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Impaired Cognitive Performance and Metabolic Disturbance in Streptozotocin-Nicotinamide Induced Type 2 Diabetes Mellitus and the Protective Effect of Nigerian Propolis

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ABSTRACT

Defects in insulin signaling and oxidative stress are implicated in cognitive dysfunction in diabetes. This study evaluated the effects of *propolis* on cognitive impairment in streptozotocin-nicotinamide model of type 2 diabetes mellitus in Wistar rats. Diabetes was induced by single intraperitonieal administration of streptozotocin (65 mg/kg) 15 min after nicotinamide (110 mg/kg) had been adminstered. Diabetic animals were treated with glibenclamide (5 mg/kg), *propolis* (200 and 300 mg/kg), or normal saline for 4 weeks after which spatial memory was assessed with the Morris' water maze (MWM). At the end of the study the animals were euthanized and blood collected via cardiac puncture while the brain was homogenized. Insulin was assayed from plasma while malondialdehyde (MDA), superoxide dismutase (SOD), gluthatione (GSH) and catalase were assayed from brain homogenate. Homeostatic model assessment (HOMA) was used as marker for insulin resistance. Significant rise in blood glucose, plasma insulin, and brain MDA ($P < 0.05$) with reduction in SOD, GSH, and catalase levels were observed in the diabetic group. Treatment with 200 and 300 mg/kg *propolis* and glibenclamide significantly decreased blood glucose, plasma insulin, and MDA ($P < 0.05$) and increased brain levels of SOD, GSH and catalase. *Propolis* (200 and 300 mg/kg) also significantly ($P < 0.05$) decreased escape latency in the MWM in comparison to the diabetic group. Nigeri*an propolis* thus seems to protect against impaired cognitive performance in experimental diabetes mellitus.

Keywords: *Spatial memory, insulin resistance, oxidative stress, Morris water maze, Nigerian propolis*

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders characterized by sustained increase in blood glucose as a result of insulin deficiency caused by autoimmune death of pancreatic β-cells which is the case in type 1 DM or resulting from insulin resistance or insentivity as seen in type 2 DM. Other types of DM are mostly caused by genetic factors (Vos et al. 2012). The International Diabetes Federation (IDF) estimates that 382 million people

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had diabetes with type 2 DM accounting for 90% of the cases (Vos et al. 2012). Its prevalence is increasing rapidly, and by 2030, this number is estimated to almost double. DM occurs throughout the world, but its prevalence is higher in developed countries. The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will probably be found by 2030 (Wild et al. 2004). Although the pathogenesis of DM syndrome remains poorly understood, both type 1 and type 2 DM predisposes an individual to a similar spectrum of complications, including hypertension, macrovascular and microvascular diseases, and neuropathy among others.

Accumulating evidence points to the fact that people with DM are at risk of developing cognitive impairment probably due to the synergy between the metabolic complication associated with DM and the physiological changes that occurs within the central nervous system (Strachan et al. 2003; Cukierman et al. 2005; Alexis et al. 2008; Allan et al. 2014).

Insulin, considered until the last three decades as only a peripheral hormone unable to cross the blood brain barrier has been found to affect the central nervous system (CNS) and is also synthesized *de novo* in the CNS (Schechter et al. 1996; Laron 2009). Studies show that insulin participates significantly in neurological function such as neuroprotection, modulation of neuronal activity, modification of synaptic plasticity and lowering of threshold frequencies for eliciting long term potentiation (LTP) and long term depression (LTD) (Van der Heide et al. 2006). Defects in the insulin signaling pathway have been implicated in age-related neurodegenerative disorders such as Alzheimer's disease providing a link between the central pathology of DM and Alzheimer's dementia (Allan et al. 2014).

Oxidative stress (OS), an imbalance between the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) represents a central pathophysiological mediator of DM and progression of several neurodegenerative diseases (Dominguez et al. 2012). In DM, oxidative stress is a consequence of hyperglycemia or may result from reduced sensitivity of body tissues to insulin ultimately resulting in hyperglycemia.

