

Effects of *Papaver rhoeas* (L.) Extract on Formalin-induced Pain and Inflammation in Mice

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Abstract: Stress amelioration can improve its metabolic as well as other side effects. In the present study, the effects of hydro-alcoholic extract of *Papaver rhoeas* (L.) on formalin-induced pain and inflammation were investigated in male Swiss-Webster mice (20-25 g). Formalin injects in the plantar portion of mice hind paw and pain was studied for 60 min. The plant extract and other drugs were administered intraperitoneally 30 min before formalin. Experiments showed that administration of extract (25, 50 and 100 mg kg⁻¹) could induced analgesia in a dose-response manner in both phases of formalin test. More over, the extract inhibits inflammation induced by formalin injection. Naloxone (4 mg kg⁻¹), dextromethorphan (20 mg kg⁻¹) and NG-nitro-L-arginine-methyl-ester (L-NAME; 10 mg kg⁻¹) reduced the extract analgesia in first but not late phase. Extract administration also increased plasma corticosterone level in dose-dependent manner. It could be concluded that *Papaver rhoeas* (L.) extract could inhibits acute phase of formalin test in mice by opioidergic, glutamatergic and nitricergic mechanisms. In addition, the extract can induce corticosterone plasma level which may be responsible for inhibition of inflammation and chronic phase of pain induced by formalin.

Key words: *Papaver rhoeas* (L.), analgesia, naloxone, glutamate, nitric oxide

INTRODUCTION

Pain defined as the most important sense which caused awareness from all damaging stimulations and destructive processes in organisms (Ossipov *et al.*, 2010). Pain receptors can be simulated by mechanical, chemical and thermal stimulus (Mason, 2005). Pain is the worse human experience and now enormous expenses are paid to cure and decrease it (Coutaux *et al.*, 2005; Mason, 2005; Ossipov *et al.*, 2010). For this reason drugs which decreased pain and its effects have attended by doctors and researchers. Different studies have determined pain transmission pathways in the nervous system and now these paths and their mediator neurotransmitters have been relatively known (Coutaux *et al.*, 2005; Mason, 2005; Ossipov *et al.*, 2010). These tracks are usually blocked by analgesic drugs, which can involve spinal and supra spinal sites more than peripheral paths. The non-steroidal anti-inflammatory drugs (NSAIDs) which decrease the production of arachidonic acid by products such as prostaglandins (Simmons *et al.*, 2004) are among the first line of pain relive strategies. However, gastro-intestinal hemorrhage may limit their usage. Moreover, the possible tolerance

and dependence development which are created after long time opioid drugs consumption may decrease the opioid usage. For this reason, researchers have been trying to find new medications with lower side effects and higher potency for pain killing. Plant materials are well known by human before many times and now with attention focused to use them in this matter (Zargari, 1994).

Papaver rhoeas L. is grassy plant with red flower and height 25-90 cm with growing in different parts of world including Iran (Zargari, 1994). *Papaver* have different alkaloids for example rhoeadine, rhoeadic acid, papaveric acid, mekoid acid, mucilage and sugar (El-Masry *et al.*, 1981; Gambaro *et al.*, 2012; Gurbuz *et al.*, 2003; Kalva and Sanyar, 1989). Boiled plant are used for inflammation and sleep disorders, this plant have sedative and emollient effect, too for reason a little dose of morphine in papaver extract that called "harmless opium" (Gurbuz *et al.*, 2003; Zargari, 1994). With attention to anti opioid, anti dopaminergic and antikolinergic effects of papaver extract and role of these neurotransmitter systems in analgesia (Abbott *et al.*, 1982; Mason, 2005; Ossipov *et al.*, 2010; Zarrindast *et al.*, 2002). In the present study, the effect of *Papaver rhoeas* on the pain induced by formalin test in mice was evaluated. Previous

study focused on the effects of papaver extract on withdrawal syndrome (Pourmotabbed *et al.*, 2004), place preference paradigm (Sahraei *et al.*, 2006), morphine sensitization (Sahraei *et al.*, 2009) and morphine tolerance in mice (Shams *et al.*, 2008).

