Antihyperglycemic Activity, Antihyperlipidemic Activity, Hepatoprotective Activity and Histopathological Analysis of Natural Honey in Streptozotocin Induced Diabetic Rats

Asaduzzaman M*, Nahar L2, Hasan M2, Khatun A2, Shahedul Haque M2, Hasan N1, Tamannaa Z1, Huda N1, Fazley Rabbi M1, Ray M3, Nur Islam M4, Maniruzzaman M1, Mobassirul Islam M5, Dastagir N6 and Sarker S7

1Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi, Bangladesh
2MBBS, Gonoshasthaya Samaj vittik Medical College and Hospital, Dhaka, Bangladesh
3Department of Pharmacology, Kyoto Pharmaceutical University, Nakauchi-cho, Missasagi, Yamashina-Ku, Kyoto, Japan
4Department of Pharmacology, Kyoto Pharmaceutical University, Nakauchi-cho, Missasagi, Yamashina-Ku, Kyoto, Japan
5Department of Pharmacy, University of Rajshahi, Rajshahi, Bangladesh
6Department of Public Health and Informatics, Jahangirnagar University, Savar, Dhaka
7Department of Microbiology and Veterinary Public Health, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh

Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, secretion insufficiency and receptor insensitivity to endogenous insulin [1]. It is a complex progressive disorder characterized by impaired insulin sensitivity, reduced insulin secretion and progressive failure of β-cells. Its incidence is associated with high morbidity and mortality rates. Increased oxidative stress is believed to play an important role in the etiology and pathogenesis of chronic complications of diabetes [2,3]. Free radicals are very reactive species, capable of inducing modification of the biological membrane of phospholipids and proteins, resulting in modifications of cell function and cellular death [4-6]. The risks of diabetic complications are particularly cardiovascular diseases (CVD) and peripheral vascular disease (PVD) [7]. Complications such as coronary artery disease (CAD), stroke, neuropathy, renal failure, retinopathy amputations, blindness etc. are known to be associated with diabetes mellitus [8]. Epidemiological reports have shown that the effect of postprandial hyperglycemia on cardiovascular risk is greater than the effect of fasting hyperglycemia. Despite excellent potencies, synthetic antidiabetic drugs had offered unwanted therapeutic profiles marked by fluid retention, drug-induced hypoglycemia, and increased rate of lactic acidosis, liver malfunctioning due to cirrhosis, weight gain and cardiac dysfunction [9]. Myocardial infarction (MI) is serious manifestations of Ischemic heart disease. In the course of cardiac surgery and MI, ventricular arrhythmias such as ventricular tachycardia and ventricular fibrillation are the most important causes of mortality [10]. In management of such conditions, drug may be lifesaving. In the case of natural honey, it has been applied for medicinal purposes of cardiovascular diseases (CVD) [11]. Honey showed against cardiovascular risk factors such as hyperlipidemia by the antioxidant properties because antioxidants are neutralizing free radicals [12-14]. Diabetes mellitus is commonly associated with hepatic dysfunction or abnormalities such as elevations in serum alkaline phosphatase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), by the supplementation of honey it recover liver damage with the activation of glucokinase enzyme in the liver.

Keywords: Honey; Glucose; Antihyperglycemic; Antihyperlipidemic; Hepatoprotective

