Effects of honey on inflammation and nitric oxide production in Wistar rats

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Objective: The aim of the study is to investigate the effects of honey on acute and chronic inflammations and nitric oxide production in rats.

Methods: Carrageenan, cotton pellet and formaldehyde methods were used in quantifying the anti-inflammatory effect of honey while the effect of honey on nitric oxide (NO) production was investigated by administering N^3-nitro-L-arginine methyl ester (L-NNAME, 100 mg/kg body weight, subcutaneously) and L-arginine (300 mg/kg body weight, intraperitoneally) to groups of rats. Animals were divided into five groups each comprising of five rats in each experiment; two groups were orally administered distilled water (control) and indomethacin (5 mg/kg body weight), respectively, while the remaining three groups were administered 2, 6 and 10 g/kg honey for anti-inflammatory studies.

Results: Honey significantly (P<0.05) reduced the paw size in the carrageenan model, while in the cotton pellet model, the granuloma weight was significantly (P<0.05) reduced. Honey also significantly (P<0.05) reduced the arthritis induced by formaldehyde injection from the second day of the study. In the investigation on NO involvement, L-NNAME significantly inhibited paw oedema while the administration of L-arginine abolished the anti-inflammatory effect of honey and L-NNAME.

Conclusion: The results obtained from the study confirm that honey has an anti-inflammatory effect which may be due in part to inhibition of NO release. Therefore honey may be used to treat certain acute and chronic inflammatory conditions.

Keywords: honey; inflammation; nitric oxide; rats

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马旭辉, 张杰. 中药对内皮素及一氧化氮的调控作用研究进展. 中西医结合学报. 2004; 2(2); 152-153.


Honey is a saturated sugar solution created by bees primarily from nectars. It is used both as food and medicine since ancient time\cite{1,23}. The main use of honey is as a flavour, sweetener and an energy source\cite{13}. It is used medicinally for the treatment of skin burns, diabetic and gastric ulcers, respiratory diseases, eczema and dandruff\cite{24,25}. The use of honey for the treatment of wounds is common worldwide\cite{4,51}. It has been hypothesized that honey’s healing effect on wounds may in part be due to its antioxidant, antibacterial and anti-inflammatory effects\cite{1,7,94}. Although there have been reports indicating that honey has anti-inflammatory activities in humans and rodents, most of these studies were based on the effect of honey or parts of its constituents on mediators of inflammation and a few on carrageenan-induced paw oedema\cite{10,11,12,13}. In the present study we used three standard animal models of inflammation to investigate the anti-inflammatory effect of honey and also investigated the effect of honey on nitric oxide (NO) production in Wistar rats.

1 Materials and methods

1.1 Drugs and reagents The honey used for this study was purchased from the apiary of the Faculty of Agriculture, University of Ilorin, Ilorin, Nigeria. The honey was packed in sterile bottles containing 2 kg of honey each. Formaldehyde was obtained from BDH Chemicals Ltd. Poole, England, while carrageenan, N⁰-nitro-L-arginine methyl ester (L-NAME), indomethacin and L-arginine were products of Sigma-Aldrich, St. Louis, MO, USA.

1.2 Experimental animals and grouping Male (n = 60) and female (n = 40) Wistar rats weighing (145.0 ± 8.3) g were used for the experiment. They were maintained under standard conditions of temperature, humidity and light-dark cycle (12 h light/dark cycle). Animals had free access to water and food during the period of the laboratory experiments. Laboratory investigations on the animals were carried out in accordance with the ethical guidelines stipulated by ethical committee of the College of Health Sciences, University of Ilorin, Nigeria. These guidelines were in accordance with the internationally accepted principles for laboratory animal use and care. Animals for the various anti-inflammatory studies were divided into five groups comprising of five animals each. The animal grouping was arrived at by slightly modifying the grouping pattern used by previous studies\cite{11,16}. These groups are: (A) distilled water, (B) honey (1 g/kg body weight), (C) honey (6 g/kg body weight), (D) honey (10 g/kg body weight) and (E) indomethacin (5 mg/kg body weight). Water, honey and indomethacin were administered orally to respective groups by using a cannula.

