Original Research *SpatulaDD.2015;5(3):177-182*

The antinociceptive effect of *panax ginseng* in colorectal distension-induced visceral pain model

Panax ginseng ekstraktının kolorektal distansiyonla indüklenen viseral ağrıda antinosiseptif etkisi

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ABSTRACT

Aim: Ginseng is a traditional plant, a member of Panax class of Araliacae family. Its analgesic effect was shown, however its analgesic effect have not been studied yet in colorectal distension-induced visceral pain model. We researched the analgesic effect of Panax ginseng extract (GNS) and opioidergic interaction on its potential analgesic effect.

Methods:. Experiments were performed on 54 male Sprague-Dawley rats weighing about 250–300 g and were divided into 9 groups on the condition that ginseng extract 25, 50, and 100 mg/kg, morphine 0.5 and 5 mg/kg, naloxon 1mg/kg+ginseng 100 mg/kg, morphine 0.5 mg/kg+ginseng 25 mg/kg, morphine 5 mg/kg+ginseng 100 mg/kg and saline were given to the groups by intraperitoneal route. Enamelled nichrome electrodes were embedded into external oblique muscles of rats. After recovery period, visceral pain was constituted by colorectal distetion (CRD) aparatus situated on rectum and descending colon of each rat on experiment day. Visceromotor response, which was originated from contraction of the muscles, were evaluated electromyographically before and 10, 30, 60, 90, 120 min after drugs administration.

Results: Statistically, analgesia was observed only at a dose of GNS 100 mg/kg i.p. ($p<0,05$). Naloxone didn't affect the analgesic effect of GNS at a dose of 100 mg/kg ($p>0,05$). For the combination groups; morphine 5mg/kg+ GNS 100 mg/kg and morphine 0.5 mg/kg+ GNS 25 mg/kg, neither synergism nor antagonism was observed.

Conclusion: Our results is proved that GNS has an analgesic effect on visceral pain and this analgesic effect is not reversed by naloxone.

Keywords: *Panax ginseng*; Opioidergic receptors; Colorectal distension; Visceral pain.

ÖZET

Amaç: Ginseng Araliacae familyasından Panax sınıfına mensup geleneksel bir bitkidir. Analjezik etkisi gösterilmiştir ancak kolorektal distansiyonla indüklenen viseral ağrı modelinde analjezik etkisi henüz çalışılmamıştır. Biz Panax ginseng ekstraktının (GNS) analjezik etkisini ve bu etkide opioiderjik sistemin rolünü araştırdık.

Yöntemler: Toplamda 54 erkek, Spraque-Dawley cinsi, 250-300 g ağırlındaki sıçanlar 9 'ar grup olacak şekilde; ginseng eksraktı 25, 50, ve 100 mg/kg, morfin 0.5 ve 5 mg/kg, nalokson 1mg/kg+ginseng 100mg/kg, morfin 0.5 mg/kg+ginseng 25 mg/kg, morfin 5 mg/kg+ginseng 100 mg/kg ve salin grubu olarak ayrıldı ve bütün ilaçlar intraperitoneal yoldan verildi. Emaye kaplı nikrom elektrotlar sıçanların sağ ya da sol eksternal kaslarına implante edildi. İyileşme periyodundan sonra, deney günü viseral ağrı sıçanların kolorektal bölgesine yerleştirilen bir aparatın şişirilmesi ile oluşturuldu. Viseromotor cevap, abdominal eksternal kaslarının kasılmasından kaynaklanmaktadır ve ilaçlar verilmeden 10, 30, 60, 90 ve 120 dakika önce alındı.

Bulgular: Panax ginseng 100mg/kg da anlamlı analjezik etki gösterdi ve bu etki nalokson ile geri dönmedi. Morfin 0.5mg/kg+ginseng 25 mg/kg, morfin 5 mg/kg+ginseng 100 mg/kg kombinasyon gruplarında ne sinerjizma ne de antagonizma gözlendi.

Sonuç: GNS viseral ağrıda analjezik etkilidir ve bu etki nalokson ile geri döndürülmemektedir.

