In vitro antidiabetic activity and glycemic index of bee honeys

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Honey has a plethora of researches that related to its use in many disease conditions along with its recognition as a complete food for improving general health among humans irrespective of their age group. Literature so far highlights the floral specificity in the therapeutic properties of honey. In the present investigation honeys from different bee origin were collected in the raw and processed form and their therapeutic effects were ascertained. The antidiabetic property of the honeys was analyzed using the in vitro alpha amylase and alpha glucosidase enzyme inhibition assays. Among the honeys analyzed raw (77.61 % and 80.46 % at 500 µg/ml) and processed (64.84 % and 78.29 % at 500 µg/ml) Trigona honeys were found to have highest percentage of inhibition against alpha amylase and alpha glucosidase enzyme, respectively. The honeys analyzed were having moderate glycemic index and glycemic load values. This opens up scope for its utilization by diabetic people who prefer to have sugar occasionally.

Keywords: Alpha amylase enzyme, Alpha glucosidase, Anti-diabetic, Honey, Glycemic index, Glycemic load.

IPC Int. CI: C12N 9/00, C12N 11/00, A61K 38/43, A01D 16/02, A23L 21/25, C07K 5/062, C07K 5/103

Diabetes mellitus (DM) is a chronic metabolic disorder in which prevalence has been increasing steadily all over the world. Accordingly, it is fast becoming an epidemic in some countries of the world with the number of people affected expected to double in the next decade due to increase in ageing population, thereby adding to the already existing burden for healthcare providers, especially in poorly developed countries. Framing and reframing of the treatment modalities for diabetes is an ongoing issue from time to time which include lifestyle modifications, treatment of obesity, oral hypoglycemic agents, and insulin sensitizers like metformin, and a biguanide that reduces insulin resistance. Type 2 diabetes mellitus (T2DM) is characterized by multiple pathophysiologic abnormalities. Gradually, multiple glucose-lowering medications are commonly required to reduce and maintain plasma glucose concentrations within the normal range. Type 2 diabetes mellitus individuals also are at a very high risk for microvascular complications and the incidence of heart attack and stroke is increased 2-3 fold compared with non-diabetic individuals. The evidence based on the possibility of cardiovascular outcomes in the type 2 DM are still incomplete and must await large, long term clinical trials in patients at low risk modern treatment strategies.

Changeability in the medications along with the persistence of DM as one of the major cause of morbidity and mortality globally, has resulted in a renewed interest in research that investigates the health benefits of herbs and natural products including honey in the management of diabetes mellitus. Honey is a natural substance produced by bees from nectar. It is considered one of the last un-treated natural food substances. The composition of honey is influenced by a number of factors such as geographical origin, botanical sources of nectar, environmental and climatic conditions as well as processing techniques. The various varieties of honey may be grouped into monofloral or multifloral. But therapeutic efficacy of the honeys from bee origin is meagrely defined with specific to their antidiabetic role. In this context honeys collected from five bee species in their raw and processed forms were subjected to antidiabetic analysis which comprises of measuring their glycemic index, glycemic load and in vitro antidiabetic assays, viz. alpha amylase inhibition assay and alpha glucosidase inhibition assay.

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Materials and methods

Sample collection
A total of five multifloral honey samples (in quadruples each) of, the Indian hive bee, Apis cerana indica F. (Apidae) (Ac), the European or Italian bee, Apis mellifera L. (Apidae) (Am), the rock bee, Apis dorsata F. (Apidae) (Ad), the little bee, Apis florea F. (Apidae) (Af) and Trigona irridipennis S. (Stingless bee) (Ti) were collected from the local beekeepers of different areas of southern zone of Kerala in raw as well as processed form. The processed form of Apis florea bee honey was not available due to the paucity of inventory. The samples collected in January – February of 2015 were stored in half liter pet containers duly labeled with name codes and date of collection. All chemicals used in this study were of analytical grade.

