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Research Article

Effects of the phosphodiesterase type-5 inhibitor tadalafil on nociception, morphine analgesia and tolerance in rats

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ABSTRACT

Aim: Tadalafil is a potent, selective and reversible inhibitor of phosphodiesterase type 5 (PDE5) enzyme breakdowning cyclic guanosine monophosphate (cGMP). In this study, we aimed to investigate the effects of tadalafil on nociception, morphine analgesia and tolerance.

Methods: In this study, 54 Wistar Albino (230-250 g) male rats were used. First of all, four different doses (2, 4, 8, 16 mg/kg) were used to determine the optimum effective dose of tadalafil on nociception. Optimum activity was found at 8 mg/kg and animals were divided into six groups: Saline (S), 8mg/kg tadalafil, 5mg/kg morphine (M), M+ tadalafil, morphine tolerance (MT) and MT+ tadalafil. Saline was given to the control group, tadalafil intraperitoneally and morphine subcutaneously administered at the indicated doses. To develop tolerance to morphine, 10mg/kg morphine was injected daily in the morning and evening for five days and tolerance was evaluated with single dose of morphine on sixth days. The resulting analgesic effect was measured with hot plate and tail flick analgesia tests and recorded at 30th, 60th, 90th and 120th minutes.

Results: Tadalafil showed anti-nociceptive effect when given alone at different doses ($p<0.05$). However, tadalafil significantly decreased the analgesic effect of morphine ($p<0.05$). In addition, tadalafil significantly increased the tolerance to morphine ($p<0.05$).

Conclusions: The phosphodiesterase type 5 inhibitor tadalafil have anti-nociceptive properties and it decreases analgesic effect of morphine, in addition improves tolerance development. These effects probably may occur via NO/cGMP pathway.

Keywords: Phosphodiesterase type 5 inhibitors; tadalafil; nociception; morphine analgesia; morphine tolerance.

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Introduction

Morphine is an opiate receptor agonist and analgesic that is routinely administered in cases of strong and chronic pain in clinics. The duration of morphine's effect is reduced by the development of tolerance to its anti-nociceptive properties. Despite the many studies that have been made examining the development of opioid tolerance, underlying mechanisms of tolerance is still unclear. Recent studies propose that an endogenous molecule nitric oxide (NO), linked to the regulation of many physiological and pathological pathways, is involved in the morphine tolerance development [1, 2, 3]. Moreover, it has been seen that these effects of NO may be associated with cyclic guanosine monophosphate (cGMP) [4].

Tadalafil, a selective phosphodiesterase-5 (PDE5) inhibitor, enables the removal of cGMP, one of the main factors bringing about smooth muscle relaxation. Given their effectiveness in improving nitric oxide-driven cGMP collection and maintaining the causes of vasodilatation in corpus cavernosum, PDE5 inhibitors are generally prescribed for the treatment of erectile dysfunction (ED) in male [5]. Many studies have been made recently into PDE-5 suggesting their possible applications in areas other than ED [6, 7]. It has been shown to have anti-oxidant properties in that it decreases 3,4-Methylenedioxyamphetamine and increases superoxide dismutase and catalyzes enzyme activity in rat ovary tissue following ischemia/reperfusion injury [8]. In addition, it has an anti-oxidant effect in ischemia/reperfusion injury in rat brain tissue [9]. Furthermore, it has been demonstrated that the anti-inflammatory cytokine IL-10 was increased in the tadalafil-treated diabetic model in mice [10]. It has also been demonstrated that tadalafil is useful in the treatment of structural detrusor changes, hypoxia, and bladder compliance after cavernous nerve injury [11].

Sildenafil and vardenafil are other known PDE5 inhibitors. Several studies into sildenafil and vardenafil using various experimental nociceptive models have shown that they have anti-nociceptive properties [12, 13]. The aim of current study was to examine the possible involvement of tadalafil in nociception, morphine analgesia and morphine tolerance development in rats.

Methods

Animals

Wistar Albino rats (230-250 g, n=6 for each group) were acquired from the Laboratory Animal Center of Cumhuriyet University (Sivas, Turkey). The rats were maintained under standard situations: 12-h light–dark cycle (lights turn on at 08:00 A.M.) with ad libitum food and water and a constant temperature (22±2 °C). All the experiments were performed between 09:00 and 17:00. The animals were handled and the procedures were carried out in accordance with the guidelines provided by the National Institute of Health detailed in the "Principles of animal laboratory care". The experimental protocols were approved by the Cumhuriyet University Animal Ethics Committee.

