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# HYPERICINS CONTENT, ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF IRANIAN *HYPERICUM PERFORA TUM*

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## ABSTRACT

*Hypericum perforatum* is a medicinal plant which has been known in traditional medicine as anti-inflammatory and healing agent. The total content of hypericins was determined 0.101% in Iranian *H. perforatum*. The anti-inflammatory and analgesic effects of *H. perforatum* extract, a native plant of Iran, were studied using carrageenan induced edema, formalin, hot plate and writhing tests. 3h after carrageenan injection, similar activity against carrageenan-induced rat paw edema was observed with *H. perforatum* extract (100 and 150 mg/kg) and indomethacin (4 mg/kg). In the formalin test, the extract (25-250 mg/kg, i.p.) caused graded inhibition of both phases of formalin-induced pain (*P<0.001*).- In the hot plate test, the i.p. administration of the extract at the doses of 25- 250 mg/kg significantly raised the pain threshold at an observation time of 30 min in comparison with the control group (*P<0.001*). In the writhing test, the extract at doses of 25 mg/kg *(P<0.05)*, 50, 75, 100 and 150mg/kg (*P<0.001*) produced a significant decrease in the number of writhing in comparison with the control group. The extract, at antinociceptive doses, did not affect motor coordination of animals when assesed in the rotarod model.

**Keywords:** *Hypericum perforatum*, Hypericins content, Anti-inflammatory activity, Analgesic activity; Rotarod test

## INTRODUCTION

*Hypericum* is a genus of about 400 species, wide-spread in warm temperature areas throughout the world and well represented in the mediterranean area. Some species of this genus are used in folk medicine as anthelmintics, diuretics, on wounds, scalds and herpes (Trovato *et al.*, 2001). One of the most important species of this genus is *Hypericum perforatum* L. (Hypericaceae). This plant is a perennial herb, which widely distributed in Europe, Asia (e.g. Iran) and Northern Africa and naturalized in U.S.A. (Rechinger, 1968; Gambarana *etal.*, 1999). *Hypericum perforatum* (St. John's Wort) can reach a height of up to 60 cm, has yellow, star-shaped flowers and opposite leaves with characteristic translucent glandular dots (Ganzera *et al.*, 2002). Numerous compounds with documented biological activities have been reported from this species, e.g. naphthodianthrones, hypericin and pseudohypericin, different flavonoids like quercetin, hyperin, etc., phloroglucinol, essential oils and xanthones have been reported to produce antidepressant, antimicrobial, antioxidant, and anti-inflammatory activity (Mukherjee *et al.*, 2000). *H. perforatum* has recently gained popularity as an alternative treatment for mild to moderate depression (Greeson *et al.*, 2001).

In continuation of studies of Iranian plants (Morteza-Semnani *et al.*, 2002), the medicinal properties attributed to *H. perforatum* L., prompted us to investigate anti-inflammatory, antinociceptive activity and acute toxicity of this plant for the first time in Iran. The present paper describes quantitative determination of hypericins content and some of the pharmacological activities of this plant using carrageenan-induced rat paw edema, formalin, hot plate, writhing and rotarod tests.

## MATERIALS AND METHODS

### Plant material

*Hypericum perforatum* L. was collected from the suburb of Yasuj, in the west of Iran, in June 2001 and identified by Dr Gh. Amin, Department of Pharmacognosy, Tehran University of Medical Sciences. A voucher specimen (No. 131) was preserved at the herbarium of Faculty of Pharmacy, Mazandaran University of Medical Sciences. The aerial parts of plant were dried in the shade and powdered so that all the material could be passed through a mesh not larger than 0.5 mm.

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**Preparation of extract**

100 g powder was Soxhiett-extracted with methanol for 16 h., then this extract was evaporated to dryness and weighed (24 g, 24%) (Denke *et al*., 1999).

