J. Ginseng Res.
Vol. 29, No. 2, 94-99 (2005)

Effects of Ginsenoside Total Saponins on Experimental Irritable Bowel Syndrome in Rats

Jong-Hoon Kim and Seung-Yeol Nah#

Research Laboratory for the Study of Ginseng Signal Transduction and Dept. of Physiology, College of Veterinary Medicine, Konkuk University, Seoul 143-701

(Received June 1, Accepted June 11, 2005)

Abstract : In the previous study, we reported that the *in vitro* inhibitory effect of ginsenosides, active ingredient of *Panax ginseng*, on 5-HT_{3A} receptor channel activity is coupled to *in vivo* anti-vomiting and anti-nausea effect. In the present study, we further investigated that the inhibitory effect of ginsenosides, active ingredient of *Panax ginseng*, on 5-HT3A receptor channel activity is also coupled to attenuation of irritable bowel syndrome (IBS), which is induced by colorectal distention (CRD) and 0.6% acetic acid treatment. The CRD-induced visceral pains induced by CRD and acetic acid treatment are measured by frequency of contractions of the external oblique muscle in conscious rats. Treatment of GTS significantly inhibited CRD-induced visceral pain with dose-dependent manner. The EC_{50} was 5.5 ± 4.7 mg/kg (95% confidence intervals: 1.2-15.7) and the antinociceptive effect of GTS on visceral pain was persistent for 4 h. We also compared the effects of protopanaxadiol (PD) ginsenosides and protopanaxatriol (PT) ginsenosides with saline on acetic acid- and CRD-induced visceral pain, and found that protopanaxatriol (PT) ginsenosides was much more potent than PD ginsenosides in attenuating CRD-induced visceral pain. These results indicate that PT ginsenosides of *Panax ginseng* are components for attenuation of experimentally CRD-induced visceral pains.

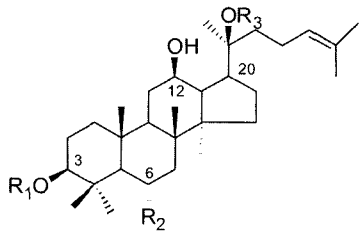
Key wards : Ginsenoside total saponins; 5-HT_3 receptor; IBS; Visceral nociception

INTRODUCTION

Irritable bowel syndrome is one of the most common gastrointestinal disorders seen in primary care and specialist practice. Visceral pain is the most common symptom experienced by patients suffering from IBS. Although the precise mechanism of IBS has not been fully investigated, it is believed that 5-hydroxytryptamine receptor$_3$ (5-HT_3) is involved in some symptoms of IBS. For example, Zacopride, a specific 5-HT_3-receptor antagonist, is effective in diarrhea-predominant IBS patients with abdominal pain and bowel discomfort.[1] However, 5-HT3 receptor antagonists demonstrate a wide heterogeneity of potency and efficacy against visceral pain. Granisetron[2] but not ondansetron[3] is able to reduce rectal sensitivity in patients with irritable bowel syndrome. Thus, 5-HT_3 receptors are related to the nociceptive processes of visceral pain. But the precise mechanisms by which 5-HT_3-receptor antagonists inhibit the visceral pain remain unclear, although among the 5-HT receptor subtypes, 5-HT_3 receptors have been implicated in visceral nociception pathways both in animals and humans[4]. Currently, the development of new 5-HT_3-receptor antagonists received many attentions related to pathologies involving abnormal visceral perception without side effects.

Ginseng, the root of *Panax ginseng* C.A. Meyer, is well known as a tonic for restoring and promoting human health. In traditional medicine, ginseng alone has been used for the alleviation of symptoms such as anorexia, dyspepsia, pain and vomiting[5]. The main molecular components responsible for the actions of ginseng are ginsenosides, which are also known as ginseng saponins. Ginsenosides have a four-ring, steroid-like structure with sugar moieties attached, and about 30 different forms have been isolated and identified from the root of *Panax ginseng*. They are classified into protopanaxadiol or protopanaxatriol ginsenosides according to the position of sugar moieties at carbon-3 or-6 (Fig. 1)[6]. Ginsenosides regulate several types of ligand-gated ion channel activity. In cells expressing nicotinic acetylcholine receptors, such as bovine chromaffin cells, protopanaxatriol (PT) rather

