



## Effects of Caffeine on Morphine Tolerance and Analgesia in Mice

Bohloul Habibi Asl\*, Reza Abdelalipur, Mehrdad Shahidi

*Department of Pharmacology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran*

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### Abstract

Caffeine, an adenosine A<sub>1</sub>, A<sub>2A</sub>, and A<sub>2B</sub> receptor antagonist, is frequently used as an adjuvant analgesic in combination with non steroidal anti-inflammatory drugs or opioids. The aim of this study was to evaluate the effects of caffeine on preventing the development of morphine tolerance and analgesia in mice. In this study, different groups of mice received morphine (30 mg/kg) + saline (10 ml/kg), or morphine (30 mg/kg) + caffeine (10, 15, 25, 50, 75, or 100 mg/kg) i.p. 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. Analgesic effects of caffeine also were evaluated alone or in combination with different doses of morphine. It was found that pretreatment with caffeine (75, 100 mg/kg) decreased the degree of morphine tolerance significantly ( $p < 0.01$ ). Combination of caffeine (10, 50 mg/kg) with morphine (3, 6, 9 mg/kg) caused a significant decrease in morphine analgesic effect ( $p < 0.01$ ). But, in high doses of caffeine (100 mg/kg) the analgesic effect of morphine increased significantly ( $p < 0.01$ ). This effect was inhibited by atropine (5 mg/kg, SC). These effects can be related to different mechanisms of caffeine in different doses and the effects of caffeine to the release of acetylcholine.

*Keyword:* Analgesia; Caffeine; Morphine sulfate; Tolerance.

*Received:* January 14, 2011; *Accepted:* March 15, 2011.

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### 1. Introduction

It is known that continuous or long term use of opiate drugs may cause tolerance and dependence which limits the therapeutic efficacy of these drugs [1-3]. With repeated administration of these drugs, adaptive mechanisms are initiated. One such mechanism is the development of tolerance. Another results from development of counter

adaptations such that once the drug is removed a sequence of rebound signs and symptoms are manifested. Recent work suggests that these adaptive processes at the cellular, synaptic, and network levels downstream from the receptor may hold the keys to understanding of addiction [4]. Perhaps the most important adaptations that develop as a result of chronic opioid administration occur in neural systems responsible for the transition from casual to compulsive drug use. At the cellular level, adaptive mechanisms that occur with repeated and/or continuous morphine treatment to

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\*Corresponding author: Bohloul Habibi Asl, Department of Pharmacology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran  
Tel, Fax: (+98)411-4422443  
Email: bhabibi@iran.ir

mediate associative tolerance are probably mediated by separate mechanisms [4, 5]. Although compounds of extremely high potency have been produced, the problem of tolerance and dependence on these agonists persists.

Several studies have indicated that different neurotransmitters and receptors are involved in morphine tolerance [6, 7], and the influence of receptors and neurotransmitters on the morphine tolerance and other opioids are contradictory [6, 8]. One of the neurotransmitters involved in morphine tolerance is adenosine. Caffeine, a mild stimulant, is the most widely-used psychoactive drug in the world (Goodman). Caffeine is rapidly distributed throughout all tissues and easily crosses the placenta and brain barrier. Caffeine has intrinsic antinociceptive properties and is frequently used as an adjuvant analgesic drug [8, 9]. It is thought that caffeine analgesia is produced, at least in part, through adenosine receptor antagonism. The adenosine receptor family comprises four subtypes: A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, and A<sub>3</sub> [10]. They are widely distributed in CNS and peripheral tissues. The A<sub>1</sub> receptors are found in high density in the brain (cortex, cerebellum, and hippocampus), the dorsal horn of the spinal cord, at lower levels in

other brain regions, and in peripheral tissues [10, 11]. Caffeine is an adenosine A<sub>1</sub>, A<sub>2A</sub>, and A<sub>2B</sub> receptor antagonist. In this study, we examined the role of caffeine on morphine tolerance and analgesia in mice. We demonstrated analgesic and anti-analgesic effects of caffeine in different doses in mice.

