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Original Article

Therapeutic Potential of Black Seed and Opium Poppy Oils in Mitigating Morphine Withdrawal Syndrome in an Animal Model

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Abstract

Background: Dependence and withdrawal syndrome caused by opioids are the most important and unambiguous factors in the clinical use of narcotic painkillers. Some evidence indicates the beneficial effects of herbal fragments in attenuating this complication. In the current study, the impact of black seed oil (BSO) and opium poppy oil (OPO) was investigated on the symptoms of morphine withdrawal syndrome in mice.

Methods: For three days, morphine was administrated to induce dependence in mice. To induce the withdrawal syndrome, on the 4th day, naloxone was injected. Thirty minutes before naloxone administration, various doses of BSO (250, 500 mg/kg) and OPO (150, 300 mg/kg) were given as active treatments, along with saline and clonidine, to 6 groups. The data obtained were compared to those from other groups that received clonidine and saline separately (n = 8). The levels of excitability, anxiety-like behavior, and pain threshold were assessed using the open field test, elevated plus maze (EPM), and hot plate tests.

Findings: Clonidine and both studied doses of BSO significantly increased the presence of mice in the light arm of the EPM ($P < 0.05$). The irritability and locomotion in animals with withdrawal syndrome in the groups that received BSO and clonidine considerably reduced and the pain sensitivity was elevated ($P < 0.05$). There was no significant difference between the BSO and clonidine groups. OPO did not significantly improve symptoms.

Conclusion: The present results revealed that the administration of BSO is effective in relieving the manifestations of morphine withdrawal syndrome, including anxiety, irritability, and motor activity, in a manner comparable to clonidine.

Keywords: Black seed oil, Locomotion, Morphine withdrawal syndrome, Opium poppy oil, Pain threshold

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Introduction

Morphine and other related opioids are potent analgesics that are widely used in the market, especially for the control of moderate to severe pains. However, they are also among the most commonly abused substances.[1] In recent years, addiction to opioids, which are generally prescribed to relieve acute and even chronic pain, has significantly increased, imposing substantial costs on the healthcare systems of various countries.[2] It is estimated that 2-10% of patients who receive long-term prescription opioids (for neuropathic pain and pain due to malignancy) manifest addiction to varying degrees.[3]

Chronic use of opioids is often associated with dependence, and their sudden withdrawal causes symptoms of withdrawal syndrome, including restlessness, irritability, insomnia, muscle pain, nausea, vomiting, and diarrhea, which can limit the clinical use of this class of drugs.[4] Despite extensive research, compounds that possess the analgesic properties of opioids without causing addiction and dependence have not yet been identified. Furthermore, despite numerous studies aimed at understanding the mechanisms involved in opioid dependence, the definitive cause of this phenomenon remains unclear.[5]

Resources of traditional medicine and recent research on medicinal plants have revealed that these agents can be useful in treating different degrees of addiction with fewer complications and costs. Plants, such as Oats, St John's wort, Passionflower, and Valerian root have shown promise in alleviating withdrawal symptoms, reducing dependence, and potentially detoxifying opioid addicts. Some of these agents have also entered clinical

trials.[5,6] Nowadays, due to the increased accessibility of these factors, many investigations have focused on the use of effective components and their phytochemical metabolites, particularly for the management of widespread disturbances in the central and peripheral nervous systems.[6]

The beneficial effects of *Nigella sativa* as a neuroprotective and anti-inflammatory agent have been considered in several investigations.[7] The oil gained from the seeds of *N. sativa* (black seed oil) modulates the discharge of some neurotransmitters, such as glutamate and gamma-aminobutyric acid (GABA), in cultured cortical neurons.[8,9] The exceptional analgesic effects of *Papaver somniferum* (opium poppy) have also been noted in conditions of hyperalgesia.[10] Also, opium poppy oil possesses antioxidant and sedative properties.[11]

So far, there has been no evaluation or comparison of the effects of black seed and opium poppy oils in relieving the manifestations of opioid withdrawal syndrome. Presently, most protocols for managing opioid withdrawal syndrome involve the use of sympathetic blockers, particularly clonidine.[3] Therefore, the aim of this study was to determine the effects of oral administration of black seed and opium poppy seed oils on the incidence of opioid withdrawal syndrome in an animal model, in comparison with clonidine.

