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Preliminary comparative study of anti-inflammatory effect of unheated and heat-treated Sahara honey: *In vivo* approach

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ABSTRACT

Objective: To investigate the effect of unheated and heat-treated of Sahara honey.**Methods:** A total of 24 Swiss albino mice weighing 25–35 g were divided into four groups ($n = 6$). Anti-inflammatory effect was assessed at 1, 2, 3, 4, 5 and 6 h after subplantar injection of carrageenan (0.5 mL of a 1% solution in normal saline). In addition, total phenolic content was determined by modified Folin-Ciocalteu method.**Results:** The total phenolic content capacity of the Sahara honey before and after heat treatment was between 72 and 97.9 mg of gallic acid equivalents/100 g of honey respectively. Administration of unheated honey (oral administration) reduced significantly ($P < 0.05$). The carrageenan induced mice paw edema model at 1, 3 and 6 h for 21.85%, 5.43% and 80.43%, respectively. Administration of heat-treated honey showed insignificant inhibition of carrageenan and induced paw edema at 1 h (31.16%), 3 h (0.25%) and 6 h (34.19%). The 50 mg/kg diclofenac exhibited percent reduction in paw volume 16.12%, 8.90% and 15.32% after 1 h, 3 h and 6 h, respectively, when compared with control animals. No toxicity was identified.**Conclusions:** Our results suggest that unheated Sahara honey has anti-inflammatory effects by reducing the mice paw edema size while heat-treated Sahara honey decreases the anti-inflammatory activity.

1. Introduction

For many centuries, honey has been used in vital alternative medicine of Ayurveda. Several biological activities like immunostimulatory, anti-oxidant, hepatoprotective and anti-inflammatory activities were reported on the natural honey[1-3]. The composition and physicochemical quality of honey varies among different types of honey depending on geographical locations, floral source, harvesting, purity and storage conditions[4]. Heating of honey is contraindicated according to Ayurveda as it causes deleterious effects[5]. The influence of different heat treatments on the quality parameters of various types of honey is well investigated.

The influence of thermal treatment at various temperatures 25, 50, 75 and 100 °C for 15, 30 and 60 min on the antimycotic activity, polyphenol content and colour of Sahara honey was investigated by Algerian scientists[6]. Similar studies were obtained by Jahan *et al.*, who investigated the influence of heat treatment on the biological proprieties in Bangladesh honey[7].

In Algeria, there are several types of honey, including *Euphorbia*, Sidr and jujube. In an animal model, a topical dressing of unheated Sahara honey is believed to have several positive effects on wound healing[8-10]. A search of the available literature revealed no data on the anti-inflammatory activity of Sahara honey. This study aimed to investigate the effect of unheated and heat-treated of Sahara honey.

2. Materials and methods

2.1. Honey samples and heat treatment

Euphorbia honey is a local monofloral honey produced by

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Apis mellifera bees from the flora source of *Euphorbia*. In the experiment, the Sahara honey was heated at 90 °C for 120 min and diluted in water until the final concentration was 80% (w/v). The samples were cooled and immediately used to determine their total polyphenol content and their anti-inflammatory activity.

2.2. Total phenolic content using the Folin-Ciocalteu method

Foline-Ciocalteu method was used to assay the total phenolic content, which was described by Singleton *et al.*[11]. About 30 µL honey solution (0.1 g/mL) was mixed with 2.37 mL milli-Q water and 150 µL of 0.2 mol/L Folin-Ciocalteu reagent. The solution was thoroughly mixed by vortexing and incubated for 2 min at ambient temperature. About 450 µL sodium carbonate solution (0.2 g/mL) was added to the reaction mixture and further incubated for 2 h at ambient temperature. The absorbance was measured at 765 nm using a spectrophotometer. The total phenolic content was determined by comparing with a standard curve prepared using gallic acid (0–200 mg/L). The mean of at least three readings was calculated and expressed as mg of gallic acid equivalents/100 g of honey.

2.3. Experimental animals

For the experiment, 24 Swiss albino mice of 4–5 weeks of age, weighing 25–35 g, were purchased from the animal house of the Department of Biology, Mostaganem University, Algeria. The animals were housed under standard environmental conditions of temperature (20–23 °C) and 12 h light: 12 h dark cycle.