Alpha amylase inhibition assay
Alpha-amylase activity can be measured in vitro by hydrolysis of starch in presence of alpha amylase enzyme. This process was carried out by quantifying the reducing sugar (glucose equivalent) liberated under the assay condition. The enzyme inhibitory activity was expressed as decrease in units of glucose liberated. This assay was carried out using a modified procedure of McCue & Shetty (2004). A non-proteinaceous alpha amylase inhibitor (acarbose) was utilized as a standard or reference amylase inhibitor to compare the activity of the bee honeys against this enzyme.

Alpha glucosidase inhibition assay
Alpha-glucosidase activity can be measured in vitro by determination of the reducing sugar (glucose) arising from hydrolysis of sucrose by alpha-glucosidase enzyme. A-Glucosidase inhibitory activities were evaluated according to the chromogenic method, with some modifications. Absorbance of the honey samples was measured with a microplate reader at 405 nm, while the reaction system without honey samples was used as control. The system without a-glucosidase was used as blank. Each experiment was conducted in quadruples. Inhibitory activity was expressed by subtracting relative:

\[
\text{Inhibition (\%)} = \left[ \frac{\text{Abs (control)} - \text{Abs (honey)}}{\text{Abs (control)}} \right] \times 100
\]

Glycemic index
The glycemic index (GI) concept is based on the difference in blood glucose response after ingestion of the same amount of carbohydrates from different foods, and possible implications of these differences for health, performance and well-being. GI is defined as the incremental blood glucose area (0 - 2 hrs) following ingestion of 50 gm of available carbohydrates in the test product as a percentage of the corresponding area following an equivalent amount of carbohydrate from a reference product. A high GI is generally accompanied by a high insulin response. Computation of GI: GI of the test food (honey) was calculated as per the method suggested by Jenkins et al., (1982). Healthy subjects, from whom prior informed consent was obtained, was given portions of test food and glucose containing 50 gm available carbohydrate on separate occasions after an overnight fasting. Blood samples were taken from finger tips before administration of test food and glucose to access the fasting blood sugar level of the subjects. After the administration of test meal, blood glucose levels of the subjects were analyzed independently at half an hour interval up to two hours (1/2 hr, 1 hr, 1 1/2 hrs, and 2 hrs). Blood glucose concentration of the subjects was determined by using a glucometer and the results were validated with the help of a physician in diabetology clinic. The response of the reference food mainly glucose and also that of the test food administered on the subjects were plotted against time ‘t’. The area under curve (AUC) thus obtained was found out from test food as well as for reference food (glucose). The GI of test food is computed as the ration of AUC of test food and AUC of reference food.

\[
\text{GI} = \left( \frac{\text{AUC(test food)}}{\text{AUC(Reference food)}} \right) \times 100
\]

Glycemic load
In practice, the actual carbohydrate load from a normal portion size varies considerably between food products. In order to address this problem, the concept of glycemic load (GL) was introduced representing both the quality and the quantity of the carbohydrates in a food or a meal. GL allows comparisons of the likely glycemic effect of realistic portions of different foods, calculated as the amount of carbohydrate in one serving times the GI of the food (i.e. GL = gm carbohydrate * GI/100).

Statistical analysis
Presentation of results
The antidiabetic assays, glycemic index and glycemic load were analyzed in triplicates and
presented here as the mean, reported to the same number of significant figures as per original analytical results. The results of the antidiabetic assays were reported as percentage of inhibition while the glycemic index and glycemic load values were reported.

Statistical analysis of the data

The results obtained from the analysis were further analyzed using a complete randomized design (CRD–ANOVA-one way) for the interpretation of the results. The regression analysis was utilized for obtaining the concentration of the honey required to attain 50% of inhibition ($IC_{50}$ value) on the alpha amylase enzyme and $p$ value (at 5%) represent the statistical significance of the data obtained.

