

Honey Reduces Anxiety in Morphine-Dependent Rats: An Exploratory and Locomotor Model

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Abstract: Withdrawal from chronic opiates is associated with an increase in anxiety behaviours but the anxiety activity in the morphine-dependent and tolerance animals is not clear. Therefore, aim of this study was to examine the effect of honey on the anxiety activity in both morphine-dependent animals. Rats were injected with bi-daily doses (10 mg/kg, at 12 h intervals) of morphine over a period of 7 days. Honey (200 mg/kg, i.g.) was given 15 min. before morphine. Following these injections, anxiety behaviours were tested in the open field test. We found the open test, the morphine-dependent and tolerance in group Morp+Sal made significantly reduce anxiety activity compare with control group. Centre square duration, stretch attend postures and grooming shown a significant value in Morphine+Honey (Morp+H), Morphine+Methadone (Morp+Met), Morphine+Methadone+Honey (Morp+Met+H) compare with Morphine+Saline (Morp+Sal), $p < 0.05$. However, there was no significant difference in another score of measurement in morphine-dependent rats as compared to the control groups. We conclude that honey decreases the severity of the anxiety behaviours in both morphine-dependent and tolerance rats. Thus, honey could be a potential natural method to decrease some of the deleterious behavioural consequences of opiate abuse.

Key words: Honey, reduce, anxiety level, morphine tolerance dependence, potential natural method, deleterious

INTRODUCTION

Anxiety is potent stimulators of drug cravings and drug-seeking behaviours in opioids addiction. Brain anxiety systems are thought to play a significant role in generating the negative emotional state characteristic of drug dependence with dysregulation of anxiety systems also underlying the persistence of drug-seeking and relapse (Bali *et al.*, 2015; Kreek *et al.*, 2012).

Thus, reversing or preventing of the anxiety behaviours induced by drugs of abuse might be useful in the treatment of relapse after periods of abstinence. Treating patient's co-occurring mood disorders may reduce their substance craving and taking and enhance their overall outcomes (Logrip *et al.*, 2012). Animal studies have shown that both spontaneous and antagonist-precipitated

morphine withdrawal result in significant increases in anxiety behaviours (Zhang and Schulteis, 2008; Grasing *et al.*, 1996). Systemic or central administration of the μ -opioid receptor agonist morphine induces anxiety effects which are reversed by the opioid receptor antagonist naloxone (Schulteis *et al.*, 1998; Motta and Brandao, 1993; Zarrindast *et al.*, 2005). These findings indicate that acute morphine has anxiety actions but the underlying mechanisms are not well known.

Recent studies have shown that polyphenol in honey is associated with a reduction in anxiety in humans and rodents (Rahman *et al.*, 2014; Xu *et al.*, 2014). Some evidence indicates that honey as antioxidant might be useful as a natural substitute for drug reward and help in the reduction of drug abuse and the treatment of addicts (Hashim *et al.*, 2015; Zakaria *et al.*, 2015; Bakar *et al.*, 2015).

In fact, honey in rodents is associated with a number of adaptive behavioural and physiological effects including improved cognitive function in rats a reduction in anxiety-associated behaviours (Rahbi *et al.*, 2014) enhanced neurogenesis and angiogenesis and an increase in neurotrophic factors such as IGF-I and BDNF (Rahman *et al.*, 2014).

Both the Holy Qur'an and Hadith refer to honey as a healer of disease. And thy Lord taught the bee to build its cells in hills on trees and in (men's) habitations there issues from within their bodies a drink of varying colours wherein is healing for mankind. Verily in this is a sign for those who give thought.

Narrated Ibn'Abbas (The Prophet said), "Healing is in three things: A gulp of honey, cupping and branding with fire (cauterising)". But I forbid my followers to use (cauterisation) branding with fire.

Narrated Ibn'Abbas: The Prophet said, "Healing is in three things: cupping, a gulp of honey or cauterisation (branding with fire) but I forbid my followers to use cauterisation (branding with fire)". Narrated Aisha: The Prophet used to like sweet edible things and honey.

Narrated Jabir bin Abdullah: I heard the Prophet saying, "If there is any healing in your medicines then it is in cupping, a gulp of honey or branding with fire (cauterisation) that suits the ailment but I don't like to be (cauterised) branded with fire".

Narrated Abu Said Al-Khudri: A man came to the Prophet and said, "My brother has some abdominal trouble". The Prophet said to him "Let him drink honey". The man came for the second time and the Prophet said to him, "Let him drink honey". He came for the third time and the Prophet said, "Let him drink honey". He returned again and said, "I have done that the Prophet then said, "Allah has said the truth but your brother's abdomen has told a lie. Let him drink honey". So, he made him drink honey and he was cured.

However, the effects of honey on chronic morphine-induced anxiety behaviours are unknown. In the present study we tested the specific hypothesis that honey can diminish the severity of naloxone-precipitated withdrawal responses and reduce anxious behaviours in morphine-dependent and withdrawn rats.