## 2. Materials and methods

### 2.1. Animals

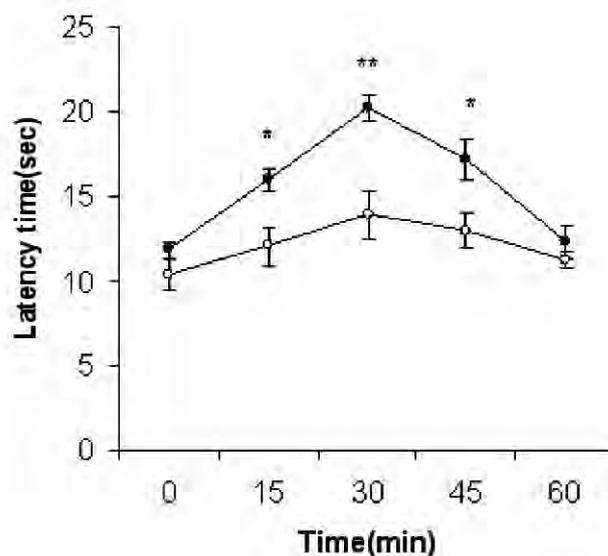
Male albino Swiss-Webster mice (20-30 g, Razi Institute, Tehran, Iran) were used throughout the study (9 mice for each experiment). Animals were housed under a 12/12-h light cycle (07:00–19:00), with food and water available *ad libitum*. The animals were randomly allocated to different experimental groups.

### 2.2. Drugs

Morphine sulfate (Darupakhsh, Iran), powder of anhydrous caffeine (Hunan Pharmaceutical Factory), atropine (Darupakhsh, Iran) were used.

### 2.3. Method

Hot-plate test: each animal was placed on a surface (23×23 cm) maintained at 55±2 °C



**Figure 1.** Analgesic effects of morphine on (○) tolerant and (●) non-tolerant mice. Animals received either saline (10 ml/kg, i.p.) or morphine (30 mg/kg, i.p.) 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 (30 mg/kg, i.p.) in tolerant and non-tolerant mice. Each group had at least 9 mice. Results are expressed as Mean±SE. \*Significantly different from tolerant group ( $p<0.05$ ), \*\* ( $p<0.01$ ).

**Table 1.** Analgesia induced by i.p. administration of caffeine.

Drug/dose(mg/kg)	Base line (sec)	Latency Time(sec)			
		15 (min)	30 (min)	45 (min)	60 (min)
Saline normal (10 ml/kg)	11±0.72	10.78±0.87	10.89±0.63	10.68±0.68	9.88±0.61
Caffeine (5 mg/kg)	11.05±1.25	11.05±1.32	11.23±1.38	10.5±1.33	10.27±1.22
Caffeine (10 mg/kg)	11±1.15	11.5±0.99	11.93±1.19	10.69±1.06	10.62±0.78
Caffeine (15 mg/kg)	11.33±1.56	11.43±1.75	12±1.77	10.31±1.31	9.16±1.34
Caffeine (25 mg/kg)	11.44±0.76	12±0.78	12.38±0.89	10.77±1.02	10.22±1.05
Caffeine (50 mg/kg)	11.12±1.37	11.75±1.55	12.38±1.23	10.75±1.01	9.75±1.05
Caffeine (75 mg/kg)	11.5±0.77	11.98±0.63	13.06±0.43	12.12±0.31	11±0.57
Caffeine (100 mg/kg)	11.25±1.21	12.93±1.39	14.25±1.03*	12.06±1.10	10.63±0.74

All administrations were i.p. Latency time was measured as explained under materials and methods by hot plate test; \*Significantly different from Base line ( $p<0.01$ ).

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 intraperitoneally (i.p.) by morphine (30 mg/kg) in combination with either caffeine or saline or both once a day for four 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 in combination with caffeine or saline or both.