Anahtar kelimeler: Panax ginseng; Opioiderjik reseptörler; Kolorektal distansiyon; Viseral ağrı.

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Received October 06, 2015 ; **Accepted** November 13, 2015
DOI 10.5455/spatula.20151118060835
Published online in ScopeMed (www.scopemed.org).
Spatula DD..

INTRODUCTION

Panax ginseng (Asian ginseng, GNS) has been a traditionally used herbal medicine in eastern Asia for many years [1]. So far, more than 30 different types of ginsenosides that mediate the main therapeutic effect of ginseng have been defined [2-4]. It has been shown that GNS extract has an antinociceptive effect in somatic and visceral pain models such as the tail flick [5], hot plate, and acetic acid abdominal constriction tests [6] according to their ginsenoside contents. Despite these findings, the net antinociceptive mechanism of GNS is not sufficiently clear. In some studies, this has been associated with the inhibition of Ca2+ channels [7,8]. Moreover, the antinociceptive mechanism of ginseng extract and its interaction with the opioidergic system has been researched; the antinociceptive effect of different types of ginsenosides in writhing and formalin tests, for instance, has been shown not to be inhibited by naloxone, an opioid antagonist [9]. Furthermore, Vietnamese ginseng has been shown to decrease morphine-induced antinociception [10], while it has been found that ginsenoside Rc blocks B-endorphin-induced antinociception [11]. These studies show that the antinociceptive interaction of GNS may also depend on the type of extract and its ingredients.

Visceral pain is a very serious condition for patients, and research has focused on developing a remedy. Because of its poor localization and characterization, visceral pain differs from somatic pain, which arises from relatively superficial organs such as the skin and muscle [12,13]. Although in previous studies, the acetic acid constriction test (writhing test) was used as a visceral pain model, this model has some limitations. Here, acetic acid is administered to the peritoneum; however, the peritoneum also receives somatic innervation [14]. Thus, a different visceral pain model, namely the colorectal distension (CRD)-induced model, has been used as an alternative method. Here, distension of the colon causes a noxious stimulus, and the visceromotor response (VMR) is seen as a noxious response in the external abdominal muscles [15,16].

In this study, we aimed to elucidate the possible antinociceptive effect of GNS in the visceral pain model induced by CRD in rats and to assess the involvement of the opioidergic system in the possible antinociceptive effect of GNS.

MATERIALS AND METHODS

Animals

Ninety 3- to 4-month-old male Sprague Dawley rats weighing 250 to 300 g were used. These rats were kept in cages under controlled temperature (22±1°C) and humidity (55±10%). The room had a 12-h light/dark cycle from 7:00 a.m. to 7:00 p.m. Food and tap water were available ad libitum. After the surgical procedure, one rat was housed per cage to avoid surgical stress and wound formation. All animals were obtained from the Ondokuz Mayıs University Laboratory Animals Research and Application Center, ethic decision was approved by the Institutional Animal Care and Use Committee of the Ondokuz Mayis University (in 01/04/2015) and the protocols adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain. After the approving of the ethical decision, the study was performed in the next five months.

Table 1. Summary of the effects produced by drugs alone on the area under the curve (AUC) of the time–response function of VMR to noxious CRD (see Materials and methods).

Drug (dose)	AUC (means±S.E.M)
Saline (0.1 ml/100gr)	-26±57
Ginseng (25mg/kg)	-41±65
Ginseng (50mg/kg)	164±197
Ginseng (100mg/kg)	727±167
Morphine (0.5 mg/kg)	278±94
Morphine (5 mg/kg)	1662±342
Morphine (0.5 mg/kg)+ Ginseng (25mg/kg)	397±179
Morphine (5 mg/kg)+ Ginseng (100mg/kg)	1668±340
Naloxone (1mg/kg)+ Morphine (5 mg/kg)	-39±52
Naloxone (1mg/kg)+ Ginseng (100mg/kg)	702±131

Surgical Procedures

Under ketamine (100 mg/kg) and chlorpromazine (0.75 mg/kg intraperitoneally [i.p.]) anesthesia, enameled nichrome electrodes (diameter: 80 μm; Driver-Harris, Cedex, France) were implanted in the left external oblique muscles just above the inguinal ligament. To obtain electromyographic (EMG) activity records, the rats undergoing surgery were adapted in Bollman cages to reduce restraint stress and motion artifacts related to EMG activity [17].