To whom correspondence should be addressed.
(Tel) +82-2-450-4154; (Fax) +82-2-450-3037
(E-mail) synah@konkuk.ac.kr



Ginsenosides	R_1	R_2	R_3
Rb_1	-Glc_2-Glc	-H	-Glc_6-Glc
Rb_2	-Glc_2-Glc	-H	-Glu_6-Ara(pyr)
Rc	-Glc_2-Glc	-H	-Glc_6-Ara(fur)
Rd	-Glc_2-Glc	-H	-Glc
Re	-H	-O-Glc_2-Rha	-Glc
Rf	-H	-O-Glc_2-Glc	-H
Rg_1	-H	-O-Glc	-Glc
Rg_2	-H	-O-Glc_2-Rha	-H
Rg_3	-Glc_2-Glc	-H	-H

Fig. 1. Structures of the nine representative ginsenosides. They differ at three side chains attached the common steroid ring. Abbreviations for carbohydrates are as follows: Glc, glucopyranoside; Ara (pyr), arabinopyranoside; Rha, rhamnopyranoside. Superscripts indicate the carbon in the glucose ring that links the two carbohydrates.

than proptopanaxadiol (PD) ginsenosides, especially ginsenoside Rf and Rg2, more potently inhibit acetylcholine-stimulated Na^+ influx[7]. More directly, Choi *et al.* (2002) and Sala *et al.* (2002) showed that PT ginsenosides more potently inhibit acetylcholine-induced inward current in *Xenopus* oocytes expressing several subtypes of neuronal muscle-type nicotinic acetylcholine receptors[8,9]. Lee *et al* (2005) and Jeong *et al* (2005) showed that PT ginsenosides and PT ginsenoside metabolite, M4 more potently inhibit 5-HT-mediated inward currents in *Xenopus* oocytes expressing 5-HT_3A receptors than PD ginseosides and ginsenoside metabolite, Compound K[10]. Interestingly, it is known that 5-HT_3A receptors existing in peripheral nervous systems such as intestines are involved in IBS. Thus, the regulations of 5-HT_3A receptor channel activity by ginsenosides suggest a possibility that ginsenosides also could attenuate IBS but until now it remains to be elucidated.

In the present study, we investigated *in vivo* study whether the inhibitory effects of ginsenosides on 5-HT_3A receptor channel activity are also coupled to alleviate visceral pains. For this, we induced a experimental visceral pain using colorectal distention (CRD) and 0.6% acetic acid treatment in rat[11] and investigated the effect of GTS on acetic acid- and CRD-induced visceral pain in rats.[12] We have also compared the effect of GTS with zacopride, a specific 5-HT_3 receptor antagonist, to provide evidences regarding its ability to modulate 5-HT receptor and alleviate visceral hypersensitivity[11]. We found that GTS reduced CRD-induced visceral pains with dose-dependent manner and that PT ginsenosides rather than PD ginsenosides was more potent in the inhibition of CRD-induced visceral pain.

2. MATERIALS AND METHODS

Materials

Ginseng total saponins (GTS) and PD and PT ginsenosides compounds were provided from the Korea Ginseng and Tobacco Research Institute (Korea). PD ginsenosides contained Rb_1 (34.2%), Rb_2 (18.1%), Rc (19.3%), Rd (16.5%), and Rg_3 (7.6%) and other minor PD ginsenosides. PT ginsenosides contained Re (38%), Rf (9.6%), Rg_1 (39.4%), Rg_2 (8.4%), and other minor PT ginsenosides. Fig. 1 shows the structures of the representative ginsenosids. The stock GTS solution was diluted with saline before use. Other chemical agents were obtained from Sigma (St. Louis, MO). GTS was given at doses ranging from 0.1 to 1000 mg/kg via s.c., zacopride (4-amino-N-(1-azabicyclo[2.2.2.]oct-3-yl)-5-chloro-2-methobenza-mide) at doses ranging from 0.1 to 300 μg/kg via p.o., and 100 mg/kg PD or 100 mg/kg PT ginsenosides. All drugs were dissolved in saline, adjusting pH 7.4, and administered in a volume of 1 ml/kg body weight.

