Antinociceptive Effects of St. John's Wort, *Harpagophytum Procumbens* Extract and Grape Seed Proanthocyanidins Extract in Mice

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Hypericum perforatum extract (St. John's wort, SJW), *Harpagophytum procumbens* extract (HPE) and Grape seed proanthocyanidin extract (GSPE) have a broad spectrum of biological activities including antidepressant, anti-inflammatory or anti-oxidant effects. The aim of this study was to clarify antinociceptive properties of SJW, HPE and GSPE in mice with mechanisms that might potentially underlie these activities. Also, the effects of these herbal extracts on the antinociception and plasma and brain concentrations of morphine were examined. Oral pretreatment with SJW (100–1000 mg/kg) and HPE (30–300 mg/kg) attenuated significantly times of licking/biting both first and second phases of formalin injection in mice in the dose-dependent manner, and GSPE (10–300 mg/kg) suppressed second phase. Naloxone (5 mg/kg, s.c.) significantly attenuated antinociceptive effect of HPE but not SJW and GSPE. Formalin injection resulted in significant increase in the content of nitrites/nitrates (NO_x) in mouse spinal cord. The rise of spinal NO_x content by formalin was significantly attenuated by HPE and SJW. The pretreatment with SJW significantly potentiated an antinociceptive effect of morphine (0.3 mg/kg, s.c.), although concentrations of morphine in plasma and brain were not significantly changed by these herbal extracts. In conclusion, the present study has shown that SJW, HPE and GSPE exert significant antinociceptive effects in the formalin test of mice. In addition, opioidergic system seems to be involved in the antinociceptive effect of HPE but not SJW and GSPE. Furthermore, SJW potentiates morphine-induced antinociception possibly by pharmacodynamic interaction.

Key words antinociceptive effect; St. John's wort; *Harpagophytum procumbens* extract; Grape seed proanthocyanidin extract; morphine; interaction

Currently, the consumption of dietary supplement containing botanical products and foods is growing at a remarkable speed, in terms of the promotion of health or prevention and treatment of diseases. The extract from *Hypericum perforatum* (St. John's wort, SJW) possess clinical efficacy in the therapy of mild to moderate depression.^{1,2)} The most important constituents of SJW are phloroglucinols such as hyperforin and pseudohyperforin, naphthodianthrone such as hypericin and pseudohypericin, in addition to flavonoids such as rutin, quercetin, quercitrin. Several *in vitro* studies have indicated that SJW and hyperforin may act *via* a blockade of reuptake of serotonin, noradrenaline and dopamine in similar manner as most of the current antidepressants such as tricyclic antidepressants,^{3–5)} which have been known to exhibit antinociceptive properties by monoamine reuptake blockade.

Harpagophytum procumbens extract (HPE) and Grape seed proanthocyanidins extract (GSPE) have been reported to exert anti-inflammatory activity in rodents.^{6–8)} *Harpagophytum procumbens* commonly known as Devil's claw is an herbaceous plant, growing specifically in Southern Africa. Preparations of its secondary roots contain iridoid glycosides, mainly harpagoside, harpagogide and procumbide. HPE have been shown to possess clinical efficacy in the treatment of degenerative rheumatoid arthritis, osteoarthritis and tendonitis.^{6–8)} In addition, experimental study has revealed anti-inflammatory activity of HPE in Freund's adjuvant-induced arthritis model.⁹⁾ Proanthocyanidins are naturally occurring polyphenolic compounds widely available in fruits, vegetables, nuts, seeds, flowers and bark. Grape seed proanthocyanidins,

a combination of biologically active polyphenolic flavonoids including oligomeric proanthocyanidins, have been shown to exert a novel spectrum of biological, pharmacological, therapeutic and chemoprotective properties against oxygen free radicals and oxidative stress. GSPE protects against free radicals models and has exhibited superior antioxidant performance as compared to vitamin C, E and β -carotene.¹⁰⁾

Previous studies with antidepressant activity of SJW and anti-inflammatory effects of HPE and GSPE have led to the idea that these herbal products exert antinociceptive action. Thus, the aim of this study was to clarify the antinociceptive properties of SJW, HPE and GSPE after oral administration to mice with mechanisms that might potentially underlie these activities. The effects of these herbal extracts on the antinociception and plasma and brain concentrations of morphine were also examined.

