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Differential Effect of Honey on Selected Variables in Alloxan-Induced and Fructose-Induced Diabetic Rats

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ABSTRACT

Honey contains a high concentration of fructose, a monosaccharide, capable of raising blood sugar level after oral ingestion. It is thus a paradox that nutritional experts have advocated its use as a nutrition supplement in patients with diabetes mellitus. It has also been used, over the years, as a sweetener by those who wish to avoid the use of sugar. The effective use of sugar in diabetes may be due to its other constituents, especially the various antioxidants that are abundant in honey. Glycemic effect of honey on alloxan-induced diabetes and with concomitant administration of fructose was studied in male rats of the Wistar strain. Alloxan was injected into the rats through a tail artery and three days later, a confirmation of successful induction of diabetes was made by demonstration of hyperglycemia in the rats. Another group of rats received daily oral ingestion of fructose. At the end of three weeks it was found that daily ingestion of honey for three weeks progressively and effectively reduced blood glucose level in rats with alloxan-induced diabetes. Honey also caused a reduction in hyperglycemia induced by long-term ingestion of fructose, albeit to a lesser degree than its effect on alloxan-induced hyperglycemia. Honey could not reduce blood glucose in controlled rats that received neither alloxan treatment nor fructose ingestion, even though it caused an increase in body weight, irrespective of other substances concomitantly administered to the rats. It is thus apparent that honey may be a useful adjunct in the management of diabetes, while serving as a sweetener, especially if taken in moderate quantities.

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Key Words: *Honey, Alloxan-induced diabetes, Fructose-induced diabetes, Rats*

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INTRODUCTION

The non-communicable chronic diseases prevalent in the developing countries of Tropical Africa include sickle cell disease, hypertension and diabetes mellitus. There is an increasing advocacy of alternate therapy in the management of these and other diseases, as the use of Western medicines are costly and at best are useful only for control of these diseases. Several alternate therapies include honey as an important component in the management of diabetes but the mechanism for its hypoglycaemic effect has not been clearly understood. Elevation of plasma insulin levels and lowering of blood glucose levels have been observed in patients with diabetes after administration of honey (Al-Waili N, 2003; Al-Waili NS, 2004). Other studies have also demonstrated that honey may be important in reduction of some biochemical markers that are linked to an increased risk of heart disease (Shambaugh *et al* 1990). Hyperlipidaemia and insulin resistance have also been shown to be better after consumption of honey (Katsilambros *et al* 1988).

Ingestion of high doses of fructose over a prolonged period has been used to induce persistent hyperglycaemia rats with features similar to those seen in patients with type 2 diabetes mellitus (DM), hence its use in type 2-like DM induction in animals (Ostos *et al* 2002). Natural honey contains a high concentration of fructose and so it is puzzling that honey would lower blood glucose levels in humans with DM. This study was thus designed to study the glycaemic effects of honey on alloxan-induced DM (akin to type 1 DM), and fructose-induced DM (akin to type 2 DM) in male Wistar rats. It is assumed that honey would not reduce the level of hyperglycaemia induced by fructose, even if it does in the rats with alloxan-induced DM. Attempts will be made to explain the differential effects thus elucidated.

MATERIALS AND METHODS

48 matured male Wistar rats, each weighing about 200g, were used for the study. They were allowed

2-week acclimatization to laboratory environment and were divided randomly into six groups of eight rats as follows:

- Group 1a served as control rats. They were given standard rat chow for three weeks.
- Group 1b rats were given honey along with standard rat chow for three weeks.
- Group 2a rats were administered alloxan according to standard procedure (Szkudelski *et al*, 1998) on day 1 of the study and those that developed diabetes after three days were thereafter given standard rat chow for three weeks.
- Group 2b rats were treated as those of group 2a but were given honey along with standard rat chow after diabetes induction with alloxan.
- Group 3a rats were given standard rat chow and fructose for three weeks.
- Group 3b rats were given standard rat chow fructose for three weeks. Thereafter honey was given along with standard rat chow and fructose for a further period of three weeks.

In effect, corresponding subgroup a served as control for subgroup b and group 1a also served as overall control for all the groups.

D-Fructose (BDH, Poole, England) with a molecular weight of 180.16 was used for the study. Each rat, regardless of weight, consumed a solution containing 6.6g of fructose/5ml of distilled water (through an oral cannula) daily.

The standard rat chow given was obtained from a commercial outlet in Ibadan, Nigeria as pellets, which contain 67.9% of starch, 21.0% of protein, 3.5% of fat, 6.0% of fiber, 0.8% of minerals and 0.8% of vitamins. Each rat consumed 3.4g of the pellets per day.

Fructose (67.9%) was substituted for starch in the rats that were given fructose (Comte *et al*, 2004; Grover *et al*, 2005).

Honey was obtained from, and certified pure by the Wildlife Unit of the Department of Forestry, University of Ibadan. Each rat on honey received a daily dose of 10ml honey/kg/5ml of distilled water (Busserolles *et al*, 2002) through an oral cannula.

