Antidiarrheal, Analgesic, and Anthelmintic Activities of Honeys in the Sundarbans Mangrove Forest, Bangladesh

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ABSTRACT: This study evaluated the antidiarrheal, analgesic, and anthelmintic activities of honey samples from the Sundarbans mangrove forest of Bangladesh. Composite raw honey (RH), and its diethyl ether (DEH), ethanol (ETH), methanol (MEH), and distilled water (DWH) fractions were investigated. RH and its fractions strongly inhibited castor oil-induced diarrheal episodes in mice at a concentration of 250 mg/kg body weight (b.w.) (P<0.05). At this concentration, RH, DEH, ETH, MEH, and DWH showed inhibitory activity on diarrheal episodes at 43.8, 47.4, 29.8, 12.3, and 38.5%, respectively, whereas for the inhibitory activity for the positive control (PC, 3 mg loperamide/kg b.w.) was 47.4%. Similarly, DEH (250 mg/kg b.w.) showed strongest inhibition (63.5%) of acetic acid-induced writhing in mice, followed by RH (55.7%), ETH (46.2%), MEH (37.6%), and DWH (32.9%). In a hot plate test, mice treated with DEH at a concentration 250 mg/kg b.w. showed the greatest increase in response time, followed by treatment with RH. RH was further used in an anthelmintic test, where it showed a strong dose-dependent reduction in both the paralysis time and the time until death of the parasite, Paramphistomum cervi. Honeys in the Sundarbans could therefore be of great use as nutraceuticals.

Keywords: analgesic, anthelmintic, diarrhoea, honey, the Sundarbans

INTRODUCTION

Diarrhea is the passage of abnormal liquid or unformed stool at an increased frequency. Infectious agents, certain medications, plant and animal toxins, gastro-intestinal disorders, and substances that increase gastrointestinal tract secretions may cause diarrhea. Diarrheal diseases are one of the major causes of mortality and morbidity in the world, especially in developing countries. Diarrhea accounts for more than 5 ~ 8 million deaths each year, mainly in infants and children under 5 years old (1,2). Pain or analgesia is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (3). Excessive pain may be unbearable and cause other effects such as a sinking sensation, apprehension, sweating, nausea, palpitation, or a rise or fall in blood pressure (4). Analgesic is an agent that reduces or eliminates pain by acting on the sensory nervous system, either centrally or peripherally without significantly altering consciousness.

Helminthiasis, also referred to as a worm infection, is caused by parasites in both human and livestock animals. Although it is generally restricted to tropical regions, this infection is one of the most prevalent infections worldwide. Helminthiasis plays a great role in the prevalence of anaemia, blindness, eosinophilia, gastroenteritis, lameness, pneumonia, and stunted growth (5,6). Diseases caused by parasitic infection may cause severe economic losses from domestic and farm yard animals, and may have detrimental effects on both animals and humans in the endemic areas. Various kinds of severe diseases such as schistosomiasis, ascariasis, ancylostomiasis are caused by parasitic infections, which promote untold suffering to millions of people worldwide (6). Therefore, it is very important to control these diseases.

The majority of people in developing countries rely on traditional medicines to treat diseases such as diarrhea, analgesia, and helminthiasis. The World Health Organization has emphasized the use of traditional medical practices in its diarrhea control program (7,8). Many kinds of synthetic drugs are available to combat against diarrhea, analgesia, and helminthiasis but may have severe side effects; therefore, there is increasing demand for natural medicines, since these are mainly free from adverse effects.

Honey is a complex mixture of simple sugars, such as...
glucose (31%) and fructose (38%), and minor amounts of proteins, phenolic compounds, free amino acids, carotenoids, organic acids, minerals, enzymes, vitamins, and aroma compounds (9-11). Honey has more than 500 active components and is considered part of many traditional medicines and cultures. These components have antibacterial, anti-oxidant, anti-inflammatory, anti-browning, anti-allergic, anti-parasitary, anti-ulcer, anti-tumor, and anti-viral activities (10,12). Honey has been consumed over many years for both its high nutritional value and for its constructive effects on human health as a curative agent (13).

Sundarbans is the world's largest contiguous tract of mangroves forest located in the South-Western regions of Bangladesh. The forest is one of the largest areas of honey production in the world (14). This mangrove ecosystem produces more than 200 metric tons of honeys each year, which is mainly consumed in South-Asian countries, especially in Bangladesh and India. A physico-chemical study showed that the honeys produced in the Sundarbans are of excellent quality and contain a high content of polyphenols and antioxidants (15). Honey-producing mangrove plants in the Sundarbans show various health promoting properties, however the honeys from the Sundarbans are still totally unexplored in regard to their pharmacological effects. Therefore, the present study evaluated the antidiarrheal, analgesic, and anthelminthic activities of the honeys from the Sundarbans.

