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Effects of Ginseng Saponins on the Development and Loss of Morphine Tolerance and Dependence

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Abstract—Ginseng saponins(GS), protopanaxadiol saponins(PD) and protopanaxatriol saponins (PT) were tested for the inhibition of the development of morphine tolerance and dependence[1] antagonism of morphine analgesia[2] and the loss of morphine tolerance and dependence in mice[1] The results were as follows: 1. Inhibition of the development of morphine tolerance and dependence. 2. Antagonism of morphine analgesia. 3. Increase in the loss of morphine tolerance and dependence. Antagonism of morphine by ginseng saponins and its reversal by L-DOPA and 5-HTP suggest some possibility that catecholamines and serotonin levels might be associated with the results.

Keywords—Morphine · ginseng saponin · tolerance · dependence · analgesia

The analgesic action of morphine is so remarkable, but the repeat treated morphine produces physical dependence, characterized by withdrawal symptoms and tolerance develops to most of its effects, when used it continuously. The continuing search for morphine type compounds has failed to produce an analog that exhibits most of the useful properties of morphine, but which is devoid of addiction liabilitly. Similarly, long acting and orally effective narcotic antagonists with minimum secondary effects are being sought to treat narcotic addicts.

A folk medicine prescribed by seven herbal drugs including *Panax ginseng* has been used as antidotes in the treatment of morphine tolerant-dependent patients and its effective component is keratin of Manis squama without telling any effects of *Panax ginseng* on morphine tolerant-dependent patients[3]. But it has been reported that there are analgesic and hypothermic effects in ginseng extract and saponins[4,5] development of analgesic and hypothermic tolerance[6] inhibition of the development of morphine induced tolerance and dependence in ginseng butanol fraction[7] protopanaxadiol fraction and protopanaxatriol fraction[8] and inhibition of the development of morphine induced dopamine receptor supersensitivity[9]. Since each of the above saponin fractions was not pure saponin from *Panax ginseng*, the present study was undertaken to determine 1) inhibition of the development of morphine tolerance or not, 2) antagonism of morphine analgesia or not and 3) increase in the loss of tolerance and dependence or not by pure red ginseng saponins (GS), protopanaxadiol saponins(PD) and protopanaxatriol saponins (PT).

Materials and Methods

White ICR male mice weighing 18~20 g were used in all experiments, in a group of 10~15 mice. Ginseng total saponins (GS), protopanaxadiol saponins (PD) and protopanaxatriol sapon-

ins (PT) (Korea Ginseng & Tobacco Research Institute) dissolved in distilled water were administered intraperitoneally to mice except morphine hydrochloride (Dae-Won Pharm. Co.,) which was given subcutaneously.

Induction of Morphine Tolerance and Dependence

Morphine hydrochloride 40 mg/kg (s.c.) was injected to mice every 8 hr for a period of 6 days by Way and his coworker's method[1] and 25 and 100 mg/kg of GS, PD and PT were injected i.p. to the respective mice groups once a day 2 hr prior to the first injection of morphine daily for a period of 6 days except saline in the morphine control group.

Measurement of Analgesic Tolerance

The inhibition degree of morphine tolerance development by the administration of each saponin was evidenced by the increase in analgesic response to morphine hydrochloride (10 mg/kg s.c.) as analgesic percent (at 30, 60 and 90 min) estimated by the tail flick method 8 hr after the final injection of morphine[7] and calculated as area under the curve by Kaneto and his coworker's method[10].

Our previous studies have shown that morphine 10 mg/kg s.c. produced consistant and reliable analgesia, 100% and hypothermia, about $-2°C$ in none treated normal mice while showed around 10% analgesia and about $-1°C$ hypothermia in morphine tolerant-dependent mice treated by Way *et al.*, method.

The tail flick latencies to thermal stimulation were determined in seconds prior to and at 30, 60 and 90 min after the injection of morphine. A value of 10 sec was used as the cut-off point to avoid damage to the tail. The analgesic response for each mouse was calculated by the following formula:

$$\text{Percent analgesia}=\frac{T_t-T_0}{T_c-T_0}\times 100$$

Where T_0 is base line or pre-morphine tail flick reaction time; T_t is the reaction time at t min after morphine injection, and T_c is cut off time. The base line tail flick latencies in different groups were around 2 ± 0.2 seconds. The effect was calculated as area under the curve (AUC) that obtained by plotting the analgesic percent on the ordinate and the time intervals (min) on the abscissa, and expressed as percent of the effect obtained in morphine alone treated control animals.