Alloxan (Sigma Aldrich, St Quentin-Fallavier, France) with a pH of 7.0 was kept at 37°C before injection through a vein of the penis at a dose of 65mg alloxan/kg (Gruppuso *et al* 1990; Boylan *et*

al 1992) after a 24-hr fast. Prior, light anaesthesia was induced in the rats (with 0.6ml of 25%w/v/100g of rat) before intravenous administration of alloxan. Blood sugar was evaluated 72hr later to confirm effective induction of DM (as hperglycaemia).

The rats were weighed weekly from the start of the study (i.e. at the end of the two weeks of acclimatization to the laboratory environment), with a laboratory scale (Harvard Trip Balance, Florham Park, NJ, USA) to the nearest gram.

Blood was collected from the tail of the rats by nipping with a pair of fine scissors. Blood sugar was estimated from a drop of blood so collected with a glucometer (PRESTIGE SMART SYSTEM™, Home Diagnostics, Inc, Ft Lauderdale, USA). Glucose estimation was made weekly throughout the period of the study.

The measured glucose values and the weights of the rats were subjected to statistical analysis, using the SPSS v 11 statistical package and Microsoft Excel 2007. Data were expressed as mean ± SEM. Values were compared using student – t test and differences in the values were considered statistically significant when P<0.05.

RESULTS

Six of the rats in group 3a died before the end of the experiment and supplemental rats were provided and similarly treated until there were eight that stayed alive until the end of the study. Two rats died in group 2a but the rats in the other groups survived the period of the study.

Rats in groups 1a, 1b, 3a and 3b progressively gained weight, whereas those of groups 2a and 2b, i. e. the ones that had alloxan-induced diabetes had progressive weight loss after an initial slight weight increase. However, the alloxan-induced diabetic rats that were given honey had less weight loss. The weight changes of the rats are shown in Table 1.

Changes in the blood glucose levels in the rats are shown in Figure 1. There was a steady rise in the blood glucose level in all animal groups during the course of the study. In group 1a, the blood glucose rise was not significant. Addition of honey as supplement to the diet (group 1b) led to a steady significant rise in blood glucose. At the end of the third week, blood glucose had risen to double the value at the start of the study.

Table 1:
Changes in body weight (in grams) of the rats

Treatment Group	Body weight at basal level	Body weight at end of wk 1	Body weight at end of wk 2	Body weight at end of wk 3
1a – Control: on rat chow	197.04 ± 10.17	196.75 ± 22.91	220.87 ± 28.68	251.25 ± 36.02*
1b – Rat chow + honey	204.63 ± 12.94	243.87 ± 33.59#	285.37 ± 38.94*#	307.50 ± 40.69*#
2a – Alloxan treated + rat chow	219.72 ± 7.30	220.12 ± 9.04	215.62 ± 12.08	185.00 ± 14.51*
2b – Alloxan + rat chow + honey	211.62 ± 14.08	229.75 ± 20.78	220.12 ± 17.59	198.75 ± 15.97*
3a – Fructose + rat chow	207.11 ± 8.71	181.25 ± 8.75	198.37 ± 11.76	200.00 ± 13.49*
3b – Fructose + rat chow + honey	201.14 ± 9.32	213.75 ± 5.64#	237.37 ± 4.64*#	240.00 ± 11.95*#

All values are mean ± S.E.M, n (no of rats per group) = 8

* = statistically different from basal level (p<0.05)

= statistically different from corresponding values in subgroup a (p<0.05)

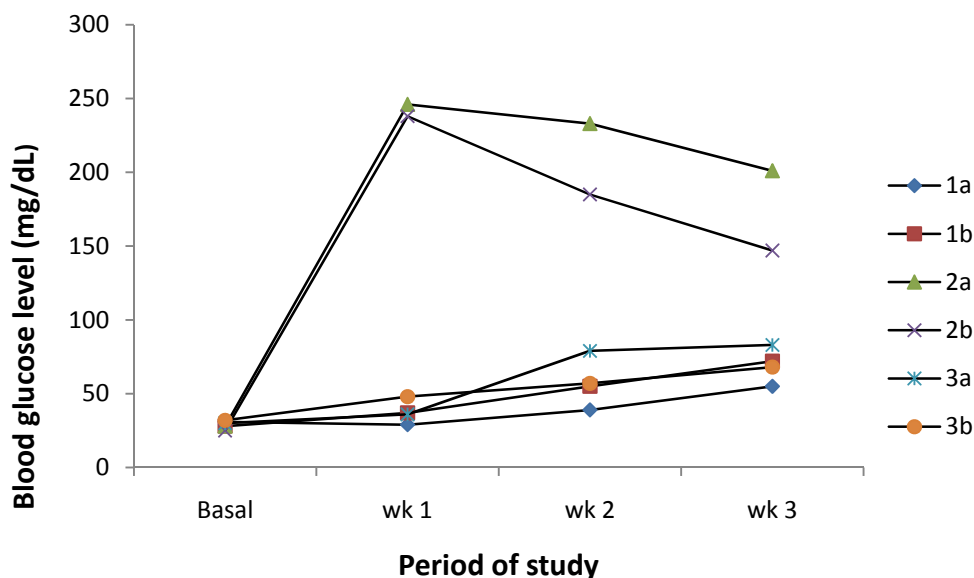


Figure 1.

