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HYPERICUMPERFORATUM L. SHOWED ANTINOCICEPTIVE EFFECTS THROUGH OPIOIDERGIC AND SEROTONERGIC SYSTEMS

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Abstract

There are some reports concerning the antidepressant effects of *Hypericumperforatum L.(Hp)*, but there are few studies indicating its antinociceptive effects of *Hp*. For assessment of its site of antinociceptive action and related systems, *Hp* was filtered and administered both intrathecally *(i.t.)* and intracerebroventriculary*(i.c.v.)*. For evaluation of probable role of serotonergic and opioidergic systems, Methysergide (non-selective antagonists of serotonergic systems) andnaloxone hydrochloride (antagonist of opioidergic system) were used respectively. In central administration, the *Hp*(1 and 2 mg/rat, i.t.) induced analgesia in the tail flick and both phase of formalin test. The *i.c.v.* administration of *Hp* (2mg/rat) produced analgesia in both phases of formalin test, while it had no effect on tail flick latency. Methysergide pretreatment inhibited antinociceptive effects of *Hp* in both phases of formalin and tail flick tests. Naloxone inhibited antinociceptive effects of *Hp* in both phase of formalin test, while it had no effect on tail flick latency. The results showed that central administration its spinal effect seems be more potent than its cerebral effect. It seems that part of antinociceptive effect of *H*p it's related to serotonergic and opioidergic systems.

Keywords: Opioidergic, serotonergic, Hypericumperforatum L, antinociceptive, Rat.

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1. Introduction

Pain is an unpleasant sensory and emotional experience which is developed associated with actual or potential tissue damage (Saarto, & Wiffen, 2010). Around the world, millions of people suffer from the pain and hope to find drugs with higher efficacy and fewer side effects to overcome their pain (Millan, 2002). The pain is described as one of the vital signs that lack of attention will have enormous negative consequences. Stimulation of certain areas of the brain stem can reduce or inhibit pain sensation. These areas include the abdominal area of diencephalon, periaqueductal gray matter of the brain, and the midline nuclei of brain stem (Sepehri, 2011).

Serotonin is an important mediator of pain and its important role in the pain management has repeatedly been investigated. $5HT_1$ and $5HT_2$ and $5HT_3$ receptors have been identified in the spinal cord and there are different and sometimes contradictory reports about the role of these receptors in pain transmission and control pain in different tests (Abe et al. 2009). Increase in the activity of serotonergic pathway is associated with increased analgesic effect and reduction of the activity of these neurons leads to increased sensitivity to painful factors (Berne et al., 2004).

2. Problem Statement

One of the most important factors in pain controlling is in the opioids system and analgesia induced by opioids is due to the presynaptic effects of C fiber neurons and the second degree neurons, as well as the impact on the interneurons of the spinal cord. Analgesic effects of opioids are applied through raphe Magnus nucleus and gray matter around the Sylvian aqueduct (Wagner et al. 2013). Frequent use of opioids leads to three states of tolerance, psychological dependence and physical dependence (Udenfriend, & Meienhofer, 2014).

Since standard therapies have potential side effects and no effectiveness (the use of chemical and synthetic drugs) then the use of other complementary medicines, particularly herbal treatments for pain management is increasing (Sepehri, 2011). *Hypericum* is a genus of the *Hypericaceae* family which has about 400 different species. This plant grows in warm regions including the Mediterranean region and some of the species of this plant are used in traditional medicine as an anti-worm, diuretic, wound healing and the treatment of herpes (Trovato, 2001). Previous studies have reported analgesic effects of *Hp* (Khaksarian et al. 2004; Raak et al. 2012). Additionally in recent years, reports of this herbal alternative treatment have been published for treatment of mild to moderate depression mediated by serotonergic and adrenergic mechanisms (Jean, Pouligon, & Henriot, 2006).

3. Research Questions

Considering that Hypericum has analgesic and antidepressant effects of and former study in which we reported analgesic effects is in the spinal cord as the main effect of the aqueous extract then the probability of analgesic effects of serotonergic and opioidergic systems in the spinal cord can be mentioned (Biranvand, & Khaksarian, 2008).

4. Purpose of the Study

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Therefore this study aimed to investigate the role of serotonergic and opioidergic systems in the analgesic effects of aqueous extracts of *HP*at the level of the spinal cord.