Materials and Methods

Devices and Chemicals

The tools and chemicals used in this research included the following: black seed (standardized based on 71% W/V linoleic acid) and opium poppy oils (standardized based on 63% W/V linoleic acid) purchased from Barij Essential Pharmaceutical Company (Iran), clonidine hydrochloride manufactured by Tolid Darou Pharmaceutical Company (Iran), morphine sulfate manufactured by Daroupakhsh Company (Iran), naloxone made by Caspian Tamin Company (Iran), insulin syringes, the digital scale made by Noavaran Tajhiz Company (Iran), mice restraints, standard animal cages for mice measuring 24x13.5x13 cm, a hot plate device, the set of cameras, an open field box, and behavior monitoring software manufactured by Tajhiz-Gostar-Omid-Iranian Equipment Company (Iran).

Animals and the Procedure of Morphine Withdrawal Induction

Mice have been a popular animal model for addiction research.[12,13] They have a robust genetic predisposition to exhibit addictive behaviors, making them an ideal model for studying the genetic components of addiction. Also, they have been selectively bred to display exaggerated behavioral responses to addictive substances, such as cocaine and morphine, allowing researchers to isolate specific traits and study their mechanisms.[14]

To carry out this research, male NMRI (Naval Medical Research Institute) mice, which are an excellent choice for behavioral studies, were used. These mice weighed between 20 to 30 grams and were approximately three months old. The mice were sourced from the animal house of Baqiyatallah University of Medical Sciences (Iran) and were maintained in the pharmacology laboratory of the Faculty of Pharmacy. The animals were housed in five groups of eight (according to similar studies) in special cages and weighed before the experiment. It should be mentioned that all the experiments were conducted during the light phase of the day.[12]

To induce opioid dependence in mice, morphine was administered with the following schedule and amounts: morphine was injected intraperitoneally at doses of 50, 50, and 75 mg/kg three times a day (for three days at 9:00 am, 1:00 pm, and 5:00 pm, respectively) to induce dependence in mice. The afternoon dose was higher to minimize withdrawal during the night. Also, on the morning of the fourth day (2 hours before the naloxone injection), 50 mg/kg of morphine was administrated.[13]

To induce withdrawal syndrome, naloxone (2 mg/kg) was injected 2 hours after the last morphine administration on the fourth day. Animals were then evaluated by behavioral tests as described below. Different doses of black seed oil (250, 500 mg/kg) and opium poppy oil (150, 300 mg/kg) were administered as active treatments, along with clonidine (0.2 mg/kg) as a positive control and saline as a negative control, injected 30 minutes before naloxone in different and separate groups (Figure 1). Table 1 displays the animal groups that were examined.

Elevated Plus Maze

The murine elevated plus maze (EPM) is a type of unconditioned anxiety test that is mostly used to check the level of induced anxiety, memory, and depression. The basis of this test lies in the animal's natural reluctance to enter open arms, reflecting the conflict between exploration and aversion to open spaces. This apparatus consists of two open arms and two closed arms arranged in a cross formation, elevated half a meter above the ground. The dimensions of the light (open) and dark (closed) arms of this device are 50 x 40 x 10 cm. These arms are connected by a square area measuring 10 x 10 cm. The appropriate light for this test in this research was provided by a 100-watt lamp positioned 120 cm above the

Table 1. Groups of Studied Mice Addicted to Morphine (n = 8)

