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Original Article

The Effect of Pretreatment with the Hydroalcoholic Extract of Ginger on the Modulation of Dopamine D2 Receptor Agonist and Antagonist Impacts on Pain Sensitivity in Male Rats

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Abstract

Background and Aim: Zingiber officinale (ginger) extract increased dopamine concentration in various brain areas. Therefore, the present study aimed to investigate how ginger extract can affect the efficiency of D2 receptor agonist (bromocriptine) and antagonist (chlorpromazine) on pain sensitivity in rats.

Materials and Methods: Forty-eight adult male rats in standard conditions divided into eight groups, i.e. the control, the ginger sham1, sham2, ginger, bromocriptine 10 or 30 µM + ginger, and chlorpromazine 20 or 40 µM + ginger. The cannulation of the lateral ventricle was conducted unilaterally by the stereotaxic procedure. A pain sensitivity test was carried out in all the groups by formalin test on the 16th day.

Results: The results of this study indicated that ginger could remarkablly ($P<0.01$) reduce pain sensitivity in all stages of the formalin test. As the data revealed, bromocriptine 10 or 30 µg/rat and chlorpromazine 20 or 40 µg/rat significantly ($P<0.01$) decreased the pain sensitivity in all phases of the formalin test in comparison with the control and sham groups. However, chlorpromazine 20 or 40 µg/rat noticeably ($P<0.01$) increased the pain sensitivity when compared to ginger and bromocriptine groups.

Conclusion: According to the results of the present study, ginger mimics the analgesic effect of bromocriptine. Moreover, ginger has attenuated the hyperalgesic effect of chlorpromazine. It seems that ginger has a synergistic effect with the analgesic effect of the dopamine D2 receptor.

Keywords: Ginger, Dopamin D2 receptor, Pain, Male Rat

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Introduction

Pain is an unpleasant experience, and it has emotional, cognitive, and social aspects mediated by neural networks in the brain (1). The mechanisms of neural pain sensation are complex and begin with stimulating the nociceptive nerve endings and transferring them to the pain centers in the nervous system, and psychological reactions to it are different in all individuals in the world (2).

The dopaminergic system has an important role in pain control at several different levels of the central nervous system (3). Based on animal models of pain behavior and some clinical data, pain modulation and regulation were investigated by the dopamine D2 receptors (4). In some human investigations, pain sensation reduction by dopamine D2 receptors agonist has been reported (5).

Herbal medications have drawn the attention of scientists because of their natural ingredients and fewer side effects. Zingiber officinale (ginger) root has active ingredients such as gingerols, and shogaols are responsible for anti-nociceptive and anti-inflammatory effects (6). Specific compounds of ginger increase serum levels of interleukin-10 and enhance its anti-inflammatory effect. However, they inhibit the production of inflammatory prostaglandins that are highly effective in reducing acute and chronic pain (7).

Ginger in various forms is effective in releasing and increasing dopamine levels. Dopamine deficiency in Parkinson's disease can be treated by ginger consumption. It increases central dopamine levels, enhances antioxidant activity, and protects dopaminergic neurons (8). Ginger components, zingerone, and 6-shogaol can prevent the death of dopamine-containing neurons by blocking the function of a 6-hydroxy dopamine (9).

Since ginger affects the release of dopamine in the brain, it can affect the analgesic impacts of dopamine through the dopamine D2 receptor. Therefore, the question arises: Can the oral hydroalcoholic extract of ginger 50 mg/kg/day for 15 days affect the dopamine function through dopamine agonist and antagonist on pain in male rats?

Materials and Methods

Animals and study design

Forty-eight male Sprague Dawley rats weighing 200-250g were used in this study. The rats were kept in standard condition temperature 20±2 °C and a light-dark cycle of 12/12h. They were fed standard food and water ad libitum. The rats were randomly divided into eight groups (n=7): 1- Control (intact rats); 2- sham 1 (administration of water orally 0.4 ml for 15 day); 3- sham 2 (received 4 µl intracerebroventricular (ICV) injection of artificial cerebrospinal fluid (ACSF); 4- ginger (received 0.4 ml ginger); 5 and 6- bromocriptine (received 0.4 ml ginger + 0.75 µl bromocriptine 10 or 30 µg/rat); and 7 and 8- chlorpromazine (received 0.4 ml ginger + 4 µl chlorpromazine 20 and 40 µg/rat). The ginger dose was 50 mg/kg/day, and it was administrated orally for 15 days in all the groups (10). Bromocriptine, chlorpromazine, and ACSF were microinjected 15 minutes before the formalin test by ICV administration. The fresh rhizomes of ginger (herbarium code no. 1483) were used. Preparation of the ginger hydroalcoholic extract was carried out according to the reference (10).

Stereotaxic Procedure

The rats were anesthetized via the IP injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). They were fixed in the stereotaxic apparatus using blunt ear bars. The coordinates for the lateral ventricle were: 3.5 mm bellow cerebral cortex, 0.5 mm anterior to Bregma, and 1.5 mm lateral to the midline. The guide cannula was fixed to the skull via dental acrylic cement and two tiny stainless steel screws. Eventually, the animals underwent a 7-day recovery period.

