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Original article

Evaluation of the hypoglycaemic effect of honey in alloxan-induced diabetic wistar rats

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A R T I C L E   I N F O

A B S T R A C T

The use of honey in diabetes have been highly controversial, although studies have shown that honey may be beneficial in diabetic subject because of its low glycaemic index, other researchers have disagreed, largely because it is a source of dietary carbohydrate. This study was designed to determine the hypoglycaemic effect of honey in experimentally induced-diabetic and normoglycemic Wistar rats. Fifty (50) healthy adult rats of both sexes with average weight of 180 g were used for this study. They were assigned into two groups: Diabetic and Normoglycaemic group, each group was sub grouped into five groups of five animals each. Hyperglycemia was induced in the diabetic group by single intraperitoneal injection of 150 mg/kg b w of alloxan dissolved in cold normal saline (0.9%). 72 hours later blood sample was collected and blood glucose measured using one touch glucometer. Rats with blood glucose level greater than 200 mg/dl were considered to be diabetic. Varying doses of honey (250, 500, and 1000 mg/kg b w) were administered orally once daily to both groups. The result obtained showed significant decrease ($p<0.05$) in blood glucose levels in the diabetic animals that received all doses of honey when compared to the control animals. The results obtained in the normoglycaemic group showed no significant difference ($p>0.05$) in

blood glucose levels between the control and the animals administered with graded doses of honey when compared. The result of this study demonstrated that honey possesses anti-hyperglycemic activities in wistar rats and can be recommended to diabetics and non diabetics for its beneficial effect on glycaemic control.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder of impaired carbohydrates, fat and protein metabolism. It is characterized by hyperglycaemia expressed as abnormal glucose value, which is due to insulin deficiency and/or insulin resistance which results in decrease utilization of carbohydrate and excessive glycogenolysis and gluconeogenesis from amino acid by fatty acids (Guyton and Hall, 2004; Pontiroli *et al.,* 1994). This disease is on the increase all over the world, especially in Africa where it was formerly regarded as the disease of the affluent (Amos *et al.,* 1997). Recent estimates indicate there were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 366 million by 2030 (WHO, 2007). Diabetes is associated with reduced life expectancy, significant mortality and diminished quality of life, in 2005 an estimated 1.1 million people died from diabetes. Mortality and morbidity has increased in diabetes, due to the associated chronic complications; specific microvascular retinopathy, nephropathy and non-specific macrovascular artherosclerosis. Acute metabolic complications (e.g. diabetic ketoacidosis) continue to be a cause of mortality in developing countries (Ayesha *et al.,* 2002). The increased rates of mortality and morbidity are associated with high blood glucose levels and high glycaemic index (GI) diets in both diabetic and non-diabetic patients (Chepulis and Starkly, 2008). It is now recognized that dietary carbohydrate components influence the prevalence and severity of diabetes. Renewed attention to alternative medicines and natural therapies has stimulated a new wave of research interest into traditional practice and nutrition-oriented interventions. Furthermore, natural preparations and herbal products are preferred in developing countries, because they are cheaper than conventional medicines and have lesser side effects associated with them (Alzahrani and Bakhotmah, 2010). Honey is one of the oldest known medicines, and sweetener. It is a carbohydrate – rich syrup produced by bees, primarily from floral nectars (Honey Scientific Report, 1999). Fructose and glucose are the major components but a larger number of other chemical compounds are present, its moisture content and water activity are low. For a long time in human history it was an important carbohydrate source and the only largely available sweetener until industrial sugar production began to replace it after 1800 (Crane, 1975). Honey was highly valued in the Middle East. It is mainly used in Islamic culture, it was mentioned in the Holy Qur'an fourteen hundred years ago (1400) in Surah Al-Naml, verse 68-69 as a cure for human beings irrespective of their race, colour, religion or beliefs (Kandil *et al*., 1987). Honey has been used for treatment of respiratory diseases, urinary diseases, gastrointestinal diseases, skin ulcers, psoriasis (Zaghlow *et al.,* 2001, Al-waili *et al.,* 1999 and Molan, 1999). Honey is also known to have an inhibitory effect on aerobic and anaerobic bacteria, yeast, fungi and viruses (Molan, 1999; Zaina *et al.,* 1990; Al-Waili *et al.,* 2002a; Al-wail, 2001b; Al waili, 2000c). Honey is reported to reduce inflammation, edema, and exudation, promotes healing and stimulates tissue regeneration, accelerates wound healing and improves epithelial growth (Molan, 1999; Oryan and Zakar, 1998; Bergman *et al.,* 1983). Honey has been acknowledged to be a cure to many diseases, with effect to this claims, research works have been done on the medicinal uses of honey, and have confirmed its beneficial uses, but the claims on the use of honey in the management diabetes mellitus has not been clearly elucidated. This study aims to evaluate the hypoglycemic effect of honey in alloxan-induced diabetic wistar rats.