5. Research Methods

5.1. Animals

70 male Sprague-Dawely rats weighing 200-230 g were used for all tests.

Extract preparation method, determination of the tolerable dose range, and cannula replacing into the spinal cord and *i.c.v* is available in our previous report (Biranvand, & Khaksarian, 2008).

5.2. Drugs

Methysergide (non-selective antagonists of serotonergic systems) and naloxone hydrochloride (antagonist ofopioidergic system) were purchased from Sigma Co.

5.3. Formalin test

All tests were carried out between 9 am to 5 pm at the laboratory temperature (21-23ºC) and in a relaxed and stress-free environment. 20µl formalin2.5% was injected by an insulin syringe subcutaneously into the right metatarsus. Then the animals were placed inside transparent plexiglass box with dimensions of 30*30*30cm, below which there was a 45 degree angle mirror. According to the animal dominant movement a score was given every 15 seconds and pain of animals was divided into four grades according to the following paragraphs:

0: animal sits regardless of the injected foot or walks, or stands on both legs evenly

1: The animal's foot is in contact with the compartment, but animal throws its body weight more on the healthy leg

2: Animallifts its feet completely from the floor.

3: Animal licks, chews, or severely moves the injected foot.

Zero to five minutes as the first phase (acute pain) and from 16 to 60 minutes as the second phase (chronic pain) were analyzed.

5.4. The Tail-flick test

To perform this test, the device of Tail-flick model812 made by HSE company was used. Animal were put in the Restrainer box horizontally with the hanging tail and after animal relaxed, the light intensity of 7 was used while 10 seconds was considered as cut off time to prevent tissue damage. Tail withdrawal latency was measured for each rat after light exposure (predrug-latency) and after injection (postdrug- latency) three times at intervals of 1 minute and the average response time delay (LT) was

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recorded. The analgesic amount created by the maximum possible analgesia (% MPE) was calculated according to the following formula.

% MPE = [(LT-BL) / 100BL] × 100

MPE (Maximum possible effect) is the maximum possible effect, BL (Base line)

5.5. Basic latency time and LT (Latency time) and Intrathecal injection drug (IT)

Methysergidedrugs were prescribed as 30 micrograms per animal and naloxone HClas 5 micrograms per animal 15 minutes before the intraperitoneal administration of aqueous extracts of *Hp* (400 mg/kg) and animals were examined by formalin test and tail flick test.

5.6. Intrathecal injection of aqueous extracts of *Hp* in the formalin test

The aqueous extract at doses of 1 and 2 mg per mouse and saline by volume of 10 ml were injected within one minute using a 25-ml Hamilton syringe. 10 minutes later, 50 µl of formalin 2.5% was injected into the right metatarsus andthe animals were immediately placed in the formalin test box and their pain behaviors were evaluated for 1 hour.

5.7. Intrathecal injection and ICV injection of aqueous extracts of *Hp*in tail flick test

At first the latency time of test was measured before treatment and then the aqueous extract and saline in doses of 1 and 2 mg/rat with a volume of 10µL within a minute using a 25-ml Hamilton syringe was injected into the spinal and intracranial. 5 minutes later, the delay time after treatment was measured through tail flick testthe ICV injection was carried out at the same way.

Each of Methysergideand naloxone hydrochloride drugs were injected for separate groups with 10 µL/min duration using the Hamilton syringe 25 ml for intrathecal injection and 15 minutes later, aqueous extracts of *Hp* was injected intraperitoneally, while after 30 minutes formalin test, and after 25 minutes, tail flick test were conducted.

5.8. Statistical analysis

Results were expressed as Mean ± SEM. Statistical calculations were conducted based on the paired t-test, unpaired t-test, one-way ANOVA, and Tukey test. $P < 0.05$ was considered as significant level.

6. Findings

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3.1. Sereonegic system

3.1.1. Sereonegic system in formalin test

The role of the serotonergic system in the analgesic effect of *Hp*extract in the formalin test: pre-treatment of intrathecal administration of Methysergideat a dose of 30µg/rat reduced a portion of the analgesic effect of intraperitoneallyinjection of aqueous extract (at a dose of 400 mg/kg) in both phases of Formalin test (P <0.001) (Figure 01 & 02). It should be noted that the choice of the dose for the extract was because this plant inhibited pain in both phases of Formalin test.

