

PRE-EMPTIVE ANALGESIC EFFECTS OF TRAMADOL, ST. JOHN'S WORT, AND PANAX GINSENG EXTRACT IN MICE

CENGİZ KAYA¹, YASEMIN BURCU USTUN¹, FATİH İLKAYA², ERSİN KOKSAL¹

¹Anesthesiology and Reanimation Department, Faculty of Medicine, Ondokuz Mayıs University, Samsun - ²Pharmacology Department, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey

ABSTRACT

Aim: Pre-emptive analgesia, has been popularised by demonstration of its important decremental effects on the severity and duration of pain. Our aim was to evaluate the pre-emptive analgesic efficacies of St. John's Wort (SJW), Panax ginseng (GNS), and tramadol (TRM) by constructing a surgical pain model in rats.

Material and methods: Ninety-six rats were divided into three groups: preoperative, postoperative, and control. The preoperative group received the study drug or placebo (10 ml/kg physiological saline via the intraperitoneal route) one hour before and two hours after the incisions. The postoperative group was given the study drug or placebo 2 hours after the incisions. Finally, the placebo was administered to the control group one hour before and two hours after the incisions. The drugs (25, 50, and 100 mg/kg SJW; 100 mg/kg GNS; and 20 mg/kg TRM) were administered intraperitoneally. Their analgesic efficacies and motor activities were evaluated using a hot-plate test and locomotor activity tests.

Results: The locomotor activities of SJW were lower than those of the control and TRM groups. The study drugs were compared among groups, and preoperative hot-plate test latencies following administration of 100 mg/kg SJW were longer than those of the postoperative group. When compared irrespective of the groups, the hot-plate latencies of SJW 100 mg/kg, GNS, and TRM were longer than that of placebo.

Conclusions: In our study, SJW 100mg/kg, GNS, and TRM demonstrated an antinociceptive effect in the hot-plate test in mice, while the drugs apart from TRM suppressed locomotor activity. In addition, SJW, GNS, and TRM did not manifest pre-emptive analgesic efficacy in this study.

Key words: St. John's Wort, Panax ginseng, tramadol, pre-emptive analgesia.

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Introduction

Preoperative administration of analgesia in order to prevent or alleviate postoperative pain is called pre-emptive analgesia⁽¹⁾. Some studies have demonstrated that application of various antinociceptive techniques effectively suppressed central sensitisation that developed following surgical incisions, while others have displayed inefficiency⁽¹⁻⁵⁾. In order to provide effective pre-emptive analgesia, an adequate level of analgesia should be ensured, which must encompass the early postoperative period⁽⁶⁾.

Hypericum perforatum (St. John's Wort, SJW) has been used for years as an herbal medicine in the treatment of eczema, burn wounds, anxiety, and

depression⁽⁷⁾. Studies have demonstrated its anti-inflammatory, antibacterial, antitumoural, antiangiogenic, and analgesic properties⁽⁷⁻⁹⁾. The antinociceptive effect is exerted via central and peripheral mechanisms^(7, 10), and hypericin and hyperforin are considered responsible for the analgesic properties⁽¹¹⁾. Inhibition of the analgesic activity of hyperforin with naloxone indicates the place of the opioid system in the mechanism of antinociceptive activity⁽¹¹⁾. In addition, hyperforin blocks synaptosomal reuptake by monoamines (serotonin, dopamine, and noradrenaline), which suggests its contribution to its pain-alleviating effects⁽⁷⁾. Hypericin selectively inhibits protein kinase C found in the brain and spinal cord, with a resultant impact on the modulation of pain^(11, 12).

In addition to these effects, increase in GABAergic transmission, decrease in nitric oxide levels, and inhibition of lipooxygenase enzyme are among other mechanisms of the antinociceptive activities of SJW⁽¹³⁾.

In studies on ginseng *Panax ginseng* (Asian ginseng, GNS), *Panax quinquefolius* (American ginseng), and *Panax japonicus* (Japanese ginseng) have been used most often. More than 30 species of ginsenosides (ginseng glycosides) have been defined; they are the basic molecules responsible for the effects of ginseng⁽¹⁴⁻¹⁶⁾. GNS is a significant, traditional Chinese herbal medicine used for thousands of years for its tonic effects⁽¹⁷⁾. It is also used in the treatment of neuropathic and somatic pain⁽¹⁵⁾.