Group number	Agents administrated after addiction induction
1	Saline (0.2 mL) + Naloxone (2 mg/kg)
2	Clonidine (0.2 mg/kg) + Naloxone (2 mg/kg)
3	Opium poppy oil (150 mg/kg) + Naloxone (2 mg/kg)
4	Opium poppy oil (300 mg/kg) + Naloxone (2 mg/kg)
5	Black seed oil (250 mg/kg) + Naloxone (2 mg/kg)
6	Black seed oil (500 mg/kg) + Naloxone (2 mg/kg)

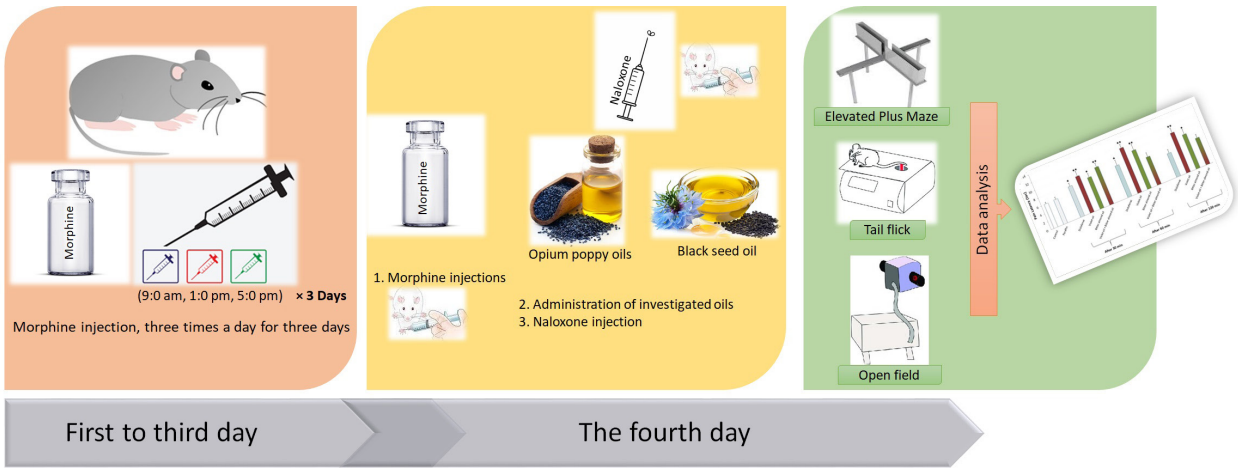

Figure 1. The process and timeline graph of creating an addiction to morphine and subsequently inducing withdrawal syndrome in the studied small laboratory mice

central area.

In the current study, after naloxone injection, the animals were allowed to explore the device freely for five minutes, during which their behavior was filmed and recorded for subsequent analysis. To standardize conditions, all animals were placed in the central area facing the open arm to avoid forcing them into the closed arm. The duration of time spent in the light and dark arms, as well as the total number of entries into the open and closed arms, were recorded based on the video footage. An entry into an arm was defined as the mouse placing all four paws inside, and the time spent in the arm was measured accordingly.[15]

Tail Flick

The tail flick test is one of the examples of a thermal reflex pain test, primarily driven by spinal responses. In this test, the painful stimulus was generated by focused light radiation directed at a specific point on the mice's tails. The thermal stimulus from the lamp rays was set to 9 V. The light intensity was adjustable, ranging from levels 1 to 10. In all experiments, the intensity of emitted light was set to 10, and the cut-off time was 15 sec.[16]

Open Field

The open field is one of the most well-known behavioral paradigms used in rodents for testing anxiety-like behavior, exploration, and habituation. Data collected from the open field test contain significant information about the rodent's behavioral constitution. This tool includes a square surrounded by long walls to prevent the animal from escaping, typically measuring 40 x 40 x 30 cm for small laboratory mice. The floor of this square is divided into 16 smaller square units. A camera positioned above the open field records the animal's movements, including the number of squares traversed, resting time, the duration the animal stands on its hind legs (rearing time), and the frequency of licking and scratching (grooming time).