#### 2.5. Evaluation of analgesia

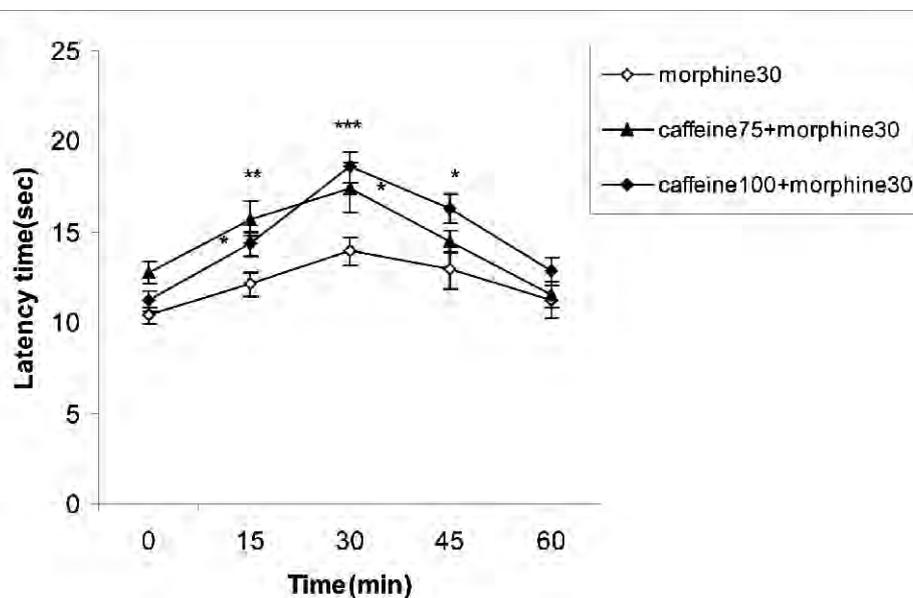
Analgesic effects of caffeine (10, 15, 25, 50, 75, 100 mg/kg, i.p.) were evaluated alone or in combination with different doses of morphine (3, 6, 9 mg/kg, IP).

#### 2.6. Statistical analysis

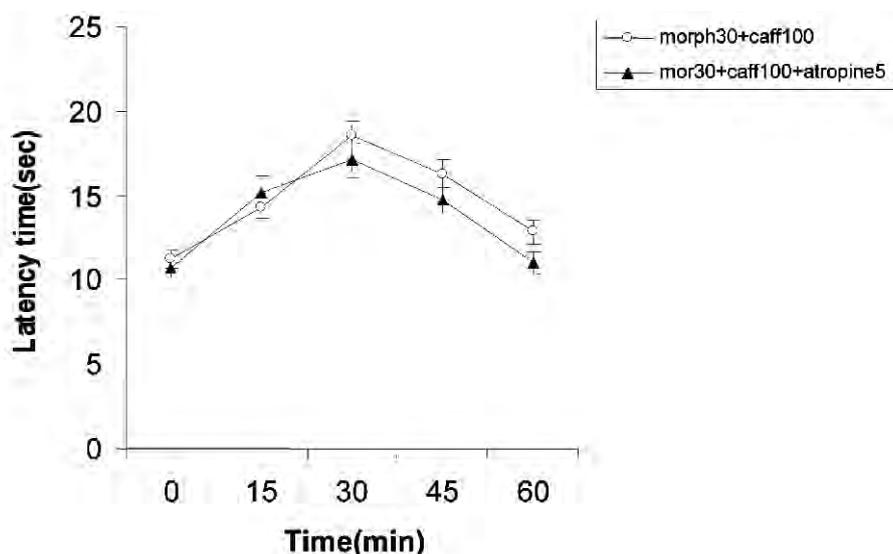
The results are expressed as the Mean±SE. Differences between the individual mean values in different groups were analyzed by one-way analysis of variance (ANOVA). Differences with a  $p<0.05$  were considered significant.

### 3. Results

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



**Figure 2.** Effects of different doses of caffeine (75, 100 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±SE. \*( $p<0.05$ ), \*\*( $p<0.01$ ), \*\*\*( $p<0.001$ ) Significantly different from the control group [morphine (30 mg/kg)].