In other to understand the molecular basis and the pathogenesis of complications in DM with the view to finding potential therapy, several animal models have been developed to closely mimic what is observed in humans. The streptozotocin (STZ) - nicotinamide model is used extensively to mimic type 2 DM and also test substances with potential antidiabetic/ hypoglycemic effects. Nicotinamide helps protect against β-cytotoxicity of STZ resulting in moderate changes in hyperglycemia, reduction of β-cells (Szkudelski 2012), reduction in pancreatic insulin store (Islam and Wilson 2012), impaired insulin secretion resulting in glucose intolerance (Szkudelski 2012), and impaired glucose-stimulated secretion of insulin (L'abbate et al. 2007). This model also responds well to sulphonylureas.

Synthetic oral hypoglycemic drugs such as biguanide (metformin), sulphonylureas (glibenclamide) which are insulin secretagogues, meglitinides (repaglinide), thiazolidinediones (pioglitazone), α-glucosidase inhibitors (acarbose) are used in the management of DM and its complications, they fail to restore the observed impairment in cognitive function in diabetic patients probably because of their poor penetration of the blood brain barrier (Annadurai et al. 2012). However, in recent years, a number of natural products including *propolis* have been shown to ameliorate DM complications.

Propolis is a hive product of the honeybee that consists of resinous materials and secretions. Its chemical composition depends on its location of origin owing to variations in species of bees and plants and time of collection. In general, raw *propolis* has been found to be composed of about 30% wax, 5% pollens, 50% resins, 10% essential oil, and 5% of various organic compounds. With more than 300 constituents presently identified from different samples, the list keeps growing by the day. Diterpenic acids, polyphenols, and aromatic acids are the essential compounds responsible for the biological activities of *propolis*. In general, *propolis* has been reported to possess various biological activities such as anticancer, anti-inflammatory, antioxidant and hypolipidemic activities among others (Russo et al. 2006). Previous studies showed that aqueous extract of *propolis* has higher polyphenol content in comparison to the ethanol extract. This difference accounts for the higher activity of aqueous extract of *propolis* in all the antioxidant assay systems in comparison to the ethanolic extract (Laskar et al. 2010).

Several studies have been carried out to examine the effect of *propolis* from different geographical regions on experimentally induced diabetes and its complications but there is presently no study on the effect of *propolis* in general and Nigerian *propolis* in particular on cognitive dysfunction in experimental DM induced with streptozotocin-nicotinamide.

This study sought to evaluate the effect of *propolis* of Nigerian origin on cognitive dysfunction in streptozotocin-nicotinamide induced experimental diabetic rats and the possible mechanisms underlying such effects.

MATERIALS AND METHODS

Animals

Forty male Wistar rats weighing between 200-280 g were used for this study. The animals were housed (8 rats per cage) in the animal holding of the Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria at room temperature with 12 hour light and

dark cycles. They were allowed free access to food and water and acclimatized to their new environment for three weeks prior to the start of experiment. All experimental protocols were approved by the University of Ilorin Ethical Review Committee (UIERC) which is in accordance with International Guidelines for the Care and Use of Laboratory Animals.

Extract Preparation

Propolis was obtained from Fihi Shi-fau Iinasi bee therapy clinic, Biological Garden, Kwara State College of Education Ilorin, Kwara state, Nigeria. Raw *propolis* was air dried and then blended into finer particles which was dissolved in water at 50°C. Following filtration, the filtrate was evaporated to dryness in a water bath set at 50°C. The dry sample was weighed and dissolved in warm distilled water according to doses to be administered to different groups of experimental animals.

Induction of DM

After acclimatization, the animals were fasted overnight, streptozotocin was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in distilled water. Diabetes was induced by single intraperitoneal administration of nicotinamide (110 mg/kg) 15 min prior to single intraperitonial administration of streptozotocin (65 mg/Kg). Using a one touch ultra-glucometer, animals with blood glucose level ≥ 250 mg/dl, a week after diabetes induction were considered diabetic and recruited for the experiment.

Groupings

The animals were divided into 5 groups of 6 rats each. Normal control received normal saline); Diabetic received 5 mg/kg glibenclamide; Diabetic treated and *propolis* (200 mg/kg of *propolis*); Diabetic treated and *propolis* (300 mg/kg of *propolis*); Diabetic untreated (received normal saline). The treatment lasted for 4 weeks.

