INTRODUCTION

Diabetic mellitus (DM) has long been defined as a heterogeneous group of metabolic disorders characterized by glucose intolerance and fasting hyperglycemia [1]. Type 1 DM accounts for approximately 10% of all cases and it is essentially immune-mediated with Type 2 DM afflicting about 6% of adult population, and it is essentially a result of insulin resistance and impaired β-cell function [2]. Besides fasting hyperglycemia, several organs develop complications in diabetes. Key organs in this respect include the kidney (nephropathy), eye (retinopathy), brain and peripheral nerves (neuropathy), liver (glycogen storage disease and steatohepatitis), blood vessels (atherosclerosis and microangiopathy) [3-5].

In long-standing DM, the morphology and function of the liver are disturbed. Liver biopsy findings in Type 1 diabetics with hepatomegaly are comparable to hepatic findings in Mauriac’s syndrome and include marked glycogen deposition in hepatocytes [6]. Clark and Diehl [7] reported that in Type 2 diabetes, impaired insulin action usually result in non-alcoholic fatty liver disease, including steatosis and steatohepatitis.

There is ample evidence of an important role of oxidative and glycooxidative stress in the pathogenesis of diabetic complications [8]. The exact contribution of antioxidant enzyme to oxidative stress in diabetes is not fully understood. Learning and memory impairment has also been associated with oxidative stress in streptozotocin-induced diabetes rats [9].

Propolis is a natural product derived from plant resins and collected by honeybees (workers) to be used as glue and as draught-extruder for beehives [10]. Honey another product derived from honeybees was almost the only source of sugar available to the ancients and was valued for its medicinal benefits. It has been reported that propolis contain at least 200 compounds with more than 100 being present in any given sample [11]. These include fatty and phenolic acids and esters, substituted phenolic esters, bioflavonoids (flavones, flavanones, flavonols and others), terpenes, β-steroids, aromatic aldehydes and alcohols, and derivatives of sesquiterpenes, naphthalene and stilbenes [12-14].

The main types of flavonoids that have been reported include rutin (an antihypertensive agent), quercetin (a potent anti-diabetic material), galangin [15] and caffeic acid phenethyl.
ester [16]. These entire constituents give propolis the ability to perform many functions. Propolis is reported to have hepatoprotective [17], antioxidant [18], antimicrobial [19], anti-inflammatory [19] and anticancer [20] properties.

Several factors are responsible for the variability in the propolis chemical components which includes: Unpredictability of growing plant species around the beehive, condition of the climate, beekeeper actions and nature of the soil [21]. Propolis has been reported to be geographically sensitive and each area has its own peculiar constituent [10]. Gómez-Caravaca et al. [22] reported that despite geographical differences, most propolis samples contain 50% resin, 30% wax, 10% essential oils, 5% pollen and 5% of other organic compounds in their chemical composition. Several studies have been carried out to examine the effects of propolis from different geographical regions on experimentally induced diabetes especially from the Arab world, Brazil and Croatia, but little or no information is available on the anti-diabetic properties of Nigerian propolis (N. propolis).

Currently, botanical drugs are being screened for their efficacy, safety and dosage in the management of DM because they are cheap and readily available. In this work, we report the blood glucose responses of hyperglycemic rats to a regimen of N. propolis. Besides this, histology of the liver and pancreas, activities of hepatic alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) and the antioxidant superoxide dismutase (SOD) and malondialdehyde (MDA) were reported.

MATERIALS AND METHODS

Animals

A total of thirty adult male Wistar rats were bred at the animal holdings of the Department. Animals weighed between 190 and 230 g and were 8 weeks old at the start of the experiment. They were exposed to 12 h light, 12-h dark cycle at 22-24°C. All animals were maintained on a pelletized growers feed (Flour Mill Ltd., Ibadan, Nigeria). Rat pellets and water were given freely. Study was performed in accordance with the ethical guidelines stipulated by the Ethical Committee of the College of the institution. These guidelines were in accordance with the internationally accepted principles for laboratory animal use and care.

Collection and Extraction of Propolis

N. propolis was purchased from the Federal University of Agriculture, Abeokuta, Ogun state in Nigeria. Raw propolis was obtained by scraping it off its hive frames. Ethanolic extract was prepared according to the method described by Ivan et al. [23].

