Title: Effects of pretreatment with ginseng extract on dopamine D2 receptor analgesia

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Abstract

**Purpose of the study:** The ginseng extract is an herb that has been used for many purposes such as analgesic effect. Dopamine D2 receptors are involved in the regulation of pain in humans. Therefore, the aim of present investigation was to study how pretreatment with aqueous alcoholic extract of ginseng can affects dopamine D2 receptors pain sensitivity.

**Methods:** Forty-five adult male rat weighing 250±20 were used. Animal were maintained in standard condition temperature 21-24°C. Experimental groups were as follow: 1- Sham1 (intraperitoneal (IP) injection of normal saline); 2- Sham2 (intracerebroventricular (ICV) injection of artificial cerebrospinal fluid (ACSF)); 3- experimental 1(IP injection of ginseng extract) 4 and 5 experimental 2 and 3 (IP injection ginseng extract + bromocriptine 10 and 30 ug/rat by ICV injection) 6 and 7 experimental 4 and 5 (IP injection of ginseng extract + chlorpromazine 20 and 40 ug/rat by ICV injection). Ginseng extract 100 mg/kg/day were used for 7 days. Pain sensitivity test were done in all group by formalin test. Lateral ventricle was cannulated unilaterally by stereotaxic procedure.

**Results:** Our data showed that ginseng 100 mg/kg/day significantly (p<0.05) decreased pain sensitivity compared to sham1. Bromocriptine in two doses significantly decreased pain sensitivity compared to sham 2. Chlorpromazine in high doses significantly increased pain sensitivity compared to sham2.

**Conclusion:** The present results indicate that ginseng can modulate the D2 receptor of dopamine system in control of pain sensitivity in formalin test; because bromocriptine and ginseng had similar effect it seems that they had synergistic effect.

**Key Words:** ginseng, agonist D2, antagonist D2, formalin test
Introduction:
Pain is a physical and mental suffering that may arise by internal or external stimuli. Regulation of pain is a complex process that depends on interaction of many physiological, neurological and hormonal factors. By changing at level of chemical mediators, some environmental events reduce or increase pain sensitivity. These chemical mediators have great importance in pain relief (Garland, 2012).

Dopamine is one of the main neurotransmitters of the central nervous system and many neurological and psychiatric diseases are related to its secretion and functions (Dauer & Przedborski, 2003). It seems that analgesic effect of dopaminergic system is through endogenous opioid receptor (Volkow, 2010). These effects on pain processing are mainly done by striatal dopaminergic D2 receptor (Becker et al., 2013). In addition, it is reported that activation of dopamine D2 receptor through descending endogenous pain-control pathways is important (Dauer & Przedborski, 2003).

Ginseng is an herbal plant belonging to *panax* genus of the family *Araliaceae* (Rhim, Kim, Lee, Oh, & Nah, 2002). The roots of this plant contain a class of steroid glycosides called ginsenoside that is responsible for pharmacological activity of the plant (Chang, Seo, Gyllenhaal, & Block, 2003). The scientific names of ginsenoside are *Saponins tripterpenoid* or sometimes *panaxoside* in ginseng root (Sun, 2004). Ginseng has antioxidant, anti-inflammatory, anti-apoptotic and anti-aging properties. These effects include increased neuronal survival, growth and development of neuron and prevention from neuronal death (Rausch, Liu, Gille, & Radad, 2006). Kim and colleagues (2006) stated that ginseng saponins not only cause release of dopamine directly or indirectly through cholinergic system, but also they can directly affect dopamine D2 receptors (S. E. Kim, Shim, Chung, & Lee, 2006). On the other hand, in dopaminergic neuron in cultures ginsenosides have partial neurotrophic and neuroprotective effects. Therefore, they increase cell survival by decreasing the release of
lactate dehydrogenase and preventing mitochondrial membrane potential loss (Sandoval-Avila et al., 2018).

In spite that there are many investigations on dopamine D2 receptor and pain, on the other hand, relation between ginseng extract and pain was investigated; but the analgesic effect of these two factors simultaneously was not evaluated. Therefore, we aims to investigate the effect of dopamine D2 receptor agonist (bromocriptine) and antagonist (chlorpromazine) on pain sensitivity after ginseng extract 100 mg/kg/day administration for 7 days.

**Materials and Methods:**

A total number of 56 adult male Wistar rats weighing approximately 250±20 g in standard conditions (12-hour light/dark cycle at 22±2 °C) were used. Access to food and water were ad libitum.

The rats were randomly divided into 7 groups (n=8), as follows: Sham 1: Intact rats were received IP injection of normal saline. Sham 2: rats were unilaterally received artificial cerebrospinal fluid (ACSF) by intracerebroventricular (ICV) injection into the lateral ventricle.

Experimental 1: rats were received IP injection of ginseng extract. Experimental 2 and 3: rats were received IP injection of ginseng extract + unilateral ICV injection of bromocriptine 10 or 30 µg/rat. Experimental 4 and 5: rats were received IP injection of ginseng extract + unilateral ICV injection of chlorpromazine 20 or 40 µg/rat.

