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Research Article

Evaluating the Effects of Chronic Administration of Natural Honey on the Development of Dependence on Morphine in the Male Rats

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ABSTRACT

Background: According to the previous studies, the exact mechanism of dependence on opioids and withdrawal syndrome has not been fully understood but one of the most important mechanisms is the increase of pro-inflammatory cytokines in CNS. On the other way, previous studies showed that natural honey (NHO) has anti-inflammatory properties. This study was aimed to evaluate the effects of chronic administration of natural honey on the development of morphine dependence in male rats.

Methods: Honey was prepared from Tarom Oliya region in Zanjan province. Experiments were performed on male Wistar rats weighing 225-275 g, randomly divided into 6 groups (n=8). The study groups included morphine group, the three doses of morphine plus honey group (at doses of 200,400 and 800 mg/kg, i.p.), the morphine plus vehicle group, and the saline group. The subcutaneous injections of additive doses of morphine were used for 9 days to create morphine dependency. On the 9th day, one hour after the morning dose of morphine, naloxone (4 mg/kg, i.p.) was injected, and symptoms of withdrawal syndrome were assessed for 60 minutes. Then, blood samples were taken to measure TNF-α. One-way ANOVA and Tukey tests were used to compare the results. P- Value of <0.05 was considered as statistically significant.

Results: The results of this study showed that intraperitoneal injection of honey at 3 doses (200, 400 and 800 mg/kg with p <0.001) could significantly decrease the total score of the symptoms compared to the morphine-vehicle control group. Natural honey (NHO) could significantly decrease TNF-α at dose of 400 mg/kg.

Conclusion: The results indicated that chronic administration of NHO had beneficial effects in reducing symptoms of morphine withdrawal syndrome, and this effect is probably due to the anti-inflammatory effect caused by the polyphenolic compounds in honey.

Introduction

Opioids have an essential role in treatment of pain. They are used for the management of acute and chronic pain throughout the world. Opioids also influence the treatment of chronic non- cancer pain. Effectiveness, safety, and abuse liability of opioids are important issues, which requires spending further time to approve them.[1] Morphine works through opioid receptors named plasma membrane-bound G protein-coupled receptors.[2-4] However, evidence showed that these receptors and their signaling ways are important.

There are three groups of endogenous opioid peptides (endorphins) having a unique effect in CNS.[5] Their mechanisms in the CNS have not been understood correctly. However, studies showed that monoamine neurotransmitters, serotonin, and norepinephrine are necessary to control pain.[6-8] There are many studies on opiate dependence and its withdrawal symptoms, including studies on increasing inflammatory cytokines (such as IL-1β, IL-6 and TNF-α,[9] nitric oxide (NO)[10] and induction of oxidative stress in the central amygdala,[11] increase in the NMDA (N-methyl-D-aspartate)[8,6] and glucocorticoid receptors.[12] Also, different uncontrolled processes are related to morphine oxidative stress.[13-16]

Honey is a traditional food product, which has been known as the highest and most nutritious foods for centuries, and it has also been used to treat most diseases among all nations due to its healing properties. Many studies showed that honey could have several effects on our body[17] like anti-oxidant,[18] anti-inflammatory,[19] anti-bacterial,[20] anti-diabetic[21] as well as protective effects on respiratory, gastrointestinal, cardiovascular[17,22] and nervous systems.[17] Honey has anti-oxidant,[23,24] anti-bacterial and anti-inflammatory properties. It can be used as a wound dressing to promote rapid and improved healing.

Some recent studies have emphasized the use of natural honey in modern medicine along with the use of its anti-

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oxidant effects.[25]
Therefore, the present study was designed to investigate the possible effects of natural honey in reducing the withdrawal symptoms of morphine due to its proven anti-inflammatory effects, and also to find a way to control the withdrawal syndrome of morphine in the body.