MATERIALS AND METHODS

Study duration: The study was conducted from Jan 2008 until March 2009. All studies were done in the behavioral laboratory section of Neuroscience Research Center, Baqiyatallah (A.S.) University of Medical Sciences, Tehran, Iran. Experimental duration in this study was one hour and animals' response was recorded at same time.

Animals: Male albino Swiss-Webster mice (20-25 g, Pasture Institute, Tehran, Iran) were used (8 mice for each experiment). The animals were housed 8/cage with 12 h light/dark cycle with food and water available *ad libitum*.

Plant material: *Papaver rhoeas* was collected from Kermanshah region (western Iran). The plant was authenticated by M. Kamalinejad (Department of Pharmacognosy, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran) and a voucher specimen coded P-147 has been deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences (Tehran, Iran).

Preparation of the extract: The extract preparation method was as follows: 50 g of the total plant (including fruit, petal, root, stem and leaf) powder was added to 500 mL of 50% ethanol (v/v) and 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 a temperature of 33°C and the extract was later freeze-dried. The yield of extraction was 15 mg of freeze-dried powder for 100 mg of the dry plant. The extract was dissolved in normal saline and was immediately administered intraperitoneally (i.p.) to the mice, later expressed as mg of extract per kg body weight.

Pain assessment in animals: Animals were transferred to the experiment room 1 h before the experiments were beginning. After 30 min for adaptation, mice were given 2 μ L formalin solutions by subcutaneous injection in their paws and immediately placed on 30×30×30 cm boxes made of Plexiglas. Each mouse was tested for analgesia with method used elsewhere (Abbott *et al.*, 1982). Drugs were injected to the animals subcutaneously or intraperitoneally 30 min before the experiments were began. If more than

one drug were used, 30 min interval was observed between injections.

Drugs: In research dextromethorphan hydrobromide, indomethacin hydrochloride, L-NAME, naloxone hydrochloride (Sigma-USA) was used. All drugs were solved in saline, injection intraperitoneal with considering weight of mice. The extract of papaver solved in saline, and injection intraperitoneal with considering weight of mice.

Statistical analysis: All data were represented as mean \pm SEM. In order to test the hypothesis, Un-paired Student t-test or one way analyses of variance (ANOVA) followed by Tukey test were performed to assess specific group comparisons. Differences with $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Effect of different doses of *Papaver rhoeas* extract on formalin-induced pain: Animals received saline (10 mL kg⁻¹, i.p.), or different doses of *P. rhoeas* extract (25, 50 and 100 mg kg⁻¹; i.p) 30 min before formalin injection. Animal responses were evaluated 30 min later. Results indicated that the extract could suppress the acute phase of formalin-induced pain [F(4, 18) = 7.3, $p < 0.01$] (Fig. 1). Compared to morphine, dexamethasone, indomethacin and *P. rhoeas* extract suppressed pain to a similar degree in phase 2 of the formalin test [F(4, 18) = 10.28, $p < 0.0001$] (Fig. 1).

Effects of opioid and glutamate receptor inhibition and nitric oxide synthesis inhibition on *P. rhoeas* extract induced analgesia: The effect of naloxone, dextromethorphan and L-NAME on extract-induced analgesia in phases 1 and 2 of the formalin test is shown in Fig. 2. As is clear in the figures, pretreatment of the animals with the antagonists inhibit the efficacy of the extract-mediated pain inhibition [F(16, 84) = 9.81, $p < 0.001$], [F(16, 84) = 11.3, $p < 0.001$] (Fig. 2).

Present study demonstrates the ability of *P. rhoeas* extract for reduction of both acute and chronic phases of pain arising from formalin injection in mice. These findings are in contrast with previous study which reports no pain reduction after extract administration in mice as investigated by tail flick method (Shams *et al.*, 2008). Moreover, opioid, glutamate and nitric oxide mechanisms appear to be involved in this regard.