MATERIALS AND METHODS

Drugs SJW and HPE were kindly donated by Indena (Milan, Italy). SJW was standardized to the content of hypericin (0.3%) and hyperforin (3.2%) and HPE was also standardized to the content of harpagoside (1.9%). GSPE was kindly supplied by Kikkoman Co. (Chiba, Japan), and standardized to the content of proanthocyanidin (83.9%). Morphine hydrochloride was purchased from Takeda Pharmaceutical Co. (Osaka, Japan). All other drugs and materials were obtained from commercial source. Morphine, naloxone and formalin were dissolved in 0.9% NaCl. SJW was suspended

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in distilled water and sonicated for 10 min before oral administration. HPE and GSPE were dissolved in distilled water.

Animals Male ICR mice (Japan SLC Inc., Shizuoka, Japan) weighting 20–30 g were used. Animals were housed under a 12-h light/dark cycle in a room with controlled temperature ($24 \pm 1^\circ\text{C}$) and humidity ($55 \pm 5\%$). They were allowed free access to food and water prior to the experiments. All animal procedures were in strict accordance with the guideline approved by the Experimental Animal Ethical Committee of University of Shizuoka.

Formalin Test In the formalin test, mice were adapted in open Plexiglas observation chambers at 1 h before injection of formalin. Formalin (20 μl of a 2.5% solution in saline) was injected subcutaneously into the dorsal surface of right hind paw of mice using a Hamilton microsyringe with a 30-gauge needle, as previously described.¹¹⁾ Each mouse was immediately returned to the observation chamber after formalin injection. A mirror was placed behind the chamber to allow the unhindered observation of formalin-injection paw. The time spent for licking or biting of injected paw (nociceptive response) was measured with stopwatch at 5 min intervals until 40 min post formalin injection and considered as a quantitative indication of nociception. The sum of time of licking/biting from 0 to 5 min was considered as the first phase, whereas the second phase was taken as the sum of time for licking/biting from 10 to 30 min. SJW (100–1000 mg/kg), HPE (30–300 mg/kg), GSPE (10–300 mg/kg) or vehicle (control group) were orally administrated to different groups of mice 60 min before formalin injection. Naloxone (5 mg/kg), yohimbine (3 mg/kg) and methysergide (3 mg/kg) were subcutaneously administered just before oral administration of these herbal extracts and the formalin test was performed 60 min after the administration of herbal extracts.

To examine effects of these herbal extracts on the antinociception of morphine, animals received SJW (300 mg/kg), HPE (30 mg/kg), GSPE (30 mg/kg) or vehicle at 45 min before the treatment with morphine (0.3 mg/kg, s.c.). Formalin test was performed at 15 min after morphine administration.

Tail-Flick Test Tail flick latency was measured using the automated tail flick analgesia meter (MK-330B, Muromachi kikai, Tokyo, Japan), as previously described¹²⁾ with minor modification. A noxious beam of light was focused on the tail about 4 cm from the tip, and the tail flick latency was recorded automatically to the nearest 0.1 s. The intensity of the radiant heat source was adjusted to yield baseline latencies between 2 and 4 s. Each mouse was given one test to determine baseline latency to tail-flick with a cutoff of 10 s set to avoid tissue damage. Animals were administrated SJW (1000 mg/kg), HPE (1000 mg/kg), GSPE (1000 mg/kg) or vehicle, and tail flick latencies were determined at 30, 60, 90, 120, 150 and 180 min after administration.

Measurement of Locomotor Activity The locomotor activity of mice was assessed in the open-field test as described previously.¹³⁾ Open field was a 30 $\times$ 30 cm acrylic area with 30 cm high black walls surrounding the field. Thin black stripes were painted across the floor dividing the field into 9 squares of equal area and the number of squares crossed with all paws crossing was counted in a 5-min session.

Determination for Contents of Nitrites/Nitrates (NO_x) in Brain and Spinal Cord SJW (1000 mg/kg), HPE

