Journal of Experimental Pharmacology

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ORIGINAL RESEARCH

Role of Cannabinoid Receptors in Crocin-Induced Hypoalgesia in Neuropathic Pain in Rats

This article was published in the following Dove Press journal:
Journal of Experimental Pharmacology

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Purpose: Neuropathic pain involves injury or alteration of the normal sensory and modulatory nervous systems to produce a set of symptoms that are often difficult to treat. Previous study indicates that crocin has anti-inflammatory properties that may be mediated by the neurotransmitter system. In this study, we determine if there is an interaction between crocin and the cannabinoid system on chronic constriction injury (CCI)-induced neuropathic pain in male rats.

Materials and Methods: In this experimental study, adult male Wistar rats (220–250 g) were used. CCI was induced by setting four loose ligatures around the sciatic nerve. In part 1, after nerve lesion, vehicle, crocin (60 mg/kg) or Win 55-212-2 (0.1 mg/kg) as an agonist and AM 251 (0.1 mg/kg) as an antagonist of cannabinoid receptors were injected intraperitoneally daily in separate groups for 2 weeks. In part 2, two weeks after nerve lesion, vehicle (5 µL), crocin (6 µg/5 µL), Win 55-212-2 (0.1 µg/5 µL), AM 251 (0.1 µg/5 µL) were administered intracerebroventricularly (ICV) in separate groups. Mechanical allodynia and thermal hyperalgesia were measured using Von Frey filaments and plantar test device, respectively, at day 14. Data were analyzed by two-way ANOVA and Sidak's multiple comparisons post-test.

Results: Results indicated that centrally administered crocin significantly decreased thermal hyperalgesia and mechanical allodynia. Also, peripheral injection of crocin significantly decreased mechanical allodynia but not thermal hyperalgesia. Central or peripheral administration of Win 55-212-2 or AM 251 modulates the analgesic effect of crocin significantly.

Conclusion: Our findings showed that crocin has significant analgesic effects that are probably mediated by an endocannabinoid mechanism.

Keywords: neuropathic pain, crocin, rat, AM 251, Win 55-212-2

Introduction

Neuropathic pain as a type of chronic pain can enhance nerve sensitivity to noxious stimuli (hyperalgesia) and non-noxious mechanical and thermal (allodynia) stimuli, which are associated with a series of central and peripheral nervous system injuries.[1–3] The mechanisms involved in the induction and continuation of neuropathic pain are not exactly clear, but various studies have shown that involvement of the neuroimmune system and neuroinflammation due to glial activation in the peripheral and central nerves leads to the production of pro-inflammatory cytokines.[4] Several studies have documented that proinflammatory cytokines such as TNF-α and some interleukins play an important role in the development, spreading, continuity, and severity of neuropathic pain.[1,5] The pain is intractable and rarely responded to treatments. There is plenty of evidence that researchers have been looking for safer

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DovePress
http://doi.org/10.2147/JEP.S250738

approaches to deal with pain, with special attention being paid to the use of medicinal plants. One of the plants whose therapeutic effects are excessively raised in traditional medicine, and has a high frequency of consumption everyday as a food flavoring, is *Crocus sativus*, whose stigma consists of saffron.[6] Saffron is a mixture of bitter taste, aromatic aroma, and red color.[7] Its bitter taste is related to picrocrocin, which is a glycosylated hydroxyl safranal. Other components include monotropoids, which are volatile and aromatic compounds. The color of saffron is due to crocin, which is a type of glycosylated carotenoid.

Generally, the saffron extract contains a variety of carotenoids such as crocetin, crocetin di-glucoester, crocetin di-gentibiose, and crocin.[8] Various studies have shown beneficial effects of saffron administration against tumor growth and genotoxicity of a chemotherapeutic agent in vitro and in vivo, respectively.[9,10] Evidence suggests that cellular stress produces free radicals which lead to various central nervous system (CNS) neurodegenerative diseases such as Alzheimer's and Parkinson's and crocetin and crocin in saffron extract inhibit the effects of free radicals.[11]

