



## Effects of Quercetin and ACTH on Morphine-Induced Tolerance and Dependence in Mice

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### Abstract

The goal of this study was to evaluate the effects of quercetin and ACTH injection on prevention of development of morphine tolerance and dependence in mice. In this study different groups of mice received morphine (40 mg/kg, i.p.) plus quercetin (5, 10, 25 mg/kg, ip), ACTH (1, 2.5, 5 IU/mice, i.p.), or combination of quercetin (5 mg/kg) and ACTH (1 IU/mice) once a day for four days. Tolerance was assessed by administration of morphine (9 mg/kg) and using hot plate test on the fifth day. It was found that pretreatment with quercetin or ACTH decreased the degree of tolerance. Co-administration of quercetin and ACTH before morphine did not decrease the tolerance, significantly. From these results it may be concluded that administration of quercetin or ACTH alone could prevent the development of tolerance to the analgesic effects of morphine. These effects may be related to as nitric oxide inhibitor (eNOI) behavior of quercetin and the ability of morphine and their receptors in the control of the secretion of CRH.

*Keywords:* ACTH; Morphine; Quercetin; Tolerance.

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### 1. Introduction

Drug addiction can be defined as the compulsive seeking and taking of a drug despite adverse consequences. Although addiction involves many psychological and social factors, it also represents a biological process; the effects of repeated drug exposure on a vulnerable brain. To understand addiction, it is important to define the types of molecular and cellular adaptation at the levels of neurons and synapses that account for tolerance,

sensitization and dependence, which are often used to define an addicted state. Tolerance describes diminishing sensitivity to a drug's effects after repeated exposure; sensitization describes the opposite. Dependence is an altered physiological state caused by repeated drug exposure, which leads to withdrawal when drug use is discontinued. Each is seen in human addicts and is believed to contribute to continued drug use during addiction. Considerable progress has been made in identifying the molecular and cellular adaptations that mediate these processes [1-3].

Quercetin is a flavonoid that forms the "backbone" for many other flavonoids,

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including the citrus flavonoids rutin [4, 5], hesperidin [6], naringin and tangeritin. Quercetin is found to be the most active of the flavonoids in studies, and many medicinal plants owe much of their activity to their high quercetin content. Quercetin has demonstrated significant anti-inflammatory activity [7] because of direct inhibition of several initial processes of inflammation. For example, it inhibits both the manufacture and release of histamine [8] and other allergic/inflammatory mediators. In addition, it exerts a potent antioxidant activity and vitamin C-sparing action [4, 5, 9]. Quercetin also shows remarkable anti-tumor properties [10, 11]. In a previous study, the ability of quercetin to reverse the development of morphine tolerance and dependence in mice has been suggested [12]. On the other hand a decreased release of pro-opiomelanocortin-derived peptides in chronic treatment has been shown.

Evidence suggests a close relationship between ACTH and opioids. ACTH pretreatment has been reported to influence subsequent response to chronic morphine administration and demonstrated significant analgesia in response to the first administration. Tolerance to this effect had been developed following four days of repeated exposure to morphine [13]. A major focus of current research was to explore the tolerance prophylaxis of ACTH and quercetin and the influence of co-administration of ACTH and quercetin on morphine induced tolerance in mice.

## 2. Materials and methods

### 2.1. Animals

Male albino mice (20-30 g) were used in this study. Pain sensitivity was evaluated by hot-plate test. Animals were kept in a temperature-controlled with 12 h dark 12 h light cycle, and had access to water and rodent diet ad libitum.

### 2.2. Drugs

Morphine sulfate was obtained from Darupakhsh Pharmaceutical Company, Tehran, Iran. ACTH was purchased from Hetero Drugs Limited, India; and naloxone hydrochloride was obtained from Tolid Daru Pharmaceutical Company, Tehran, Iran.

### 2.3. Hot - plate test

Each animal was placed on a surface (23×23 cm) maintained at  $55\pm 2$  °C surrounded by a Plexiglas wall 20 cm high. Licking of hands was used at the end point for determination of response latencies. Failure to respond by 45 seconds was a marker for termination of the test (cut off).