Changes in blood glucose levels in the rats (1a – Control: on rat chow; 1b – Rat chow + honey; 2a – Alloxan treated + rat chow; 2b – Alloxan + rat chow + honey; 3a – Fructose + rat chow; 3b – Fructose + rat chow + honey)

The rise in blood glucose in group 2a rats was about 10 times the basal value. There was a slight drop at the end of week 2 and a further drop at the end of week 3, but the difference in values of the blood glucose at the end of each week was highly significant ($p < 0.01$). Addition of honey as a supplement to the diet of rats treated with alloxan (group 2b) caused a significant drop in blood glucose level. This fall was also steady from the start to the end of the study. The difference in corresponding blood glucose levels between groups 2a and 2b was significant ($p < 0.05$).

The rats that received fructose as part of their diet (group 3a) had a slight but steady rise in blood glucose levels, such that at the end of the third week, the blood glucose level had risen to double the basal value. Honey supplementation in the rats receiving fructose (group 3b) resulted in slight reduction in blood glucose levels. The reduction was, however, not statistically significant ($p > 0.05$).

DISCUSSION

Pancreatic β cell destruction with alloxan has been successfully used in the induction of type 1-like diabetes mellitus in laboratory animals (Szkudelski et al 1998). Induction of type 2-like diabetes has been more difficult. Most of type 2 diabetes models [e.g. Kyoto (York 2004); Koletsky (Takaya et al 1996); Ob/ob (York and Hansen 1997) Tubby Mouse (Coleman and Eicher 1990; Zucker (Harris et al 1987)] have been genetically derived. The method of (Comte et al 2004) used in this study is acceptable in that there was a significant hyperglycemia following chronic ingestion of fructose. It is not known, however, if the hyperglycemia so induced is a reflection of type 2-like diabetes.

Keeping the rats in the laboratory for a period of three weeks resulted in progressive increase in weight and blood glucose level, irrespective of treatment given to them. It could be that the regular consumption of the rat chow and aging

process are important factors to consider in this study.

It is apparent, however, that addition of honey, as a supplement to the diet results in further increase in weight in all the rats, irrespective of treatment, and a reduction in blood glucose level in the rats subjected to hyperglycemic maneuvers. Honey is widely used in medical practice, principally as a topical antibacterial agent and for effective healing of ulcers, irrespective of the cause of the ulcer (Dumronglert 1983). In treating diarrhea, honey promotes the rehydration of the body and more quickly clears up the diarrhea and any vomiting and stomach upsets. The antibacterial properties of honey, both the peroxide and non-peroxide, are effective against several strains of bacteria which are notoriously resistant to antibiotics (Hegggers 1987). Other topical uses of honey include treatment and healing of eczema and masking of acne (Green 1988). Health benefits of honey use include anti-allergic properties. Honey is also a sweetener, and in certain situations it has replaced sugar. This is especially so in patients with diabetes mellitus.

The distinct qualities of honey as a useful agent in medical practice may be due to its unique components. Honey is composed of minerals like magnesium, potassium, calcium, sodium chlorine, sulphur, copper, iodine, zinc, iron and phosphate. It also contains vitamins B1, B2, C, B6, B5 and B3, all of which change according to the qualities of the nectar and pollen. The anti-oxidant effects of honey (Gheldof et al 2002) would thus make it a useful adjunct in the management of diabetes mellitus.

The mechanism for the hypoglycemic effect of honey is, however, not well understood. Honey is a mixture of sugars – fructose (about 38.5%) and glucose (about 31.0%), maltose, sucrose and other complex carbohydrates. One would thus expect that consumption of honey would raise the blood sugar and that in fact the glycemic index of honey should approach that of glucose. The finding in several studies that honey causes a reduction in blood glucose levels in both normal and diabetic patients is an indication that honey has a mechanism, probably insulin sensitization effect. This has been suggested by Al – Waili (2004).

These studies were carried out over a period of few hours in contrast to the present study which spanned a period of three weeks. The failure of honey to reduce blood glucose to appreciable levels in the control rats (group 1a) despite a great reduction in rats made hyperglycemic in this study further buttresses the fact that the hypoglycemic effect of honey may be as a result of multifactorial mechanisms, including insulin sensitization and anti-oxidant activity. Otherwise similar effect should have been observed in the rats that were given fructose, a major constituent of honey, along with the standard rat chow, especially as fructose administration caused a significant weight gain in the rats, similar to the effect of honey on weight of the rats.

The results of animal experimentation may not be truly extrapolated to human situation, but the result of this study provides further evidence that honey consumption, at least in moderate quantities, may be a useful adjunct in the management of diabetes mellitus.

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