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# Effects of Ginseng Leaf Saponins on the Development of Morphine Tolerance and Dependence in Mice

Hack-Seang Kim, Sun-Hye Kim, Myung-Koo Lee, Kang-Ju Choi* and Suk-Chang Kim*
*College of Pharmacy, Chungbuk National University, Cheongju 360-763, and *Korea Ginseng and Tobacco Research Institute, Taejon 302-345, Korea*
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**Abstract** □ The effects of orally administered ginseng leaf saponins(GLS) on the analgesic action of morphine, the development of morphine induced tolerance and physical dependence, and the hepatic glutathione contents in mice were investigated. GLS antagonized the analgesic action of morphine and inhibited the development of morphine induced tolerance and physical dependence. It also inhibited the decrease in hepatic glutathione level induced by multiple injections of morphine.

**Keywords** □ *Panax ginseng* C.A. Meyer, morphine, glutathione, ginseng leaf saponin

## Introduction

Morphine is a potent analgesic, but multiple administration of morphine produces physical dependence and tolerance to most of its effect. The problem of addiction to morphine has stimulated a continueing research for morphine type compounds which are free of addiction liability, and for long-acting and orally effective narcotic antagonists with minimum secondary effects. A folk medicine composed of seven herbal drugs including *Panax ginseng* has been used as antidote in the treatment of morphine tolerant and dependent patients[1]. Researchers have reported the analgesic and the hypothermic activities in ginseng extract and saponins.[2-4] It was reported that ginseng saponins antagonized morphine analgesia in mice and this action might be attributed to decrease in dopamine and serotonin levels.[5] It has been also reported that orally administered ginseng saponins(GS), protopanaxadiol saponins, protopanaxatriol saponins and ginseng ether fraction inhibit the development of morphine induced tolerance and physical dependence, and the hepatic glutathione level decrease induced by multiple injections of morphine.[6] In addition to root saponins (ginsenoside $Rb_1$, $Rb_2$, Rc, Rd, Re and $Rg_1$)[7] a number of new saponins (ginsenoside $F_1$,$F_2$,$F_3$,$F_{6a}$ and $F_{6bc}$) were isolated. The yields of alcohol extract and crude saponin from the leaves were higher than those of the roots.[8] However, there have been no reports about the effects of ginseng leaf saponins(GLS) in morphine treated mice yet despite of abundance of ginseng leaves.

The present study was undertaken to observe the inhibitory effects of orally administered GLS on the development of morphine induced tolerance and physical dependence, and also on the changes in the hepatic glutathione contents which are closely related to the degree of detoxication of morphinone, a novel metabolite of morphine.[9]

## Materials and Methods

White ICR male mice weighing 18-22g, in a group of 10-15, were used in all experiments. GLS (supplied from the Korea Ginseng and Tobacco Research Institute) dissolved in distilled water were administered to mice orally once a day 30 min prior to the injection of morphine. To induce morphine tolerance and dependence, morphine hydrochloride

(Dae-Won Pharm. Co.) 10 mg/kg was administered subcutaneously(s.c.) to mice once a day for 6 days by Kaneto's method.[10]

**Measurement of antagonism of morphine analgesia**

GLS 50, 100 and 200 mg/kg were administered orally daily for 6 days and the antagonistic effects of GLS against morphine 5 mg/kg (s.c.) were determined by the tail flick method[11] on the 7th day, 24 hrs after the final administration of GLS. The tail flick latencies to thermal stimulation were determined in sec prior to and at 30, 60 and 90 min after morphine injection. A value of 10 sec was used as a cut-off point to avoid damage to the tail. The analgesic response for each mouse was calculated by the following formula;

$$\text{Analgesia}(\%) = \frac{\text{Tt-To}}{\text{Tc-To}} \times 100$$

where To is base line or pre-morphine tail flick reaction time, Tt is the reaction time at t min after morphine injection and Tc is cut-off time. The base lines of tail flick latencies in different groups were around $2 \pm 0.2$ sec. The effects were calculated as area under the curve (AUC) that was obtained by plotting the analgesic percent on the ordinate and the time intervals(min) on the abscissa, and expressed as a percent of the effect obtained in control animals treated with morphine 5 mg/kg alone.