Experimental Protocol

CRD values were assessed as an index of visceral nociception in rats [15-17]. To generate a certain distension into the colorectal region of the rats, first, a colorectal apparatus was designed from a flexible Tygon plastic tube inserted into a 6-cm latex balloon tied carefully to the tube. Thus, while one end of the tube was covered with the balloon, the other end was open to allow inflation by the injector. Then, the balloon was lubricated with ultrasound gel and inserted through the descending colon; thus, it was approximately 11–12 cm from the anus. To avoid slipping of the colorectal apparatus, the tube was taped to the base of the tail. Because the rats were

accustomed to the Bollman cages, they were awake during the experimental procedure. The colorectal apparatus was inflated with air to bring about CRD. The tube was attached to a bridge amplifier (ML221, AD Instruments, Australia) via a pressure transducer (MLT380, AD Instruments, Australia). The intracolonic pressure was monitored and recorded by a data acquisition system (ML870/P, 128 PowerLab 8/30, AD Instruments, Australia) connected to the bridge amplifier.

The VMR arising from the contraction of the external oblique musculature was quantified based on the EMG activity recorded by electrodes implanted in the external oblique musculature [15]. The EMG signal was amplified using a Bio Amp (ML132, AD Instruments, Australia) connected to the data acquisition system and integrated offline using the Chart program (version 5.2). Distension was given once (single distension) at 80 mmHg for 20 seconds. The EMG values were analyzed as digital numbers. The application of single distension was repeated 10, 30, 60, 90, and 120min after the administration of drugs or saline.

Drugs

All drugs were freshly prepared daily, dissolved in saline, and administered via the i.p. route at a volume of 0.5ml. Nanjing Zelang Medical Technology (Jiangsu, Nanjing, China) offered a commercial sample of ginseng, specifically extract from the root of dried GNS (batch number GN21106-GIN; high-performance liquid chromatography findings according to the report of company: Re, 9.2%; Rb1, 19.8%; Rb 2,9.7%; Rb 3,2.4%; Rc, 13.5%; Rd, 8.0%). *Panax ginseng* extract was given at doses of 5, 25, and 100 mg/kg i.p. Morphine hydrochloride (Galen Co., Turkey) was given at doses of 5 and 0.5 mg/kg i.p. and naloxone hydrochloride (St. Louis, MO, USA) was given at a dose of 1mg/kg i.p. 10 min before GNS administration.

Statistical Analysis

All data were expressed as the mean ± standard error of the mean. The VMR induced by CRD is represented as percentage of control (% control); pretreatment response to 80 mmHg is defined as 100%. The overall effect of any treatment was determined by taking the area under the curve (AUC) of the time-response function with the Excel computer program. The AUC was calculated from the time plot of post drug responses normalized to the baseline response (100%), plotted against time using the trapezoidal rule (AUC= Σresponse X 120 min).

All analyses were performed on SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). After confirmation of normal distribution of AUC data, one-way analysis of variance with the Duncan post hoc test was used for multiple comparison. Values of $p \leq 0.05$ were considered statistically significant.

RESULTS

The Antinociceptive Effect of GNS and Morphine

While *Panax ginseng* at a dose of 25 and 50 mg/kg,i.p. did not exhibit any significant antinociceptive effect, a significant reduction of VMR to CRD at a dose of 100 g/kg, i.p. was observed (Figure 1A and 1B, p=0.049). The antinociceptive effect of GNS was maintained for about 60 min (Figure 1). Morphine, as a positive control, exerted a potent antinociceptive effect at 5 mg/kg i.p. (Figure 2, $p<0.05$) but had no effect at 0.5 mg/kg i.p. (Figure 3, $p>0.05$). Naloxone at 1mg/kg, i.p. significantly reversed the antinociceptive effect of 5mg/kg i.p. of morphine (Figure 3, $p<0.05$).