Results and discussion

Alpha amylase inhibition assay

All the honeys for the assay were taken at concentrations ranging from 100 - 500 (µg/ml) and the results of which revealed that at all concentrations Trigona iridipennis honey achieved highest alpha amylase inhibition in their raw (19.19 – 77.61 µg/ml) and processed (12.73 – 64.84 µg/ml) forms. All the other honeys exhibited different rate of inhibition at different concentrations as noticed among the raw honeys. At concentrations 100 and 200 (µg/ml) the descending order of honeys exhibiting alpha amylase inhibition were as follows: Trigona iridipennis honey (19.19 %, 24.19 %) > Apis dorsata honey (15.99 %, 17.5 %) > Apis cerana honey (12.62 %, 16.91 %) > Apis florea honey (10.99 %, 14.04 %) > Apis mellifera honey (9.41 %, 12.86 %). While at concentrations 400 to 500 (µg/ml) Apis mellifera and Apis florea honeys were found to have similar alpha amylase inhibition capacity of 41.32 % and 41.30 %, respectively. The raw Apis dorsata honey had 44.34 % of alpha amylase inhibition at 500 (µg/ml).

A similar trend in alpha amylase inhibition was noticed among the processed honeys analyzed with Trigona (19.19 % - 64.84 %) honey being the highest at all concentrations and Apis dorsata (8.18 %) and Apis mellifera (8.69 %) having comparable percentage of inhibition at 100 (µg/ml). But at further concentrations 200 - 300 (µg/ml) the percentage inhibition of Apis dorsata (12.77 %, 16.55 %) was found to be higher than Apis mellifera (9.19 %, 13.91 %) honey. On contrary at 500 (µg/ml) processed Apis mellifera and Apis dorsata had similar levels of percentage inhibition 32.19 % and 32.09 %, respectively. The processed Apis cerana honey also had relatively comparable percentage of inhibition 33.94 %. The antidiabetic activity of honeys under the study was compared with a standard antidiabetic drug acarbose and it could be noted that the alpha amylase inhibition capacity for acarbose ranged from 36.08 % to 91.97 %. Even though the honeys under the study had moderate inhibition capacity, the raw as well as processed Trigona iridipennis honeys proved to have comparatively good antidiabetic effect with regards to the standard acarbose (Table 1).

The efficacy of the honeys against alpha amylase was studied by fitting the regression equations and obtaining the $IC_{50}$ value which denotes the amount of honey to obtain 50% of inhibition over alpha amylase enzyme. The $IC_{50}$ values of all the honeys analyzed were in range from 348.9 to 872.6 (µg/ml). From the obtained $IC_{50}$ values for different honeys it was clear that raw Trigona honey had the least $IC_{50}$ value of 348.9 (µg/ml) followed by processed Ti (423.87 µg/ml). The highest $IC_{50}$ values were noticed in processed Apis mellifera and Apis dorsata honeys of 872.6 and 870.5 (µg/ml), respectively (Fig. 1).

Alpha glucosidase inhibition assay

The percentage inhibition of alpha glucosidase was analyzed in ten microliters of each honey samples.
The highest percentage inhibition was observed in raw *Trigona iridipennis* honey (80.46%) followed by raw *Apis mellifera* (79.86%) and *Apis dorsata* (79.67%) honeys. The percentage inhibition of *Apis florea* honey was viewed to be 79.65%. The least percentage of inhibition was noted in *Apis cerana* honey (76.14%). All the raw honeys analyzed were significantly different from raw Ti honey whereas, the honeys *Apis mellifera*, *Apis dorsata* and *Apis florea* exhibited non significance in their percentage of inhibition against alpha glucosidase.

In general a reduction in the activity of processed honeys against alpha glucosidase enzyme was noticed. The highest percentage inhibition among the processed honeys was in *Trigona* honey (78.29%) followed by *Apis dorsata* (70.55%) and *Apis cerana* honey (70.22%). The least activity among the processed honeys was viewed in *Apis mellifera* honey (69.17%). The alpha glucosidase inhibition of all the honeys decreased significantly on processing. Among the processed honeys significant difference was not viewed in *Apis dorsata* and *Apis cerana* honeys, while all the honeys decreased significantly in their percentage inhibition on comparison with *Trigona* honey. Similarly significant difference was observed between the raw and processed form of each honey.