Measurement of Naloxone Induced Jumping Response

The inhibition of naloxone induced withdrawal jumping in morphine alone dependent mice and in morphine ginseng saponin treated mice was estimated by the decreased number of the naloxone (4 mg/kg) induced withdrawal jumping mice for 30 min 8 hr after the final injection of morphine. The abstinence syndrome was quantified by placing the animals on a circular platform 35 cm in diameter and 70 cm in height and counting the number of jumping animals for 30 min[1].

Measurement of Antagonism of Morphine Analgesia

Antagonism of morphine analgesia by GS, PD and PT was determined by the decrease in analgesic response to morphine 5 mg/kg (s.c.) by the measurement of analgesic tolerance. L-DOPA hydrochloride (Sigma) and 5-HTP hydrochloride (Sigma) in 0.5% CMC suspension, ginseng total saponins (GS) Protopanaxadiol saponins (PD) and Protopanaxatriol saponins (PT) (Korea Ginseng Tobacco Research Institute) dissloved in distilled water were administered intraperitoneally to mice except morphine hydrochloride (Dae-Won Pharm. Co.,) which was given subcutaneously. Ginseng Saponins (i.p.) were administered 0, 2, 4, 8, 16hr advance to morphine (s.c.) to test the influences of the time intervals of ginseng treatments but in the recovery tests of morphine

analgesia, mice pretreated with 100mg/kg of each ginseng saponin once a day for a period of 6 days were tested for ginseng saponins antagonisms of morphine analgesia (5 mg/kg) and influences of L-DOPA (50 mg/kg and 100 mg/kg), 5-HTP(50 mg/kg and 100 mg/kg) on the ginseng saponins antagonisms of morphine analgesia.

Measurement of Analgesic Tolerance Loss

Morphine hydrochloride 40 mg/kg was injected S.C. to mice every 8 hr for a period of 6 days before the following tests started[1]. The lost degree of morphine tolerance by the administration of ginseng saponins was evidenced by the increase in analgesic response to morphine hydrochloride (10 mg/kg s.c.) as analgesic percent (at 30, 60 and 90min) estimated by tail flick method[11], on the 5th and on the 7th day after the pretreatment in morphine tolerant mice with 100 mg/kg of each saponin (i.p.) once a day for 4 or 6 days and 24 hr after the final injection of each saponins, and calculated as area under the curve[10] by the measurement of analgesic tolerance.

Measurement of Abrupt Inhibition of Naloxone Induced Jumping Response

The abrupt inhibition of naloxone induced withdrawal in morphine dependent mice by GS, PD and PT was estimated by the decreased number of the naloxone (4 mg/kg i.p.) induced withdrawal jumping mice for 30 min 8 hr after the final injection of morphine and after pretreatment with each dose of saponins 30 min prior to the naloxone test[1] by the measurement of naloxone induced jumping response.

Measurement of Dependence Loss

After the pretreatment in morphine dependent mice with 100 mg/kg of each saponin (i.p.) once a day for 7 days and 24 hr after the final injection of each saponin, the lost degree of morphine dependence was estimated, on the 8th day, by scoring of the naloxone (10 mg/kg i.p.) induced withrawal in morphine dependent mice for 30 min and scored as follows: jumping 2, diarrhea 2, defecation, wetdog shake, writhing syndrome, rearing, grooming and ptosis 1 each in all or none response by the modified Tagashira and Dewey's method[12].

Statistics

The differences in the means for different responses in different treatment groups were analyzed by student's t test except Fischer's probability test in naloxone induced jumping response[7].

Results

Inhibition of Analgesic Tolerance Development

The analgesia of each group to morphine 10 mg/kg calculated as the AUC was observed by 1.9 times in GS 25 mg/kg, 3.1 times in GS 100 mg/kg, 1.8 times in PT 25 mg/kg, and 2.1 times in PT 100 mg/kg as compared with that of morphine control group while no significant difference was shown in both doses of PD (Fig. 1).