**Quantitative determination of hypericins content**

The determination of total hypericins content in plant material was carried out by spectrophotometric method at 590 nm (USP, 2002). Three determinations were made from sample and the mean value was calculated. The total content of hypericins was determined 0.101% in Iranian *H. perforatum.*

**PHARMACOLOGICAL ASSAYS**

**Carrageenan-induced rat paw edema**

The anti-inflammatory activity of extract was determined by the Carrageenan-induced edema test in the hind paws of rats. Male Albino Wistar rats (8 per group), 150-200g, were fasted for 24h before the experiment with free access to water. 50 µ1 of a 1% suspension of carrageenan (Sigma Co., USA) in saline was prepared Ih before each experiment and was injected into the plantar side of both hindpaws of the rats. Just prior to use, the dried extract was dissolved in a mixture of propylene glycol and water (1:4). The extract at the doses of 25, 50, 75, 100 and 150 mg/kg and indomethacin at the dose of 4 mg/kg were administered intraperitoneally (i.p.). Drugs or drugless vehicle were injected Ih before the carrageenan treatment. Paw volume was measured immediately after carrageenan injection and at 1-, 2-, 3- and 4-h intervals after the administration of the edematogenic agent using a plethysmometer (model 7159, Ugo Basile, Varese, Italy). The degree of swelling induced was evaluated by the ratio a/b, where a and b are total volumes of both hind paws after and before carrageenan treatment, respectively. A ratio smaller than 1.5 after drug administration was considered as a significant inhibitory effect of the drugs (Chi and Jun, 1990).

**Formalin test**

Male Albino Wistar rats (8 per group), 150-200g, were kept in plexiglas cages with free access to food and water. Testing took place in the middle of the light period of a 12h/12h light/dark cycle. Just prior to use, the dried extract was dissolved in a mixture of propylene glycol and water (1:4). Each animal was tested once only. Plant extract (25, 50, 75,100, 150, 200 and 250 mg/kg) and morphine sulphate (5 and 10 mg/kg) were administered i.p.. Control groups received only drugless vehicle. The antinociceptive activity of the drugs was determined using the formalin test described by Farsam *et al*. (2000). One hour before testing, the animal was placed in a standard cage (30xl2xl3cm), that served as an observation chamber. 50 µ1 of 2.5% formalin injected to the dorsal surface of the left hindpaw. The rat was observed for 60 min after the injection of formalin, and the amount of time spent licking the injected hindpaw was recorded. The first 5 min post formalin injection is known as the early phase and the period between 15-60 min as the late phase. The drugs were, administered 30 mm before the injection of formalin.

**Hot plate test**

Male Swiss mice were placed on an aluminium hot plate kept at a temperature of 55 ± 0.5°C for a maximum time of 30 s (Franzotti *et al.*, 2000). Reaction time was recorded when the animals licked their fore- and hind paws and jumped; at (before) 0 and 15, 30, 45 and 60 min after intraperitoneal administration of 25, 50,75,100,150, 200 and 250 mg/kg of extract to different groups. Morphine 10 mg/kg was used as the reference drug.

**Writhing test**

The test was carried out according to the method described by Pieretti et al. (1999). The extract at the doses of 25, 50, 75, 100 and 150 mg/kg was administered i.p.. 30 min after treatment, the mice were given an intraperitoneal (i.p.) injection of 0.6% v/v acetic acid in a volume of 10 ml/kg to induce the characteristic writhings. The number of writhings occurring between 5 and 15 min after acetic acid injection was recorded. The response of the extract treated animals was compared with that of the animals receiving indomethacin (5 mg/kg), morphine (1 mg/kg) as well as with the control group.

**Rotarod test**

The integrity of motor coordination was assessed with a rotarod apparatus, at a rotating speed of 16 r.p.m. A preliminary selection of mice was made on the day of experiment excluding those that did not remain on the rotarod bar for two consecutive periods of 45 s each (Pieretti *et al.*, 1999). The number of falls from the rod was counted for 45 s (male Swiss mice 20-25g, 8 per group). The performance time was measured before and 15, 30, 45 and 60 min

after extract administration.

**Statistical analysis**

ANOVA followed Student-Newman-Keuls test was used to determine significant differences between groups and $P<0.05$ was considered significant.

