

Original Article

# Interactive effect of aqueous-alcoholic extract of ginger as well as GABA<sub>A</sub> receptor agonist and antagonist on pain sensitivity in male rats

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## Abstract

**Introduction:** Ginger has shown anti-nociceptive effects. Here we investigated the possible involvement of GABA<sub>A</sub> receptors in anti-nociceptive effect of ginger using muscimol (GABA<sub>A</sub> agonist) and picrotoxin (GABA<sub>A</sub> antagonist) in rats that received ginger. The pain sensitivity was evaluated by formalin test.

**Methods:** Thirty-five male Sprague-Dawley rats were randomly divided into 7 groups (n=5): sham1 (received distilled water. PO); sham2 (received water + 0.75μl artificial cerebrospinal fluid by intracerebroventricular (ICV) injection); experimental1 (received ginger at 50 mg/kg/day, PO); experimental2 and 3 (received ginger+ 0.75μl of 250 or 500ng/rat muscimol by ICV injection); experimental4 and 5 (received ginger+ 0.75μl of 250 and 500ng/rat picrotoxin by ICV injection). On day 16 and 30min after ICV injections, formalin test was performed on all rats.

**Results:** Ginger significantly reduced pain sensitivity in both phases of formalin test in comparison to sham1 and 2. In early and late phase, both doses of muscimol reduced pain sensitivity as compared to the ginger group. Picrotoxin at 250ng/rat+ ginger reduced pain sensitivity as compared to the group that received ginger, in both the early and the late phases of the formalin test. Picrotoxin at 500ng/rat+ ginger increased pain sensitivity in the early phase and late phase of formalin test as compared to the ginger group.

**Conclusion:** Aqueous- alcoholic extract of ginger has significant analgesic effects in late phase of formalin test and GABA<sub>A</sub> receptors may be involved in this regard.

## Keywords:

Ginger;  
Muscimol;  
Picrotoxin;  
Pain sensitivity;  
Rat

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## Introduction

Pain, at all forms including mental, emotional and physical is one of the most frequent complains of patients and it is almost always a sign of imbalance in one or more parts of the body (Vachon-Preseu

et al., 2016). Accordingly, the design of therapeutic interventions to reduce pain is perhaps the physician's greatest challenge (Bonakdar and Sukiennik, 2016).

Previously, it has been shown that *Zingiber officinale* (ginger) owns a number of beneficial effects for attenuating pain. Ginger is widely used as a spice in

cooking and in traditional medicine as a treatment for cold, fever, headache and nausea following pregnancy and digestive problems. It also induces sleep and sedation. It has been demonstrated that ginger may diminish the symptoms of cancer therapy as well as motion sickness and there is suggestive evidence that ginger reduces inflammation. It has also been documented that ginger extract evokes anti-nociceptive effect and enhances morphine-induced analgesia and can be an efficacious adjunct therapy for pain control (Jagetia et al., 2004; Sepahvand et al., 2010).

The main constituents of ginger are gingerols, shogaols, paradols and zingeron. These compounds are known to have properties including antipyretic, analgesic, anti-apoptotic, anti-tumorigenic, anti-inflammatory and anti-oxidant effects (Jiang et al., 2006).

Gamma-aminobutyric acid (GABA) is the most abundant inhibitory neurotransmitter in the central nervous system (Chebib and Johnston, 2000; Carter et al., 2011). GABA produces neuronal inhibition by acting on two major types of receptors: ionotropic receptors that are ligand-gated ion channels (GABA<sub>A</sub> and GABA<sub>C</sub> receptors), and metabotropic receptors that are G-protein coupled receptors (GABA<sub>B</sub> receptors) that act via second messengers (Chebib and Johnston, 2000).

Loss of GABA-mediated inhibition of nociception may be a key process in the development of inflammatory and neuropathic pain (Munro et al., 2011; Munro et al., 2013). Microinjection of GABA antagonists into the ventrolateral periaqueductal gray matter leads to global activation of neurons and produces anti-nociceptive effects to noxious stimuli (Morgan et al., 2008). In the previous study we observed that intrahippocampal injection of GABA<sub>A</sub> agonist and antagonist respectively had analgesic and hyperalgesic effects (Taherianfard and Mosavi, 2012).

