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Effect of Tualang (*Apis dorsata*) Honey on Development of Morphine Tolerance in Rats

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Research Article

Abstract

Background: Morphine tolerance leads to decrease in analgesic effect of the drug, causing a need to increase the dose and eventually increased dependency. Previous studies demonstrated the involvement of morphine tolerance with decrease in antioxidant activities and increased oxidative stress. Honey, a traditional natural remedy possesses analgesic, antioxidant and neuroprotective effect against oxidative stress, suggesting its potential in reducing morphine tolerance. This study aimed to assess the effect of different *A. dorsata* honey doses on development of morphine tolerance in Sprague-Dawley rats.

Methods: To induce morphine tolerance, rats were subcutaneously injected with 10 mg/kg morphine 30 minutes after *A. dorsata* honey oral administration (0.5, 1.5 and 2.5 g/kg). Analgesic effects (MPE %) were then assessed via hot plate test and mean difference between groups were analysed using Repeated Measure ANOVA.

Findings: Our results showed that dose 0.5, 1.5 and 2.5 g/kg significantly prevented the development of morphine tolerance compared to control group ($p<0.05$, $p<0.001$ and $p<0.05$, respectively).

Conclusion: *A. dorsata* honey can prevent development of morphine tolerance. This property highlights the potential of this honey type in the treatment of opioid tolerance and dependence.

Keywords: *A. dorsata* honey; Analgesic effects; Morphine tolerance

1. Introduction

Morphine is a commonly used opioid in acute and chronic pain management [1]. Its functions as analgesic includes before surgery, in regional anaesthesia and to treat joint pain. The administration however is limited to certain dose due to its tolerance effect, which is a critical problem related to repeat or chronic usage of morphine [2]. Tolerance in opioid usage is defined as the reduction of the morphine effect after repeated administration, causing the need to increase the dose to obtain the same effect [3]. Tolerance also leads to several other adverse effects such as sedation, decrease in physical activity, respiratory depression, constipation, nausea and possible addiction potential [4]. Thus, the search for alternative to reduce the morphine tolerance and to maintain the morphine analgesic effect has become crucial. Most of the previous studies focused on the mechanism of morphine tolerance involving brain mu opioid receptor [5]. However, emerging studies also showed the involvement of oxidative stress and damage in the development of morphine tolerance, as morphine itself acts as oxidative stress causing agent [6]. Production of nitric oxide and nitric oxide synthase (NOS) are proved to be involved in the development of morphine tolerance [7,8]. An increased release of the free radical in response to tolerance led to decrease in cell defense systems such as Glutathione (GSH) and antioxidant enzymes, indicating elevated level of oxidative stress. More interestingly, oxidative damage in neurons due to morphine treatment resulted increase in tolerance [9]. Thus, morphine tolerance is highly associated with oxidative stress and damage.

Honey, a natural supersaturated solution of sugars

produced by honeybees, are known as food and traditional remedy for illnesses such as wound, sore throat, cough, cold, stomach ulcer and asthma [10,11]. Islamic history encouraged the use of honey for healthy life, as described in Holy Quran, “And thy Lord taught the bee to build its cells in hills, on trees, and in (men’s) habitations; Then to eat of all the produce (of the earth), and find with skill the spacious paths of its Lord there issues from within their bodies a drink of varying colors, wherein is healing for men: verily in this is a sign for those who give thought" [12]. Honey possesses important biological activities in managing nociception such as antioxidant, analgesic effect and anti-inflammatory properties [11,13-15]. More importantly, it is concentrated with bioactive compounds including phenolic acids and flavonoids for example quercetin and ellagic acid,which were proven to reduce morphine tolerance [16,17]. Chlorogenic and ellagic acid acted as NO inhibitor while apigenin was found to inhibit depletion of GSH beside its neuroprotective effect [18,19]. To date, there is no study conducted to investigate the effect of *Apis dorsata* honey on morphine tolerance. This type of honey has higher phenolic content and antioxidant capacity compared to gelam, Indian forest and pineapple honey [20].

Based on these findings, this study is planned to study the preliminary effect of *A. dorsata* honey on development of morphine tolerance by focusing on the assessment of its behavioural effects on rats.

