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Reduction of Metabolic Signs of Acute Stress in Male Mice by *Papaver rhoeas* Hydro-alcoholic Extract

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Abstract: In the present study, effects of hydro-alcoholic extract of *Papaver rhoeas* L. (Papaveraceae) on the metabolic changes induced by electro foot shock stress in male NMRI mice (25-30 g) has been investigated. The mice were received electric foot shock (40 mV) for 100 sec. Plasma corticosterone levels, food and water intake and delay to eating (Anorexia) were assessed 20 min later. Different doses of the plant extract (15, 30 and 60 mg kg⁻¹), or saline (10 mL kg⁻¹) was injected to the animals intraperitoneally 30 min before the stress. The control groups received saline (10 mL kg⁻¹) or the extract (15, 30 and 60 mg kg⁻¹) and 30 min later were exposed to the apparatus but did not received stress. Our results indicated that stress can increase plasma corticosterone level significantly and the extract can exacerbate the stress effect. However, stress could reduce food and water intake and increase delay to eating times which were inhibited by the extract pretreatment. The results indicate that administration of the extract of *Papaver rhoeas* can reduce the side effects of stress but increases plasma corticosterone level which may be due to its effects on the adrenal gland.

Key words: *Papaver rhoeas*, anorexia, water intake, food intake, electro foot shock, corticosterone

INTRODUCTION

Over the past decade, there was a great achievement in the use of herbal products for treatment of psychotic disorders such as anxiety and depression (Sarris *et al.*, 2011). Herbal products such as saffron extract are used for treatment of mild to moderate depression (Sarris, 2007). In addition, data indicated that herbal medicine may be useful for Alzheimer disease (Akhondzadeh *et al.*, 2010). However, there is few data concerning the efficacy of herbal products on stress side effects amelioration. For example, Halataei *et al.* (2011), have shown that saffron extract can reduce stress-induced anorexia in mice (Halataei *et al.*, 2011). It is also interesting that saffron extract can reduce stress side effects in rats (Hooshmandi *et al.*, 2011). In this regard, it seem be useful to use other plant extract for stress management. *Papaver rhoeas* L. (Papaveraceae) is an annual herb with reddish flowers that indigenous to many regions in the world including Iran, Turkey, India and Europe (Zargari, 1995). The plant is used for diarrhea, sleep disorders, cough and analgesia in Iran for several centuries (Zargari, 1995). According to some data, the *P. Rrhoeas* may also have sedative and emollient effects

(Zargari, 1995). Investigations on the major chemical components of the plant extract have revealed the presence of rhoeadine, rhoeadic acid (Kalva and Sanyar, 1989; Slavik *et al.*, 1989), Papaveric acid (Zargari, 1995), rhoeagenine (Rey *et al.*, 1992) and anthocyanins (Matysik and Benesz, 1991).

Previous data have indicated that the *P. rhoeas* can reduce the expression and development of morphine-dependence in male morphine dependence mice (Pourmotabbed *et al.*, 2004). Furthermore, the extract can interact with morphine-induced conditioned place preference (Sahraei *et al.*, 2006), behavioral sensitization (Sahraei *et al.*, 2006), behavioral (Sahraei *et al.*, 2007) and analgesia tolerance (Shams *et al.*, 2008) in mice. However, there is no data concerning the effect of the extract of *P. rhoeas* on metabolic signs of acute stress.

The present study was undertaken to evaluate the effects of *P. Rhoetas* extract on the metabolic signs of acute stress in male mice.

MATERIALS AND METHODS

Study duration: These studies were followed from Jan 2009 to Jun 2010. All studies were done in the behavioral

laboratory section of Neuroscience Research Center, Baqiyatallah (a.s.) University of Medical Sciences, Tehran, Iran. Stress duration in this study was one hour and then after the variables was recorded.

Animals: Male albino Swiss-Webster mice (25-30 g, Pasture Institute, Tehran, IRAN) were used (8 mice for each experiment). The animals were housed 10% with 12/12 h light-cycle with *ad libium* food and water available.

Plant material: *P. rhoeas* was collected in may-2009 from Kermanshah province (west of 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 Department of Pharmacognosy, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Preparation of the extract: One hundred gram of the total plant (including fruit, petal, root, steam and leaf) powder was added to 1000 mL of 50% ethanol (V/V) and were left to macerate at room temperature (25°C) for 18 h. 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, which later expressed as mg of extract per kg body weight.