Following each experiment, 0.4 µL methylene blue was microinjected unilaterally into the lateral ventricle to specify the location of the inserted quid cannula (Fig 1). Brains were removed and submerged in 10% phosphate-buffered formalin for 24 h. The fixed brains were then cut into 300 µm-thick sections, and the blue spots were histologically compared to the schematic sections in the atlas of Paxinos and Watson (Fig.1) (11).

The Formalin Test

50µl formalin (2.5% in normal saline) was subcutaneously injected into the dorsal surface of the right hind paw. The pain score was recorded every 15 seconds (20 records in 5 minutes) as follows. If the animal did not react, the score would be 0; if the rats did not rely on the injected paw, the score would be 1; if the animals hold their foot up, the score would be 2; and finally if the rast lick and bite the injected paw, the score would be 3. The pain measurement was performed for 60 minutes (Tjølsen et al., 1992).

Animal ethics and welfare were approved by the Institutional Research Ethics Committee of Shiraz University (94GCU4M1755).

Statistical Analysis

We used SPSS (version 22) to analyze the data. Data analysis was carried out using the one-way, the repeated measure ANOVA, and the post-hoc Tukey test. $P<0.05$ was considered as a significant level. The data were shown as Mean±SEM.

Results and Discussion

Based on one-way ANOVA, the mean nociceptive score in the ginger group was significantly (P <0.001) lower than the control and sham groups at the early and late stages of the formalin test (Fig. 2).

It was shown that bromocriptine 10 and 30 could significantly (P<0.001) reduce the mean nociceptive score in the early and late phases of the formalin test compared to the sham 2 group. Bromocriptine 10 and 30 ug/rat had no difference with the ginger group (fig. 3).

Chlorpromazine 20 and 40ug/rat remarkably (P<0.05) decreases the mean nociceptive score in the early and late phases of the formalin test compared to the sham 2 group. According to Fig. 4, chlorpromazine 20 and 40 groups have significantly (P<0.01) increase the mean nociceptive score in the early and late phases of the formalin test compared to the ginger group.

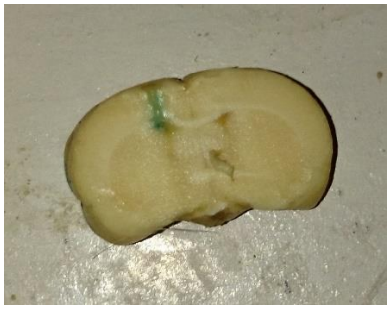

Figure 1. A depiction of the brain section and the location of the lateral ventricle injection site.

Chlorpromazine (P<0.001) had a significantly higher mean nociceptive score than bromocriptine. However, bromocriptine and chlorpromazine had significant (P<0.001) lower mean nociceptive scores than sham 2.

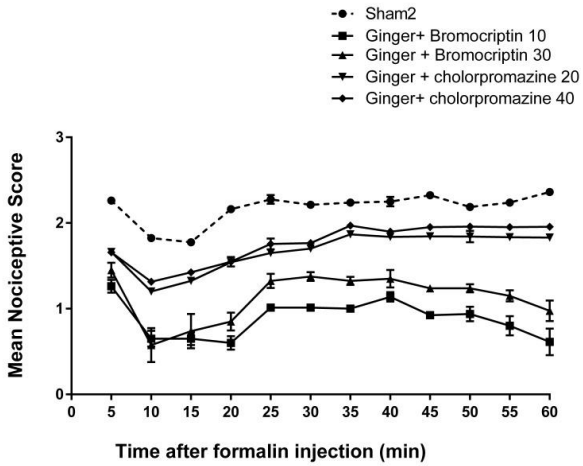

Figure 5. Effects of ginger (50mg/kg/day) + chlorpromazine 20 and 40 µg/rat and ginger (50mg/kg/day) + bromocriptine 10 and 30µg/rat on the mean nociceptive score. The data were shown as Mean±SEM.

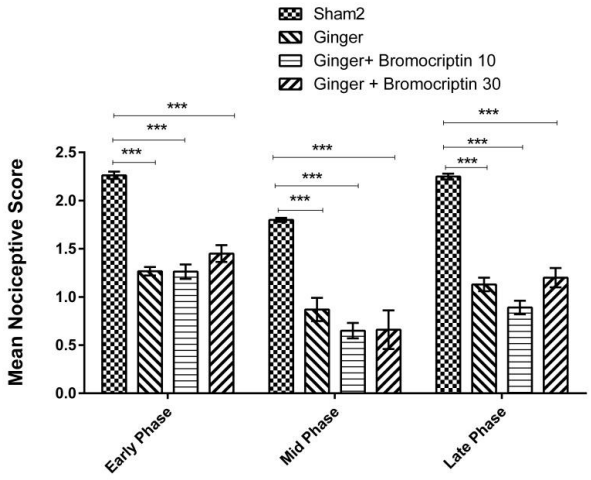

Figure 3. Effects of ginger (50mg/kg/day) + bromocriptine 10 and 30 µg/rat on the mean nociceptive score. The data were shown as Mean±SEM.
*** P<0.001 was considered as a significant difference compared to the sham 2 group.