This test was performed after naloxone injection, and to standardize the conditions for all animals, the mice were first placed in the central square. The duration of this experiment was 5 minutes, during which the animals were allowed to exhibit their behaviors freely while being recorded by the camera.[17]

Statistical Analysis

All data were reported as the mean ± standard deviation of the tested mice in each group, and a one-way variance was applied for statistical analysis. SPSS version 24.0 and Microsoft Excel 2016 were used to analyze data and generate graphs. In each case, a *P*-value of less than 0.05 was considered significant. The researchers also performed a Kolmogorov-Smirnov test and obtained a *P*-value of 0.077, which is higher than the alpha level of 0.05, indicating that the data follow a normal distribution.

Results

Assessment of Pain Response Threshold

In the tail-flick test, 30 minutes after naloxone administration, the groups receiving black seed oil (both studied doses) produced significant analgesic effects compared to the control group ($P<0.05$). In this context, the administration of opium poppy seed oil did not have a remarkable effect on increasing the pain threshold in the tail flick test. The groups receiving 250 and 500 mg/kg doses of black seed oil did not differ significantly from each other, and there was no considerable difference between the black seed oil and clonidine groups (Figure 2).

Assessment of Motor Activity

The data of the open field test are presented in Figure 3. In the groups receiving black seed oil (both 250 and 500 mg/Kg) the average duration of resting time (immobility) was considerably higher compared to the control group ($P<0.05$). However, this effect was not as great as that observed with clonidine administration.

Bipedal standing time (rearing) was significantly reduced in morphine withdrawal syndrome mice

receiving black seed oil. In this case, the greatest decrease in rearing time due to morphine withdrawal syndrome was observed in mice receiving clonidine, although there was no significant variation between the groups receiving black seed oil and clonidine.

In the animals receiving 500 mg/kg of black seed and clonidine, grooming time was significantly lower than that of the control group ($P<0.05$). Also, in mice receiving 250 mg of black seed oil and both groups receiving opium poppy oil, no significant difference was observed in comparison with the controls.

Regarding motor activity, the administration of black seed oil was able to reduce the total number of crossed squares in mice with withdrawal syndrome ($P<0.05$). There was no significant difference between clonidine and black seed oil in reducing the locomotion rate in mice suffering from morphine withdrawal syndrome. Opium poppy oil had no significant effect on any of the evaluated factors in the open field test.

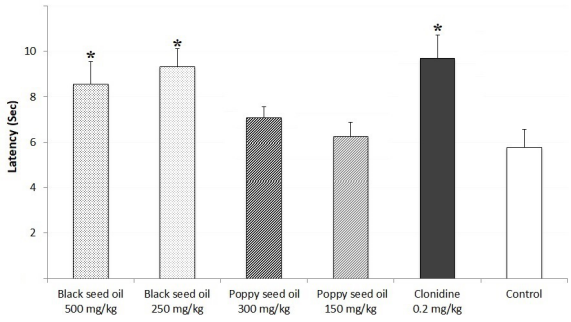

Figure 2. Comparison of the time taken to initiate a reaction to the heat stimulus in the tail-flick test after naloxone injection in mice addicted to morphine and receiving different amounts of opium poppy and black seed oils. Data are reported as mean ± standard deviation (n = 8), and the results obtained from each group were analyzed independently against the control group using one-way analysis of variance and SPSS software. * indicates a significant difference compared to the control group ($P<0.05$)

Assessment of Anxiety-Like Behavior

Data from the EPM test revealed that in morphine-dependent mice, the administration of either 250 or 500 mg/kg of black seed oil (similar to clonidine) before inducing withdrawal syndrome with naloxone significantly decreased the total number of arm crossings compared to the controls ($P<0.05$). Also, the administration of 500 mg/kg of black seed oil or clonidine led to an increase in the presence of animals with withdrawal syndrome in the open arms. The administration of the investigated doses of opium poppy seed oil did not have a significant effect on the time spent in the light arms or the total number of arm crossings in this test (Figure 4).