Along with enzyme inhibition capacity certain other established mechanisms responsible for the antidiabetic activity of honey are its effect on prolongation of gastric emptying\(^1\), reduced rate of intestinal absorption and reduced food intake\(^1\).\(^2\)

As reported by Nasrolahi *et al.*, (2012)\(^4\), antidiabetic drugs in combination with honey improved glycemic control, enhanced antioxidant defences and reduced oxidative damage thereby, reversed the degeneration of beta cells of pancreas and also enhancing the insulin production as well as reducing the insulin resistance towards the glucose moieties in circulation (Table 2).

### Glycemic index and Glycemic load

The glucose tolerance tests of 10 healthy subjects were conducted to analyze the GI of the different bee honeys in their raw and processed forms. The GTT values of the subjects revealed that, on fitting quadratic regression equation \((y = -0.002x^2 + 0.276x + 87.2)\) for raw *Apis cerana* honey with \(R^2 = 0.53\); \((y = -0.002x^2 + 0.313x + 77.6)\) for raw *Apis mellifera* honey with \(R^2 = 0.31\); \((y = -0.002x^2 + 0.314x + 85.22)\) for raw *Apis dorsata* honey with \(R^2 = 0.78\) and \((y = -0.002x^2 + 0.284x + 85.22)\) for *Apis florea* honey with \(R^2 = 0.78\) and \(y = -0.001x^2 + 0.145x + 85.17\) with \(R^2 = 0.65\) for *Trigona iridipennis* honey.

The \(R^2\) values depict the regression lines are much fitting. It can be noted that, when compared to glucose, subjects attained peak blood sugar levels much slower while consuming the honeys. On contrast processed *Apis cerana* (53.5 min) and *Apis dorsata* (53.5 min) honeys attained the maximum much in advance on consumption.

### Table 2—Alpha glucosidase inhibition activity of bee honeys

<table>
<thead>
<tr>
<th>Species</th>
<th>Raw honey</th>
<th>Processed honey</th>
<th>t value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ac</td>
<td>76.14</td>
<td>70.22</td>
<td>48.33**</td>
</tr>
<tr>
<td>Am</td>
<td>79.86</td>
<td>69.17</td>
<td>23.48**</td>
</tr>
<tr>
<td>Ad</td>
<td>79.67</td>
<td>70.55</td>
<td>27.28**</td>
</tr>
<tr>
<td>Af</td>
<td>79.65</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Ti</td>
<td>80.46</td>
<td>78.29</td>
<td>5.98**</td>
</tr>
<tr>
<td>CD*</td>
<td>0.511</td>
<td>0.759</td>
<td></td>
</tr>
</tbody>
</table>

* * Significant difference among species (p<0.05) **Significance at 1%
From the above mean GTT values of the subjects, GI of each honey was obtained and it was ranged from 54 - 67 which shows that all the honeys under the study were belong to medium GI category except for raw *Trigona iridipennis* honey which had GI of 55 hence raw Ti honey belong to low GI foods. But processed *Trigona iridipennis* honey had GI of 56 hence it belong to the category of medium GI foods. The raw and processed form of *Apis mellifera* honey had equivalent GI value of 63 and a similar trend was noticed among all raw and processed honeys in their GI levels. The highest GI was viewed to be in processed *Apis cerana* honey of 67 this was followed by raw *Apis cerana* and *Apis florea* honeys with GI value of 65 each. The raw and processed *Apis dorsata* honeys followed Ti honey with lower GI values of 61 and 62, respectively (Table 4).