Induction of colorectal distention (CRD) in rat

Male Sprague-Dawley rats (Orient, Korea) weighing 300 - 350 g were used. Prior to the experiments, they were housed communally at 22 ± 2°C and had free access to food and water. After an overnight fast, each animal was placed in a transparent plastic cage lined up with sawdust and allowed 45 min to get used to its surroundings. Procedures for the maintenance and use of the experimental animals were carried out in accordance with the guidelines of the International Association for the Study of Pain. The visceral stimulus employed in all experiments was a distension of the descending colon by inflation of a 5-cm-long latex balloon inserted via the anal route and kept in place by taping the polyethylene tube holding the balloon to the base of the tail, in order to ensure that the tip of the balloon remained 10cm from the anal verge. In all experiments, pressure within the balloon was continuously monitored by a pressure transducer (Bioblock, Illkirch, France) and care was taken to apply constant

pressure distension. Acetic acid (0.6%, 1.5 ml) was then injected intracolonically through a small catheter mounted along the balloon assembly. After 1 h, a first period of 30 mmHg distension was applied for 10 min (control period)[11]. This distension period was followed by oral administration of vehicle, GTS, PD or PT ginsenosides. After 20 min, a second period of distension (30 mmHg for 10 min) was again applied (treatment period). Pain was scored by visual counting of abdominal contractions over the two 10-min distension periods.

Data analysis

Results are expressed as means ± S.E.M. Statistical significance between control and treatment periods was assessed using the Wilcoxon test. Differences were considered statistically significant at $P < 0.05$. The antinociceptive effect of 5-HT_3 receptor antagonists was expressed by the following equation: % antinociception=100 × [1-(AC after treatment/AC before treatment)] (AC=cumulative abdominal contraction). The ED_{50} was calculated using the method of Litchfield and Wilcoxon[12].

3. RESULTS AND DISCUSSION

5-Hydroxytryptamine$_{3A}$ (5-HT_{3A}) receptor is not only involved in vomiting and nausea central nervous system but also related with the irritable bowel syndrome (IBS) in peripheral nervous system. We have shown that the inhibitory effect of ginsenosides, active ingredient of *Panax ginseng*, on 5-HT_3A receptor channel activity is coupled to anti-vomiting and anti-nausea effect. In the present study, we further investigated that the inhibitory effect of ginsenosides, active ingredient of *Panax ginseng*, on 5-HT_3A receptor channel activity is also coupled to attenuation of IBS for this, we utilized *in vivo* visceral pain animal model using rats and tested the effect of GTS on visceral pain. As shown in Fig. 2, we could observe a small number of abdominal contractions less than ten times after application of distension procedure (30 mmHg for 10 min) following intracolonic administration of saline. Thus, this procedure produced cumulative responses by 8.1 ± 2.4 abdominal contractions during the distension period. In contrast, the same procedure produced a dramatic increase in the pain score by 197.8 ± 8.3 for 10 min in abdominal contractions ($P < 0.01$, significantly different from saline alone treatment) 1 h after intracolonic administration of 0.6% acetic acid instead of saline. This response was stable and reproducible as shown by the number of abdominal contractions obtained during the second period of distension applied 20 min later (203.9 ± 5.3 abdominal contractions) (data not shown).

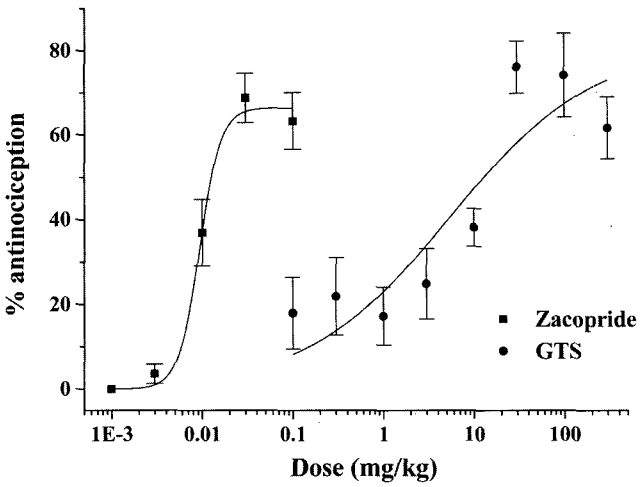


Fig. 2. Dose-response relationships of antinociceptive actions produced by oral administration of GTS (●) and subcutaneous injection zacpride (■) on abdominal contractions during colonic distension (30 mmHg, 10 min) in acetic acid- and CRD-induced visceral pain in rats. Results are expressed as means ± S.E.M of 11~12 animals per dose.