2.4. Grouping of animals

For the anti-inflammatory activity against the acute inflammation, 24 animals were divided into four groups ($n = 6$). Group A (carrageenan control) received 500 µL of distilled water; Group B received 80% of unheated Sahara honey; Group C received 80% of heat-treated Sahara honey and Group D received 50 mg/kg of diclofenac (positive control).

2.5. Acute toxicity study

Unheated and heat-treated Sahara honey in 80% was administered to the mice orally (*p.o.*). All the groups were observed for possible behavioral changes, allergic reactions and mortality for the next 24 h.

2.6. Carrageenan-induced acute inflammatory model

Carrageenan (0.1 mL of 1%) was injected into the footpad of the hind paws of each mouse in all groups. The volume of the carrageenan injected into the foot was measured at 0, 1, 2, 3 and 6 h using a plethysmometer. The percentage of inhibition (PI) at each time interval was calculated[12].

$PI = [(V_t - V_0)_{\text{control}} - (V_t - V_0)_{\text{treated}}] / (V_t - V_0)_{\text{control}} \times 100$ where V_0 expressed mean paw volume at 0 h and V_t expressed mean paw volume at a particular time interval.

2.7. Statistical analysis

The results were expressed as the mean \pm SD. Data were analyzed with One-way ANOVA using Statistica version 7 software and the differences at level $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Acute toxicity study

Oral administration of unheated and heat-treated Sahara honey at the doses of 80% did not produce any mortality or noticeable behavioral changes in mice within 24 h observation period.

3.2. The effects of unheated and heat-treated Sahara honey on total polyphenol contents

The total polyphenol content of the of Sahara honey investigated before and after heat treatment was between 72.0 and 97.9 mg of gallic acid equivalents/100 g of honey. The polyphenol content was found to increase by 26.45% following heat treatment at 90 °C for 120 min. The total phenolic contents were calculated using the following linear equation based on the calibration curve of gallic acid ($Y = 2.842X + 0.009$, $R^2 = 0.9888$).

3.3. Carrageenan-induced acute inflammatory

The anti-inflammatory activities of unheated and heat-treated Sahara honey were further evaluated by the inhibition of carrageenan-induced hind paw edema in mice (Figure 1). PI of paw edema by the unheated Sahara honey was observed as 21.85% (1 h), 46.43% (5 h) and 80.43% (6 h) at the concentration of 80% *p.o.*, respectively (Figure 2). At 2 h, it decreased to 20.01%, and at the end of 3 and 4 h, it was 5.43% and 10.95%, respectively.

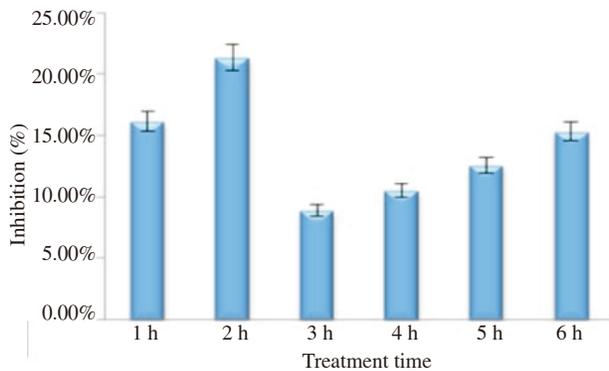


Figure 1. Percentage of anti-inflammatory effect of diclofenac at different time intervals.

Values were expressed as mean \pm SD.

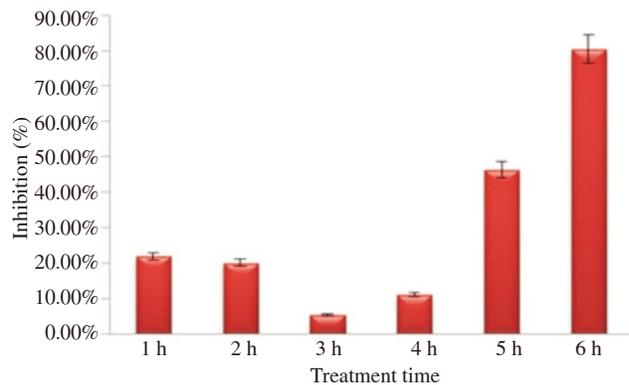


Figure 2. Percentage of anti-inflammatory effect of unheated Sahara honey at different time intervals.

Values were expressed as mean \pm SD.