Hence, it could be concluded that honey is a better option of sweetening agent for people who should control their blood sugars levels to minimum. Among the honeys analyzed the raw *Trigona iridipennis* honey could be stated as the best honey for diabetics as the GI content was observed to be 55 which belong to the low GI category. Using the glycemic index, glycemic load was also calculated. The glycemic load (GL) for portion size of 25 gm with an average available carbohydrate of 20 gm of five different bee honeys in their raw and processed forms were noticed to be from 11.8 to 13.5, which signifies that all the honeys under the study had medium glycemic load. Among the honeys analyzed the lowest GL was viewed in raw and processed *Trigona iridipennis* honeys of 11.8 and 12.1, respectively. Raw *Apis dorsata* honey had the second lowest GL of 12.3 on processing it had increased to 13.1. A slightly higher level of GL was reported in raw and processed form of *Apis mellifera* honey of 13.5, related trends were also noticed in raw and processed *Apis cerana* and *Apis florea* honeys. It is evident that, the GI and GL of all the honeys under the study fall under the category of medium GI foods having the glycemic index ranging from 56 to 69 with an exemption to raw *Trigona* honey with GI value 54, which could be considered as a low GI food. In the same way according to the glycemic load (GL) values obtained all the honeys fall in to the category of medium GL food with GL ranging from 11 to 19.

Significant difference was noted among all the raw honeys in the glycemic index levels, while the *Apis mellifera* and *Apis florea* honeys had similar levels of GI. On processing the glycemic index of the honeys had increased and significant difference in the GI was viewed among all the processed honeys. The Glycemic load also varied significantly among the raw honeys analyzed except for *Apis mellifera* and *Apis florea* honeys. A similar trend was noticed among the processed honeys where significant difference was not observed among the processed *Apis cerana* and *Apis dorsata* honeys. As honey has equal sweetening capacity like that of the commonly used table sugar, it would be appropriate to compare both sweetening agents. The GI value of raw and processed *Trigona* honey in the present study is 54 and 56, respectively, whereas the average GI value for the table sugar is $68\pm5$. This means that Ti honey

| Table 3—Mean glucose tolerance test (GTT) values of the respondents |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | Ac | Am | Ad | Af | Ti |
| Time | Raw | Processed | Raw | Processed | Raw | Processed | Raw | Raw | Processed |
| Initial (0 min) | 88±2.6 | 90±5.3 | 80±2.3 | 83±5 | 85±7.2 | 85±4.1 | 85±3.1 | 86±5 | 84±5.3 | 81±6.1 |
| 30 min | 89±5 | 94±1.3 | 82±4.1 | 89±5 | 91±6.4 | 86±5 | 90±3.1 | 87±6.2 | 89±5.4 | 118±5.8 |
| 60 min | 101±2.4 | 103±5.6 | 83±6.1 | 99±5.4 | 96±6 | 94±1.3 | 95±3.5 | 88±3.9 | 95±5.2 | 122±2.3 |
| 90 min | 84±3 | 89±2.4 | 98±4.7 | 84±4.3 | 87±5.3 | 84±4.6 | 98±4 | 90±4.6 | 93±5 | 103±2.4 |
| 120 min | 82±4.2 | 85±4.1 | 79±5.8 | 80±1.4 | 84±4.2 | 81±2.4 | 88±4.2 | 84±2.1 | 86±5 | 94±1.9 |
| Observed peak time (min) | 60 | 60 | 90 | 60 | 60 | 60 | 90 | 90 | 60 | 60 |
| Actual peak time (min) | 69 | 54 | 78 | 65 | 71 | 53 | 88 | 83 | 73 | 63 |

| Table 4—Glycemic index and Glycemic load of bee honeys |
|---|---|---|---|---|
| Species | Glycemic index (GI) | Glycemic load (GL) |
| | Raw | Processed | Raw | Processed |
| Ac | 65 | 67 | 13.00 | 13.14 |
| Am | 63 | 63 | 13.54 | 13.54 |
| Ad | 61 | 62 | 12.35 | 13.17 |
| Af | 65 | - | 13.32 | - |
| Ti | 54 | 56 | 11.88 | 12.18 |
| CD* | 0.673 | 0.667 | 0.338 | 0.031 |