## RESULTS AND DISCUSSION

Since hypericin and pseudohypericin, naturally occurring red pigments, are chemotaxonomically important for the infrageneric classification of *Hypericum* (subfamily Hypericoideae) (Kitanov, 2001), thus we investigated hypericins content in Iranian *H. perforatum.* The total content of hypericins was determined 0.101% in this plant. The carrageenan test is highly sensitive to non-steroidal anti-inflammation drugs. It has long been accepted as a useful phlogistic tool for investigating new anti-inflammatory drugs (Just *et al.*, 1998). The results obtained with extract and indomethacin in the carrageenan-induced edema test are shown in **Fig. l.** The degree of swelling of the carrageenan-injected paws was maximal 3h after carrageenan injection. Statistical analysis shows that 3h after carrageenan injection, the extract at the doses of 25, 50, 75, 100 and 150 mg/kg produced a significant decrease in the degree of swelling in comparison with the control group ($P<0.001$). The degree of swelling (a/b) during 3h after carrageenan injection was < 1.5 at the doses of 25-150 mg/kg of the extract. Similar activity against carrageenan-induced rat paw edema was observed with *H. perforatum* extract (100 and 150 mg/kg) and indomethacin 3 hr after carrageenan injection. The degree of swelling (a/b) at the doses of 100 and 150 mg/kg of the extract was non-significant.

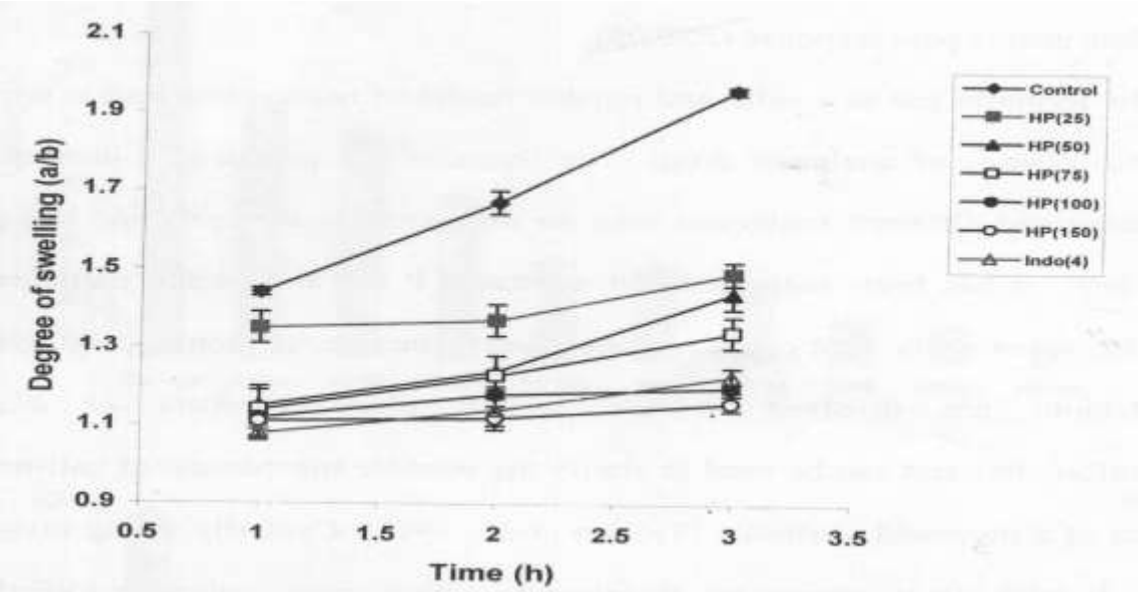


Fig.l. Effect of i.p. administration of indomethacin (4mg/kg), *H. perforatum* ^extract (HP 25, 50, 75, 100 and 150 mg/kg) and vehicle on the rat paw edema induced by intraplantar carrageenan injection. Each point represents the mean ± S.E.M. for eight animals. Student-Newman-Keuls analysis shows that, 3h after carrageenan injection, all test groups are significantly different from control group *(P<0.001).*

Effects of extract on the formalin test have been shown in **Fig.2.** Statistical analysis shows that all test groups are significantly different from control group on the early and late phases ($P<0.001$). The extract (25-250 mg/kg, i.p.) caused graded inhibition of both phases of formalin-induced pain *(P<0.001).* Anti-nociceptive activity of the extract at the dose of 150 mg/kg in the early phase was more than morphine at the dose of 5 mg/kg and less than morphine at the dose of 10 mg/kg ($P<0.05$). Similar anti-nociceptive activity was observed with *H. perforatum* extract (200 mg/kg) and morphine at the dose of 10 mg/kg in the early phase *(P>0.05).* Anti-nociceptive activity of the extract at the dose of 250 mg/kg in the early phase was more than morphine at the dose of 10 mg/kg ($P<0.05$). Similar anti-nociceptive activity was observed with *H. perforatum* extract (200 and 250 mg/kg) and morphine at the dose of 10 mg/kg in the late phase. The response of the extract at the doses of 200 and 250 mg/kg had not statistically significant difference to each other on the early and late phases pain response.

