ABSTRACT

**Purpose:** The study investigated honey's hepatoprotective effect and lipid profile on alloxan-induced diabetic rats.

**Methods:** Thirty-six (36) male Wistar rats were assigned into six (6) groups with six (6) animals each, group 1 (Normal control), group 2 (Negative control), group 3 (Glibenclamide), group 4 (treated with 0.2mls of honey) group 5, (treated with 0.5mls of honey and group 6 (treated with 0.8mls of honey). The rats were fed with standard feed and drinking water ad libitum. The diabetic control, diabetic glibenclamide and the treated groups (0.2 mL/kg, 0.5 mL/kg and 0.8 mL/kg) were induced with diabetes by intraperitoneal injection of 120 mg/kg bodyweight alloxan monohydrate, and confirmation was done using a glucometer. Treatment lasted for three weeks (21 days), after which rats were sacrificed by cervical dislocation under light ether anaesthesia. Blood was collected for biochemical evaluation using standard techniques (Randox kits). Blood glucose levels were monitored on days 0, 7, 14, and 21.

**Results:** Administration of honey to the diabetic rats significantly reduced (p < 0.05) glucose level (119.50 mg/dl), total cholesterol (TC), triglyceride (49.5 mg/dl) and low-density lipoprotein cholesterol (LDL-C), while significantly increasing (p < 0.05) high-density lipoprotein cholesterol (HDL-C) when compared to the diabetic untreated rats. Liver enzyme parameters: alanine
Keywords: Alloxan monohydrate; diabetes; glibenclamide, lipid profile; liver function.

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder that has emerged as a significant challenge in the 21st century which its incidence is on the rise globally, particularly in Africa [1]. This metabolic disorder affects protein, lipid, and carbohydrate metabolism, which are essential biochemical pathways of the body [2]. Diabetic status can result in additional metabolic abnormalities and complications, including dyslipidemia, hepatomegaly, liver disease, weight loss, renal disease, and coma [3]. Excess glycoprotein in the hepatic tissue may cause diseases in patients with diabetes. Patients who only have excessive glycogen deposition can suffer from hepatomegaly and liver enzyme abnormality, which can be improved with sustained glucose control. In the untreated diabetic population, the activities of liver damage markers, including serum alanine aminotransferase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP), are increased [4]. Diabetes-related complications include diabetic nephropathy and renal dysfunction, and about 20-40% of patients with diabetes (type 1 and type 2) develop nephropathy [4].

Serum lipids abnormalities are also associated with diabetes [5]. Previous research has linked high levels of total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and low concentrations of high-density lipoprotein cholesterol (HDL-C) to coronary heart disease [5]. Current stream treatment modalities utilizing chemo-drugs such as metformin and sulfonylurea dissimulate multidrug resistance and other side effects, including; gastrointestinal effect, body fluid accumulation and heart disease [6]. This urges the quest for alternate options that will be efficaciously safe and harmless to reduce sugar levels and ameliorate other diabetic complications. Natural products are considered a practical alternative, and recently honey has caught the interest of researchers as an alternative therapeutic agent [7].

Honey is one of the oldest known medicines and sweeteners. It is a natural product formed from the nectar of flowers by honeybees (Family: Apidae). It’s thought to be one of the remaining unprocessed natural foods [8]. Honey, which comprises monosaccharides and oligosaccharides predominantly, contains 181 constituents [9]. Honey includes roughly 200 components, according to Rahman et al. [10], glucose, fructose, amino acids, flavonoid, vitamins, and minerals. Honey contains several enzymes, including glucose oxidase, diastase, invertase, phosphatase, catalase, and peroxidase [11]. The use of honey in folk medicine dates back to 2100-2000 BC 4, [12]. Most of the health advantages attributed to honey in the past were based on hunches or generalizations with little scientific support [13]. In recent years, however, there has been a revived interest in studying the potential health advantages of natural and unprocessed honey in managing numerous disorders. As a result, various medical properties of honey have been discovered. Cardioprotective [14], hepatoprotective [15], hypoglycemic [16], antioxidant [17], and antihypertensive [18] actions are among them. Honey’s antifungal [19], antiviral [20], anti-inflammatory [21], and anticancer properties have also been documented and attributed to it [22].

2. MATERIALS AND METHODS

The fresh honey was bought from Fibers Global Farms, Isuochi in Umunneochi Local Government Area of Abia State. It was evaluated at the Beekeeping Extension Society, Umuahia, Abia state, to have a moisture content of 18.7% certifying it to be pure, unadulterated honey.

