Original Article

Effects of Different Doses of Tualang Honey on Pain Behavior in Rats with Formalin-Induced Inflammation

Che Badariah Abd Aziz, Che Aishah Nazariah Ismail, Mohd Ibariyah Iberahim, Mahaneem Mohamed, Shamsul Kamaruljan

Abstract

Honey was reported to reduce pain and inflammation. However, very few studies have investigated on the effective dose of honey that reduces pain and inflammation. The aim of this study was to compare the effects of different doses of Tualang honey on pain behavior and inflammation induced by formalin injection. Twenty-four Sprague-Dawley male rats (250-300 g) were randomly allocated to four different treatment groups (n=6 per group). Each group was force-fed either with distilled water, 0.2 g/kg, 1.2 g/kg or 2.4 g/kg Tualang honey for 5 days. Intraplantar 1% formalin (0.1 ml) was given on the fifth day and rat's pain behavior was recorded using a digital camera for an hour. Paw diameter and circumference were recorded before and four hours after formalin injection. Pain behavior score was tabulated at each minute and averaged at five minutes interval. Repeated measures analysis of variance (ANOVA) followed by post hoc Bonferroni test was used to analyze the data and significance level was accepted at $P < 0.05$. There was no difference in the pain behavior score between all groups in the first 10 minutes post-formalin injection. However, the pain behavior score was significantly reduced from minute-35 to 60 post-formalin in the group receiving 1.2 g/kg honey compared to control group. The reduction of pain behavior score was not significant in the groups receiving 0.2 g/kg and 2.4 g/kg of honey. There was no significant difference in the paw diameter and circumference between the groups. It is concluded that Tualang honey at 1.2 g/kg significantly reduced pain behavior score in the rats with formalin-induced inflammation, while the low and high dose did not give significant response. The results throw some light for the possible use of honey as a pre-emptive analgesic agent.

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Various studies have investigated the antimicrobial and anti-inflammatory properties of honey. It is used both in modern medicine and important components in traditional medicines in wound treatment. Its antibacterial properties prevent infection of the wound and the anti-oxidant properties promote wound healing.[1-3] Honey enhances antibody production against thymus-dependent and thymus-independent antigens during primary and secondary immune responses. Honey stimulated proliferation of B and T lymphocytes in cell culture and stimulated monocytes to release cytokines such as TNF-α, IL-1β and IL-6 which activated immune responses.[4] Honey was found to reduce plasma prostaglandin (thromboxane B_2, PGE_2 and PGF_{2a}) in normal human subjects and prostaglandin is well known to mediate inflammation and pain.[5,6]

From the Department of Physiology (C.B.A.A., C.A.N.I, M.M.) and Department of Anaesthesiology (M.I.I., S.K.), School of Medical Sciences, Universiti Sains Malaysia Health Campus, Kubang Kerian, Kelantan, Malaysia.

Corresponding author:
Dr Che Badariah Abd Aziz, MBBS, MSc and PhD
Department of Physiology, School of Medical Sciences,
Universiti Sains Malaysia Health Campus,
16150Kubang Kerian, Kelantan, Malaysia.
E-mail: badariah@kb.usm.my

Honey has been shown to reduce pain from burn wound when applied locally and it probably reduces inflammatory pain by suppressing inflammation and hastens subsidence of passive hyperaemia.[7] Gelam and Acacia honey have been shown to increase the pain threshold toward noxious thermal stimuli in animal studies.[8,9] To date, the antinociceptive effects of Tualang honey (a wild multifloral Malaysian honey) towards formalin-induced pain has never been investigated. Hence, this study was aimed to determine the effects of different doses of Tualang honey on pain behavior score and inflammation induced by formalin injection.

Materials and Methods

Honey

Tualang honey was supplied by Food and Agricultural Marketing Authority, Malaysia. The honey was freshly prepared in three different doses; low dose (0.2 g/kg body weight), medium dose (1.2 g/kg body weight) and high dose (2.4 g/kg body weight) using distilled water as vehicle.

