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Natural honey lowers plasma glucose, insulin and C-peptide in streptozotocin-induced diabetic rats: Comparison with metformin

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ABSTRACT

In spite of increasing incidence of diabetes still it could not be effectively controlled with only one medication. In the present study, the effect of honey compared to metformin was investigated on glycemia in the streptozotocin-induced diabetic rats. Forty two rats were randomly divided into seven groups ; C: non-diabetic rats received distilled water, CH: non-diabetic rats received honey, CD: diabetic rats received distilled water, DM: metformin treated diabetic rats, DH: honey treated diabetic rats either in 1.2 mg/kg (DH1) or 2.4 mg /kg dose (DH2) , and DMH: diabetic rats treated with a combination of metformin and honey. Diabetes was induced by a single dose of streptozotocin (50 mg/kg; i.p.). After 30 days , blood samples were withdrawn to measure blood glucose, insulin and serum C peptide. Treatment with metformin or honey at the two studied doses (1.2 and 2.4 mg/kg) significantly decreased the glucose level in diabetic rats. Treatment with metformin slightly increased serum insulin level and had no significant effect on serum C-peptide. While treatment with honey either in a dose of 1.2 mg/kg or 2.4 mg/kg markedly increased serum insulin and C-peptide levels. The best result was in the combination of honey with metforminthat almost reached the non- diabetic control values. This study indicated that consuming honey in a dose of 1.2 or 2.4 mg/kg improves glycemic control and reduces both insulin and C peptide levels more than metformin alone. The best results were in the combination of honey and metformin.

Key words: Insulin, C reactive protein, honey, metformin, rats.

INTRODUCTION

Diabetes is a metabolic disease resulting in chronic hyperglycemia and caused by defect in insulin secretion, action or both and consequently disturbances in carbohydrates, proteins, and lipids metabolism [1]. In spite of increasing incidence of diabetes still it could not be effectively controlled with only one medication[2]. Moreover, the available drugs have undesirable side effects such as weight gain, hypoglycaemia, deterioration in beta cell function, poor compliance and limitations in preventing diabetes complications associated with oxidative stress[3].

The need for a new natural drug is always a request. Honey has been used in traditional medicine since ancient Egyptians as natural antibiotic to treat wounds, burns, gastrointestinal disorders, asthma, and cataracts [4] and has recently become a subject of renewed research interest in the last few years [5].

Honey is a natural product of bees formed after uploading and modifying plants nectar inside bees themselves. Honey is composed mainly of glucose and fructose sugars in addition to water, minerals, proteins and acids [6]. It is God’s present to the universe. Both human and animal kinds feed on it and get medicinal and nutritional benefits. Among these benefits are antimicrobial, anti-inflammatory and antioxidant effects [7]. Several studies proved the value of honey for diabetics either in experimental animals[8-9] or in humans[10].

Several antioxidant were found in honey as enzymes (catalase, glucose oxidase), acids (ascorbic, phenolic , organic and amino acids) and other antioxidant components (flavonoids , carotenoid derivatives, Maillard reaction products and proteins) [11-12]. Various polyphenols are reported in honey as caffeic acid, caffeic acid phenyl ester, pinocembrin, acacetin, chrysin, quercetin, galangin and kaempferol[13]. C-peptide is a good marker of insulin secretion. Its measurement can be considered indirectly a validated means of quantifying insulin secretion. [14]. Metformin is an oral hypoglycemic drug for regulating blood sugar. Its main role is to increase insulin sensitivity in liver and facilitate glucose transport in hyperglycemia and insulin resistance [15].

The aim of this study is to investigate the effect of natural honey on plasma glucose, insulin and C-reactive protein in streptozotocin-induced diabetic rats and compare this effect with metformin the common oral hypoglycemic drug.

