Research Paper: Intra-cerebroventricular Administration of Crocin Attenuates Sleep Deprivation-induced Hyperalgesia in Rats

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ABSTRACT

Introduction: Sleep deprivation can cause hyperalgesia and interfere with analgesic treatments. The aim of the present study was to establish an obligatory sleep-abstinence model and also evaluate the effects of Intracerebroventricular (ICV) injection of crocin on pain perception in Wistar rats.

Methods: In this experimental study, 35 adult male Wistar rats were randomly divided into 5 groups (n=7). The intra-ventricular cannulation was done for all rats before sleep deprivation. Sleep deprivation was performed by placing animals on a chamber equipped with an automatic animated conveyor (5 s with an interval of 3 min) for 72 h. Subsequently, the sleep-deprived animals received ICV injection of saline (MOD), Morphine 10 µg (MOR), Crocin 10 ug (Cr10), and Crocin40 µg (Cr40) using a microsyringe. Besides, a non-sleep-deprived group was allocated as a Control Group (NC) and only received an ICV injection of saline. Fifteen minutes after the ICV injections, pain perception was evaluated by the hot plate test (54±0.4°C).

Results: Compared with the NC group, latency significantly decreased in the MOD group (6.28±0.48 vs. 4.28± 0.48, P<0.0001). In comparison with the MOD group, both morphine (8.42±1.53) and crocin (7.60±1.45 for Cr10 and 8.14±0.89 for Cr40) could significantly increase latency in the sleep-deprived animals (P<0.0001). There was no statistically significant difference between the Cr10 and Cr40 (P=0.42), Cr10, and MOR (P=0.059) and Cr40 with MOR (P=0.86) groups.

Conclusion: Our results indicated that crocin could attenuate hyperalgesia induced by sleep deprivation in rats.

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Highlights

- Central analgesic effect of Crocin is comparable with morphine.
- Sleep deprivation for 72 hours in a customized less harmful deprivation device decreases pain threshold.
- Social behavior of a group of sleep-deprived rats can be studied using our sleep deprivation device.

Plain Language Summary

Enough sleep is vital for human health and productivity. Chronic pain is a very common condition, which not only is annoying during wake time but also affects the quality and duration of sleep. Treating pain-related sleep problems is a challenging issue for physicians due to the side effects of hypnotics. A pain killer, which concomitantly can support sound sleep, will be very helpful in chronic pain. Crocin is one of the ingredients of saffron with the considerable analgesic property. In this study, we simulated chronic sleep loss in a group of sleep-deprived animals in a specific conveyor belt equipped with a chamber for 72 h. Then, we tested their pain threshold both with and without crocin injection into their brain ventricles. We also compared the effect of crocin with morphine, which is well known for its potent painkilling properties. Our results indicated that saffron may be the main source for developing drugs addressing pain-related sleep problems.

1. Introduction

Pain is a distressing feeling often caused by intense or damaging stimuli to escaping from threats, and an alert indicating there is a minor problem somewhere in the body. Meanwhile, this life-saving alarm is annoying when becomes chronic. Chronic pain with a prevalence of about 46.5% not only decreases the quality of life by depressing mood directly (Elliott, Smith, Penny, Smith, & Chambers, 1999) but also its role in sleep disruption imposes more emotional, physical, and economic burden (Magni, Marchetti, Moreschi, Merskey, & Luchini, 1993; Turk & Rudy, 1988). Chronic pain is considered as an important issue in health care systems and is the most common cause of seeking medical service (Black, 1975).

On the other hand, a great body of evidence shows that insomnia, loss of sleep, or Sleep Deprivation (SD) is associated with several neurological disorders, like memory deficits, Alzheimer disease, Parkinson disease, pain perception, etc. (Atkinson, Ancoli-Israel, Slater, Garfin, & Gillin, 1988). Primary insomnia prevalence is estimated at about 15 – 30% in different societies (Campbell et al., 2015; Cheatle, Foster, Pinkett, Lesneski, Qu, & Dhingra 2016; Modalen et a l., 2016). Comorbid insomnia prevalence in patients suffering from chronic pain is around 50%. On the other hand, depression is concomitant with sleep disturbances in a bidirectional relationship (Augustinavicius, Zanjani, Zakzanis, & Shapiro, 2014; Murphy & Peterson, 2015). These facts highlight the importance of a medical approach, by which these three common interrelated pathologies can be addressed. Common hypnotics, such as benzodiazepines increase total sleep time; however, they are unable to provide a refreshing sleep and also may increase pain sensitivity. Accordingly, physicians may choose opioids as an alternative (Lamberg, 1999). On the other hand, the chronic use of opioids is a medical challenge. Trazodone, an antidepressant medication, decreases sleep-onset latency, and increases sleep efficiency (O'malley et al., 2000). In spite of these effects of Trazodone, it can lead to muscle contraction and hypersensitivity to touch (Arnold, Keck, & Welge, 2000).