Some studies have shown that saffron has a moderating effect on neurobehavioral activities, glutathione, glutathione peroxidase, glutathione reductase, glutathione transferase, superoxide dismutase, catalase, and Na, K-ATPase, and the content of glutamate and aspartate.[12] Because one of the causes of neuropathic pain is inflammation and oxidative stress, and crocin has an anti-inflammatory and antioxidative effect,[13] the crocin may therefore be effective in attenuating the neuropathic pain. Recently, our colleagues in a new study have shown that crocin increases analgesic effects of morphine in neuropathic pain male rats.[14]

Previous studies have shown that the cannabinoids are potentially effective in the treatment of neuropathic pain. In this regard, the use of tetrahydrocannabinol as a synthetic and non-specific cannabinoid receptor agonist has been effective in reducing allodynia associated with neuropathic pain in an animal model.[15]

Our previous study revealed that the systemic administration of crocin could relieve neuropathic pain in the chronic constriction injury (CCI) model in rats.[16]

Amin and Hosseinzadeh showed the beneficial effects of aqueous and alcoholic extracts of saffron on neuropathic pain induced by sciatic nerve CCI.[17] In another study on the spinal cord contusion rats, treatment with crocin significantly reduced injury-related pain. Someone believed that the mechanism of action of crocin in controlling neuropathic pain is a reduction in serum levels of CGRP.[18] The activity of cannabinoids and endocannabinoids in the CNS operate with specificity and occurs through interactions with CB1 receptors.[19] The CB1 receptors are widely distributed in the CNS and are predominantly present in the presynaptic terminals.

Extensive studies have been carried out on the effects of cannabinoids and their receptors on neuropathic pain; for example, Palazzo and his colleagues showed that CB1 receptors in CNS have a moderating role in inflammatory and neuropathic pains.[20]

Toniolo and colleagues reported that coping with neuropathic pain using hemopressin, a CB1 receptor agonist, has beneficial effects in neuropathic pain which indicates the role of CB1 receptors in modulating pain. The effect of hemopressin is mediated by reducing the calcium inflow in the DRG neurons and opening up of potassium channels in the peripheral nerves.[21]

The study of Svizenska and colleagues on CB2 receptors in DRG showed that these receptors also play a role in modulating neuropathic pain.[22] It has been reported that saffron plays a protective role in retinal damage induced by the cannabinoid system.[23] Regarding the effect of the cannabinoid system on neuropathic pain, and its interaction with the protective effect of saffron on retinal injury, and also the hypoalgesic effect of crocin which is an important component of saffron, the aim of this study is whether crocin's effect on neuropathic pain is mediated through cannabinoid receptors.

Materials and Methods

Animals

In the present study, 105 adult male Wistar rats (15 groups with seven rats in each group) weighing 220–250 g were used. They were kept at 12:12 h light/dark cycle at constant temperature of 22 ± 2 °C and controlled humidity and had free access to water and food.

The samples were selected randomly from the total population of animals in the physiology research center of Semnan University of Medical Sciences, Semnan city, Semnan province and randomly assigned to control and experimental groups. All experiments were performed in accordance with the rules of the local Ethical Committee of the Semnan University of Medical Sciences for Animal Studies under permit IR. Semums. REC 1395.147.

Drugs

The chemicals which were used in the present study were included crocin (Sigma Co.), Win 55-212-2 (Tocris Co.), and AM 251 (Tocris Co.).

Crocin was administered at the intraperitoneal dose of 60 mg/kg[24] and the ICV dose of 6 μg/rat. Win 55-212-2 as a cannabinoid receptor agonist was administered intraperitoneally (dose of 0.1 mg/kg) and the ICV (dose of 0.1 μg/rat). AM 251 as a cannabinoid receptor antagonist was administered at the intraperitoneal dose of 0.1 mg/kg and the ICV dose of 0.1 μg/rat. Doses of drugs were selected based on previous studies.[15,25-27] Physiologic saline was used as crocin solvent and dimethyl sulfoxide (DMSO) 40% solution was used as solvent of Win 55-212-2 and AM 251.[15] AM 251 was injected 0.5 hour before Win-55-212-2 and crocin in separate groups. Determination of neuropathic pain was performed one hour after crocin injection on the 14th day of treatment.