MATERIALS AND METHODS

Animals:
This study was conducted on 42 healthy female albino rats weighing 110-130 g. All animals were maintained under standard laboratory conditions of temperature (22±2 °C), humidity (45±5%) and 12 h day: 12 h night cycle and were allowed free access to food (standard pellet diet) and water ad libitum. Rats were obtained from the Experimental Animal House of National Research Centre, Cairo Egypt. The use of rats and all the experimental procedures were approved by the Animal Ethics Committee of the National Research Centre and were handled in accordance with the Institutional Guidelines for the Care and Use of Animals for Experimental Purposes.

The animals were acclimatized to the animal room condition for at least one week prior to the experiment.

Preparation of honey and metformin:
Natural honey (clover) was purchased from the Ministry of Agriculture Market, Dokki, Egypt. This honey has the following composition: pH (2.7), ash (0.37%), moisture (16.3%), total protein (0.96), total lipids (0.72), total sugars (82.1%) and total reducing sugar (72.2%) [fructose (38.3 %), glucose (28.2 %), sucrose (4.8 %)] . Honey was freshly diluted with distilled water just before each administration. Metformin (100 mg/kg body weight) was dissolved in distilled water before administration.

Induction of diabetes:
Diabetes was induced in 42 fasted rats by intraperitoneal (i.p.) injection of streptozotocin (STZ) at a dose of 50 mg/kg body weight, dissolved in 0.1M cold citrate buffer (pH = 4.5) [16]. Diabetes was stabilized in these STZ treated rats over a period of 3 days. After that time the blood was collected by sinocular puncture and the plasma glucose level of each rat was determined. Rats with a fasting plasma glucose range of ≥ 250 mg/dl were considered diabetic and included in the study [17].

Treatment:
Rats were randomly divided into seven groups, six rats in each as follows:
Group 1: Non-diabetic control rats treated with distilled water (C)
Group 2: Diabetic control rats treated with distilled water (CD)
Group 3: non-diabetic rats treated with 4.8 mg/kg of honey (CH)
Group 4: Diabetic rats treated with 100 mg/kg of metformin (DM)
Group 5: Diabetic rats treated with 1.2 mg/kg of honey (DH1)
Group 6: Diabetic rats treated with 2.4 mg/kg of honey (DH2)
Group 7: Diabetic rats treated with 4.8 mg/kg of honey + 100mg/Kg of metformin (DMH)

Treatment with honey and metformin was administered orally using an intra-gastric tube once daily for 30 days

Biochemical assays:
At the end of the experiment (30 days), rats were fasted overnight and blood samples were withdrawn through the retro-orbital plexus under light ether anaesthesia using a heparinized glass capillary and collected in tubes. Blood was allowed to clot and serum separated by centrifugation at 4000 rpm for 10 min.

Determination of glucose, insulin, serum C peptide:
Serum glucose was determined by the glucose oxidase method [18]. Serum insulin levels were determined by Biosource –INSEASIA [19]. Serum C peptide was measured by radioimmunoassay method [20].

Statistical analysis

All values were expressed as mean± SEM. The differences were compared using one way analysis of variance (ANOVA) followed by Tukey tests and $p<0.05$ were considered statistically significant. Statistics was performed using SPSS for windows version 16.

RESULTS

Serum glucose:

Table 1 summarizes serum glucose level in different groups of animals at start and after 30 days of treatment period. As expected ,the serum glucose concentration of the diabetic control rats after 30 days treatment period was significantly higher (249.3± 17.69 mg/dl) than those of the non-diabetic control rats(79.83± 3.75 mg/dl). Treatment with Metformin or honey at the two studied doses (1.2 and 2.4 g/kg) significantly decreased the glucose level with increment of -167.5 ± 45.97,-121.33 ± 42.26 , or -129.16 ± 31.27mg/dl)respectively in diabetic rats. Combination of Metformin and honey was the best treatment to reduce the glucose concentrations in diabetic rats with increment of -214.8± 31.009mg/dl.