Tramadol (TRM) is a synthetic, centrally acting (μ - moderate, δ , and κ weak analgesic) opioid receptor agonist classified as an atypical or weak opiate analgesic drug^(18,19). Apart from these effects, activation of supraspinal descending pain inhibitory pathway plays a role in its mechanism of action⁽²⁰⁾. This non-opioid mechanism is realised via inhibition of the reuptake of noradrenaline and serotonin⁽⁶⁾. TRM has a lower incidence rate of the side effects commonly associated with opioids (respiratory depression and dependence potential), making this drug an advantageous alternative in postoperative analgesia^(21,22).

In our study, we aimed to compare the preemptive analgesic effects of these three drugs using a hot-plate test by constructing a surgical pain model in mice. In addition, in order to evaluate the analgesic effects accurately, we evaluated the effects of the drugs on motor function using locomotor activity tests.

Materials and methods

Animals

Male Swiss albino mice (24–26 g) from Ondokuz Mayıs University Laboratory Animals (Samsun, Turkey) were used. Ten mice were housed per cage. The cages were placed in the experimental room 24 hours before the test for acclimatization. The animals were fed a standard laboratory diet and tap water ad libitum and kept at $23 \pm 1^\circ \text{C}$ with a 12-hour light/dark cycle, with light at 7 am. All experiments were carried out in accordance with the NIH Guide for the Care and Use of Laboratory animals. All efforts were made to minimize animal suffering, and to reduce the number of animals used.

All experimental protocols were approved by the Animal Care and Use Committee of the Ondokuz Mayıs University, and were carried according to Helsinki Declaration.

Drugs

Nanjing Zelang Medical Technology (Jiangsu, Nanjing, China) offered a commercial sample of St John's Wort dried extract (Batch Number; ZL-20120928: High-performance liquid chromatography report; Hypericin:0.3%, Hyperforin: 3%) and ginseng, the root of *Panax ginseng* dried extract (Batch Number GN21106-GIN: High-performance liquid chromatography report; Re:9.2%, Rb1: 19.8%, Rb2: 9.7%, Rb3: 2.4%, Rc: 13.5%, Rd: 8.0%). Tramadol hydrochloride was provided by Abdi Ibrahim (Contramal amp, 100 mg/amp). All drugs were dissolved in physiological saline (PS) solution immediately before use. Drug concentrations were prepared so that the necessary dose could be administered in a volume of 10 mL/kg by intraperitoneal (IP) injection. Control animals received an injection of an equal volume of PS.

Surgery

Skin incision surgery was performed as previously described with minor modification⁽²³⁾. All mice were anaesthetized and the plantar surface of the left hind paw was prepared in a sterile manner with 10% w/v povidone-iodine solution, 1-cm longitudinal incision was made with a number-11 blade, through skin and fascia of the plantar aspect of the foot, starting 0.5 cm from the proximal edge of the heel and extending toward the toes. The plantaris muscle was elevated and incised longitudinally. Following homeostasis with gentle pressure, the skin was opposed with two single interrupted sutures using 5-0 mersilk sutures. The wound site was covered with gentamycin antibiotic cream and the animals were allowed to recover individually in a cage.

Postoperatively, the animals were housed individually and the incision was checked daily; any sign of wound infection or dehiscence excluded the animal from the study. All animals were euthanised at the end of the protocol.

Hot-Plate Test

The hot-plate test was performed as previously described⁽²⁴⁾. Mice were placed inside a stainless-steel container, which was set thermostatically at $52.5 \pm 1^\circ \text{C}$ (Ugo Basile, Thermal Plantar TM,

Verase, Italy). Reaction times (in seconds) were measured with a stopwatch before and 4 h, day 1, day 2, day 3 after administration of the drugs. The endpoint used was the licking of the fore or hind paws. Those mice scoring less than 12 and more than 18 seconds in the pretest were rejected. An arbitrary cutoff time of 45 seconds was adopted.