**Figure 3.** Effect of atropine (5 mg/kg, s.c.) on tolerance induction by morphine + caffeine co-administration. Each group had at least 9 mice. Results are expressed as Mean ± SE.

As shown in Figure 1, animals received morphine (30 mg/kg, i.p.) once a day for four days, and in each mice antinociceptive response to a test dose of morphine (9 mg/kg, i.p.) was assayed 24 h after the last dose of morphine. Animals that became tolerant to effects of morphine exhibited only a small antinociceptive effect.

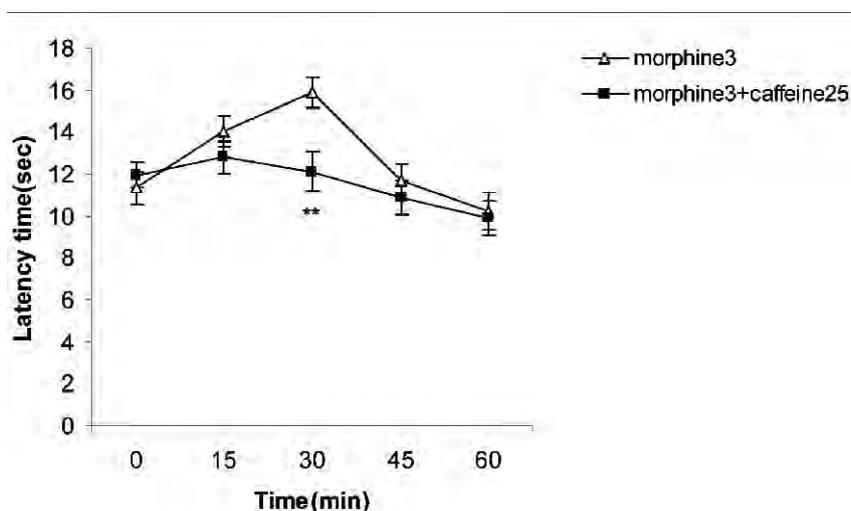
doses of administration of caffeine (i.p.) in hot-plate test. Animals received saline (10 ml/kg, i.p.) or different doses of caffeine (5, 10, 15, 25, 50, 75, 100 mg/kg, i.p.). As shown in Table 1, only caffeine with a dose of 100 mg/kg, produced a significant ( $p < 0.05$ ) antinociceptive effect as compared to the saline in hot-plate test.

### 3.2. Analgesia induced by administration of caffeine

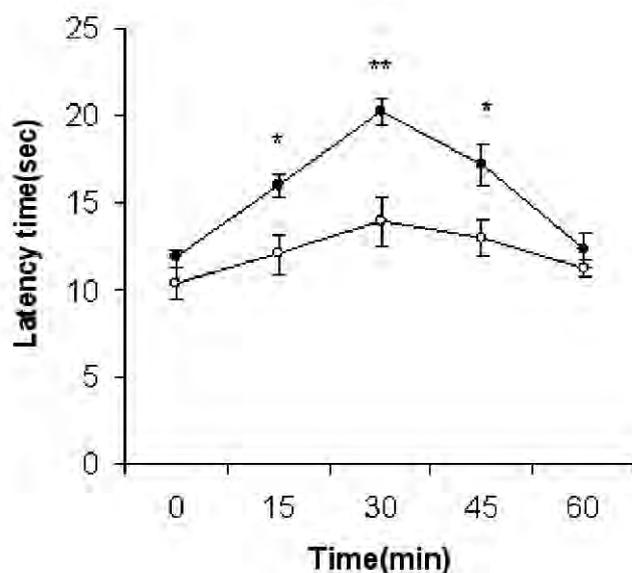
Table 1 shows the response of various

### 3.3. Effect of caffeine on tolerance to chronic morphine therapy

As shown in Figure 2, caffeine injection



**Figure 4.** Effects of caffeine (25 mg/kg, i.p.) + morphine (3 mg/kg, i.p.) on analgesia determined by hot-plate test. Results expressed as Mean±SE of 9 mice.;\*\* ( $p < 0.01$ ) Significantly different from the control group [morphine (3 mg/kg)].



