

Effects of *Papaver rhoeas* Extract on the Tolerance Development to Analgesic Effects of Morphine in Mice

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Abstract

Previous studies have shown that the extract of *Papaver rhoeas* reduces morphine dependence, locomotor activity and reward. In present study, the effects of hydro-alcohol extract of *Papaver Rhoeas* on the tolerance to analgesic effects of morphine in mice have been investigated using tail flick method. Subcutaneous (s.c.) administration of morphine (1, 2, 5 and 10 mg/kg) induced analgesia. However, intrapretoneal administration of the hydro-alcohol extract of *Papaver rhoeas* (25, 50 and 100 mg/kg) had not an effects on analgesia. Reduction of analgesic in mice pretreated with morphine (50 mg/kg, twice daily; for 3 days), alone, indicated that tolerance has been developed. Hydro-alcohol extract of *Papaver rhoeas* (25, 50 and 100 mg/kg, i.p.) administration, 30 min before each of three daily doses of morphine, attenuated the morphine tolerance dose-independently, indicating that administration of the extract reduces morphine tolerance in mice.

Keywords: *Papaver rhoeas*; Morphine; Analgesia; Tolerance; Mice.

Introduction

Tolerance to opioids, defined as a loss of effect following repeated treatments which limits the clinical usefulness of morphine (1-3). On the

basis of investigations, it is thought that chronic exposure of spinal and supraspinal areas to morphine and other opioids can cause neuronal adaptations, which compromise opioid activity (3-6). From the molecular perspective, opioid tolerance is associated with an up-regulation of the cyclic adenosine mono phosphate (cAMP) pathway in the target cells (4, 6-7), opioid

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receptor down-regulation, phosphorylation, internalization and desensitization (3, 8). It has also been shown that G-proteins phosphorylation also occurs in the cells exposed to opioids (6-7). Other studies have proposed a role for dopamine (9-11), acetylcholine (12), glutamate (13-14) and nitric oxide (15) in morphine tolerance.

However, since morphine tolerance remains unresolved, attempts were made world wide to find out the new ways for its inhibition. *Papaver rhoeas* L. (Papaveraceae) is an annual herb indigenous to Iran and many other regions in the world (16). In Iranian folk medicine, the plant extract has been used in treatment of a wide range of diseases including inflammation, diarrhea, sleep disorders, treatment of cough and pain, and also reduces the withdrawal signs of opioid addiction (16). It is also claimed that *P. rhoeas* exhibits sedative, narcotic, and emollient effects (16). Also, it is claimed to cause intestinal and urinary irritation and to be useful in various conditions such as bronchitis, pneumonia, and rash fever (16). Chemical studies have demonstrated the presence of rhoeadine, rhoeadic acid (17-18), papaveric acid (16), rhoeagenine (19) and anthocyanins (20) as the major compounds of the extract of *P. rhoeas*.

Our previous research has focused on the effects of the *P. rhoeas* on expression and development of morphine-dependence (21), morphine-induced conditioned place preference (22) and morphine-induced behavioral sensitization (23) in mice while, the effects of the extract of *P. rhoeas* on morphine tolerance is not yet clear and it needs further investigation.

Considering the role of opioidergic (1-3, 8), dopaminergic (9-11) and cholinergic (12) systems on morphine tolerance and the anti-opioidergic, anti-dopaminergic and anti-cholinergic activities of the extract of *P. rhoeas* (16), the present work was undertaken to evaluate the effects of *P. rhoeas* extract on the tolerance to analgesic effects of morphine in mice.

Experimental

Animals

Male albino Swiss-Webster mice (20-25 g, Pasture Institute, Tehran, Iran) were used (8 mice

for each experiment). The animals were housed 10 per cage with 12/12 h light-cycle with *ad-lib* food and water available.

Drugs

The morphine sulfate (TEMAD company, Iran) was used in the present study. Morphine sulfate and the extract were dissolved in normal saline and given in a volume of 10 ml/kg and were prepared immediately before use.

Plant Material

P. rhoeas was collected from Kermanshah region (west of Iran). The plant was authenticated by M. Kamalinejad (Department of Pharmacognosy, School of Pharmacy, Shaheed Beheshti University of Medical Sciences) and a voucher number P-147 has been deposited at the herbarium of Department of Pharmacognosy, School of Pharmacy, Shaheed Beheshti University of Medical Sciences.