2. Materials and methods

2.1. Animals

Fifty (50) Wistar strain albino rats of both sexes weighing between 110-260 grams, were obtained from the Department of Biology, Federal College of Education (F.C.E.)., Zaria. The rats were acclimatized and maintained

under laboratory condition of temperature, humidity and light for two weeks. The animals were randomly divided into two groups of 25 rats each (experimental and control).

2.2. Chemicals

Alloxan monohydrate purchased from Sigma-Aldrich (Jos, Nigeria), Glibenclamide (Gliben-clamide[®], B.P 5mg, NAFDAC Reg. No 04-7261) and Glucose were purchased from a reputable pharmacy. All chemicals were of analytical grade.

2.3. Honey

Honey was obtained from Herbal point, Zaria, Nigeria. It was evaluated at the Beekeeping Extension Society, Zaria to have a moisture content of 18.7% certifying it to be pure, unadulterated honey. 2g of honey was diluted with 10 ml distilled water and a light brown liquid was obtained. Honey was administered to the animals per body weight.

2.4. Induction of experimental diabetes mellitus

All the animals were fasted for 12 hours, but were allowed free access to water, before commencement of the experiments. The rats were injected with alloxan dissolved in cold normal saline (0.9%) at a dose of 150mg/kg intraperitoneally (Al-shamanoy *et al.,* 1994). Since alloxan is capable of producing fatal hypoglycaemia as a result of the intense pancreatic insulin release, rats were treated with 20% glucose solution orally after 6 hours. The rats were then kept for 24 hours on 5% glucose solution in their cages to prevent hyperglycaemia (Gupta *et al.,* 1984). After period of 72 hours the rats with blood glucose level greater than 200 mg/dl were considered diabetic and were used for this study.

2.5. Experimental design

After induction of diabetes in the Wistar rats, the animals were randomly assigned to 5 groups of 5 rats each as follows:

2.5.1. Diabetic group

Group 1: received 1000mg/kg body weight of honey orally.
Group 2: received 500mg/kg body weight of honey orally.
Group 3: received 250mg/kg body weight of honey orally.
Group 4: received 5mg/kg body weight of Glibenclamide orally.
Group 5: received Normal saline orally.

2.5.2. Normoglycaemic group

Group 1: received 1000mg/kg body weight of honey orally.
Group 2: received 500mg/kg body weight of honey orally.
Group 3: received 250mg/kg body weight of honey orally.
Group 4: received 5mg/kg body weight of Glibenclamide orally.
Group 5: received Normal saline orally.
Fasting blood glucose level was taken for all the groups, before administration of the treatment doses.

2.6. Determination of blood glucose level

All blood samples were collected from the tail vein of the rats at interval of 7 days within the two weeks of study. Determination of the blood glucose levels was done by the glucose-oxidase principle (Beach and Turner, 1950) using the one touch basic (lifescan, mulpital CA instrument) and the result were expressed as mg/dl (Rheney and Kirk, 2000).

2.7. Statistical analysis

Blood glucose levels were expressed in mg/dl as mean ± SEM. The data were statistically analyzed using ANOVA followed by Tukey's post-hoc test. The values of $p < 0.05$ were considered as significant.

3. Results

Figure 1 shows the blood glucose level for the diabetic group after treatment with honey for two weeks. Statistical analysis using ANOVA revealed that the treatment with honey caused a significant decrease in the blood glucose levels after first week (7 days) and second week (14 days) when compared to the blood glucose level before treatment. Mean blood glucose levels for group 1 that received 1000 mg/kg of honey were 323 ± 27 mg/dl at the onset of the study, 120 ± 20 mg/dl after one week and 66 ± 15 mg/dl after two weeks. For the group 2 that received 500 mg/kg of pure honey, mean blood glucose level were 313 ± 20 mg/dl before treatment, 192 ± 38 mg/dl and 66 ± 38 mg/dl after first and second week of treatment respectively. The mean and SEM for the group 3 that received 250 mg/kg of pure honey, were 334 ± 41 mg/dl before treatment, 234 ± 25 mg/dl after one week of treatment and 93 ± 5 mg/dl after second week of treatment. For the group 4 that received glibenclamide, the mean and SEM were, 331 ± 25 mg/dl before treatment, 233 ± 21 mg/dl after first week of treatment and 155 ± 24 mg/dl second week of treatment. The mean and SEM for the group 5 received normal saline, were 304 ± 18 mg/dl before treatment , 280 ± 26 mg/dl and 257 ± 29 mg/dl after first week and second week of treatment respectively.