Discussion

The development of tolerance, dependence, and the occurrence of withdrawal syndrome, characterized by symptoms, including anxiety, irritability, and reduced pain threshold are the major and common problems associated with the therapeutic use or abuse of opioid

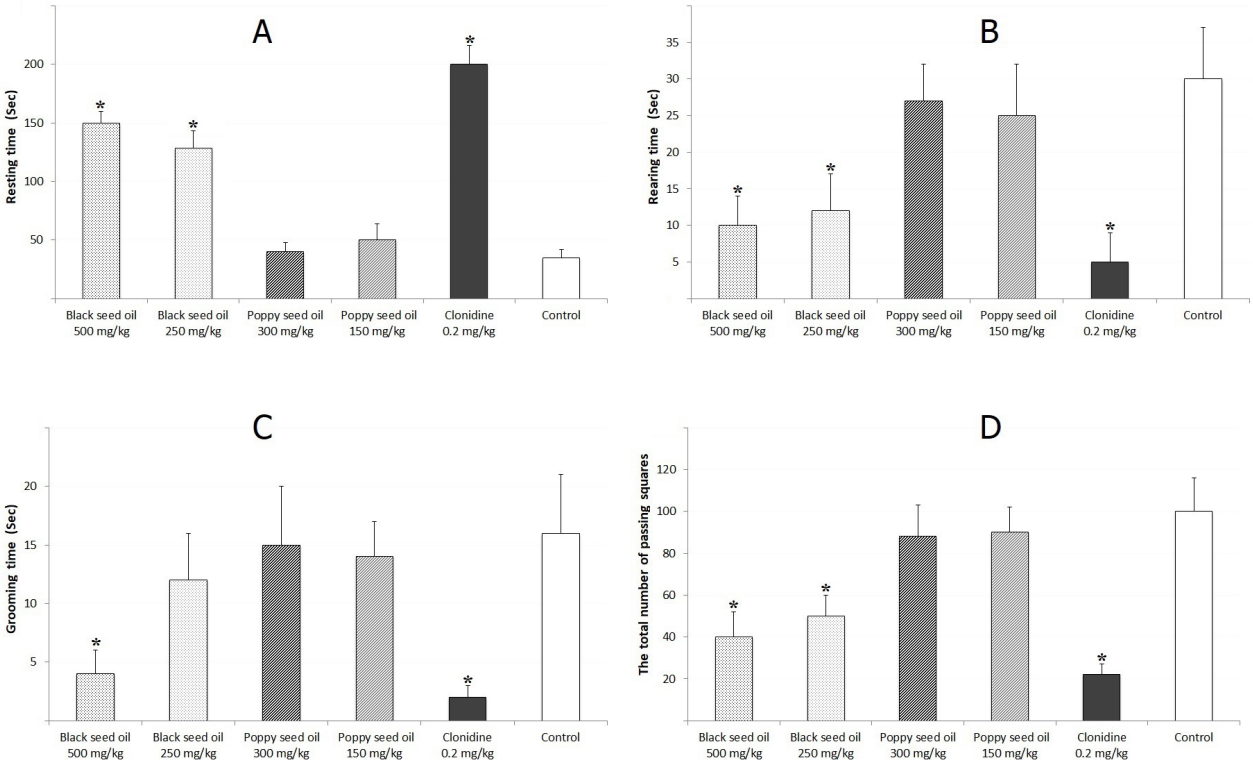

Figure 3. Evaluation of data obtained from the open field test after naloxone injection in mice addicted to morphine and receiving different amounts of opium poppy and black seed oils. Data are presented as mean ± standard deviation (n = 8), and the results from each group were analyzed separately against the control group using one-way analysis of variance and SPSS software. * indicates a significant difference compared to the controls ($P<0.05$)

