Gastroprotective effects of dietary honey against acetylsalicylate induced experimental gastric ulcer in albino rats

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Abstract: Honey is a substance produced by bees from the nectar of plants. It is used as a medicine. It is known for its biological properties, having antibacterial, antifungal and healing properties. This work was designed to explore the effect of honey extracts on healing of gastric ulcer in experimental rats. Thirty male albino rats (170±5g bw) were used and allocated into 6 equal groups. Group1 (Gp-1) used as negative control while the Gp2-5 were given aspirin orally (200mg/kg bw), and Gp3-5 were treated with honey at doses of 3.5, 7 and 14 ml/kg bw, for seven days respectively. The length of gastric ulcer, volume of gastric juice, total acidity, pH value, and histopathological changes of the stomach were examined. The results revealed that treated orally with honey extracts reduced the length of gastric ulcer, total acidity, volume of gastric juice, and ameliorate histopathological changes caused by Acetylsalicylate. It is concluded that, honey could be used for healing acute gastric ulcer.

Key words: Honey, Aspirin, Gastric ulcer, Histopathological

1. Introduction  

Gastric and peptic ulcer can occur at any age with higher incidence during the last decade (Suadicani et al., 1999). Peptic ulcers are erosions or open sores in the mucous lining of the stomach or duodenum. Peptic ulcer should never be treated without proper diagnosis (Bukhari et al., 2011). They are usually caused by infection with Helicobacter pylori (H. pylori). Moreover, ulcers can also be caused or aggravated by stress, alcohol, smoking, and dietary factors. Patients with peptic ulcer due to infection should receive conventional treatment directed toward eradicating the organism, various combinations of antibiotics, acid blockers, as well as medical supervision (Shearman and Crean, 2001, Suadicani et al., 1999).

The symptoms of peptic ulcer are occasionally painless. However, the most common symptom is a dull ache in the upper abdomen that usually occurs two to three hours after a meal; the ache is relieved by eating (David et al., 2002, et al., 2014). Other common symptoms include weight loss, bloating, belching, and nausea. Untreated, peptic ulcers often bleed and may cause sharp burning pain in the area of the stomach or just below it (Shearman and Crean, 2001). Gastric ulcers are less common than duodenal ulcers and more patients' age of both sexes (David et al., 2002). It occurs usually along the lesser curvature of the stomach and its size varies from millimeters to centimeters. However, not improving gastric ulcers, could be resulting in death (Süe, 1990, Shearman and Crean, 2001).

Honey is a natural product collected by honeybees from different plants. Modern studies recommend to use honey because it displays antibacterial, antifungal, antiviral, hepatoprotective, and anti-inflammatory properties Muriel et al. (2007). The antimicrobial properties of honey, possibly attributable to its high flavonoid content. Matongo and Nwodo (2014). Honey has been reported to be effective in gastrointestinal disorders, in the healing of wounds, burns and as antimicrobial agents and to have gastric protection against acute and chronic gastric lesions in animals (Bukhari et al., 2011). The honey is effective in the treatment of various wounds and infections because of its antimicrobial properties (Ali and Al Swayeh, 2003). Scientifically, honey was reported for its ability to enhance the healing of topical wounds (Al-Mazrooa, et al., 1999). Furthermore, modern research shows that honey is
effective when used in the treatment of gastric pain, as well as gastric and peptic ulcers (Bukhari et al., 2011).

The present study was designed to investigate the effect of bee honey on healing of acute gastric ulcer induced experimental by Acetylsalicylate in rats.

2. Material and Methods

Material

A- Material: Pure, un-boiled, commercial honey (known as cider, honey) was used in these experiments.

Rats: Thirty, male albino rats (175±g, bw) of Sprague Dawley strain were obtained from the laboratory of animal house, Faculty of Medicine, Umm al Qura University. Rats were housed in wire cages in a room temperature maintained at 25 ±°C and kept under normal healthy conditions. All rats were fed for one week on basal diet before starting the experiment for acclimatization.

Diet: The rats were fed on ration (a basal diet devoid of starch) composed of wheat bran, soya bean powder 44%, fish meal, molasses, fibers 3.3%, sodium chloride, calcium carbonate, calcium phosphate, methionine and ash (net protein 22% and fats 4.7 (Dennis et al., 2009). The diet was fed and water was provided ad libitum for the experimental period.