Experimental Groups

In the present research 105 rats were andomly divided into 15 groups as explained in Table 1. In the present study we intended to detect the effect of crocin administration immediately following injury until maximum pain response time (14 days post-surgery) and its interaction with the cannabinoid system. In the first experiment (i.p. treatments), crocin i.p. was injected repeatedly during 14 days post-surgery and at days 14, saline, Win 5-212-2 (non-selective CB1 receptor agonist) and AM 251 (CB1 cannabinoid receptor antagonist)[28] were administered 30 minutes before crocin injection in separate groups and then 60 minutes later behavioral tests were performed according to the timeline in Figure 1.

In the second experiment (ICV treatments), nine days after CCI, ICV cannulation was performed through stereotaxic surgery and five days later (recovery time) at 14 days post-CCI, saline, Win 5-212-2 and AM 251 were administered 30 minutes before ICV injection of crocin in separate groups and then 60 minutes later behavioral tests were performed according to the timeline in Figure 2.

Table 1 Experimental Groups

Days Groups	1-13 i.p. Injection	14 ICV Injection	Test
Intact	–	–	✓
Sham	Veh + Sal	–	✓
CCI	Veh + Sal	–	✓
CCI	Win + Sal	–	✓
CCI	AM + Sal	–	✓
CCI	Veh + Cro	–	✓
CCI	Win + Cro	–	✓
CCI	AM + Cro	–	✓
Sham	–	Veh + Sal	✓
CCI	–	Veh + Sal	✓
CCI	–	Win + Sal	✓
CCI	–	AM + Sal	✓
CCI	–	Cro + Veh	✓
CCI	–	Cro + Win	✓
CCI	–	Cro + AM	✓

Abbreviations: i.p., intraperitoneal; ICV, intracerebroventricular; CCI, chronic constriction injury; Sal, saline; Veh, vehicle; Win, Win 5-212-2; AM, AM 251; Cro, crocin.

Surgical Procedure and Neuropathic Pain Induction

Induction of neuropathic pain was performed through CCI of the sciatic nerve, which was described by Bennet and Xie.[29]

After anesthetizing the animal using intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg), a 2-cm long incision was made at the place of sciatic nerve of the left thigh. After moving aside the muscles of the area, the sciatic nerve was separated from the surrounding tissues. Four loose moveable ligatures at a distance of 1 mm apart were created around the sciatic nerve before branching nerve trifurcation using Catgut Plain # 4.0 sutures and then the skin was stitched using silk suture # 4.0. Animals in the sham group received all procedures except nerve ligation. All animals were kept in solitary cages for 24 hours after surgery to fully become conscious and start eating and drinking.

Intracerebroventricular Cannulation

Seven days following sciatic nerve CCI, the skull of animal was fixed in the stereotaxic apparatus and a stainless steel guide cannula (No. 23 and 10 mm in length) was implanted (coordinate of AP = −0.6, DV = −3.5 and ML = 1.5 from the skull level)[30] according to the rat brain atlas of Paxinos and Watson.[31] The implanted cannula was fixed on the skull with dental cement. The cannula was kept open using sterile oil-impregnated copper wire. The rats were placed in a room with controlled temperature until they regained consciousness. Five days following stereotaxic surgery were considered as recovery time and the experiments were carried out at 14 days post-CCI. The ICV injection was performed by using a Hamilton syringe through the guide cannulas.

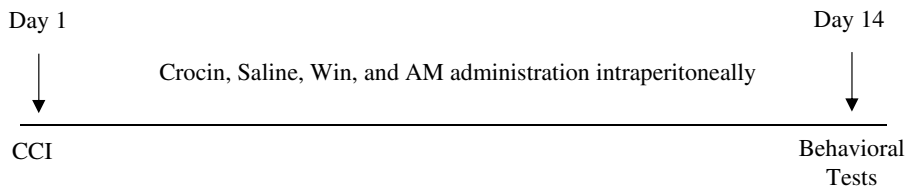

Figure 1 Timeline of intraperitoneal administration of treatmentss.