3.1.2. Sereonegic system in Tail flick test

The role of the serotonergic system on analgesic effect of *Hp* extract in Tail flick Test: intrathecal injection of Methysergide had no effect on the Tail flick latency. However the intrathecal pretreatment with Methysergide 30µg/rat inhibits the analgesic effect of intraperitoneal injection of aqueous extracts of *Hp* (*P <0.05) (Figure03 A& B). Intraperitoneal administration of aqueous extract of *Hp* increased latency of test.

3.2. Opioidergic system

3.2.1. Sereonegic system in formalin test

The effect of Opioidergic system on the analgesic effect of Hp extract in the formalin test: intrathecal injection of naloxone 5µg/rat could substantially increase the pain in the first phase of the formalin test Figure 04(*P <0.05), but had no impact in the second phase Fig.5. The pre-treatment intrathecal dose of naloxone at a dose of 5µg/rat reversed analgesic effects of intraperitoneal administration of aqueous extract significant with P <0.001 in the first and second phases. The difference between naloxone - saline group and naloxone - extract group test was significant in both phases (***P<0.001) (Figure 04 & 05).

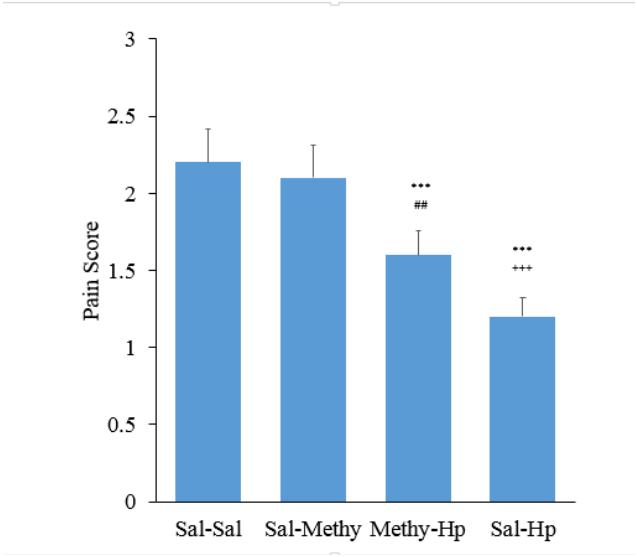

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Figure 01. Pre-treatment with intrathecal injection of Methysergide(Methy)30µg /rat on analgesia induced by intraperitoneal injection of 400mg/kg aqueous extract *Hp*of the first phase of the formalin test. (n=6). Methysergid significantly reduced antinociceptive effects of Hp extract in comparing Saline-Saline(sal-sal) group(***p<0.001), Sal-Methy(+++p<0.001) and Meth-Hp(##p<0.01). One-way ANOVA with post-hoc Tukey Test.

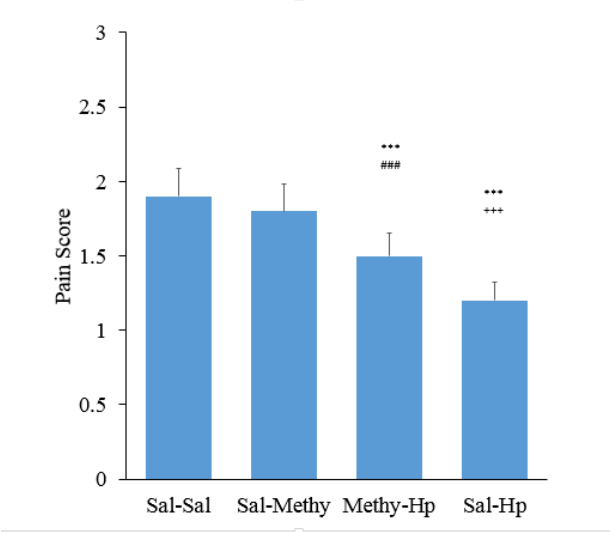
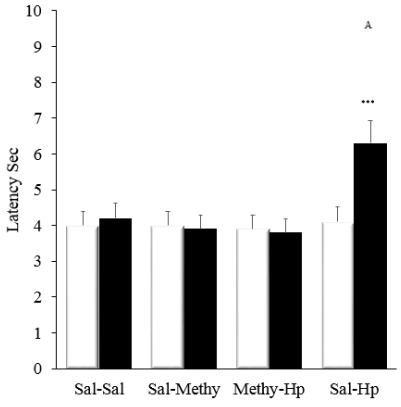


Figure 02. Pre-treatment with intrathecal injection of Methysergid (Methy)30µg /rat on analgesia induced by intraperitoneal injection of 400mg/kg aqueous extract *Hp*of the second phase of the formalin test.(n=6). Methysergid significantly reduced antinociceptive effects of Hp extract in comparing Saline-Saline (sal-sal) group(***p<0.001), Sal-Methy (+++p<0.001) and Meth-Hp(###p<0.01). One-way ANOVA with post-hoc Tukey Test.