Behavioural study (Morris water maze)

A circular pool, black in colour, 210 cm in diameter and 50 cm in height was filled with water at 28 ± 1 °C up to the 30 cm mark. A submerged platform was hidden in a fixed position. The platform aids the animal to escape the water environment by finding and standing on the platform. During a series of trials each rat was trained to find the platform in at most 60 s. Animals unable to locate the platform during this time frame were helped to find it. The animals were given 3 trials per day for 5 consecutive days corresponding to days; 22, 23, 24, 25 and 26 of the experimental period. On day 27 of the experiment the water in the Morris maze was coloured with skimmed milk to make the platform invisible. Rats were placed in the coloured water to find the platform and rats unable to find the platform in 60 s were helped to it. Time taken to find the platform was taken as the escape latency and a measure of spatial memory. Long term (the first trial on the day of the test) and short term (30 min after the first trial) spatial discrimination were recorded (Bromley-Brits et al. 2011).

Sample Collection and Biochemical Assay

After termination of the treatment (on day 28 of treatment), animals were anaesthetized with ketamine (5 mg/kg). Blood samples were collected via cardiac puncture and the brains excised. Each brain was homogenized in 0.2 M sucrose. Insulin was assayed from plasma while catalase, gluthathione (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA) in supernatant from the brain homogenates.

Estimation of Blood Glucose

Weekly change in blood glucose was measured with digital glucometer (Accu-chek® Active, Roche Diagnostic, Germany).

Estimation of Insulin and Homeostasis Model Assessment (HOMA-IR) for Insulin Resistance

Plasma insulin level was estimated using insulin ELISA kit for rats (Linco Research Inc.) following the procedure outlined in the kit. HOMA-IR was calculated and used as a marker for insulin resistance according to the formula below (Yogendra et al. 2014):

$$\frac{\textit{fasting blood glucose}\ (\frac{mg}{dl}) \times \textit{fasting plasma insulin}\ (\frac{\mu U}{ml})}{450}$$

Estimation of Total Protein

The method of Lowry et al. (1951) was employed using bovine serum albumin (BSA) as standard. In summary, this method is based on the reaction between phosphomolybdic-phosphotungestic acid present in the Folin-Ciacalteu reagent and aromatic amino acids residues of the protein in the sample. Absorbance at 750 nm was used in estimating the amount of protein in the sample.

Estimation of Catalase, SOD, GSH, and MDA

The level of catalase, SOD, GSH, and MDA were assayed in brain homogenates by modifying the methods of Beers and Sizer (1952), Misra and Fridovich (1972), Jollow et al. (1974), and Reilly and Aust (1999).

Catalase was measured using the principle of the disappearance of peroxide which was followed via spectrophotometry at 240 nm. At a pH of 7.0, 1 unit of catalase decomposes 1 µl of H_2O_2 per min at a temperature of 25°C. Before use, the enzyme source was diluted in phosphate buffer (0.05 M and pH 7.0). The assay mixture contained 1.9 ml of distilled water, 1.0 ml H_2O_2, and 0.1 ml of diluted enzyme source. This mixture was incubated in spectrophotometer to achieve equilibration of temperature and establishment of blank rate for 5 min. The decrease

in absorbance was monitored at 240 nm for 2 min after the addition of the diluted enzyme source. Catalase activity was calculated according to the

$$(\Delta A_{240}/\text{min} \times 1000) \,/\, (43.6 \times \text{mg protein/ml reaction mixture})$$

following expression:
(ΔA_{240}/min)= change in absorbance calculated from the initial 45 s.

SOD: The principle behind this assay is the ability of SOD to inhibit the auto-oxidation of epinephrine at a pH of 10.2. Reagents employed include 0.05 M carbonate buffer (pH 10.2) and 0.3 mM adrenaline. 1 ml of sample was diluted in 9 ml of distilled water to give a 10 ml dilution. To 2.5 ml of 0.05 M carbonate buffer, an aliquot of the diluted sample was added to equilibrate in the spectrophotometer. Addition of 0.3 mM adrenaline to the mixture triggered the reaction. In the reference cuvette is 2.5 ml buffer, 0.3 ml of adrenaline, and 0.2 ml of distilled water. Increase in absorbance at 480 nm every 30 s for 150 s was monitored. SOD activity was calculated according to the expression below:
Increase in absorbance/minute = $\frac{A3 - A0}{2.5}$ (A3 and A0 are absorbance after 150s and 30s)
% Inhibition = $\frac{\textit{increase in absorbance for substrate}}{\textit{increase in absorbance for blank}} \times 100$

1 unit of SOD activity is the amount of SOD required to cause 50% inhibition of the oxidation of adrenaline to adenochome per min.