### 2.4. Induction of tolerance

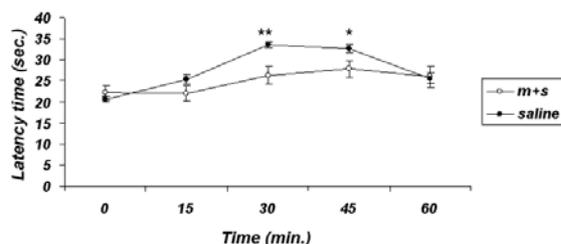
In order to induce tolerance, groups of 9 mice were chosen randomly. Mice were treated by morphine (40 mg/kg; i.p.) and quercetin or ACTH or both of them once a day for four consecutive days. To evaluate the degree of tolerance, the antinociceptive effect of a test dose of morphine (9 mg/kg) was measured 24 h after the last dose of morphine.

### 2.5. Quercetin preparation

The powdered material plants (*Allium cepa*) were extracted by 50% EtOH. The hydroalcoholic extract was concentrated by rotatory evaporator and defatted with petroleum ether twice. Residue was hydrolyzed at 100 °C by 1 N HCl for 1 h and then the extract was allowed to cool down, and quercetin crystals were formed. The quercetin crystals were separated by centrifuge from hydrolyzed solution and were recrystallized by acetone and finally determined by <sup>1</sup>HNMR and <sup>13</sup>CNMR.

### 2.6. Statistical analysis

The results are expressed as the Mean±SE. Differences between the individual mean values in different groups were analyzed by



**Figure 1:** Effects of morphine on (○) tolerant and (●) non-tolerant mice. Animals received either normal saline (10 ml/kg, i.p.) or morphine (40 mg/kg, i.p.) + normal saline (10 ml/kg) for 4 days. Antinociception of a test dose of morphine (9 mg/kg, i.p.) was tested 24 h after the last dose of morphine (40 mg/kg) in tolerant and non-tolerant mice. Each group had at least 9 mice. Results are expressed as Mean±SE. \*\* $p<0.01$ , significantly different from the respective non-tolerant control group.

one-way analysis of variance (ANOVA) and tukey test as a post hoc analysis. Differences with a  $p<0.05$  were considered significant.

### 3. Results

#### 3.1. Development of tolerance to the morphine antinociception

Animals received morphine (40 mg/kg, ip) once a day for 4 days. In each group antinociceptive response of a test dose of morphine (9 mg/kg, i.p.) was assayed 24 h after the last dose of morphine (40 mg/kg, i.p.). Animals that became tolerant to the effects of morphine exhibited only a small antinociceptive effect (Figure 1).

#### 3.2. Effect of pretreatment with ACTH on tolerance to chronic morphine therapy

As shown in Figure 2, ACTH injection (1, 2.5, 5 IU/mice, i.p.) 30 min. before daily morphine administration, decreased tolerance to the analgesic effects of morphine significantly ( $p<0.01$ ).

#### 3.3. Effect of pretreatment with quercetin on tolerance and dependence to chronic morphine therapy

As shown in Figure 3, injection of quercetin (5, 10, 25 mg/kg, i.p.) 30 min. before daily morphine administration

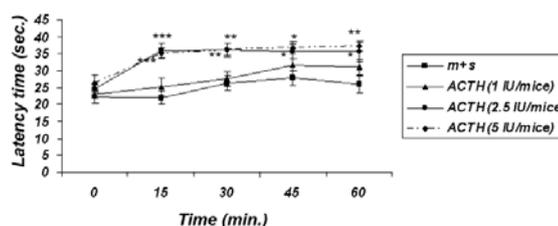
decreased tolerance to the analgesic effect of morphine, significantly ( $p<0.01$ ).

#### 3.4. Effect of pretreatment with quercetin and ACTH on tolerance to chronic morphine therapy

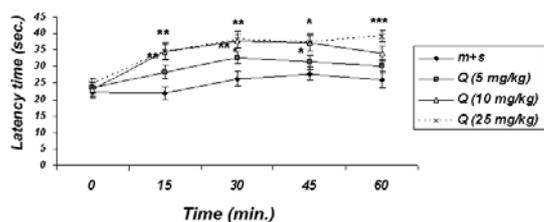
As shown in Figure 4, co-administration of ACTH (1 IU/mice, i.p.) and quercetin (5 mg/kg, i.p.) 30 min. before daily morphine administration decreased tolerance phenomenon, significantly ( $p<0.01$ ).