Materials and Methods

Animals

Male Wistar rats, weighing 225-275 g were obtained from the laboratory animals of the Pasteur Institute (Iran). The rats were kept in cages with enough water and food in a room with good air conditioning, constant temperature (23±2°C) and 12hour light/dark cycle. The experiments were carried out to evaluate morphine tolerance, and they were divided randomly in 6 groups of 8 rats. 2 days before performing the tests, they were moved regularly to the lab environment, to minimize their stress, which may influence the test results. After completion of the experiments, the rats were killed by intraperitoneal injection of pentobarbital (150 mg/kg).
This study was carried out based on the ethical standards of "Principles of Laboratory Animal Care" and was approved by the Ethics Committee of Tabriz University of Medical Sciences (ethical code: IR.TBZMED.VCR.REC.1396.1215).

Drugs

Morphine sulfate and naloxone hydrochloride were obtained from Darupakhsh Company, Tehran, Iran.

Natural Honey

Natural honey (NHO) was obtained from a local beekeeper in Tarom Olya (Zanjan, Iran) and was prepared freshly for intraperitoneal injection (so that honey was dissolved in normal saline and, pH was adjusted in 7.4 using phosphate buffer).

Experimental Groups

48 male wistar rats were randomly assigned in 6 different experimental groups (n=8). Group 1 (morphine group) received an increasing dose of morphine. Group 2 (saline group) received only saline (1ml/kg) 30 minutes after daily morphine injection, groups 3-5 received 200, 400 and 800 mg/kg of NHO intraperitoneally with vehicle twice a day, respectively. Group 6 (morphine+vehicle) as a control group received an increasing dose of morphine and vehicle of NHO (normal saline, 1ml/kg, i.p.).

Induction of the Morphine Withdrawal and Measurement of the Withdrawal Behaviors

Additive doses of morphine were administrated subcutaneously for 9 days to induce the morphine dependence. The performance procedure was as follows; day 1: 5 mg/kg/12h, days 2 and 3: 10 mg/kg/12h, days 4 and 5: 15 mg/kg/12h, days 6 and 7: 20 mg/kg/12h ,and days 8 and 9: 25 mg/kg/12h. This morphine administration protocol demonstrated a high dependence in the rats [26]. The rats in the saline group received only saline on the ninth day. One hour after the morning morphine injection on the ninth day, the rats received naloxone (4mg/kg)[27] intraperitoneally to induce the withdrawal signs. The rats were studied in a clear plexiglass chamber, and after naloxone injection, withdrawal signs were evaluated by an observer, who was not aware of the nature of the treatments received by animals, during a 60 minute period, and 11 distinct behaviors were recorded. The chamber was equipped with a digital camera to record the behaviors of the rats. The score of each behavior was divided by weighing factor attributed to it (Table 1). The results were accumulated, and Total Withdrawal Score (TWS) was calculated for each animal. TWS was used as an index of the withdrawal intensity.[28]

Table 1. Weighting factors of morphine withdrawal symptoms.

Behavior signs	Weighting factor
Jumping	4
Wet-dog shake	5
Head shakes	5
Paw tremor	5
Abdomen writhing	5
Genital grooming	5
Body grooming	10
Face wiping	10
Teeth grinding	10
Standing on feet	20

Locomotor Activity Test

The locomotor activity was evaluated by the modified open-field test in morphine plus honey (800 mg/kg) and morphine control groups, on the ninth day before morphine and naloxone injections. In this test, the number of crossing the lines was determined based on drawing on the underside floor of the plexiglass behavioral cage (100×100cm) by each rat.[29]

Analysis of TNF-α

The levels of TNF-α were analyzed in the serum of rats using Enzyme Linked-Immune-Sorbent Assay (ELISA) techniques (Rat Tumor necrosis factor α, ELISA, Shanghai Crystal Day Biotech Co., Ltd., Shanghai, China). The serum was incubated inside the well. After washing, a particular antibody for TNF-α was added, and it was attached to TNF-α during incubation. After a second washing, the enzyme Streptavidin-peroxide was added to the antibody during the third incubation, and after third washing and destroying unbound proteins, the substrate was added which connects to the catalyst in the previous step and produces a color. The color intensity is related to the TNF-α concentration, thus it was measured by spectrometry.[30]

Statistical analysis

The results obtained by recording the withdrawal syndrome signs were expressed as (n=8) mean ± SEM. Students T-test , One-Way ANOVA , and Post-Hoc Tukey test were used to compare the results. In all analyses, p-values of <0.05 represented a significant difference.