The maximum PI of paw edema by the heat-treated Sahara honey was observed as 31.16% (1 h), 31.21% (5 h) and 34.19% (6 h) at the concentration of 80% *p.o.*, respectively (Figure 3). At 2, 3 and 4 h, it decreased to 12.63%, 0.25% and 8.55%, respectively. Unheated and heat-treated Sahara honey significantly reduced the edema as shown in Figures 2 and 3. About 50 mg/kg diclofenac *p.o.* showed a maximum PI of 21.37% at 2 h after its administration (Figure 1).

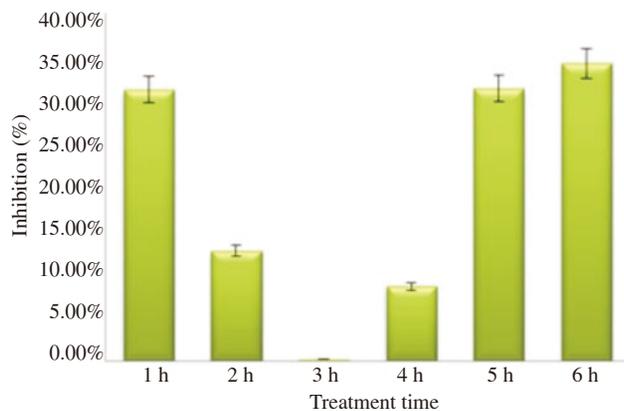


Figure 3. Percentage of anti-inflammatory effect of heat-treated Sahara honey at different time intervals.

Values were expressed as mean \pm SD.

4. Discussion

Our primary aim was to assess the anti-inflammatory effects

of unheated and heat-treated of Sahara honey by carrageenan-induced paw edema.

Edema is one of the fundamental actions of acute inflammation and is an essential parameter to be considered when evaluating compounds with potential anti-inflammatory activity[13,14]. The anti-inflammatory activity of unheated and heat-treated Sahara honey is clearly observed in carrageenan-induced mice paw edema, with a maximal effect at 6 h after carrageenan injection. The ranking order for the anti-inflammatory activity of Sahara honey was established as unheated > heat-treated > diclofenac.

The injection of carrageenan-induced edema is biphasic. The first phase (1 h) involves the release of serotonin and histamine and the second phase (> 1 h) is mediated by cyclooxygenase products and the continuity between the two phases is provided by kinins[15,16]. Prostaglandins (PG) (PGE₂) play an essential role in inflammation[17].

Also, tumor necrosis factor- α is a major mediator in inflammatory responses[18]. Nitric oxide (NO) and cytokines are another important pro-inflammatory molecules that are released in an acute and chronic inflammatory response and are related to exudation and cellular chemotaxis[13,19]. The results indicated that unheated and heat-treated Sahara honey inhibited the PGE₂ and NO in inflammatory tissues of inflammation model.

Previously, some researchers have also proposed the anti-inflammatory property of unheated honey *in vivo*[20]. Recently, Hussein *et al.* reported that Gelam honey reduces the production of pro-inflammatory mediators NO, PGE₂, tumor necrosis factor- α and interleukin-6 in plasma of carrageenan-induced paw oedema inflammation[21]. In a recent study, Timm *et al.* investigated the effect of four different honeys including manuka honey on the release of important pro-inflammatory cytokine (interleukin-6) from cell line Mono Mac (MM6 cells)[22].

Phenolic compounds are clinically important inhibitors of inflammatory mediator's production *in vivo*[23].

The total phenolic content and flavonoids are predictive markers of the anti-inflammatory activity in honey[24]. Nevertheless, thermal treatment can result in some compounds changes in honey. In a recent study, Jahan *et al.* observed that the total phenol content increased with treatments at temperatures between 50, 70 and 90 °C for 12 h of drying process for Bangladesh honey[7]. Akhmazillah *et al.*, however, reported an increase in the phenolic content of manuka honey from New Zealand in treatment temperatures between 50 and 70 °C[25].

To our knowledge, this is the first report on the anti-inflammatory effect of heat-treated of Sahara honey. The novel observation made in this study was a strong and significant ($P < 0.05$) relationship between heat treatment and PI of the inflammation.

In conclusion, this is the first study reporting the results of a representative screening of Sahara honeys for anti-inflammatory effect and further studies are needed to isolate the pharmacological active compounds responsible for this activity.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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