For 7 days IP injection of normal saline and ginseng extract in dose 100 mg/kg/day were done. Formalin test in sham1 and experiment 1 were performed 30 min after IP injection of normal saline or ginseng extract and in groups that received ICV injection drugs or ACSF; 30 min after ICV injection. Hydro-alcoholic extract of ginseng preparation was done according to reference (Palaniyandi, Suh, & Yang, 2017).

Formalin test: to evaluate the pain sensitivity, subcutaneously injection of 50 μL formalin solutions into the dorsal surface of the animal’s right hind paw (2.5% in normal saline) was
done. Every 15 seconds during 60 minutes the pain score was recorded. The score would be zero if the animal showed no reaction, (0); the score would be one if the animal did not rely on the injected paw; and the score would be two if the animal holds its paw up; and finally the score would be three if the rat licks and/or bites the injected paw. The results of every 15 seconds were averaged for every 5 minutes and for data analysis were considered.

Stereotaxic procedure: animals anesthetized with IP injection of ketamine 10% (100 mg/kg) and xylazine 2% (10 mg/kg). Rats fixed in the stereotaxic apparatus using blunt ear bars. The skull was carefully exposed and stainless steel guide cannula (23-gauge needle), were inserted unilaterally in the lateral ventricle. The coordinates for lateral ventricle according to bregma were 0.5 mm anterior and 1.5 mm lateral to midline and 3.5 mm below to cortex. The guide cannula via dental acrylic cement and two tiny stainless steel screws fixed to the skull. At the end, animals were given 7-days recovery period.

**Statistical analysis:** for data analysis, SPSS (version 21) used. The data analyzed by the one-way ANOVA, repeated measure ANOVA and post-hoc test was Tuckey. P value considered p< 0.05.

**Results:**

According to ANOVA measuring IP injection of ginseng 100 mg/kg/day for 7 days significantly reduced pain sensitivity in early phase \( [F(9)=61.2, p=0.0] \), intermediate phase \( [F(9)=16.8, p=0.0] \) and late phase \( [F(9)=92.8, p=0.0] \) of formalin test relative to the control and sham1 groups (fig1).

One way ANOVA represented that ICV injection of bromocriptine 10 µg/rat after IP injection of ginseng 100 mg/kg/day for 7 days significantly (P<0.0001) reduced pain sensitivity during 60 minutes of formalin test relative to control and sham1 groups. ICV injection of bromocriptine 30 µg/rat after IP injection of ginseng 100 mg/kg/day for 7 days had similar
effect except that in 25th and 45th minutes there were no difference relative to control and sham2 groups. Bromocriptine in two doses significantly decrease pain sensitivity in early phase \[ F(9) = 61.2, p=0.0 \], intermediate phase \[ F(9) = 16.8, p=0.0 \] and late phase \[ F(9) = 92.8, p=0.0 \] of formalin test relative to control and sham2 groups but does not have any difference with ginseng group (Fig 2).

Chlorpromazine 20 µg/rat had no significant effect on pain sensitivity relative to control and sham2 groups; while chlorpromazine 20 µg/rat significantly increased pain sensitivity in early phase \[ F(9) = 61.2, p=0.0 \], intermediate phase \[ F(9) = 16.8, p=0.0 \] and late phase \[ F(9) = 92.8, p=0.0 \] of formalin test relative to ginseng group (fig3). Chlorpromazine 40 µg/rat significantly increased pain sensitivity in early phase \[ F(9) = 61.2, p=0.0 \], intermediate phase \[ F(9) = 16.8, p=0.0 \] and late phase \[ F(9) = 92.8, p=0.0 \] of formalin test relative to control, sham2 and ginseng groups (fig3).

Fig1: Effect of ginseng 100 mg/kg/day on nociceptive scores (mean±SE).

* Difference between ginger group in comparison to control, sham 1 and sham2 groups

* P<0.05, ** P<0.01, *** P<0.001
Fig 2: Effect of bro (bromocriptine) 10 and 30 µg/rat on nociceptive scores (mean±SE) in rat pretreated by ginseng 100 mg/kg/day for 7 days.
* Significant difference between bro 10 and 30 µg/rat group in comparison to sham2 group
* P<0.05, ** P<0.01, *** P<0.001.

Fig 3: Effect of clo (chlorpromazine) 20 and 40 µg/rat on nociceptive scores (mean±SE) in rat pretreated by ginseng 100 mg/kg/day for 7 days.
* Significant difference between clo 20 and 40 µg/rat groups, in comparison to sham2 group;
# Significant difference between clo 20 and 40 µg/rat groups, in comparison to ginseng 100 mg/kg/day group.
Discussion:

Present study was shown that hydro-alcoholic extract of ginseng 100 mg/kg/day has analgesic effect in phase 1 and phase 2 of the formalin test. Previous studies revealed that ginseng saponins have analgesic effects in the Writhing test and in phase 2 of formalin test (Nabata, Saito, & Takagi, 1973; Shin et al., 1997).