Next, we tested the antinociceptive effect of GTS in the range of 0.1 to 300 mg/kg. As shown in Fig. 2, GTS inhibited significantly the abdominal contractions with dose-dependent manner and with a sigmoidal dose-response curve. Percent antinociceptive effect of GTS was 17.9 ± 8.4, 21.9 ± 9.1, 17.2 ± 6.8, 24.9 ± 8.3, 38.1 ± 4.4, 76.1 ± 6.2, 74.2 ± 9.9 and 61.78 ± 7.34% at doses of 0.1, 0.3, 1, 3, 10, 30, 100 and 300 mg/kg, respectively but at dose of GTS (>300 mg/kg) the effect of GTS produced a lower degree of antinociception than 100 mg/kg (Fig. 2). The EC_{50} of GTS was 5.5 ± 4.7 mg/kg (95% confidence intervals: 1.2-15.7). Zacopride also significantly inhibited abdominal contractions with a sigmoidal dose-response curve. Percent antinociceptive effect of zacopride was 0.0, 3.6 ± 2.3, 36.9 ± 7.8, 68.8 ± 5.86, 63.4 ± 6.7 and 36.5 ± 7.6% at doses of 0.001, 0.003, 0.01, 0.03, 0.1 and 0.3 μg/kg, respectively. The EC_{50} of zacopride was 9.3 ± 0.7 μg/kg (95% confidence intervals: 3.7-19.7) (Fig. 2). This result using zacopride is well consistent with previous report[11]. Interestingly, in both groups, abdominal contractions totally disappeared at the end of distension period. Adiministration of GTS and zacopride significantly inhibited abdominal contractions induced by colonic distension, with a sigmoidal dose-response curve as also reported from several other studies with 5-HT_3 receptor

antagonists[13,14].

As a next step, we investigated time-course effect of GTS on CRD-induced visceral pain. As a previous method, after injection of saline (control), 30 mg/kg zacopride or 100 mg/kg GTS, a second period of distension (30 mmHg for 10 min) was again applied (treatment period) 5, 10, 20, 60, 120, 240 and 480 minutes later injection, respectively. Percent antinociceptions were 3.7 ± 4.2, 8.9 ± 5.3, 4.8 ± 4.7, 12.5 ± 3.9, 10.8 ± 9.1, 13.9 ± 5.8 and 15.9 ± 9.7% in saline treated control group, 34.4 ± 3.4, 65.4 ± 2.7, 68.8 ± 5.9, 60.2 ± 3.2, 55.4 ± 5.8, 19.1 ± 8.2 and 8.9 ± 4.7% in zacopride-treated animals, and 30.5 ± 2.6, 62.7 ± 4.9, 76.1 ± 6.2, 75.5 ± 5.0, 71.1 ± 2.8, 59.7 ± 8 and 19.9 ± 4.8% in GTS-treated group (n = 12-13, each group, respectively). Further increase in the time of GTS (> 240 min) produced a lower degree of antinociception (Fig. 3A). For 480 min, zacopride or GTS produced a statistically significant antinoceptive effect (44.6 ± 9.01% or 56.5 ± 8.5% of antinociception, respectively) in CRD-induced visceral pain compared with control group (10.1 ± 6.1%) (Fig. 3B).