Aspirin: Aspegic, (Acetylsalicylate) prepared by dissolving one vial in 25ml distilled water (Amirya Pharmaceutical Industries, Cairo, Egypt). A volume of 1ml of this solution was contained about 200 mg, of Acetylsalicylate

Methods:

Experimental design

Rats were divided into 5 equal groups (each =6). The first group, was fed on the basal diet only and served as a negative control. The second groups, was oral administrated aspirin, at a dose of 200 mg/kg, bw, once time, for induction of acute gastric ulcer, according to (Agrawal et al., 2000). Rats groups (3-5) were administrated orally aspirin, at a dose of 200mg/kg, Bw plus honey solution at doses of 3.5, 7 and 14 ml/kg bw, respectively, for seven successive days according to the dose carried out by Al-Mazrooa, et al. (1999).

Measure the length of gastric ulcer:

At the end experimental period, all rats were fasted for 12-14hrs. All rats were sacrificed, and their stomachs were tied around both openings (cardiac & pyloric sphincters) and injected with distilled water (3ml). The gastric juice was then collected in sterilized tube. The stomachs were opened longitudinally, washed with saline and examined under dissecting microscope for ulcer. The length of gastric ulcer was measured and expressed as mean ±SE for each group. The curative ratio was then calculated for each treated group, according to the method described by Akhtar and Ahmed (1995) using the following equation:

\[
\text{Curative ratio (CR) } = \frac{(LC - LT \times LC)}{LC} \times 100.
\]

LC = length of ulcer in control positive group.
LT = length of ulcer in treated group

Measurement the volume of gastric juice:

Gastric juice was collected in tubes and centrifuged at 500 RPM., for 5 minutes. The volume of gastric juice was measured by graduated cylinder.

Determination of the standard normality of NaOH:

The standard normality of NaOH was determined according to the method described by A.O.A.C. (1995).

Histopathological studies

Specimens from stomachs were collected from rats of all experimental groups at the end of the experimental period, fixed at 10% neutral buffered formalin (pH=7. 0), dehydrated in ethyl alcohol, then cleared in xylol and embedded in paraffin; 4-6 microns thickness sections prepared and stained with heamtoxylin and eosin for examining both fore and glandular parts of the stomach Bancroff et al. (1990).

Statistical analysis:

The data were analyzed using Statistical Package for Social Science (SPSS) 20 for Window. Data are expressed as the mean ± standard error (SE); by using analysis of variance (ANOVA) between different treated group followed by least significant difference (LSD), and the differences were considered significant at \( P \leq 0.05 \).

3. Results

The effect of honey on the length of gastric ulcer in experimental rats:

The obtained results in a table (1) showed that a single oral administration of aspirin (200 mg/kg bw.) induced gastric ulcer in rats; the mean length of gastric ulcer in the aspegic treated alone, increased compared with control respectively. The data revealed that oral administration of honey at a dose of 14 ml /kg bw, for 7 days after aspirin (200 mg/kg bw.) decreased in the length of gastric ulcer, which were 1.88±0.07 mm with curative rate 58.68.

The effect of honey on the volume of gastric juice in rats:

As shown in table (2) the volume of gastric juice obtained from rats given aspirin-induced gastric ulcer was 0.75±0.09 ml when compared with 0.51±0.07 ml in normal rats. Oral administration with honey at a dose of 14 ml /kg bw, with aspirin, for 7 days caused the highest significant decrease in the volume of gastric juice, but it was non-significant changed in the group treated with honey at a dose of 3.5 ml /kg bw.

The effect of honey on pH of gastric juice in rats:

It is clear from data illustrated in table (3) that oral administration of aspirin at a dose of 200 mg/kg bw., decreased the pH of gastric juice (4.10±0.08) as compared with normal rats (5.72±0.31). Data showed
that oral administration of honey at a dose of 14 ml/kg bw, for 7 days after aspirin (200 mg/kg bw.) reflected the maximal value of pH level (5.35±5.55).
The effect of honey on the total acidity (%) of gastric juice in experimental rats:

Data presented in table (4) show the effect of the honey at three doses on the total acidity of gastric juice in rats. It was found that oral administration of honey at a dose of 14 ml/kg bw., for 7 days after aspirin (200 mg/kg bw) caused significantly decrease in total acidity of gastric juice (3.41±0.08\(^a\)) compared with aspirin positive control (6.83±0.39).

Microscopically, the stomach of a rat from control negative group showing normal gastric epithelium with normal gastric gland (Figs.1 & 2). Stomach of rats from the group treated with aspirin only, revealed documentation of the lamina epithelium of gastric mucosa associated with leukocyte cell infiltration in the lamina propria, in addition to loss of arrangement of the layers of the stomach (Figs 3-5). However, stomach of rats treated with honey at a dose 3.5 ml / kg bw showed slight sloughing of the mucosa with minimal infiltration (Fig. 6). The rat group treated with at a dose of 7 ml / kg bw showing loss of architecture of superficial epithelium and gastric pits (Fig.7). Meanwhile, the apparent normal gastric mucosa was observed in examining stomach of rats treated with honey at a dose of 14 ml /kg bw, minimum loss of architecture of superficial epithelium and gastric pits (Fig 8).