A mechanism associated with analgesic action of ginseng by formalin test has been specified, yet. Nevertheless, it is likely that saponins of ginseng attach with nonopioid receptors at cell surface and regulate the voltage-gated calcium channels. In other words, the calcium channels are inhibited by ginseng saponins. Voltage-gated calcium channels play an important role in releasing pain neurotransmitters in pre-synaptic nerve endings of efferent neurons and blocking of it lead to decrease pain to some extent (S. Y. Nah & McCleskey, 1994).

The other analgesic mechanism of ginseng in the formalin test is that ginseng saponins or ginsenosides probably affect the dopaminergic activity of the central nervous system (Jun, Bae, Kim, Koo, & Kim, 2015). In addition to the postsynaptic effect of ginseng on voltage-gated channels, it may also affect presynaptic signal pathway in dopamine system and thereby can increase dopamine release from nucleus accumbens (NAc) as a result causes pain relief in the formalin test (Mancusoa & Santangelob, 2017; S.Y. Nah, Bhatia, Lyles, Ellinwood, & Lee, 2009).

Present investigation was shown that bromocriptine 10 and 30 µg/rat following to hydro-alcoholic extract of ginseng 100 mg/kg/day for 7 days had analgesic effect as great as ginseng alone in phases of formalin test. Therefore, it seems that ginseng extract may be applies its analgesic effects through dopaminergic system receptors same as dopamine. After injection of selective dopamine agonist in nucleus accumbens core it exert analgesic effects of
dopaminergic drugs in the formalin test (Faramarzi, Zendehdel, & Haghparast, 2016). Haghparast and colleagues (2012) were suggested dopamine D2 receptors that located in the nucleus accumbens have an important role in the adjustment of acute and chronic inflammation pain in the formalin test (Haghparast, Ghalandari-Shamami, & Hassanpour-Ezatti, 2012). Lintas and colleagues (2011), were reported that bilateral microinjection of dopamine D2 receptor agonist in the nucleus accumbens leads to inhibition of chronic phase caused by formalin test (Lintas et al., 2011).

There are several mechanisms for analgesic effects of bromocriptine; one of them pointed that it prevents nitric oxide (NO) release by affecting α2-adrenergic receptors (Beck et al., 2004). Some investigation suggested that NO is a modulator of the nervous system in many activities. One of the NO roles has taken into pain consideration, because following nerve damages in the affected area, an increase in the level of NO was occurred (Cury, Picolo, Gutierrez, & Ferreira, 2011; Levy & Zochodne, 2004). Moreover, production of NO, which increases following the formalin test, is inhibited by flavonoids and phenolic compounds of ginseng; thereby a decrease in NO leads to analgesic activity (Jang et al., 2016; Y. O. Kim et al., 2015). Therefore, ginseng extract and bromocriptine synergistically can reduce the NO production.

Current study was shown that chlorpromazine in two doses following to IP injection of ginseng 100/mg/kg/day had hyperalgesic effect relative to ginseng group; while hyperalgesic effect of it was in dose 40 µg/rat but not in dose 20 µg/rat in comparison to control and sham2 groups. Various studies showed that Raclopride (dopamine D2 antagonist) causes hyperalgesia in rat and pet animals (DaSilva et al., 2017; Dias et al., 2015). Prescription of dopamine antagonist can inhibit the analgesic effect caused by release of dopamine (Yazdi-Ravandi, Razavi, Haghparast, & Goudarzvand, 2014). Selective prescription of dopamine D2 receptor antagonist systematically reduces analgesic effect caused by amphetamines, morphine and cocaine in the formalin test (Pelissier, Laurido, Hernandez, Constandil, & Eschalier, 2006). Quinpirole as a
dopamine D2-like receptor agonist reduces pain in both phases of the formalin test and Sulpiride as an antagonist can potentially reverse analgesic effects observed by this agonist (Shamsizadeh, Pahlevani, Haghparast, Moslehi, & Zarepour, 2013). These results were supported our findings.

According to the present results: 1- ginseng 100 mg/kg/day for 7 days had analgesic effect. 2- Bromocriptine in two doses after pretreatment with ginseng 100 mg/kg/day had analgesic effect the same as ginseng alone. Therefore, it seems that bromocriptine and ginseng had synergistic but not additive effect. 3- Chlorpromazine in two doses after pretreatment with ginseng 100 mg/kg/day had hyperalgesic effect relative to ginseng alone. Therefore, hyperalgesic effect of chlorpromazine is more potent than analgesic effect of ginseng.

Ethical Considerations:

Compliance with ethical guidelines? All animal procedures performed according to the Institutional Research Ethics Committee of the School of Veterinary Medicine of Shiraz University.

Conflict of interest? All the authors confirm that, there is no financial or other relationship, which could cause a conflict of interest.

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