Abstract

Honey contains a variety of phytochemicals and other substances, such as organic acids, vitamins and enzymes, which may serve as a source for dietary antioxidants. Antioxidants shows improve insulin level and protect insulin resistant in diabetes mellitus. Honey bee venom reduces blood glucose level as well as lipid profile through increased insulin secretion and glucose take-up. For that reason, honey could be considered as a potential remedy for diabetes as well as Cardiovascular Disease (CVD). The objective of this study was to determine the medicinal activity of honey and its role on antihyperglycemic, antihyperlipidemic, hepatoprotective activity and histopathological analysis of natural honey in Streptozotocin- induced diabetic rats. The experimental rats were divided into six groups (n = 6). Diabetes Mellitus (DM) was induced by single intraperitoneal injection (65 mg/kg BW) of freshly prepared Streptozotocin hydrate solution in 0.9% saline solution. Hyperlipidemia was induced by mixture of cholesterol (1.5%) and cholic acid (0.5%) with diet of rats. At the end of treatment, the blood glucose level and lipid profile was measured by using commercial kits. Histopathology of liver and heart were performed of observed any changes in the cellular structures (degradation and regeneration) of the rats after receiving the sample for 28 consecutive days with respect to control group. Honey bee-treatment significantly decrease blood glucose level in diabetic rats. TC, TG, LDL, VLDL are significantly (p < 0.05) decrease whereas HDL significantly increase (p < 0.001). The SGPT and SGOT were significantly decrease (p < 0.05) which showed hepatoprotective activity of honey. Honey has favorable effect on the histopathological changes in Streptozotocin- induced diabetic rats. On the basis of above findings, it can be concluded that, supplementation of honey could significantly contribute to control blood glucose level as well as lipid profile in diabetic subjects. Honey showed prevent various complications of diabetes and improve some haematological parameters.

*Corresponding author: Asaduzzaman MD, Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh, Tel: +88-01719248007; E-mail: asad09bmb@gmail.com

Received December 05, 2015; Accepted March 22, 2016; Published March 30, 2016.


Copyright: © 2016 Asaduzzaman M, et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
process of glycogen synthesis [15]. There is no systematic data available on the physicochemical and medicinal properties of Bangladeshi honey. This study was undertaken to analyse Bangladeshi honey for better understanding of its medicinal properties.

Materials and Methods

Sample Collection: Multifloral honey samples were collected from the largest mangrove forest of the world, Sundarban, Bangladesh, in February 2015.

Animals care

Adult male and female Wistar rats (180-210 g) were used in this study. Animals were bred and reared in the animal house facility of the Department of Biochemistry and Molecular Biology, University of Rajshahi, at a constant room temperature of 25°C and in an environment with the humidity ranging between 35% and 65%. The rats were housed in plastic cages with soft wood-chip bedding and received a normal day-night cycle. The rats were provided with a standard laboratory pellet diet and water ad libitum. The experiments were conducted according to the ethical guidelines approved by the Institute of Biological Science (IBSc), Rajshahi University, Bangladesh.

Induction of diabetics

Diabetes was induced by single intraperitoneal administration of streptozotocin (65 mg/kg) body weight dissolved in 0.1 M citrate buffer, pH 4.5 in rats fasted for 16 hours. Another group of rats was injected with citrate buffer alone without streptozotocin. This group served as control. Two days after streptozotocin injection, development of diabetes was confirmed by measuring blood glucose levels in blood samples taken from tail vein. Rats with blood glucose concentrations of 11 mmol/L or higher were considered to be diabetic. Blood glucose levels of the control rats remained normal (< 4.2 mmol/L). Glucose measurement was performed with an Accu-Chek glucometer (Roche, Germany).

Blood collection

Blood samples from all groups were collected on days 1, 7, 14, 21 and 28 in a fasting state from rat’s marginal ear vein by 26G needle and syringe. Plasma concentrations of triglyceride (TG), total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), VLDL, SGPT, SGOT were measured using a quantification kit (Linear chemicals, Barcelona, Spain) by automatic Bioanalyzer (Hitachi 7180, Hitachi, Tokyo, Japan). Statistical analysis was carried out using Science (SPSS) version 17.0. The data are expressed as mean ± SEM. P value < 0.05, p < 0.001 were considered statistically significant.