Ginger supplementation has been associated with an increased GABA concentration in the hippocampus as well as cerebral cortex of senile female rats (Hegazy and Ali, 2011). For determination of ginger analgesic mechanism, we designed the present study to test the hypothesis that whether GABAergic system is directly involved in anti-nociceptive effects of ginger extract, by evaluating interactive effects of muscimol (GABA<sub>A</sub> receptor agonist) and picrotoxin

(GABA<sub>A</sub> receptor antagonist) and ginger extract in formalin test on male rats.

## Materials and methods

### Preparation of ginger extract

Fresh rhizomes of ginger were purchased from a local market in Shiraz and authenticated by a botanist (sample of the rhizome was deposited at the herbarium of the Shiraz Herbal Medicine Research Center). Two kilograms of ginger rhizomes thoroughly washed, sliced, grated and dried in shadow. The dried rhizome of ginger was then finely ground. The powder was extracted with equal proportions of 70% ethyl alcohol (1:1, w:v) for 48 hours. The extract filtered and the filtrate evaporated to dryness and then freeze-dried (3 times during 24h). The powder reconstituted in distilled water at the time of use (Mirazi and Karami, 2016).

### Animals and study design

Thirty-five male Sprague-Dawley rats weighting 220-280g were housed under standard temperature 20±2°C and 12/12 hours light/dark cycle. After a week of adaptation, animals were randomly divided into 7 groups (n=5 each). Sham1: normal rats that received distilled water orally for 15 days; sham2: received distilled water for 15 days and 0.75µl artificial cerebrospinal fluid (ACSF) by intracerebroventricular (ICV) injection on day 16; experimental1: received 50 mg/kg/day ginger extract (Li et al., 2012) orally by gavage for 15 days; experimental2 and 3: received 50 mg/kg/day ginger extract orally for 15 days and muscimol (Sigma, USA) at 250 and 500ng/rat in the volume of 0.75µl (Taherianfard and Mosavi, 2012) by ICV injection on day 16, respectively; experimental4 and 5: received 50 mg/kg/day ginger extract orally for 15 days and picrotoxin (Sigma, USA) at 250 and 500 ng/rat in the volume of 0.75µl (Taherianfard and Mosavi, 2012) by ICV injection on day 16, respectively. At the end of the experiment (day 16<sup>th</sup>), formalin test performed on all animals 30min after ICV injection of drugs or ACSF and simultaneously in sham1 group.

### Stereotaxic surgery

For ICV injections, stereotaxic surgery performed as follows. Animals positioned in a rodent stereotaxic device after anesthetic sedation with a mixture of

ketamine (100mg/kg) and xylazine (10mg/kg) (Moazen et al., 2018). The skull adequately exposed using an electric high-speed drill at the previously labeled appropriate position. GABA<sub>A</sub> agonist and antagonist were administered unilaterally at two doses of 250 or 500 ng/rat by ICV injection, at the following coordinates from the bregma; -0.96 mm anterior (A/P), 1.87 mm lateral, and 3 mm depth from cerebral cortex and skull thickness was considered 1mm using a 5µl Hamilton syringe. To confirm proper injection, rats exposed to a lethal concentration of ether after formalin test. At the end of experiments, the microinjection site marked by injection of cresyl violet into the lateral ventricle. Subsequently the brain removed and placed in 10% formaldehyde solution. Coronal sections made to check the precision of injection. When the microinjection site was not correct, the animal was excluded from the experiment and replaced.

All procedures used in the present study were according to institutional research ethics committee guidelines of Shiraz University for care and use of laboratory animals in experimental studies (Animal Ethics Committee approval number= GCU2M1755).

