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The Effects of Peripheral and Central Administration of *Hypericum perforatum* L. on Chronic and Acute Pain in Male Rats

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Abstract: In this study, *Hypericum perforatum* L. (HP) aqueous extract was administered intraperitoneally (i.p.) for evaluation of its antinociceptive effect. For assessment of its site of action, HP was filtered and administered both intrathecally (i.t.) and intracerebroventricularly (i.c.v.). Antinociceptive effects of HP extract were evaluated using formalin and tail-flick pain models. Peripheral effects of HP extract were assessed in three doses (200, 400 and 800 mg kg⁻¹, i.p.) and compared with the antinociceptive effect of Sodium Salicylate (SS) as a positive control. Administration of 300 mg kg⁻¹ of SS i.p. had no effect on tail-flick latency, while all doses of extract increased it. In both phases of formalin test, all doses of extract alleviated the animal's nociception, but SS (300 mg kg⁻¹) produced antinociception only in the second phase of formalin test. In central administration, the HP (1 and 2 mg/rat, i.t.) induced analgesia in the tail-flick and both phases of formalin tests. The i.c.v. administration of HP (2 mg/rat) produced analgesia in both phases of formalin test, but not on tail flick latency. The results showed that peripheral and central administration of HP has antinociceptive effect and its spinal effect seems to be more potent than its cerebral effect.

Key words: Antinociceptive effect, hypericum perforatum, rat

INTRODUCTION

The study of plants that have been traditionally used as a painkiller should still be seen as a fruitful and logical research strategy, in the search for new analgesic drugs^[1].

HP belongs to the genus *Hypericum* of family Hypericaceae native to Europe, West Asia and North America. HP is a perennial herb growing up to a height of 80 cm and has been used extensively as a medicinal plant over centuries. The aerial portions that have bright yellow flowers have been used for treatment aspects. It is commonly known as St. John's Wort and has been used in folk medicine for a variety of clinical uses such as wound healing, diuretic, antihelminthic and antiseptic^[2-4]. Many scientific papers have reported about antidepressant effect of HP^[5-8], but there are few studies indicating its antinociceptive and anti-inflammatory effects. In the present study the effects of peripheral and central administration of aqueous extract of Iranian HP on chronic and acute pain have been investigated.

MATERIALS AND METHODS

Collection and identification of the plant: HP was collected from the province of Tehran, Iran: Shemshak-Meigoun, Roadside, with 2300 m altitudes, on July 3, 2002. Mainly, the aerial parts of the plants that have a high proportion of buds and flowers were selected. The plant was identified by Dr. Sonboli, Faculty of Science, Institute of Medicinal Plant. It was identified and stored in the herbarium of the Institute of Medicinal Plants, Shaheed Beheshti University (voucher No. 2002-001).

Preparing of extract: One hundred milligram of dried aerial portions that have bright yellow flowers, were added to 1500 mL of boiling water and boiled for 20 min, then filtered through a filtering mesh. The water extract was concentrated on a boiling bath to the desired level, cooled and stored at refrigerator. The aqueous extract was dissolved in distilled water at the desired concentration just before use.

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Moisture measurement: In order to determine the moisture extract, 2 g of final extracts was placed in an oven in 60°C for 72 h, then the weight loss was calculated and used as a moisture indicator. The final extract contained 10% of water.

Animals: Male Sprague-Dawely Wistar rats weighing 200-230 g were used for all experiments and were housed in groups of six in plexyglass cages. Housing conditions were maintained at 24±2°C and 12:12 light dark cycle. Food and water were available *ad libitum*.

Analgesic tests:

Formalin paw test: 2.5% formalin (50 µL) was subcutaneously (s.c.) injected into the plantar surface of the animal's right hind paw. Nociception was rated using the original protocol^[9]. Briefly, the pain scoring measurement was as follows; 0: normal weight bearing on the injected paw, 1: limping during locomotion or resting the paw lightly on the floor, 2: elevation of the injected paw, 3: licking or biting of the injected paw, or grooming. Behavioral responses were observed and recorded for 1 h after the formalin injection. The first 5 min was considered as the early phase and the period between 16-60 min, as the second phase. Following s.c. intraplantar injection of formalin, the animals were immediately placed in a chamber with a mirror placed underneath the floor to allow an unobstructed view of the formalin injected paw. All animals were brought to test chamber 30 min prior to the experiment. The rats were not tested more than once and testing took place between 09:00 and 15:00 h.