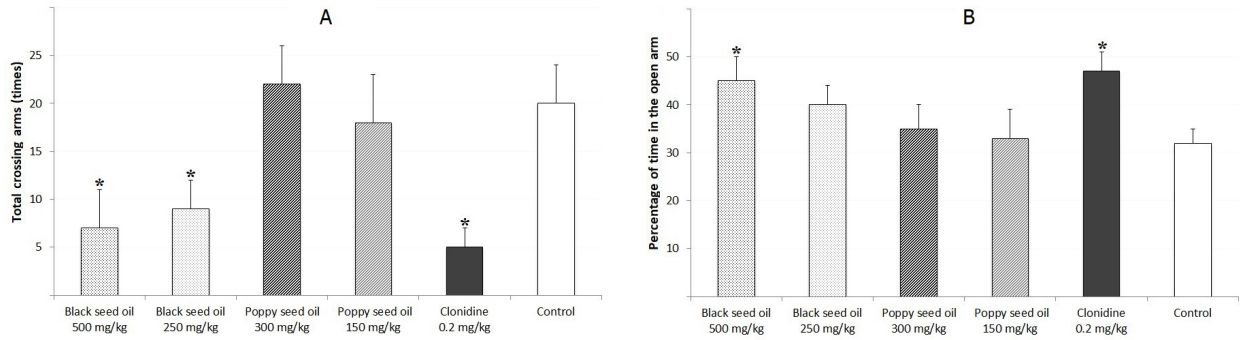

Figure 4. Data from the elevated plus maze (EPM) test after naloxone injection in morphine-addicted mice receiving different doses of opium poppy and black seed oil. Data are presented as mean ± standard deviation (n = 8), and the results obtained from each group were analyzed independently against the control group using one-way analysis of variance and SPSS software. * indicates a considerable difference compared to the controls ($P < 0.05$)

agents.[12] Withdrawal syndrome among users of these agents is a challenge that can be controlled to some extent with autonomic system inhibitors, such as clonidine and its derivatives. However, the side effects of this class of drugs have also created restrictions.[18] There is some promising evidence regarding the use of herbal agents and their derivatives in treating this complex psychosomatic condition.[5]

Recent preclinical research has elucidated that the phytochemical components of the *N. sativa* plant, particularly the benzoquinone compounds (such as thymoquinone), are effective in addressing various neuropsychiatric complications, including anxiety and depression.[19] In addition, in many studies, benzylisoquinoline alkaloids and other components found in the oil of the poppy plant (*Papaver somniferum*) have shown various medicinal effects, including antioxidant, anti-inflammatory, and analgesic properties.[20] Considering that the primary source of opioids is the poppy plant, the initial hypothesis is that the oil of the poppy plant may be beneficial in alleviating the manifestations of morphine withdrawal syndrome, which was investigated in the present study.

The emergence of anxiety as one of the symptoms of withdrawal syndrome has made it difficult for addicts to cease chronic morphine use. In the EPM test, the two factors of duration of presence and repetition of entering the light arms are considered measures to evaluate anxiety-like behavior. The results of the current study confirmed that the administration of black seed oil can decrease the anxiety behavior caused by withdrawal syndrome. In line with our results, Ajao et al. showed that black seed oil can increase open-arm explorations in rats.[21] Previous studies have identified thymoquinone in black seeds as the primary active component responsible for reducing anxiety caused by stimulants and toxic agents.[22,23] Also, thymoquinone can prevent the development of and dependence on morphine.[24]

Despite previous findings indicating that poppy seed oil can reduce anxiety behaviors, the current study revealed that anxiety symptoms resulting from morphine withdrawal syndrome cannot be alleviated with poppy oil.[25] This oil is ineffective for alleviating anxiety symptoms of opioid withdrawal due to its lack of necessary compounds for treating underlying neurochemical imbalances. Opium poppy oil is derived from the opium poppy plant and generally does not contain opioid alkaloids, like morphine and codeine.[26]

In the present study, black seed oil (unlike poppy seed oil) was able to improve the acute reduction in pain threshold following the stress of morphine withdrawal syndrome (about 80% of the latency observed with clonidine). The role of inflammatory mediators and oxidative stress in morphine withdrawal syndrome is evident.[27] Also, pain sensation is centrally regulated by different neural complexes, such as opioidergic, noradrenergic, and serotonergic pathways.[28] Chemical analyses in previous studies have clearly revealed that the amount of opioid alkaloids in poppy oil is minimal and can be disregarded.[29]