Induction of stress: The apparatus used for stress induction was explained in detail elsewhere (Halataei *et al.*, 2011; Hooshmandi *et al.*, 2011; Rosales *et al.*, 2002) with modifications.

Experimental design: For these propose, each animal was randomly assigned in stressed or control group. Animals were transferred to the experimental room 1 h before the experiments were begun for environmental adaptation. Then the stressed animals were placed in the compartments individually and 30 min later an electro foot shock was applied. After electro foot shock termination, the animals were left in the compartment for additional 30 min and then they removed to their home cages. Controls were just placed in the compartment for 60 min

without any foot shock. The amount of food and water used by each animal was recorded. The delay to eating time (the time elapsed by the animals to starting chow eating when they return to their cages) also was recorded as an index of stress. Blood samples from retro-orbital sinus were taken for blood plasma corticosterone evaluations.

Blood sampling and plasma corticosterone levels detection in stressed mice: Blood samples were taken from retro-orbital sinus (0.3 mL of the blood in 0.7 mL sodium citrate 3%) of the mice, 60 min after stress termination. The samples were centrifuged in 5000 rpm for 3 min in 4°C and the supper natant serum was collected for corticosterone detection. Corticosterone concentration was determined by ELISA kit (Rat Corticosterone ELISA kit; EIA-4164; DRG Instruments GmbH, Germany) in 450 nm.

Statistical analysis: Data showed as mean±SEM. One-Way analyses of variance followed by Tukey test were performed to assess specific group comparisons. Differences with $p < 0.05$ were considered statistically significant.

RESULTS

Effects of *P. rhoeas* extract on the stress-induced plasma corticosterone level elevation: The effects of stress on blood corticosterone elevation and effectiveness of *P. rhoeas* water alcoholic extract (15, 30 and 60 mg kg⁻¹, i.p.) to enhancing this effect is shown in Fig. 1. As it is clear in the figure, foot shock stress can increase plasma corticosterone level in the control group and the extract can exacerbate the effect of stress [F(7, 60)=11.3, $p < 0.0001$], (Fig. 1).

Effects of *P. rhoeas* extract on the stress-reduced food and water intake delay to eating: Animals' food intake also was recorded after the experiments. Results showed that food intake increases after stress application in groups which received saline and extract (15, 30 and 60 mg kg⁻¹, i.p.) [F(7, 60) = 6.54, $p < 0.0001$], (Fig. 2).

It was interesting that animals' water intake also was increased in animals which received stress and it was inhibited by extract pretreatment [F(7, 60) = 4.31, $p < 0.01$] (Fig. 3).

After stress termination, when the animals were returned to their home cages, the time elapsed for

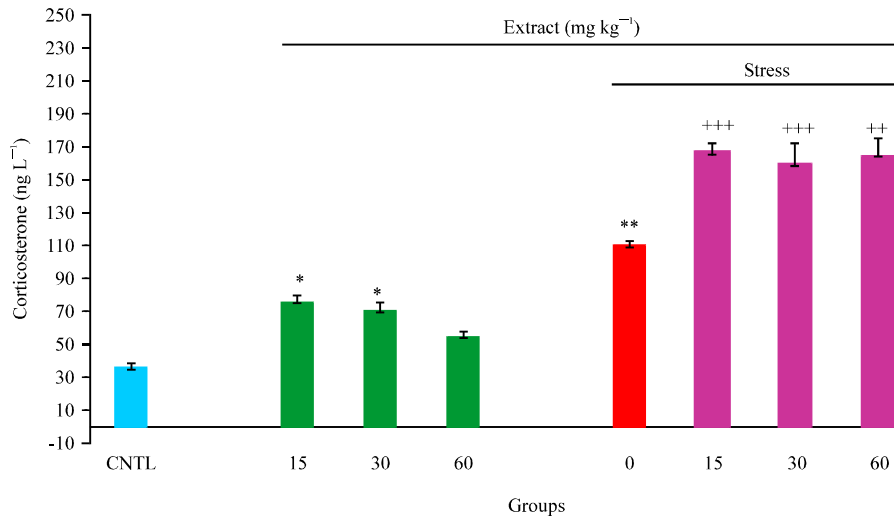


Fig. 1: Plasma corticosterone level after foot shock stress in mice that received i.p., of *P. rhoeas* extract. Plasma corticosterone levels were increased in the stressed and extract-treated groups in comparison with non stressed group. Data are shown as the mean±SEM for 7-9 mice. *p<0.5, **p<0.01, Significantly different from the non-stressed group. ++p<0.01 and +++p<0.001 significantly different from the stressed group