MATERIALS AND METHODS

Animals: All experiments were carried out on male Sprague-Dawley rats, weighing 300-350 g were

housed in groups of six per cage in inside a room maintained at $22\pm 1^{\circ}\text{C}$ with an alternating 12/12 h light/dark cycle (lights on 7:00 a.m.-7:00 p.m.) and free access to food and water except during testing. Animals were handled daily (between 9:00 and 10:00 a.m.) for 4 days before the experiment days in order to adapt them to manipulation and minimise nonspecific anxiety responses. Rats were divided randomly into several experimental groups, each comprising 10 animals. All experiments followed the guidelines on ethical standards for investigation of experimental anxiety in animals and approved by the animal experimentation ethic committee of UniSZA.

Procedure: Stingless bee honey was provided by HoneyGold Lab, Kelantan. Stingless bee honey was also dissolved in physiological saline. It was freshly prepared by diluting in physiological saline prior to administration to the animals.

Honey and methadone were given intragastrically (i.g.) by gavage and morphine was injected intraperitoneally (i.p.). These drugs and honey were given in the volume of 1 mL/kg (i.g. and i.p.). control animals received saline in the equal volume (1 mL/kg).

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 min before morphine. Honey or saline was given according to the same schedule as a control group. On day 7, naloxone (3 mg/kg i.p.) was given 5 h 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 (diarrhoea, teeth chattering, ptosis) for which only presence or absence was evaluated (Mahani *et al.*, 2012). On day 7, naloxone (3 mg/kg i.p.) was given 5 h after the last injection of morphine. Animals were observed for 60 min. injection of naloxone. 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 (diarrhoea, teeth chattering, ptosis) for which only presence or absence was evaluated.

Open field test: Rats were carried to the test room in their home cages and were handled by the base of their tails at all times. The rat was placed in the centre or one of the four corners of the open field and allowed to explore the apparatus for 10 min. After the 10 min test, rats were returned to their home cages and the open field was cleaned with 70% ethyl alcohol and permitted to dry between tests. To assess the process of habituation to the novelty of the arena, rats were exposed to the apparatus for 10 min on 4 consecutive days. Each animal was then given a score for total locomotor activity that was calculated as the sum of line crosses and number of rears.

Behaviours scored: The behaviours scored (Brown *et al.*, 2001) included:

- Line crossing, frequency with which the mice crossed one of the grid lines with all four paws
- Center square entries, frequency with which the mice crossed one of the red lines with all four paws into the central square
- Center square duration, duration of time the mice spent in the central square
- Rearing frequency with which the mice stood on their hind legs in the maze
- Stretch attend postures frequency with which the animal demonstrated forward elongation of the head and shoulders followed by retraction to the original position
- Grooming, duration of time the animal spent licking or scratching itself while stationary

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 Tukey's test. The difference in grade signs between experimental groups was determined by one-way ANOVA followed by the Tukey's test. Checked sign

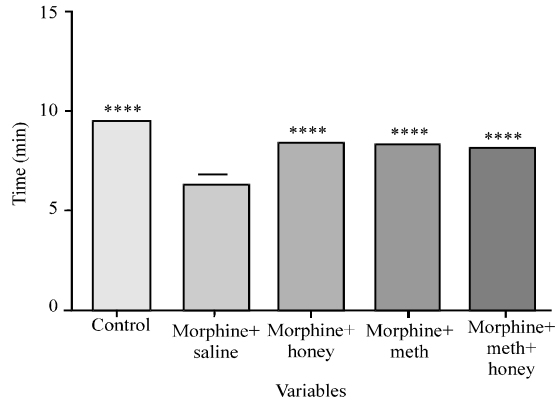


Fig. 1: The effect of honey on centre square duration upon anxiety behavioural activation in an open field of morphine-dependent rats; **** $p < 0.05$ Morp+H, Morp+Met, Morp+Met+H and control compare with Morp+Sal

behaviours were quantified as the number of animals exhibiting the sign/total number of animals observed and data obtained were analysed non-parametrically with the fisher exact test. $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Honey decreases the severity of anxiety behaviours in morphine-dependent and tolerance rats: Table 1 shown centre square duration and grooming shown a significant value, $p < 0.05$ compare with control group. In Morp+H (Centre square duration and grooming) it demonstrated significant value, $p < 0.05$ compared with group Morp+Sal. Anxiety activity behavioural activation was reducing in this group.

Whereas in Morp+Met it showed significant value in grooming, $p < 0.05$ compared with group Morp+Sal. In a group of Morp+Met+H, it showed significant value in centre square duration, stretch attends postures and grooming, $p < 0.05$ compared with group Morp+Sal. Both groups also are shown in reducing anxiety activity after consuming methadone and honey.

Figure 1 shows a significant value in centre square duration for all group of animals compare with a control group and Morp+Sal. The numbers of central square duration of time spent in the central square are measures of exploratory behaviour and anxiety. A high frequency or duration of these behaviours indicates high exploratory behaviour and low anxiety levels.