Tail flick test: Nociceptive response was assessed with a tail-flick apparatus (HSE, Germany) using a method initially described by D'Amour and Smith^[10]. Animals responded to the focused heat-stimulus by flicking or removing the tail. The baseline latency time (reaction time) was obtained with three measurements. The average of these measurements was considered as the pre-drug latency time. Extract or drugs were administrated immediately after the third pre-drug measurement. Another set of three measurements was taken 5 or 25 min afterwards for intrathecal or intracerebroventricularly, or intraperitoneal administrations, respectively and their average was considered as the post-drug latency time. A cut-off time of 10 sec was used to prevent tissue damage.

Surgical procedures:

Intrathecal catheterization: Animals were initially anesthetized with ketamin hydrochloride (70 mg kg⁻¹, i.p.) plus chlorpromazine (10 mg kg⁻¹, i.p.) and placed in a stereotaxic frame. A catheter constructed of PE-10 tubing

was inserted to subarachnoid space at foramen magnum and threaded 8 cm caudal to the lumbar spinal cord according to a modification of method introduced by Yaksh and Rudy^[11]. Animals were individually housed in the cages and a 48 h period was considered for recovery. After the recovery period those that displayed motor deficits were omitted from the study.

Intracerebroventricular cannulation: Animals were initially anesthetized and placed in a stereotaxic frame. A 23-G needle, as a guide cannula, was inserted in left lateral ventricle: AP = -0.8 mm, Lat. = 1.4 mm, DV = 3.3 mm^[12].

For preventing obstruction of guide cannula, a G-27 needle was threaded into it. Animals were individually housed and a 48 h period was considered for recovery.

Pontamine sky blue as a color substance was intracerebroventricularly injected for histological confirmation. Brains of the animals were fixed in 10% formalin and sliced for confirmation. The results were considered valid if the color substance was diffused to the left lateral ventricle.

Drugs: Peripheral effects of HP extract were assessed in three doses (200, 400 and 800 mg kg⁻¹, i.p.) and compared with SS 300 mg kg⁻¹ i.p. and saline (2 mg kg⁻¹, i.p.) treated groups. For studying the central effect of HP extract, the effects of doses 1 and 2 mg/rat (i.t.) and 1 and 2 mg/rat (i.c.v.) were evaluated. The volume of injection, in central administrations was 10 µL.

Statistical analysis: Results are presented as mean±SE. Statistical significance of differences between groups for formalin test were analysed by one way ANOVA followed by Tukey's Post test and the result of tail flick tests was evaluated using paired t-test. p<0.05 was considered as significant.

RESULTS

Formalin test:

Peripheral administration of HP extract: HP extract significantly inhibited both phases of formalin test, dose dependently (p<0.01). Administration of SS (300 mg kg⁻¹) i.p. had no effect on early phase of formalin test, while it produced analgesia in the second phase of formalin test (Table 1).

Central administration of HP extract: Filtered HP extract (1 and 2 mg/rat, i.t.) produced analgesia in both phases of formalin test (at least p<0.05) (Table 2). In (i.c.v.) administration, only the dose 2 mg/rat of filtered HP extract showed antinociception in both phases of formalin test (p<0.01) (Table 3).

Table 1: The effects of peripheral administration (i.p.) of *Hypericum perforatum* L. (HP) and Sodium Salicylate (SS) on Formalin test

Groups	N	First phase	Second phase
Saline	10	2.34±0.04	1.97±0.05
SS (300 mg kg ⁻¹ , i.p.)	6	2.14±0.21	1.64±0.08**
HP (200 mg kg ⁻¹ , i.p.)	6	1.63±0.08***	1.61±0.08**
HP (400 mg kg ⁻¹ , i.p.)	6	1.37±0.16***	1.33±0.05***
HP (800 mg kg ⁻¹ , i.p.)	6	1.08±0.19***	1.05±0.13***

