Fluid and glucose transport across intestinal epithelium following honey intake in rats

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Abstract
The effect of honey intake on fluid and glucose absorption in rats was studied using everted sacs intestine techniques. Twelve rats (180-200 gm) were randomly selected and grouped into the control (A) and honey-fed (test) group (B) with six rats in each group. The control group was fed with rat chow and water while the test group was fed with rat chow, water and honey. The experiment lasted for 4 weeks excluding two weeks of acclimatization. The animals were scarified at the end of study and the abdomen opened immediately, and the intestine dissected out. 10 cm long of the intestine (2 from jejunum, 2 from ileum) were cut out and used for the everted sacs experiment. The results showed that honey intake did not affect fluid transport but affected glucose transport across the small intestine. There were no significant differences observed in initial wet weight (IWW), initial serosal volume (ISV) and final serosal volume (FSV) in both the test and control groups (p>0.05). No significant differences were also observed in mucosal fluid transfer (MET), serosal fluid transfer (SET) and gut fluid uptake (GFU) in both the test and control groups (p>0.05). However, mucosal glucose transfer (MGT), serosal glucose transfer (SGT) and GGU, while MGT and SGT were significantly higher in the test group compared to the control (p<0.05), the gut glucose uptake (GGU) was however significantly reduced in the test compared with the control (p<0.05). It is therefore concluded that honey intake significantly reduced GGU while it significantly increased MGT and SGT in the intestinal epithelium of Wister rats.

1 Introduction
Honey is a natural product that has been widely used for its therapeutic effects. It has been reported to contain about 200 substances. Honey is composed primarily of fructose and glucose but also contains fructo-oligosaccharides and many amino acids, vitamins, minerals and enzymes. The composition of honey varies depending on the plants on which the bee feeds. However, almost all natural honey contains flavonoids (such as apigenin, pinocembrin, kaempferol, querce tin, galangin, chrysin and hesperetin), phenolic acids (such as ellagic, caffeic, p-coumaric and ferulic acids), ascorbic acid, tocopherols, catalase, superoxide dismutase, reduced glutathione, Millard reaction products and peptides1,2. Most of those compound works together to provide a synergistic antioxidant effect.

Honey has had a valued place in traditional medicine for centuries. However, it has a limited use in modern medicine due to lack of scientific support. For a long time, it has been observed that honey can be used to overcome liver, cardiovascular and gastrointestinal problems. Scientists have revealed that honey has powerful anti-bacterial properties on at least sixty species of bacteria, and unlike antibiotics, which are often useless against certain types of bacteria, honey is nontoxic and has strong effects. The benefits of honey have been extolled since ancient times by many religious faiths and recorded in ancient scriptures3-5. There is paucity of the report about the effect of honey on fluid and glucose absorption by the small intestine. Honey has nutritional and
medicinal values. It is accepted by all ages and generations, traditions and civilization, both ancients and modern as food and medicine. It is unique viscous syrup made by bees from nectars of different flowers and species of plants. The effect of this viscous syrup intake on the fluid and glucose transport across the small intestine was studied using everted sac technique of Wilson and Wiseman (1954) and also used by Barry et al (1961) and modified by Adeniyi and Olowokooren (1987). In the present study we planned to determine the glucose transport across intestinal epithelium following honey intake in rats.

2 Materials and Methods

2.1 Methodology

The four segments of the small intestine (I, II, III and IV) each 10 cm long (2 from jejunum, 2 from ileum) were cut out in a manner shown below for the sac experiment.

Twelve adult Wister rats were used, grouped randomly into control (6 rats) and honey fed (6 rats). The animals weighed between 180-200 gm, at the start of the experiment which lasted 4 weeks. The animals were bred in the animal house of Physiology Department, Faculty of Basic Medical Sciences, Anambra State University Uli, Anambra State. The control group was fed with rat chow and water while the test group was given rat chow, water and honey (1 ml everyday via 5 ml syring orally). At the end of the experiment, the animals were killed by stunning after being starved overnight. The abdomen was opened and intestine dissected out and put in a dish containing normal saline solution for the experiment.