(300 mg/kg), GSPE (300 mg/kg) or vehicle was administered to mice orally, and thereafter, brain and spinal cord were dissected 80 min after the administration. Other groups of animals received herbal extracts at 60 min before formalin injection in the same way as formalin test, and brain and spinal cord were removed 20 min after formalin injection. The content of NO_x in mouse brain and spinal cord were determined as previously described with minor modification.¹⁴⁾ Brain and spinal cord of mice were homogenized in 3 volume distilled water and centrifuged at 15000 g for 20 min at 4°C . Fifty microliters of supernatant of tissue homogenate was mixed with 20 μl of 0.31 M potassium phosphate buffer (pH 7.5), 10 μl of 0.86 mM β -nicotinamide adenine dinucleotide phosphate (β -NADPH), 10 μl of 0.11 mM flavin adenine dinucleotide (FAD) and 20 mU of nitrate reductase. Samples were incubated for 60 min at room temperature in the dark. Then, 5 μl of 1 M ZnSO_4 was added to the sample and centrifuged at 15000 g for 10 min 4°C . One hundred microliters of Griess reagent (1:1 mixture of 1% sulphanilamide in 5% H_3PO_4 and 0.1% N -(1-naphthyl)ethylenediamine) was added to 80 μl of supernatant and the mixture incubated for 10 min at room temperature. Absorbance was measured at 550 nm by a micro plate reader (Perkin-Elmer Life Sciences) and converted to NO_x content by using a nitrate standard curve.

Measurement for Morphine Concentration in Plasma and Brain SJW (300 mg/kg), HPE (30 mg/kg), GSPE (30 mg/kg) or vehicle was orally administered to mice 45 min before the treatment with morphine (0.3 mg/kg, s.c.). Mice were sacrificed 30 min after morphine administration, and blood and brain were collected. Plasma was separated by centrifugation. Morphine concentrations in plasma and brain were determined by HPLC with electrochemical detector as previously described.¹⁵⁾ Plasma sample (200 μl) was mixed with 50 μl of 2 μM naloxone (internal standard) and 800 μl of 0.5 M ammonium sulfate (pH 9.3). Brain sample was homogenized in 4 volumes of saline. The homogenate (1 ml) was mixed with 50 μl of 2 μM naloxone and 100 μl of 1 M perchloric acid and centrifuged at 2000 g for 10 min at 4°C . The supernatant was transferred to another tube containing 2 ml of 0.5 M ammonium sulfate (pH 9.3). The mixture from plasma or brain sample was then applied to the Oasis HLB cartridge (Waters, Milford, MA, U.S.A.), which was pretreated with 1 ml methanol and 1 ml distilled water. Morphine was eluted with 1 ml methanol after the cartridge was washed with 4 ml of 15% methanol in 5 mM ammonium sulfate (pH 9.3). The elute was evaporated under a stream of nitrogen at 40°C . The residue was dissolved in 200 μl of the mobile phase and 50 μl of this solution was injected into HPLC system. The HPLC analysis was constructed with a pump (LC-20AD, Shimadzu, Kyoto, Japan), an electrochemical-detector (Coulochem III, ESA Inc., Chelmsford, MA, U.S.A.) and a injector (SIL-20AC, Shimadzu, Kyoto, Japan). The separation was performed on an analytical column (CAPCELLPAK SCX UG80, 5 μm , 100 $\times$ 3 mm, Shiseido, Tokyo, Japan). The mobile phase consisted of 67% acetonitrile and 33% 20 mM potassium dihydrogen phosphate (pH 2.1) at a flow rate of 0.5 ml/min. The HPLC column was maintained at 40°C and the electrochemical detector was set to +250 mV for detector 1, +600 mV for detector 2 and 800 mV for the guard cell.

Statistical Analysis All values are expressed mean $\pm$ S.E. Data were analyzed by Student's *t*-test or one-way analysis of variance followed by Dunnett's *post hoc* test. For all comparisons, differences were considered statistically significant at $p < 0.05$.

RESULTS

Effects on Nociceptive Responses in the Formalin and Tai-Flick Test The s.c. injection of 2.5% formalin into the right hind paw of mice induced a biphasic licking/biting nociceptive response. SJW at doses of 500 and 1000 mg/kg reduced significantly the licking/biting time both first phase (20.0 and 24.9%, respectively) and second phase (37.2 and

56.5%, respectively) in the dose-dependent manner (Figs. 1a, b). Similarly, HPE at doses of 100 and 300 mg/kg also reduced significantly the licking/biting time in both first phase (18.1 and 27.1%, respectively) and second phase (42.5 and 59.0%, respectively) (Figs. 1c, d). GSPE at doses of 30, 100 and 300 mg/kg reduced significantly (31.7, 38.3 and 48.1%, respectively) the licking/biting time in the second phase but not in the first phase (Figs. 1e, f).