**Figure 5.** Effects of caffeine (15 mg/kg, i.p.) + morphine (6 mg/kg, i.p.) administration on analgesia determined by hot-plate test. Results expressed as Mean $\pm$ SE of 9 mice. \*( $p < 0.05$ ), \*\*\*( $p < 0.001$ ) Significantly different from the control group [morphine (6 mg/kg)].

(75, 100 mg/kg, i.p.), 30 min. before daily morphine administration, significantly decreased tolerance to the analgesic effects of morphine ( $p < 0.01$ ).

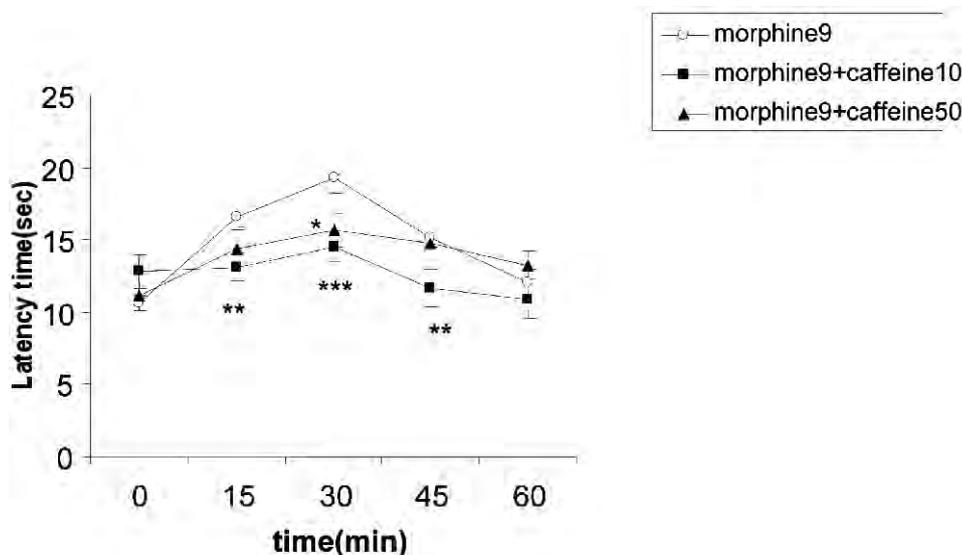
#### 3.4. Effect of atropine in tolerance induced by co-administration of morphine + caffeine

Figure 3 shows that pretreatment with atropine (5 mg/kg, s.c.), 30 min. before daily

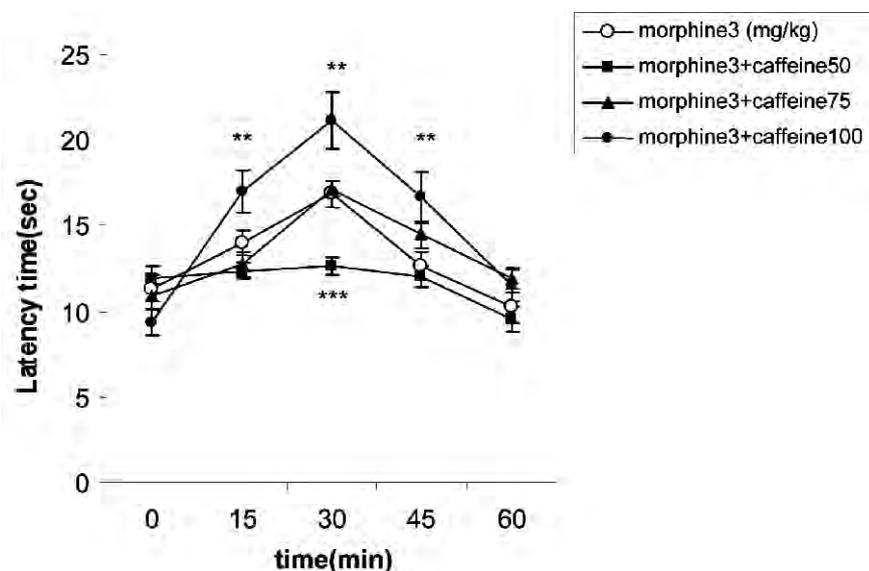
morphine and caffeine co-administration, changed morphine tolerance, significantly.