MATERIALS AND METHODS

Chemicals and reagents
Acetic acid, diethyl ether, dimethyl sulfoxide, ethanol, methanol, and Tween-80 were obtained from Merck KGaA (Darmstadt, Germany). Morphine was obtained from Gonoshasthaya Pharmaceuticals Ltd. (Dhaka, Bangladesh).

Honey samples
Ten composite samples of honeys (S1, S2, S3, S4, S5, S6, S7, S8, S9, and S10) were obtained from the honey collectors in the Sundarbans mangrove forest between March and July 2015. The flowering time of most of the nectar-producing plants in the Sundarbans is March to June, and the honey flow continues until July. The Sundarbans Forest Office, Khulna, Bangladesh only permits people to harvest honey for three months each year (April to June). Two composite samples were collected in each month (S1 and S2 in March; S3 and S4 in April; S5 and S6 in May; S7 and S8 in June; S9 and S10 in July) from different parts of the Sundarbans. The collected honey samples were taken to the laboratory and kept in a refrigerator at 4°C in air-tight glass containers.

Extraction of honey
Five grams of each sample were placed in a beaker at a total weight of 50 g, and the honey was mixed thoroughly. Composite honey (10 g) was placed in airtight containers, and was extracted by adding 200 mL of 100% diethyl ether, following vigorous shaking for 30 min. The mixture was filtered through Whatman filter paper no. 1, air-dried, and the extract was stored at 4°C in a refrigerator as the diethyl ether fraction. Ethanol, methanol, and distilled water fractions were successively prepared following the same procedure, using the residues on the filter paper. The diethyl ether, ethanol, methanol, and distilled water fractions were designated as DEH, ETH, MEH, and DWH, respectively, with yields of 0.07 ±0.01, 29.53±0.43, 55.25±1.42, and 5.95±0.91%, respectively. For each experiment, extracts were dissolved in water to defined concentrations, with Tween-80 at a maximum concentration of 0.1%. The control was prepared by the same method but without addition of extract.

Animals
Young Swiss albino mice (16-22 g) of either sex were purchased from the International Centre for Diarrheal Disease Research' Bangladesh (ICDDR’B). Mice were provided with ICDDR’B formulated food and tap water ad libitum, and maintained in polypropylene cages (6 mice per cage of 30 cm×15 cm×15 cm dimension) with a natural day-night cycle. All experiments were conducted in isolated and noiseless conditions in the animal house, in accordance with the guidelines of the Animal Ethics Committee, Khulna University, Khulna, Bangladesh (Research Ref. No.: KUAEC-2017/07/02), which are consistent with the EU Directive 2010 for animal experiments.

Antidiarrheal activity assay
Castor oil-induced diarrhea was studied as described by Shoba and Thomas (2). Animals fasted with water for 24 h, and were randomly divided into control, positive control (PC), and test treatment groups (n=6). Each mouse was placed in an individual cage, the floor of which was lined with absorbent paper. Suspensions of each fraction were prepared in water with 0.1% Tween-80. The control group received distilled water containing 0.1% Tween-80; the PC received loperamide [3 mg/kg body weight (b.w.)]; the test groups received the fractions and raw honey (RH) at varying concentrations (100–500 mg/kg b.w.). After 1 h, each mouse was given 0.5 mL of castor oil with the help of a feeding needle. The total numbers of diarrheal droppings were noted every half an hour over a time period of 5 h.

Analgesic activity assay
Acetic acid-induced writhing was studied as described
by Koster et al. (16). Animals fasted for 18 h with water, and were randomly divided into control, PC, and test groups (n=6). Each mouse was placed in an individual cage, the floor of which was lined with absorbent paper. Suspensions of the fractions and RH were prepared in water with 0.1% Tween-80; solutions were orally administered to mice 30 min before intraperitoneal injection of 0.7% (v/v) acetic acid, at a dose of 10 mg/kg b.w.; the control group received distilled water containing 0.1% Tween-80, and the PC received diclofenac sodium (25 mg/kg b.w.). Writhings, such as stretching or bending of the body, were counted between 5 to 15 min after acetic acid administration. Dose-dependent inhibition of writhings was also measured for DEH.