Each sac was made by tying the distal end of the segment with a dry thread, weighed in a balance. From the tied end, a rod was placed to push the end inward, thereby everting the sac (Mucosa side outside, serosa side inside). The sac was then filled with 1 ml Krebs solution (serosa fluid) the free end was tied afterward with a similar dry thread which was also weighed.

The krebs bicarbonate solution at volume of 40 ml was put in incubating flask labeled I, II, III and IV respectively, each flask was aerated using 95% oxygen and 5% carbon dioxide gas mixture in a Galen Kamp shaker bath for 30 minutes. The sacs were immersed in the aerated fluid and aerated further for 2 minutes after which they were incubated in an incubator for another 28 minutes. After incubation, the sacs were blotted and weighed as follows:

- Weight of empty dish + 2 ligatures = W1
- Weight of empty dish + 2 ligatures + empty sac = W2
- Weight of empty dish + 2 ligatures + initial weight of full sac = W3
- Weight of empty dish + 2 ligatures + final weight of full sac = W4

The units for fluid transfer expressed in this study were those of Parsons et al (1958) where fluid transferred was determined as measure of volume transferred by a unit wet weight of intestine for a given period. The mucosal fluid transfer (MFT), the serosal fluid transfer (SFT) and Gut fluid uptake (GFU) were determined by using the results obtained from weighing in the following formulae.

- Initial wet weight (IWW) = W2 - W1
- Initial serosal volume (ISV) = W3 - W2
- Final serosal volume (FSV) = W4 - W5
- SFT = (W4 - W5) - (W3 - W2)
- GFU = W5 - W2
- MFT = SFT + GFU

SFT, GFU and MFT are expressed as Vol/g sac/30 minutes where serosal fluid transfer is defined as a change in serosal lumen during incubation. GFU is defined as increase in the fluid content of the intestine tissue owing to the increase in the water content of intestine tissues and the swelling of epithelial cells. MFT is the decrease in the volume of fluid on the mucosal side during absorption.

For glucose transfer, the method was the same in calculation as the fluid transfer.

The terms used for glucose transfer are, mucosal glucose transfer (MGT), serosal glucose transfer (SGT) and gut glucose uptake (GGU).

The MGT is the amount of glucose that disappeared from the Mucosal Fluid. SGT is the amount of glucose that entered the serosal fluid that is increase glucose concentration in the serosal fluid. GGU is the difference in glucose concentration between the MGT and the SGT + glucose metabolized and glucose present in the gut wall at the end of the experiment (Jervis and Smyth (1960)).

A glucose kit (Ames/MBI Blood analyzer, glucose kit UK) for blood and glucose was used. The initial and final concentrations of glucose in the kreb’s bicarbonate solution in the intestinal sac (serosal fluid) and the suspending fluid (Mucosal Fluid) before and after incubation were determined. The units for glucose transfer are the same for fluid transfer (mg/g sac/30 minutes). The physiological solution was bubbled continuously with a 95%:5% oxygen : Carbon dioxide mixture. The pH was between 7.35 - 7.40 and the temperature was 37°C

2.2 Statistical analysis

The experimental results were expressed as the Mean ± SEM for six animals in each group. The parameters were analysed statistically.
using one-way analysis of variance ANOVA, followed by Dunnett’s multiple comparison test (DMCT). P value of < 0.05 was considered as statistically significant.

### 3 Results

Fluid transfer in the small intestine following honey ingestion in Wister Rats, and data are presented in table 1. In IWW, there was no significant differences observed between the control (0.71± 0.05) and the test (0.72±0.10) at p>0.05. In ISV, there was no significant differences observed between the control (0.78±0.10) and the test (0.74±0.11) at p>0.05. In FSV, there was also no significant differences observed between the control (0.38±0.07) and the test (0.42±0.05) at p>0.05.

**Table 1: Mean values for IWW, ISV and FSV in the control and the test groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>IWW</th>
<th>ISV</th>
<th>FSV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.71±0.05</td>
<td>0.78±0.10</td>
<td>0.38±0.07</td>
</tr>
<tr>
<td>Test</td>
<td>0.72±0.01</td>
<td>0.74±0.11</td>
<td>0.42±0.05</td>
</tr>
</tbody>
</table>

Values are expressed as the Mean ± SEM

The values of GU, SFT and MFT are displayed in table 2. There was no significant difference observed in the mean values of GFU for the control (0.2±0.04) and test group (0.18±0.04) at p>0.05. For SFT, there was no significant different observed between the control (0.338±0.08) and the test (0.39±0.05) at p>0.05. Also for the MFT, there was no significant different observed between the control (0.29±0.11) and the test (0.31±0.02) at p>0.05.