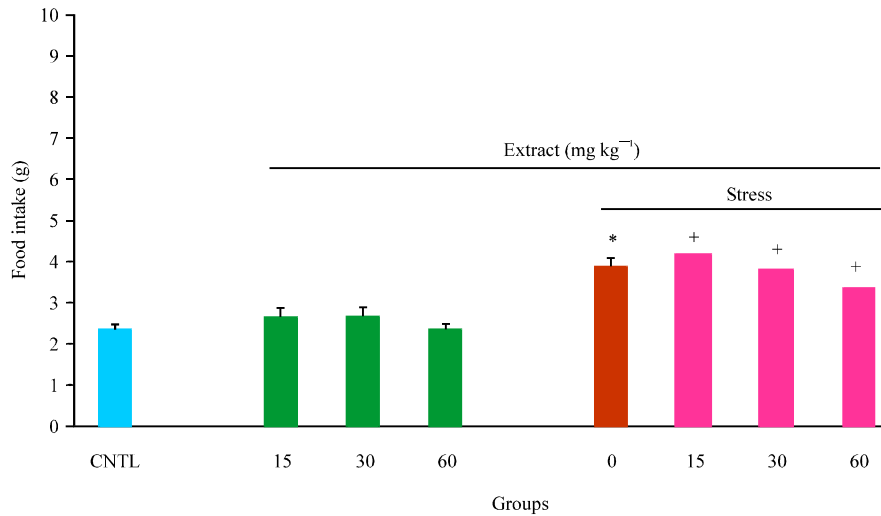


Fig. 2: Food intake after stress in mice that received i.p., *P. rhoeas* extract. Food intake was increased in the saline treated group, but in groups that received intraperitoneal extract, food intake decreased. Data are shown as the mean±SEM for 7-9 mice. *p<0.05, Significantly different from the non-stressed group. +p<0.05 different from stressed group

initiation of food consumption was recorded. Our data indicated that the stress group needed more time for initiation of food intake [F(7, 60)=12.23, p<0.0001], (Fig. 4).

DISCUSSION

Possible inhibition of stress signs by the *P. rhoeas* extract was examined in the present experiments. Stress

signs including changes in the food and water intake and anorexia are among the side effects which are initiated by the brain stress system (Adam and Epel, 2007). These signs are thought to be attributed in the metabolic syndrome (Lupien *et al.*, 2009). Our data indicated that stress can increase food and water intake and the extract can reduced these signs. In addition, stress increases the delay to eating time and the extract can reduce the time to normal.

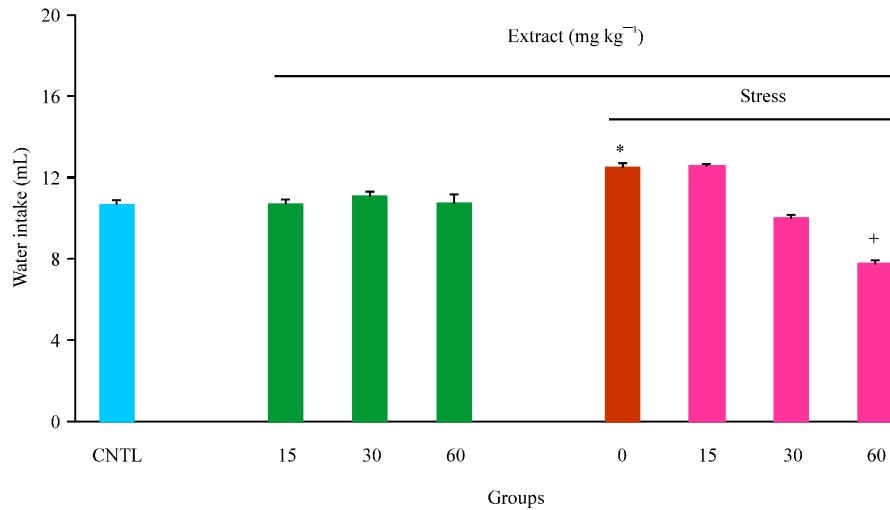


Fig. 3: Water intake after stress in mice that received i.p., *P. rhoeas* extract. Water intake was increased in the saline treated group, but in groups that received intraperitoneal extract, water intake decreased. Data are shown as the mean±SEM for 7-9 mice. * $p < 0.05$, significantly different from the non-stressed group. + $p < 0.05$ different from stressed group