Drugs

The morphine sulfate and tadalafil (Cialis® 20 mg tablets, Lilly) were dissolved in saline solution. The drugs were freshly dissolved on the days of experimentation. Morphine (5 mg/kg) was administered subcutaneously (s.c.) [4] and tadalafil (2, 4, 8, 16 mg/kg) intraperitoneally (i.p.) before the analgesia tests.

Induction of morphine tolerance

In order to induce morphine tolerance, the rats were selected at random and treated s.c. with 10 mg/kg morphine twice a day (09:00 and 17:00) for five days. Furthermore, morphine (10 mg/kg) applied 30 min after each tadalafil injection for five days in order to determine the effects of tadalafil (8 mg/kg, i.p.) on morphine tolerance. The optimal analgesic dose of morphine (5 mg/kg, s.c.) was given on the sixth day without saline or tadalafil and the tail-flick and hot-plate tests were measured at 30-min intervals (30, 60, 90, and 120 min) in order to evaluate the degree of tolerance.

Antinociception tests

Tail-flick test

We made use of a standard tail flick device (May TF 0703 Tail-flick Unit, Commat, Turkey) to measure thermal nociception. The radiant heat source was focused on the distal portion of the tail at a distance of 3 cm in each measurement after the administration of saline or the study's drugs. Tail-flick latencies (TFL) were obtained once the saline or drugs had been administered. The cut-off latency time was adjusted to 15 s to prevent tissue injury. The hyperalgesic response in the tail-withdrawal test is generally attributed to the central mechanisms of pain [14, 15].

Hot-plate test

The anti-nociceptive reaction on the hot-plate is thought to stem from a combination of central and peripheral mechanisms [14]. In this test, the animals were placed individually on a hot-plate (May AHP 0603 Analgesic Hot-plate Commat, Turkey) with the temperature calibrated to 54±3°C. The latency to the first sign of paw licking or jump reaction to avoid the heat was taken as an indicator of the pain threshold. The cut-off time was 30 s to prevent damage to the paw.

Experimental protocols

The analgesic effects of tadalafil and morphine were evaluated at 30-min intervals (30, 60, 90, and 120 min) using tail-flick and hot-plate tests. Doses administered i.p. at 2, 4, 8, 16 mg/kg were administered in order to evaluate the effective dose of tadalafil on nociception. Optimum activity was found at a dose of 8mg/kg. The animals were divided into six groups: Saline (S), 8mg/kg tadalafil, 5mg/kg morphine (M), M+ tadalafil, morphine tolerance (MT) and MT+ tadalafil. Saline and tadalafil were administered i.p. and morphine was administered s.c. at the indicated doses (volume of administration, 1 ml/kg).

Data analysis

To calculate the percentages of maximum anti-nociceptive effect (% MPE) the tail-flick and hot-plate latencies (which are in seconds) were converted to the percentage of anti-nociceptive effectiveness using this equation: % MPE = [(Post drug latency – Baseline latency) / (Cutoff value – Baseline latency)] ×100.

Statistical analysis

All experimental results were expressed as mean ± SEM (standard error of the mean). The anti-nociceptive effect was measured and the mean % MPEs were calculated for all groups. The data were analyzed using analysis of variance (one-way anova) followed by a post-hoc Tukey test. A significant difference was defined as a p value <0.05.