Inhibition of Naloxone Induced Jumping Response

Depending on the doses, ginseng saponins produced significant inhibition of naloxone induced jumping responses by 40% in GS 25 mg/kg, 50% in GS 100 mg/kg, 50% in PD 100 mg/kg, 50% in PT 25 mg/kg and 70% in PT 100 mg/kg group but not significant in PD 25 mg/kg and 0% inhibition in morphine control group (Fig. 2).

Antagonism of Morphine Analgesia

Each 100 mg/kg of GS, PD and PT showed its own weak analgesic activity corresponding to 12%, 22% and 10% of morphine 5 mg/kg in analgesic activity respectively.

No significant potentiation or antagonism of concomitant administration of morphine 5 mg/kg with each 100 mg/kg of GS and PT was observed

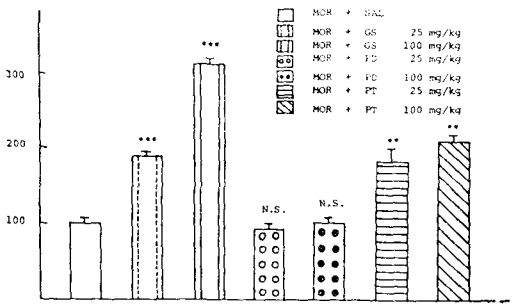

Fig. 1. Effects of GS, PD and PT on tolerance to the analgesic action of morphine in mice.
Morphine 40 mg/kg s.c. was injected to the mice every 8 hr for 6 days and saline or daily dose, 25 and 100 mg/kg of GS, PD and PT was injected to the respective group.
The inhibitory degree of tolerance development by GS, PD and PT were evidenced by the increase in analgesic response to morphine hydrochloride 10 mg/kg s.c. as estimated by the tail flick method and calculated as the AUC method as described in the text.
* $P<0.05$ ** $P<0.01$ *** $P<0.001$

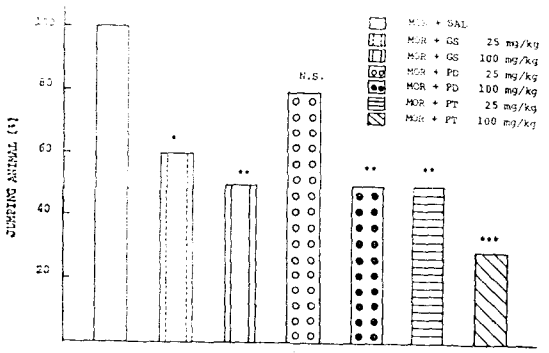

Fig. 2. Effects of GS, PD and PT on the development of morphine dependence in mice by the naloxone induced jumping response.
Each group of mice was injected with morphine hydrochloride 40 mg/kg s.c. at 8 hr intervals and with 25 and 100 mg/kg GS, PD and PT for the respective group at 24 hr intervals for 6 days. The withdrawal test was made 8 hr after the final morphine injection, by challenging with naloxolne 4 mg/kg i.p. The difference in different groups were analyzed by Fischer's probability test.
* $P<0.05$ ** $P<0.01$ *** $P<0.001$