To study analgesic activity using the hot plate test, the method described by Eddy and Leimbach (17) was followed, with a slight modification. Animals fasted with water for 12 h, and were randomly divided into control, PC, and test groups (n=6). Prior to experimentation, the hot plate was set to 55°C. Mice of different groups were treated with the control vehicle (0.1% Tween-80 in distilled water), morphine (10 mg/kg b.w.), different experimental fractions (250 mg/kg b.w.), or RH (250 mg/kg b.w.), administered with a feeding needle. Animal were placed on a hot plate and the time before licking paws or jumping on the hot plate, which ever appeared first, was recorded using a stop watch. To observe the effect over time, mice were placed on the hot plate at 0, 30, 60, 90, 120, 180, and 240 min after administration of the respective fractions, and the response times were recorded. The cut off time for the response recording was set to 20 s to minimize undue tissue damage resulting from over exposure to heat.

Anthelmintic activity

The anthelmintic assay was carried out as per the method of Tandon et al. (18), with necessary modifications. Live parasites Paramphistomum cervi (Trematoda) were collected from freshly slaughtered cattle at local abattoirs. After cleaning, the parasites were stored in 0.9% phosphate-buffered saline (PBS) at pH 7.4. Ten mL of control solution (1% Tween-80 in PBS), and 10 mL of albendazole, standard drug solution (15 mg/mL) were placed into separate petri-dishes. Four different doses of RH solutions (100, 200, 300, and 400 mg/mL in PBS) were also placed in each petri-dish (10 mL). Ten (10) parasites were incubated (37±2°C) in treatment condition, and were observed for paralysis, and time until death. Three replications were carried out for each treatment.

Statistical analysis

Statistical analysis was performed using SPSS (version 16, SPSS Inc., Chicago, IL, USA). Results were expressed as mean±standard deviation (SD). One way analysis of variance (ANOVA) was used to analyze statistical differences between multiple groups. Differences with P-values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Antidiarrheal activity

At a dose of 250 mg/kg, all the fractions and RH significantly (P<0.05 vs. control, calculated using the Student’s t-test) inhibited castor oil-induced diarrhea in mice. DEH showed the highest rate of inhibition (47%), followed by RH (44%), DWH (38%), ETH (30%), and MEH (12%); loperamide (PC, 3 mg/kg), an antidiarrheal drug, showed 47% inhibition (Fig. 1A). The average number of characteristic diarrheal stools for control group was 16±2. Significant difference (P<0.05) was observed between the PC and the test group treated with MEH. Fig. 1B shows the dose dependent inhibition of diarrhea with DEH, ETH, and RH. At the concentration of 500 mg/kg, only DEH strongly (P<0.05) inhibited diarrhea compared with PC, ETH, and RH. In this study, castor oil was used to in-
duce diarrhea in mice; castor oil contains ricinoleic acid, which causes irritation and inflammation of the intestinal mucosa, resulting in release of prostaglandins that induce motility and secretion (19). This increases the permeability of the mucosal cells, and decreases Na⁺ and K⁺ absorption, stimulating peristaltic activity and diarrhea. The observed antidiarrheal activities of both RH and the various fractions may be due to inhibition of prostaglandin biosynthesis and/or reduction of gastrointestinal motility. It has been previously shown that polyphenol extract from apples inhibits cholera toxin-induced diarrhea in a dose-dependently manner, and that fractions containing polymerized catechins most effectively inhibit toxin-mediated fluid secretion (20).

