbal medicinal product with broad anti-inflammatory actions. *J Med Food* 2005; 8: 125-32.
- Grzanna R, Phan P, Polotsky A, Lindmark L, Frondoza CG.

- Ginger extract inhibits β -amyloid peptide-induced cytokine and chemokine expression in cultured thp-1 monocytes. *J Altern Complement Med* 2004; 10: 1009-13.
- Guo Z, Zhang L, Wu Z, Chen Y, Wang F, Chen G. In vivo direct reprogramming of reactive glial cells into functional neurons after brain injury and in an alzheimer's disease model. *Cell stem cell* 2014; 14: 188-202.
- Ha SK, Moon E, Ju MS, Kim DH, Ryu JH, Oh MS, et al. 6-shogaol, a ginger product, modulates neuroinflammation: A new approach to neuroprotection. *Neuropharmacology* 2012; 63: 211-23.
- Hashemian M, Anissian D, Ghasemi-Kasman M, Akbari A, Khalili-Fomeshi M, Ghasemi S, et al. Curcumin-loaded chitosan-alginate-stpp nanoparticles ameliorate memory deficits and reduce glial activation in pentylenetetrazol-induced kindling model of epilepsy. *Prog Neuropsychopharmacol Biol Psychiatry* 2017; 79: 462-71.
- 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: 411-9.
- Hosseini A, Mirazi N. Alteration of pentylenetetrazole-induced seizure threshold by chronic administration of ginger (*zingiber officinale*) extract in male mice. *Pharm Biol* 2015; 53: 752-7.
- Hosseini A, Mirazi N, Gomar A. Protective effect of ginger against the pentylenetetrazole-induced seizure threshold model in streptozocin treated-diabetic mice. *Physiol Pharmacol* 2016; 20: 108-16.
- Hussein UK, Hassan NEY, Elhalwagy MEA, Zaki AR, Abubakr HO, Nagulapalli Venkata KC, et al. Ginger and propolis exert neuroprotective effects against monosodium glutamate-induced neurotoxicity in rats. *Molecules* 2017; 22: E1928.
- Ikonomidou-Turski C, Cavalheiro EA, Turski L, Bortolotto ZA, Kleinrok Z, Calderazzo-Filho L, et al. Differential effects of non-steroidal anti-inflammatory drugs on seizures produced by pilocarpine in rats. *Brain Res* 1988; 462: 275-85.
- Kaur H, Patro I, Tikoo K, Sandhir R. Curcumin attenuates inflammatory response and cognitive deficits in experimental model of chronic epilepsy. *Neurochem Int* 2015; 89: 40-50.
- Lantz RC, Chen GJ, Sarihan M, Solyom AM, Jolad SD, Timmermann BN. The effect of extracts from ginger rhizome on inflammatory mediator production. *Phytomedicine* 2007; 14: 123-8.
- Lehrmann E, Guidetti P, Löve A, Williamson J, Bertram EH, Schwarcz R. Glial activation precedes seizures and hippocampal neurodegeneration in measles virus-infected mice. *Epilepsia* 2008; 49: 13-23.
- Liu B, Hong JS. Role of microglia in inflammation-mediated neurodegenerative diseases: mechanisms and strategies for therapeutic intervention. *J Pharmacol Exp Ther* 2003; 304: 1-7.
- Liu W, Ge T, Pan Z, Leng Y, Lv J, Li B. The effects of herbal medicine on epilepsy. *Oncotarget* 2017; 8: 48385-97.
- Maragakis NJ, Rothstein JD. Mechanisms of disease: Astrocytes in neurodegenerative disease. *Nat Clin Pract Neurol* 2006; 2: 679-89.
- Marchi N, Granata T, Freri E, Ciusani E, Ragona F, Puvenna V, et al. Efficacy of anti-inflammatory therapy in a model of acute seizures and in a population of pediatric drug resistant epileptics. *PLoS One* 2011; 6: e18200.
- Mashhadi NS, Ghiasvand R, Askari G, Hariri M, Darvishi L, Mofid MR. Anti-oxidative and anti-inflammatory effects of ginger in health and physical activity: Review of current evidence. *Int J Prev Med* 2013; 4: S36-42.
- Masuda Y, Kikuzaki H, Hisamoto M, Nakatani N. Antioxidant properties of gingerol related compounds from ginger. *Biofactors* 2004; 21: 293-6.
- Moghadamnia Y, Mousavi Kani SN, Ghasemi-Kasman M, Kazemi Kani MT, Kazemi S. The anti-cancer effects of capparispinosa hydroalcoholic extract. *Avicenna J Med Biotechnol*; In press.
- Naeimi R, Safarpour F, Hashemian M, Tashakorian H, Ahmadian SR, Ashrafpour M, et al. Curcumin-loaded nanoparticles ameliorate glial activation and improve myelin repair in lyolecithin-induced focal demyelination model of rat corpus callosum. *Neurosci Lett* 2018; 674: 1-10.
- Oboh G, Ademiluyi AO, Akinyemi AJ. Inhibition of acetylcholinesterase activities and some pro-oxidant induced lipid peroxidation in rat brain by two varieties of ginger (*zingiber officinale*). *Exp Toxicol Pathol* 2012; 64: 315-9.
- Pelvig DP, Pakkenberg H, Stark AK, Pakkenberg B. Neocortical glial cell numbers in human brains. *Neurobiol Aging* 2008; 29: 1754-62.
- Penna SC, Medeiros MV, Aimbire FS, Faria-Neto HC, Sertie JA, Lopes-Martins RA. Anti-inflammatory effect of the hydralcoholic extract of *zingiber officinale* rhizomes on rat paw and skin edema. *Phytomedicine* 2003; 10: 381-5.
- Schmidt D. Efficacy of new antiepileptic drugs. *Epilepsy Curr* 2011; 11: 9-11.
- Shanmugam KR, Mallikarjuna K, Kesireddy N, Reddy KS. Neuroprotective effect of ginger on anti-oxidant enzymes in streptozotocin-induced diabetic rats. *Food Chem Toxicol* 2011; 49: 893-7.
- Shim S, Kim S, Choi DS, Kwon YB, Kwon J. Anti-inflammatory effects of [6]-shogaol: Potential roles of hdac inhibition and hsp70 induction. *Food Chem Toxicol* 2011; 49: 2734-40.
- Vezzani A, Aronica E, Mazarati A, Pittman QJ. Epilepsy and brain inflammation. *Exp Neurol* 2013; 244: 11-21.
- Vezzani A, Balosso S, Ravizza T. The role of cytokines in the pathophysiology of epilepsy. *Brain Behav Immun* 2008; 22: 797-803.
- Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. *Nat Rev Neurol* 2011; 7: 31-40.
- Vezzani A, Granata T. Brain inflammation in epilepsy:

- experimental and clinical evidence. *Epilepsia* 2005; 46: 1724-43.
- Yao J, Ge C, Duan D, Zhang B, Cui X, Peng S, et al. Activation of the phase ii enzymes for neuroprotection by ginger active constituent 6-dehydrogingerone in pc12 cells. *J Agric Food Chem* 2014; 62: 5507-18.
- Zhu X, Dong J, Shen K, Bai Y, Zhang Y, Lv X, et al. Nmda receptor nr2b subunits contribute to ptz-kindling-induced hippocampal astrogliosis and oxidative stress. *Brain Res Bull* 2015; 114: 70-8.