Original article

Honey potentially mitigates morphine analgesic tolerance and physical dependence in rats

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Abstract

Introduction: Honey has been used traditionally in medicine as well as food supplements. Honeybees are said to be able to cure many diseases. However, its influences on opioid tolerance and dependence have not yet been clarified.**Materials and Methods:**Adult male Sprague-Dawley rats were rendered tolerant to the analgesic effect of morphine by injection of morphine (10 mg/kg, i.p.) twice daily for 14 days. To develop morphine dependence rats given escalating doses of chronic morphine. To determine the effect of stingless bee honey on the development of morphine tolerance and dependence. The hotplate and naloxone precipitation tests were used to assess the degree of tolerance and dependence, respectively.**The results:** Our results showed that chronic morphine-injected rats displayed tolerance to the analgesic effect of morphine as well as morphine dependence. Methadone+morphine (MetM), methadone+morphine+ honey (MetMH) and morphine+Honey (MH) significantlylower the development of morphine tolerance with p-value $p<0.05$. In addition, concomitant treatment of morphine with MH and MetMH attenuated almost all of the naloxone-induced withdrawal signs which include abdominal contraction, diarrhea, pertussis, teeth chattering, and jumping. **Conclusion:** The data indicate that honey has a potential to reduce tolerant and dependence property.

Keywords: Honey, mitigate, morphine tolerance dependence

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Introduction

In Malaysia, opiates dependence has been incriminated in the country's social and economic development. Harm Reduction Program was initiated by Malaysia Ministry of Health in 2006, in which Methadone Maintenance Therapyhas been used as a therapeutic method to control opioid dependence problems. Withal, the user of methadone itself would cause dependency and the curative dose has been increasing over the period of time to make the same

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results1.

Recently using of non-addictive materials have been applied to attenuate opiate dependency.These let in natural elements such as honey, habbatusauda, herb roots and so on[2, 3,4].In the advancement of oxidative stress there are two components included; arrangement of free radicals and reduction of antioxidant activity[5]. The instrument of oxidative stress in the brain overstated by opiate admissions. Previous consider assigning that oxidative stress grounds opiate reliance [6, 7].

Recently, anti-tolerance and antiaddictive impacts of characteristic herbal items has strained energetic intrigued [8]. Natural items, therefore, require specific scientific exploratory testing as well as clinical trials before they can assume inescapable choice in the controlling of opioid side impacts.

Nectar bees require to be been recognized as facilitating therapeutic values essentially as an anti-cancer, burn wound healing, antibacterial moreover needs certain impacts on the brain, for example, enlightening memory.That is due to the fact honey bees own bioactive compounds consisting of flavonoids and phenolics [9, 10,11,12,13].

Antioxidant properties in nectar bees can provide a sense of stability to a number of antioxidants and free radicals in the body [14, 15]. Nectar conceivably has beneficial shrewdness of morphine-induced oxidative stress, to be able to enhance the reliance and tolerance in opiate dependency, human beings, some other contemplate designated that lessening in oxidative stress in opiate addicts will lower dependence and withdrawal indications[7].

We as a great deal as this time precise that nectar fortifies a vital antinociceptive impact[16] and has a positive impact on morphine analgesia and can be a sturdy association with pain control [17]. Withdrawals from opiates can cause digestive problems which include nausea and diarrhea similarly to muscle pain and anxiety.

Therefore, the present study was designed to test the speculation that honey ought to exert restraining consequences against chronic morphine side effects along with the development of antinociceptive tolerance and physical dependence in rats.

Materials And Methods

Animal

All experiments had been carried out on male Sprague-Dawley rats, weighing 250–350 g, that was housed two/four consistent with cage beneath 12 hours light/dark cycle in a room with managed temperature (22 ± 1 ◦C). Food and water have been available ad libitum. Animals had been treated each day (among 9:00 and 10:00 a.m.) for four days, earlier than the experiment days, which will adapt them to manipulate and limit nonspecific stress responses. Rats were divided randomly into several experimental groups, every comprising five-6 animals. All experiments accompanied the suggestions on ethical requirements for research of experimental pain in animals18 and accepted by way of the Animal Experimentation Ethic Committee of UniSZA.

Protocol

Stingless bee honey was additionally dissolved in physiological saline. Honey and methadone were given intragastrically (i.g.) by way of gavage and morphine was injected intraperitoneally (i.p.). These drugs had been given inside the volume of 1 ml/kg (i.g. and i.p.). Control animals received saline within the equal volume (1 ml/kg).