**Table 2: Mean values for MFT, SFT and GFU in the control and test group (mg/g sac/30 minutes)**

<table>
<thead>
<tr>
<th>Group</th>
<th>GFU</th>
<th>SFT</th>
<th>MFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.2±0.04</td>
<td>-0.38±0.08</td>
<td>-0.29±0.11</td>
</tr>
<tr>
<td>Test</td>
<td>0.18±0.04</td>
<td>-0.39±0.05</td>
<td>-0.21±0.02</td>
</tr>
</tbody>
</table>

Values are expressed as the Mean ± SEM

The rate of glucose transfer in small intestine of rats is exhibited in table 3. Values of glucose concentration in mucosal fluid before incubation in the control and test were 184.25±1.48 mg/dl and 185.58±1.07 mg/dl respectively. There was no significant difference observed between the control group and the test group at p>0.05. Concentration of glucose in mucosal fluid after incubation in the control group and the test group were 184.25±2.71 mg/dl and 182.00±0.86 mg/dl respectively. There was significant difference observed between the control and the test group, p<0.05. Again, concentration of glucose in the lumen after incubation in the control group and test group were 58.55±1.30 mg/dl and 77.25±0.28 mg/dl respectively. There was significant difference observed between the control and the test group at p<0.05.

**Table 4 shows the mean values of MGT, GGU, and SGT in the control and the test group, after incubation. MGT in the control and test groups were -5.08±1.68 mg/g sac/30 minutes and 3.58±0.47 mg/g sac/30 minutes. MGI was significantly higher in the test group compared to the control group (p<0.05). GGU in the control and test groups were 130.67±1.72 mg/g sac/30 minutes and 105.17±0.13 mg/g sac/30 minutes, respectively. GGU was significantly lower in the test group compared with the control group (p<0.05). SGT in the control group and test group were -17.42±1.30 mg/g sac/30 minutes and 1.25±0.28 mg/g sac/30 minutes respectively.**

There was no significant difference between the control (184.25±1.48) and the test (185.58±1.07) in glucose concentration in the serosal fluid before incubation. There was significant difference between the control group (189.25±2.71) and the test group (182.00±0.86 mg/dl) in glucose in the serosal fluid after incubation (p<0.05). There was significant difference between the control (58.55±1.30 mg/dl) and the test (77.25±0.28 mg/dl) in the intestinal lumen after incubation (p<0.05). MGT is significantly higher in the test compared with the control P<0.05. GGU is significantly lower in the test compared with the control P<0.05. SGT is significantly higher in the test compared with the control P<0.05.

### 4 Discussions

The study was carried out to access fluid/Glucose transport across the small intestine following honey intake in Wister rats. Food and other nutrients enter the blood across the intestinal epithelium. Epithelial membrane offers a barrier to these substances that are being absorbed, serving as a sieve, allowing some substances to pass while excluding others. Fluid and glucose transport across the intestine may be carrier mediated or by diffusion. For glucose, it may depend on luminal sodium ion concentration and membrane potentials. Anything that affects these properties affects transport across the intestine. The results obtained in this study showed that GGU, SFT and MFT in both the control and test groups were not statistically different. Also the IWW, ISV and final serosal volume in both the control group and test group were also not statistically different except in the percentage weight gain where there was a statistical increase in the test group compared to the control group. In the glucose transport across the intestinal epithelium, there was no significant difference in the mean volumes of mucosal glucose concentration between the control (184.25±1.48 mg/dl) and the honey fed (test) group (182.00±0.80 mg/dl) before incubation, however, there was a significant difference in the glucose concentration following incubation.

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was significant difference in the mean volume of mucosal glucose concentration after incubation between the control and the test group. Furthermore, also there was significant difference between the control and the test group after incubation in the intestinal lumen. Was MGT was significantly higher on the test compared to the control. Similarly, SGT was significantly higher among the test group compared to the control. In contrast, the GGU in the test group was significantly decreased compared to the control group. The percentage weight gain was significantly higher on the test compared to the control.