Pain score: mean±SE, **p<0.01 and ***p<0.001. The differences in pain score analysed by ANOVA and followed by Tukey test

Table 2: The effects of central administration (i.t.) of *Hypericum perforatum* L. on Formalin test

Groups	N	First phase	Second phase
Saline	10	2.26±0.07	1.94±0.06
HP (1 mg kg ⁻¹ , i.t.)	6	1.54±0.12***	1.47±0.16*
HP (2 mg kg ⁻¹ , i.t.)	6	1.00±0.11***	1.39±0.11**

Pain score: mean±SE, *p<0.05, **p<0.01 and ***p<0.001. The differences in pain score analysed by ANOVA and followed by Tukey test

Table 3: The effects of central administration (i.c.v.) of *Hypericum perforatum* L. on Formalin test

Groups	N	First phase	Second phase
Saline	10	2.03±0.04	2.05±0.05
HP (1 mg kg ⁻¹ , i.c.v.)	6	1.93±0.08	1.93±0.07
HP (2 mg kg ⁻¹ , i.c.v.)	6	1.58±0.12**	1.68±0.06**

Pain score: mean±SE, **p<0.01. The differences in pain score analysed by ANOVA and followed by Tukey test

Table 4: The effects of peripheral administration (i.p.) of *Hypericum perforatum* L. and Sodium Salicylate on tail-flick latency

Groups	N	Latency (sec)	
		Pre-drug	Post-drug
Saline	10	4.40±0.31	
4.26±0.33			
Sodium salicylate	6	4.25±0.33	
4.43±0.41			
HP (200 mg kg ⁻¹ , i.p.)	6	3.88±0.11	
5.40±0.66*			
HP (400 mg kg ⁻¹ , i.p.)	6	3.38±0.13	
6.88±0.73**			
HP (800 mg kg ⁻¹ , i.p.)	6	3.46±0.31	
7.85±0.81***			

Latency Period: mean±SE, *p<0.05, **p<0.01 and ***p<0.001. The difference between pre and post drug latencies were analysed by the paired t-test

Table 5: The effects of central administration (i.t. and i.c.v.) of *Hypericum perforatum* L. on tail-flick latency

Groups	N	Latency (sec)	
		Pre-drug	Post-drug
Saline (i.c.v.)	10	4.16±0.24	
4.3±0.35			
HP (1 mg/rat, i.t.)	6	4.15±0.19	
5.05±0.36*			
HP (2 mg/rat, i.t.)	6	3.92±0.20	
5.45±0.36**			
Saline (i.c.v.)	6	3.52±0.17	
3.73±0.14			
HP (1 mg/rat, i.c.v.)	6	3.85±0.23	
3.48±0.31			
HP (2 mg/rat, i.c.v.)	6	3.8±0.29	4.18±0.40

Latency period: Mean±SE, *p<0.05 and **p<0.01. The differences between Pre and post drug latencies were analysed by the paired t-student test

Tail-flick test: Peripheral administration of HP extract: Intraperitoneal administration of HP extract in doses (200, 400, 800 mg kg⁻¹) had a significant effect on tail-flick

latency (p<0.05, p<0.01 and p<0.001, respectively), but SS 300 mg kg⁻¹ did not show any significant effect on tail-flick latency (Table 4).

Central administration of HP extract: As it is shown in Table 2, intrathecal administration of filtered HP extract in doses 1 and 2 mg/rat had a significant effect on tail-flick latency (p<0.05 and p<0.01, respectively), but intracerebroventricular administration of HP extract (1 and 2 mg/rat) had no significant effect (Table 5).

DISCUSSION

Tail flick and formalin tests are proven to be effective in evaluating the antinociceptive activity^[13]. Few numbers of studies indicating the antinociceptive and anti-inflammatory effects of HP and in fact, there is no study indicating antinociceptive effects of HP using formalin test.