### Formalin test

After injection of formalin (50µl, 2.5%) in hind paw, the rats' behaviors evaluated in blocks of 5 minutes every 15 seconds, during 60 minutes. The scores

were as follows: the injected paw was not favored, "0"; the injected paw had little or no weight on it, "1"; the injected paw was elevated and was not in contact with any surfaces, "2"; and the injected paw was licked, bitten, or shaken, "3". Two distinct periods of licking activity were identified, the early response (neurogenic phase) and the late (inflammatory phase), which were recorded during 0-15 or 15-45 minutes, respectively (Yosefifard and Hassanpour-Ezatti, 2014)

### Statistical analysis

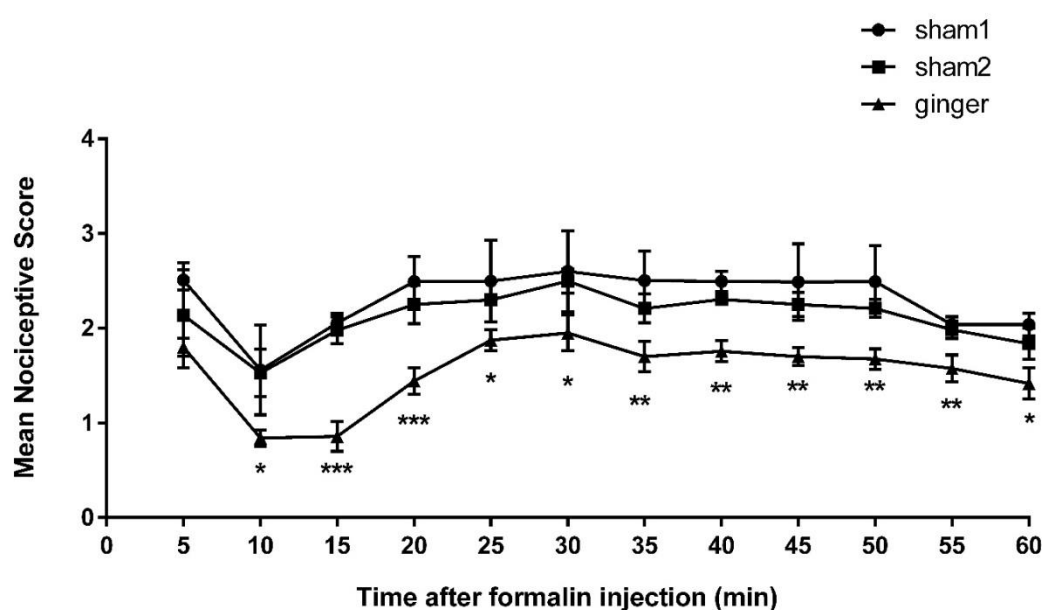
Statistical procedures performed using SPSS software (version 22). To analyze data repeated measures ANOVA and one way-ANOVA followed by Tukey test as post-hoc used. The data were presented as mean±SEM and level of significant was  $P<0.05$ .

## Results

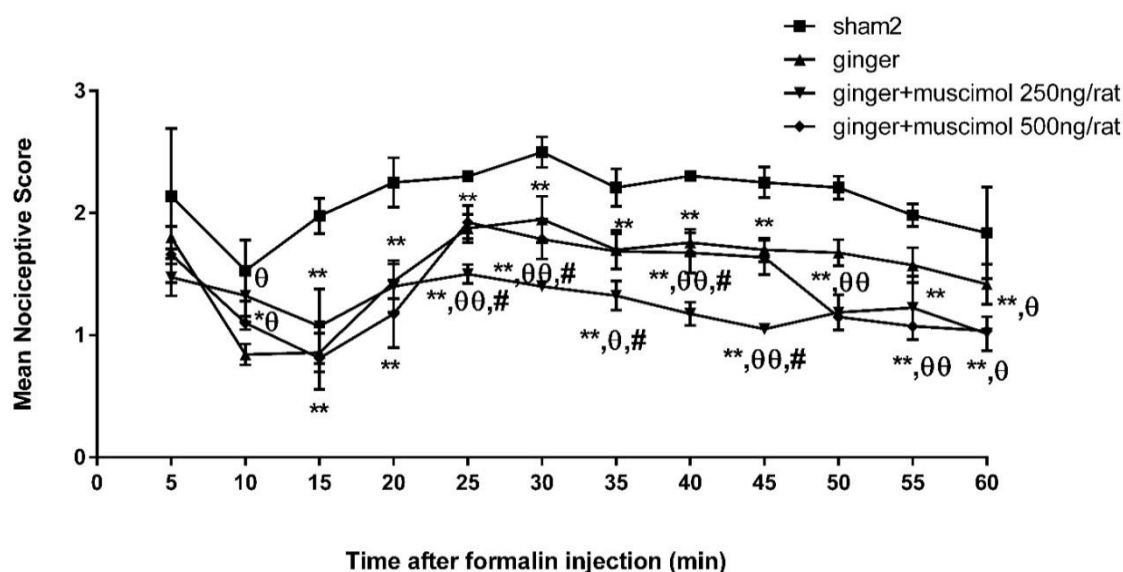
### The effect of ginger extract on pain sensitivity