2. Materials and Methods

2.1 Animal

Animals were housed in Faculty of Medicine, University of Sultan Zainal Abidin (UniSZA). Sprague-Dawley male rats (200-250 g each) were used. The rats were housed at controlled temperature 22 ± 2°C and 12 h light/dark cycle with free access to food and water *ad libitum*. The animals were habituated to the testing environment for at least 2 weeks before experimenting in order to adapt the animals to the manipulation and to minimize nonspecific stress responses. All experiments carried out were approved by the Animal Experimentation Ethic Committee of UniSZA (ID: UAPREC/16/003).

2.2 Drugs and honey

Morphine (10 mg/ml in 1 ml ampoule) was purchased from Hameln, Germany and stored in 4°C. The solution was sterilized before use. *A. dorsata* honey was collected in 2016 from *Apis dorsata* beehives on Tualang tree (*Koompassia excels*) in Pasir Akar, Besut, Terengganu, Malaysia by Department of Agropolis, Faculty of Biosource and Food Industry, UniSZA.

3. Protocol

3.1 Tolerance study

This experiment consisted of five treatment groups (n=8); saline, morphine (10 mg/kg)+saline, morphine (10 mg/kg)+honey 0.5 g/kg, morphine (10 mg/kg)+honey 1.5 g/kg and morphine (10 mg/kg)+honey 2.5 g/kg. Saline is the negative control for morphine group. Morphine (10 mg/kg) is the negative control for morphine+honey dose groups. The honey doses 0.5, 1.5 and 2.5 g/kg is equal to 1-2 tablespoons, 4-5 tablespoons and 7-8 tablespoons an individual may use [21]. Toxicity test on maximum 5 g/kg dose of *A. dorsata* honey was carried out based on Organization for Economic Co-operation and Development (OECD) guideline for testing of chemicals (OECD, 2001) and the result demonstrated no sign of toxicity on the animals.

3.2 Induction of morphine tolerance

For induction of morphine tolerance, animals were injected (subcutaneously using 23G needle) with morphine (10 mg/kg) twice daily (9 am and 5 pm) for 14 days [22]. Our preliminary study confirmed that this dose can cause profound analgesia without causing side effects in normal rat. Saline group received 1ml/kg saline subcutaneously. To determine the effect of *A. dorsata* honey on the development of morphine tolerance, *A. dorsata* honey (0.5, 1.0 and 2.5 g/kg) were administered orally using oral gavage 30 min prior to each injection of morphine. Control groups received saline (1 ml/kg).

3.3 Hot plate test

Hot plate test was carried out 30 min after morphine administration. Each animal was placed in plexi-glass box on hotplate (Bioseb, France) with temperature 55°C and surface area of 23 × 23 cm. Latency time for the animal to lick hind paw or jump (whichever came first) was recorded as post-drug latency. Before drug administration, the latency (measured in second, s) was measured and average of second and third reading was taken as baseline latency. The cut-off time was set at 30s to avoid tissue damage. Before experiment, the animal was tested for 4 days to obtain stable latency response and animal who fail to respond within 30 s was excluded from experiment [7].The result was based on percentage of Maximum Possible Effect (%MPE):

%MPE=(Post drug latency-Baseline latency)/(Cutoff time-Baseline latency) × 100

Hotplate test was carried on day 1 and day 14 of the experiment in the morning.

3.4 Data analysis

Statistical analysis was performed using the Statistical Package for Social Sciences version 20.0 for Windows. Statistical comparisons in %MPE between groups over the time course of study was determined using Repeated measure ANOVA followed by Scheffe's post hoc test at 95% confidence intervals.

4. Results

On day 1, morphine administration in morphine-treated groups caused significant analgesic MPE% as compared to saline group. On day 3, the MPE% significantly decreased, indicating development of the morphine tolerance analgesic effect started (data not shown). Repeated administration of morphine in respective morphine alone and morphine (10 mg/kg) co-administered with *A. dorsata* honey (0.5, 1.5 and 2.5 g/kg) groups caused a significant decrease in MPE% on day 14 compared to day 1 (refer Table 1 for confidence interval, CI). As a control group, rats treated with saline gave no significant analgesic effect from early to end of the treatment. Morphine control group produced non-significant MPE% compared to saline group, confirming establishment of morphine tolerance on Day 14. Co-administration of morphine (10 mg/kg) with 0.5 g/kg, 1.5 g/kg and 2.5 g/kg *A. dorsata* honey significantly prevented morphine tolerance effect on Day 14 compared to morphine control group (CI:-14.494,-0.110; -26.148,-11.764; -19.078,-4.694). Dose 1.5g/kg *A. dorsata* honey produced highest MPE% on Day 14 (Figures 1 and 2). Compared to morphine control group. Graphs were obtained using GraphPad Prism 6.