Groups: The control group received a placebo 1 hr before and 2 hr after surgical incision; the preoperative group received the active drug 1 hr before and placebo 2 hr after surgery; the postoperative group received placebo 1 hr before and active drug 2 hr after surgery. 6 mice per group were used (Figure 1).

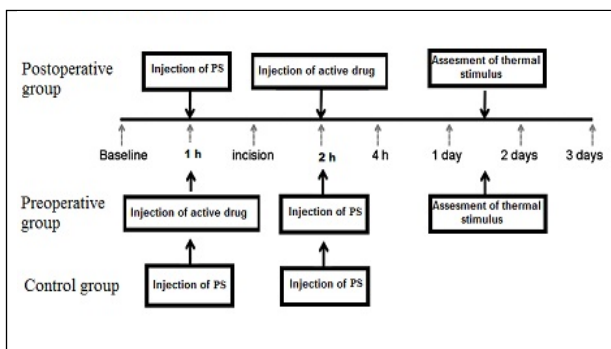


Fig. 1: Study procedure.

Active drugs; SJW 25, 50, 100 mg/kg, TRM 20 mg/kg, GNS 100 mg/kg was administered to animals via IP route. The observers were blind to the experimental and treatment conditions. To minimize experimental variability, all behavioral and operations were done by the same person.

Locomotor Activity Test

The mice were assigned to 6 groups. 5 mice per group were used. Each group received IP, 10 ml/kg PS, TRM 20 mg/kg, SJW 25, 50, 100 mg/kg, GNS 100 mg/kg. The spontaneous motor activity of the animals was assessed in an activity cage (controller model 7441 and Grid-Floor Detecting Arrangement Cage model 7432; Ugo Basile, Italy) at 30 and 120 min after administration of PS and drugs. Prior to treatment with PS and drugs, the animals were placed in the activity cage for 2 min for acclimatization. The activity of each mice was automatically recorded for 5 min.

Statistical analyses

Statistical software SPSS 21.0 for windows was employed in all statistical tests. Data were expressed as means \pm standard deviation of the mean. Data analysis was performed using the

untransformed data of hot-plate latencies and locomotor activities. Antinociception was expressed as mean of time spent biting and licking. Locomotor activity value were the total number of pulses measured in the activity cage. Data were analyzed by analysis of variance (Two-factor ANOVA) followed by post hoc Tukey analysis. A P-value of 0.05 was considered significant.

Results

Locomotor activity test

No statistically significant differences in locomotor activity were detected at 30 and 120 minutes among the drug and control group.

In comparisons among the study groups, locomotor activity with SJW 25, 50, and 100 mg/kg was lower than that of the TRM and control groups (121.00 ± 39.51 , 101.63 ± 125.70 , 152.83 ± 118.13 , 279.83 ± 97.37 , and 381.41 ± 100.54 min, respectively) ($p < 0.0001$). Locomotor activity of the GNS group was only lower than that of the control group (261.08 ± 74.64 and 381.41 ± 100.54 min, respectively) ($p < 0.05$) (Figure 2).

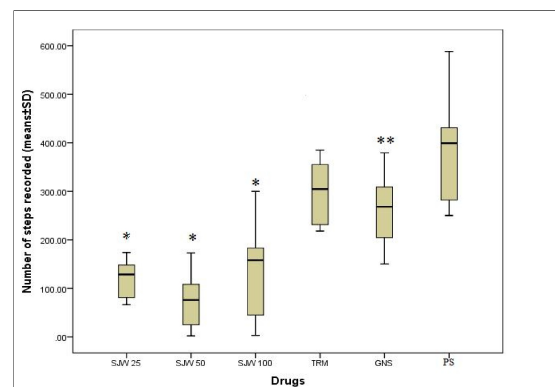


Fig. 2: Effects of drugs and PS on locomotor activity in mice. The parameters evaluated were the total number of pulses measured in the activity cage. Values are the mean \pm S.D (n = 6).

*: $p < 0.0001$ when compared to TRM and PS.

** : $p < 0.05$ when compared to control PS.

Hot-plate latency test

Intergroup comparisons of hot-plate latencies

A statistically significant difference in hot-plate latencies was found among the preoperative, postoperative, and control groups (13.91 ± 6.27 , 16.22 ± 7.12 , and 9.74 ± 3.39 sec, respectively) ($p < 0.05$) (Figure 3).