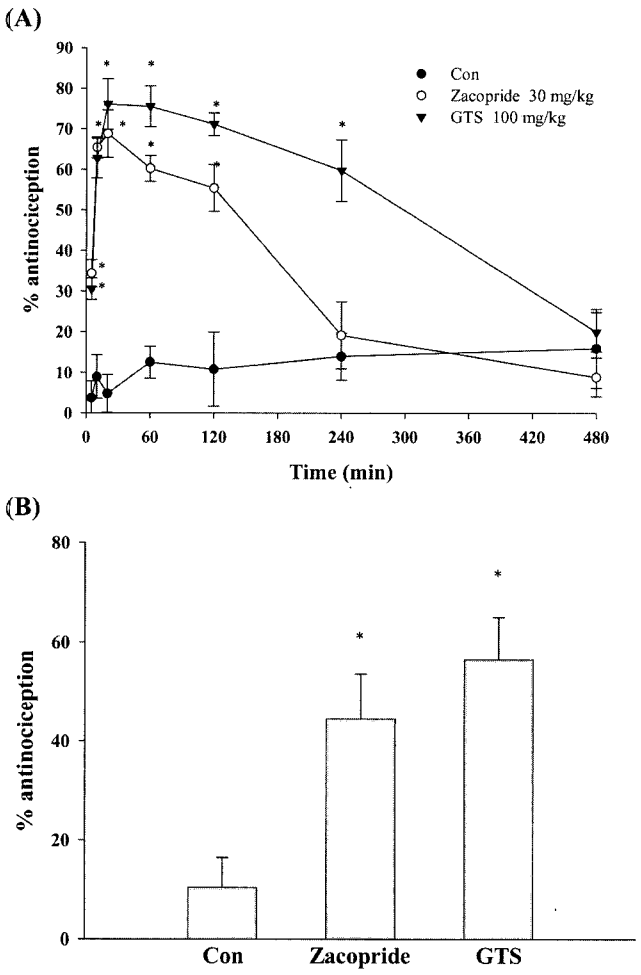


Fig. 3. **(A)** Time-response relationships of antinociceptive actions produced by GTS. GTS (100 mg/kg, p.o.) and zacopride (30 mg/kg, s.c.) were pre-admnistered, respectively, with the indicated time (5, 10, 20, 60, 120, 240 and 480 min before) before induction of acetic- and CRD-induced visceral pain. Percent antinociceptive effects of GTS and zacopride on acetic acid- and CRD-induced visceral pain were determined as described in Materials and Methods. GTS (▼)- and zacopride (○)-treated groups were significantly different from control saline-treated group. *$P < 0.001$, compared with GTS animals. (n = 11~12, per time). GTS (▼) and zacopride (○) group also exhibited significantly improved percent antinociceptions compared with control group (●).
(B) The histograms summarize time-dependent effect of GTS and zacopride for 8 h on acetic acid- and CRD-induced visceral pain. Data represent the mean ± S.E.M. *$p < 0.01$ compared with saline treatment alone, n = 11 - 12 animals in each data point.

We also investigated time-dependent effect of PD or PT ginsenosides on CRD-induced visceral pain. After intraperitoneal injection of saline (control), PD (100 mg/kg) or PT ginsenosides (100 mg/kg), a second period of distension (30 mmHg for 10 min) was again applied (treatment period) 5, 10, 20, 60, 120, 240 and 480 minutes later injection, respectively. Percent antinociceptions were 11.5 ± 3.1, 4.3 ± 2.7, 9.6 ± 5.3, 7.8 ± 3.9, 15.3 ± 4.8, 22.9 ± 7.1, and 12.0 ± 6.8% in saline treated control group, 5.8 ± 4.8, 6.5 ± 7.3, 5.0 ± 6.9, 10.3 ± 7.1, 26.5 ± 5.9, 28.7 ± 6.9 and 5.8 ± 8.8% in PD ginsenosides-treated animals, and 2.0 ± 3.1, 27.8 ± 9.1, 39.5 ± 6.2, 47.7 ± 8.8, 41.5 ± 7.4, 32.8 ± 5.4 and 3.2 ± 2.9% in PT ginsenosides-treated group (n=12-13, each group, respectively). Further increase in the time of GTS (>240 min) produced a lower degree of antinociception (Fig. 4A). Maximal antinociceptive times were 240 min (28.7 ± 6.9% of antinociception) and 60 min (47.7 ± 8.8% of antinociception) respectively, in PD and PT after injection (Fig. 4B). For 480 min, PD and PT produced significant antinoceptive effect in CRD-induced visceral pain. Average percentages of antinocicepton were 12.6 ± 3.9% and 27.7 ± 6.9%, respectively, in PD and PT-treated animals. In the other hand, average percentage of antinociception was 11.8 ± 2.3% in saline treated control group (Fig. 4B).