Preparation of the extract

The extract preparation method was prepared as follows: 500 ml of 50% ethanol in water (v/v) was added to 50 g of the total plant powder (including fruit, petal, root, stem and leaf) and the mixture was left to macerate at room temperature for 20 h. The basin was slowly rotated during this time. After filtration, ethanol was evaporated at low pressure at 33 °C and the extract was freeze-dried. The extraction yield was 15 g of freeze-dried powder for 100 g of the dry plant. The extract was dissolved in normal saline and was immediately administered intraperitoneally (i.p.) to the mice expressed as mg of extract per kg body weight.

Calculation of ID_{50}

Inhibitory Dose 50% (ID50) of the extract was calculated using the GraphPad Prism version 2 computer software. According to our previous results (24) the ID50 values by 95% confidence interval was: 29.8 mg (8.35 mg-51.68 mg). The doses of the extract used in the present study were chosen according to ID50 values.

Assessment of analgesia

Tail flick latency was obtained using a tail

flick unit (Hugo-Sachs Elektronik, Model 230, Germany). Briefly, radiant was focused on a blackened spot -2 cm from the tip of the tail, and the latency until the mouse flicked its tail was recorded. Beam intensity was adjusted to give a tail flick latency of 2-3 sec in control animals (9, 25). Each mouse was tested 60 and 30 min before drug administration for the baseline latency determination and only once after drug administration. The measurement was terminated if latency exceeded the cut off time (10 sec) to avoid tissue damage.

Experimental design

Experiment 1: morphine and P. rhoeas extract analgesia

Animals were put in the special cages and transferred to the experiment room by the experimenter, 1 h before the experiments were beginning. After 30 min for adaptation, each mouse was tested for analgesia on 1st and 30th min. Then, each animal received different doses of morphine or *P. rhoeas* extract (25, 50 and 100 mg/kg) and 30 min later third analgesia test preformed. Control groups received saline.

Experiment 2: effects of morphine on the induction of tolerance

Mice were transferred from the vivarium to the laboratory. Two groups of animals were given morphine (50 mg/kg, s.c.; twice daily) or saline (10 ml/kg, s.c.) and placed in their home cages. At the end of this period, they were returned to the vivarium. This period was repeated for day 2 and day 3. On the day 4, mice were transferred to the laboratory, received morphine (10 mg/kg, s.c.), placed in the apparatus and evaluated for analgesia.

Experiment 3: effects of P. rhoeas extract on the analgesic tolerance induced by morphine

Mice were injected with various doses of *P. rhoeas* extract (25, 50 and 100 mg/kg, i.p.) or saline (10 ml/kg, i.p.) and immediately placed back in their home cages in the vivarium. Thirty minutes later, mice were received morphine (50 mg/kg, s.c.) in their home cages. This period was repeated for day 2 and day 3. All of the groups were transferred to the laboratory on day 4,

received a challenge dose of morphine (10 mg/kg, s.c.), immediately placed in the apparatus and evaluated for analgesia.

Statistical analysis

All data were represented as mean±SEM. In order to test the hypothesis, Un-paired t-test (effects of morphine on the induction of analgesic tolerance) or One-way analyses of variance (ANOVA) followed by Tukey test (Morphine and extract induced analgesia and effects of *P. rhoeas* extract on the analgesic tolerance induced by morphine) were performed to assess specific group comparisons. Differences with $P < 0.05$ were considered statistically significant.

Results

Experiment 1: Analgesia included by Morphine and P. rhoeas extract

The effects of morphine and extract on analgesia are shown in Figure 1 and 2. Results indicated that morphine (1, 2, 5 and 10 mg/kg, s.c.) administration induced nociception in the animals [$F(4, 36) = 6.51, P < 0.0001$] (Figure 1). However, administration of different doses of the *P. rhoeas* extract (25, 50 and 100 mg/kg, i.p.) did not induced nociception in the animals [$F(3, 28) = 0.94, P > 0.05$] (Figure 2).

Experiment 2: Effects of morphine on the induction of analgesic tolerance

As shown in Figure 3, analgesia significantly decreased in mice pretreated with morphine (50 mg/kg, twice daily X 3 days), compared with mice pretreated with saline [$t_{14} = 7.34, P < 0.0001$].

Experiment 3: Effects of P. rhoeas extract on the analgesic tolerance induced by morphine

Pretreatment of the animals with different doses of the *P. rhoeas* extract (25, 50 and 100 mg/kg, i.p.) significantly decreased the analgesic tolerance induced by morphine [$F(5, 44) = 5.2, P < 0.0001$] (Figure 4).