Study design

This study was approved by Animal Ethics Committee of Universiti Sains Malaysia [USM/Animal Ethics Approval/2011/(70)/(328)]. Sprague-Dawley adult male rats with the weight of

250-300 g (n=24) were obtained from Laboratory Animal Research Unit, Universiti Sains Malaysia. They were maintained in a 12-hour dark-light cycle and permitted with free access to food and water. All the rats were kept in the individual cages and acclimatized to the laboratory environment for at least 4 days.[10] The experiments were carried out from 08.00 to 16.00 in the physiology laboratory.

Groups

The rats were randomly allocated into four treatment groups; distilled water (as control), Tualang honey at 0.2, 1.2 or 2.4 g/kg body weight. The doses chosen were based on a previous study done by Omotayo *et al.*[11] The dose of honey at 0.2 g/kg is the dose that is usually consumed by Malaysian and therefore it was chosen as low dose in this study. From this dose, we multiplied it by 6 and 12 to get 1.2 and 2.4 g/kg which are considered as medium and high doses, respectively. Each rat was force-fed once per day by gavaging with distilled water or Tualang honey from Day 1 to Day 5.

Formalin injection

On Day 5, one hour after treatment, 0.1 ml of 1% formalin (HmbG® Chemicals, Hamburg, Germany) was injected by using 25-gauge needle at subplantar region of the rat's right hind paw. The behavior of each rat was recorded for 60 minutes using a digital video camera (Panasonic, Japan) and behavioral assessment was based on the four behavioral categories (0 to 3).[10,12] The mini compact disc was later viewed and the pain behavior score was tabulated every minute as described by Dubuisson & Dennis, and averaged at 5-minute intervals.[12,13] The categories were:

- 0 = the injected paw is not favored (foot flat on the floor with toes splayed)
- 1 = the injected paw has little or no weight on it with no toe splaying
- 2 = the injected paw is elevated and the heel is not in contact with any surface
- 3 = the injected paw is licked, bitten or shaken

All the rats in this investigation were used only once and were sacrificed at the end of the experiments.

Paw circumference

In order to evaluate for peripheral edema, a thread was used to measure paw circumference at the metatarsal level.[14] Each rat was placed in a plastic cylinder (20 x 30 cm), and its tail and paw were pulled through a hole at the base of the cylinder. The paw circumference was then measured (to the nearest mm) before and four hours after the formalin injection. The length of the thread which corresponded to the circumference was measured using a ruler. The changes in the paw circumference were calculated and recorded for each rat using the formula below:

Change in paw circumference = paw circumference after injection - paw circumference before injection.

Statistical analysis

Statistical Package of Social Science (SPSS) version 18.0 (Chicago, USA) was used for data entry and statistical analysis. Pain behavior scores obtained by the formalin test were analyzed using repeated measures analysis of variance (ANOVA) with one within-subjects factor (time; 13 levels) and one between-subjects factor (drug; 4 levels). Repeated measures ANOVA was also used to analyze the effects of phase 1 formalin test (mean score at 5 minutes) and phase 2 formalin test (mean of scores from 10 to 60 minutes) with one within-subjects factor (time, 2 levels) and drug (honey 0.2 g/kg, honey 1.2 g/kg, honey 2.4 g/kg) as the between-subjects factor. A post hoc Scheffe test was performed. The changes in paw circumference data were analyzed using one-way ANOVA. Significance level for all data was accepted at $P < 0.05$.

Results

Biphasic pain response was demonstrated in the control group receiving distilled water. An increase in the behavior score was shown in the first 5 minutes (phase 1) followed by 5 to 10 minutes of decreased score. An increase in the pain behavior score was also observed from 15 minutes to 60 minutes post formalin injection (phase 2). The study demonstrated a significant difference between control group to the groups receiving different honey concentrations, F(4.032, 80.636 = 82.260, $P < 0.001$). Post hoc Bonferroni test showed a significant difference between group receiving Tualang honey 1.2 g/kg compared to control group ($P < 0.05$), while other groups given 0.2 and 2.4 g/kg Tualang honey showed no significant difference compared to control group (Figure 1).