Tolerance test (Antinociceptive test)

Antinociception was assessed with a hot plate maintained at 54±0.1℃. The latency to licking a hind paw was measured with an accuracy of 0.1 second and the reduce-off time became 30 seconds to keep away from tissue harm. The rats have been trained on the recent plate for 4 days before drug administration with a view to obtaining a solid baseline reaction within the range of 3–6 seconds. On the day of the test, two control latencies were measured before the rats were injected subcutaneously (s.c.) with morphine (10 mg/kg) and methadone (5 mg/kg) or saline was given intragastrically (i.g.) by using gavage.

Morphine tolerance

To induce analgesic tolerance, morphine at a daily dose of 20 mg/kg in two equally divided doses was administrated at 8.00 a.m. and 6.00 p.m. from days 1 to 8. Honey or saline was given according to the same schedule as control groups. Analgesic tolerance testing was performed both before and 30 minutes after morphine administration on days 1, 3, 9,12 and 14. To determine the effect of honey on the development of morphine tolerance, honey (200 mg/kg i.g.) was injected concomitantly with morphine but in days that analgesic tolerance testing was measured, morphine was injected first and analgesic tolerance was measured 30 minutes after morphine administration and then, honey was given[19].

Morphine dependence and induction of withdrawal syndrome

To develop morphine dependence, rats were injected intraperitoneally with morphine twice daily for 7 days. The dose of morphine on day 1 and 2 was 2.5 mg/kg; this dose was doubled every day thereafter to

reach a total dose of 40 mg/kg on day 6. On day 7, the animals received the last injection of morphine, 50 mg/kg. To determine the effect of honey on the development of morphine dependence, honey (200 mg/kg i.g.) was given 15 minutes before morphine. Honey or saline was given according to the same schedule as acontrol group. On day 7, naloxone (3 mg/kg i.p.) was given 5 hours after the last injection of morphine. Immediately after naloxone injection, each animal was placed in a transparent acrylic cylinder to observe the frequency of withdrawal manifestations. Two classes of signs were distinguished: graded signs (abdominal contraction, grooming and jumping), which were quantified numerically; and checked signs (diarrhea, teeth chattering, ptosis) for which only presence or absence was evaluated[4].

On day 7, naloxone (3 mg/kg i.p.) was given 5 hours after the last injection of morphine. Animals

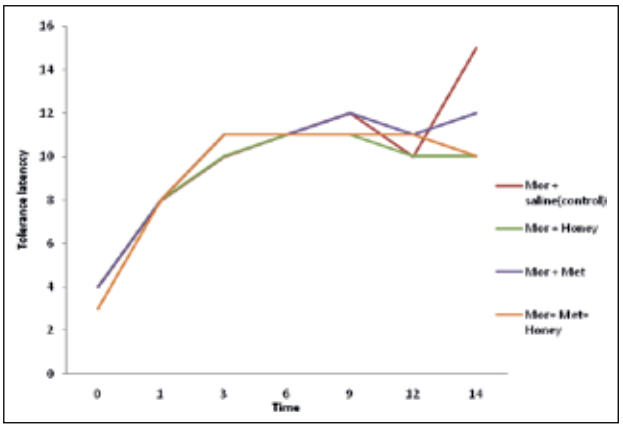

Figure 2. The effect of honey on the development of tolerance in chronic opioids rats.

As shown in Fig. 2, following chronic administration of morphine and methadone twice daily for 14 days, a significant decrease occurred to its analgesic effect (tolerance). Concomitant treatment of MH and MetM-Hlower the tolerance of morphine on day 14 compare with MS and MetM, p-value<0.005.

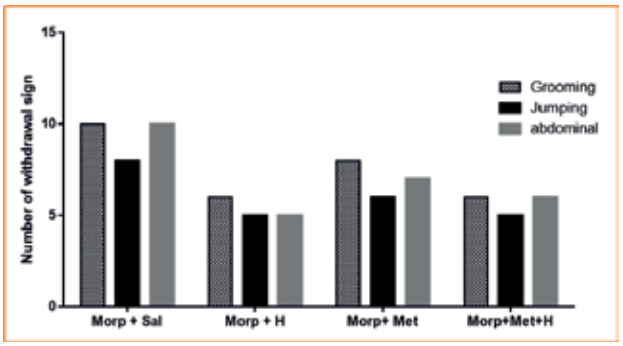

Figure 3. Effect of different types of treatment (honey and methadone) on a mean number of naloxone-induced grooming, jumping and abdominal contraction during a 60 min observation period after naloxone injection.