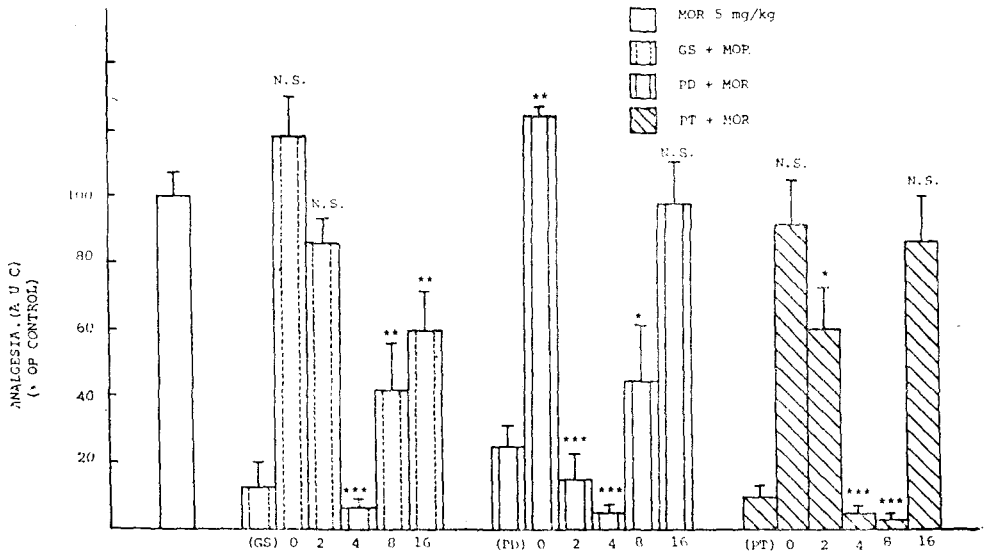

Fig. 3. Antagonism of morphine at time intervals after the administrations of GS, PD and PT.
Mor: morphine hydrochloride 5 mg/kg (s.c.)
GS: ginseng total saponins 100 mg/kg (i.p.)
PD: protopanaxadiol saponins 100 mg/kg (i.p.)
PT: protopanaxatriol saponins 100 mg/kg (i.p.)
0, 2, 4, 8 and 16: time intervals of morphine injection after ginseng treatment. The values are the means ± S.E. of 10 experiments.
* $P<0,05$ ** $P<0.01$ *** $P<0.001$

except the potentiation of PD with morphine.

But significant antagonism of morphine showed by 40% in PT group and by 83% in PD group pretreated with the respective saponin 2 hr advance except in GS group, but one tenth of morphine analgesic activity, strong antagonism was seen in all groups pretreated with the respective saponin 4 hr advance (Fig. 3).

A single injection of L-DOPA and 5-HTP up to 200 mg/kg had no significant effects on the analgesic effect of morphine. In a primary experiment, 5 mg/kg of morphine analgesia was reduced to about one tenth of the analgesic activity by the antagonisms of GS, PD and PT on the analgesic activity of morphine in mice pretreated with each saponin for 6 days (Fig. 4, 5, 6). When 50 or 100 mg/kg of L-DOPA and 5-HTP were injected 30 min prior to the administration of morphine to mice pretreated with GS 100 mg/kg, once a day for 6 days, some more active analgesic effects in both doses were observed than this of morphine alone (Fig. 4).

But L-DOPA 50 mg/kg, 100 mg/kg and 5-HTP 50 mg/kg in PD treated mice enhanced the reduced analgesic activity of morphine to 58%, 84% and 68% of morphine alone activity, while 5-HTP 100 mg/kg recovered the reduced analgesic activity of morphine to over 100% of morphine alone activity. 5-HTP in both doses of 50 mg/kg and 100 mg/kg showed more enhanced analgesic activity than those of L-DOPA treated group (Fig. 5). Meanwhile, L-DOPA 50 mg/kg in PT treated mice enhanced the reduced analgesic activity of morphine to 62% of morphine alone activity but L-DOPA 100 mg/kg, 5-HTP 50 mg/kg and 100 mg/kg recovered the reduced analgesic

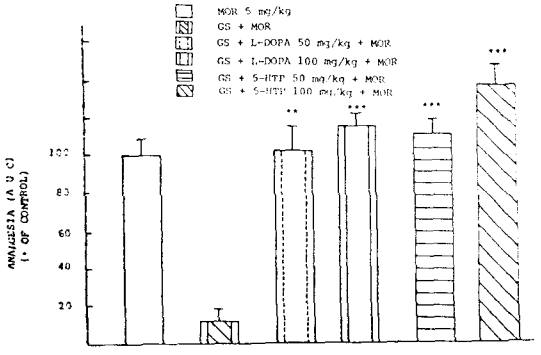

Fig. 4. Analgesic activity of morphine alone and after pretreatment with GS, L-DOPA and 5-HTP

GS: ginseng total saponins pretreated with a dose of 100 mg/kg (i.p.) once a day for 6 days and 24 hr after the test was measured.