The effects of naloxone, yohimbine and methysergide on antinociception of SJW, HPE or GSPE in the formalin test were examined. Naloxone (5 mg/kg, s.c.) attenuated significantly (65.9%) antinociceptive effect in the second phase of formalin test by HPE (300 mg/kg, *p.o.*), but not by SJW (1000 mg/kg, *p.o.*) and GSPE (300 mg/kg, *p.o.*) (Fig. 2). None of these agents-induced antinociceptive responses in the first phase was attenuated by naloxone. In addition, naloxone at this dose effectively reversed morphine (0.3 mg/kg)-induced antinociceptive response both first and second phase. Yohimbine (3 mg/kg, s.c.) and methysergide (3 mg/kg, s.c.) did not significantly influence the antinociceptive effect of SJW (1000 mg/kg) both first and second phase (data not shown).

In the tail-flick test, there were little significant differences of tail-flick latencies between vehicle-treated group and each group treated with SJW (1000 mg/kg), HPE (1000 mg/kg) or GSPE (1000 mg/kg) (Fig. 3). Morphine increased significantly the latency at 30 and 60 min after administration.

Effects on the Locomotor Activity in Mice In open-field test, the numbers (counts/5 min) of crossing in mice 60 min after the pretreatment with vehicle, SJW (1000 mg/kg, *p.o.*), HPE (300 mg/kg, *p.o.*) and GSPE (300 mg/kg, *p.o.*) were 106 ± 7 , 118 ± 8 , 110 ± 7 and 114 ± 8 , respectively. Thus, these herbal extracts had little significant effect on the locomotor activity.

Effects on the Contents of NO_x in Brain and Spinal Cord The formalin injection induced a significant (1.8 fold) increase of NO_x contents in mouse spinal cord but not in the brain. The formalin-induced increase of NO_x content in the spinal cord was significantly reversed by the pretreatment with SJW or HPE but not GSPE (Table 1).

Effects on the Antinociceptive Effect and Concentration in Plasma and Brain of Morphine Morphine at the dose of 0.3 mg/kg significantly reduced the licking/biting time in the first phase (24.8%) and the second phase (36.1%) of formalin test in mice. The antinociceptive effect (reduc-

Fig. 1. Effects of SJW, HPE and GSPE on Licking and Biting Responses in First (a, c, e) and Second Phases (b, d, f) of Formalin Test in Mice

Mice received orally SJW (100, 300, 500, 1000 mg/kg) (a, b), HPE (30, 100, 300 mg/kg) (c, d) and GSPE (10, 30, 100, 300 mg/kg) (e, f) 60 min before the formalin injection to mouse paw. The licking/biting time (s) was measured. Each value represents mean $\pm$ S.E. ($n=6$). Asterisks show a significant difference from control mice, * $p < 0.05$, ** $p < 0.01$.

Fig. 2. Effect of Naloxone on Antinociception of SJW, HPE, GSPE and Morphine in First (a) and Second (b) Phases of Formalin Test in Mice

Mice received naloxone (5 mg/kg, s.c.) before oral administration of herbal extracts (SJW: 1000 mg/kg, HPE: 300 mg/kg, GSPE: 300 mg/kg) and then the formalin test was performed 60 min after the administration of herbal extracts. Naloxone was treated 45 min before the administration of morphine (0.3 mg/kg, s.c.), and the formalin test was performed 15 min after the administration of morphine. Each value represents mean $\pm$ S.E. ($n=6$). Symbols show a significant difference from control mice (***, ****) or from the corresponding mice without naloxone (###, ####, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Fig. 3. Effects of SJW, HPE, GSPE and Morphine on Nociceptive Responses in Tail-Flick Test of Mice

Tail-flick latency was measured every 30 min after oral (s.c. for morphine) administration of each agent (SJW, 1000 mg/kg; HPE, 1000 mg/kg; GSPE, 1000 mg/kg; morphine, 3 mg/kg). Each value represents mean $\pm$ S.E. ($n=7-8$). Asterisks show a significant difference from control mice at the same time, $*p<0.05$.