Serum insulin and C-peptide:

Table 2 shows the serum levels of insulin and C-peptide of control and diabetic rats. Serum insulin was significantly decreased in diabetic control (0.30± 0.02 µU/ml) in comparison to non- diabetic rats (0.60± 0.02 µU/ml). Treatment with Metformin slightly increased serum insulin level to 0.38± 0.01µU/ml. While treatment with honey either in a dose of 1.2 g/kg or 2.4 g/kg markedly increased insulin levels to 0.51± 0.04µU/ml and 0.48± 0.02µU/ml respectively. The best result was in the combination of honey with metformin**(**0.53± 0.03µU/ml).

Similarly the level of serum C-peptide significantly decreased in diabetic control (6.41± 0.39nmol/L) compared to non- diabetic rats (9.63± 0.37 nmol/L). Treatment with Metformin had no significant effect on serum C-peptide compared to diabetic control rats. While treatment with honey in a dose of 1.2 g/kg or 2.4 g/kg significantly increased C-peptide levels to7.85± 0.31 nmol/L or8.36± 0.34nmol/L respectively. The best result was in the combination of honey with metformin **(**9.26± 0.38nmol/L) that almost reached the non- diabetic control level (9.63± 0.37 nmol/L).

Treatment with honey in the two doses improves glycemic control and reduces both insulin and C peptide than metformin alone. The best results were in the combination of honey and metformin.

Table 1. Effects of natural honey, metformin, and their combinations on serum glucose in STZ-induced diabetic rats at start (zero) and after 30 days (end)

Group	Serum glucose (mg/dl) Zero	end	Serum glucose increment (mg/dl)
Non-Diabetic control (C)	85.16± 4.33	79.83± 3.75	- 5.33±7.06
Non-diabetic+ Honey (4.8 g/kg) (CH)	84.66± 4.58[b]	92.66± 6.28[b]	8.0±6.94[b]
Diabetic Control (CD)	386.0±12.7[a]	249.3± 17.69[a]	- 136.66±12.14[a]
Diabetic+ Honey (1.2 g/kg) (DH1)	274.6± 33.2[ab]	153.3± 13.4[ab]	-121.33±42.26[c]
Diabetic+ Honey (2.4 g/kg) (DH2)	294.6± 34.1[ab]	165.5± 11.9[ab]	-129.16±31.27 [ac]
Diabetic+ Metformin (100 mg/kg) (DM)	325.7± 41.5[a]	158.1± 15.6[ab]	-167.5±45.97 [a]
Diabetic+ Metformin (100 mg/kg) + Honey (4.8 g/kg) (DMH)	314.6±32.4[a]	99.38± 3.52[ab]	-214.8±31.009[ab]

Data are expressed as mean ±SEM. Each group consisted of 6 animals. Values are statistically significant at p<0.05, [a] compared with non-diabetic control (C), [b] compared with diabetic control(CD), [c] compared with diabetic+metformin+ honey (DMH) , [d] compared with diabetic+metformin (DM)

Table 2. Effects of natural honey, metformin, and their combinations on serum insulin and C peptide in STZ-induced diabetic rats

Group	Serum Insulin (µU/ml)	Serum C peptide (nmol/L)
Non-Diabetic control (C)	0.60± 0.02	9.63± 0.37
Non-diabetic+ Honey (4.8 g/kg) (CH)	0.53± 0.03[b]	8.86± 0.17[ab]
Diabetic Control (CD)	0.30± 0.02[a]	6.41± 0.39[a]
Diabetic+ Honey (1.2 g/kg) (DH1)	0.51± 0.04[bd]	7.85± 0.31[abc]
Diabetic+ Honey (2.4 g/kg) (DH2)	0.48± 0.02[abd]	8.36± 0.34[ab]
Diabetic+ Metformin (100 mg/kg)(DM)	0.38± 0.01[abc]	7.53± 0.32 [ac]
Diabetic+ Metformin (100 mg/kg) + Honey (4.8 g/kg) (DMH)	0.53± 0.03 [b]	9.26± 0.38 [b]

Data are expressed as mean ±SEM. Each group consisted of 6 animals. Values are statistically significant at p<0.05, [a] compared with non-diabetic control (C), [b] compared with diabetic control(CD), [c] compared with diabetic+metformin+ honey (DMH) , [d] compared with diabetic+metformin (DM).