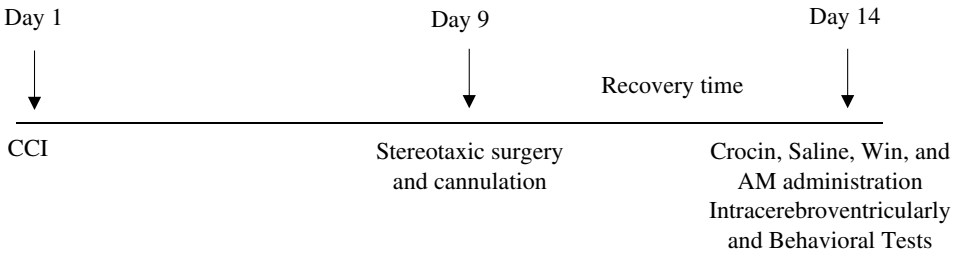

Figure 2 Timeline of intracerbroventricular administration of treatments.

Pain Assessment

Mechanical allodynia and thermal hyperalgesia were evaluated on the 14th day after surgery (initiation of maximum pain-like response time) by Von Frey hairs (Stoelting, Wood Dale, IL, USA) and a plantar test device (Ugo Basile Biological Apparatus, Italy), respectively. Both mechanical allodynia and hyperalgesia were evaluated in the same animals.

Evaluation of Mechanical Allodynia

Mechanical allodynia was assessed by Von Frey hairs through the method described by Ren.[32] Von Frey hairs (Stolting Co., the USA) are elastic filaments which apply a different force to the surface depend on their diameters. The filaments are applied to the dorsal surface of the injured paw between fingers 2 and 3 in order of increasing diameter and forces, until a withdrawal response is obtained. Filaments were used in an ascending manner. Three withdrawal responses from five stimulations (1 second for each stimulation with a 10 second time interval between each) were considered a pain-like threshold response. Sixty-gram force was considered as the cut-off point.[33]

Evaluation of Thermal Hyperalgesia

Thermal hyperalgesia was assessed through the method described by Bennett.[29] To determine thermal hyperalgesia, plantar test device (Ugo Basile Biological Apparatus, Italy) was used. After habituation in the plantar test device (about 15 minutes), a source of infrared radiation was adjusted below the injured foot and exposure to the beam began with an intensity of 60 Hz. The radiation was exposed three times consecutively with an interval of 5 minutes and the average of three withdrawal responses was regarded as the pain-like response of each animal. The cut-off time of each test was considered to be 60 seconds for preventing tissue damage due to radiation.

Statistical Analysis

Regarding the normal distribution of data (using the Kolmogorov–Smirnov test), two-way analysis of variance (ANOVA) was used. Sidak's multiple comparisons test was used to compare the mean of groups to each other and $P < 0.05$ was considered significant. All groups that received intraperitoneal injection were compared with each other and also all groups that received ICV injection were compared with each other. All data were expressed as mean ± SEM of measured parameters. The Graphpad prism 8.0 statistical software (GraphPad, San Diego, CA, USA) was used to analyze the data.

Results

The present results showed that crocin can suppress neuropathic pain induced by CCI in rats. The results were divided in three parts: approving neuropathic pain, evaluation of mechanical allodynia and thermal hyperalgesia following peripheral injection of crocin, and evaluation of mechanical allodynia and thermal hyperalgesia following central injection of crocin. Crocin has no significant increase in paw withdrawal threshold in naïve rats (data not shown).

Induction of Neuropathic Pain Using Chronic Constriction Injury

Figure 3 shows the paw withdrawal threshold response of CCI rats against that in sham and intact rats. CCI of the sciatic nerve significantly led to mechanical allodynia ($F_{2, 18} = 16.25$, $P < 0.001$) (Figure 3A) and thermal hyperalgesia ($F_{2, 18} = 18.31$, $P < 0.01$) (Figure 3B).