Figure1 shows pain sensitivity scores in sham1 and experimental1 groups. Ginger administration significantly ( $P<0.05$ ) decreased pain sensitivity in both early and late phase of formalin test in comparison to sham1 and 2 groups.

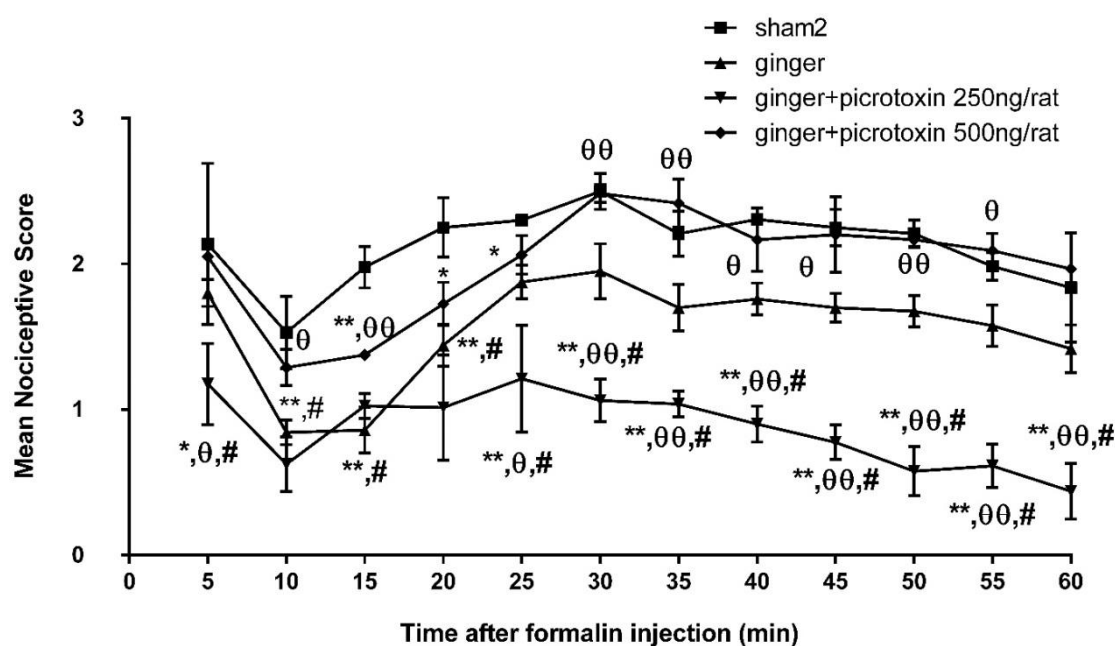
### The interactive effects of ginger extract and



**Fig.1.** Nociceptive scores (mean±SE) of rats in sham1, sham2 and ginger 50 mg/kg/day treated groups after formalin test. \*Difference between ginger group in comparison to sham1 or sham2 groups; \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ .



**Fig.2.** Nociceptive scores (mean±SE) of rats in sham2, ginger 50 mg/kg/day and ginger 50 mg/kg/day + muscimol 250 and 500ng/rat treated groups after formalin test. \*difference between ginger+muscimol 250 or 500ng/rat in comparison to sham2 group; <sup>θ</sup>difference between ginger+muscimol 250 or 500ng/rat in comparison to ginger group; #difference between ginger+muscimol 250ng/rat in comparison to ginger+muscimol 500ng/rat. \* $P<0.01$ , \*\* $P<0.001$ , <sup>θ</sup> $P<0.01$ , <sup>θθ</sup> $P<0.001$ , # $P<0.001$



**Fig.3.** Nociceptive scores (mean±SE) of rats in sham1, sham2, ginger 50 mg/kg/day and ginger 50 mg/kg/day + picrotoxin 250 and 500ng/rat treated groups after formalin test. \*difference between ginger+picrotoxin 250 or 500ng/rat in comparison to sham2 group, <sup>θ</sup>difference between ginger+picrotoxin 250 or 500ng/rat in comparison to ginger group, #difference between ginger+picrotoxin 250ng/rat in comparison to ginger+picrotoxin 500ng/rat. \* $P<0.01$ , \*\* $P<0.001$ , <sup>θ</sup> $P<0.01$ , <sup>θθ</sup> $P<0.001$ , # $P<0.001$

### muscimol on pain sensitivity

In the early (0-5 min) phase of formalin test, there was no significant difference between the muscimol-treated groups in two doses and sham2, but in the late phase of formalin test, the group treated with muscimol in two doses showed significant ( $P<0.05$ )

reduction in the pain sensitivity in comparison to sham2 (Fig. 2). In late phase of formalin test, muscimol 250ng/rat reduced pain sensitivity as compared to the cases that received only ginger extract ( $P<0.05$ , Fig. 2).



### The interactive effects of ginger extract and picrotoxin on pain sensitivity

As observed in Figure 3, in the early phase of formalin test, ginger extract+picrotoxin at 250 ng/rat resulted in significant ( $P<0.05$ ) reduction in formalin-induced pain with respect to the all other groups. Both doses of picrotoxin showed no significant differences ( $P>0.05$ ) in the middle phase of formalin test, but a significant reduction ( $P<0.05$ ) on pain sensitivity was observed in rats treated with ginger extract+picrotoxin 500 ng/rat as compared to the sham2 group in the late phase of formalin test.

Picrotoxin at 250ng/rat reduced pain sensitivity significantly as compared to the group that received ginger, in the late phases of the formalin test ( $P<0.05$ ). While picrotoxin at 500ng/rat resulted in increased pain sensitivity in the late phase when compared with the ginger group ( $P<0.05$ ), while having no significant alteration in the early phase of formalin test ( $P>0.05$ , Fig. 3).

## Discussion

The formalin test is the most accepted chemical test for evaluation of nociception. It requires the injection of an adequate amount of formalin into the surface of the hind paw. Formalin test consists of early phase (0-10 min) and late phase (15-60) in which the animal shows painful behaviors. These phases are separated with a quiet phase named interphase, in which the nociceptive responses are decreased or completely disappeared (Mohammad-Zadeh et al., 2014). Unlike reflex tests such as tail flick and hotplate tests, formalin test is persistent and more closely related to clinical pain conditions (Abbott et al., 1999).

Consistent with previous investigations, the present study shows that oral administration of ginger extract induces analgesia in both the early and late phases of the formalin test. It seems that ginger-induced L-type calcium channel blockage is mainly responsible for its pharmacological properties, including analgesia (Khalid et al., 2011). Moreover, it has been demonstrated that anti-inflammatory effect of ginger occurs through the NF- $\kappa$ B signalling pathway and ginger has decreased cytokine gene TNF $\alpha$  and IL-6 expression *in vivo* (Li et al., 2011).