Table 1. Effects of Oral Administration of SJW, HPE and GSPE on Contents of NO_x in Mouse Brain and Spinal Cord

Treatment	Contents of NO_x	
	Brain (nmol/g)	Spinal cord (nmol/g)
Without formalin		
Vehicle	122.6 $\pm$ 15.4	89.2 $\pm$ 9.7
SJW	89.3 $\pm$ 3.3	91.7 $\pm$ 5.0
HPE	87.3 $\pm$ 4.2	87.4 $\pm$ 4.3
GSPE	113.2 $\pm$ 20.4	120.0 $\pm$ 7.5
With formalin		
Vehicle	130.9 $\pm$ 7.3	161.7 $\pm$ 11.5**
SJW	112.4 $\pm$ 5.1	119.8 $\pm$ 13.6#
HPE	121.1 $\pm$ 7.8	116.0 $\pm$ 9.9#
GSPE	106.6 $\pm$ 4.7	152.1 $\pm$ 8.3**

Formalin (2.5%, 20 μ l) was injected 60 min after oral administration of SJW (1000 mg/kg), HPE (300 mg/kg) and GSPE (300 mg/kg). Brain and spinal cord were removed 20 min after formalin injection. Each value represents mean $\pm$ S.E. ($n=6-8$). Symbols show a significant difference from vehicle group without formalin (**) or from vehicle group with formalin (#), $**p<0.01$, $#p<0.05$.

tion of licking/biting time) of morphine in the second phase of formalin test was significantly potentiated by pretreatment with low dose (300 mg/kg) of SJW (Fig. 4). On the other hand, HPE (30 mg/kg) and GSPE (30 mg/kg) had little significant effect on the antinociceptive effect of morphine. The antinociceptive effect of morphine in the first phase was unaffected by these herbals.

The concentrations of morphine in plasma and brain 30 min after s.c. injection of morphine (0.3 mg/kg) in mice pretreated with SJW, HPE and GSPE were not significantly different from those in morphine-treated mice without these herbals (Table 2).

DISCUSSION

In the present study, we investigated antinociceptive properties of SJW, HPE and GSPE in mice with mechanisms that might potentially underlie these activities. SJW and HPE attenuated significantly nociceptive (licking/biting) responses in both first and second phase of formalin test. In contrast, GSPE was significantly efficacious only against the second phase. In the formalin test, it is considered that first phase of formalin-induced behavior reflects direct activation of A-

Fig. 4. Effects of SJW, HPE and GSPE on Antinociception of Morphine in First (a) and Second (b) Phases of Formalin Test in Mice

Mice received orally herbal extracts (SJW, 300 mg/kg; HPE, 30 mg/kg; GSPE, 30 mg/kg) 45 min before morphine administration (0.3 mg/kg, s.c.). The formalin test was performed at 15 min after morphine administration. Each value represents mean $\pm$ S.E. ($n=6-7$). Symbols show a significant difference from control mice (**), or from mice treated with morphine (##), $*p<0.05$, $**p<0.01$, $##p<0.01$.

Table 2. Effects of Oral Administration of SJW, HPE and GSPE on Plasma and Brain Concentration of Morphine in Mice

Treatment	Concentration of morphine	
	Plasma (ng/ml)	Brain (ng/g)
Vehicle	29.4 $\pm$ 2.9	13.0 $\pm$ 1.2
SJW	31.1 $\pm$ 3.8	10.5 $\pm$ 1.1
HPE	33.3 $\pm$ 7.0	15.3 $\pm$ 0.8
GSPE	33.8 $\pm$ 5.0	13.5 $\pm$ 1.9

Morphine (0.3 mg/kg, s.c.) was administered to mice 45 min after oral administration of SJW (300 mg/kg), HPE (30 mg/kg) and GSPE (30 mg/kg). Blood and brain samples were collected at 30 min after morphine administration. Each value represents mean $\pm$ S.E. ($n=3-4$).

delta and C afferent fibers while the second phase reflects both ongoing peripheral sensory input and central sensitization. Therefore, it is suggested that antinociceptive effects of SJW and HPE are mediated through central or both central and peripheral antinociceptive effects while the effect of GSPE is mainly due to the peripheral effect.