Results

The Effect of NHO on the Rats' Locomotor Activity

The locomotor activity test was performed in morphine+honey (800 mg/kg) and morphine+vehicle groups. The results of Independent Samples T-test showed no significant difference between the two groups.

The Effect of NHO on the Morphine Withdrawal Syndrome

The intraperitoneal injection of naloxone significantly increased the TWS (21.21±1.8) in the control group (morphine+vehicle) compared to the saline group (3.2 ±0.6, $p<0.05$) (Figure 1).

The comparison of the morphine and the morphine+vehicle groups with saline control group indicated a significant difference between them ($p<0.05$), thus all groups were compared with the morphine+vehicle group. NHO administration (200, 400 and 800 mg/kg) reduced the naloxone-induced TWS in a dose-independent manner, and significant differences were found compared to the morphine+vehicle group ($p<0.001$ for 200 mg/kg, $p<0.001$ for 400 mg/ kg and $p<0.001$ for 800 mg/kg, respectively) (Figure 2).

Data analysis showed that, the most effective doses of NHO were 200 mg/kg and 800 mg/kg.

Table 2 depicts that, NHO reduced the morphine withdrawal symptoms compared to the group received morphine and vehicle.

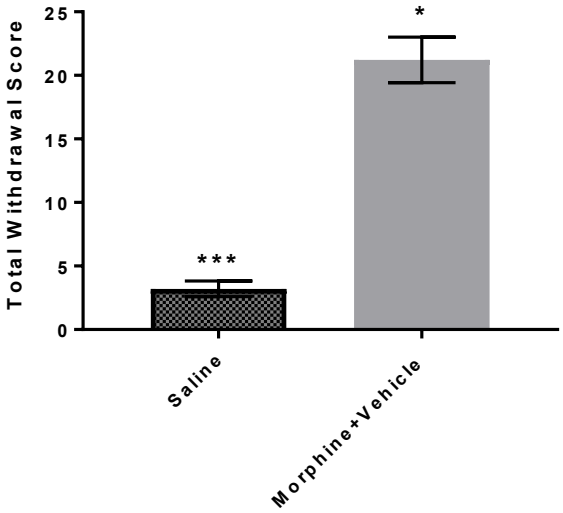

Figure 1. Naloxone (4mg/kg) – induced TWS in the control group in comparison to the saline group during 60 min of the experiment. Data showed as mean± S.E.M.

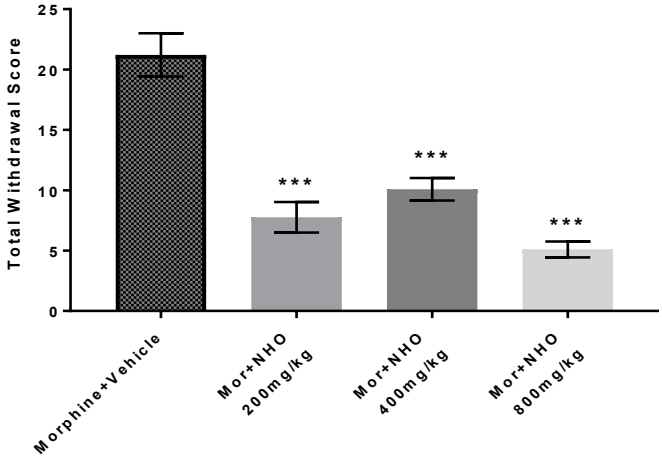

Figure 2. Effects of intraperitoneal injection of natural honey (NHO) on the expression of naloxone-induced TWS in morphine-dependent rats in comparison to the control group (morphine+vehicle). Data showed as mean± S.E.M. **: $p<0.01$ and ***: $p<0.001$.