Although research on herbal remedies is still in its early stages, many herbs are thought to provide pain management and improve sleep quality (Uritu et al., 2018). Crocus sativus (Sarris, Panossian, Schweitzer, Stough, & Scholey, 2011), commonly known as saffron, is an appropriate candidate, which may target all these three conditions "pain, insomnia, and depression" at the same time. Traditionally, saffron has been used for depression, fear, sleep disorders, pain relief, etc. (Safakhah et al., 2016) which has been approved in different experimental studies (Vahdati Hassani, Naseri, Razavi, Mehri, Abnous, & Hosseinzadeh, 2014; Schmidt, Betti, & Hensel, 2007; Shafiee, Arekhi, Omranzadeh, & Sahebkar, 2018). The analgesic effect of saffron has also been indicated (Ahmad Dar, Brahaman, Tiwari, & Pitre, 2012). Crocin, picrocrocin, and safranal are three main biologically active ingredients of saffron. Crocin has reported responsible for the analgesic activity of saffron. The pain-relieving effect of crocin in orofacial pain has been documented by

injecting crocin into the cerebral fourth ventricle (Tamaddonfard, Tamaddonfard, & Pourbaba, 2015).

However, to the best of our knowledge, there is no evidence addressing the analgesic effect of crocin on sleep deprivation-induced hyperalgesia. The aim of the present study was to investigate the analgesic effect of crocin in a sleep deprivation condition.

2. Methods

2.1. Animals

Healthy male Wistar rats (60 days old) were purchased from the laboratory animal facility in Birjand University of Medical Sciences, Birjand, Iran. The rats were housed in a temperature-controlled room (22±2°C) with a 12 h light/dark cycle and had free access to standard laboratory animal diet (Behparvar, Iran) and tap water.

Experimental design and sleep deprivation

The animals were divided randomly into five equal groups (n=7) as follows:

- Control (CON): Intra-cerebroventricular (ICV) injection of saline without Sleep Deprivation (SD).
- Model (MOD): ICV injection of saline after SD.
- Morphine (MOR): ICV injection of morphine (40 µg/rat) after SD.
- Crocin 10 µg/rat (Cr10): ICV injection of crocin (Sigma, USA) (dissolved in saline) at a dose of 10 µg/rat after SD.
- Crocin 40 µg/rat (Cr40): ICV injection of crocin (dissolved in saline) at a dose of 40 µg/rat after SD.

2.2. ICV injection

To deliver the chemical agents into the brain, a permanent guide cannula was implanted in each lateral ventricle of the brain. In brief, each rat was anesthetized with an intraperitoneal injection of a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), and a 3-mm stainless-steel guide cannula (25 gauge) was stereotaxically (Stoelting Stereotaxic Apparatus, Wood Dale, IL, USA) placed in the lateral ventricle of the brain.

The stereotaxic coordinates, according to the Paxinos and Watson (1997) were: -1.08 mm posterior to the bregma, 2 mm lateral to the midline, and 4 mm below the top of the skull. The guide cannula was anchored with two screws and dental acrylic. To verify the correctness of the cannula implantation procedure, three rats were subjected to an ICV injection of 5µl Hematoxylin into each ventricle. Animals were then euthanized by the administration of a high dose of ether, and their brains were removed and placed in a formalin solution (10%). After 24 h, the brains were sectioned coronally (50-100 µm) and viewed under a loop to observe the distribution of Hematoxylin in the lateral ventricles according to the atlas of Paxinos and Watson (1997).