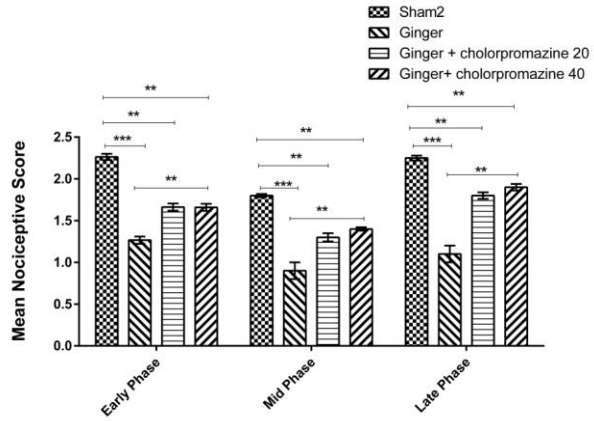

Figure 4. Effects of ginger (50mg/kg/day) + chlorpromazine 20 and 40 µg/rat on the mean nociceptive score. The data were shown as Mean±SEM. ** P<0.01 and *** P<0.001 was considered as a significant difference.

Formalin is a valid and reliable test in rodents. This test occurs principally in two phases. The early phase is short (~5 min) followed by a brief (~10 min) period of relative silence after which the late phase lasts an additional 45 minutes (12).

The present study showed that ginger lowered the mean nociceptive score in all phases of the formalin test compared to the control and sham groups. The two main components of ginger that are central to the prevention of oxidative stress and inflammation are 6-gingerol and 6-shogaol (13). Our findings were similar

to the analgesic effect of ginger, which has been reported by various researchers (14). Researchers have suggested that the analgesic effect of ginger may be done by reducing inflammation (15). Asami et al. indicated that 6-shogaol [1-(4-hydroxymethoxyphenyl) 4-decen-one], isolated from ginger, has several neurobiological and anti-inflammatory impacts (16). The other analgesic mechanism of ginger is through the dopaminergic system. Park et al. reported that 6-shogaol, a pungent compound isolated from ginger, protects dopaminergic neurons by inhibiting inflammatory pathways in Parkinson's disease (8).

The dopaminergic system can suppress the pain through the descending pathways (17). In this study, bromocriptine in both doses, following 15 days of the oral administration of ginger, had strong analgesic effects. Injection of D2 dopamine agonist into the isolated cortex and the nucleus accumbens significantly decreased the pain sensation in different models that were investigated (18). Fajrin et al. reported that 6-shogaol decreases TRPV1 and NMDAR2β expressions in the spinal cord, hence reduces pain symptoms in the diabetic neuropathy. Activation of dopamine D2 receptors could regulate the function and trafficking of NMDA receptors. Therefore, it seems that ginger can act as a dopamine D2 receptor agonist (19).

Microinjection of chlorpromazine by ICV at both doses of 20 and 40 µg/rat decreased nociceptor score compared to the control and sham 2. But ICV microinjection of chlorpromazine increased nociceptor score compared to the ginger and bromocriptine groups.

Injection of dopamine D2 receptor antagonist alone does not affect the nociceptor score in inflammatory pain tests (20). Chlorpromazine showed non-competitive inhibition kinetics, and it could affect TRPV1 by calmodulin-dependent manner and change the Ca^{++} channel (21). Therefore, in the groups receiving chlorpromazine + ginger, the ginger analgesic effects were individually and predominantly applied, and pain sensitivity was decreased in male rats compared to the control and sham groups. Nevertheless, chlorpromazine has shown hyperalgesia in rats compaed to ginger alone. Hence, in the present study, it seems that chlorpromazine had hyperalgesic effect, but ginger reduced the effects of chlorpromazine hyperalgesia.

Chlorpromazine has paradoxical effects according to the action on presynaptic or postsynaptic receptors. It induced hyperalgesia via the postsynaptic receptor and led to analgesia through the presynaptic receptor (22). In the present study, it seems that chlorpromazine acted through the postsynaptic receptors. However, the powerful analgesic effect of ginger reduced chlorpromazine hyperalgesia.

Conclusion

According to the results of this study pretreatment with the aqueous-alcoholic extract of ginger has a synergistic effect on bromocriptine. Pretreatment with the aqueous-alcoholic extract of ginger attenuates the hyperalgesic effect of chlorpromazine. Therefore, it seems that ginger acts as a dopamine D2 receptor agonist and mimics the effects of dopamine via the inhibition of TRPV1. Pretreatment with ginger diminished the hyperalgesic effect of the antagonist.

Acknowledgment

Every individual who contributed to this study was considered as an author.

Conflict of Interest

The authors declare that they have no conflict of interest.

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