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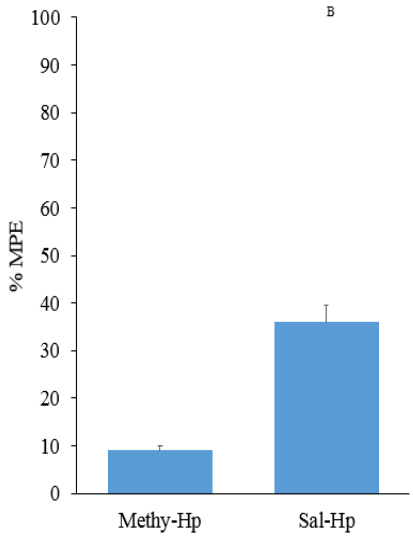

Figure 03. Pre-treatment with intrathecal injection of Methysergide(Methy) 30µg /rat on analgesia induced by intraperitoneal injection of 400mg/kg aqueous extract *Hp* on Tail flick test.(n=6). A. Predrug □ and post drug ■ latency were measured(by paired t-test). B.Maximum possible effect (MPE) inhibited by Methysergid (Methy) pretreatment (*p<0.5, un-paired t-test).

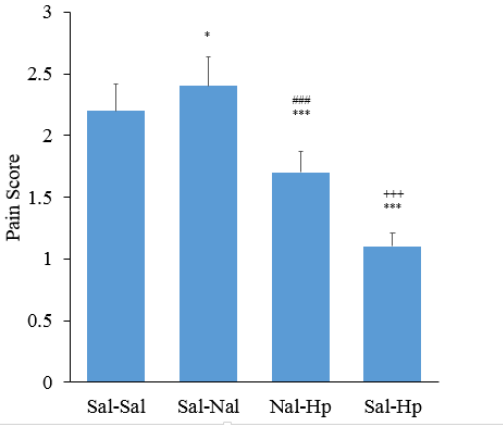

Figure 04. Pre-treatment with intrathecal injection of Naloxane(Nal)5µg /rat on analgesia induced by intraperitoneal injection of 400mg/kg aqueous extract *Hp*of the first phase of the formalin test.(n=6). Naloxane (Sal-Nal group) significantly produced pain score in comparing Saline-Saline(sal-sal) group(*p<0.05), however Naloxane(Nal-Hp) in comparing Sal-Hpat least +++p<0.001) I nhibited some part of aninociceptive effects of Hp. One-way ANOVA with post-hoc Tukey Test.

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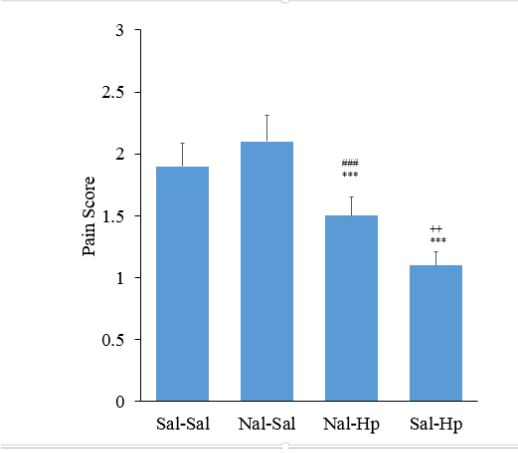

Figure 05. Pre-treatment with intrathecal injection of Naloxane (Nal)5µg /rat on analgesia induced by intraperitoneal injection of 400mg/kg aqueous extract *Hp*of the second phase of the formalin test.(n=6). Naloxane significantly reduced antinociceptive effects of Hp extract in comparing Saline-Saline(sal-sal) group(***p<0.001), Sal-Nal(++p<0.01) and Meth-Hp(###p<0.01). One-way ANOVA with post-hoc Tukey Test.