Results

The effect of different doses of tadalafil on nociception

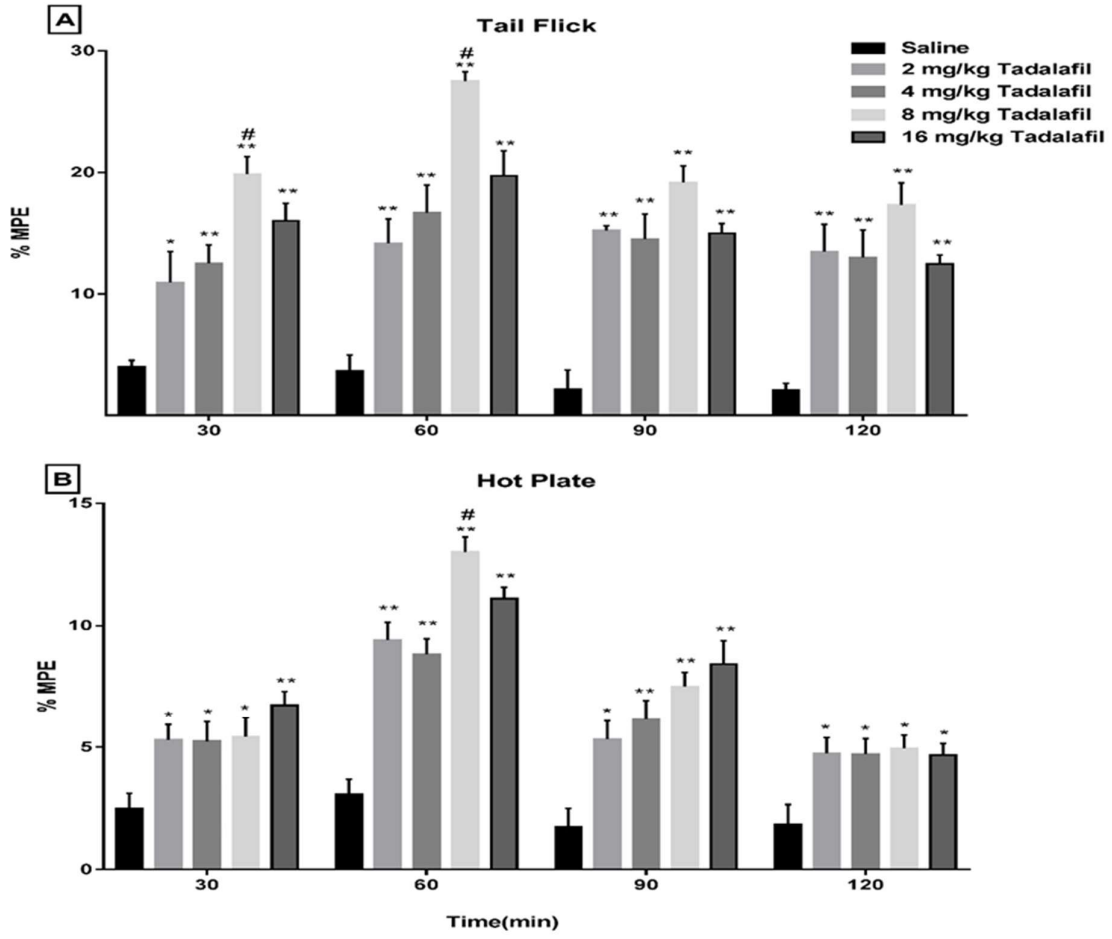
To examine optimal dose of tadalafil, the analgesic response were evaluated for 2, 4, 8 and 16 mg/kg doses of tadalafil at 30 min intervals during two hours by the analgesia tests. All the dose groups showed anti-nociceptive effects compared to the saline group at 30, 60, 90, and 120 minutes in both tail flick test ($p<0.05$; Figure 1A) and hot plate test ($p<0,05$; Figure 1B). However, the maximum analgesic effect was found at 60 min after the administration of 8 mg/kg tadalafil (27.5±0.7 for tail-flick and 13.0±0.6 for hot-plate test).

Antinociceptive effect of 8 mg/kg dose of tadalafil was significantly higher than 2 mg/kg, 4 mg/kg, 16 mg/kg tadalafil and saline groups in both tail-flick test ($p<0.05$; Figure 1A) and hot-plate test ($p<0.05$; Figure 1B).

The effect of tadalafil on morphine analgesia

The findings demonstrated that tadalafil significantly reduces the antinociceptive effect of morphine in both tail-flick test ($p<0.05$; Figure 2A) and hot plate test ($p<0.05$; Figure 2B) compared to the morphine group. Furthermore the maximum decreasing effect of

Figure 1. The effect of different doses of tadalafil on nociception . (A) shows effect of different doses of tadalafil in the tail-flick test; (B) shows effect of different doses of tadalafil in the hot-plate test. Each point symbolizes the mean±SEM of % MPE for 6 rats. *p<0.05 and **p<0,01 compared to the saline group,#p<0,05 compared to other dose groups.