Experimental animals grouping and treatment

The experimental rats were six groups and each group contain six rats (n=6). Distilled water, honey and glibenclamide were administered once daily by oral gavage. The animals were treated for 4 weeks as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood sample drawing days</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (control)</td>
<td>5.6 ± 0.1</td>
<td>5.8 ± 0.2</td>
<td>5.8 ± 0.1</td>
<td>5.8 ± 0.2</td>
<td>5.7 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Diabetic control</td>
<td>20.1 ± 0.5*</td>
<td>21.5 ± 0.4*</td>
<td>21.6 ± 0.7*</td>
<td>23.4 ± 0.5*</td>
<td>24.5 ± 0.5*</td>
<td></td>
</tr>
<tr>
<td>Diabetic + Glibenclamide</td>
<td>20.3 ± 0.9*</td>
<td>16.4 ± 0.4</td>
<td>13.5 ± 0.5*</td>
<td>11.5 ± 0.7*</td>
<td>9.3 ± 0.3*</td>
<td></td>
</tr>
<tr>
<td>Diabetic + Honey</td>
<td>21.2 ± 1.0*</td>
<td>18.6 ± 0.8*</td>
<td>16.7 ± 1.3</td>
<td>14.9 ± 1.1</td>
<td>12.3 ± 0.5*</td>
<td></td>
</tr>
</tbody>
</table>

* Body weight decreases with injection of STZ. Body weights were measure with day 1 interval for 28 days. Each value is the mean ± SEM n = 6. Blood glucose level in the treated rats were significantly different from normal and diabetic control groups at *P < 0.001, whereas **P < 0.001 and ***P < 0.05 indicated the significantly difference from diabetic control group.

Table 1: Effect of honey on the blood glucose level of experimental rats (mmol/L).

**Figure 1:** Changes in lipid profile after honey treatment of diabetic and hypercholesteremic rats in 28 days.

**Group-6:** Diabetic rats + glibenclamide (0.5 mg/kg body weight).

**Experiment of histopathology of liver and heart**

According to [16] processing and staining technique was as follow: Tissue (liver and Heart) obtained from all experimental groups were washed immediately with saline and then fixed in 10% buffered neutral formalin solution. After fixation, the tissue was processed by embedding in paraffin. Then, the tissues were sectioned and stained with hematoxylin and eosin (H&E) and examined under high power microscope (200X,400X) and photomicrographs were taken.

**Results**

Table 1 showed blood glucose and body weight changes in experimental rats. The blood sugar measurements in diabetic rats after a 4-week treatment period showed significant variations among different groups (P < 0.001). Days 1, 7th, 14th, 21st, 28th, the diabetics rats group blood sugar level increasing maximum 23-25 mmol/L. In 28 days supplementation of honey blood glucose level significantly decrease 47.25% whereas Glibenclamide 63.47% (P < 0.001) (Figure 1).

Figure 1 showed the antihyperlipidemic activity of honey. In 28 days the level of Total cholesterol (TC), Triglycerides (TG), LDL, VLDL increase in diabetic rats group 23.05%, 11.73%, 27.30%, 12.35% respectively whereas the level of HDL decrease 30.43%. By the supplementation of honey the level of TC, TG, LDL, VLDL were decrease whereas HDL increase significantly (P < 0.05). In hypercholesterolemic rats group the Total cholesterol (TC), Triglycerides (TG), LDL, VLDL increasing as 46.55%, 82.31%, 38.03%, 82.80%, respectively whereas
Deposition of cholesterol in this group. Original magnification 25X.

For glibenclamide treated group shown the normal histopathology of heart. Honey treatment: Liver remained in apparently normal architecture. The structure was quite similar to the normal control group. (A) Normal control: Showing normal-appearing of hepatocytes, portal space (PS), sinusoids (arrows), and Kupffer cells. (B) Diabetic control: Diabetic control histopathology of rats liver shown the micro fat droplet deposition (Black arrow) and the onset of sinusoidal enlargement (arrows) and small amount of fatty vacuoles, respectively. (C) Glibenclamide (10 mg/kg): Standard drug treated group shown histopathology similar to the normal control group. (D) Honey showed apparently normal architecture. The structure was quite similar to that of the control group and tissue damage and necrosis were of less extent in these groups than the DC group. (E) Liver from hypercholesterolemic rats sacrificed showing progressive worsening of sinusoidal enlargement (arrows) and liver fatty degeneration. (F) Honey treatment: Liver remained in apparently normal architecture with no deposition of cholesterol and triglyceride and no vesicular steatosis in the liver tissues (Figures 3 and 4).