Interactions of Crocin and Cannabinoid Receptors on the Mechanical Allodynia and Thermal Hyperalgesia Following Peripherally Injected Crocin

Figure 4A shows the effects of peripheral injection of crocin as one of the main constituents of saffron on the mechanical allodynia (Von Frey test) in the presence of Win 55-212-2 as a cannabinoid receptor agonist and AM 251 as an antagonist of cannabinoid receptor. The animal behavior was evaluated on day 14 to investigate the interaction of crocin and cannabinoid receptors on the neuropathic pain. CCI decreased the paw withdrawal threshold significantly ($P < 0.05$), which was increased by intraperitoneal injection of crocin in neuropathic pain rats compared to the saline treated group. Pretreatment with AM 251 significantly ($P < 0.001$) prevented the effect of crocin on the paw withdrawal threshold in CCI rats. Pretreatment with Win 55-212-2 did not change the effect of crocin on the paw withdrawal threshold in CCI rats.

Two-way ANOVA indicated a significant difference between groups (vehicle, Win 55-212-2, and AM 251) ($F_{2, 36} = 13.43$, $P = 0.0001$), significant differences between treatments (saline and crocin) ($F_{1, 36} = 5.95$, $P = 0.01$) and significant interaction between groups and treatments ($F_{2, 36} = 5.34$, $P = 0.009$). Win-55-212-2 has a similar effect in saline and crocin groups. On the other hand, the effect of AM 251 is completely different in saline and crocin treatments groups.

Figure 4B displays the effects of peripheral injection of crocin on the thermal hyperalgesia in the presence of Win 55-212-2 as a cannabinoid receptor agonist and AM 251 as an antagonist of cannabinoid receptor.

Intraperitoneal injection of crocin decreased (increased latency) thermal hyperalgesia in neuropathic pain rats-with respect to the saline-treated group, but this decrease was not significant. However, pretreatment with Win 55-212-2 increased the crocin effect so that effect of the sum of them was significantly ($P < 0.05$) greater than the effect of saline pretreatment Win 55-212-2. Pretreatment with AM251 significantly ($P < 0.05$) reversed the effect of crocin + Win on paw withdrawal latency in CCI rats.

Two-way ANOVA indicated a significant difference between groups (vehicle, Win 55-212-2, and AM 251) ($F_{2, 36} = 4.949$, $P = 0.0126$), and a significant difference between treatments (saline and crocin) ($F_{1, 36} = 14.81$, $P = 0.0005$).

Interactions of Crocin and Cannabinoid Receptors on the Mechanical Allodynia and Thermal Hyperalgesia Following Centrally Injected Crocin

Figure 5A shows the effects of ICV injection of crocin on mechanical allodynia in presence of Win 55-212-2 as a cannabinoid receptor agonist and AM 251 as an antagonist of cannabinoid receptor.

ICV injection of crocin significantly ($P < 0.0001$) decreased mechanical allodynia in neuropathic pain rats compared to the saline-treated group. Pretreatment with AM251 significantly ($P < 0.01$) reversed the effect of crocin

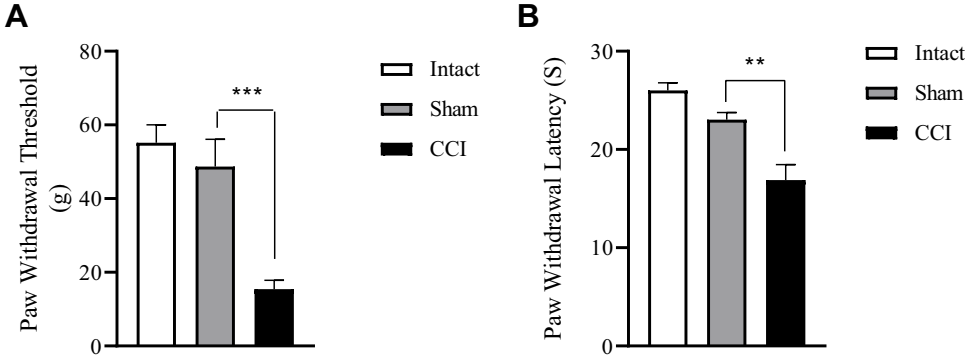

Figure 3 Induction of neuropathic pain using chronic constriction injury. Chronic constriction injury (CCI) of the sciatic nerve significantly reduced paw withdrawal threshold (**A**) and paw withdrawal latency (**B**) in male rats. Data are expressed as mean ± SEM of the evaluated parameter. n = 7. $^{**}P < 0.01$, $^{***}P < 0.001$.