Figure 2 shows the blood glucose level normoglycaemic group after treatment with honey for two weeks. Results obtained revealed that honey had no significant effect on blood glucose level in normoglycemic wistar rats. The mean and SEM for the group 1 received that 1000 mg/kg of honey were 65 ± 2.0 mg/dl before treatment, 62 ± 1.0 mg/dl and 53 ± 3.0 mg/dl after treatment for the first and second week respectively. For the group 2 that received 500 mg/kg of honey, the mean and SEM, were 67 ± 3.0 mg/dl before treatment, 63 ± 1.0 mg/dl and 51 ± 2.0 mg/dl after first and second week of treatment respectively. The mean and SEM for the group 3 received 250 mg/kg of honey, were 66 ± 3.0 mg/dl before treatment, 47 ± 6.0 mg/dl after one week of treatment and 53 ± 4.0 mg/dl after the second week of treatment. For the group 4 that received glibenclamide, the mean and SEM were, 64 ± 4.0 mg/dl before treatment, 51 ± 5.0 mg/dl after first week of treatment and 44 ± 3.0 mg/dl second week of treatment. The mean and SEM for the group 5 received normal saline, were 61 ± 1.0 mg/dl before treatment, 61 ± 5.0 mg/dl and 42 ± 2.0 mg/dl after first week and second week of treatment respectively.