Intraventricular injections of the drugs were performed using a 13-mm length injection needle connected via a 20-cm polyethylene catheter to a 5-µl Hamilton syringe. The intra-cerebral injection was performed over 5 min with a total volume of 5 µl per ventricle. After injections, the injection needle was left in place for a further 30 s to facilitate diffusion of the drug solution. Crocin and morphine were injected 15 min before the Hot Plate (HP) test.

2.3. Sleep deprivation setup

In this study, we designed a Programmable Sleep Deprivation Device (PSDD). The PSDD consisted of a chamber (130×45 cm) equipped with an automatic animated

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Figure 1. The Programmable Sleep Deprivation Device (PSDD)

A. PSDD consisted of a water chamber; and B. An automatic animated conveyor belt

conveyor belt. In one side of the cage, a water chamber (25 cm in depth) was placed (Figure 1). When animals did not walk contrary to the conveyor direction, they fell into the water chamber. PSDD was set up on 3 min immobility followed by a 5 s transition time consequently. Accordingly, when rats were immobile more than a certain time (3 min), the conveyor belt moved them, therefore, they had to walk or fall into the water.

2.4. HP Test

Nociceptive sensitivity was assessed using the HP test. The HP surface heat was set at 55°C and a plexiglass cage was fixed over the HP. All rats were placed in the plate 15 min after ICV infusion. The start time was determined, and as soon as the hands or feet were started licking, animal tolerance was recorded. The cutoff point time was considered as 40 s. The apparatus was thoroughly cleaned between trials.

2.5. Statistical analysis

Statistical comparisons were performed using GraphPad Prism software v. 5 (GraphPad Software, San Diego, CA, USA). The differences among experimental groups were compared using the One-way ANOVA followed by Tukey's test. Values were expressed as Mean±SD. P<0.05 was considered statistically significant.

3. Results

No death was observed during the research procedure. The Shapiro-Wilks test indicated the normal distribution of data (P=0.062); therefore, the One-way ANOVA was used. The results of the HP test are depicted in Figure 2. Accordingly, SD caused a significant decrease in the latency of rats compared with non-SD animals (4.2±0.48 vs. 6.2±0.47, P<0.0001). As expected, the latency of SD rats receiving ICV injection of morphine (40 µg/rat) significantly improved even more than the control non-SD group (8.4±0.53, p<0.0001). Likewise morphine, crocin at both doses (10 and 40 µg/rat) significantly (P<0.0001) increased latency of the SD rats (7.6±0.45 and 8.14±0.89 respectively). There was no significant difference between crocin doses regarding latency in the HP test (P=0.42). Compared with the morphine-treated animal, there was no statistical difference in the latency of rats received Cr10 (P=0.059) and Cr40 (P=0.86).

4. Discussion

In this study, we measured the pain threshold in sleep-deprived rats in sham or ICV crocin-injected groups. The aim of sleep deprivation in this study was to produce a natural sleep deprivation model, not a total sleep deprivation. Fortunately, no rats died due to exposure to the PSDD. Sleep-deprived rats showed decreased latency to noxious thermal stimulus compared with the control group. All rats chose the farthest locations from the water chamber, indicating their awareness about the rules of

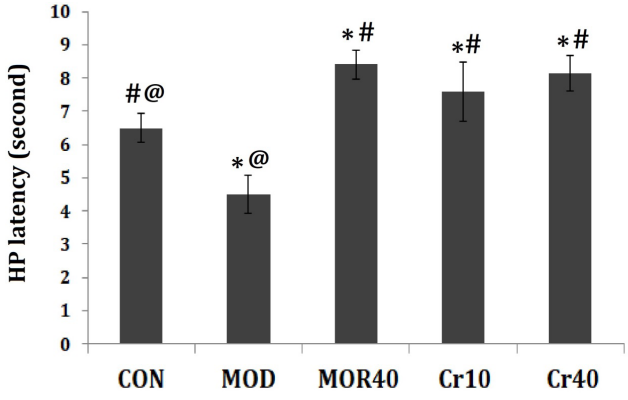

Figure 2. Effects of Intracerebroventricular (ICV) injection of crocin on sleep deprivation-induced hyperalgesia in rats

Values are presented as Mean±Standard Deviation.