1.3 Carrageenan-induced paw oedema Paw inflammation was produced in all groups according to the method described by Winter et al\cite{15}. One hour after the administration of honey, indomethacin or distilled water, 0.1 mL of 1% carrageenan was injected into the left hind paw of each animal under the sub plantar aponeurosis.

Measurement of paw size was carried out as described previously\cite{11} by wrapping a piece of cotton thread round the paw and measuring the circumference with a metre rule. Paw size was measured immediately before carrageenan injection and 3 and 5 h after. Oedema inhibitory activity was calculated according to the following formula\cite{16}.

\[
\text{Percentage inhibition} = \frac{(C_t - C_e)_{control} - (C_t - C_e)_{treatment}}{(C_t - C_e)_{control}} \times 100\%
\]

Where \(C_t\) = paw circumference at time \(t\), \(C_e\) = paw circumference before carrageenan injection and \(C_t - C_e\) = oedema.

1.4 Cotton pellet-induced granuloma The model described by Ismail et al\cite{17} was used in this study. A sterilized cotton pellet weighing 30 mg was implanted subcutaneously into the groin region of rats after the five groups were treated (once daily) with honey, indomethacin or distilled water for 7 consecutive days. The animals were sacrificed on the 8th day with an over dose of ether. Thereafter, the pellets surrounded by granuloma tissue were dissected out carefully and oven dried at 60 °C to a constant weight. The mean weight of the granuloma tissue formed around each pellet was obtained and the percentage inhibition was determined.

1.5 Formaldehyde-induced arthritis This study was carried out as previously described by Owoyele et al\cite{18}. The left paws of all the animals in all groups were injected with 0.1 mL of 4% formaldehyde on the first and third day of the experiment. Honey, distilled water or indomethacin was administered orally to the animals once daily for 10 consecutive days starting from the first day of formaldehyde injection. The daily changes in the paw size were measured by using cotton thread and metre rule as in the carrageenan model. Changes in paw size of the treatment groups were compared with that of the control group.

1.6 Involvement of NO This study was carried out to investigate the involvement of NO in carrageenan-induced oedema. Carrageenan was injected into the paw of animals 1 h after honey (2 and 6 g/kg body weight, respectively), distilled water, L-NAME (100 mg/kg body weight, subcutaneously) or indomethacin were orally administered to the animals. All the animals were administered L-arginine (300 mg/kg, intraperitoneally) 1 h after carrageenan administration according to the method used by Foyet et al\cite{18}. Paw oedema was measured every 1 h for a period of 6 h after carrageenan administration as described earlier.

1.7 Statistical analysis The results are expressed as \(x \pm s_x\). Data were subjected to analysis of variance followed by Waller-Duncan post hoc test by using SPSS 16.0 statistical package. \(P < 0.05\) was
considered statistically significant.

2 Results

2.1 Carrageenan-induced oedema  The results of the carrageenan-induced oedema test showed that honey significantly reduced the oedema from (10.4 ± 1.4) mm (in the control) to (4.8 ± 1.0) mm (in the 10 g/kg group) \( (P < 0.05) \). The results are shown in Table 1.

2.2 Cotton pellet-induced granuloma  Table 2 shows the results of the cotton pellet test. The result indicates that honey and indomethacin produced significant inhibition of granuloma formation by 20% to 40% \( (P < 0.05) \).

2.3 Formaldehyde-induced arthritis  The results of the formaldehyde-induced arthritis test are shown in Table 3. The results showed that honey and indomethacin significantly \( (P < 0.05) \) inhibited arthritis during the ten-day period of the study.

2.4 Involvement of NO  Table 4 shows the effect of L-arginine injection on anti-inflammatory effect of honey in carrageenan test. L-arginine injection completely abolished the anti-inflammatory effects of honey, L-NAME and indomethacin.