Discussion

In the present experiments, we examined the

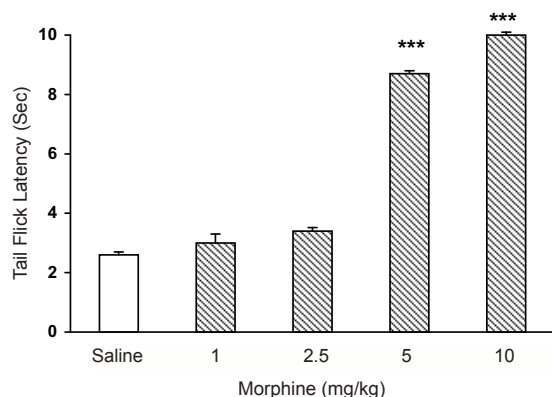


Figure 1. Effect of different doses of morphine on analgesic response. Animals received morphine (1, 2, 5 and 10 mg/kg, s.c.) or saline (10 ml/kg, s.c.) as described in the method section. Each point is the mean±SEM for eight mice. ***p<0.001 different from the saline control group.

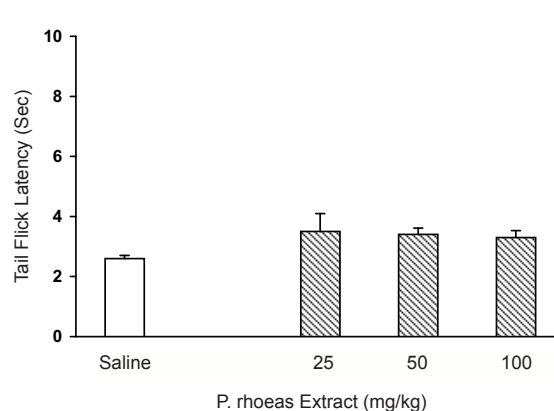


Figure 2. Effect of *P. Rhoeas* extract on the analgesic response. Mice were received either different doses of the extract (25, 50 and 100 mg/kg, i.p.) or saline (10 ml/kg, i.p.) as described in the method section. Each point is the mean±SEM for eight mice.

effects of *P. rhoeas* extract on the analgesic tolerance induced by morphine.

In accordance with the previous studies, our data shows that acute administration of morphine induces analgesia in the animals (9-15) in the morphine-naïve mice. The analgesic effects of morphine are generally attributed to its effects on different opioid receptors, especially μ -opioid receptors within the central nervous system (1-2). However, another opioid receptor sub-types including δ - and κ -opioid receptors are also considered in analgesic responses of morphine (3-4, 8). The same mechanisms would be responsible for results obtained in the present study. In addition, our results showed that the extract did not induce analgesia in doses used in the present study. Our previous studies have shown that the extract did not induce place preference (22), dependence (21) and behavioral activity (23), which are in agreement with the present study. As mentioned above, the extract exhibits mild anti-opioid and anti-dopaminergic activity (16). Considering the role of opioid (1-2) and dopamine (25) systems in analgesia, one may conclude that the extract might have a hyperalgesic effect. However, remembering our previous results on the lack of effectiveness on place preference, dependence and locomotion (21-23), it is expected to observe no effects from the extract

on analgesia in the present study.

Results obtained in next part of the experiments, showed that repeated injections of morphine induced tolerance to analgesic effect of the opioid, which is indicated by a lower latency in response to the challenge dose of morphine. Our present data are consistent with the previous findings that indicate development of tolerance to the analgesic effects of morphine [9-10, see 3 for review]. Previous investigators have demonstrated a postsynaptic decrease in opioid receptor number (2-3, 8) and sensitivity (8) following the chronic administration of morphine. In addition, an increase of cyclic-Adenosine-Mono-Phosphate (cAMP) concentration also was detected in different brain areas of morphine-tolerated animals (4, 7), which is due to the enzyme adenylate cyclase (AC) overactivity following chronic morphine treatment regimen. Moreover, endogenous opioids including enkephalines are shown to be involved in this regard (25). In addition, studies revealed that inhibition of AMPA and NMDA glutamate receptors could inhibit morphine tolerance (13-14). Also, involvement of dopamine D_2 receptors (9), as well as nitric oxide (15) is well established in this regard, which might be considered in interpreting the results obtained in present study.