4. Discussion

Poor glycaemic control is an important factor in the pathogenesis of Diabetes mellitus. Recent research shows that diet containing high glycaemic index diet may be detrimental to health because of prolonged hyperglycaemia. Studies have also shown that high glycaemic index diet has been associated with high mortality and morbidity rates in diabetics and non-diabetics. (Chepulis and Starkey,2008). Low glycaemic index foods are now being suggested as a replacement for high glycaemic index foods as they induce a lower glycemic response, and this is thought to equate to a lower insulin demand, better long-term blood glucose control, and a reduction in blood lipids (Brand-Miller 2003). The results of this study appear to demonstrate the beneficial effect associated with the ingestion of honey by both diabetic and non-diabetics. Blood glucose level was considerably reduced in diabetic animals after two weeks of administration with honey. The decrease in blood glucose level was observed to be dose and time dependent, with the most effective response seen in the group administered the highest dose of honey (1000 mg/kg) . The reduction in the blood glucose levels honey administered groups were also shown to be lower than the group receiving glibenclamide. This result substantiates evidence that honey is beneficial in type I and type II diabetes (Al-Waili, 2004). Alloxan administration induces diabetes by destroying the beta cells of the islet of Langerhans of the pancreas leading to reduction in the synthesis and release of insulin, thereby inducing hyperglycaemia leaving residual or less active beta cells (Szkudelski, 2001). Results obtained from this study suggest regenerative properties of honey on the islets of langerhan, thus serving as a nutritional-diet based therapeutic interventions in diabetes. Although, definite mechanism and explanation on how honey reduces blood glucose levels and its regenerative property on pancreatic cells still remain unclear and is subject to further investigations, possible suggestions may be associated with its antioxidative properties, since Alloxan have been reported to induce tissue damage by causing generation of reactive oxygen species similarly found in Type I diabetes (Eze *et al*., 2012). Furthermore, the hydrogen peroxide ($H_2O_{2)}$ produced when honey is dissolved in water, helps in stimulating the remaining β-cells to secrete insulin which suggest and further provides possible mechanism of the hypoglycaemic activity of honey could be used in diabetes. The hypoglycaemic effect of honey recorded in this study, is consistent with results by Oztasan *et al.* (2005) who reported that, mad honey decreases blood glucose and lipid levels in streptozotocin-induced diabetic Wistar rats. Al-Waili (2004) also demonstrated that the use of honey was associated with significantly low glycaemic index and significantly lowered blood glucose levels in diabetic subjects.
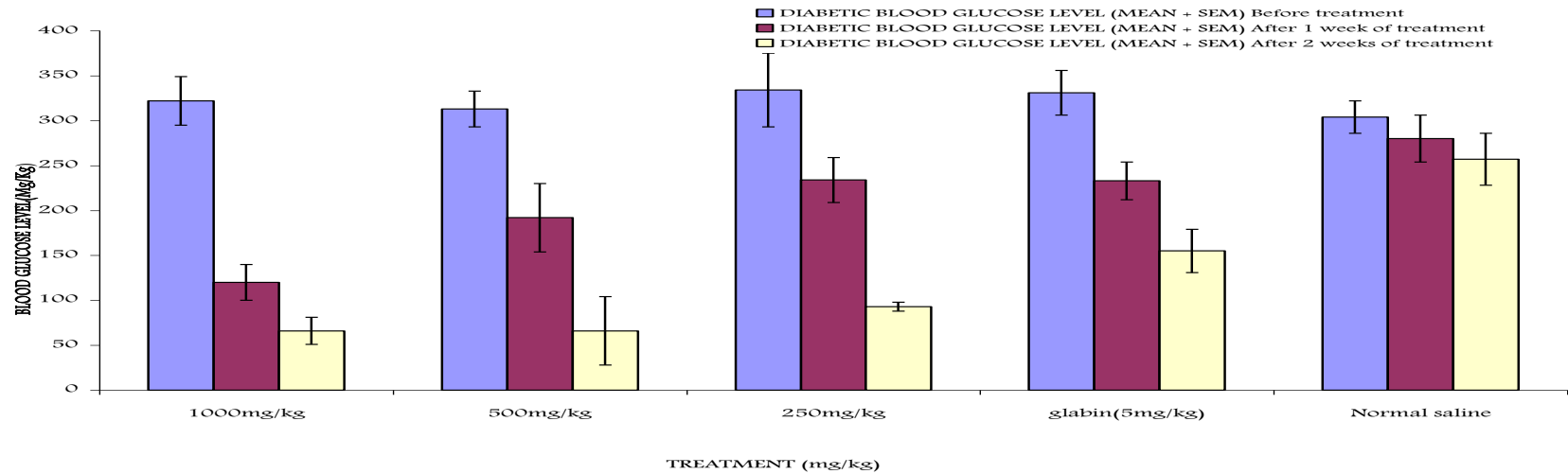
Fig. 1. Significant decrease in blood glucose level (Mean ± SEM) in diabetic Wistar rats after two weeks of treatment with honey, p<0.05 is significant when compared to control group.
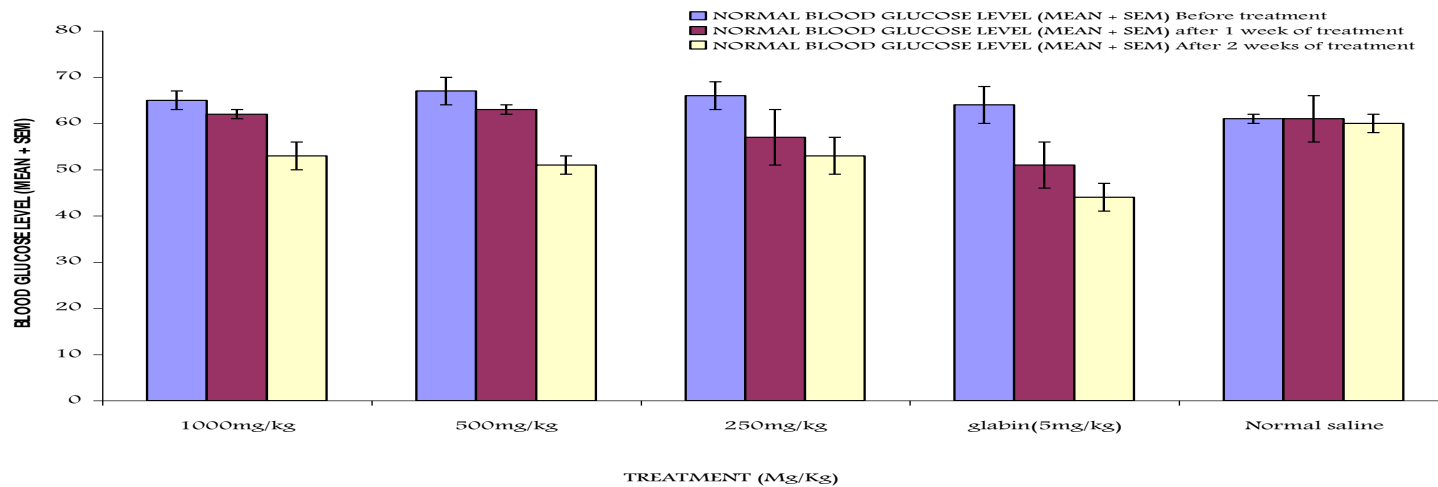

Fig. 2. Blood glucose level (Mean ± SEM) of Normoglycaemic Wistar rats administered honey for two weeks (14 days), p < 0.05 is significant when compared to control group.

This result agrees with the study carried out on normoglycemic wistar rats by Akhtar and Khan, (1989) and Isa *et al*., (2012) who reported that pure honey did not significantly decrease in blood glucose level in an non-diabetic Wistar rats, which suggest that honey could be used in diabetes, with beneficial effects as well serve as substitute for sugar. It also suggested that honey has a gentler effect on glycemic responds and can therefore be appropriate for glycemic control and may be recommended as source of carbohydrate and good for patient suffering from diabetes.

5. Conclusion

The result of this study demonstrated that honey possesses anti-hyperglycemic activities in wistar rats and can be recommended to diabetics and non diabetics for its beneficial effect on glycaemic control.

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