Table 1  Effects of honey on carrageenan-induced paw oedema in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>Initial paw size before carrageenan administration ( (X \pm s_x, \text{mm}) )</th>
<th>Increase in paw size 3 h after carrageenan administration ( (X \pm s_x, \text{mm}) )</th>
<th>Increase in paw size 5 h after carrageenan administration ( (X \pm s_x, \text{mm}) )</th>
<th>Inhibition 3 h after carrageenan administration (%)</th>
<th>Inhibition 5 h after carrageenan administration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (distilled water, 10 mL/kg body weight)</td>
<td>5</td>
<td>2.50 ± 0.04</td>
<td>10.4 ± 1.4</td>
<td>9.6 ± 1.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Honey (2 g/kg body weight)</td>
<td>5</td>
<td>2.43 ± 0.05</td>
<td>6.2 ± 0.6*</td>
<td>4.8 ± 1.5*</td>
<td>40.4</td>
<td>50.0</td>
</tr>
<tr>
<td>Honey (6 g/kg body weight)</td>
<td>5</td>
<td>2.48 ± 0.05</td>
<td>4.6 ± 0.4*</td>
<td>4.8 ± 1.3*</td>
<td>55.8</td>
<td>50.0</td>
</tr>
<tr>
<td>Honey (10 g/kg body weight)</td>
<td>5</td>
<td>2.41 ± 0.03</td>
<td>4.8 ± 1.0*</td>
<td>1.8 ± 0.4*</td>
<td>55.8</td>
<td>81.3</td>
</tr>
<tr>
<td>Indomethacin (5 mg/kg body weight)</td>
<td>5</td>
<td>2.50 ± 0.05</td>
<td>3.4 ± 1.0*</td>
<td>1.4 ± 0.9*</td>
<td>67.3</td>
<td>85.4</td>
</tr>
</tbody>
</table>

\* \( P < 0.05 \), vs control group.

Table 2  Effects of honey on cotton pellet-induced granuloma in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>Increase in weight of pellet ( (X \pm s_x, \text{g}) )</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (distilled water, 10 mL/kg body weight)</td>
<td>5</td>
<td>0.26 ± 0.02</td>
<td>—</td>
</tr>
<tr>
<td>Honey (2 g/kg body weight)</td>
<td>5</td>
<td>0.16 ± 0.02*</td>
<td>38.5</td>
</tr>
<tr>
<td>Honey (6 g/kg body weight)</td>
<td>5</td>
<td>0.12 ± 0.02*</td>
<td>53.8</td>
</tr>
<tr>
<td>Honey (10 g/kg body weight)</td>
<td>5</td>
<td>0.08 ± 0.03*</td>
<td>69.2</td>
</tr>
<tr>
<td>Indomethacin (5 mg/kg body weight)</td>
<td>5</td>
<td>0.14 ± 0.02*</td>
<td>46.2</td>
</tr>
</tbody>
</table>

\* \( P < 0.05 \), vs control group.

Table 3  Effects of honey on formaldehyde-induced arthritis in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (distilled water, 10 mL/kg body weight)</td>
<td>5</td>
<td>7.0 ± 0.2</td>
<td>8.2 ± 0.2</td>
<td>10.8 ± 0.5</td>
<td>11.6 ± 0.5</td>
<td>10.8 ± 0.4</td>
<td>8.2 ± 0.4</td>
<td>7.0 ± 0.7</td>
<td>6.2 ± 0.2</td>
<td>5.8 ± 0.2</td>
<td>5.8 ± 0.2</td>
</tr>
<tr>
<td>Honey (2 g/kg body weight)</td>
<td>5</td>
<td>6.4 ± 0.4</td>
<td>4.4 ± 0.1*</td>
<td>6.0 ± 0.4*</td>
<td>4.6 ± 0.6*</td>
<td>3.2 ± 0.5*</td>
<td>2.8 ± 0.4*</td>
<td>2.2 ± 0.2*</td>
<td>1.8 ± 0.2*</td>
<td>1.6 ± 0.2*</td>
<td>1.6 ± 0.2*</td>
</tr>
<tr>
<td>Honey (6 g/kg body weight)</td>
<td>5</td>
<td>5.4 ± 0.2*</td>
<td>6.2 ± 0.4*</td>
<td>7.2 ± 0.4*</td>
<td>8.0 ± 0.3*</td>
<td>1.8 ± 0.2*</td>
<td>1.4 ± 0.2*</td>
<td>1.4 ± 0.2*</td>
<td>1.2 ± 0.2*</td>
<td>1.2 ± 0.2*</td>
<td>1.2 ± 0.3*</td>
</tr>
<tr>
<td>Honey (10 g/kg body weight)</td>
<td>5</td>
<td>3.6 ± 0.4*</td>
<td>3.8 ± 0.4*</td>
<td>3.8 ± 0.4*</td>
<td>3.6 ± 0.4*</td>
<td>5.8 ± 0.2*</td>
<td>3.0 ± 0.3*</td>
<td>2.0 ± 0.3*</td>
<td>1.8 ± 0.2*</td>
<td>1.4 ± 0.2*</td>
<td>1.4 ± 0.2*</td>
</tr>
<tr>
<td>Indomethacin (5 mg/kg body weight)</td>
<td>5</td>
<td>3.6 ± 0.4*</td>
<td>4.0 ± 0.4*</td>
<td>5.2 ± 0.2*</td>
<td>3.0 ± 0.3*</td>
<td>2.0 ± 0.3*</td>
<td>1.6 ± 0.2*</td>
<td>1.8 ± 0.2*</td>
<td>1.8 ± 0.2*</td>
<td>1.6 ± 0.2*</td>
<td>1.4 ± 0.2*</td>
</tr>
</tbody>
</table>