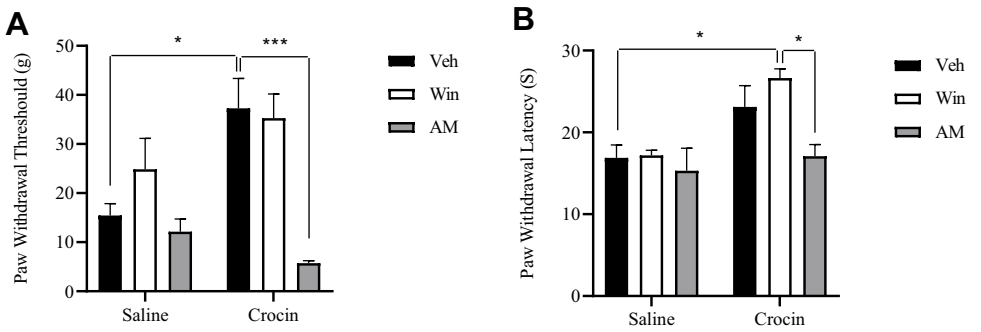

Figure 4 Interaction of peripherally injected crocin and cannabinoid receptor on the mechanical allodynia and thermal hyperalgesia. Crocin significantly increased paw withdrawal threshold in neuropathic pain rats and pretreatment with AM 251 as an antagonist of cannabinoid receptor significantly prevented thecrocin effect (**A**). Intraperitoneal injection of crocin increased paw withdrawal latency in neuropathic pain rats compared to saline-treated rats, althoughthis increase was not significant. Crocin pretreatment with Win 55-212-2 significantly increased paw withdrawal latency with respect to bothsaline + Veh and saline + Win 55-212-2. Pretreatment with AM 251 significantly prevented the effect of crocin + Win on the thermal hyperalgesia (**B**). Data are expressed as mean ±SEM of the evaluated parameter. n = 7. *P < 0.05, ***P < 0.001.

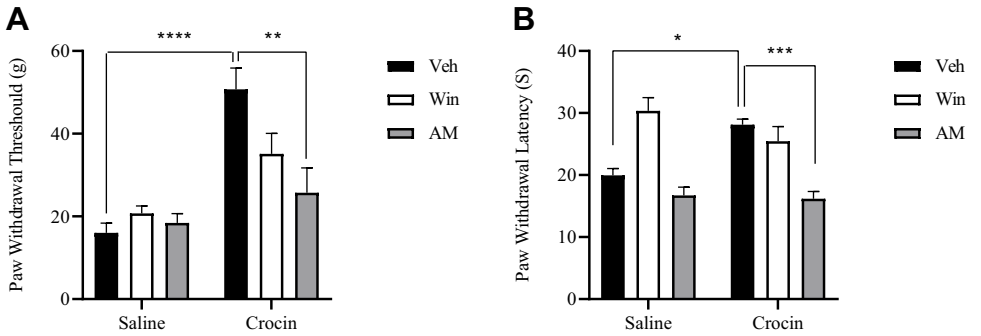

Figure 5 Interaction of crocin and centrally injected cannabinoid receptor on mechanical allodynia and thermal hyperalgesia. Intracerebroventricular administration of crocin significantly increased paw withdrawal threshold in neuropathic pain rats and pretreatment with AM 251 significantly prevented the crocin effect. Win 55-212-2 as an agonist of cannabinoid receptor along with crocin decreased the effect of crocin on the paw withdrawal response of neuropathic pain rats, but this effect was not significant (**A**). Intracerebroventricular administration of crocin significantly changed paw withdrawal latency in neuropathic pain rats compared to the saline + vehicle group and pretreatment with AM 251 significantly prevented the crocin effect. In the saline-treated group, administration of Win 55-212-2 significantly increased paw withdrawal response of neuropathic pain rats compared to the saline-treated group and AM 251 significantly reversed the Win effect (**B**). Data are expressed as mean ± SEM of the evaluated parameter. n = 7. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

on the paw withdrawal threshold in CCI rats. Pretreatment with Win 55-212-2 reduced (but not significantly) the effect of crocin on the paw withdrawal threshold in CCI rats.