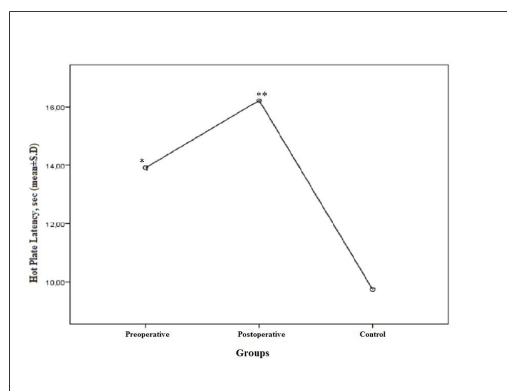


Fig. 3: Hot-plate latencies according to groups.

*: $p < 0.05$ when compared to control group

** : $p < 0.05$ when compared to control and preoperative groups

Comparison of hot-plate latencies of drugs

The hot-plate latency of the SJW 100 mg/kg group was statistically significantly longer relative to the SJW 25 mg/kg, SJW 50mg/kg, and control groups (17.43 ± 8.50 , 12.00 ± 3.63 , 12.56 ± 4.35 , and 9.74 ± 3.39 sec, respectively) ($p < 0.0001$). The hot-plate latencies of the GNS and TRM groups were significantly longer than those of the control group (14.60 ± 5.42 , 14.81 ± 6.81 , and 9.74 ± 3.39 sec, respectively) ($p < 0.0001$) (Figure 4).

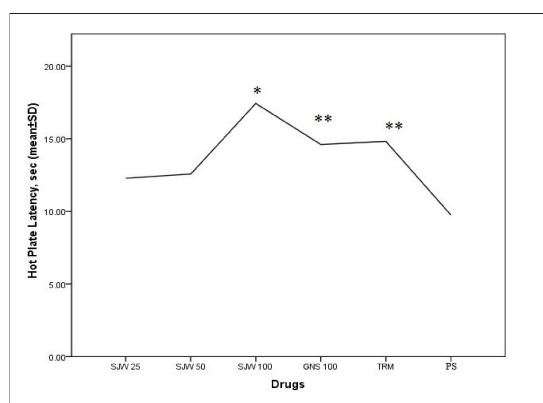


Fig. 4: Influence of drugs and PS on the latency time to pain reaction in the hot plate assay.

*: $p < 0.0001$ when compared to PS and SJW 25, 50mg/kg

** : $p < 0.0001$ when compared to PS

Comparison of measurement times of hot-plate latencies

A statistically significant difference in reaction times detected at four hours and two and three days was found among the groups (11.66 ± 3.51 , 18.69 ± 7.31 , 13.57 ± 5.91 , and 11.53 ± 5.82 sec, respectively) ($p < 0.0001$). In addition, a statistically significant difference was found between one-day estimates compared with baseline and three-day measurements (15.78 ± 7.01 , 13.57 ± 5.91 , and 11.53 ± 5.82 sec, respectively) ($p < 0.0001$).

Intergroup comparisons of drugs

Hot-plate latency of the postoperative SJW 100 mg/kg group was statistically significantly longer than that of the preoperative group (20.33 ± 9.49 and 14.53 ± 6.29 sec, respectively) ($p < 0.05$). The hot-plate latency test results of the other groups did not differ statistically significantly (Figure 5).

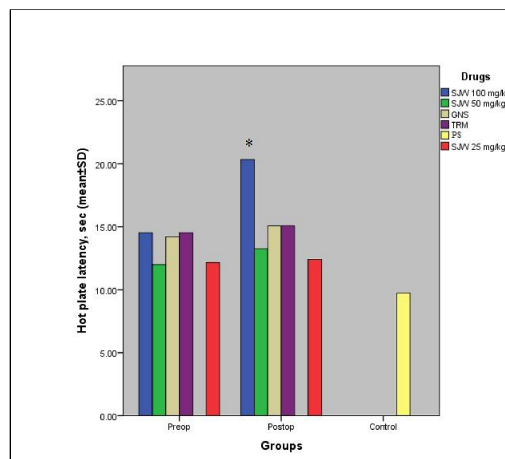


Fig. 5: Intergroup comparisons of the drugs.