* Significant difference among species (p<0.05)
may have lower blood glucose response than table sugar. But the present investigation revealed that the GI values of the other honeys, viz. raw and processed *Apis dorsata* (61, 62), raw and processed *Apis mellifera* (63), *Apis florea* (65), raw and processed *Apis cerana* (65, 67) falls in to the intermediate glycemic index range and these honeys were considered to be better substitute for sugar because they possesses natural antioxidants such as polyphenols, flavonoids, flavonols and vitamin C that can decrease oxidative stress in humans. Thus, persons with diabetes mellitus can include moderate amounts of honey in a balanced diet.

The results of the present study were in confirmation with the studies conducted on the glycemic index of other honeys by various researchers for example, the glycemic index for thyme honey and clover honey were 65.9 and 64.9, respectively and both were significantly lower than glucose. Similar observations were also reported in the 5 *manuka* honey samples, selected from different geographical locales around North Island of New Zealand for having moderate GI values.

Processing did not found to have an impact on the glycemic index, glycemic load and the peak absorption time of the honeys. Even then, the alpha amylase and alpha glucosidase enzyme inhibition capacity of the honeys were found to be reduced on processing. This might be due to the reduction in the potency of the volatile compounds that present in the honeys which contribute the antidiabetic property. This could be rationalized by the improved antidiabetic activity of *Trigona* honey among the other processed honeys under the study. Hence, it could be stated that among the honeys analyzed the raw as well as processed form of *Trigona* honey had better antidiabetic property in comparison with the other honeys in the present in investigation.

**Significance of the study**

The therapeutic effect of honeys had been explored thoroughly, but use of honey by diabetic is still a myth. Recent studies have carried out in understanding the glycemic index and proved honey to be a moderate GI food, while the mechanism of action of honey in curbing the rise in the blood sugar is not understood. The present study is the first of its kind which establishes the antidiabetic activity of honey in terms of their crucial role in regulating the sugar/carbohydrate metabolism. Hence, in the present the antidiabetic mechanism of honey was studied through two in vitro media, viz. alpha amylase enzyme inhibition assay and alpha glucosidase enzyme inhibition assay. The study is also novel as the honey samples are collected with bee specificity where most the therapeutic analysis is done according to the floral base of honey from which it is obtained. The results of the present study offers strong reason for the utilization of honey among diabetic for the reason it can curb their feeling to have sweet along with umpteen health benefits. Synthetic α amylase inhibitors like acarbose cause side effects such as abdominal pain, diarrhea and soft faces in the colon. Hence, the present study recommends the utilization of honey a natural nutraceutical for maintaining the blood sugar level at normal levels without any side effects.

**Conclusion**

Several studies had come up with the therapeutic effect of honeys in maintaining the degenerative as well as life style diseases. But its utilization in diabetic condition continued to be a myth, until research proved honey to be moderate glycemic index. Even after wards, the probable mechanism of action honey was vague. Hence, the present established two possible mechanism of action of honeys against controlling diabetes mellitus in the in vitro medium. As alpha amylase and alpha glucose are one of the key enzymes which present in the brush border epithelium of intestine for converting the complex starch molecules into simple sugars, a competitive inhibition of these enzymes by the phytochemicals in the honeys could help in counteracting the rise in blood sugar at faster rates. Considering the research around the issue of exclusion of sugar by the diabetic it is encouraging to showcase that honey can be a good substitute for sugar among the diabetic. Because the type of carbohydrate consumed can only affect how quickly blood glucose levels rise. Honey can not only act as a substitute for sugar among the diabetic, in fact it also contributes to curb the diabetic associated micro vascular complications and angiopathies as honey could revitalize the blood components along with its composition.

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**References**