Two-way ANOVA indicated a significant difference between groups ($F_{2, 36}$ = 3.782, P = 0.0323), a significant difference between treatments ($F_{2, 36}$ = 13.43, P = 0.0001), and a significant interaction between groups and treatments ($F_{2, 36}$ = 6.010, P = 0.0056).

Figure 5B illustrates the effects of ICV injection of crocin on thermal hyperalgesia in the presence of Win 55-212-2 as a cannabinoid receptor agonist and AM 251 as an antagonist of cannabinoid receptor. ICV injection of crocin significantly increased the latency of thermal hyperalgesia in neuropathic pain rats (P < 0.05) with respect to saline received CCI rats and administration of AM 251 before crocin, significantly (P < 0.001) prevented the effect of crocin.

Pre-administration of Win 55-212-2 did not change paw withdrawal latency of neuropathic pain rats compared to the crocin-treated group.

Two-way ANOVA indicated a significant difference between groups ($F_{2, 36}$ = 26.36, P < 0.0001), and a significant interaction between groups and treatments ($F_{2, 36}$ = 8.600, P = 0.0009).

Discussion

To our knowledge, this is the first study about the interaction of crocin and the cannabinoid system in improving neuropathic pain. In the first stage of this study, neuropathic pain was induced by CCI and the result was compared to a sham group. The Von Fray (evaluation of mechanical allodynia) and plantar (evaluation of thermal hyperalgesia) tests were used to assess neuropathic pain.

The results of this study showed that CCI led to mechanical allodynia and thermal hyperalgesia. These results are in consistent with the results of other studies.[16,34]

The pain intensity in different groups in the present study was assessed following the injection of crocin alone and in combination with a cannabinoid receptor agonist and its antagonist. Our results showed that crocin reduces neuropathic pain induced by chronic constriction injury of the sciatic nerve in male rats.

This result is consistent with the findings of previous studies evaluating the effect of crocin on neuropathic pain. Safakhah and colleagues showed that crocin reduced mechanical allodynia and thermal hyperalgesia in CCI male rats.[16] On the other hand, it has recently been shown that crocin increases paw withdrawal threshold in morphine-treated neuropathic pain rats.[14]

Amin and Hosseinzadeh examined the effects of aqueous and alcoholic extracts of saffron on the neuropathic pain induced by CCI on the sciatic nerve. They reported that the aqueous and alcoholic extracts of saffron significantly decreased CCI-induced neuropathic pain in male rats.[17] Because of the antitumor and antioxidant effects of saffron have been investigated in in-vitro and in-vivo studies,[35,36] so it is possible to attribute the hypoalgesic effect of saffron to its antioxidant properties. On the other hand, the antioxidative effect of saffron has been attributed to crocin, which is one of the active constituents of saffron.[37]

Also, some studies have shown that saffron has anti-inflammatory, antidiabetic, and anticancer activities.[17,38-41] Clinical findings indicated that saffron also has antiepileptic effects.[42]

In the present research, CCI-induced mechanical allodynia did not change following intraperitoneal and intracerebroventricular administration of the cannabinoid receptor agonist. In consistent with these results, it has been reported that the synthetic administration of cannabinoid agonist (Win 55-212-2) reduces allodynia and hyperalgesia in the neuropathic pain rats.[15]

Further, a study by Costa and colleagues in 2004 showed that repeated administration of the synthetic cannabinoid agonist (Win 55-212-2) as a therapeutic agent led to a simultaneous reduction in hyperalgesia and the production of algogenic mediators in the rats with neuropathic pain.[43] Possible reasons for this controversy could be: (1) a different route of Win 55- 212-2 administration in their study with respect to ours, and (2) repeated administration of Win 55- 212-2 in their research versus a single administration in our study.