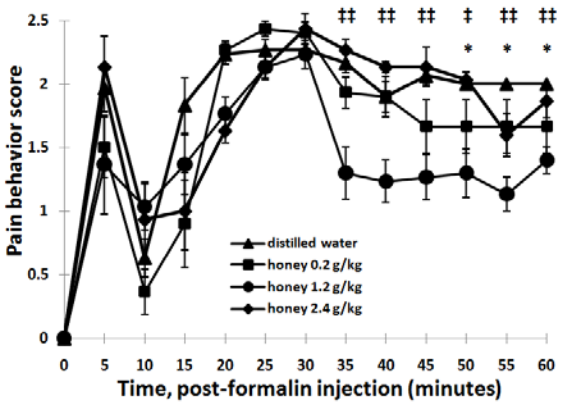

Figure 1 Pain behavior score in groups receiving distilled water and different doses of Tualang honey. Significant reduction of pain behavior score was seen between all treatment groups at 50 to 60 minutes, as compared to control (*$P < 0.05$). Honey 1.2 g/kg significantly suppressed the nociceptive response from 35 to 60 minutes, compared to control. (‡ $P < 0.05$ and ‡‡ $P < 0.001$).

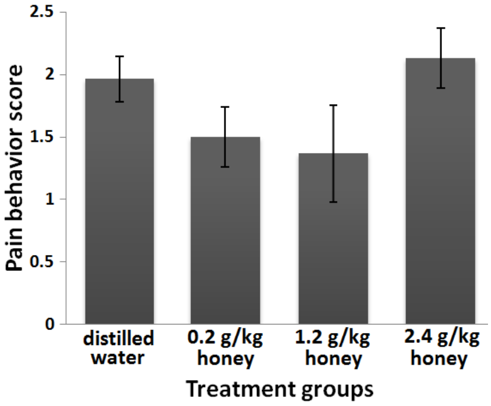

Figure 2 Pain behavior scores of all groups in phase 1. The pain behavior scores were not significantly different when compared between the four treatment groups ($P > 0.05$).

There was a reduction in pain behavior score in the group receiving 1.2 g/kg of Tualang honey in phase 1 but it was not statistically significant (Figure 2). In the later part of phase 2, the pain behavior score was significantly reduced in this group compared to control group ($P < 0.001$ at the 35th, 40th, 45th, 55th and 60th minutes; $P < 0.05$ at the 50th minute) (Figure 3). The pain behavior score was not significantly inhibited in the group which received the low (0.2 g/kg) and high (2.4 g/kg) doses of Tualang honey (Figure 3). There was also no significant change in paw circumference when compared between the groups although the change was lower in the group which received 1.2 g/kg of honey ($P > 0.05$) (Table 1).

Discussion

The present study investigated the effects of different doses of Tualang honey on pain behavior score induced by formalin injection in male Sprague Dawley rats. Formalin test produces two phase of pain behavior and the pain behavior in phase 1 was partly due to the formation of prostaglandin that generates the signs and symptoms of inflammation.[15] Phase 2 of the formalin pain behavior score is known

Table 1 Changes in circumference in all groups. Minimal change was seen in the group receiving 1.2 g/kg of honey, but the difference was not significant when compared with the other groups ($P > 0.05$). Data on the changes in paw circumference were analyzed using one-way ANOVA.

Group	Changes in paw circumference (mm)
Control	3.50 ± 0.56
0.2 g/kg	3.67 ± 0.80
1.2 g/kg	2.60 ± 0.75
2.4 g/kg	3.00 ± 0.45

to be contributed by local inflammatory response and functional alterations in the spinal cord and at higher levels of the nervous system.[16] The study had demonstrated that Tualang honey at 1.2 g/kg significantly reduced pain behavior score in later part of phase 2 pain while other doses showed no significant effect. The findings suggest that administration of Tualang honey at 1.2g/kg for five days inhibited inflammatory induced pain and the inhibition was more marked in phase 2. Vitamin C, which is one of the components of Tualang honey, has been shown to inhibit both phases of formalin induced behavior (paw licking) and these effects were suggested as due to inhibition of the ionotropic glutamate receptors at the spinal cord level.[17] Tualang honey has been shown to have higher vitamin C (36.09 ± 0.41 mg per 100g) compared to other types of honey (14.64 ± 0.72 to 32.14 ± 0.68 mg per 100g) and this property might contribute to the reduction in nociceptive response induced by the inflammation in the present study.[18] However, this study did not show significant result in phase 1, most probably because of the different type of behavior assessment and animals used.