The formalin test is a valid and reliable model of nociception and is sensitive for various classes of analgesic

drugs. The formalin test produced a distinct biphasic response and different analgesics may act differently in the early and late phases of this test. It has been suggested that substance P and bradykinin participate in the manifestation of the first phase response, and histamine, serotonin, prostaglandin and bradykinin are involved in the second phase (Shibata *et al.,* 1989). Therefore, this test can be used to clarify the possible mechanism of anti-nociceptive effect of a proposed analgesic (Tjolsen *et al.,* 1992). Centrally acting drugs such as opioids inhibit both phases but peripherally acting drugs such as indomethacin and dexamethasone only inhibit the late phase. The late phase seems to be an inflammatory response with inflammatory pain that can be inhibited by anti-inflammatory drugs (Hunskaar and Hole, 1987; Rosland *et al.,* 1990). The effect of *H. perforatum* extract on the first and second phases of the formalin test suggests that its activity may have resulted from its central action when compared with morphine activity in this respect.

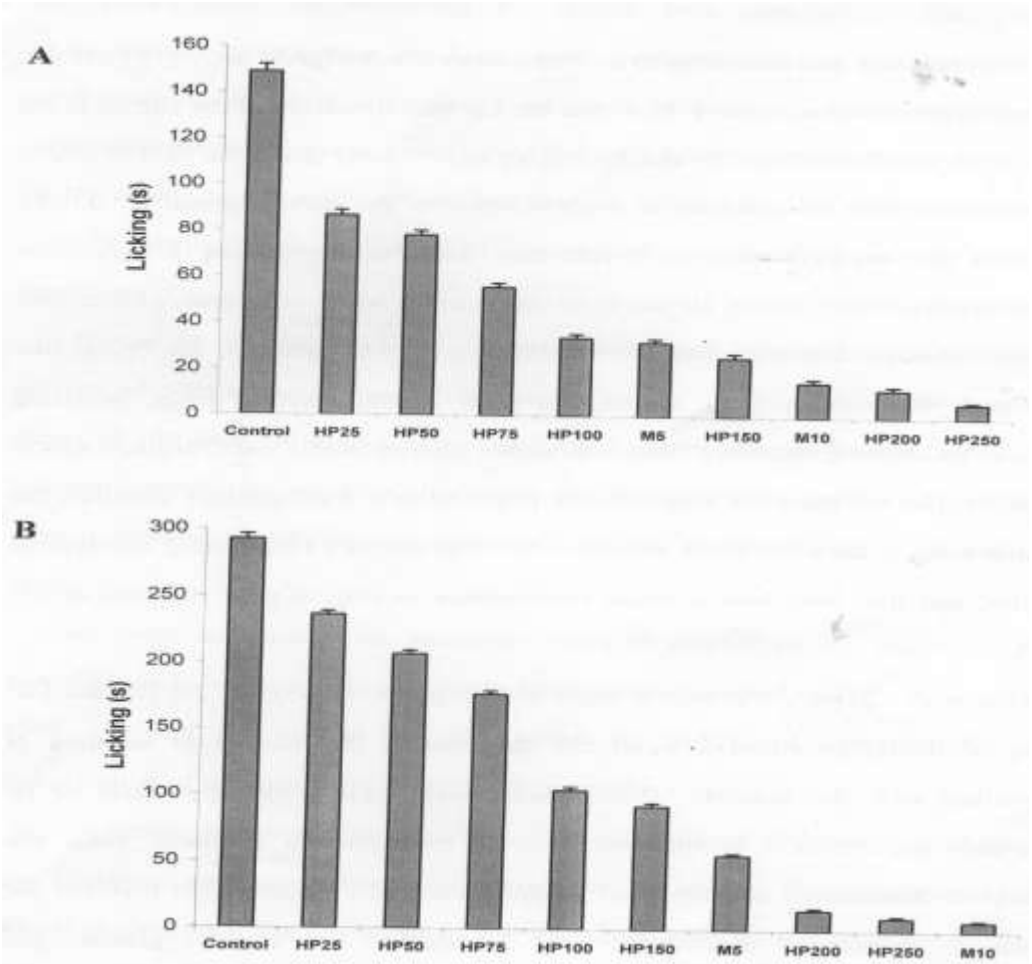


Fig.2. Effect of *H.perforatum* extract (HP 25, 50, 75, 100, 150, 200 and 250 mg/kg) and morphine )M 5 and 10 mg/kg) on the scores of the first phase (A) and the second phase (B) of the formalin test. The scores were calculated by summing the time (s) spent in formalin-induced hindpaw licking behaviour during a period of 300 s (0-5 min after formalin injection) for the first time and a period of 2700 s (15-60 min after formalin injection) for the second phase. Values represent the mean $\pm$ S.E.M. of eight animals. Student-Newman-Keus test shows that all test groups are significantly different from control group (*P<0.001*).

Anti-inflammatory effects of the extract on the carrageenan test suggests that antinociceptive effect of the extract in the second phase of formalin test could have been partially due to its peripheral action. In the carrageenan test, *H. perforatum* extract at the doses of 25-150 mg/kg caused a significant inhibition during the 3$^{rd}$ h that is the phase of prostaglandin release (Franzotti *et al.,* 2000). Thus, it seems that the extract relieved pain through both central and peripheral mechanisms.