In the other part of the present study we examined the effect of cannabinoid receptor agonist and antagonist on the hypoalgesic effect of crocin. Administration of Win 55-212-2 before crocin caused no significant difference compared to the group that received vehicle and crocin, and the observed allodynia and hyperalgesia were the same in both groups (Win + crocin in comparison with vehicle + crocin). On the other hand, pre-administration of AM 251 (cannabinoid receptor antagonist) suppressed the hypoalgesic effect of crocin, which revealed an interaction of crocin with the cannabinoid system. In line with the results of this research, it has been reported that the analgesic effect of paracetamol inhibited following pretreatment with AM 251 (cannabinoid antagonist).[44] On the other hand, Maccarone and colleagues showed that similar to cannabinoid receptor blocking, saffron suppresses retinal damage due to high-intensity brightening light through downregulation of cannabinoid receptors.[23] This report shows that the protective effect of saffron on the retina could be due to cannabinoid system activation.

In another part of this study, peripheral and central administration of the cannabinoid receptor antagonist (AM 251) on the CCI-induced neuropathic pain revealed no significant change compared to the vehicle group. In consistent with our finding, Costa and colleagues in 2005 reported that SR141716 (CB1 receptor inverse agonist) not only decreased the neuropathic pain but also improved repair of the myelin sheath.[45] This discrepancy could be due to several reasons, including duration of treatment (a single administration in our study versus repeated administration in the study of Costa et al), different doses of antagonists (0.1 μg/kg and 0.1 mg /kg in our study versus 1, 3, and 10 mg/kg in the study of Costa et al), and different routes of administration (i.p. and ICV administration in our study against oral administration used in the study by Costa et al).

As chronic pain, importantly neuropathic pains, is one of the main concerns of healthcare providers such as physicians and rehabilitation authorities, and has also shown a poor response to a variety of therapeutic methods, and besides various studies which have proved the analgesic effects of crocin, therefore the main challenge in this study was to find the mechanism of crocin's analgesic effects and comprehensively the role of cannabinoid receptors following systemic and intracerebroventricular injection of crocin on the behavioral responses of the neuropathic pain. The present study suggests that the systemic and intracerebroventricular injection of cannabinoid receptor antagonist (AM 251) has an inhibitory effect on the

crocin-induced hypoalgesia. Because administration of the cannabinoid receptor antagonist reduced the hypoalgesic effects of crocin, it can be concluded that the crocin at least exerts some of its hypoalgesic effects through cannabinoid receptors. It was found that crocin alone induced the hypoalgesic effect on the neuropathic pain, but pre-administration of Win 55-212-2 had no synergistic effect on the crocin-induced hypoalgesia. Regarding this effect, it could be supposed that the observed effect is the maximum response due to crocin-stimulated endogenous cannabinoid release or pain-modulating mediators.

With regard to our present results and previous studies of cannabinoid receptors and their analgesic effects, it seems that the cannabinoid receptor is one of the possible mechanisms by which crocin exerts its analgesic effects. In addition, it should be noted that there are various mechanisms other than the cannabinoid system that play a role in the effects of crocin on the CCI-induced neuropathic pain, including the anti-inflammatory or antioxidative effect of crocin,[37,46,47] or the reducing effect of crocin on the CGRP level,[18] or the crocin-mediated release of analgesic mediators that needs to be further studied.

Regarding repeated administration of crocin in the first experiment of this study, it is possible that the crocin led to histological or even gene expression changes that in turn led to hypolagesia. Due to financial constraints we could not evaluate histological changes or gene expression evaluation, which is one of the limitations of this research.

According to our results, the observed hypoalgesia following ICV administration of crocin was more significant than intraperitoneal administration. One possible explanation for this observation could be due to the first-pass effect following i.p. administration of crocin. Also it may be that some degradation of crocin following i.p. administration led to a less significant effect against that ICV administration. It is possible that after intraperitoneal injection, crocin was partially involved with hepatic first-pass metabolism, which led to reducing its concentration at the target sites.

Conclusion

Because the administration of the cannabinoid receptor antagonist reduced the analgesic effects of crocin, it can be concluded that at least crocin exerts its analgesic effects partly through the cannabinoid receptors.

Acknowledgment

The present research was supported by a grant (ethical number: IR.Semums.REC 1395.147) from the Semnan University of Medical Sciences, and the authors announce their appreciation.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure

All authors have nothing to disclose and declare that they have competing financial interests.

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