\* \( P < 0.05 \), vs control group; \* \( P < 0.05 \), vs honey (2 g/kg) group; \* \( P < 0.05 \), vs honey (6 g/kg) group.
Table 4 Effects of L-arginine on the anti-inflammatory activities of honey, indomethacin and L-NAME in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>0.5 h (x ± s)</th>
<th>1 h (x ± s)</th>
<th>2 h (x ± s)</th>
<th>3 h (x ± s)</th>
<th>4 h (x ± s)</th>
<th>5 h (x ± s)</th>
<th>6 h (x ± s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (distilled water, 10 mL/kg body weight)</td>
<td>5</td>
<td>0.60±0.07</td>
<td>0.92±0.13</td>
<td>0.98±0.22</td>
<td>0.98±0.12</td>
<td>0.98±0.12</td>
<td>0.74±0.15</td>
<td>0.80±0.13</td>
</tr>
<tr>
<td>Honey (2 g/kg body weight)</td>
<td>5</td>
<td>0.66±0.14</td>
<td>0.68±0.14</td>
<td>0.84±0.14</td>
<td>1.00±0.22</td>
<td>1.00±0.25</td>
<td>0.90±0.19</td>
<td>0.84±0.17</td>
</tr>
<tr>
<td>Honey (6 g/kg body weight)</td>
<td>5</td>
<td>0.32±0.13* △ △</td>
<td>0.46±0.07* △ △</td>
<td>0.74±0.11</td>
<td>0.86±0.18</td>
<td>0.78±0.04</td>
<td>0.52±0.22</td>
<td>0.46±0.12</td>
</tr>
<tr>
<td>L-NAME (100 mg/kg body weight)</td>
<td>5</td>
<td>0.10±0.07* △ △</td>
<td>0.33±0.12* △ △</td>
<td>0.42±0.13</td>
<td>0.85±0.17</td>
<td>0.65±0.15</td>
<td>0.50±0.22</td>
<td>0.53±0.13</td>
</tr>
<tr>
<td>Indomethacin (5 mg/kg body weight)</td>
<td>5</td>
<td>0.64±0.17</td>
<td>0.76±0.20</td>
<td>0.78±0.15</td>
<td>0.88±0.13</td>
<td>0.72±0.09</td>
<td>0.60±0.12</td>
<td>0.72±0.17</td>
</tr>
</tbody>
</table>

* P<0.05, vs control group; △ P<0.05, vs honey (2 g/kg) group; △△ P<0.05, vs indomethacin (5 mg/kg) group. L-NAME: Nω-nitro-L-arginine methyl ester.

3 Discussion

The present study looked at the anti-inflammatory effect of freshly produced and packaged honey. The laboratory models used were carefully selected to investigate the effects of honey on acute and chronic inflammations. In the acute inflammation which was represented by the carrageenan model, honey was able to inhibit the paw oedema. This showed that honey can inhibit the involvement of inflammatory mediators such as prostaglandins, histamine and cytokines which are prominent in carrageenan-induced oedema.[10, 11]. The observation from this model further confirms the results of Kassim et al.[12]. They reported similar results in the carrageenan model. Kassim et al.[12] went further to show that honey reduced the concentration of NO and prostaglandins E2 in tissue exudates from inflamed paws. In contrast, we used L-NAME (a nitric oxide inhibitor) and L-arginine (a nitric oxide synthase (NOS) substrate) to determine the involvement of NO in the anti-inflammatory effect of honey.