Flavonoids are present in ginger rhizome and leaves. In order for flavonoids to enter the brain, they must

first cross the blood-brain barrier (Ghasemzadeh et al., 2010). Both *in vitro* and *in vivo* studies have indicated that the flavanones hesperetin, naringenin and their relevant metabolites, as well as some dietary anthocyanins, cyanidin-3-rutinoside and pelargonidin-3-glucoside are able to traverse the blood-brain barrier (Youdim et al., 2004a; Youdim et al., 2004b). Experiments with some natural and synthetic flavones and flavanones have shown that they can modulate GABA generated chloride currents (Goutman et al., 2003; Campbell et al., 2004; Kavvadias et al., 2004). Flavonoids have a range of activities on GABA<sub>A</sub> receptors, acting as positive as well as negative modulators of GABA<sub>A</sub> receptor function. Binding studies first demonstrated that flavones are ligands for benzodiazepine binding sites on GABA<sub>A</sub> receptors (Marder and Paladini, 2002). Therefore, in addition to the peripheral analgesic and anti-inflammatory effects of ginger, flavonoid content of the leaves and rhizomes of ginger can pass through the blood-brain barrier and due to the different central mechanisms including impact on GABA<sub>A</sub> receptor, may control pain centrally (Ghasemzadeh et al., 2010). In our study, by considering the fact that the extract exerted significant inhibition of both phases in the formalin-induced pain model, it seems that overall anti-nociceptive effect of the ginger extracts involves central mechanism.

The conjugated forms of ginger metabolites, glucuronide in humans and (S)-[6]-gingerol-4-O-h-glucuronide in rats, are majorly responsible for the analgesic property of ginger after oral administration (Jiang et al., 2008). Present investigation showed that muscimol in the early phase of the formalin test at both doses in animals which received 50 mg/kg/day ginger for 15 days, had no significant effect on pain sensitivity. However, muscimol especially at the dose of 250ng/rat in the late phase of the formalin test, significantly decreased pain sensitivity in comparison to ginger alone. Previous studies have reported that the stimulation of GABA<sub>A</sub> receptors, by the administration of muscimol can cause analgesic effects (Gilbert and Franklin, 2001; Mahmoudi and Zarrindast, 2002). Muscimol, as an agonist of GABA<sub>A</sub> receptors, can hyperpolarize the neuron's membrane for hours and open the Cl<sup>-</sup> channels, and in doing so, exerts its analgesic effects (Reis et al., 2007). Previously it was shown that ICV and

intrahippocampal injection of muscimol at similar doses which were used in the present study had analgesic effects during estrous cycle of rat (Taherianfard and Mosavi, 2012).

Ginger compounds affect the glutamine-glutamate-GABA metabolism, reducing the glutamate level and increase GABA synthesis (Waggas, 2009). Ginger also increases GABA in the hippocampus and the frontal cortex. In addition to this, ginger prevents the suppression of the function of GABA receptors by inhibiting eicosanoids (Schwartz-Bloom et al., 1996). As a result, at the beginning, a large amount of GABA is released following ginger administration and then muscimol prevents its reabsorption (Schwartz-Bloom et al., 1996; Waggas, 2009). Therefore, enough neurotransmitters will not be reabsorbed by the glial and GABAergic cells for the normal functioning of the glutamine-glutamate-GABA cycle. Despite the severe release of GABA, the glutamine-glutamate-GABA cycle is disturbed and after that, GABA release terminates. This process may be helpful in explaining the weaker analgesic effect of muscimol at 500ng/rat compared to 250ng/rat as observed in the present study.

In the present study, ICV injection of picrotoxin at 250ng/rat in rats that received ginger at 50 mg/kg/day for 15 days led to analgesia in early and late phase of formalin test in comparison to ginger alone group. In a study by Yokoro et al. (2001), sub convulsant doses of picrotoxin decreased the phenobarbital-induced hyperalgesic response in different algosimetric assays. The anti-nociceptive effect of picrotoxin has also been described by Tatsuo et al. (1997) and may be related to its action on GABA<sub>A</sub> receptors present in the descending inhibitory system.

In our study, picrotoxin at 500ng/rat resulted in pain sensitivity increase in the early phase of formalin test while having no significant alteration in the late phase of formalin test. Previously it was shown that ICV and intrahippocampal injection of picrotoxin at similar doses which were used in the present study has hyperalgesic effect during estrous cycle (Taherianfard and Mosavi, 2012). In the present study, it seems that analgesic effect of ginger was dominant which led to suppression of hyperalgesic effect of picrotoxin.

Finally, it should be mentioned that we performed a preliminary study and the involvement of GABA<sub>A</sub> receptors in anti-nociceptive effect of ginger needs to be more precisely investigated by complementary

methods at molecular, physiological, behavioral and genetic levels.