were observed for 60 minutes injection of naloxone. Immediately after naloxone injection, each animal was placed in a transparent acrylic cylinder toobserve the frequency of withdrawal manifestations. Two classes of signs were distinguished: graded signs (abdominal contraction, grooming and jumping), which were quantified numerically; and checked signs (diarrhea, teeth chattering, ptosis) for which only presence or absence was evaluated.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences version 22.0 for Windows. The results are expressed as mean ± SEM. The difference in antinociception between groups over the time course of study was determined by two- or one-way analysis of variance (ANOVA) followed by the Newman–Keuls test.The difference in grade signs between experimental groups was determined by one-way ANOVA followed by the Newman–Keuls test. Checked sign behaviors were quantified as the number of animals exhibiting the sign/total number of animals observed, and data obtained were analyzed non-parametrically with the Fisher exact test. $P < 0.05$ was considered significant.

Results

Repeated co-administration of honey just in dose 4 mg/kg with morphine significantly decreased ($P < 0.005$) the frequency of abdominal contraction as compared with morphine-treated rats and methadone-treated rats. It also shows the significantly decreased ($P < 0.005$) in MetH and a MetMgroup of animal (Figure 3).

In morphine-dependent animals, naloxone-induced jumping (a withdrawal graded sign). A group of MH and MetH could suppress the mean number of naloxone-induced jumping (Figure 3). Experimental animals receiving chronic morphine also displayed grooming, following naloxone injection. The frequency of naloxone-induced grooming was significantly reduced in groups which MH and MetMH (Figure 3).

Group of animal	Withdrawal sign		
	Diarrhea	Ptosis	Teeth
Morphine + saline	8/8	8/8	8/8
Morphine + Honey	4/8	4/8	5/8
Morphine + Methadone	6/8	6/8	5/8
Morphine +Methadone + Honey	5/8	4/8	4/8

Table 1. The number of animals exhibiting the signs/total number of animals observed in each group.

As shown in Table 1, naloxone-induced diarrhea, ptosis, and teeth chattering in all of the morphine-treated rats. In animals that had morphine plus saline, the effects of naloxone were similar to those in morphine-treated rats. Logically, those withdrawal signs were not observed in control animals. Co-administration of honey with morphine and honey with Methadone and Morphine significantly reduced the number of animals that exhibited diarrhea ($P < 0.05$) and teeth chattering ($P < 0.05$).

Discussions

Morphine tolerance, dependence and withdrawal sign

Despite the variety of papers published on honey, none has centered on its interaction with opioid side-consequences. Our outcomes confirmed that honey had significant anti-tolerant effects towards morphine-brought antinociception in rats. Similarly, honey should diminish a series of behavioral responses to morphine withdrawal.

Aforesaid reviews indicated that free radicals and nitric oxide signaling play critical roles in the growth of opioid analgesic tolerance and dependence, and radical scavenging agents, as well as nitric oxide synthase inhibitors, can be possibly implemented within the avoidance of morphine tolerance and withdrawal syndrome[5,21,22,2,4].

The excessive antioxidant activity of honey and its compounds have been discovered in common reports[23,24,26,27,28]. Moreover, a bioactive component of honey along with a phenolic compound, adequately overthrows gathering of reactive oxygen and nitrogen species and re-establishes endogenous antioxidant glutathione degrees and up-regulates the countenance of antioxidant enzymes. Therefore, evidently the antioxidant belongings of honey and its nitric oxide synthase inhibitory effect can be, as a minimum in an element, liable for such deterring response in this examine. However, this possible mechanism desires to be clarified with the aid of the in addition, complementary investigation.

Our results showed that naloxone induces diarrhea and increases the abdominal contraction in continual morphine-treated rats and co-administration of honey with morphine substantially reduces the range of animals that unveiled diarrhea and additionally decreases the frequency of abdominal contraction. It is well known that the abnormal enablement of gastrointestinal motility and disproportionate fluid secretion could be the primary grounds of diarrhea.

Remarkably, honey is a natural medication for the dealing of gastrointestinal problems. Honey has inhibitory results on colonic motility through direct motion on lean muscle tissues and has the potential to reduce fluid secretion[29,30,31,32]. Therefore, honey seems to be positive all through the withdrawal duration for addicted patients with gastrointestinal hyperactivity and diarrhea.

Conclusions

In decision, this study disclosedthat honey has noteworthy inhibitory effect in contradiction of morphineantinociceptive tolerance and physical dependence in rats. The anti-tolerance/dependence ability of honey may be viaantioxidant properties of honey from the effect of their bioactive compound such as phenolic.

Acknowledgments

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Conflict Of Interest

The authors have declared that no conflict of interest exists.

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