Endogenous Antioxidant Responses to Dietary Honey Supplementation in Alloxan induced Diabetic Wistar Rats

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Abstract:

Background

Dietary management has been a source of concern both to the diabetics and to their attending physicians. Cellular injury resulting from oxidative stress is implicated in the pathogenesis of diabetes and its complications. The aim of the present study was to determine the impacts of honey on the endogenous antioxidants of alloxan-induced diabetic wistar rats.

Methods

The study involved 32 male wistar rats separated into groups of 8. Diabetes mellitus was induced in each animal by intra-peritoneally injecting a single dose of 200mg/kg of 2% alloxan solution after an overnight fast and confirmed after 72 hours with plasma glucose ≥ 12.0mmol/l. Group 1 served as the diabetic control. Groups 2, 3 and 4 received 10ml/kg/day of 10%, 30% and 50% honey respectively. At the end of 8 weeks, blood samples were collected from the animals to determine the biomarkers of oxidative stress using standard methods.

Results

The results showed that dietary supplementation of moderately diluted honey caused significant increases in serum glutathione and superoxide dismutase but no significant change in catalase of diabetic rats.

Conclusion

Honey supplementation in moderately diluted forms ameliorates alloxan induced oxidative stress by inducing the endogenous antioxidants. Therefore the blood glucose lowering and organo-protective effects of honey may be attributed to its anti-oxidative properties

Key Words; Honey, endogenous antioxidants, alloxan, diabetic rats.

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I. Introduction

Diabetes mellitus is associated with multi-organ damage usually due to oxidative stress posed by persistent hyperglycemia (1). Oxidative stress causes cellular injury as a result of increased levels of free radical due to an imbalance between radical-generating and radical-scavenging functions (2) such that the disease will not occur until the free radicals outweigh the antioxidants. Oxidative stress thus would inhibit both the pancreatic β-cell output of insulin as well as impaired cellular uptake and utilization of glucose (3).

The rising prevalence of diabetes mellitus in Africa (4) may not be unconnected with the gradual replacement of unprocessed and natural foods with highly refined foods in today’s African diets. Some of these natural foods are known to increase the activities of naturally occurring antioxidants in the body and thus increasing the radical scavenging function that prevents oxidative stress and its consequent cellular damage (5). Honey contains phenolic compounds and flavonoids which largely determine its antioxidant activity (6,7,8). Phenolic compounds are essential ancillary plant metabolites that determine the dietary qualities of fruits and vegetables. Daily dietary consumption of these compounds form part of healthy eating habits and play vital role in prevention of some diseases (9).

The aim of the present study was to determine the effect of dietary honey supplementation on the endogenous antioxidants of alloxan induced diabetic wistar rats.

METHODS

The research experiment was performed in the animal house of the department of Human Physiology, faculty of Basic Medical sciences after an approval by the Research Ethics Committee of the Centre for Research Management and Development, University of Port Harcourt, Nigeria. A total of 32 matured male wistar rats bred from the department of Human Physiology were used for the experiment and divided into 4

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groups (n=8) and acclimatized under laboratory settings of temperature, humidity and light in their respective cages for two weeks prior to the experiment.

Diabetes mellitus was induced in each animal by intra-peritoneally injecting a single dose of 200mg/kg of 2% alloxan solution after an overnight fast. The animals were confirmed diabetic 72 hours later with plasma glucose ≥ 12.0mmol/l using Accu-check Active glucometer (Roche Diagnostics, Germany). Natural honey was harvested from a bee farm and reconstituted by diluting it with distilled water to produce 10% (v/v), 30% (v/v) and 50% (v/v) honey respectively. Group 1 served as diabetic control. Groups 2, 3 and 4 received 10ml/kg/day of 10%, 30% and 50% honey respectively. At the end of week 8, each animal was anaesthetised and blood samples collected by cardiac puncture into specimen containers to determine the biomarkers of oxidative stress using standard methods. Superoxide dismutase was determined using the method described by (10). Reduced glutathione (GSH) was measured by the method described by (11) using GSH Assay kit while Catalase was measured according to the method described by (12).

Statistical analysis was done using spss vs. 20.0. Tables and graphs were used to represent data. Comparison of means was done using one-way ANOVA test and paired sample t test. Differences in values were considered statistically significant at p<0.05.

### II. Results

**Table 1: Effect of honey on superoxide dismutase (SOD) and catalase (CAT)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (U/mg of protein)</th>
<th>CAT (U/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic control</td>
<td>3.62±0.25</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>Diabetic + 10% honey</td>
<td>6.09±0.71*</td>
<td>0.08±0.03</td>
</tr>
<tr>
<td>Diabetic + 30% honey</td>
<td>5.31±0.35*</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>Diabetic + 50% honey</td>
<td>6.91±1.27*</td>
<td>0.05±0.02</td>
</tr>
</tbody>
</table>

N=8. *Significant change compared to diabetic control (p<0.05).

**Table 1**

![Effect of honey on the GSH of alloxan induced diabetic rats (*Significant compared to control, p<0.05)](image)

**Fig.1**

**III. Discussion**

In the present study alloxan administration was used to induce diabetes mellitus (and oxidative stress). Several studies suggest that reduced glutathione (GSH) and other endogenous antioxidants are depleted in diabetes mellitus (13,14,15,16) resulting in oxidative stress and its consequent cellular effects (17). The present study showed that the level of GSH significantly increased in the diabetic groups fed with 30% and 50% honey respectively in a dose-dependent fashion (Fig.1) while administration of 10% honey had no significant effect on the GSH of diabetic rats. Therefore the GSH synthetic effect of honey as reported in previous studies (18,19) could be enhanced when honey is consumed in moderately diluted forms. Again, the phenolic compounds
present in honey have been reported to elevate the expression of γ-glutamylcysteine synthase which is involved in the synthesis of glutathione both invivo and invitro (20).

The levels of the endogenous antioxidant enzyme, SOD increased significantly in the honey fed diabetic groups compared to the diabetic control (Table 1), although not in a dose dependent pattern. Endogenous catalase did not show any significant response to dietary honey supplementation in diabetic rats. However, a slight dose-dependent decline in the serum levels of catalase was observed (Table 1). This could suggest that increasing the concentration of honey might further decrease the serum catalase so that the optimal anti-oxidative benefits of honey in diabetic rats would be derived only when honey is consumed in the moderately diluted forms. The findings of the present study to a large extent support the potential restorative effect of endogenous antioxidant enzymes in diabetes (21).

Some antioxidants found in honey are reported to strongly inhibit fructose and glucose transport mediated by glucose transporter-2 (22) resulting in decreased absorption of glucose from the gut and reabsorption from renal tubules, thus decreasing the plasma glucose. This is one possible explanation to the blood glucose lowering effect of honey observed in some studies (23).

The antioxidant properties of honey especially when taken in diluted forms by diabetic rats could result in the regeneration of pancreatic beta cells (24,25,26) with its associated rise in insulin output and subsequent blood glucose control. The preservation of antioxidants and prevention of oxidative stress as shown in the present study has also been suggested to be responsible for the organo-protective effect of honey (27, 28).

IV. Conclusion

Honey supplementation in moderately diluted forms ameliorates alloxan induced oxidative stress by inducing the endogenous antioxidants. Therefore the blood glucose lowering and organo-protective effects of honey may be attributed to its anti-oxidative properties.

References


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