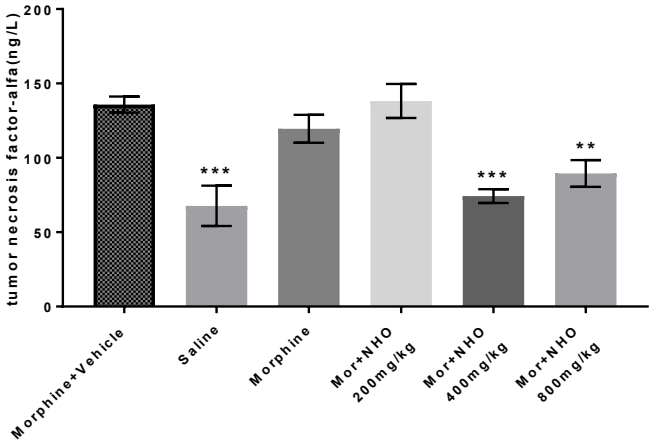

Figure 3. Effects of intraperitoneal injection of natural honey (NHO) on the tumor necrosis factor-alfa (TNF-α) in morphine-dependent rats in comparison to the control group (morphine+vehicle). Data showed as mean± S.E.M. ***: $p<0.001$.

Table 2. A comparison of the morphine withdrawal behaviors precipitated by naloxone (4 mg/kg) between the experimental groups during the 60-min observation.

Groups	Signs										
	Jumping	Abdomen writhing	Wet-dog shake	Standing on feet	Paw tremor	Genital grooming	Body grooming	Face wiping	Teeth chattering	Head shakes	Stool weight
Mor	19.1±3.5	7.4±2.1*	9±1.53	39.1±5.6	13.4±3.1	9.9±2.8	16.7±4.21	13.7±2.7	62.6±15.1	20.6±3.8	4.87±0.71
Saline	0±0***	0.5±0.26	0.25±0.2**	15.1±1.5*	2.2±1.6**	2.5±0.53	6.4±0.8	5.5±1.18	0.62±0.42**	1±0.7**	0.87±0.22***
Mor + NHO (200mg/kg)	3.7±1.5**	2.6±1.1	1.2±0.6**	22.5±5.3	3.5±1.2**	2.6±0.5*	12.75±6	9.12±1.4	3.9±0.9***	3±1.7**	4.87±0.63
Mor + NHO (400mg/kg)	14±3.45	2.2±0.9	2.6±0.82	8.1±1.7***	9.1±2.3	2.5±0.5	3.9±1.2	7.5±1.1	3.4±1.2***	7±2.5	4.37±0.62
Mor + NHO (800mg/kg)	5.1±2.2**	2.5±0.8	0.62±0.3**	14.7±2.4*	4.4±0.96**	1.8±0.2*	3±1.12	4.75±1.6	2.1±0.8***	1.1±0.4***	3.50±0.6
Mor + Veh	18.1±2.7	3.87±1.2	6.1±1.6	32±5.9	18.4±4.1	8.8±3.2	11.4±3.1	9.75±2.3	24.25±6.5	15.5±3.3	5.12±0.35

All data are shown as mean ± SEM
Mor: morphine, Sal: saline, NHO: natural honey, Veh: vehicle
*p<0.05; **p<0.01; ***p<0.001 compared to the control group (morphine + vehicle)

The Effect of NHO on the Rats' Serum Level of TNF-α

The results of this study showed that the chronic administration of NHO could significantly reduce morphine-induced dependence in the rats in a dose-independent manner (Figure 3). The doses of 200 and 800 mg/kg were found to be more effective than the dose of 400 mg/kg, and they significantly improved the withdrawal signs, but the results showed that, the administration of NHO at the dose of 400 mg/kg was more effective than other treatments in decreasing the TNF-α.

Discussion

Despite the high rate regarding the use of opioid analgesics and especially morphine in the relief of acute and chronic pain with moderate to severe degrees in the clinic, there are still barriers to their long-term use; the tolerance to opioid analgesia and the need for higher doses in tolerated patients is considered as one of the most critical problems in this regard. Also, excessive and prolonged use of opioids in the clinic or its abuse has a high risk of dependence, and even in some cases due to the difficulty of withdrawal, opiate addiction, especially morphine is unavoidable. In this study, it was found that, NHO as a most valuable and useful food in the world has significant effects on reducing the morphine dependence. The results showed that administrating different doses of NHO inhibited morphine dependence by significantly reducing the morphine withdrawal symptoms, while preventing an increase in serum levels of TNF-α caused by chronic morphine use.