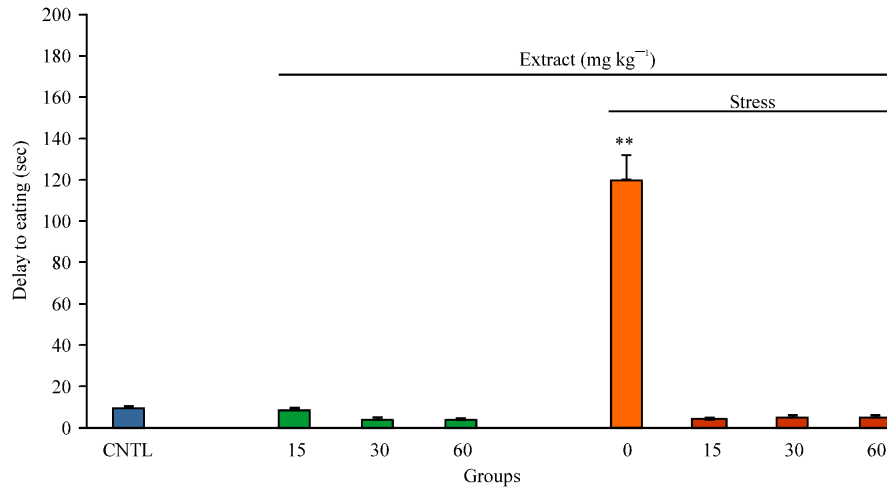


Fig. 4: The delay to eating (the time elapsed before the initiation of food consumption) after foot shock stress in mice that received i.p., *P. rhoeas* extract. The delay to eating was increased in the stressed, but in groups that received intraperitoneal extract, the delay to eating did not change. Data are shown as the mean±SEM for 7-9 mice. ** $p < 0.01$, Significantly different from the non-stressed and extract treated groups

In accordance with the previous studies, our data shows that stress can increase plasma corticosterone level in the mice (Halataei *et al.*, 2011) and rats (Hooshmandi *et al.*, 2011). The mechanism(s) by which stress can increase plasma corticosterone level is well understood (McEwen, 2007). According to previous findings, stress can activate the Hypothalamus-Pituitary-Adrenal (HPA) axis which in fact elevated the plasma

corticosterone level dramatically (Miller and O'Callaghan, 2002). In addition, stress increases the hormone Corticotropin-Releasing-Hormone (CRH) in the hypothalamus and induced anorexia and/or hyperphagia (Adam and Epel, 2007). Considering our results it is clear that stress may induce hyperphagia and elevate plasma corticosterone level by such mechanism(s).

It was interesting that the extract administration can increase plasma corticosterone level in the non-stress animals. This finding is somehow interesting because the extract may induce its effects via such mechanism. Whether this finding is useful for some human disease or not? Cannot be responded at the time and some experiments are needed in this regard. However, there is no study concerning the effects of papaver R. extract on the corticosterone release from adrenal glands. Investigators have shown that the extract may contain little amounts of opioids (Zargari, 1995). On the other hand, data are available that opioids including morphine can induce corticosterone release from rat adrenal glands (Degli *et al.*, 1995; George and Way, 1955). So it is likely that the extract may increase plasma corticosterone in the animals via a similar mechanism. On the other hand, none of the other signs measured in the present study were not changed in these animals. These findings also express the fact that the extract increases the plasma corticosterone via a peripheral but not central mechanism. In our experiments the extract can interact with the effects of stress and reduces all of the signs of stress except the plasma corticosterone level increment. Our data indicate that the extract have a constituent(s) that can interact with stress signs and subsequently ameliorates these signs.

The extract exhibited mild opioid and also anti-dopaminergic and anti-cholinergic activities (Zargari, 1995; Soulimani *et al.*, 2001). These neurotransmitters are involved in the expression of stress signs (McEwen, 2006), it is more likely that the extract attenuates these signs via one or more of these systems. However, since there is no study regarding the effects of extracts on the stress signs, it is difficult to interpretation of data.

As we mentioned above, the extract contains several constituents (Zargari, 1995) and may account for responses reported in the present study. However, the role of these substances should be examined separately in the future experiments.

CONCLUSION

From the results from these studies, one can recommend that the extract may be effective to impair the mechanisms which activated by stress under acute administration. Results, all together, show that the extract of *P. rhoeas* inhibits the signs of stress in mice.

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