**Measurement of analgesic tolerance**

The inhibition of morphine tolerance development by administration of GLS was evidenced by the increase in analgesic response to morphine 5 mg/kg as analgesic percent, estimated at 0, 30, 60 and 90 min by the tail flick method[11] 24 hrs after the final injection of morphine and calculated as AUC by Kaneto's method.[10]

**Measurement of naloxone induced withdrawal**

The inhibition of naloxone induced withdrawal syndrome in morphine alone treated mice and in morphine-GLS treated mice was estimated by the decrease of withdrawal scores induced by naloxone 1 mg/kg (i.p.) for 30 min, 24 hrs after the final injection of morphine on the 7th day. The abstinence syndrome was quantified by placing the animals on a diaphanous circular platform, 35 cm in diameter and 70 cm in height. The withdrawal syndromes induced by naloxone were counted as follows; jumping and diarrhea 2 point; defecation, wetdog shake, writhing syndrome, rearing, grooming and ptosis 1 point by all or none response by the modified Tagashira and Dewey's method.[12]

**Measurement of hepatic glutathione levels**

The mice treated with morphine the test drugs for 6 days were sacrificed by decapitation on the 7th day, 24 hrs after the final injection of morphine. The liver was removed immediately and the glutathione concentration was determined by the method of Ellman.[13] The removed liver was homogenized in 4 volumes of 0.5 M ice-cold phosphate buffer, pH 7.4, to give a suspension equivalent to 250 mg/kg of wet liver. For estimation of reduced glutathione, an aliquot was deproteinized by addition of an equal volume of 4% trichloroacetic acid containing 1 mM Na-EDTA and centrifuged 2000 × g for 5 min. The supernatant 0.5 ml was added to 4.5 ml of 0.1 mM 5.5'-dithiobis(2-nitrobenzoic acid). After mixing for 20 min at room temperature, absorbance at 412 nm was recorded against a reagent blank. All the operation were carried out at 0-4 °C.

**Statistics**

The data were expressed as mean of changes ± S.E. The differences of the means for different responses in different groups were analyzed by the Student's *t*-test.

## Results

**Antagonism of morphine analgesia**

The analgesic effects of morphine showed 86% in GLS 50 mg/kg, 53% in GLS 100 mg/kg and 30% in GLS 200 mg/kg treated group, compared with that of the morphine control group (Fig. 1).

**Inhibition of analgesic tolerance development**

The analgesia of each group to morphine 5 mg/

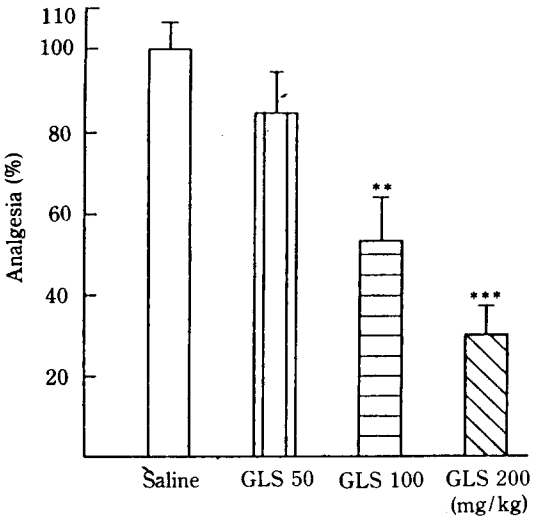


**Fig. 1.** Effect of ginseng leaf saponin (GLS) administered orally on the morphine analgesia. GLS 50, 100 and 200 mg/kg were administered orally 24 hrs for 6 days and the antagonistic effects of GLS against analgesic action of morphine 5 mg/kg s.c. were determined by the tail flick method on the 7th day 24 hrs after the final administration of GLS.
**: $p<0.01$, ***: $p<0.001$

kg calculated as the AUC showed 3.8 times in GLS 100 mg/kg and 6 times in GLS 200 mg/kg treated group, compared with that of morphine control group, but no significant differences were observed in GLS 50 mg/kg treated group (Fig. 2).

### Inhibition of withdrawal syndrome induced by naloxone

The inhibition degree of naloxone induced withdrawal scores were 20% in GLS 100 mg/kg and 28% in GLS 200 mg/kg treated group, but no significant inhibition was observed in GLS 50 mg/kg treated group (Fig. 3).