* $P<0.05$ ** $P<0.01$ *** $P<0.001$

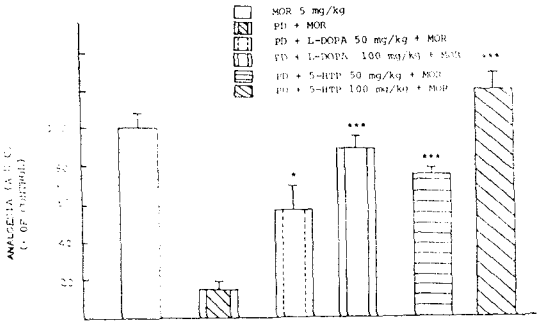

Fig. 5. Analgesic activity of morphine alone and after pretreatment with PD, L-DOPA and 5-HTP

PD: protopanaxadiol saponins pretreated with a doses of 100 mg/kg (i.p.) once a day for 6 days and 24 hr after the test was measured.

* $P<0.05$ ** $P<0.01$ ** $P<0.001$

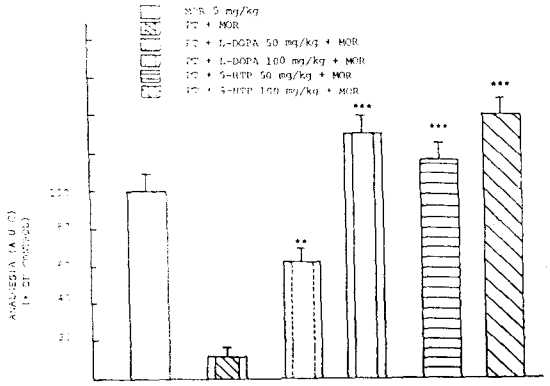

Fig. 6. Analgesic activity of morphine alone and after pretreatment with PT, L-DOPA and 5-HTP

PT: protopanaxatriol saponins pretreated with a dose of 100 mg/kg (i.p.) once a day for 6 days and 24 hr after the test was measured.

* $P<0.05$ ** $P<0.01$ *** $P<0.001$

activity of morphine to over 100% of morphine alone activity (Fig. 6). L-DOPA and 5-HTP produced suppressive effects on GS, PD and PT antagonisms of morphine analgesia. 5-HTP in the same dose of L-DOPA showed a tendency of greater activity than that of L-DOPA.

Analgesic Tolerance Loss

Pre-morphine treatment base lines of tail flick latencies in different groups were as follows: saline (2.22±0.05 sec), GS (2.11±0.06 sec), PD (2.10±0.04 sec), PT (2.01±0.07 sec) on the 5th day and saline (1.97±0.05 sec), GS (2.10±0.12 sec), PD (2.06±0.04 sec) and PT (2.28±0.07 sec) on the 7th day.

Similarly there was no difference in the base line latencies on the 5th day and on the 7th day.

The AUC values of tolerance loss in morphine tolerant control group with time are 8.94±1.33 (n=56), 17.94±3.73 (n=11) and 28.41±7.47 (n=10) respectively at 8 hr, 104 hr (on the 5th day) and 152 hr (on the 7th day) after the final injection of morphine.

The degree of tolerance loss in morphine and ginseng saponins treated mice on the 5th day to morphine hydrochloride (10 mg/kg) was observed by 5 times in GS treated group and 7.5 times in PD treated group, compared with that of morphine alone treated control group as shown in Fig. 7, but no significant loss was in PT treated group while significant loss of tolerance on the 7th day (recovery of analgesia) appeared in all of GS, PD and PT treated groups as shown by 3.8 times in GS 100 mg/kg, 4.7 times in PD 100 mg/kg and 2.1 times in PT 100 mg/kg, compared with this of morphine control group (Fig. 8).