There are different kinds of honey with varying compounds in nature; accordingly there are various effects of NHO on biological systems. The results of the locomotor activity test showed that, there was no statistically significant difference between the morphine+NHO group and the morphine+vehicle group. Therefore, no correlation was found between the inhibitory effects of NHO on withdrawal syndrome and the locomotor activity test results. The results of the locomotor activity test for the morphine+vehicle group showed the pharmacological effects of morphine is different from drug withdrawal signs, and it is a unique index in drug dependence.[31] Also, some studies showed that the NHO could induce inhibitory effects on locomotion only at high doses.[32]

Morphine is known as one of the significant opioids, and the possibility of its addiction is high. Previous studies on opioids, especially morphine have shown that long-term use of morphine stimulated glia cells in the spinal and supra-spinal cord,[33] and immune system and increased production of pro-inflammatory cytokines such as IL-1β, 6 and TNF-α , as well as the occurrence of neuroinflammation. Inflammation in the CNS is associated with more sensitive pain transfer pathways and counteracts the analgesic effect of opioids.[34] On the other hand, many studies indicated the involvement of the glutamatergic system in tolerance and dependence on opioids.[35-37] Some researchers have also showed that the pro-inflammatory cytokines, especially TNF-α through NF-κB inhibits the expression of Glutamate Carriers (GCs), especially the Glutamate-Aspartate (GLAST) vector, leading to increased neurotoxicity due to increases in synaptic glutamate levels. TNF-α promotes the expression of AMPA receptors in the CNS and also influences the activity of the glutamatergic nervous system. Also, many studies have shown that NHO with numerous flavonoid and phenol compounds has inhibitory effects on the pathways responsible for producing various inflammatory factors such as IL-1, IL-10, IL-6, COX-2, TNF- α, IκBα, NF PDGF, TGF-β, LOXs, NO, iNOS, and PGs.[38,39] According to these findings, it can be concluded that in the present study, one of the mechanisms which is introduced regarding the effect of NHO on reducing the incidence of chronic morphine dependency and reducing the withdrawal symptoms, may result from the influence of chronic consumption of honey on preventing an increase in the level of pro-inflammatory factor TNF-α.

In addition, several studies have shown that the acute and chronic administration of opioids, especially morphine, causes oxidative stress in the body by increasing the production of free radicals (such as O_2 and peroxynitrites) and reducing the activity of immune systems such as

endogenous antioxidant enzymes (GSH, SOD, CAT, and GSHPx).[40,41] Nitric oxide and superoxide are the main precursors of peroxynitrites in the body, and they are exaggerated by chronic administration of morphine and stimulation of NOS, and they have been shown to play an essential role to stimulate pain transfer pathways, opiate hyperalgesia, and tolerance to their analgesic effect.[4,42-44] NHO with various antioxidant compounds, especially phenolic compounds, is one of the most nutritious foods, which its effects on many disorders and diseases have been discussed with particular attention in many studies.[17,45-48] Thus, the present study was designed to confirm the beneficial effects of the chronic administration of NHO in combination with morphine in modulating the dependence and withdrawal symptoms of opioids, as well as determining the role of antioxidant compounds in NHO in preventing the development of oxidative stress and its effects on the long term use of morphine.

The results showed that the chronic administration of NHO could attenuate morphine dependence and serum level of TNF-α, however further studies are needed to achieve complete evidence and to find the exact mechanisms of action regarding the main components of NHO influencing morphine dependence.

Conclusion

The inhibition of the inflammatory responses and glia activation induced by morphine and prevention of NO formation were the probable mechanisms for the NHO effects on the morphine dependence. The results of this study showed that, NHO could attenuate the severity of morphine withdrawal syndrome, and no correlation was found between the effects of natural honey injections on the morphine withdrawal signs and the motor activity disturbance in the rats. Also, natural honey could reduce the serum level of TNF-α especially at the dose of 400 mg/kg.

In addition to contributing in reduction of pain tolerance in patients, this study will also save a lot of economic savings by lowering the use of opioid derivatives without causing any effect on low or even better doses.

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Conflict of interests

The authors declare that they had no conflict of interests.

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