**Analgesic activity**

During the acetic acid-induced writhing test, RH and each of the four fractions were given orally to mice (250 mg/kg b.w.), and each significantly \((P<0.05)\) inhibited the frequency of acetic acid-induced abdominal constrictions (Fig. 2). However, only DEH strongly inhibited \((P<0.05)\) abdominal constrictions to a greater extent than the PC, diclofenac sodium (25 mg/kg b.w.). At this concentration, ETH and RH showed similar inhibitory effects to that of PC, whereas MEH and DWH showed smaller effects (Fig. 2A). The control group showed an average amount of writhing of 50±4. Since DEH showed the most potent effect, its dose-dependent inhibition of acetic acid-induced writhing in mice is shown in Fig. 2B. Increasing the response time of mice (paw licking/jumping) following treatment with RH and the different fractions was tested using the hot plate test. Fig. 3A shows the increase in mouse response time following treatment with RH and the different fractions at a dose of 250 mg/kg b.w.. All the fractions increased the response time, with the highest effect observed at 90 min following treatment, whereas the greatest response for the PC group was recorded at 60 min. The response times for all groups started to decline after 120 min. All treatment groups significantly \((P<0.05)\) increased the response times compared with the control group. Since DEH showed the highest response time, its dose-dependent effects are shown in Fig. 3B. Intraperitoneal administration of acetic acid causes tissues to produce prostaglandins (PGs), and sympathomimetic mediators like PGE2 and PGF2α (21), bradykinin, histamine, and 5-hydroxytryptamine (22) in the peritoneal fluid, which stimulate peripheral nociceptive neurons. The hot plate test measures nociceptive response latencies in response to a thermal stimulus, which is sensitive to opioid receptors located in the central nervous system (23). All the fractions (administered at 250 mg/kg) inhibited acetic acid-induced writhing, and significantly delayed the response time in mice. Among the fractions, DEH showed the highest analgesic activity through both the peripheral and the central nervous system. These observations suggest that honeys of the Sundarbans have both peripheral and central analgesic properties. The peripheral analgesic action of these fractions may be mediated via inhibition of cyclooxygenases (COXs), and/or lipoxygenases, whereas the central analgesic action may be mediated through inhibition of central pain receptors. Honeys of the Sundarbans are rich with both non-phenolic and phenolic antioxidants, such as (+)-catechin, (−)-epicatechin, p-caumeric acid, syringic acid, trans-cinnamic acid, and vanillic acid (15); these may be able to effectively ameliorate inflammatory responses, through reducing oxidative stress and inhibiting the activities of pro-inflammatory enzymes. Polyphenols in honey, such as chrysin, suppress COX-2 enzymes (24), whereas quercetin attenuates cold allodynia and hyperalgesia (25).
Almasaudi et al. (26) reported that Manuka honey inhibits the inflammatory cytokines TNF-α, IL-1β, and IL-6. However, involvement of autonomic receptors in the anti-nociceptive and anti-inflammatory effects of honey has also been reported (27). Antihistaminic and/or anti-inflammatory activities of citrus fruit peels and various edible fruits have also been reported in various studies (28,29).

**Anthelmintic activity**

The anthelmintic activity of fresh honeys was evaluated by observing both the paralysis time and the time until death time for *Paramphistomum cervi*. At a dose of 100, 200, 300, and 400 mg/mL, the paralysis times were 15.8, 8.6, 5.6, and 4.7 min, respectively, and the times until death were 34.0, 15.8, 9.2, and 8.0 min, respectively. Albendazole, a standard drug, showed a paralysis time of 8.3 min and a time until death of 17.2 min; corresponding times for the control group were 83.5 min and 161.8 min, respectively (Fig. 4). All doses of honey used in this study significantly (*P*<0.05) reduced both the paralysis time and the time until death, compared with the control group. In comparison with PC, honey at concentrations of 300 and 400 mg/mL strongly (*P*<0.05) reduced the paralysis time, but had no effect on the time until death. In helminthiasis, different parts of the body are infested with worms, such as pinworm, roundworm, or tapeworm, which can grow into the liver and other organs away from their primary residence is the gastrointestinal tract (30). Hosts experience varying detrimental effects, such as from food deprivation, blood loss, and toxin secretion (4). Polyphenolic compounds (31), in particular tannins (32), show anthelmintic activities by either binding to glycoproteins present on the cuticles of the parasites (33), by inhibiting tubulin polymerization, or by hampering energy generation in helminthic para...
sites through uncoupling oxidative phosphorylation (34). Natural compound may also show anthelmintic activities through hyperpolarization of the nerve membrane, and by inhibiting acetylcholinesterase enzymes, damaging neuromuscular coordination in the parasite (35,36). It is widely believed that peptides in honey consist of a major class of compounds that shows anthelmintic activity (37) and, recently, a 5.8-kDa bioactive peptide has been isolated from Manuka honey with anthelmintic properties (38). However, a study by Sajid and Azim (39) showed that the major sugar components in honey are not involved in nematidic activity, but that a glycoconjugate with the molecular mass of 5511 is the major nematidal component of honey.

Every year about 150 million pounds of honey are used as an ingredient (40) in the cereal, baking, meat, and syrup industries. Honey is additionally widely used in traditional medicine across the world, since it is very effective for dressing wounds, burns, ulcers, and inflammations. Methanol, ethanol, and ethyl acetate extracts from honey potentially inhibit growth of pathogenic bacteria that lead to food spoilage (41). It has been reported that the highest antioxidant capacity is present in the methanol extract of honey (174.2 mg gallic acid equivalent/g RH or 81.9 mg ascorbic acid equivalent/g RH) (15). Therefore, usage of honey extracts, especially methanol, in food industries will enhance the stability and quality of processed foods, which may lead to better consumer acceptance. Moreover, the present study revealed that honeys produced in the Sundarbans have antidiarrheal, analgesic, and anthelmintic activities. Regular consumption of the honey may therefore prevent diarrhea, pain, and helminth diseases. Further in vitro and in vivo studies are essential to elucidate the potential bioactive component(s) in the honeys from the Sundarbans, and their utilization as dietary supplements, and in preventive medicine and drug development.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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