### 4. Discussion

The main goal of this study was to evaluate the effects of quercetin as nitric oxide inhibitor (eNOI) [14] and ACTH (adrenocorticotrophic hormone) on development of tolerance and dependence to morphine effects. Tolerance and dependence may be viewed as a result of neuronal adaptation which is induced by repeated drug exposure, and NMDA receptors have been consistently implicated in establishment of such long term changes [1-3, 5, 6, 15-18]. It has been shown that morphine withdrawal precipitates glutamate release [1-3, 15-17]. Conversely intra-cerebroventricular glutamate or NMDA administration produces withdrawal signs in morphine-dependent rats [5-8, 10, 17, 19, 20]. Excitatory synaptic input to ventral tegmental area (VTA) mediated by glutamate is a key component of the regulation of dopaminergic cells. The glutamate afferents arise from three primary sources: The medial prefrontal cortex, the pedunculopontine region



**Figure 2:** Effects of different doses of ACTH injection (1, 2.5, 5 IU/mice, i.p.) on tolerance determined by hot-plate test in morphine-tolerant mice. Each group had at least 9 mice. Results are expressed as Mean±SE. \* $p<0.05$ ; \*\* $p<0.01$ ; \*\*\* $p<0.001$ ; significantly different from the control group.



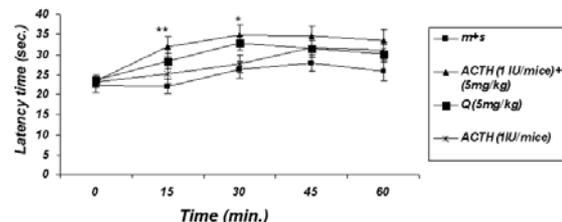
**Figure 3:** Effects of different doses of quercetin (5, 10, 25 mg/kg, i.p.) on tolerance determined by hot-plate test in morphine-tolerant mice. Each group had at least 9 mice. Results are expressed as Mean $\pm$ SE. \* $p$ < 0.05; \*\* $p$ <0.01; \*\*\* $p$ <0.001; significantly different from the control group.

and the subthalamic nucleus [20]. Glutamate acts on AMPA, NMDA and mGluRs to depolarize dopamine neurons [22, 23].

Synaptically released glutamate may cause rapid and slow changes in the activity of dopaminergic cells. One role of glutamate innervation of the VTA is to mediation of a switch from pacemakerlike firing in dopaminergic cells to burst-firing pattern [23-25]. Recent studies propose that repeated administration of opiate may activate the NMDA-receptor through G protein associated with opioid receptor and/or may have intracellular mechanisms [1-3, 5, 15-18]. This opiate related activation of NMDA-receptors may initiate subsequent intracellular changes such as production of nitric oxide (NO) and/or activation of protein kinas C (PKC) [15-17]. Both NO and PKC have been shown to be critical for development of morphine tolerance [15-18].

Previous studies [23-25] have shown that administration of nitric oxide inhibitor (eNOI) attenuated intracellular Ca influx in both NMDA receptor gated channel and voltage gated Ca channel. The results of the present study show that quercetin (5, 10, 25 mg/kg, i.p.), as nitric oxide inhibitor, attenuates development of morphine tolerance. These effects may be related to nitric oxide inhibitor (eNOI) behavior of quercetin.

As shown in the present study, ACTH injection 30 min. before daily morphine



**Figure 4:** Effects of the use of quercetin (5, mg/kg, i.p.) + ACTH (1 IU/mice, i.p.) on tolerance determined by hot-plate test in morphine-tolerant mice. Each group had at least 9 mice. Results are expressed as Mean $\pm$ SE. \* $p$ <0.05; \*\* $p$ <0.001; significantly different from the control group.

administration, decreased tolerance to the analgesic effects of morphine. Previous works demonstrated that the adrenocorticotrophic hormone (ACTH) but not corticosterone (CORT) response to stress is altered by prenatal morphine exposure in adult male rats [25]. In a previous study, the results indicated a decreased release of pro-opiomelanocortin-derived peptides after chronic treatment with morphine in the rat [26]. The results possibility indicate that morphine change HPA activity by acting on specific receptors in the hypothalamus and raise the possibility that opioid peptides and their receptors are physiologically important in the control of the secretion of CRH.

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