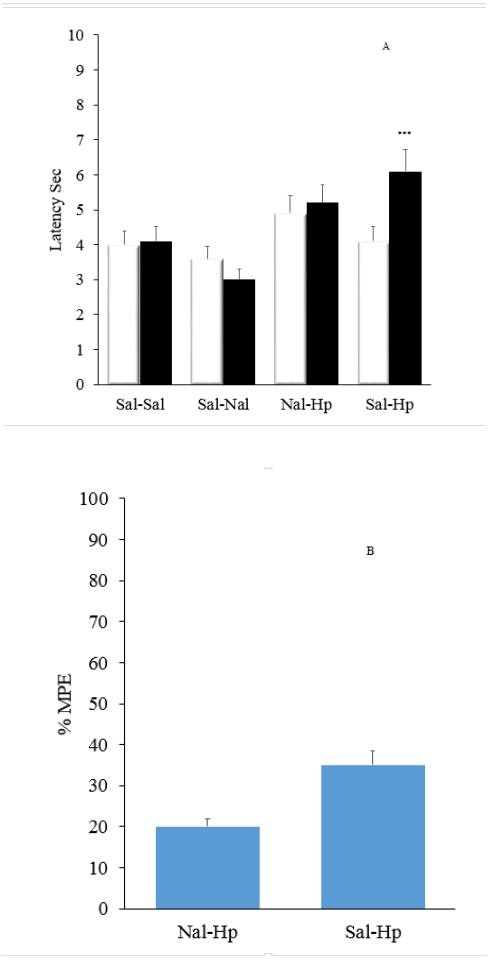

Figure 06. Pre-treatment with intrathecal injection of Naloxane(Nal) 5µg /rat on analgesia induced by intraperitoneal injection of 400mg/kg aqueous extract *Hp* on Tail flick test.(n=6). A. Predrug □ and post drug■ latency were measured. Nalderceased latency time significantly(*p<0.05 by paired t-test) AND Hp increased latency time (***p<0.001). B. Maximum possible effect (MPE) unchanged by Nalpretreatment (*p<0.5, un-paired t-test).

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Pretreatment with intrathecal injection administration of naloxone before intraperitoneal injection of extract could not change the time delay of Tail flick test. Naloxone alone increased pain in Tail flick test. Figure 06.

7. Conclusion

The results of this study showed that pain reduction at the first phase of the formalin test is mediated by serotonin receptors in some parts and by opioid receptors in other parts. Pretreatment with intrathecal injection administration of MethiSerjid before intraperitoneal injection of extract removed a part of the analgesic effect of the extract the first phase of the formalin test. This issue can suggest blocking the analgesic effects of extract through serotonergic receptor antagonists in the first phase of the formalin test. Apadins and colleagues in 1999 proposed serotonin and noradrenaline reuptake and inhibition properties for the analgesic effect of triquetrifolium Turrafrom Hypericaceae family (Sanchez-Mateo et al. 2006). 5HT1A receptor agonists reduce pain caused by the formalin (Millan, 2002). Intrathecal serotonin administration facilitates pain at lower doses and at high doses could antagonize the pain (Zeitz et al. 2002). It seems that a part of the analgesic effect of *Hp* which is applied through serotonergic system is related to the 5HT1A receptors that actually needs more accurate investigations. MthySerjide can change pain behaviours of the animal (Kümper, 1989). Naloxone increases animal pain. Pretreatment with intrathecal injection of naloxone before intraperitoneal administration of extract removed a part of the analgesic effect of the extract in the first phase of the test. This proposes involvement of the opioid system in the analgesic effects of *Hp* extract in the formalin test. But it should be considered that naloxone alone increases the pain at the first phase of the formalin test that is a part of adverting effects of the extract of due to the hyperallergenic effect of naloxone. However, the increase in pain is significant compared to saline by blocking the analgesic effect (8% vs. 57%).This proves the interference of opioid system in the analgesic effects of the extract. It has been reported that intrathecal naloxone injection increased pain by formalin (Al, 1995). It seems that the analgesic effect of the *Hp* extract through opioid system is probably due to binding to the μ receptor is probably that needs to be investigated. Finally, experimental results have shown that formalin increases the activity of C fibers in the first phase of the formalin test. Reducing the activity of C fibers through *Hp* extract can be mentioned that this effect may be associated with increased levels of monoamines such as serotonin through descending pathways of pain suppression by the extract.