In previous studies and animal models, the administration of black seed oil has reduced neuropathic pain by strengthening the serotonergic pathways involved in the pain inhibitory system of the brainstem.[30] Thymoquinone and other quinolones from black seed oil are cyclooxygenase inhibitors and reduce prostaglandins involved in pain.[31] It has been confirmed that various components of *N. sativa L.* oil, such as unsaturated fatty acids and essential oils, inhibit the generation of reactive oxygen species, which are produced under stress conditions and lead to a decrease in pain threshold.[32]

N. sativa extract has shown efficacy in the ventral tegmental area (VTA) of the brain, which is involved in reward and drug dependency.[33] The study by Sedaghat et al. on rats reported that thymoquinone has a protective effect on dopaminergic neurons.[34] In addition, using Western blotting, the researchers identified changes in the levels of dynorphin and enkephalin, two endogenous opioids that regulate the perception of pain and pleasure.[35] However, specialized studies showed a specific increase in dopamine and a decrease in glutamate secretion with *N. sativa*, suggesting a relaxing effect or even a reduced propensity for addiction.[36] Previously, immunohistochemical

assessments demonstrated that black seed oil decreased astrogliosis (a pathological hallmark of CNS injury) in the caudate and accumbens nuclei.[37] Similar benefits of black seed oil have been documented in the pyramidal neurons located in the prefrontal and frontal cortex.[21]

In seizure threshold assessment models, black seed oil reduced motor activity and muscle contractions.[38] Consistent with previous findings, the present study showed that black seed oil has significant effects comparable to clonidine in reducing the number of instances of bipedal standing, grooming time, and overall motor activity, which significantly increase after opioid withdrawal syndrome.[39] In this case, the administration of poppy oil had no significant effects on locomotion and other behaviors associated with distress following morphine withdrawal syndrome.

It has been demonstrated that chronic administration of black seed oil increases the levels of serotonin, which induces the coordination of behavior and reduces anxiety.[40] The oil contains some flavonoids that have been widely studied for their potential effects on memory, learning, and cognition.[41] However, the earlier investigation with EPM and open field tests revealed an augmentation in explorative activity and non-sedative anxiolytic effects under non-stress conditions.[42]

Conclusion

The administration of black seed oil is effective in relieving the manifestations of morphine withdrawal syndrome, including anxiety, irritability, and motor activity, in a manner comparable to clonidine. Given the availability of various pharmaceutical dosage forms of black seed oil, it can be considered for human studies. It can be clearly stated that the components in black seed oil have promising effects in improving the reduction of pain threshold following morphine withdrawal syndrome. More research is needed to determine the exact pharmacological mechanism of black seed oil in controlling these symptoms, and it is recommended to evaluate other withdrawal situations, including alcohol withdrawal syndrome.

Limitations

This study only examined the impacts of the investigated oils in an animal model of anxiety-like behavior and pain threshold and did not address other symptoms of opioid withdrawal syndrome. Also, the exact components of the oils and their quantities were not precisely described.

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Authors' Contribution

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Writing-original draft: Mahdi Mashhadi Akbar Boojar, Reza Kazemi.
Writing-review & editing: Mehdi Saberi.

Competing Interests

The authors reported no conflicts of interest.

Ethical Approval

The mice had free and continuous access to standard pelleted food and water, with a light/dark cycle of 12 hours each, a temperature of 25 ± 1°C, and a relative humidity of 45-55% maintained from the beginning to the end of the experiments. The number of mice in each cage was kept below ten, and all other ethical protocols approved by the Ethics Committee of Baqiyatallah University of Medical Sciences were adhered to during the transportation and housing of the animals. This study was approved by the Ethics Committee of Baqiyatallah University of Medical Sciences (IR.BMSU.REC.1400.087; letter number: 91000017 dated 2021-11-24).

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