2.3 Induction and assessment of DM

To induce hyperglycemia, 25 animals were fasted overnight. Diabetes was induced by single intra-peritoneal (ip) injection of alloxan monohydrate (100 mg/kg) (Sigma, St. Louis, USA) in sterile normal saline (0.9%). Five Control animals were injected intra-peritoneally with citrate buffer alone at a single dose of 1.2 ml/kg. All animals were allowed free access to feed and water after alloxan injection, and they were left undisturbed for a minimum of 72 h for hyperglycemia to develop [24]. Animals were identified as diabetic on the basis of blood glucose levels (higher than 230 mg/dL) in tail blood using a one touch ultra mini Glucometer (LifeScan Inc., Milpitas, USA) 5 days after alloxan treatment. Weekly record of blood glucose level was taken afterwards.

Administration of Drugs

A total of 25 adult Wistar rats (twenty surviving diabetic rats and five normal rats) were randomly assigned into one of the following treatment groups of five animals each: Control, diabetic, diabetic + N. propolis (200 mg/kg, p.o.), diabetic + N. propolis (300 mg/kg, p.o.) and diabetic + metformin. Metformin (Merck, Germany) was given orally at 150 mg/kg. Administration of both drugs lasted for 28 days and was carried out at 09:00-10:00 daily.

Blood Glucose, Feed Intake and Body Weight (BW)

A One Touch Ultra Mini Glucometer (LifeScan Inc., Milpitas, USA) was used to estimate the blood glucose of treated and control animals. Blood was obtained from the dorsal vein of the animals. Blood glucose was estimated at day 0 and at 1, 3, 5, and 7 h after the first dose of the extract. Thereafter, measurement was done twice a week for 4 weeks. Feed intake was monitored on a daily basis during the experimental period (data not show). BW of the animals was also recorded twice a week.

Termination of Treatment

All animals were fasted and killed under diethyl ether anesthesia 24 h after the last treatment day. Blood was collected into heparinized tubes and centrifuged at 5000 r/min for 20 min in a desktop centrifuge model 90-1 (Jiangsu Zhangji Instruments Co., China). Plasma was stored at −20°C until analyzed. Laparotomy was performed on each animal; the liver and pancreas were excised, rinsed in normal saline and fixed in Bouin’s fluid.

Histological Studies

The livers and pancreases fixed in Bouin’s fluid were processed and stained with hematoxylin and eosin for histological studies. Photomicrographs were taken with a JVC color video digital camera (JVC, China) mounted on an Olympus light microscope (Olympus UK Ltd, Essex, UK).

Assessment of Liver Function

Biochemical analysis of the serum enzymes for AST and ALT was by the method of Reitman and Frankel [25].

Markers of Oxidative Stress Assessment

Serum level of MDA and SOD were assayed by the method of Ohkawa et al. [26] and Misra and Fridovich [27] respectively.
Determination of Serum Lipids

Triglycerides (TG) and total cholesterol (CHOL) were determined by enzymatic methods according to Diniz et al. [28] using commercial diagnostic kits (Randox, UK).

Statistical Analysis

Data obtained were presented as mean ± standard error of mean and analyzed for statistical significance using one-way analysis of variance, followed by Waller-Duncan post-hoc test. P < 0.05 was considered statistically significant.

RESULTS

To evaluate the effect of the ethanolic extract of N. Propolis on alloxan-induced diabetic rats, several biochemical estimations were carried out in all experimental animals for the estimation of plasma glucose, serum cholesterol, serum TG, liver function tests and oxidative stress markers. The histology of the liver and pancreas was also compared. The following pharmacological effects were observed:

Effect on Hyperglycemia

The mean blood glucose level of normal control rats fed on a normal diet was almost invariable throughout the experimental study. On the contrary, the blood glucose level of diabetic untreated rats was significantly increased (P < 0.05) when compared with the normal control group. When alloxan-induced diabetic rats were treated with metformin (150 mg/kg) and N. propolis at doses of 200 and 300 mg/kg, lowering in blood glucose was observed from the second week onwards when compared to the diabetic control group [Figure 1].

Effect on BW

Table 1 shows the initial and final BW of animals in all groups after the 28 day experimental period. BW of animals in the diabetic group decreased after 28 days while there was significant (P < 0.05) increase in the BW of animals in other groups.

Effect on lipid profile

Level of serum TG

Increase in level of serum TG was observed in the diabetic group when compared to the non-diabetic control group. Decrease in serum triglyceride levels was observed between metformin and extract treated groups when compared to the diabetic untreated group [Table 2].