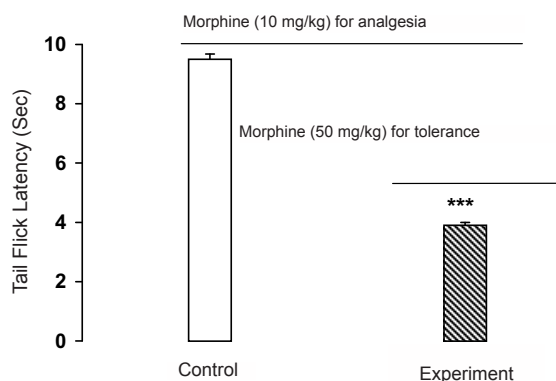


Figure 3. Effect of morphine on the development of analgesic tolerance. Two groups of animals received 50 mg/kg morphine, twice daily X 3 days or saline (in their home cage). On the day 4 of the experiments, the animals received morphine (10 mg/kg) in the apparatus and were evaluated for analgesia. Each point is the mean \pm SEM for eight mice. *** p <0.001 different from the control group.

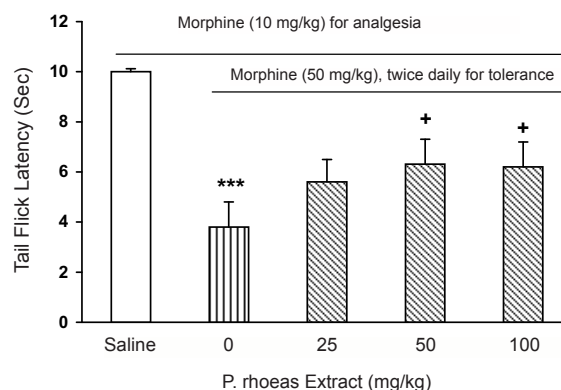


Figure 4. Effects of various doses of the extract of *P. Rhoeads* on the analgesic tolerance induced by morphine. Mice were received saline (10 ml/kg) or the extract (25, 50 and 100 mg/kg, i.p.) 30 min prior to pretreatment with 50 mg/kg of morphine, twice daily X 3 days (in their home cage). On the fourth day, all mice received a morphine challenge (10 mg/kg, s.c.) in the apparatus and were evaluated for analgesia. Values are the mean \pm SEM for eight animals. *** P <0.001, compared with the saline control group, + P <0.05, compared with the morphine control group.

Our observations indicated that pre-administration of the animals by extract, attenuates morphine tolerance. The data showed that the *P. rhoeas* extract was effective to reduce morphine tolerance. The effect of the extract was not dose-dependent. In the previous studies, however, administration of extract reduced the development of morphine dependence (21), conditioning place preference (22) and behavioral sensitization (23) in mice, which are in agreement with the results obtained in this study.

Morphine tolerance is a complex phenomenon and need the activation or inhibition of several neurotransmitter systems including opioidergic, dopaminergic and GABAergic within the brain (3, 6). In addition, different areas in the central nervous system including the spinal cord and several supra-spinal areas such as pre-aqueductal gray matter are also involved in the morphine tolerance (1-2). The extract exhibited mild anti-opioid activity (16). It is clear that inhibition of opioid receptors leads to morphine tolerance inhibition (3). The extract also exhibits anti-dopaminergic activity (26). In an interesting study, Zarrindast and co-workers have shown that dopamine D₂ receptor inhibition could attenuate morphine tolerance in mice (9). Considering these facts, one can conclude that

the extract inhibited morphine tolerance by inhibition of opioid and/or dopamine systems with in the central nervous system (CNS). However, the exact nature of this inhibition and the sites involved in the CNS, must be explored in future studies. Some investigators have shown that brain cholinergic system may have a role in morphine tolerance (12). This mechanism may also explain inhibitory effect of the extract on morphine tolerance (16), as observed in present study. Taken together, the inhibitory effects of the extract on morphine tolerance may be due to anti-opioidergic, anti-dopaminergic and/or anti-cholinergic properties of the extract. However, the role of un-known properties of the extract and also un-known pharmacodynamic as well as pharmacokinetic interaction between the extract and morphine could not be excluded.

As mentioned above, the extract contains rhoeadine, rhoeadic acid (17-18), papaveric acid (16), rhoeagenine (19) and anthocyanins (20), which may account the responses reported in present study. However, the role of these substances should be examined in the future experiments. It is suggested recommend that the effect of each these substances should be examined separately to understand the exact mechanism of the effects of the extract.

In conclusion, considering the results from this study, we suggest that the extract may be more effective to impair the mechanisms, which activated by morphine under chronic administration. Overall, the results, show that the extract of *P. rhoeas* inhibits morphine tolerance in mice, and this may be applicable to human as well.

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