### Inhibition of the decrease of hepatic glutathione levels

The hepatic glutathione levels of the mice showed $3.84 \pm 0.14$ $\mu$mol/g of tissue in GLS 50 mg/kg, $4.04 \pm 0.10$ $\mu$mol/g in GLS 100 mg/kg and $4.20 \pm 0.11$ $\mu$mol/g in GLS 200 mg/kg treated group. However, the glutathione level in the morphine control group was decreased to $3.20 \pm 0.07$ $\mu$mol/g of

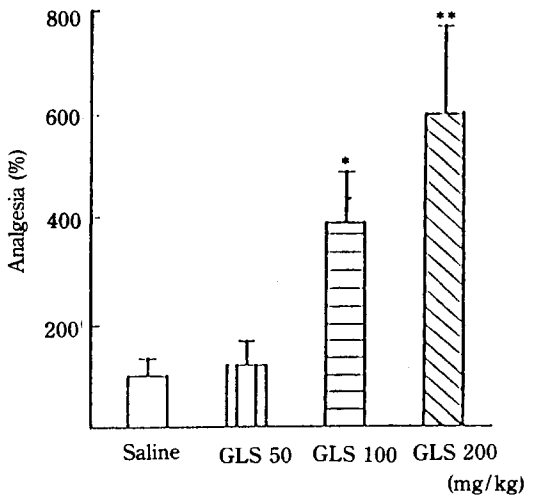


**Fig. 2.** Effects of GLS administered orally on tolerance to the analgesic action of morphine in mice. Morphine 10 mg/kg was injected into the mice every 24 hrs for 6 days. Saline or daily dose of 50 mg/kg, 100 mg/kg and 200 mg/kg of GLS were administered to the each group. The inhibition degree of tolerance development by GLS was evidenced by the increase in analgesic response to morphine 5 mg/kg s.c.
*: $p<0.05$, **: $p<0.01$

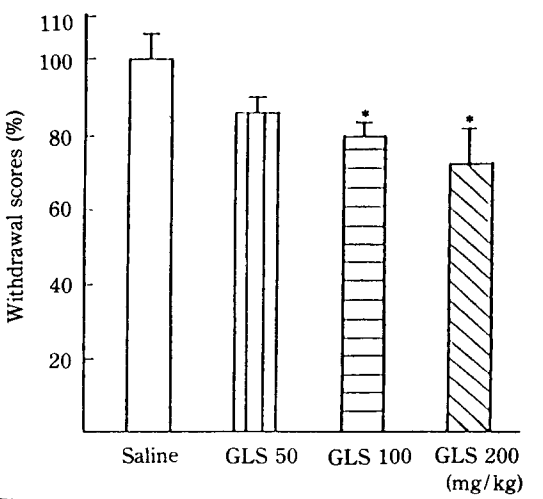


**Fig. 3.** Effects of GLS on the morphine withdrawal induced by naloxone. Morphine 10 mg/kg was injected into the mice every 24 hrs for 6 days. GLS 50, 100 and 200 mg/kg were administered orally 30 min prior to morphine injection. The withdrawal test was made 24 hrs after the final injection of morphine by challenging with naloxone 1 mg/kg i.p.
*: $p<0.05$

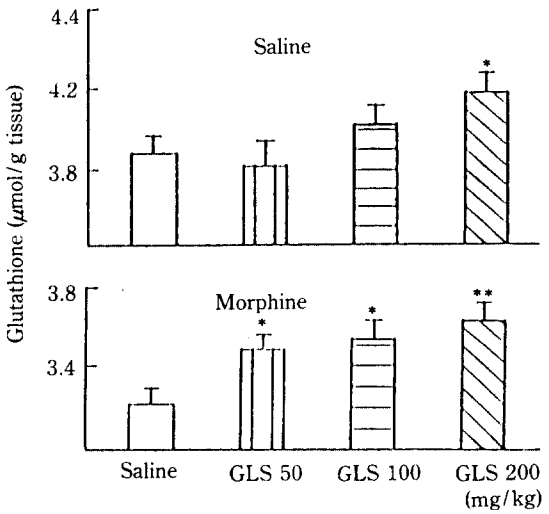


**Fig. 4.** Preventive effects of GLS on the morphine-induced decrease of heaptic glutathione level in mice. Morphine 10 mg/kg was injected into the mice every 24 hrs for 6 days. GLS 50, 100 and 200 mg/kg were administered orally into the mice once a day 30 min prior to the morphine injection for 6 days.
*: $p<0.05$, **: $p<0.01$

tissue compared with $3.89 \pm 0.08$ μ mol/g of tissue in saline group.