Table 3: Glucose transfer in small intestine of rats fed with honey and the control

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration of Glucose in serials fluid before incubation (mg/dl)</th>
<th>Concentration of Glucose in serials fluid after incubation (mg/dl)</th>
<th>Concentration of Glucose in the human after incubation (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>184.25±1.48</td>
<td>189.25±2.71</td>
<td>58.55±1.30</td>
</tr>
<tr>
<td>Test</td>
<td>185.58±1.07</td>
<td>182.00±0.86</td>
<td>77.25±0.28</td>
</tr>
</tbody>
</table>

Values are expressed as the Mean ± SEM

Table 4: Mean values for MGT, GUT, GGU, SGT, after incubation in the control and test groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mucosal Glucose Transfer (MGT) mg/gsac/30 minutes</th>
<th>Gut Glucose Uptake (GGU) mg/gsac/30 minutes</th>
<th>Several Glucose mg/gsac/30 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-5.08±1.68</td>
<td>130.67±1.72</td>
<td>-17.42±1.30</td>
</tr>
<tr>
<td>Test</td>
<td>3.58±0.47</td>
<td>105.17±0.13</td>
<td>1.25±0.28</td>
</tr>
</tbody>
</table>

Values are expressed as the Mean ± SEM

The mechanism by which honey caused a significant increase in MGT and SGT in the test group and decrease in GGU, still in the test when compared to the control was not clear. GGU reflects net diffusion of glucose across the intestinal epithelium into the blood stream. It is known that crude extracts of medicinal plants exert their effect by suppressing glucose absorption from the intestine. Serving as a remedy in the ameliorating diabetes in diabetic patients, Shimizu el at (1999)17. Osim et al (2009)12 reported inhibition of small intestine and hepatic bile Duct with the decrease bile flow in Wister rats fed with honey. Alagwu et al (2013)13 reported decrease intestinal motility in Wister rats fed with honey. It may be possible that inhibition of the small intestine by honey in this present study, may have affected the cell membrane potentials, the glucose transporters and Na permeability and other transport systems concerned about the transport of substances across the intestine, including glucose and Na. Ganony (1977)14 reported that when a cell relaxes, the membrane potential tends to increase as membrane permeability to Na decreases; Na slowly enters the cell. Reduction in membrane potential causes increased in Na permeability as its entry into the cell increases. Anything that affects these properties affects transport across the epithelium positively or negatively15,16. Ganong (2003)17 reported that glucoseentereds Cells by facilitated diffusion or in the intestine and kidneys by secondary active transport with Na. In muscles, fats and some other tissues, insulin facilitates glucoseentry too the cell by increasing the number of glucose transporters in the cell membrane. In the present study, inhibitory action of honey on the intestine may have increased the membrane potential thus decreasing Na permeability, hence the decrease in GGU observed during the study18-20. It could also be said or suggested that honey may have reduced the glucose transporters contrary to the action of insulin on glucose transporters thus leading to the decrease in glucose uptake as shown by the increase in luminal glucose concentration in this study21-23. The effect of honey on glucose uptake across the intestinal epithelium could only be explained upon the basis of inhibitory action of honey on the intestine, not on the basis of glucose transporters and membrane potentials. Further study is required to properly evaluate the action of honey on these properties before drawing any conclusion.

5 Conclusions

There is paucity of the report about the effect of honey on fluid and glucose absorption by the small intestine. The effect of honey intake on fluid and glucose absorption in rats was studied using everted sacs intestine techniques. This study suggest that honey added as additive in food or as sweetener or took by diabetics could be beneficial in lowering the blood sugar level and may thereby ameliorate diabetes in diabetic patients as shown by the present study. It is therefore concluded that honey intake has no significant action on fluid intake rather statistically reduced GGU and increased MGT and SGT across the intestinal epithelium in Wister rats.

6 Conflict of interests

None

7 Author’s contributions

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Aea and Ake: Concept, data collection, draft manuscript
Ac and Ugc: Data analysis, draft manuscript
Ad and Oee: Concept; data analysis, final manuscript

8 References