Inhibition of Naloxone Induced Jumping Response by GS, PD and PT

The significant inhibitory effect of naloxone induced jumping response in single dose treatment of each saponin was observed by 60% in GS

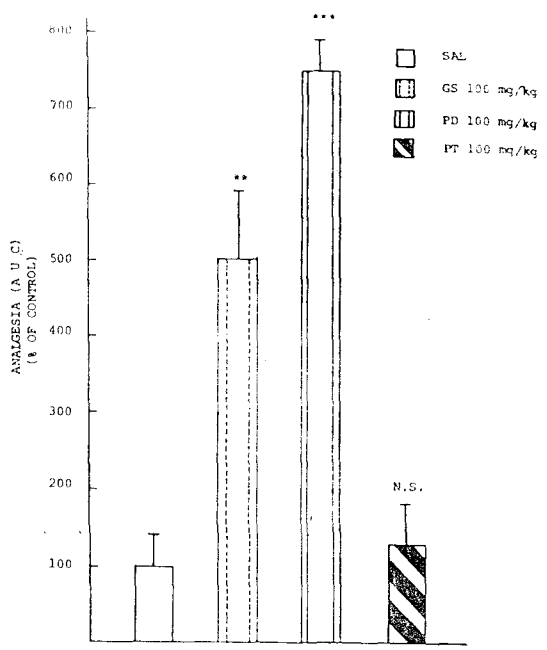

Fig. 7. Loss of analgesic tolerance in morphine tolerant mice by the administration of GS, PD and PT for 4 days.
Loss of analgesic tolerance was determined 24 hr after pretreatment with daily dose, 100 mg/kg of each GS, PD and PT for 4 days. The lost degree of morphine tolerance by GS, PD and PT was evidenced by the increase in analgesic response to morphine hydrochlorde (10 mg/kg s.c.) as estimated by the tail flick method and calculated as the AUC method as described in the text. Each group of mice was toleranced with morphine hydrochloride 40 mg/kg s.c. at 8 hr intervals for 6 days before the test started.
SAL; Saline.
GS; Ginseng Total Saponins.
PD; Protopanaxadiol Saponins.
PT; Protopanaxatriol Saponins
The values are the means of 10 experiments.
* $P<0.05$ ** $P<0.01$ *** $P<0.001$

100 mg/kg, 50% in PD 50 mg/kg and 60% in PT 25 mg/kg treated group (Table I).

Dependence Loss

Significantly less withdrawal scores by 2 in GS 100 mg/kg, 3.1 in PD 100 mg/kg and 1.9 in PT 100 mg/kg treated group than that of morphine control group appeared in all of ginseng saponins treated mice for a period of 7 days (Fig. 9).

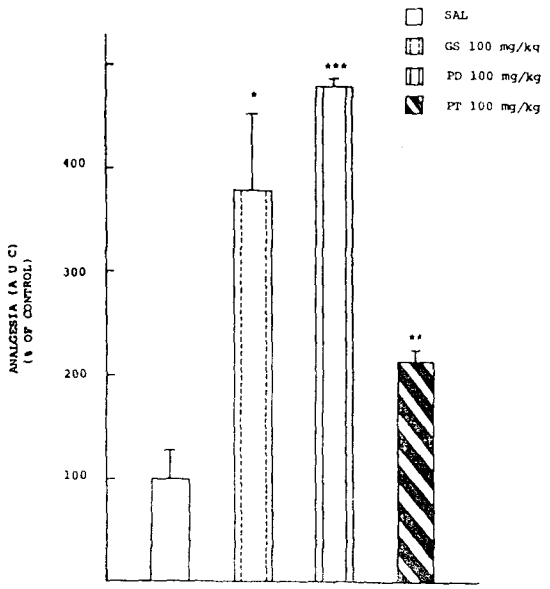

Fig. 8. Loss of analgesic tolerance in morphine tolerant mice by the administration of GS, PD and PT for 6 days.
Loss of analgesic tolerance was determined 24 hr after pretreatment with daily dose, 100 mg/kg of each GS, PD and PT for 6 days. The lost degree of morphine tolerance by GS, PD and PT was evidenced by the increase in analgesic response to morphine hydrochloride (10 mg/kg s.c.) as estimated by the tail flick method and calculated as the AUC method as described in the text. Each group of mice was toleranced with morphine hydrochloride 40 mg/kg s.c. at 8 hr intervals for 6 days before the test started. The values are the means of 10 experiments.
* $P<0.05$ ** $P<0.01$ *** $P<0.001$