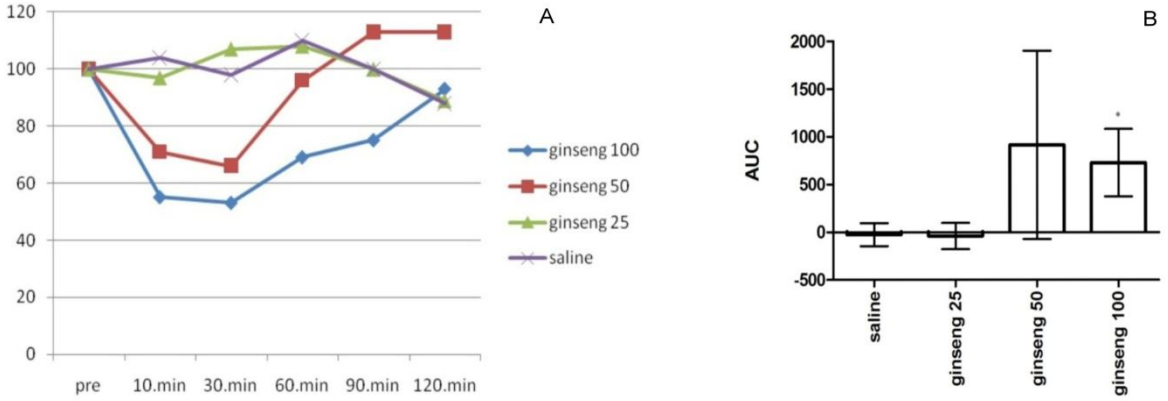

Figure 1A and 1B: Effects of GNS (25–100 mg/kg, i.p.) on the visceromotor response (VMR) to 80 mm Hg CRD. **A**: Administration of GNS dose-dependently attenuated the VMR to noxious CRD. VMRs were recorded 10, 30, 60, 90 and 120 min after GNS administration. VMRs are represented as percentages of the control (% control), and the baseline response prior to the administration of the drugs is defined as 100%. **B**: Data presents as areas under the curve (AUC) (see Materials and methods). *p< 0.05 compared to the saline-administered control group.

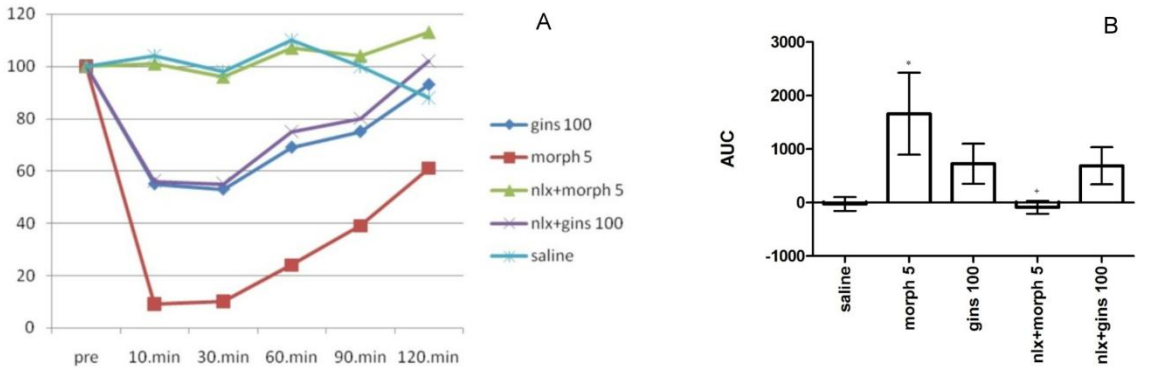

Figure 2A and 2B: Effects of naloxon (1mg/kg, i.p.) on the significant antinociceptive dose of GNS (100 mg/kg, i.p) and on the antinociceptive dose of morphine (5 mg/kg, i.p), on the visceromotor response (VMR) to 80 mm Hg CRD. **A**: Administration of naloxone significantly attenuated the antinociceptive effect of morphine but not GNS. VMRs were recorded 10, 30, 60, 90 and 120 min after naloxone administration. VMRs are represented as percentages of the control (% control), and the baseline response prior to the administration of the drugs is defined as 100%. **B**: Data presents as areas under the curve (AUC) (see Materials and methods). *p<0.05 compared to the saline-administered control group.+ p< 0.05 compared to the morphine-administered group.