*: $p < 0.05$ when compared to preoperative SJW 100mg/kg

Discussion

In our study we constructed an incisional pain model in mice to evaluate the pre-emptive analgesic efficacies of TRM, GNS, and SJW extracts. The experimental animals were allocated to three groups (control, pre-, and postoperative) and given various medications, after which hot-plate latency and locomotor activity levels were individually analysed.

Analysis of the outcomes showed that SJW given at a dose of 100 mg/kg demonstrated pain-relieving effects, whereas lower SJW doses of 25-50 mg/kg lacked analgesic efficacy^(10, 11). In a study wherein mice were given SJW extracts in oral 500-1000 mg/kg doses, SJW showed analgesic activity in the formalin test and suppressed locomotor activity to an extent⁽¹⁰⁾.

It has been reported that the pharmacokinetic properties of SJW are alike in human beings and mice, with an approximate bioavailability of 20%⁽¹²⁾. In that study, an acceptable level of bioavailability at doses of 25-50 mg/kg can be assumed. In our study, we used an IP administration route, but we were unable to find any study investigating IP bioavailability in the medical literature. The inability to achieve analgesia at an IP dose of 25-50 mg/kg SJW can be attributed to lower blood

levels of SJW, due to inadequate bioavailability of the drug. In addition, the antinociceptive effects of SJW vary with the intensity of the nociceptive stimuli. For example, in a comparison using exposure to chemical stimuli, the analgesic effect of SJW was more prominent when exposed to thermal stimuli⁽⁷⁾.

Therefore, although an analgesic effect was achieved in the cited study, we were unable to obtain analgesic efficacy in our study due to the stated reasons. In the same cited study, oral doses of 1000 mg/kg SJW decreased locomotor activity. In another study, at 6-24 mg/kg IP doses of SJW, locomotor hyperactivity induced by caffeine suppressed locomotor hyperactivity in mice⁽²⁵⁾. This effect had been associated with the inhibitory effect of SJW on the nitric oxide system⁽²⁵⁾. Similarly, we detected comparable effects of SJW on the locomotor system.

In another study, which conducted formalin and acetic acid abdominal constriction tests, IP doses of 100 mg/kg SJW extract given to mice were found to be more effective than the same doses of ibuprofen⁽²⁶⁾. In our study, IP doses of 100 mg/kg SJW were found to be more effective even for overcoming adverse effects of thermal stimuli.

However, in another study, IP doses of 10–20 mg/kg SJW were found to be effective in an acetic acid constriction test, and its effect was antagonised by naloxone⁽²⁷⁾. In our study, we were unable to show analgesic effects of SJW, even at a dose of 50 mg/kg, which might be associated with variations in the antinociceptive effect of SJW dependent on the nociceptive stimuli applied (greater analgesic effects are obtained with chemical stimuli).

Many studies have found analgesic efficacies of ginsenosides in mice⁽²⁸⁻³⁰⁾. Even though their analgesic mechanism of action is not fully known, some mechanisms can be presumed to be responsible based on their analgesic effects⁽³¹⁾. These mechanisms of action include inhibitory effects on voltage-dependent Ca²⁺ channels in sensorial neurons and the binding of bradykinin and neurokinin to B₂, and NK1 receptors, as well as the suppression of glutamate/substance P release from primary sensorial nerve endings and stimulation of alpha₂ adrenoreceptors and opioid and muscarinic receptors in the intrathecal region^(7, 8, 14, 15, 28, 31, 32).

In another study, antinociceptive activity was demonstrated in a capsaicin test performed on mice with 50-100 mg/kg IP doses of GNS (its contents was not indicated), but motor coordination disorders could not be shown in a rotarod test⁽¹⁵⁾.

However, in our study, an analgesic effect was observed at a dose of 100 mg/kg, and significant suppression occurred in the locomotor test when compared with the control group. This difference might stem from the use of ginsenosides Rb and Rc in our study, instead of ginsenoside Rg. Ginsenosides Rb and Rc produce a sedative effect, whereas Rg ginsenosides show stimulatory actions on the central nervous system⁽³³⁾.