In the present study, we demonstrated that GTS and PT rather than PD ginsenosides attenuated CRD-induced visceral pain with dose-dependent manner in rats. These results indicate that the main components for the attenuation of CRD-induced visceral pain in GTS are derived from PT ginsenosides. These results are well consistent

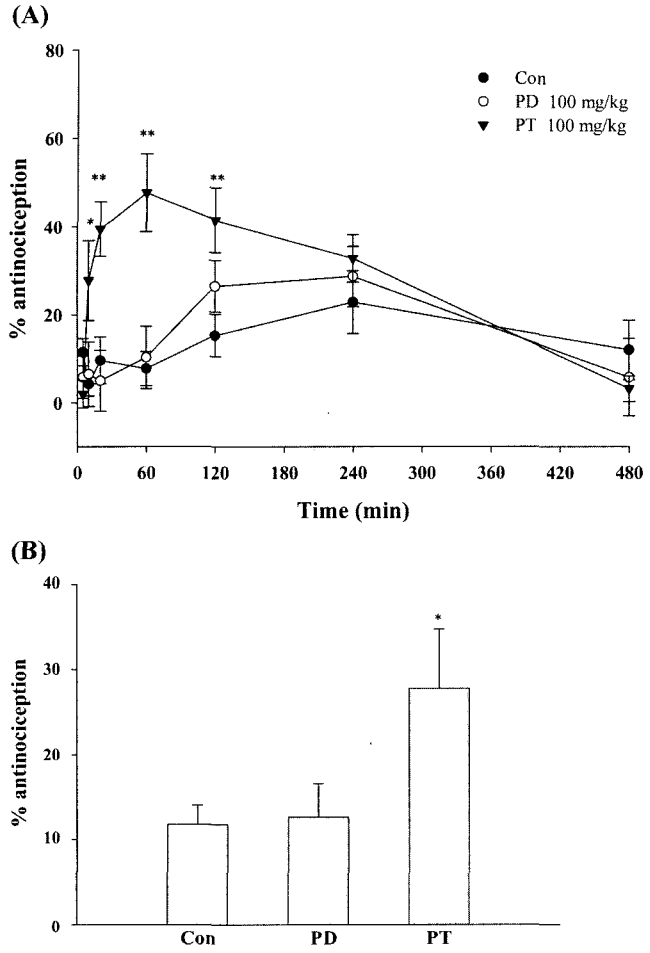


Fig. 4. (A) Time-response relationships of antinociceptive effect preduced by PT and PD. PT (100 mg/k, p.o.) saponins inhibit the visceral pain in rats, but PD (100 mg/kg, p.o.) show a relatively weak inhibition. PD and PT were orally pre-admnistered with the indicated time (5, 10, 20, 60, 120, 240 and 480 min before) before induction of acetic- and CRD-induced visceral pain. Maximal percent inhibition for PT was 47.7 ± 8.8% at 60 min pre-treatment and for PD 28.7 ± 6.85% at 240 min pre-treatment. PT (▼)- and PD (○)-treated groups were significantly different from control saline-treated group. $*P < 0.05$, $**P < 0.001$ compared with control animals. (n = 11~12, per time).
(B) The histograms summarize time-dependent effect of PD saponins and PT saponins for 8 h on CRD-induced visceral pain. Data represent the mean ± S.E.M. $*p<0.01$ compared with saline treatment alone, n = 11-12 animals in each data point.

with previous reports that PT rather than PD ginsenosides inhibit 5-HT-mediated currents in oocytes expressing 5-HT_3 receptors. As a positive control, we also used zacopride, a specific 5-HT_3 receptor antagonist, which blocked CRD-induced visceral pain. Interestingly, in comparison of potency for the inhibition of CRD-induced pain between GTS and zacopride, the EC_{50} of GTS values were 500 times less than that of zacopride. However, we could observe that GTS treatment maintained the antinociceptive effects more than 4 h, whereas zacopride-induced inhibition of CRD-induced visceral pain dramatically decreased after 2 h (Fig. 3A). Thus, it seems that GTS rather than zacopride has a long period of inhibition of CRD-induced visceral pain. Moreover, since GTS is natural component and not synthetic compound like zacopride and mild in its properties and does not much show side effects compared to other plant-derived saponins, the dosage in the range of EC_{50} might be useful for the attenuation of clinically observed IBS.