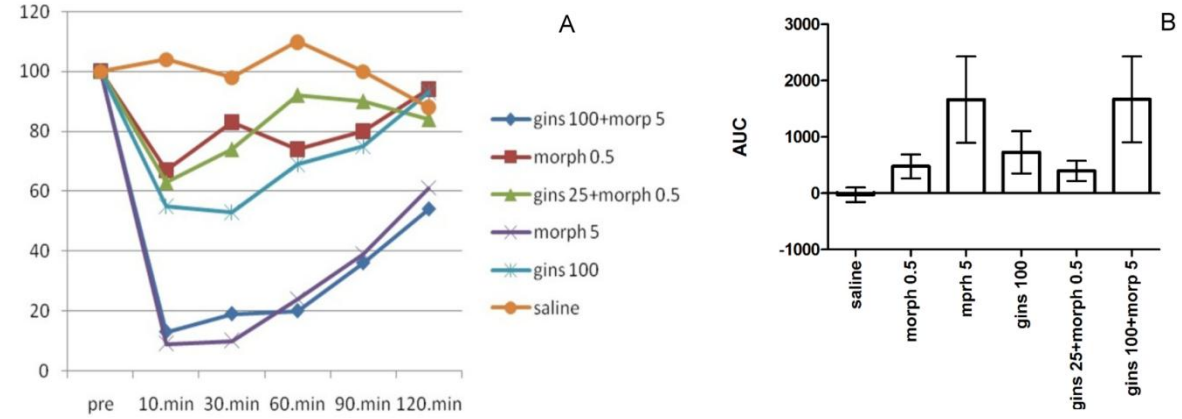

Figure 3A and 3B: Effects combination groups of morphine and GNS on the visceromotor response (VMR) to 80 mm Hg CRD. **A**: Combination of the uneffective doses of GNS (25 mg/kg,i.p) and morphine (0.5mg/kg,i.p), and combination of the effective doses of GNS (100mg/kg,i.p) and (morphine 5mg/kg,i.p) didn't exert any enhancement in comparation to GNS 25 and 100 mg/kg,i.p alone doses. VMRs were recorded 10, 30, 60, 90 and 120 min after combination drug administration. VMRs are represented as percentages of the control (% control), and the baseline response prior to the administration of the drugs is defined as 100%. **B**: Data presents as areas under the curve (AUC) (see Materials and methods). There are no statistically signification between the combination groups of morphine sulphate and GNS.

Effect of Naloxone on the Antinociceptive Effect of GNS

Administration of i.p. naloxone (1mg/kg) had no effect on the antinociceptive effect of GNS (100 mg/kg; Figure 3, p>0.05). The i.p. administration of naloxone alone did not affect any nociceptive or antinociceptive effect (data not shown).

The Effect of Combined Treatment with Morphine and GNS

In the combination of uneffective doses; GNS 25 mg/kg+ morphine 0.5 mg/kg and in the combination of effective doses; GNS 100 mg/kg+morphine 5 mg/kg, there was no statistically significant synergism or antagonism (Figure 2, p>0.05).

DISCUSSION

The data demonstrated that GNS administered i.p. had an antinociceptive effect by reducing the VMR to CRD, and this antinociceptive effect was not reversed by naloxone. In addition, no interaction was observed in the combination of GNS and morphine at high or low doses. According to our results, the

antinociceptive effect of high-dose GNS exerted a clear antinociceptive effect, although this did not reach the effectiveness of the morphine done.

So far, nearly 150 types of ginsenosides have been identified [18]. It has been established that GNS has a clear antinociceptive effect in certain pain tests [4,5,9]. Regarding the antinociceptive mechanism of GNS, some claims have been proposed according to the ingredient of different ginsenosides and acting pathways. For instance, the inhibition of substance-P induced pain with the modulation of calcium channels [4,8], and the inhibition of capsaicin-induced pain behavior [3] was also considered to represent the antinociception mechanisms. Furthermore, it has been shown clinically that GNS extract has a beneficial effect on patients with fibromyalgia [19]. Despite these findings, there are some caveats regarding the antinociceptive mechanisms of GNS extract, especially for visceral pain.