In the model of chronic inflammation, honey was effective in reducing the cellular migration to the site of the insertion of cotton pellet (a foreign substance) hence the dose-dependent reduction in the weight of dry granuloma was observed in the study. Likewise, honey was able to produce significant inhibition of the formaldehyde-induced arthritis in the animals. This model comprises of tonic pain and inflammation together and lasts for longer days than the carrageenan and cotton pellet model. The results obtained from the formaldehyde and cotton pellet models showed that honey can relieve chronic inflammation and these represent one of the first reports to emerge on the effectiveness of honey in the two models.

In the experiment involving L-NAME and L-arginine, the results showed that honey, L-NAME and indomethacin all significantly (P < 0.05) inhibited the paw oedema until about one hour after carrageenan injection, but thereafter there were no significant changes in the paw size. This showed that honey most likely produced its anti-inflammatory activity via its inhibition of NO release in the tissues. This is obvious from the results which showed that the anti-inflammatory effect of honey was wiped out when L-arginine was injected. The present finding is in agreement with the findings of Foyet et al.[13] which showed that agents that act by NO inhibition will usually have their anti-inflammatory activities reduced after the administration of an NO synthease. Three isoforms of NOS have been identified, two of these are constitutive (neural NOS and endothelial NOS) while the other is inducible (inducible NOS, iNOS). The constitutive NOS is calcium-dependent while the inducible NOS is stimulated by bacterial endotoxins and/or inflammatory cytokines.[14, 15]. The amount of iNOS produced during inflammation is greatly elevated compared with that produced by constitutive NOS. Therefore, the inhibition of iNOS activity and expression produces significant anti-inflammatory effect in acute and chronic inflammations especially in mouse paw oedema.[16, 17].

The general findings from this study indicate that honey has an anti-inflammatory effect in addition to the variously reported wound-healing properties. The anti-inflammatory effect might be part of the mechanism by which honey produces wound-healing since inflammation is part of the early process that leads to wound development. Furthermore, this anti-inflammatory effect which is partly due to inhibition of NO can further be traced to the presence of polyphenolic compounds in honey.[6, 20, 21]. This is because polyphenolic compounds usually exhibit antioxidative activities and NO has been identified as a potent radical which can be scavenged directly by polyphenolic compounds such as flavonoids.[22].

Finally, it is hereby concluded that honey has anti-inflammatory effect not only in acute inflammation but also in chronic form of inflammation such as arthritis and its use for the treatment of wound is further encouraged.

4 Acknowledgements

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目的：研究蜂对大鼠急性炎症及一氧化氮生成的作用。

方法：使用大鼠腹腔注射法、家兔法及大鼠急性炎症大鼠药物合临床评价的抗炎作用。硝普钠可有效降低大鼠一氧化氮生成的作用。在每个实验中，大鼠每组分为5只，每组5只大鼠。空白对照组和阳性对照组大鼠分别给予蒸馏水及消炎痛（5 mg/kg）口服，其余3组大鼠分别给予2.6、及10 g/kg 蜂蜜口服。

结果：在角叉菜胶所致大鼠急性炎症模型实验中，与对照组比较，蜂蜜显著降低了大鼠后爪的肿胀程度（P<0.05）；在棉球致大鼠肉芽肿模型实验中，与对照组比较，蜂蜜显著降低了肉芽肿的重量（P<0.05）；在甲基多巴致大鼠腹腔炎症模型实验中，与对照组比较，蜂蜜显著缓解了大鼠关节炎的症状（P<0.05）。在检测一氧化碳生成的实验中，硝普钠可有效降低大鼠一氧化氮生成的作用。而硝普钠可拮抗反映蜂蜜及硝酸左旋精氨酸的抗炎作用。

结论：本实验的研究结果证明了蜂蜜的抗炎作用，这可能与其抑制一氧化氮的生成有关。据此，蜂蜜可能可以被应用于某些急性炎症的治疗当中。

关键词：蜂蜜；炎症；一氧化氮；大鼠

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