Level of CHOL

Table 2 also shows that serum cholesterol levels of untreated diabetic rats were significantly higher than those in normal control group. Treatment with metformin and N. propolis lowered the level of serum cholesterol with maximum effect seen in the 300 mg/kg N. propolis group.

Effect on Serum ALT and AST

Figure 2a and b shows the serum levels of AST and ALT in all groups. Serum levels of ALT and AST were up regulated significantly (P < 0.05) in the diabetic untreated group

Table 1: Effect of ethanolic extract of N. propolis on BW

| Group | A       | B       | C       | D       | E       |
|-------|---------|---------|---------|---------|---------|
| Initial BW (g) | 210±6.3 | 220±8.3 | 197±3.9 | 213±13.2 | 215±3.8 |
| Final mass BW (g) | 257±10.6* | 198±9.7 | 225±6.5* | 242±5.6* | 244±6.5* |

BW: Body weight, *P<0.05 significantly different from diabetic control group, A: Non-diabetic control, B: Diabetic control, C: Diabetic treated (150 mg/kg metformin), D: Diabetic treated (200 mg/kg N. propolis), E: Diabetic treated (300 mg/kg N. propolis), N. propolis: Nigerian propolis

Figure 1: Showing and comparing weekly changes in the blood glucose level of the treated groups compared to the diabetic control group. *P < 0.05 significantly different from non-diabetic control group, *P < 0.05 significantly different from normal control group, *P < 0.05 significantly different from diabetic untreated group. A: Non-diabetic control, B: Diabetic control, C: Diabetic treated (150 mg/kg metformin), D: Diabetic treated (200 mg/kg N. propolis), E: Diabetic treated (300 mg/kg N. propolis)
Table 2: Effect of ethanolic extract of N. propolis on lipid profile

| Group   | A            | B            | C            | D            | E            |
|---------|--------------|--------------|--------------|--------------|--------------|
| CHOL (mg/dL) | 41.56±3.45*  | 105.46±6.06  | 42.13±1.47*  | 56.78±1.01*  | 36.12±3.13*  |
| TG (mg/dL)   | 103.96±9.34* | 185.34±7.11  | 180.71±8.68  | 183.88±13.01 | 147.16±9.96  |

*P<0.05 significantly different from diabetic control group, A: Non-diabetic control, B: Diabetic control, C: Diabetic treated (150 mg/kg metformin), D: Diabetic treated (200 mg/kg N. propolis), E: Diabetic treated (300 mg/kg N. propolis). CHOL: Total cholesterol, TG: Triglyceride

**Histology**

**Hepatic histology**

Figure 5a-e shows histology of the livers in control and treated groups at 28 days. In these groups, hepatic histology was comparable to the control, except in untreated diabetic rats, where hepatic sinusoids had become occluded, central veins were congested and hepatocytes appeared swollen.

**Pancreatic histology**

The section of rat pancreas from normal control group reveals normal pancreatic acini and islet cells, a similar result was found in the group treated with N. propolis and metformin. Alloxan-diabetic rats demonstrate degenerative and lytic changes in the islet of Langerhans of the pancreas as seen in Figure 6a-e.

**DISCUSSION**

In this study, we examined the possible antioxidants effects of N. propolis on diabetic rats. The noted significant decrease in blood glucose level in the N. propolis treated groups [Figure 1]
suggests that long-term administration/intake of this extract may have hypoglycemic effect. The reduction in the blood glucose level may be due to the presence of certain bioactive components and the protective effects that the antioxidant components of N. propolis may have on pancreatic β-cells which could enhance their production of insulin and more importantly, the possibility that propolis may enhance cellular response to insulin. In the propolis treated groups, despite the initial hyperglycemia, the antioxidants present in propolis were able to prevent oxidative stress effect of hyperglycemia on the liver and pancreas, this finding is in line with the reports of Al-Hariri et al. [29] who reported the efficacy of Arabian propolis in hyperglycemia. Hypoglycemic agents exact their effects via direct or indirect mechanisms in diabetes rats [30]. N. propolis acted as a direct hypoglycemic agent, by producing hypoglycemic effects when administered to alloxan-treated rats due to the severe destructive effect of alloxan on the β-cells of the pancreas [24].