The hot plate test was also assayed to characterize the analgesic activity of extract. The results presented in Table 1 show that the i.p. administration of the extract at the doses of 25, 50, 75, 100, 150, 200 and 250 mg/kg

significantly raised the pain threshold at observation time of 30 min in comparison with the control group (*P<0.001*). Morphine (10 mg/kg), used as a reference drug, also produced a significant antinociceptive effect during all the observation times when compared with control values (*P<0.001*). The hot plate test is considered to be selective for opioid-like compounds in several animal species, but other centrally acting drugs, including sedatives and muscle relaxants, have also shown activity in this test (Hiruma-Lima *et al*., 2000). The hot plate test measures the response to a brief, noxious stimulus; the formalin test, on the other hand, measures the response to a long-lasting nociceptive stimulus, and thus may bear a closer resemblance to clinical pain (Rosland *et al.*, 1990)

**Table 1**

Effect of *H. perforatum* extract on the latency time of mice exposed to the hot plate

| Group | Dose (mg/kg) | n | Latency (s) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Time 0 (min) | Time 15 (min) | Time 30 (min) | Time 45 (min) | Time 60 (min) |
| Control | - | 8 | 6.65 ± 0.06 | 6.42 ± 0.02 | 6.20 ± 0.04 | 6.12± 0.06 | 6.02 ± 0.05 |
| Morphine | 10 | 8 | 7.80 ± 0.06*** | 11.42 ± 0.05*** | 13.35± 0.03*** | 13.42± 0.05*** | 13.62 ± 0.08*** |
| HP | 25 | 8 | 6.72 ± 0.10 | 6.87 ± 0.02* | 6.92 ± 0.14*** | 6.60 ± 0.10** | 6.50 ± 0.19 |
| | 50 | 8 | 7.11 ± 0.03*** | 8.5 ± 0.11*** | 10.00± 0.14*** | 8.32± 0.12*** | 7.75 ± 0.09*** |
| | 75 | 8 | 7.12 ± 0.05*** | 9.43 ± 0.17*** | 11.44± 0.13*** | 9.39± 0.11*** | 8.90 ± 0.07*** |
| | 100 | 8 | 7.22 ± 0.08*** | 13.25 ± 0.13*** | 15.25± 0.14*** | 12.875± 0.13*** | 11.00 ± 0.12*** |
| | 150 | 8 | 7.25 ± 0.09*** | 15.87 ± 0.05*** | 18.37± 0.07*** | 15.25± 0.06*** | 13.22 ± 0.06*** |
| | 200 | 8 | 7.35 ± 0.06*** | 16.65 ± 0.05*** | 19.37± 0.07*** | 16.00± 0.07*** | 14.37± 0.07*** |
| | 250 | 8 | 7.37 ± 0.07*** | 16.72 ± 0.06*** | 19.45± 0.06*** | 16.07± 0.05*** | 14.42± 0.05*** |

Each group represents the mean ± S.E.M. for eight animals.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with control; Student-Newman-Keuls test.