DISCUSSION

The results of this study demonstrate and prove the health benefits associated with consuming clove honey alone or with metformin in reducing blood glucose level and increasing insulin and C-peptide levels in diabetic rats.

Honey lowers blood glucose in agreement with previous studies [21,22]. Unlike Metformin, mechanism of action of honey is not well identified in lowering blood sugar. Metformin is a drug and an oral hypoglycemic agent for lowering blood glucose. Its main role is facilitating the transport of glucose to liver and increasing insulin sensitivity in hyperglycemia and insulin resistance [15].

There are several mechanisms that explain the hypoglycemic effect of honey one of which is its components. Fructose is one of the major components [23].Fructose is a monosaccharide that is absorbed from the gastrointestinal tract slower than glucose and its metabolism is largely independent of insulin and quickly removed by the liver. Therefore, its consumption leads to only a slight increase in blood sugar and can be appropriate sweetener for patients with diabetes type 2 [24]. Clove honey used in this study contains fructose (38.3 %) , glucose (28.2 %) , fructose/glucose ratio of 1.35:1.

In addition it was found that administration of minimal amounts of fructose increases hepatic glucose uptake and glycogen storage and reduces peripheral glycemia and consequently insulin levels. This could be due to the activation of the enzyme glucokinase which controls glucose intracellular metabolism by catalysing the conversion of glucose to glucose-6-phosphate and decreases blood glucose level [25]. This explained the euglycemic rather than hypoglycemic effect of clove honey used in our study on non-diabetic rats.

Another mechanism of honey hypoglycemic effect which is not investigated in this study is the antioxidant effect [21]. Honey increases antioxidant agents such as beta-carotene, vitamin C, glutathione reductase and serum uric acid as well as containing by itself various antioxidants [26] as phenolic antioxidants [27]. The amount and type of antioxidants depends largely upon the floral/ variety of the honey [28].

In addition, honey contains different minerals such as copper, calcium, zinc, selenium, potassium, chromium, manganese. Some of these minerals such as chromium have play vital roles in controlling glucose and insulin metabolism through maintenance of normal insulin secretion from the pancreas and glucose tolerance [29]. Other studies demonstrated that zinc and copper can improve insulin sensitivity thus decreasing levels of blood glucose [30,31]. In addition, increased serum concentrations of zinc and copper were observed after supplementation of honey [26], these elements may add another explanation of the hypoglcemic effect of honey [30,31], increased C-peptide and insulin secretion [24]

In addition honey has a GI value ranging from 32 and 87, according to the international table of glycemic index which is considered as low GI[6]. Also, it was found that honey supplementation through up-regulation of Nrf2 activity and expression, increases the expression of cytoprotective genes [32].

The Present study showed that the blood level of insulin and C peptide decreased in diabetic rats, which was in accordance with other studies [24]. C-peptide is co-secreted with insulin by the pancreatic cells as a by-product of the enzymatic cleavage of proinsulin to insulin. Consequently, serum C-peptide level is a true indicator of any change in the insulin level, which is the main determinant of plasma glucose level [14]. This improvement in insulin and C-peptide levels could be related to honey’s protective effect on β-cells of pancreas [21].

It was found that in healthy subjects, compared to dextrose, honey supplementation produced lower increments in serum C-peptide and insulin levels [33]. When comparing honey to glucose-fructose solution, significantly lower serum C-peptide and insulin concentrations were observed . [34].

It was observed that honey had reduced insulin resistance in type 2 diabetic patients [35]. Similarly, honey supplementation was associated with considerable improvement in pancreatic islets and increased serum insulin levels in STZ-induced diabetic rats [8]. On the other hand, honey improves islets and increases insulin concentrations in impaired pancreatic function [9].

CONCLUSION

This study indicated that consuming clove honey in a dose of 1.2 or 2.4 mg/kg improves glycemic control and reduces both insulin and C peptide levels more than metformin alone. The best results were in the combination of honey and metformin.

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