Discussion

Kim and his coworkers[13~17] reported possible mechanisms that GS, PD and PT depleted catecholamines and serotonin levels in brain and antagonized the analgesic activity of morphine and suggested that GS, PD and PT might have reserpine or tetrabenazine like action.[2,18~22] Inhibitions of GS, PD and PT on the development of morphine induced tolerance and dependence might be mainly due to their reserpine or tetrabenazine like action and inhibition of dop-

Table I. Abrupt inhibitory effects of GS, PD and PT in morphine dependent mice by naloxone induced jumping response.
The abrupt inhibition of naloxone induced withdrawal in morphine dependent mice by GS, PD and PT was estimated by the decreased number of the naloxone(4 mg/kg, i.p.) induced withdrawal jumping mice for 30 min 8 hr after the final injection of morphine and after the pretreatment with each dose of saponins 30 min prior to the naloxone test.
* $P<0.05$ ** $P<0.01$ *** $P<0.001$

Treatment	Dosage(mg/kg)	No. of Mice None Jumped/Tested
GS	50	$2/10^{N.S.}$
	100	$6/10^{***}$
PD	25	$1/10^{N.S.}$
	50	$5/10^{**}$
PT	12.5	$4/10^{**}$
	25	$6/10^{***}$
Morphine	—	0/15

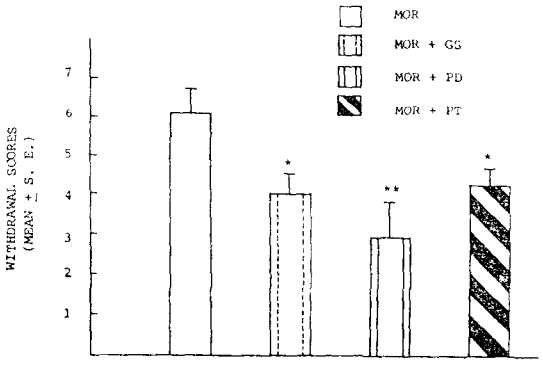

Fig. 9. Effects of GS, PD and PT on the loss of dependence in morphine dependent mice by the scoring method.
The lost degree of morphine dependence by GS, PD and PT was evidenced by the decreased scores in withdrawal response to naloxone hydrochloride 10 mg/kg i.p. as described in the text. Daily dose, 100 mg/kg of each GS, PD and PT was pretreated to the respective group for 7 days and 24 hr after, the measurment was made. The values are the means of 7 experiments.
* $P<0.05$ ** $P<0.01$ *** $P<0.001$

amine receptor supersensitivity[9]. In addition to these, the other sites such as enzymes, immune system and so on, could be thought to be pos-

sible acting points.

It is interesting to note that PD in the loss of tolerance and dependence in morphine dependent-tolerant mice is more effective than that of PT while the reversed activity appeared on the inhibition of the development of morphine induced tolerance and dependence[7,8].

The mechanisms of inhibitions of abstinence syndrome and of increases in the loss of tolerance and dependence in morphine dependent-tolerant animals by ginseng saponins remain unclear. Several neurotransmitters (Ach, D.A and c-AMP) have been implicated in abstinence syndrome. The expression of abstinence syndrome is associated with an increase in brain DA level[23] an increase in c-AMP level[24] and a decrease in brain Ach level[25].

It is possible that ginseng saponins may be inhibiting morphine withdrawal by altering the function of one or more of these substances in the brain. The studies involving the effects of ginseng saponins on whole brain neurotransmitter levels and on the neurotransmitter turnover rates in whole brain and in various regions of brain, have yielded conflicting data. Most of studies showed increases in noradrenaline, dopamine, serotonin and c-AMP in ginseng saponin treated animals[26,27] while there was no report on Ach level. Norepinephrinergic neurons in mouse brain were more influenced than dopaminergic neurons by oral chronic treatment of *Panax ginseng*[28]. Meanwhile, inhibition of abstinence syndrome and increases in the loss of tolerance and dependence in morphine dependent-tolerant mice by GS, PD and PT might be due to one or two of decreases in dopamine and serotonin levels in the brains of GS chronic treated mice (Kim and Oh 1985 unpublished).

The possible differences of the above reported effects in brain neurotransmitters levels could be originated from the different experimental methods such as administration routes, dosage sizes and tenures, animals, materials and so on.

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