Table (1): Effect of Honey on the length of gastric ulcer (mean ± SE) in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Aspirin and Extracts</th>
<th>Doses (mg/kg bw)</th>
<th>Gastric ulcer length/mm</th>
<th>CR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control –ve</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>0.00</td>
</tr>
<tr>
<td>Control +ve</td>
<td>2</td>
<td>Aspirin (Asp)</td>
<td>200 mg</td>
<td>4.55±0.10(^a)</td>
</tr>
<tr>
<td>Treated Groups</td>
<td>Honey</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (Asp) + Honey</td>
<td></td>
<td>3.5 ml</td>
<td>3.85±0.12(^a)</td>
<td>15.38</td>
</tr>
<tr>
<td>4 (Asp) + Honey</td>
<td></td>
<td>7 ml</td>
<td>2.4±0.08(^a)</td>
<td>47.25</td>
</tr>
<tr>
<td>5 (Asp) + Honey</td>
<td></td>
<td>14 ml</td>
<td>1.88±0.05(^a)</td>
<td>58.68</td>
</tr>
</tbody>
</table>

CR; Curative Ratio; Different superscript letters in the column denote high significant differences (P<0.01)

Table (2): Effect of Honey on the volume of gastric juice (mean± SD) collected from the stomachs of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Aspirin and Extracts</th>
<th>Doses (kg /bw)</th>
<th>Volume of gastric juice/ mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control –ve</td>
<td>1</td>
<td>-</td>
<td>0.51±0.07(^a)</td>
</tr>
<tr>
<td>Control +ve</td>
<td>2</td>
<td>Aspirin (Asp)</td>
<td>200 mg</td>
</tr>
<tr>
<td>Treated Honey</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (Asp) + Honey</td>
<td></td>
<td>3.5 ml</td>
<td>0.71±0.08(^a)</td>
</tr>
<tr>
<td>4 (Asp) + Honey</td>
<td></td>
<td>7 ml</td>
<td>0.62±0.04(^a)</td>
</tr>
<tr>
<td>5 (Asp) + Honey</td>
<td></td>
<td>14 ml</td>
<td>0.56±0.02(^a)</td>
</tr>
</tbody>
</table>

Different superscript letters in the column denote high significant differences (P<0.01).

Table (3): Effect of Honey at three doses doses on the pH (mean ± SE) of gastric juice collected from the stomachs of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Aspirin and Extracts</th>
<th>Doses (kg/bw)</th>
<th>pH of gastric juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control –ve</td>
<td>1</td>
<td>-</td>
<td>5.72±0.31(^a)</td>
</tr>
<tr>
<td>Control +ve</td>
<td>2</td>
<td>Aspirin (Asp)</td>
<td>200 mg</td>
</tr>
<tr>
<td>Treated Honey</td>
<td></td>
<td></td>
<td>5.13±0.11(^a)</td>
</tr>
<tr>
<td>3 (Asp) + Honey</td>
<td></td>
<td>3.5 ml</td>
<td>5.24±0.10(^a)</td>
</tr>
<tr>
<td>4 (Asp) + Honey</td>
<td></td>
<td>7 ml</td>
<td>5.55±0.12(^a)</td>
</tr>
</tbody>
</table>

Different superscript letters in the column denote high significant differences (P<0.01).

Table (4): Effect of Honey on the total acidity of gastric juice (mean ± SD) collected from the stomachs of experimental rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Aspirin and Extracts</th>
<th>Doses (kg /bw)</th>
<th>Total acidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control –ve</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control +ve</td>
<td>2</td>
<td>Aspirin (Asp)</td>
<td>200 mg</td>
</tr>
<tr>
<td>Treated Honey</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (Asp) + Honey</td>
<td></td>
<td>3.5 ml</td>
<td>4.45±0.09(^b)</td>
</tr>
<tr>
<td>4 (Asp) + Honey</td>
<td></td>
<td>7 ml</td>
<td>3.52±0.10(^a)</td>
</tr>
<tr>
<td>5 (Asp) + Honey</td>
<td></td>
<td>14 ml</td>
<td>3.41±0.08(^a)</td>
</tr>
</tbody>
</table>

Different superscript letters in the column denote high significant differences (P<0.01)
Fig. 1: A photomicrograph of a section of the stomach of a rat from control negative group showing normal gastric epithelium. H&E X 100.