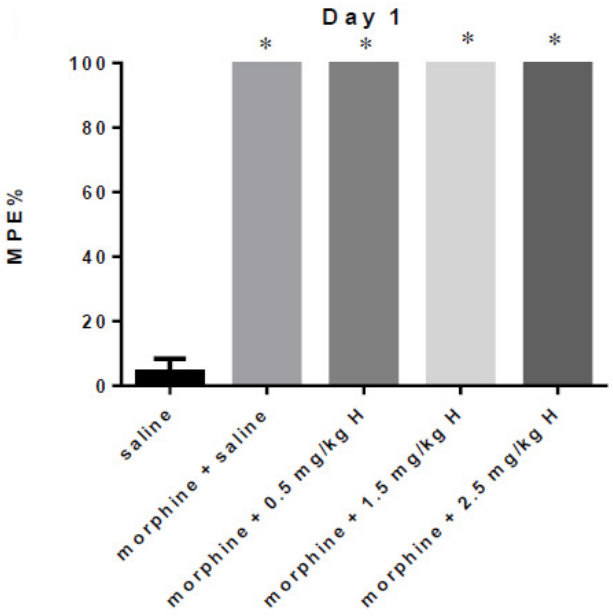

Figure 1: The effect of different doses of *Apis dorsata* honey on development of morphine tolerance in rats on Day 1.

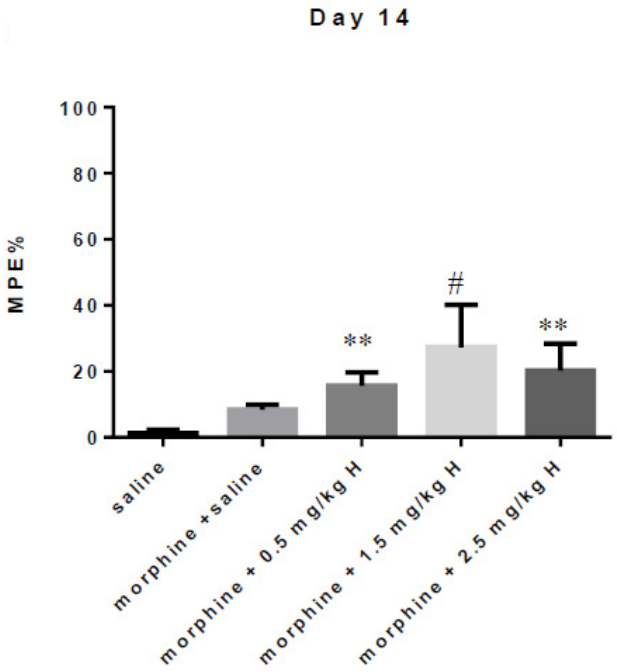

Figure 2: The effect of different doses of *Apis dorsata* honey on development of morphine tolerance in rats on day 14.
Data represents MPE% mean ± S.E.M; n=8; *p<0.001 compared to saline group; ** p<0.05 compared to morphine control group; #p<0.001

5. Discussion

This study focused on the effect of different doses of *A. dorsata* honey on the development of morphine tolerance via hotplate test. We found out the hotplate MPE% in morphine-treated groups reduced after repeated administration, indicating development of morphine tolerance, and established on Day 14. This outcome is consistent with previous study by Sharifipour [22]. More importantly, our study depicted new biological property of *A. dorsata*, in which concomitant administration of low, intermediate and high *A. dorsata* honey dose with morphine caused significant attenuation in development of morphine tolerance to analgesic effect.