#### 3.5. Analgesia induced by administration of morphine alone or plus caffeine

Table 1 shows the effects of different doses of caffeine (5, 10, 15, 25, 50, 75, 100 mg/kg, i.p.) on morphine-induced antinociception in hot-plate test.



**Figure 6.** Effects of caffeine (10, 50 mg/kg, i.p.) + morphine (9 mg/kg, i.p.) administration on analgesia determined by hot-plate test. Results expressed as Mean $\pm$ SE of 9 mice. \*( $p < 0.05$ ), \*\*( $p < 0.01$ ), \*\*\*( $p < 0.001$ ) Significantly different from the control group [morphine (9 mg/kg)].



**Figure 7.** Effects of caffeine (50, 75, 100 mg/kg, i.p.) + morphine (3 mg/kg, i.p.) administration on analgesia determined by hot-plate test. Results expressed as Mean±SE of 9 mice. \*\*( $p<0.01$ ), \*\*\*( $p<0.001$ ) Significantly different from the control group [morphine (3 mg/kg)].

Figures 4 to 7 show the results of co-administration of caffeine (5, 10, 15, 25, 50, 75, 100 mg/kg, i.p.) with morphine (3, 6, 9 mg/kg, i.p.). It was found that the combination of caffeine (10, 15, 25, 50 mg/kg, i.p.) with morphine (3, 6, 9 mg/kg, i.p.), decreased the analgesic effect of morphine, significantly ( $p<0.01$ ). But high doses of caffeine (100 mg/kg) increased the analgesic effect of morphine, significantly ( $p<0.01$ ).

### 3.6. Effect of atropine (5 mg/kg, SC) on the caffeine + morphine analgesia induction

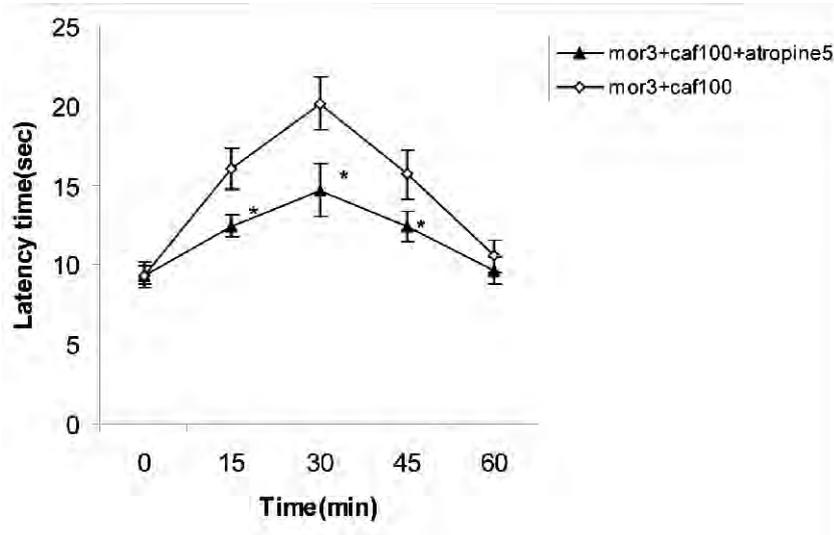
Figure 8 shows the effect of atropine (5 mg/kg, s.c.) on analgesia induced by the administration of caffeine+morphine in hot-plate test. Animals which received caffeine (100 mg/kg, i.p.) with morphine (3 /kg, i.p.), showed increased analgesic effect of morphine ( $p<0.05$ ). This effect was inhibited by atropine (5 mg/kg, s.c.) pretreatment (15 min).