In conclusion, we demonstrated that GTS attenuates CRD-induced visceral pain with dose- and time-dependent manners in rats. These results show the possibility that GTS might be utilized as an antinociceptive agent attenuating CRD-induced visceral pain.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Science and Technology through the Bio-Food and Drug Research Center at Konkuk University, Chungju, Korea.

REFERENCES

1. Camilleri, M., Northcutt, A.R., Kong, S., Dukes, G.E., McSorley, D. and Mangel, A.W. : Efficacy and safety of alosetron in women with irritable bowel syndrome: a randomised, placebo-controlled trial. *Lancet.* **355**, 1035-1040 (2000).
2. Prior, A. and Read, N.W : Reduction of rectal sensitivity and post-prandial motility by granisetron, a 5-HT_3-receptor antagonist, in patients with irritable bowel syndrome. *Aliment. Pharmacol. Ther.* **7**, 175-180 (1993).
3. Hammer, J., Phillips, S.F., Talley, N.J. and Camilleri, M : Effect of 5-HT_3-antagonist (ondansetron) on rectal sensitivity and compliance in health and the irritable bowel syndrome. *Aliment. Pharmacol. Ther.* **7**, 543 (1993).
4. Talley, N.J : 5-hydroxytryptamine agonists and antagonists in the modulation of gastrointestinal motility and sensation: clinical implications. *Aliment. Pharmacol. Ther.* Review article **6**, 273 (1992).
5. Abe, T : Clinical studies of the vitamins. *Jpn. Soc. Inter. Med.* **54**, 989-1006 (1965).
6. Attele, A. S., Wu, J. A. and Yuan, C. S : Ginseng pharmacology: multiple constituents and multiple actions. *Biochem.*

Pharmacol. **58**, 1685-1693 (1999).

7. Kudo, K., Tachikawa, E., Kashimoto, T. and Takahashi, E : Properties of ginseng saponin inhibition of catecholamine secretion in bovine chromaffin cells. *Eur. J. Pharmacol.* **341**, 139-144 (1998).
8. Choi, S., Jung, S. Y., Lee, J. H., Sala, F., Criado, M., Mulet, J., Valor, L. M., Sala, S., Engel, A. G. and Nah, S. Y.: Effects of ginsenosides, active components of ginseng, on nicotinic acetylcholine receptors expressed in *Xenopus* oocytes. *Eur. J. Pharmacol.* **442**, 37-45 (2002).
9. Sala, F., Mulet, J., Choi, S., Jung, S. Y., Nah, S. Y., Rhim, H., Valor, L. M., Criado, M. and Sala, S : Effects of ginsenoside Rg2 on human neuronal nicotinic acetylcholine receptors. *J. Pharmacol. Exp. Ther.* **301**, 1052-1059 (2002).
10. Lee, B.H., Jeong, S.M., Lee, J.H., Kim, D.H., Kim, J.H., Kim, J.I., Shin, H.C., Lee, S.M. and Nah, S.Y : Differential effect of ginsenoside metabolites on the 5-HT_3A receptor-mediated ion current in *Xenopus* oocytes. Mol Cells. 29, **17**(1), 51-56 (2004).
11. Langlois, A., Pascaud, X., Junien, J.L., Dahl, S.G. and Riviere, P.J : Response heterogeneity of 5-HT_3 receptor antagonists in a rat visceral hypersensitivity model. *Eur J Pharmacol.* **318**, 141-144 (1996).
12. Litchfield, J.T., and Wilcoxon, F.: A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther.* 96-99 (1949).
13. Moss, H.E. and Sanger, G.J : The effects of granisetron, ICS 205-930 and ondansetron on the visceral pain reflex induced by duodenal distension. *Br. J. Pharmacol.* **100**, 497-501 (1990).
14. Banner, S.E. and Sanger, G.J : Differences between 5-HT_3 receptor antagonists in modulation of visceral hypersensitivity. *Br. J. Pharmacol.* **114**, 558-562 (1995).