The phases of formalin test have obvious different properties and are a very useful tool for assessing the potency of analgesics and elucidating the mechanism of analgesia. The action of analgesics is different in the early and late phases. Drugs such as narcotics (e.g. morphine, meperidine and codeine) which primarily act centrally, inhibit both phases^[14-17], but peripherally acting drugs such as aspirin, oxyphenbotazone, dexamethazone and hydrocortisone, only inhibit the second phase of formalin-induced nociception^[15,17]. SS 300 mg kg⁻¹, i.p.) as a member of non-steroidal anti-inflammatory drugs (NSAIDs) only inhibited the late phase^[18]. These drugs attenuate the pain by inhibition of cyclooxygenase in arachidonic pathways^[19].

In the current investigation, we have demonstrated that peripheral administration of HP, dose dependently produced an antinociception in the rat by using two different experimental models (formalin and tail-flick tests). HP aqueous extract in all (i.p.) doses inhibited chemical and thermal nociception.

The first phase of the formalin test is probably due to direct chemical stimulation of nociceptors^[9,13,14]. Experimental data indicate that formalin also evokes activity in C fibers in the first phase^[20]. Therefore, HP extract may inhibit C fiber activity, but more study is needed to elucidate this view. Hyperforin and extract of HP potentially suppress 5-lipoxygenase (5-LO) and cyclooxygenase 1 (COX-1). 5LO and COX-1 are key enzymes in the formation of proinflammatory eicosanoids from arachidonic acid^[21].

Tjolsen^[13] have suggested that peripheral inflammatory processes are involved in the second phase of formalin test. COX-1 and LTD4 is product of the

5-lipoxygenase pathway of arachidonic acid^[22]. It has been reported that COX-1 inhibitor has produced a dose-dependent antinociceptive effect in the second phase of formalin test^[23]. Also Gok *et al.*^[24] have shown that leukotriene D4 (LTD4) receptor antagonists dose dependently have inhibited second phase of formalin test. It seems that HP exerts its antinociceptive activity probably through 5-LO and COX-1 inhibition in the second phase of formalin test that needs to be more evaluated.

Weak analgesics such as acetyl salicylic acid and paracetamol have little or no influence on the response in the tests with phasic stimuli such as tail flick and hot plate^[13]. In the tail flick model of nociception, SS had no effect on the latency, while HP extract caused the tail flick reaction time to increase significantly. Leukotriene B4 (LTB4) a product of the 5-lipoxygenase pathway of arachidonic acid metabolism, LTB4 also induces a thermal sensitization of cutaneous C-fiber high-threshold mechanonociceptors^[25]. It seems that the increase in the tail flick latency might be due to the 5-LO inhibition, which needs further investigation.

Central administration: Both phases of formalin test were inhibited by i.t. and i.c.v. administration of HP extract, but i.t. administration was more potent. Intrathecal administration of HP increased tail flick latency, while i.c.v. had no effect on tail flick latency. These results suggest that antinociceptive effect of HP is more potent at the spinal level.

Analgesia can be achieved both centrally and peripherally by interfering with a variety of neurotransmitter systems. In particular, the central control of pain is subject to descending modulation by brain stem cell groups such as locus coeruleus, subcoeruleus and raphe complex^[26]. These nuclei contain mainly noradrenaline and serotonin, respectively^[27]. Also, tricyclic antidepressants have been demonstrated to exhibit modest activity against neuropathic pain after systemic administration. It was stated that this effect is related to noradrenaline and serotonin reuptake inhibition^[28-31]. HP has also been shown to block noradrenaline and serotonin uptake in cortical synaptosomes^[32-34]. In this regard, there are parallels with tricyclic antidepressants, which are believed to potentiate the biogenic amines in endogenous pain-relieving systems. Since antidepressant effect is also reported for HP extract, the enhancement of noradrenaline and serotonin functions may also contribute to the analgesic effect of HP extract.

We concluded that total aqueous extract of HP exhibits antinociceptive activity on both acute and chronic pain. Intrathecal and intracerebroventricular

administration of Iranian HP aqueous extract showed that its antinociceptive effect is more potent at the spinal level. This effect is probably extracted by synaptosomal noradrenaline and serotonin reuptake inhibition. Further studies must be conducted in order

to clarify the mechanisms of antinociceptive effect of the extract and find out which constituent of the extract exerts this activity.

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