Antidepressant drugs such as tricyclics have been widely used in the treatment of patients with chronic pain. The serotonergic and adrenergic neuronal systems in CNS may be significantly involved in the descending pain-inhibitory pathways,^{16,17} and the interference of tricyclic antidepressant drugs with reuptake of serotonin and noradrenaline may be responsible for their antinociceptive activity.¹⁸ There are accumulating evidences that antidepressant activity of SJW results from the suppression of reuptake of synaptic serotonin and noradrenaline.^{3-5,19} Therefore, it is plausible that antinociceptive effect of SJW is attributable partly to the activation of descending serotonergic and adrenergic pathways. However, in the current study, the antinociceptive effect of SJW was little affected by yohimbine (α_2 adrenoceptor antagonist) and methysergide (serotonin receptor antagonist). Chatterjee *et al.*³ showed that hyperforin inhibited the uptake of GABA and L-glutamate, with similar IC_{50} values for the inhibition of uptake of serotonin, noradrenaline and dopamine. Thus, there is a possibility that the inhibition of GABA and L-glutamate uptake may be significantly associated with the antinociceptive effect of SJW. The antinociceptive effect of SJW was unaffected by naloxone, a non-specific antagonist of opioid receptors, suggesting that this ef-

fect of SJW is not mediated by opioid receptor system. Notably, relatively low dose of SJW significantly potentiated antinociceptive effect of morphine in the second phase of formalin test. It has been reported that tricyclic antidepressant drugs potentiate antinociceptive effect of morphine both animals and human and possess clinical efficacy in the treatment of chronic pain states such as an adjuvant analgesic.^{18–22)} Thus, it might be rational that SJW having antidepressant effect enhances antinociceptive effect of morphine.

Many botanical dietary supplements contain pharmacologically active phytochemicals that, when consumed concomitantly with conventional medications, may result in pharmacokinetic and/or pharmacodynamic interaction. SJW is a botanical supplement recognized for interacting with prescription medications.^{23–25)} SJW has been shown to decrease significantly blood concentrations of drugs such as indinavir, cyclosporine and midazolam by inducing particularly cytochrome P450 3A4 activity, thereby reducing the efficacy of drugs.^{23–25)} In the present study, there was no significant change in plasma and brain concentrations of morphine after oral administration of SJW, in spite that antinociceptive effect of morphine was effectively enhanced by pretreatment with SJW (Fig. 4). Therefore, this potentiation by SJW of morphine effect may be attributable to the pharmacodynamic interaction rather than pharmacokinetic interaction.

The antinociceptive effect of HPE in the formalin test (second phase) was significantly antagonized by naloxone, suggesting the involvement of opioidergic mechanism. There are three subtypes of opioid receptors (μ , δ , κ), and μ -opioid receptor activation is mainly involved in the antinociceptive effect of morphine. Our preliminary radioreceptor binding study has shown that HPE binds to μ -opioid receptors in mouse brain. Therefore, these results suggest a contribution of μ -opioid receptors in the antinociceptive effect of HPE.

Free radicals are implicated with pain^{26,27)} and some plant antioxidants have pain alleviating properties.²⁸⁾ Proanthocyanidins are major polyphenols in grape seeds and they have potent antioxidant activities *in vitro* and *in vivo* models.^{29–31)} Since GSPE also contain high amount (84%) of proanthocyanidins, antioxidant activities of proanthocyanidins might contribute to the antinociceptive effect of GSPE. In fact, this notion is supported also by the observation that *Croton celtidifolius* extract containing high amount of proanthocyanidins (75%) exhibited antinociceptive effect in the formalin test.³²⁾

SJW, HPE and GSPE did not change latencies in the tail-flick test. It is considered that tail-flick test is spinally mediated reflex to noxious stimuli. It is widely considered that analgesic effectiveness depended on the nociceptive stimulus, and that chemical stimulus (formalin test) is more sensitive than thermal one (tail-flick test).

There are considerable evidences to indicate that NO plays an important role in the processing of nociceptive transmission.^{33,34)} These include an increase in the release of excitatory neurotransmitter such as glutamate in the spinal cord following peripheral inflammation and an increase in the Ca^{2+} influx by glutamate resulting in the production of NO_x due to the activation of NO synthase. It was found that inhibition of NO synthase produced antinociception in the formalin test.^{35–37)} In this study, it was shown that formalin injection into one hind paw increased the content of NO_x in

spinal cord, in accord with previously reports using microdialysis method.^{38,39)} Moreover, oral administration of SJW and HPE significantly inhibited the formalin evoked increase of NO_x content in the spinal cord. Thus, this result suggests that antinociceptive activity of SJW and HPE is related partly to the suppression of spinal NO pathway.

In conclusion, the present study has shown that SJW, HPE and GSPE exert significant antinociceptive effects in the formalin test in mice. In addition, opioidergic system seems to be involved in the antinociceptive effect of HPE but not SJW and GSPE. Furthermore, SJW potentiates morphine-induced antinociception possibility by pharmacodynamic interaction.

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