There are very limited studies carried out to relate honey with opioid. The anti-tolerant effect of *A. dorsata* honey doses may be related with antioxidant, analgesic effect and anti-inflammatory properties of honey [13-15,23]. Honey bioactive compound such

Table 1: MPE% of different treatment groups on Day 1 and Day 14 of hotplate test.
Values are means ± SEM (n=8). p<0.05 is considered significant

Treatment	MPE%		
	Day 1	Day 14	P (CI)
Saline	2.2 ± 1.4	1.0 ± 0.4	>0.05 (-1.803, 8.041)
Morphine	100.0 ± 0.0	8.1 ± 0.5	<0.001 (89.270, 94.306)
Morphine+0.5 g/kg *A. dorsata* honey	100.0 ± 0.0	14.1 ± 0.9	<0.001 (78.068, 90.634)
Morphine+1.5 g/kg *A. dorsata* honey	100.0 ± 0.0	25.3 ± 4.5	<0.001 (53.004, 92.390)
Morphine+2.5 g/kg *A. dorsata* honey	100.0 ± 0.0	12.4 ± 2.9	<0.001 (67.137, 92.396)

as caffeic acid and chrysin were proven to have analgesic effects [24,25].

The mechanism of *A. dorsata* honey in development of morphine tolerance is still unclear; however, one possibility is that *A. dorsata* honey act as a free radical scavenger against oxidative stress [20]. *A. dorsata* honey contained higher antioxidant activity compared to widely-used Manuka honey and several Malaysian honey [14,26]. Among bioactive compound contained in *A. dorsata* honey are gallic, caffeic acids, catechin, kaempferol, naringenin, luteolin and apigenin. Studies have also shown that GSH can enhance morphine analgesia and reduce the effect on morphine withdrawal and dependence [27,28].

At the molecular level, Reactive Oxygen Species (ROS) formed by chronic morphine administration participated in signaling pathway of the drug at the mu opioid receptor (μOR). The ROS may cause change in the receptor phosphorylation (on G protein), altering it conformation and the morphine-receptor action [29]. This eventually affects cAMP level contributing to occurrence of tolerance and dependence [27]. In this mechanism, honey bioactive compounds may indirectly reduce ROS and alleviate their effect in the same signaling pathway. However, the exact mechanism is still elusive and requires more study.

Several honey bioactive compounds were reported to have properties in reducing the tolerance, for example, coadministration of ellagic acid and quercetin respectively with morphine significantly blocked development of morphine tolerance [16,17]. This finding highlighted the importance of bioactive compound of honey in maintaining the effect of morphine and prevented the use of the analgesic effects.

Inhibition of Nitric Oxide (NO) overproduction in serum, an indicator for oxidative stress formation and inflammation, was found to prevent the development of morphine tolerance [6,7,30]. Gelam honey reduced NO production when treated in inflammatory tissues [31]. The outcome suggests possible biological property of honey that may be involved in development of morphine tolerance. Honey antitolerant effect may be related with inhibition of neuroinflammation process. Involvement of glia neuroinflammation in morphine tolerance, in which inhibition of glial activation can reduce the morphine tolerance [9,32]. Honey phenol, caffeic acid was also confirmed to reduce neuronal damage and enhance depletion of glial proliferation [33].

In addition, in vitro assessment of honey flavonoids such as luteolin, quercetin, naringenin and chrysin promotes reduces in iNOS (enzyme for synthesis of NO) and NO production at the same time, inhibited neuronal death in microglia [34-38]. Some of them also confirmed to reduce the inflammatory cytokines, thus highlighting their potential in reducing morphine tolerance via lowering neuroimflammation. Apart from these supporting evidences, *A. dorsata* honey was tested before with dose of 0.2, 1.2 and 2.4 g/kg honey, showing their analgesic activity via tail flick test [26]. Our results emphasize the variation of honey doses effecting analgesic effect and the interaction of honey with morphine action.

On the other hand, stingless bee honey significantly attenuated the development of morphine tolerance via as low as 0.2 g/kg dose when combined with morphine and morphine+methadone respectively [39-46]. Thus, different types of honey possess different level in minimizing morphine tolerance. The variation of the bioactive contents in different honey leads to different biological activities. Although honey is known for its medical benefits especially in antioxidant and analgesic activities, nature factors such as climate, floral source and geographical area of honey sources contribute to variation in their chemical and bioactive composition and so their biological activity levels [14].

5. Conclusion

In conclusion, this study showed that *A. dorsata* honey significantly prevented the development of morphine tolerance. This highlights the potential therapeutic effect of *A. dorsata* honey in the treatment of pain and also opioid addiction. Further study need to be carried out to investigate the exact mechanism of *A. dorsata* honey doses in affecting morphine tolerance. Clinical study need to be carried out in order to determine specific dose prescription to obtain an effective combination of *A. dorsata* honey and opioid.

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