![Figure 3: Serum level of superoxide dismutase in the control and experimental groups. *P < 0.05 significantly different from normal control group, **P < 0.05 significantly different from normal control group, A: Non-diabetic control, B: Diabetic control, C: Diabetic treated (150 mg/kg metformin), D: Diabetic treated (200 mg/kg Nigerian propolis [N. propolis]), E: Diabetic treated (300 mg/kg N. propolis)](image)

![Figure 4: Serum level of malondialdehyde in the control and experimental groups. *P < 0.05 significantly different from the control and treatment groups. A: Non-diabetic control, B: Diabetic control, C: Diabetic treated (150 mg/kg metformin), D: Diabetic treated (200 mg/kg Nigerian propolis [N. propolis]), E: Diabetic treated (300 mg/kg N. propolis)](image)

![Figure 5: Liver of rats at 28 days of treatment. (a) Control group: The morphology of the liver is normal as indicated by intact central vein (c), sinusoids (arrowhead), and hepatocytes (arrows). (b) Diabetic group: Sinusoids have become largely occluded, perhaps due to swollen hepatocytes (arrows); central vein (c) is also congested. (c) Diabetic + metformin: The liver has normal morphology (c, central vein; arrow, hepatocyte; arrowheads, sinusoids). (d) Diabetic + 200 mg/kg of Nigerian propolis [N. propolis]: Hepatic morphology is comparable to control. (e) Diabetic + 300 mg/kg of N. propolis: The liver show normal morphology. Arrows indicate hepatocytes; arrowheads indicate sinusoids. H and E stain; ×400.](image)
could also have acted indirectly by stimulating the few surviving \( \beta \)-cells to secrete more insulin rather than aiding the regeneration of necrotic \( \beta \)-cells of the pancreas. Observation from this study shows that N. Propolis exacts its activity by both direct and indirect mechanisms.

Lipid disorders assume a position of utmost importance in patients with diabetes, because of the high risk of microvascular disease in this condition but administration of N. propolis to the rats was able to reverse hyperlipidemia seen in the diabetic rats. Similar reports of Al-Hariri [29] and El-Sayed et al. [31] shows that treatment with propolis can reduce the TG and serum cholesterol level in diabetics. The hypolipidemic effects of N. propolis observed from this study is probably due to the hypoglycaemic potential of N. Propolis, which makes it possible to ameliorate lipid and lipoprotein disorders associated with diabetes.

The ethanolic extract of N. propolis possesses antioxidant components that ameliorated the oxidative stress induced damage associated with alloxan-induced diabetes. The bioflavonoids present in N. Propolis may confer the antioxidant effect seen in this study as quecretin, a flavonoids present in N. Propolis have been reported to bring down hyperglycemia and oxidative stress in STZ-induced diabetes rats [32].

Increased levels of ALT and AST infiltrates and disturbs functioning of the hepatic cell membranes [33]. Administration of N. propolis ameliorated high levels of ALT and AST following induced-diabetes. The hepatoprotective activity of N. propolis was higher at 300 mg/kg than metformin.

A primary consideration in the assessment of the efficacy of a potential therapeutic agent for hepatic injury is its effect on liver histology. Histological sections of the liver in untreated diabetic rats show extensive occlusion of the sinusoids [Figure 5b]. Reports have revealed swelling of hepatocytes could arise from accumulation of glycogen in these cells - a condition referred to as hepatic glycogenosis of DM [34]. Treatment with the ethanolic extract of N. propolis improved hepatic injuries associated with induced-diabetes.

The pancreas is usually the main organ affected in diabetes with loss in both its exocrine and endocrine functions. This is mainly due to the close anatomical and functional links between the exocrine and endocrine pancreas [35]. Histologically, there was improvement in the islet and the acinar cells in the N. propolis treated group. This is different from what is seen in the untreated diabetic group. Pancreatic cells are usually lost because pancreatic \( \beta \)-cells are highly prone to oxidative stress and damage because they have low activity of antioxidant enzymes, which are the first line of defense against oxidative insult [27].

CONCLUSION

In conclusion, results from this study indicate that the ethanolic extract of N. propolis can ameliorate hyperglycemia, hypercholesterolemia, and hypertriglyceridemia as well as...
protect the liver and pancreas against alloxan-induced diabetes. This significant protection of N. propolis may be due to the synergistic effect of the constituents of the extract. Further biochemical and pharmacological investigations would be required to know the comprehensive mechanism of action of the N. propolis which our laboratory is currently involve in.

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