The glutathione levels of the mice treated with morphine and test drugs showed $3.84 \pm 0.09$ μ mol/g in GLS 50 mg/kg, $3.55 \pm 0.01$ μ mol/g in GLS 100 mg/kg and $3.64 \pm 0.08$ μ mol/g of tissue in GLS 200 mg/kg pretreated group. This preventive effect of GLS on the decrease of hepatic glutathione level was statistically significant compared with that of the saline control group (Fig. 4).

## Discussion

Kim *et al.* reported that GS, protopanaxadiol and protopanaxatriol antagonized morphine analgesia.[5] GS, protopanaxadiol, protopanaxatriol and ginseng ether fraction administered orally inhibited also the morphine induced tolerance and physical dependence, and the reaction of hepatic glutathione level in mice.[6] In this experiment, no major differences were observed between the effects of ginseng roots saponins and GLS as indicated above. It was suggested that GS might have reserpine- or tetrabenazine-like action and deplete catecholamines and serotonin levels.[14,15] Antagonism of morhine analgesia by GS was attributed to the reduction of catecholamines and serotonin levels in cerebral and peripheral nerve systems, and the newly equilibrated state of neurologic functions. On the other hand, L-DOPA and 5-HTP reduced this antagonism of the morphine analgesia.[16] In the liver of the mice, a portion of morphine was metabolized into morphinone, which is a novel metabolite of morphine and has 9 times toxicity of morphine based on $LD_{50}$ value (s.c. injection).[9]

Kim *et al.* reported that morphine 6-dehydrogenase which catalyzes morphinone production from morphine was inhibited by GS *in vitro*.[17] A portion of morphinone is detoxified into morphinone-glutathione conjugate, and source of morphinone is metabolized into morphinone-protein SH conjugate, which may be attributed to the development of morphine induced tolerance and physical dependence by covalent binding to the sulfhydryl group of opiate receptor[18] (Fig. 5).

The increase of hepatic glutathione level by GLS is comparable to Schole's report which shows the increase of hepatic glutathione in rats[19] by ginseng extract G115. The inhibition of the hepatic glutathione level decrease in morphine treated mice with GLS was accompanied by the inhibition of the development of morphine induced tolerance and physical dependence. These results are assumed to be caused by the dual action of GLS, the inhibition of morphinone production and the activation of morphinone-glutathione conjugation due to the increased glutathione level for detoxication. Takahasi and Kaneto proposed that the newly equilibrated state of adrenergic function as well as the inhibition of the development of morphine tolerance could be induced in mice treated daily with a small dose of reserpine.[20] Another possible explanation for the inhibition of morphine tolerance and physical dependence development by GLS might be the newly equilibrated state of neurologic function as suggested above.