In our study, the antinociceptive effect of GNS was not reversed by naloxone. The dose of naloxone used in our study was able to decrease the antinociception induced by morphine administered 5mg/kg i.p., and this was found to be capable of reversing morphine-induced antinociception in most previous studies [17, 20]. Furthermore, the combination of morphine and GNS did not exert any synergism or additive effect in their effective and ineffective doses. This finding in our study is compatible with the results reported in the other studies. In the studies considering the antinociceptive mechanism of ginsenosides, it was found that different types of ginsengs, including GNS, either work in opposition to the opioidergic drugs or are not affected by them [2,5,6,7,9]. For instance, majonoside-R2, an ocotillol-type saponin found in Vietnamese ginseng, has been shown to reduce the μ receptor agonist and κ receptor agonist U-50488H-induced antinociception in mice [10]. Based on a similar study established by Kim et al., the antinociceptive effect of the opioidergic κ receptor agonist U-50488 was antagonized by ginseng total saponins in tail-flick and tail-pinch tests, and the mechanism of this effect was attributed to the serotonergic system [9]. Ramarao and Bhargava reported that morphine-induced analgesia was reversed by GNS extract [5]. Further, ginsenoside Rc has been shown to attenuate the antinociceptive effect of supraspinally administered B endorphin, an endogenous opioid [11]. In another study performed using the acetic acid–induced writhing test, the genus Pfaffia—known as "Brazilian ginseng"—exerted a clear antinociceptive effect in mice, but this effect was not reversed by naloxone [21]. These results clearly demonstrate that ginsenosides act oppositely or have no effect on the opioidergic system in some different somatic and visceral chemical pain models. Furthermore, regarding the opioidergic interaction for mechanism of GNS on morphine tolerance, several results have been reported, and in these studies it was well established that certain ginsenosides are effective in attenuating morphine tolerance in rodents [22, 23]. Thus, the lack of effect of naloxone on GNS-induced antinociception in our study and lack of additive effect of the combination doses of GNS and morphine show that there is no opioidergic interaction in GNS-induced antinociception.

According to the high-performance liquid chromatography report established by the supplier, Nanjing Zelang Medical Technology (Jiangsu, Nanjing, China), the ingredients of *P.ginseng* used in our study were as follows: Re, 9.2%; Rb1,19.8%; Rb 2,9.7%; Rb 3,2.4%; Rc, 13.5%, and Rd, 8.0%. In previous studies, the effect of active ingredients on the antinociceptive effect of ginsenosides were also reported. In some of these, Rc, Rd [9], and Re [9, 24] were shown to be effective in the acetic acid–induced writhing test. We did not study the antinociceptive effect of GNS according to these potential active ingredients, and this represents a limitation of our study. Furthermore, we think that the relatively moderate antinociceptive effect of the GNS used in our study may have arisen from the relatively low dose of active ingredients. Li et al. reported that ginsenoside Re exerted a clear antinociceptive effect in the writhing test at a dose of 1–12 mg/kg i.p. [24], however, Shin et al. reported that Re also exerted a significant antinociceptive effect in writhing test at a dose of 300 mg/kg i.p. in mice [9]. We applied a maximal dose of GNS of 100 mg/kg, i.p.; we could not enhance this dose because of the irritant side effects on rats used in our study. Thus, the antinociceptive effect of active ingredients of GNS may be elucidated in further studies.

In conclusion, the GNS used in our study exerted a moderate antinociceptive effect and its net effect was not enhanced in combination with morphine. Thus, because of the potential use of GNS in folk medicine, other mechanisms should be researched and new drugs may be combined with GNS for visceral pain therapy as its mechanisms become better understood.

CONFLICTS OF INTEREST

All contributing authors declare no conflicts of interest.

ACKNOWLEDGMENTS

This research was performed in the laboratories of Ondokuz Mayıs University, School of Medicine, Department of Pharmacology, Samsun/Turkey and all of the drugs and materials were supplied by the authors who participated in the study.

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