2.1 Experimental Animals

Thirty-six (36) male Wistar rats (210-250g) purchased from Dr Daniel of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, were used for this study. The animals were acclimatized for two weeks and kept under natural conditions, including 12 h light and 12 h dark throughout the investigation, with free access to pellet feed and water ad libitum.
2.2 Induction of Diabetes

After acclimatization, the animals were given an 8-hour fast before receiving an intraperitoneal (IP) injection of 120 mg/kg body weight of alloxan monohydrate solution to induce diabetes. After three days of induction using the fasting blood sugar method, animals with blood glucose levels more than 150 mg/dl were declared diabetic and were chosen for the study.

2.3 Experimental Design

Rats were divided into six groups of six rats each: Group 1 (Normal control), group 2 (Negative control), group 3 (Positive/Glibenclamide), group 4 (animals treated with 0.2mls of honey), group 5 (animals treated with 0.5mls of honey), group 6 (animals treated with 0.8mls of honey) respectively. Glucose level was observed on days 0, 7th, 14th and 21st. Honey was administered orally twice for the three weeks treatment. Normal control received only food and water; Alloxan induced negative control, positive control was induced by Alloxan and treated with glibenclamide, while groups 4, 5 and 6 were induced by Alloxan, confirmed diabetic and were treated with 0.2mls 0.5mls and 0.8mls of honey respectively after 72 hours of induction. Examination of blood glucose level was performed on days 0, 7, 14 and 21.

2.4 Preparation of Drugs

Glibenclamide (500mg) manufactured by Vee Excel Drugs, and Pharmaceuticals Private Limited, India, purchased from Ludino Pharmacy shop, Ahiake, Umuahia Abia State, was made ready by mashing the tablet in a glass mortar and dissolved in distilled water (1ml) to produce a 500mg/ml solution. Glibenclamide was given orally to the animals at 500 mg/kg.

2.5 Collection and Preparation of Sera Samples

The study period lasted three (3) weeks, after which the rats fasted for 8hrs. Then rats were sacrificed by exposing them to an overdose of chloroform soaked in cotton wool placed in an anaesthetic box covered with a lid. Blood samples were drawn from the heart of each sacrificed rat from all groups by puncture, and blood samples were collected in EDTA specimen bottles. The clear serum was obtained by centrifuging the whole blood and estimating AST, ALT, ALP, Total Bilirubin and Total protein.

2.6 Determination of Liver Function Markers

2.6.1 Determination of Aspartate Aminotransferase Activity

The method of Reitman and Frankel [23]. described by Randox laboratories, the United Kingdom using Randox kits, was used for this study.

Principle: The activity was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine.

2.6.2 Determination of Alanine Aminotransferase Activity

This was also done using the method of Reitman and Frankel [23].

Principle: The activity was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4, -dinitrophenylhydrazine.

| Groups | Descriptions | Treatments |
|--------|--------------|------------|
| 1      | Normal control rats | Normal saline and feed only |
| 2      | Negative control rats | Alloxan (120 mg/kg, i.p.) untreated |
| 3      | Positive control rats | Alloxan (120 mg/kg, i.p.) + 500mg/kg/day glibenclamide |
| 4      | Diabetic treated rats | Alloxan (120 mg/kg, i.p.) + 0.2 mL/kg/day honey |
| 5      | Diabetic treated rats | Alloxan (120 mg/kg, i.p.) + 0.5 mL/kg/day honey |
| 6      | Diabetic treated rats | Alloxan (120 mg/kg, i.p.) + 0.8 mL/kg/day honey |
2.6.3 Determination of Alkaline Phosphatase Activity

The activities of alkaline phosphatase were evaluated using the methods of Kind and King [24].

**Principle:** Serum alkaline phosphatase hydrolysis yields a colourless substrate of phenolphthalein monophosphate, giving rise to phosphoric acid and phenolphthalein, which at alkaline pH, turns pink and can be determined photometrically.

2.6.4 Determination of Total Protein Estimation Activity

Total protein estimation was assayed using the direct Biuret method [25].

**Principle:** At alkaline pH value, proteins form a blue coloured complex with copper II ions which is photometrically measured.

2.6.5 Determination of Total and Conjugated Bilirubin Activity

Total and conjugated bilirubin levels were determined according to the Jendrasik and Grof method [26]. Direct (conjugated) bilirubin reacts with diazotized sulphanilic acid in an alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin-bound bilirubin by the reaction with diazotized sulphanilic acid.