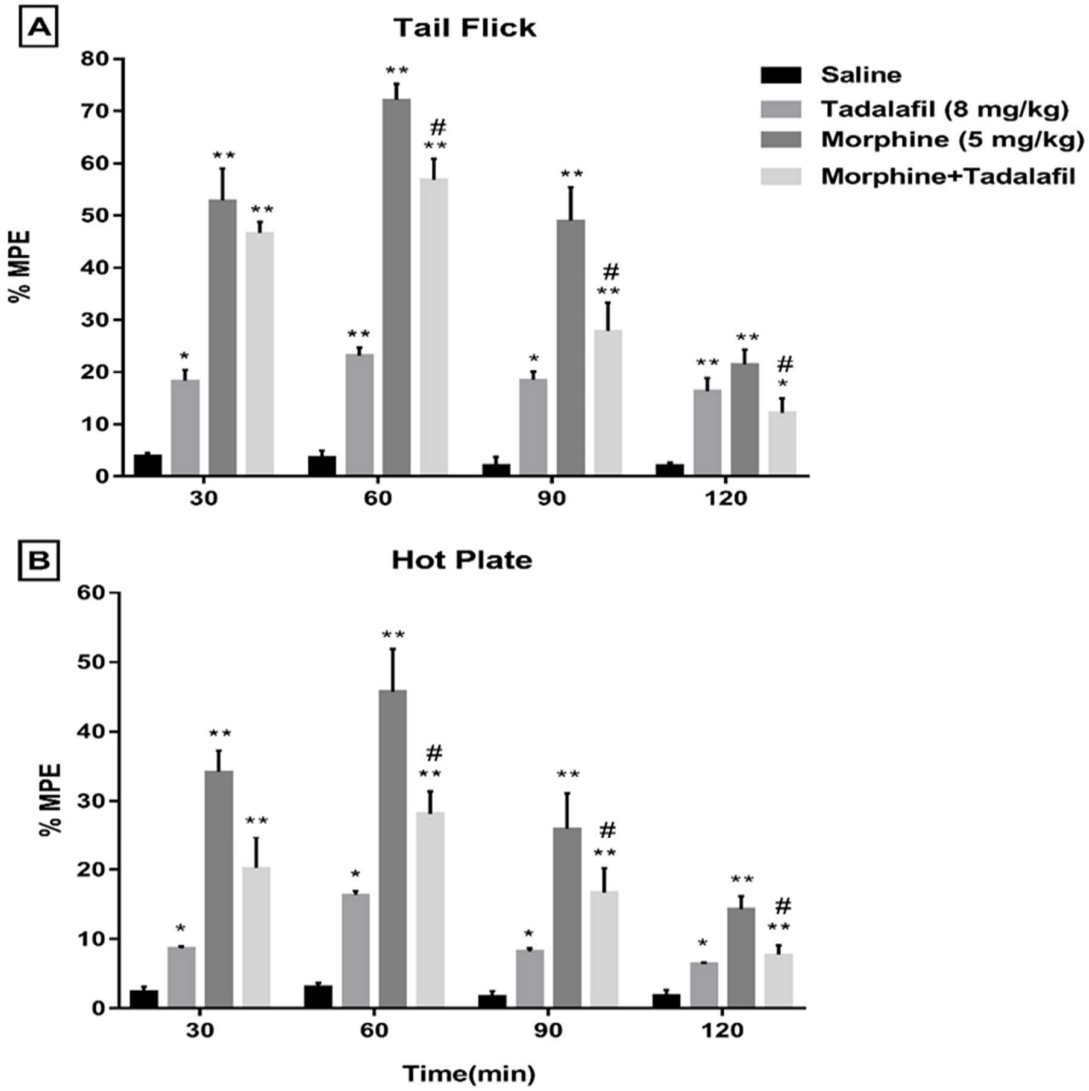


tadalafil on morphine was detected at 60 min in the tail-flick test (56.8±4.0) and hot-plate test (28.1±3.1).

The effect of tadalafil on morphine tolerance development

The morphine group's % MPE value was statistically higher than the morphine-tolerant group in both the tail-flick test ($p<0.05$; Figure 3A) and hot plate test ($p<0.05$; Figure 3B). Tadalafil in combination with morphine produced a significantly increased morphine tolerance development in both the tail-flick test ($p<0.05$; Figure 3A) and hot plate test ($p<0.05$; Figure 3B).

Figure 2. The effect of tadalafil on morphine analgesia. (A) shows effect of tadalafil on morphine analgesia in the tail-flick test; (B) shows effect of tadalafil on morphine analgesia in the hot-plate test. Each point symbolizes the mean±SEM of % MPE for 6 rats. *p<0.05 and **p<0,01 compared to the saline group, #p<0,05 compared to the morphine group.