Fig. 2: A photomicrograph of a section of the stomach of a rat from negative control group showing normal gastric gland. H&E X 100.

Fig. 3: A photomicrograph of a section of the stomach of a rat control positive (aspegic 200 mg/ kg bw) showing focal infiltration (Arrow). H&E X 400.

Fig. 4: A photomicrograph of a section of the stomach of a rat control positive showing focal infiltration and loss of arrangement of the layers of the stomach (Arrow). H&E X 400.

Fig. 5: A photomicrograph of a section of the stomach of a rat control positive showing sloughing of mucosa. H&E X 400.

Fig. 6: A photomicrograph of a section of the stomach of a rat (honey 3.5 ml / kg bw) showing slight sloughing of mucosa with minimal infiltration (Arrow). H&E X 400.

Fig. 7: A photomicrograph of a section of the stomach of a rat (honey 7 ml / kg bw) showing loss of architecture of superficial epithelium and gastric pits (arrow). H&E X 400.

Fig. 8: A photomicrograph of a section of the stomach of a rat (honey 14 ml / kg bw) showing loss of architecture of superficial epithelium and gastric pits (arrow). H&E X 400.
4. Discussion

The term peptic ulcer refers to an ulcer in the lower esophagus, stomach, duodenum, or rarely in ileum adjacent to a Meckel's diverticulum (Bukhari et al., 2011). Peptic ulcer is the general term applied to an eroded mucosal lesion in the central portion of the gastrointestinal tract. Areas affected include the lower portion of the esophagus, the stomach and the first portion of the duodenum, the "duodenal bulb". Muriel et al. (2007).

The most commonly used experimental models for the evaluation of antiulcer activity is in rats (Pillai et al., 2010). In the present study, the gastric ulcer carried out, with a single oral dose of aspirin 200 mg/kg bw (Agrawal et al., 2000). Gastric mucosal damages induced by different experimental ulcer models have different mechanisms (Samonina et al., 2004). However, the net imbalance in offensive and defensive factors brought about by them is thought to be the cause of ulcerogenesis Goel et al. (1991), Shearman and Crean (2001). The histopathological results, in our study, showed necrosis and desquamatation of lamina epithelium of gastric mucosa and necrosis in lamina propria in aspirin treated groups. Chronic or acute administration of non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, during the course of anti-inflammatory therapy, is often associated with the development of adverse gastrointestinal disorders such as gastric erosions, gastric or duodenal ulceration and other severe complications such as gastrointestinal haemorrhage or perforation that often limited their wide spread clinical use (Agrawal et al., 2000, Villegas et al., 2004).

The present results showed that, there is a reduction in gastric juice volume, pH, in the honey treated groups, in the same aspect honey solution showed protective effects against stress induced peptic ulcer in rats (Al-mazrooa et al., 1999, Bukhari et al., 2011, Math et al., 2013). Treatment with honey is much less expensive and appears to need less time. Honey was reported for its ability to enhance the healing of peptic ulcer (Lychkova et al., 2014). Helicobacter pylori is the main causes of the peptic ulcer, the protective effect of honey could be due to the antibacterial activity (Matongo and Nwodo (2014). Also, partially agreed with Muriel et al. (2007) who reported that the propolis extract displayed an anti-secretory activity, which lead to a reduction in the gastric juice volume, total acidity and pH. They indicated that Brazilian green propolis displays good anti-ulcer activity. Similar to our founding, El-Ghazaly et al. (2011) reported gastroprotective effect of the aqueous Propolis extract in rats experimental induced gastric ulcers after exposed to radiation.

Gastric mucus consists of a viscous, elastic adherent and transparent gel formed by water and glycoproteins (Shearman and Crean (2001), Iwamoto et al. (2014). The protective effects of mucous barrier depend on the gel structure and the thickness of the layer covering the mucosal surface (Iwamoto et al., 2014). The ability of the gastric mucosa to resist injury caused by endogenous secretions (acid, pepsin, and bile) and by ingested irritants such as alcohol, aspirin and NSAIDs can be attributed to a number of factors that have been generally referred as mucosal defense Wallace (2001). The gastroprotective effect of hone in the present study, approved histopathologivaly with apparent normal gastric mucosa in examining stomach of rats treated with higher doses of honey and aspirin. In accordance with our results several authors reported that the honey possesses antiulcer activities (Al-Mazrooa et al., 1999, Math et al., 2013, Lychkova et al., 2014).

Conclusion

In conclusion, the honey solution has an effective ingredient, which can protect from gastric ulcers. Honey had been tested in rats can also be used for patients with ulcer in hospitals and evaluates their nutritional status.

References


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