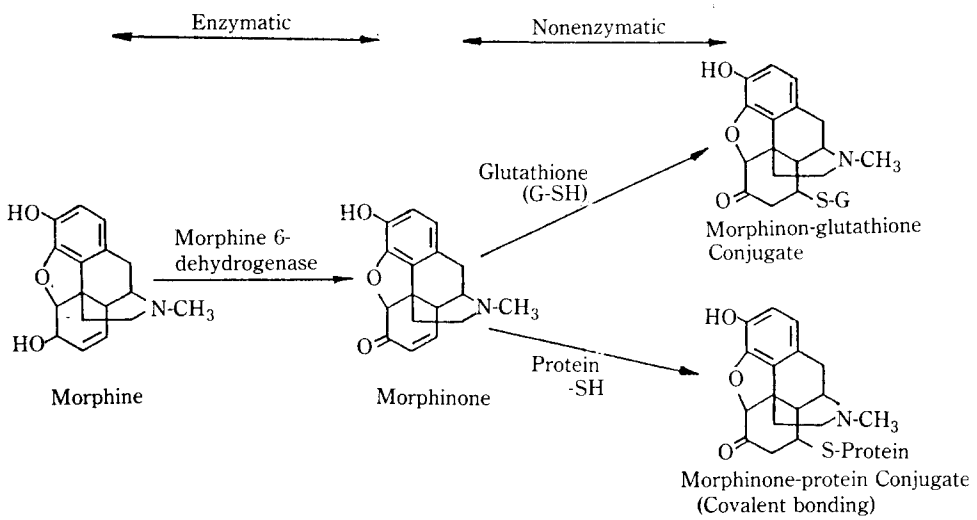


**Fig. 5.** Metabolism of morphine to morphinone and its conjugates.

The mechanism of inhibition of abstinence in morphine dependent and tolerant animals by GS remains unclear. Several neurotransmitters (Ach, dopamine and c-AMP) have been implicated in the abstinence syndrome. The expression of the abstinence syndrome is associated with an increase in brain dopamine level, [21] an increase in c-AMP level[22] and a decrease in brain Ach level.[23] Noradrenergic neurons in the mouse brain were more influenced by oral chronic treatment of *pannax ginseng* than the dopaminergic neurons.[24] The inhibition of morphine induced tolerance and physical dependence by GLS is closely related to the altered ratios of adrenaline, noradrenaline, dopamine and serotonin as well as the newly equilibrated state of neurologic function in mice. Considering these results, the inhibition of morphine induced tolerance and physical dependence by GLS was thought be the same as that of ginseng root saponins themselves even though less effective due to the difference of the components. It is proposed that GLS could be developed as a valuable natural resource for the treatment of morphine tolerant and dependent patients.

## References

1. Kim, Y.E., Such, H.S., Kim, W.K. and Kim, K.S.: *Seoul Nat. Univ. J.* **18,** 83 (1967).
2. Nabata, H., Saito, H. and Tagaki, K.: *J. Pharmacol. Exp. Ther.* **23,** 29 (1973).
3. Namba, T., Yoschizaki, M., Yomimori, T., Kobashi, K., Mitsui, K. and Hase, J.: *Yakugaku Zasshi* **94,** 252 (1974).
4. Kim, H.S. and Oh, S.K.: *Research Review of Chungbuk Nat. Univ.* **28,** 423 (1984).
5. Kim, H.S., Oh, S.K., Choi, K.J. and Lee, H.B.: *Yahak Hoeji* **29,** 188 (1985).
6. Kim, H.S., Oh, S.K., Park, W.K., Choi, J.W. and Bae, D.S.: *Arch. Pharm. Res.* **10,** 188 (1987).
7. Tanaka, O.: Proceedings of the 2nd international ginseng symposium, Korea Ginseng and Tobacco Research Institute, Seoul Korea, 145 (1978).
8. Kim, H.S.: Gun Kuk Univ. M.Sc. Thesis (1984).
9. Nagamatsu, K., Kido, Y., Terao, T., Ishida, T. and Toki, S.: *Drug Meta. Dispos.* **11,** 190 (1983).
10. Kaneto, H., Kosaka, N. and Hirota, N.: *Life Sci.* **31,** 2351 (1982).
11. D'Amour, F.E. and Smith, D.L.: *J. Pharmacol. Exp. Ther.* **72,** 74 (1942).
12. Tagashira, E. and Dewey, W.L.: The 16th symposium of drug metabolism, Pharmacology and Toxicology in Japan, p. 165 (1984).
13. Ellman, G.L.: *Arch. Biochem. Biophys.* **82,** 70 (1959).
14. Takagi, H. and Kuriki, H.: *Int. Neuropharmacol.* **8,** 195 (1969).

15. Takagi, H. and Nakama, M.: *Jap. J. Pharmacol.* **18**, 54 (1968).
16. Kim, H.S., Oh, S.K. Choi, K.J.: Proceeding of 2nd ROK-ROC symposium on natural sciences, Seoul Korea, p. 110 (1985).
17. Kim, H.S., Oh, K.W., Park, W.K., Toki, S. and Yamano, S.: *Kor. J. Ginseng Sci.* **11**, 182 (1987).
18. Nakamatsu, K., Kido, Y., Terao, T., Ishida, T. and Toki, S.: *Drug Meta. Dispos.* **11**, 190 (1983).
19. Schole, J.: Belastung Ernahrug and Resistenz-Forschritte in der Tierphysiologie and Tiererahrugn 9 ed. Paul. Parey, Hamburg/Berlin, p. 35 (1978).
20. Takahasi, M. and Kaneto, H.: The 5th Korea-Japan joint seminar on pharmacology in Cheju Korea, 1986.
21. Iwamoto, E.T., Ho, I.K. and Way, E.L.: *J. Pharmacol. Exp. Ther.* **187**, 558 (1973).
22. Collier, H.O.J. and Francies, D.L.: *Nature (London)* **255**, 159 (1975).
23. Bhargava, H.N. and Way, E.L.: *J. Pharmacol. Exp. Ther.* **194**, 65 (1975).
24. Kim, Y.C., Lee, J.H., Kim, M.S. and Lee, N.C.: *Arch. Pharm. Res.* **8**, 45 (1985).