Albert et al. (2002)showed that the extracts of *Hp* can inhibit the COX-1 and 5-LO. Therefore in addition to the explained mechanisms including opioidergicand serotonergic systems, there is another possibility for the analgesic properties of *Hp* extract in the second phase of the formalin test which is the inhibition effect of extract on COX-1 and 5-LO. It has been observed that after the release of leukotrienes from damaged tissues, the sensory fibers become more sensitive that the sensitivity occurs through D4 and B4 leukotrienes which are the metabolites of lipoxygenase pathway. 5-LO inhibitors inhibit the production of leukotriene D4 and B4. On the other hand, the release of prostaglandins and leukotrienes B4 in inflammatory processes is an amplifier for pain mechanisms (Kümper, 1989; Kaehler, 1999; Obach, 2000) that this effect is blocked by COX-1 and 5-LO inhibitors (Obach, 2000). On the other hand leukotriene D4 antagonists and inhibitors of COX-1 reduces tonic pains, such as the second phase of the

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formalin test and reduce pain caused by acetic acid (Kaehler, 1999; Nathan, 2001; Perfumi et al. 2001). 5-LO inhibitory role in the release of prostaglandins and different leukotrienes production during the second phase of the formalin test (Kümper, 1989; Obach, 2000; Perfumi et al. 2001; Dimpfel, Todorova, & Vonderheid-Guth, 1999) is extremely clear. Given the role of 5-LO inhibitors in the production of arachidonic metabolites acid and the metabolites such as leukotriene D4 (Kaehler, 1999) and prostaglandins (Perfumi et al. 2001) in tonic pains such as the second phase of the formalin test can suggest possible analgesic effect of the extract through effects inhibition of COX-1 and 5-LO introduced in the second phase of the test.

Ineffectiveness of the analgesic effect of the extract above the spinal cord obtained in the Tail flick test could be due to the different mechanisms of pain in formalin test and the mentioned test. It should be noted that the effects observed in the formalin test also confirmed more prominent spinal analgesic effects of extract in the spinal cord compared with levels above the spinal cord. MethiSerjid itself had no effect on latency of test; however pretreatment caused by intrathecal injection of MethiSerjid before injection of aqueous extracts of Hypericum intraperitoneally inhibited the analgesic effect of the extract. Therefore it can be suggested that a part of the analgesic effect of the extract in the Tail flick test is serotonergic.

On the other hand it is reported that chronic administration of *Hp* extract increases postsynaptic 5HT1A receptors (Butterweck, 2003). 5HT1 receptor agonists also increase latency in the Tail flick test but selective 5HT1A agonists do not affect Tail flick test latency (Dimpfel, & Hofmann, 1995). According to the agonistic behaviour of *Hp* extract on 5HT1A receptors and non- possible interference of 5HT1A receptors on Tail flick test response in the studied groups, it appears that inhibitory responses created in the test Tail flick is through 5HT1A receptors. It has been shown that antagonizing 5HT1A and 5HT1B receptors the no impact on latency of test. Due to multiplicity of serotonergic receptors and their role in producing analgesia (Berne et al., 2004). The presence of antinociceptive effects through the serotonergic receptors other than the 5HT1A receptor is expressed in Tail flick test which requires further investigation. Pretreatment with intrathecal injection of naloxone before extract administration did not change Tail flick test latency. Naloxone alone increased pain in Tail flick test that is similar to results of other researchers (Levine et al. 1978; Dulu et al. 2014). According to these findings interference of opioid system in the analgesic effects of the extract in Tail flick test cannot be suggested.

In the present study, the possible effects of other involved systems such as alpha-adrenergic system has not been investigated which is the limitation of this report; however, in another study the role of alpha-adrenergic system is studying. On the other hand, although the results of this study showed that much of the analgesic effects of *Hp* extract is caused by the involvement of the serotonergic and opioidergic system other mechanisms may be involved in the analgesic effects of *Hp* extract that it is not that dissimilar to the analgesic effect of tramadol. In general, it can be concluded that aqueous extract of the medicinal plant Hypericumapplies at least a part of its analgesic effects through serotonergic and opiodergicsystems in acute and chronic pain of Formalin test but the analgesic effect of aqueous extracts of *Hp* in thermal pain test of Tail-Flick is not applied through the opioidergic system but the interference of opioidergic the system through Tail flick test remains elucidated.

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