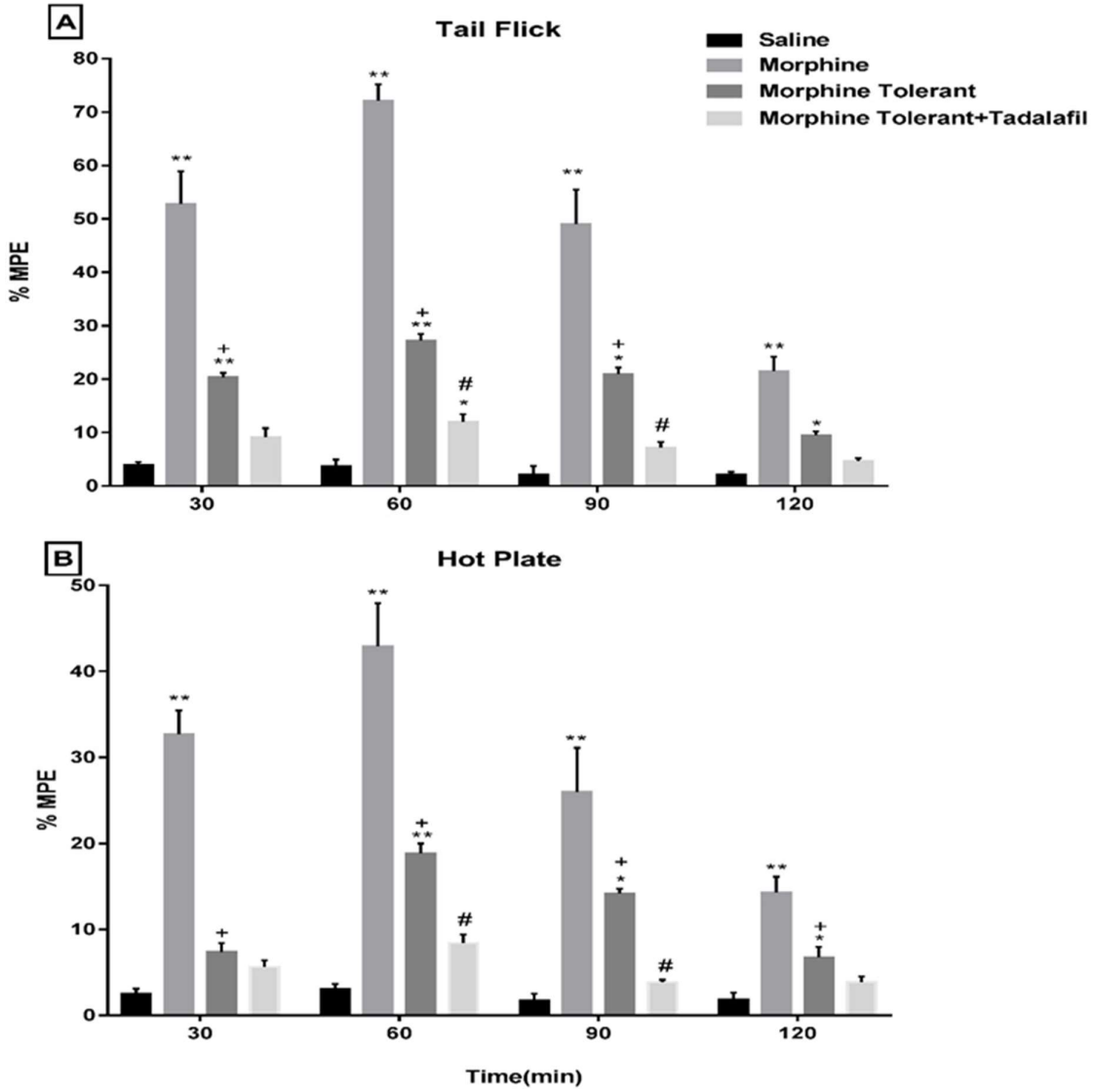


Discussion

In this study, we suggest that tadalafil is able to produce peripheral anti-nociception in both tail flick and hot plate analgesia tests. Tadalafil is a strong, selective and reversible phosphodiesterase 5 inhibitor that inhibits cyclic GMP breakdown [16].