## Conclusion

In conclusion, the results of our study indicate that oral administration of aqueous alcoholic extract of ginger has significant analgesic effects in late phase of formalin test which is regulated by CNS (Tjølsen et al., 1992) and the ginger might have a central interaction with GABA<sub>A</sub> receptors.

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## Conflict of interest

The authors declare no conflict of interest.

## References

- Abbott FV, Ocivirk R, Najafee R, Franklin KB. Improving the efficiency of the formalin test. *Pain* 1999; 83: 561-9.
- Bonakdar RA, Sukiennik AW. Integrative pain management. Valencia, California: Weil Integrative Medicine Library, 2016.
- Campbell EL, Chebib M, Johnston GA. The dietary flavonoids apigenin and (-)-epigallocatechin gallate enhance the positive modulation by diazepam of the activation by gaba of recombinant gaba(a) receptors. *Biochem Pharmacol* 2004; 68: 1631-8.
- Carter CR, Kozuska JL, Dunn SM. Insights into the structure and pharmacology of gaba(a) receptors. *Future Med Chem* 2011; 2: 859-75.
- Chebib M, Johnston GA. Gaba-activated ligand gated ion channels: medicinal chemistry and molecular biology. *J Med Chem* 2000; 43: 1427-47.
- 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: 4324-33.
- Gilbert AK, Franklin KB. Gabaergic modulation of descending inhibitory systems from the rostral ventromedial medulla (rvm). Dose-response analysis of nociception and neurological deficits. *Pain* 2001; 90: 25-36.
- Goutman JD, Waxemberg MD, Donate-Oliver F, Pomata PE, Calvo DJ. Flavonoid modulation of ionic currents mediated by gaba(a) and gaba(c) receptors. *Eur J Pharmacol* 2003; 461: 79-87.
- Hegazy HG, Ali EHA. Modulation of monoamines and amino-acids neurotransmitters in cerebral cortex and hippocampus of female senile rats by ginger and lipoic