(A) Normal control: Normal control group shown normal histopathology of the heart. (B) Diabetic control: Diabetic control histopathology shown increased interstitial space and distort the intercalated disc (Black arrow). (C) Glibenclamide (10 mg/kg): Glibenclamide treat drug shown the normal histopathology of heart. (D) Honey for three weeks led to a normal histological organization in the heart cells and nuclei. The antioxidant activity may have prevented the oxidative damage at the myocardium in STZ-induced diabetic rats. (E) Hypercholesterolemic control group heart histopathology showed degenerating muscle fibers and muscle fibers vacuolization, fibrosis, transverse striations and wide interfascicular spaces. (F) The heart cells and nuclei altered to a normal histological organization with honey treatment. Cardiac myofibres were arranged in normal structure. The samples were obtained from the same heart anatomical regions. For each group, 6 rats were examined and 50 pictures were taken. The above picture for each group was chosen randomly from the 80 pictures in this group. Original magnification 25X.

**Discussion**

The results from this study have shown that hyperglycemia Induced by injecting STZ in rats leads to cell death of neuronal cells in the hippocampus. In this study, honey treatment showed blood glucose levels lowering activity in streptozotocin induced diabetic rats. STZ monohydrate induces type-2 diabetes in experimental rats through exclusive destruction of insulin producing beta cells in pancreas [17]. Glibenclamide or honey significantly reduced blood glucose concentrations in our study which is similar to findings from previous studies [18]. Honey also contains elements such as zinc, selenium, copper, calcium, potassium, chromium, manganese, etc. [19]. Some of these minerals are reported to play vital roles in the maintenance of normal glucose tolerance and insulin secretion from the pancreatic β-cells [20]. Other ions such as copper and zinc are also known to be involved in glucose and insulin metabolism [21]. Even though the amounts of these minerals in honey may be small, it is worthwhile to note that these trace elements are not actually required in large quantities. Daily supplementation of the diabetic rats with honey for three weeks might attain sufficient concentrations of these minerals to elicit pharmacological responses which synergistically contribute to the hypoglycemic effect. Furthermore, improved secretion of insulin from the pancreas (possibly due to protective effect on beta cells) might contribute to the hypoglycemic effect of honey. The honey treatment lowered plasma glucose, cholesterol, triglyceride, and LDL levels and increased HDL levels in diabetic rats compare to untreated diabetic group. Our results were consistent with findings of Mousavi et al. which also confirmed hypoglycaemic and hypolipidemic activity of honey in diabetic mice [22]. In another study, honey reduces glycaemia and cholesterolemia in healthy subjects depending on the inoculated trace elements.

![Figure 2: Changes of SGPT and SGOT after honey treatment of diabetic and hypercholesterolemic rats in 28 days.](image1)

![Figure 3: Fluorescence Microphotograph of Liver in different groups of rats.](image2)

![Figure 4: Fluorescence Microphotograph of Heart in different groups of rat.](image3)
The assay results were expressed as mean values and the standard deviation (SD). Results were analyzed using Scientific Package of Social Science (SPSS) version 17.0. Two different sets of statistics, which is descriptive and analytical statistics, was applied. The descriptive statistic was used to analyze mean, standard deviation (SD) whereby analytical statistics, one-way ANOVA was used to determine statistical significance (p < 0.05, p < 0.001) among the groups.

**Acknowledgement**

The financial support was given by The Ministry of National Science and Technology (NST) Bangladesh, University Grant Commission (UGC) Bangladesh and Faculty of Science Rajshahi University, Rajshahi-6205, Bangladesh.

**References**