In other studies, with IP doses of 100 mg/kg GNS (ginsenosides Rb and Rc) in mice, motor coordination disorder was observed in a rotarod test, and the effective IP dose for the inhibition of spontaneous movements was found to be 92 mg/kg. In that previous study, an IP dose of 200 mg/kg GNS had an analgesic effect in a tail pressure test⁽²⁹⁾. The similarities in locomotor activity between our study and the former study might be related to the use of Rg-free ginsenosides in both studies.

Human and animal studies have demonstrated that perioperative TRM use decreased additional analgesic need and improved pain scores.⁽³⁴⁾ An equipotent dose of TRM used in mice is 12-fold higher than that used (1-2 mg/kg) in humans⁽³⁵⁾. Therefore, in our study, TRM was used at an IP dose of 20 mg/kg. Another study demonstrated that an IP dose of 20 mg/kg TRM given to rats was effective in tail-flick and hot-plate tests without altering rotarod performance⁽³⁶⁾. However, in yet another study, IP doses of 10 and 30 mg/kg TRM given to mice were found to be effective in tail-flick and hot-plate tests⁽³⁵⁾. Still, while IP doses of 25 mg/kg TRM demonstrated prominent antinociceptive effects in tail-flick and formalin tests, depression of the nervous system was not observed, even at a dose of 45 mg/kg⁽¹⁹⁾. Similar to other studies, TRM displayed an analgesic effect in our study, while no suppression of locomotor activity was observed.

In our study, in intergroup comparisons of hot-plate latency, analgesic efficacy of TRM, SJW 100 mg/kg, and GNS was observed, without any pre-emptive effectiveness. We were unable to find any studies in the literature comparing the pre-emptive analgesic efficacies of SJW and GNS. However, some studies have demonstrated that pre-emptively administered TRM was more effective than its peri- and postoperative uses⁽³⁷⁻³⁹⁾. However, in a study investigating the pre-emptive analgesic effects of TRM using an incisional model in rats, three-day cumulative pain scores with 40 mg/kg IP doses of

TRM before and after the incisions were found to be alike. In the same study, 30 mg/kg IP doses of etoricoxib, indomethacin, and naproxen were administered, and cumulative pain scores estimated on the first postoperative day in the pre-incisional etoricoxib and indomethacin groups, and on the first and second postoperative days in the naproxen group, were lower than their post-incisional scores⁽⁴⁰⁾. This phenomenon might be related to the suppression of postoperative inflammation by nonsteroidal anti-inflammatory drugs, as postoperative inflammatory mediators play a role in central sensitisation^(1, 2). Nonsteroidal anti-inflammatory drugs can prevent both central and peripheral sensitisation via inhibition of peripheral cyclooxygenase enzymes, which probably makes them more effective as pre-emptive analgesic agents⁽²⁾.

A review that analysed 12 studies on pre-emptive analgesia reported that negative results were obtained in 60% of the investigations. In three of the studies with negative outcomes, only opioids had been used. The authors of the review associated these differences in outcomes to inadequate description of pre-emptive analgesia, the effects of general anaesthetic agents on pre-emptive analgesia, the contribution of postoperative inflammation on central sensitisation, and the inability to construe appropriate placebo groups⁽²⁾.

However, we think that the central sensitising effects of postoperative inflammation might be contributing factors to the lack of pre-emptive analgesic efficacy of TRM in our study. In addition, the inability to use objective pain evaluation scales might account for the controversial results obtained, as the reactions of mice against painful stimuli are highly variable and sometimes hardly discernible. As such, observers should be very experienced in the recognition of normal and abnormal behaviours in mice; unfortunately, such evaluations are always subjective⁽⁴⁰⁾.

In conclusion, in our study, SJW 100mg/kg, GNS, and TRM demonstrated an antinociceptive effect in hot-plate tests in mice, while the drugs tested, except TRM, suppressed locomotor activity. SJW, GNS, and TRM did not exhibit pre-emptive analgesic efficacy.

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Corresponding Author

CENGİZ KAYA

Anesthesiology and Reanimation Department
faculty of medicine, Ondokuz Mayıs University
Samsun
(Turkey)