- acid. *Afr J Pharm Pharmacol* 2011; 5: 1080-1085.
- Jagetia G, Baliga M, Venkatesh P. Ginger (zingiber officinale rosc.), a dietary supplement, protects mice against radiation-induced lethality: mechanism of action. *Cancer Biother Radiopharm* 2004; 19: 422-35.
- Jiang H, Xie Z, Koo HJ, McLaughlin SP, Timmermann BN, Gang DR. Metabolic profiling and phylogenetic analysis of medicinal zingiber species: tools for authentication of ginger (zingiber officinale rosc). *Phytochemistry* 2006; 67: 1673-85.
- Jiang SZ, Wang NS, Mi SQ. Plasma pharmacokinetics and tissue distribution of [6]-gingerol in rats. *Biopharm Drug Dispos* 2008; 29: 529-37.
- Kavadias D, Sand P, Youdim KA, Qaiser MZ, Rice-Evans C, Baur R, et al. The flavone hispidulin, a benzodiazepine receptor ligand with positive allosteric properties, traverses the blood-brain barrier and exhibits anticonvulsive effects. *Br J Pharmacol* 2004; 142: 811-20.
- Khalid MH, Akhtar MN, Mohamad AS, Perimal EK, Akira A, Israf DA, et al. Antinociceptive effect of the essential oil of zingiber zerumbet in mice: possible mechanisms. *J Ethnopharmacol* 2011; 137: 345-51.
- Li XH, McGrath KC, Nammi S, Heather AK, Roufogalis BD. Attenuation of liver pro-inflammatory responses by zingiber officinale via inhibition of nf-kappa b activation in high-fat diet-fed rats. *Basic Clin Pharmacol Toxicol* 2011; 110: 238-44.
- Li Y, Tran VH, Duke CC, Roufogalis BD. Preventive and protective properties of zingiber officinale (ginger) in diabetes mellitus, diabetic complications, and associated lipid and other metabolic disorders: A brief review. *Evid Based Complement Alternat Med* 2012; 2012: 516870.
- Mahmoudi M, Zarrindast MR. Effect of intracerebroventricular injection of gaba receptor agents on morphine-induced antinociception in the formalin test. *J Psychopharmacol* 2002; 16: 85-91.
- Marder M, Paladini AC. Gaba(a)-receptor ligands of flavonoid structure. *Curr Top Med Chem* 2002; 2: 853-67.
- Mirazi N, Karami Z. The protective effect of hydroalcoholic extract from rhizome of zingiber officinale I. On carbon tetrachloride-induced hepatic injury in male rat. *Feyz* 2016; 20: 297-305.
- Moazen P, Taherianfard M, Ahmadi Soleimani M, Norozpor M. Synergistic effect of spexin and progesterone on pain sensitivity attenuation in ovariectomized rats. *Clin Exp Pharmacol Physiol* 2018; 45: 349-354.
- Mohammad-Zadeh M, Azhdari-Zarmehri H, Mosavi F, Haghdost-Yazdi H, Nazeri M, Shabani M. Modulation of different phases of formalin test by force swim stress. *Basic Clin Neurosci* 2014; 5: 303-7.
- Morgan MM, Whittier KL, Hegarty DM, Aicher SA. Periaqueductal gray neurons project to spinally projecting gabaergic neurons in the rostral ventromedial medulla. *Pain* 2008; 140: 376-86.
- Munro G, Erichsen HK, Rae MG, Mirza NR. A question of balance--positive versus negative allosteric modulation of gaba(a) receptor subtypes as a driver of analgesic efficacy in rat models of inflammatory and neuropathic pain. *Neuropharmacology* 2011; 61: 121-32.
- Munro G, Hansen RR, Mirza NR. Gaba(a) receptor modulation: potential to deliver novel pain medicines? *Eur J Pharmacol* 2013; 716: 17-23.
- Reis G, Pacheco D, Francischi J, Castro M, Perez A, Duarte I. Involvement of gaba a receptor-associated chloride channels in the peripheral antinociceptive effect induced by gaba a receptor agonist muscimol. *Eur J Pharmacol* 2007; 564: 112-5.
- Schwartz-Bloom RD, Cook TA, Yu X. Inhibition of gaba-gated chloride channels in brain by the arachidonic acid metabolite, thromboxane a2. *Neuropharmacology* 1996; 35: 1347-53.
- Sepahvand R, Esmaeili-Mahani S, Arzi A, Rasoulzadeh B, Abbasnejad M. Ginger (zingiber officinale rosc.) elicits antinociceptive properties and potentiates morphine-induced analgesia in the rat radiant heat tail-flick test. *J Med Food* 2010; 13: 1397-401.
- Taherianfard M, Mosavi M. Hippocampal gaba(a) receptor and pain sensitivity during estrous cycle in the rat. *Iran J Med Sci* 2012; 36: 289-95.
- Tjølsen A, Berge OG, Hunskaar S, Rosland JH, Holea K. The formalin test: an evaluation of the method. *Pain* 1992; 51: 5-17.
- Vachon-Preseu E, Centeno MV, Ren W, Berger SE, Tetreault P, Ghantous M, et al. The emotional brain as a predictor and amplifier of chronic pain. *J Dent Res* 2016; 95: 605-12.
- Waggas AM. Neuroprotective evaluation of extract of ginger (zingiber officinale) root in monosodium glutamate-induced toxicity in different brain areas male albino rats. *Pak J Biol Sci* 2009; 12: 201-12.
- Yosefifard M, Hassanpour-Ezatti M. Epidural administration of neostigmine-loaded nanofibers provides extended analgesia in rats. *Daru* 2014; 22: 73.
- Youdim KA, Qaiser MZ, Begley DJ, Rice-Evans CA, Abbott NJ. Flavonoid permeability across an in situ model of the blood-brain barrier. *Free Radic Biol Med* 2004a; 36: 592-604.
- Youdim KA, Shukitt-Hale B, Joseph JA. Flavonoids and the brain: interactions at the blood-brain barrier and their physiological effects on the central nervous system. *Free Radic Biol Med* 2004b; 37: 1683-93.