Present data showed that papaver extract similar to morphine, can inhibit both acute and chronic phases of pain in formalin test (Abbott *et al.*, 1982). However, effect

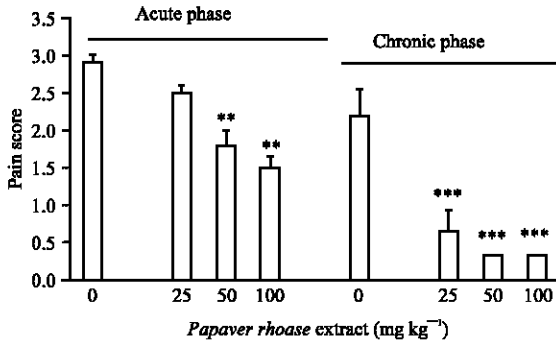


Fig. 1: Effects of *P. rhoeas* extract on acute and chronic phases of formalin test in mice. The extract can inhibit both two phases of the formalin test. Data showed as mean±SEM, for 6 mice, **p<0.01, ***p<0.001 different from experimental groups

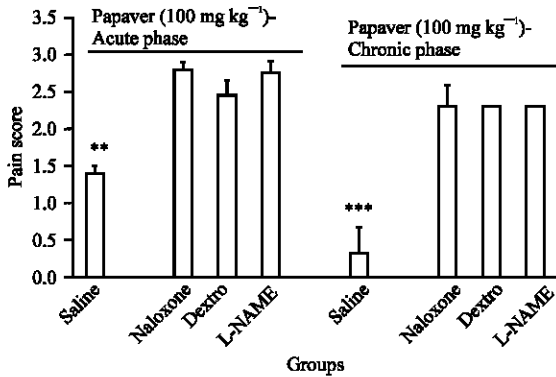


Fig. 2: Effect of opioid, NMDA receptor inhibition and nitric oxide synthase inhibition on *P. rhoeas* extracts effect on acute and chronic phases of formalin pain. The results indicated that naloxone, dextromethorphan and L-NAME did abolish the extract effects. Data showed as mean±SEM, for 6 mice, **p<0.01, ***p<0.001 different from saline treated control group

of extract disappeared after injection of naloxone, which may suggest the existence of opioid mechanism in extract function. It is important to be notice that opioid system activation is the pathway for pain reduction in the medicine. Present data also indicated that L-NAME as inhibitor of the enzyme nitric oxide synthase can reduce the extract effects on pain induced by formalin injection. Moreover, injection of dextromethorphan inhibits the extract effect which indicates the involvement of glutamate system in this regard.

Inhibition of acute phase of pain induced by formalin injection is a complex phenomenon and various

neurotransmitters pathways are involved (Coutaux *et al.*, 2005). Investigators have shown that morphine and other opioids can inhibit this phase of the formalin test (Abbott *et al.*, 1982; Zarrindast *et al.*, 2002; North, 1978). The extract action in inhibition of acute phase of formalin test indicated that the extract can interact with the pain pathways in the spinal cord (or other nervous system sites?) for inhibition of sensory information transmission. Moreover, present observation that both opioid and glutamate receptor antagonists can inhibit the extract action may indicated that the extract induce its function via such systems. In addition, inhibition of the enzyme nitric oxide synthase may indicate the involvement of nitric oxide in this regard. Previous studies also are indicate that both glutamate receptor antagonists and nitric oxide can inhibit pain in animal models and also human (Berrino *et al.*, 2003; Sawynok and Reid, 2002; Kozela and Popik, 2002; Cury *et al.*, 2011). Other findings that the extract can interact with morphine dependence (Pourmotabbed *et al.*, 2004) and sensitization (Sahraei *et al.*, 2006) may indicate that the type of the opioid receptors that activated by the extract are different with those for morphine.

CONCLUSION

Considering the results from this study, it can be suggest that the extract of *P. rhoeas* inhibits both acute and chronic phases of pain induced by formalin injection in mice which may be mediated via an opioid, glutamate and/or nitric oxide mechanisms.

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