## 4. Discussion

In this study, we evaluated the effects of systemic administration of caffeine (Adenosine  $A_1$ ,  $A_{2A}$ , and  $A_{2B}$  receptor antagonist) on morphine tolerance and

analgesia in mice. Results indicated that injection of caffeine, 30 min. before daily administration of morphine, decreased tolerance to the analgesic effects of morphine, significantly. On the other hand, administration of atropine, before daily co-administration of morphine and caffeine changed in morphine tolerance, significantly.

In several studies [12, 13] repeated injections of morphine, cocaine, and amphetamine showed an increase in presynaptic inhibition caused by endogenous adenosine. Originally done in brain slices from guinea pigs treated with either morphine or cocaine, the presynaptic regulation of the GABAB IPSP measured in dopaminergic cells of the VTA was changed in drug-treated animals, significantly. This work showed that the activation of adenylyl cyclase by either D1 receptors or forskolin had two opposing actions. One was to augment GABA release through the activation of PKA, and the second was to inhibit GABA release by activation of a presynaptic  $A_1$  adenosine receptor. In slices from drug-treated animals, the enhancement of inhibition by adenosine was so great that the effect of D<sub>1</sub> receptor activation reversed



**Figure 8.** Effects of atropine (5 mg/kg, s.c.) on analgesia induced by combination of caffeine (100 mg/kg, i.p.) + morphine (3 mg/kg, i.p.) administration, determined by hot-plate test. Results expressed as Mean $\pm$ SE of 9 mice. \* ( $p < 0.05$ ) Significantly different from the control group.

direction and inhibited, rather than augmented, GABA<sub>B</sub> IPSPs. This inhibition was blocked by adenosine receptor antagonists and agents that blocked the transport (Probenecid) or metabolism (a phosphodiesterase inhibitor) of cAMP. The opposing actions of forskolin have been reported in several brain areas [14-18]. In general, the persistent increase in extra-cellular adenosine is occurred in repeated injections of morphine. Then, effects of caffeine (morphine tolerance inhibition) are believed to occur by means of competitive antagonism at adenosine receptors. On the other hand, this effect was inhibited by atropine pretreatment (15 min.). Thus, the mechanism of caffeine on morphine tolerance seems to be dependent on cholinergic activation.

The second part of this study examined analgesia induced by treatment with caffeine. In different doses of caffeine, as shown in Table 1, only caffeine with a dose of 100 mg/kg, produced a significant antinociceptive effect as compared to the saline in hot-plate test. Combination of caffeine with morphine decreased morphine analgesic effect, but high doses of caffeine (100 mg/kg) increased the analgesic effect of morphine, significantly.

Adenosine has dual activity on nociception.

It acts centrally within the spinal cord to suppress nociceptive signaling [19], presumably through the activation of A<sub>1</sub> and A<sub>2</sub> adenosine receptors [20]. In the periphery, adenosine has algogenic activity, which is probably mediated by A<sub>2</sub> receptors [19, 21].

## 5. Conclusion

Caffeine, a virtually nonselective A<sub>1</sub>, A<sub>2A</sub>, and A<sub>2B</sub>-adenosine receptor antagonist, exhibits antinociceptive effects in high doses. Thus, caffeine acts centrally in high doses within the spinal cord to suppress nociceptive signaling. Other activities of caffeine (alteration of catecholamine or acetylcholine release and turnover, inhibition of phosphodiesterase, influence on intracellular calcium concentrations, and interaction with GABA<sub>A</sub> receptors) may contribute to its antinociceptive effects [19, 22]. Of course for clarifying the exact effects, further studies are required.

## Acknowledgements

Special thanks, and gratitude goes to those who helped us in carrying out the study successfully, especially the Pharmacology Department of Tabriz University of Medical Sciences for their helpful supports.

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