In previous studies with PDE 5 inhibitors, it was shown that they have peripheral anti-nociceptive effects by increasing intracellular cGMP levels [12,13]. In contrast to our study, one study showed that the PDE 5 inhibitor sildenafil had anti-nociceptive effects on acetic

Figure 3. The effect of tadalafil on morphine tolerance development. (A) shows effect of tadalafil on morphine tolerance development in the tail-flick test; (B) shows effect of tadalafil on morphine tolerance development in in the hot-plate test. Each point symbolizes the mean±SEM of % MPE for 6 rats. *p<0.05 and **p<0,01 compared to the saline group, +p<0,05 compared to the morphine group, #p<0,05 compared to the morphine tolerance group.



acid and carrageenan-induced hyperalgesia but not in tail flick test and hot plate test [17]. In addition to our study, another study showed that the PDE 5 inhibitor sildenafil enhanced diclofenac anti-nociceptive effects in formalin tests [18]. Furthermore, one study demonstrated that the PDE 5 inhibitor tadalafil has analgesic effects on experimental arthritis by suppressing TNF alpha release in joints [19]. In contrast to these studies, it has been suggested that zaprinast, another PDE 5 inhibitor, has no effect on hyperalgesia induced by prostaglandin E2 [20]. The differences may be a result of the various models of pain utilized, since agents with direct inflammatory stimulus like prostaglandin E might not activate the arginine-NO-cyclic GMP track. However, this pathway seems to be stimulated by agents administered with an inflammatory stimulus such as carrageenan, formalin or uric acid [21, 22], and may not even be an inflammatory pathway like in our study.

Opioid drugs like morphine activate opioid receptors, which are a family of G protein-coupled receptors that mainly signal through heterotrimeric G protein $G_{I/O}$ subunits. When an agonist binds to opioid receptors, the activation of receptors causes a signal that inhibits adenylate cyclase and calcium channels. There are four types of opioid receptors: μ, δ, κ and nociceptin/orphanin FQ. Activation of all four types of receptors could produce analgesia, whereas tolerance is mainly related to the activation of μ-opioid receptors [23]. In contrast to a previous study by ixcoatl-Zecuatl et al [17], our study showed that the PDE 5 inhibitor tadalafil decreased the analgesic effects of morphine while increasing the development of morphine tolerance [17]. Tolerance is characterized as a decrease in effect following prolonged drug administration giving rise to a loss of drug efficiency, which was sufficient to cause analgesia in the beginning [24]. The mechanisms behind the development of tolerance to the analgesic effects of opioids are unclear and more studies are needed. Opioid tolerance development could involve changes in opioid and non-opioid mechanisms. Researchers have reported various different pathways in the developmental process of morphine tolerance [25, 26, 27]. To begin with, one of these mechanisms is the NO signaling pathway [2]. NO has been implicated in the central and peripheral nervous system as a biological messenger molecule [28, 29, 30]. It is derived from the L-arginine amino acid by means of the nitric oxide synthase enzyme (NOS). NOS activation and NO release stimulate the soluble guanylyl cyclase (sGC), which causes a rise in the levels of cyclic GMP in the cells [31, 32]. In a previous study, it was demonstrated that NO-independent sGC activators (YC-1 and BAY 41-2272) and NO donor (SNAP) combined with the morphine increased the rate of morphine tolerance like our study [4]. But, another study has suggested that sGC inhibitors (LY-83,583 and methylene blue) reduced morphine tolerance development [33]. These results suggest that the cyclic GMP pathway might play a role in the development of morphine tolerance.

Furthermore, there are the other mechanisms that can contribute to opioid tolerance such as desensitization and internalization, which are major pathways for tolerance. This pathway is formed by the activation of cyclic adenosine monophosphate (cAMP) protein kinase A (PKA) [34]. In addition, glutamate has a crucial effect on the increase of cAMP in this pathway and the development of tolerance [35]. Furthermore, NO/cGMP signaling leads to an increase in glutamate release [36].

Therefore, this indirect mechanism may be the reason for the possible effects of tadalafil on tolerance.

In conclusion, we found that the PDE 5 inhibitor tadalafil has anti-nociceptive effects at different doses. Moreover, tadalafil decreases the analgesic effect of morphine and increases the tolerance to morphine. In the light of this evidence, we propose that the intracellular nitric oxide–cGMP signaling pathway may play a role in both peripheral anti-nociception and the development of morphine tolerance.

Compliance with ethical statements

Conflicts of Interest: None.

Financial disclosure: None

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