Analgesic properties of Tualang honey could also be attributed to its phenolic compounds. The high phenolic content (83.96 ± 4.53 mg of gallic acid equivalents per 100g) in Tualang honey contributes to its antioxidant property and might have a role in its anti-inflammatory effect.[18,19] Gallic acid, one of the components of Tualang honey, was reported to suppress nitric oxide synthase (iNOS), COX-2,

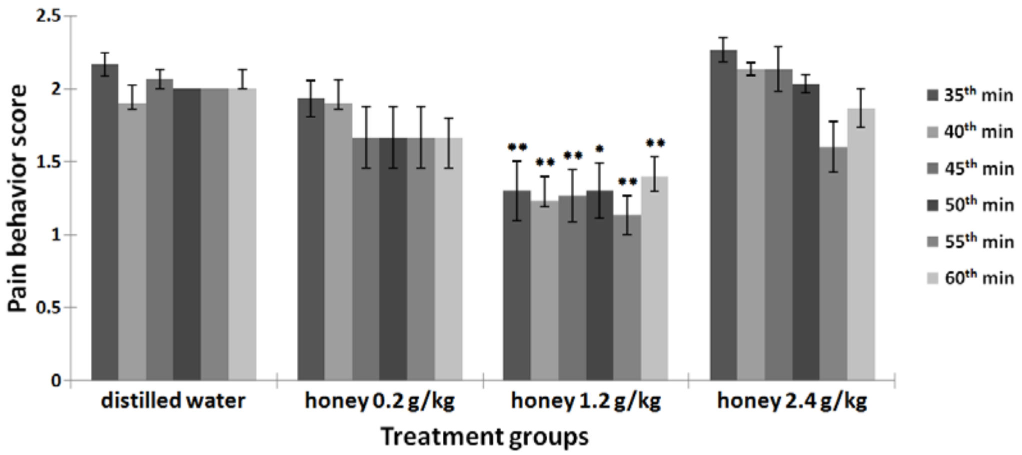

Figure 3 Pain behavior scores of all treatment groups in phase 2. The significant reduction of the score was seen in groups receiving 0.2 g/kg and 1.2 g/kg of Tualang honey (*$P < 0.05$, **$P < 0.001$). Compared to all doses, Tualang honey at 1.2 g/kg was found to significantly reduce the pain at 35th to 60th minutes.

histamine release and inhibited the pro-inflammatory cytokine production in macrophage.[20] The inhibitory activities on the inflammatory response were also shown by flavonoids and chrysin which are present in honey.[21,22] The analgesic and anti-inflammatory effects of other types of honey e.g. Gelam and Manuka honey were shown in various studies but the present study is the first study which reports the analgesic effects of Tualang honey at a specified dose.[9,23,24] The mechanisms involved in the Tulang honey's antinociceptive effects could be probably due to inhibition of release of prostaglandin, nitric oxide synthase (iNOS) or other substances into inflamed peripheral tissue or inhibition of nociceptor responses or due to inhibition of pain transmission at the central nervous system.

In good agreement with the analgesic effect, the degree of peripheral oedema in the group which received 1.2 g/kg of Tualang honey was also minimal, although not significantly different when compared to other groups.

In conclusion, different doses of Tualang honey produced different effects on the pain behavior score. It is suggested that 1.2 g/kg is the optimal concentration of Tualang honey which produces antinociceptive effects in rat model. As the mechanisms of Tualang honey in reducing inflammatory pain at 1.2 g/kg is still unclear, further research should be carried out to identify potential mechanisms in reducing inflammatory pain.

Acknowledgements

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Conflict of Interest

None to declare.

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