2.7 Determination of Lipid Profile Biomarkers

2.7.1 Determination of total cholesterol concentration

Total cholesterol was determined using the enzymatic colourimetricchoc-pad test method described by Allain et al. [27], with Randox laboratory test kits.

**Principle:** The sample’s free and esterified cholesterol originates utilizing a coupled reaction where serum cholesterol reacts with enzymes to produce a coloured complex whose intensity is proportional to the serum cholesterol concentration and is measured spectrophotometrically.

2.7.2 Determination of Triglycerides Concentration

This was also determined spectrophotometrically using the method of Tietz [28].

2.7.3 Determination of High-density Lipoprotein Concentration

This was evaluated by Grove [29] methods, as described in the Randox Laboratory test kit.

**Principle:** It involves a precipitation reaction with phosphotungstate and magnesium ion where the supernatant contains HDL, which is measured spectrophotometrically.

2.7.4 Determination of Low-Density Lipoprotein Cholesterol Concentration

Low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald’s equation (Friedewald et al., 1972). LDL-C = [TC – (HDL-C + (TG/5))] where VLDL-C = (TG/5) [30].

2.7.5 Determination of Very Low-Density Lipoprotein Cholesterol Concentration

This was calculated according to the method of Wilson et al. (1981) as VLDL = 0.2 x TG (where TG is total glycerides)

2.8 Statistical Analysis

Data obtained was expressed as mean ± SD and statistically analyzed using one-way analysis of variance (ANOVA) with Turkey’s multiple comparison post hoc tests to compare the level of significance between the test groups. The values of p<0.05 were considered significant.

3. RESULT

The data for the blood glucose level presented in fig. 1 showed that the animals in the diabetic control group experienced severe hyperglycemia compared to normal animals. The blood glucose level in the diabetic control group was 360.00±5.6 mg/dl on day 0 and 383.2±3.6 mg/dl on day 21. The standard drug glibenclamide was shown to reduce the blood glucose level significantly whereas oral administration of honey at 0.2mL/kg, 0.5mL/kg, and 0.8mL/kg significantly (P < 0.05) decreased the fasting blood serum glucose level in the diabetic rats on day 14, and day 21 as compared to the diabetic control group.
Fig. 1. Effect of Honey on blood glucose levels on days 0, 7th, 14th and 21st. Values are expressed as mean±SEM (n=6). *p<0.05 when compared with the negative control.

Fig. 2. Effect of Honey on Liver function parameter level. Values are expressed as mean±SEM (n=6). *p<0.05 when compared with the negative control. AST: Aspartate Transaminase, ALT: Alanine Transaminase ALP: Alkaline Phosphatase

Data for liver function markers is shown in fig. 2. AST and ALT activities were significantly increased in the diabetic control animals compared to the normal animals. At 0.2mL/kg, 0.5mL/kg, and 0.8mL/kg, honey decreased AST and lowered ALT activity compared to the diabetic control animals. The diabetic control animals showed a significant reduction in ALP activity compared to the normal control animals, whereas honey administration at 0.2mL/kg, and 0.8mL/kg, significantly elevated ALP activity compared to the diabetic control animals. The Glibenclamide-treated animals also showed significantly higher ALP activity than the diabetic control.
Data for bilirubin level is shown in fig. 3. TBIL, DBIL, and CBIL activities were non-significantly (p >0.05) decreased in the diabetic control animals compared to the normal animals. 0.2mL/kg, 0.5mL/kg, and 0.8mL/kg, honey elevated TBIL, DBIL, and CBIL activity compared to the diabetic control animals. The Glibenclamide-treated animals showed significantly higher TBIL, DBIL, and CBIL activity than the diabetic control.

Fig. 3. Effect of Honey on Bilirubin level
Values are expressed as mean±SEM (n=6). *p<0.05 when compared with the negative control. TBIL: Total Bilirubin, DBIL: Direct Bilirubin, CBIL: Conjugated Bilirubin

Fig. 4. Effect of Honey on Lipid function parameter level
Values are expressed as mean±SEM (n=6). *p<0.05 when compared with the negative control. CHOL: Cholesterol, TRIG: Triglyceride, HDL: High-Density Lipoprotein, VLDL: Very-Low lipoprotein, LDL: Low-Density Lipoprotein
Data for lipid function markers is shown in Fig. 4. The diabetic control animals showed a non-significantly elevated serum triglyceride and VLDL compared to the normal animals. At 0.2mL/kg, 0.5mL/kg, and 0.8mL/kg, honey was able to significantly (P<0.05) reduce serum triglyceride and VLDL in the animals compared to diabetic control. HDL and LDL activities were significantly reduced in the diabetic control animals compared to the normal animals. At 0.2mL/kg, 0.5mL/kg, and 0.8mL/kg, honey significantly increased HDL and LDL activities compared to the diabetic control animals. The Glibenclamide-treated animals also showed significantly higher ALP activity than the diabetic control. Serum cholesterol concentration was reduced significantly in the diabetic rats compared to the normal animals, and this was however reversed significantly in the animals treated with 0.2mL/kg, 0.5mL/kg, and 0.8mL/kg, honey.