**Table 2**

Effect of *H. perforatum* extract on the writhes induced by acetic acid in mice

| Group | n | Dose (mg/kg, i.p.) | No. Writhings (Mean ±S.E.M.) | Inhibition (%) |
|---|---|---|---|---|
| Control | 8 | Saline | 46.80 ±1.32 | - |
| *H. perforatum* | 8 | 25 | 42.00 ±1.83* | 10.26 |
| *H. perforatum* | 8 | 50 | 16.25 ±1.38** | 65.28 |
| *H. perforatum* | 8 | 75 | 3.50 ±0.87** | 92.52 |
| *H. perforatum* | 8 | 100 | 3.25 ±0.48** | 93.05 |
| *H. perforatum* | 8 | 150 | 0.00 ±0.00** | 100.00 |
| Indomethacin | 8 | 5 | 17.14 ±1.71** | 63.38 |
| Morphine | 8 | 1 | 7.75 ±1.49** | 83.44 |

*$P < 0.05$, **$P < 0.001$, compared with control; Student-Newman-Keuls test.

**Table 3**

Effects induced by *H. perforatum* extract (HP) administrated i.p. in the rotarod test.

| Treatment | Dose | Number of falls in 45 s (mean ± S.E.M.) | | | |
|---|---|---|---|---|---|
| | | Min after extract treatment | | | |
| | | 15 | 30 | 45 | 60 |
| Control | - | 0.25 ± 0.16 | 0.50 ± 0.19 | 0.50 ± 0.19 | 0.43 ± 0.20 |
| HP | 100 | 0.25 ± 0.16 | 0.25 ± 0.16 | 0.25 ± 0.16 | 0.25 ± 0.16 |
| | 150 | 0.25 ± 0.16 | 0.37 ± 0.18 | 0.25 ± 0.16 | 0.25 ± 0.16 |
| | 200 | 0.37 ± 0.18 | 0.50 ± 0.27 | 0.50± 0.19 | 0.50 ± 0.19 |

Each group represents the mean ± S.E.M. for eight animals. There are not significant difference between groups.

In the writhing test, the extract at doses of 25 mg/kg (*P<0.05*), 50, 75, 100 and 150 mg/kg (P0.001) produced a significant decrease in the number of writhing in comparison with the controls. These results may support the hypothesis of *H. perforatum* participation in the inhibition of prostaglandin synthesis since the nociceptive

mechanism of abdominal writhing induced by acetic acid involves the process or release of arachidonic acid metabolites via cyclooxygenase, and prostaglandin biosynthesis (Franzotti *et al.*, 2000) (Table 2).

Acetic acid writhing and hot plate tests are normally used to study the peripheral and central analgesic effects of drugs, respectively (Amabeoku *et al.*, 2001). In the present study, *H. perforatum* extract attenuated the acetic acid writhing and hot plate thermal stimulation. These observations may also support the previous hypothesis that this plant could be producing its effects both peripherally and centrally. In the current investigation, we have clearly demonstrated that i.p. injection of *H. perforatum* extract dose dependently produces an anti-nociceptive effect by using three different experimental procedures (formalin, hot plate and writhing tests). Based on the results of this study, we suggest that the anti-nociceptive effect of this extract may be attributed to inhibition of prostaglandin synthesis or release and other mediators.

Kumar *et al*. (2001) studied anti-inflammatory and analgesic activity of 50% aqueous ethanolic extract of Indian variety of *H. perforatum* at the doses of 100 and 200 mg/kg, p.o., using carrageenan induced edema, cotton pellet induced granuloma, tail flick, hot plate and writhing tests. Indian *H. perforatum* extract showed significant anti-inflammatory and analgesic activity at both dose levels.

We have also tested the analgesic doses of extract for its effect on motor coordination by rotarod test. The extract (100, 150 and 200 mg/kg) did not display any significant effect on the motor coordination of animals when tested in the rotarod test (Table 3).

In conclusion, we have demonstrated, using conventional pharmacological models, the analgesic and anti-inflammatory properties of Iranian *H. perforatum* extract. These observations support some of the traditional uses of the plant for medicinal purposes. Further studies are necessary to elucidate the mechanism behind its traditional effects.

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