Table 1: The effect of honey on the anxiety activity upon behavioural activation in an open field of morphine-dependent rats

Measure	Control (Saline)	Morp+S	Morp+H	Morp+Met	Morp+Met+H
Centre square duration	9.7±1.0	6.9±1.4	8.4±1.5*	8.4±1.2*	9.0±1.0*
Grooming	17.5±2.5	45.9±11.8	25.5±3.5*	22.5±3.0*	20.0±2.5*

*p<0.05 Morp+H, Morp+Met, Morp+Met+H and Control compare with Morp+Sal

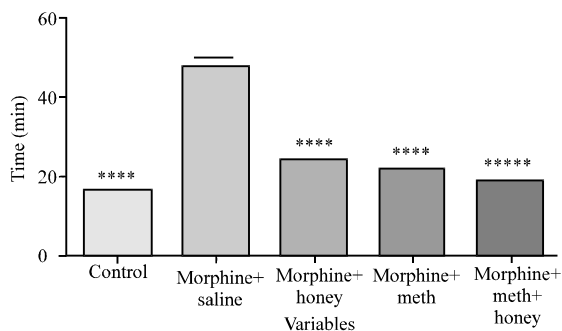


Fig. 2: The effect of honey on grooming anxiety behavioural activation in an open field of morphine-dependent rats; ** p<0.05 Morp+H, Morp+Met, Morp+Met+H and control compare with Morp+Sal**

Anxiety activity increase after given morphine and saline compare with control group. Group Morp+H, Morp+Met, Morp+Met+H demonstrated significant value, p<0.05 in comparison to Morp+Sal. Those animals reduces their anxiety activity compare with group Morp+Sal and reach almost into normal value compare with control group.

Figure 2 demonstrates a significant value in grooming for all group of animals compare with a control group and Morp+Sal. Anxiety activity increase after given morphine and saline compare with control group. Three other groups Morp+H, Morp+Met, Morp+Met+H significant value, p<0.05 compare with Morp+Sal. Those animals reduces their anxiety activity by reducing grooming activity compare with group Morp+Sal and reach almost into normal value compare with control group.

Morphine dependence enhances anxiety behaviours:

The present study indicated that the sedentary dependent rats exhibited anxiety behaviours as assessed by the open field test. In the open test, the sedentary morphine-dependent in group Morp+Sal made significantly reduce anxiety activity compare with control group. Centre square duration and grooming shown a significant value, p<0.05 compare with control group. However, there was no significant difference in another score of measurement. In the other study, they investigated the effects of physical anxiety and exogenous

corticosterone on the acquisition and expression of morphine-induced Conditioned Place Preference (CPP). Also, they tried to find out the role of Glucocorticoid Receptors (GRs) of basolateral amygdala (BLA) in this regard. It seems that anxiety exerts its effect on reward pathway via glucocorticoid receptors in the BLA (Yazdi *et al.*, 2013).

Also, further supported morphine can induce anxiety behaviour in another study. They investigated the effects of morphine-induced Conditioned Place Preference (CPP) on p-ERK/ERK ratio, p-CREB/CREB ratio and C-FOS level in the mesocorticolimbic dopaminergic system including the Nucleus Accumbens (NAc), Amygdala (AMY), Striatum (Str) and Prefrontal Cortex (PFC). They found that morphine-treated animals, acute and subchronic anxiety increased p-ERK, p-CREB and C-FOS levels in the mesocorticolimbic system (Haghparast *et al.*, 2014). RK pathway plays a critical role in the cellular adaptive responses to environmental changes. Anxiety conditions can induce the activation of activating ERK and its downstream targets, CREB and C-FOS in neural cells. Exposure to opioids has the same effect (Shiflett and Balleine, 2011).

Other study evaluated the effects of the opioid agonist, morphine on anxiety-induced anxious and the possible involvement of Nitric Oxide (NO) in such effects in rats. Restraint anxiety-induced neurobehavioral suppression was associated with reductions in brain NO oxidation products (NOx) levels which were also reversed with morphine. Interaction studies showed that sub-effective doses of morphine and L-arginine (an NO precursor) had synergistic effects on anxiety-induced elevated plus maze activity and brain NOx whereas, L-NAME (an NO synthase inhibitor) neutralised these effects of morphine (Anand *et al.*, 2012).

Previous reports indicated that free radicals and nitric oxide signalling play important roles in the development of opioid analgesic tolerance and dependence and radical scavenging agents as well as nitric oxide synthase inhibitors, could be potential tools in the prevention of morphine tolerance and withdrawal syndrome (Azman *et al.*, 2015; Abdel-Zaher *et al.*, 2010; Mori *et al.*, 2007).

Honey ameliorate anxiety behaviours in morphine dependent rats: Other important findings of this study are that honey reduces the anxious behaviours induced by morphine dependence. In other study examined the effects of Tualang honey supplement administered with the goal of preventing or attenuating the occurrence of anxiety-related behaviours in male rats subjected to noise anxiety. They found that honey can reduce anxiety activity.

CONCLUSION

In closing, our results indicate that honey can ameliorate morphine dependence, anxiety in the anxiety conditions. Since, reversing or preventing the anxiety behaviours induced by morphine might be useful in the treatment of relapse after the periods of abstinence, our findings may have a potential therapeutic application in the treatment of the anxiety and associated disorders frequently observed in addicts.

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