4. DISCUSSION

Diabetes mellitus are chronic diseases that occur due to inadequate insulin supply, which results in multiple changes in the body's metabolism. This chronic disease has drastically increased in searching for natural remedies to manage the disease. Thus, this study investigated honey's hepatoprotective and lipid profile effects in alloxan-induced diabetic rats.

In the present study, honey treatment significantly (P <0.05) decreased blood glucose levels in diabetic rats. These findings concur with the previous results of Song et al. [31], which demonstrated the glucose-lowering effect of honey in diabetic rats and diabetic patients. Honey's anti-hyperglycemic activity can be due to several nutritious ingredients such as Fructose and flavonoids, which increase insulin secretion in people with diabetes and reduce blood glucose levels [32]. Fructose in honey can catalyze the conversion of glucose into glucose-6 phosphate, lowering blood glucose levels. Moreover, fructose can stimulate insulin secretion from pancreatic cells. Blood glucose and fructose levels have increased hepatic glucose phosphorylation by activating glucokinase and inhibiting glycolysis by emphasizing phosphorylase [15].

In diabetic rats, serum lipids such as triglycerides were higher. This is because insulin activates lipoprotein lipase, hydrolyzes triglycerides [23], and inhibits lipolysis. In diabetes, however, there is an increase in lipolysis, which eventually leads to hyperlipidemia. From our study, the administration of honey significantly decreased (p <0.05) the serum triglyceride and serum very-low-density lipoprotein levels and significant increase (p <0.05) total serum cholesterol and the serum high-density lipoprotein in groups treated with honey when compared to the untreated diabetic control group.

Honey administration was found to dramatically lower high TGs and HDL cholesterol (VLDL, LDL, and cholesterol fractions). This is significant because elevated non-HDL cholesterol and hypertriglyceridemia, together with abnormal glucose metabolism, raise the risk of cardiovascular disease (CVD) [33]. As a result, the significant ameliorative effects of honey on TGs and non-HDL cholesterol suggest that honey can reduce CVD risk.

Liver disease in diabetes mellitus is caused by overworking the liver, which is responsible for maintaining normal glucose levels by storing excess glucose as glycogen. Because the cells are resistant to insulin, the liver is overworked by producing more glucose [34]. The toxic effect of Alloxan on the liver could cause it to malfunction.

In the present study, the serum elevation of liver damage biomarkers occurred due to the deleterious effect of hyperglycemia in the liver of diabetic rats. The increase in activities of these enzymes can be attributed to alloxan toxicity which leads to liver damage. However, the dose-dependent (0.2, 0.5 and 0.8mg/kg) treatment with honey for 21 consecutive days significantly (P <0.05) decreases the above enzymes’ activities. Thapa and Anuj [35] reported that ALT (10 – 55 µ/L), AST (10 – 40 µ/L), and ALP (45 – 115 µ/L) are the standard range of accepted values for liver function tests, beyond which liver disease can be suspected. Kamal and Hessah (2015) confirmed this by stating that increases in AST, ALT and ALP values above these thresholds indicate early hepatotoxicity and tissue damage detection.

From our findings, liver enzyme parameters differentials (AST, ALT, ALP, TBIL, CBIL and DBIL) were found to remain within normal range after the administration of honey in all the treated groups, which could suggest the hepatoprotective effects of the alloxan-induced Wistar rats, which is in tandem with Al-waili [18].
5. CONCLUSION

The findings in this study indicate that oral administration of honey at all doses significantly decreases the levels of blood glucose, liver function parameters, and decreased hyperlipidemia associated with diabetes. However, we recommend that further studies be carried out to determine the biomolecules that cause this effect and their mechanism of action.

DISCLAIMER

The products used for this research are commonly and predominantly used in our research area and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company; rather, it was funded by the personal efforts of the authors.

ETHICAL APPROVAL

The study was conducted by following the National Institute of Health guidelines, the USA, as approved by the College of veterinary medicine, Michael Okpara University of Agriculture, Umudike. The ethical committee's reference number is: MOUAU/CVM/REC/202015

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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