CON: Control rats with no intervention; MOD: Sleep-deprived model group; MOR40: Sleep-deprived rats that received an ICV injection of morphine (40 µg/rat); Cr10 and Cr40: Sleep-deprived rats that received ICV injection of crocin at the doses of 10 µg/rat and 40 µg/rat, respectively

* P<0.001 versus CON; # P<0.001 versus MOD; @ P<0.05 versus MOR40

the PSDD. PSDD can be used to measure pain threshold for many rats at the same time, which reduces the rats' unwanted anxiety due to animal isolation.

Consistent with the results of crocin injection to the third ventricle of the rats (Erfanparast, Tamaddonfard, Taati, & Dabbaghi, 2015), crocin injection to the lateral ventricles also showed a central analgesic effect. It has shown that the analgesic mechanism of crocin probably is not mediated by opioid receptors, because its effect is not affected by naloxone (Tamaddonfard & Hamzeh Gooshchi, 2010). Crocin plays an antagonistic role against the long-term potentiation inhibitory effect of ethanol on the N-methyl-D-aspartate (NMDA) receptor (Abe & Saito, 2000; Abe, Sugiura, Shoyama, & Saito, 1998). Such an effect has been reported about memory impairment induced by scopolamine, as well (Ghadami & Pourmotabbed, 2009). Oral administration of aqueous extract of saffron has increased dopamine and glutamate neurotransmission in the brain (Ettehadi et al., 2013), whereas crocetin has found with an anti-glutamatergic effect (Berger, Hensel, & Nieber, 2011). ICV infusion of crocin exerts antiepileptic effect when it is injected in combination with diazepam, which may be due to its effects on the GABAA receptors (Tamaddonfard et al., 2012). The molecular mechanism of action of crocin in the brain seems more complicated considering that microglial cells are also targeted by active components of saffron (Nam et al., 2010; Xie, Huo, & Tang, 2009).

In a triple-blinded clinical trial, oral administration of capsules containing 250 mg of saffron has shown a mild analgesic effect for labor pain (%11.8 reduction in pain perception) (Azhari, Ahmadi, Rakhshandeh, Jafarzadeh, & Mazlom, 2014). In a rat model for chronic constriction injury, 30 µg/kg of oral crocin but not 15 mg/kg decreased the neuropathic pain (Safakhah et al., 2016). In a rat model of neuropathic pain, 50 and 100 mg/kg of intraperitoneal injection of saffron extract in combination with 3 mg/kg amitriptyline, showed a synergistic analgesic effect (Amin, Hosseini, & Hosseinzadeh, 2017). Several studies have focused on analgesic properties of saffron extract in rat models of neuropathic pain (Amin & Hosseinzadeh, 2012), however, no comparison has been made with morphine.

Crocin, even at high doses, cannot cross the Blood-Brain Barrier (BBB), but crocin is converted to crocetin in the gastrointestinal system after oral administration and crocetin can cross the BBB (Hosseini, Razavi, & Hosseinzadeh, 2018; Lautenschläger et al., 2015; Xi, Qian, Du, & Fu, 2007; Zhang et al., 2017). Therefore, the potential central analgesic properties of crocetin, as a metabolite of crocin, should be investigated. Interestingly, according to the results of a pharmacokinetic study, crocetin at an oral dosage of 22.5 mg/kg showed a rapid absorption and a mean half-life of about 7 h with no serious side effects (Umigai et al., 2011). However, it is still unknown that crocetin at which dose can reach the brain.

In conclusion, the analgesic effect following an ICV infusion of crocin can be a promising approach for more sophisticated research, which hopefully may provide a translational therapeutic strategy for chronic pain and concomitant comorbidities.

Ethical Considerations

Compliance with ethical guidelines

All animal procedures were conducted in accordance with the guide for the laboratory animals' care and usage of Birjand University of Medical Sciences, Birjand, Iran (Ethics Code: IR.BUMS.REC.1396.47).

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Authors' contributions

Performed experiments: Faezeh Rezaei; Designed the study: Mohammad Reza Saebipour and Kazem Ghaemi, Developed sleep deprivation machine and provide animal facilities: Mohsen Foadoddini; Analyzed the data and co-wrote the first manuscript draft: Mohammad Mehdi Hassanzadeh-Taheri and Mehran Hosseini; Read and approved the final version of the manuscript: All authors.

Conflict of interest

The authors declared that there are no conflicts of interest.

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