GSH: Glutathione working standard (40 mg GSH dissolved in 0.1 M phosphate buffer, pH 7.4 and volume made up to 100 ml), Ellman's reagent, and precipitating reagent (4% sulphosalicyclic acid) were used in determining GSH level. Serial dilution of GSH working standard was prepared (shown in the table below). Absorbance was set at 412 nm with GSH concentration corresponding to this absorbance. Readings were taken in 5 min and a graph of absorbance against concentration was plotted.
To estimate GSH level, 0.2 ml of sample was added to 1.8 ml of distilled water and 3 ml of the precipitating agent. The mixture was allowed to stand for 5 min, filtered and 1 ml of filtrate was added to 4 ml of 0.1 M phosphate buffer and then 0.5 ml of Ellmans' reagent. A blank was prepared with 4 ml of 0.1 M phosphate buffer, 1 ml of precipitating solution and 0.5 ml of Ellmans' reagent. Optical density was measured at 412 nm. GSH concentration was estimated from the GSH standard curve.

MDA: MDA is produced during lipid peroxidation which reacts with thiobarbituric acid (TBA) to generate a pink coloured complex which absorbs light at 532 nm in acidic solution. The reagents used were 30% trichloroacetic acid (TCA) solution, 0.75% TBA, and 0.15 M Tris-pottassium chloride (Tris-KCl) buffer (pH 7.4). 0.4 ml of test sample was added to a mixture of 0.5 ml of 30% TCA and 1.6 ml Tris-KCl buffer. To this mixture, 0.5 ml of TBA was added and placed in a water bath for 45 min to incubate at 80^{o}C. Pink coloured mixture was produced on incubation. This mixture was cooled on ice and centrifuged at 14000 g for 15 min. The absorbance of the supernatant was read at 532 nm. To quantify MDA, the expression below was used:
MDA (units/mg protein) =

$$\frac{\textit{absorbance} \times \textit{volume of mixture}}{E_{532} \times \textit{volume of sample xmg protein}}$$

(E_{532}; molar absorptivity at 532 nm = 1.56 × 105)

Statistical Analysis
Statistical analysis was performed using SPSS 11 and Graphpad prism softwares. Two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. $P < 0.05$ was used as a measure of statistical significance. Results are expressed as mean ± S.E.M.

RESULTS

Effect of Glibenclamide and *Propolis* on Hippocampal Dependent Spatial Memory
After series of trainings 3 times daily for 5 consecutive days, 24 hours after last training, the ability of the animals to find the hidden platform for escape (escape latency) was taken as a measure of long term spatial memory (Figure 1a) and 30 min after as a measure of short term spatial memory (Figure 1b). In the long term spatial memory test, diabetic untreated animals generally took longer time to locate the hidden platform. Lowered escape latency was observed in animals treated with both doses of *propolis* compared to normal non-diabetic animals. Glibenclamide however did not significantly lower escape latency. Short term spatial memory was significantly impaired in untreated diabetic group. All treated groups recorded significantly lower escape latency ($P < 0.05$) in this latter test in comparison with the diabetic untreated group. 200 mg/kg *propolis* appeared to be the most efficacious in lowering escape latency in short term spatial memory test ($P < 0.05$) (Figure 1b).

Effects of Glibenclamide and *Propolis* Treatment on Blood Glucose Level
Weekly changes in blood glucose levels are presented in Table 1. Administration of nicotinamide and streptozotocin significantly elevated blood glucose levels ($P < 0.05$) by about 4 times on the 7th day post-induction of DM in comparison to the fasting blood glucose before diabetes induction in all diabetic groups. The final blood glucose concentration of diabetic untreated rats was about 5 times higher than in

normal control (Table 1). While both doses of *propolis* produced an early onset of action as early as first week of administration, significant blood glucose lowering effect of glibenclamide was delayed until the second week of administration. Administration of *propolis* 200 mg/kg and 300 mg/kg, and glibenclamide significantly (P < 0.05) decreased blood glucose level at the end of the experiment by 62%, 65% and 56%, respectively.

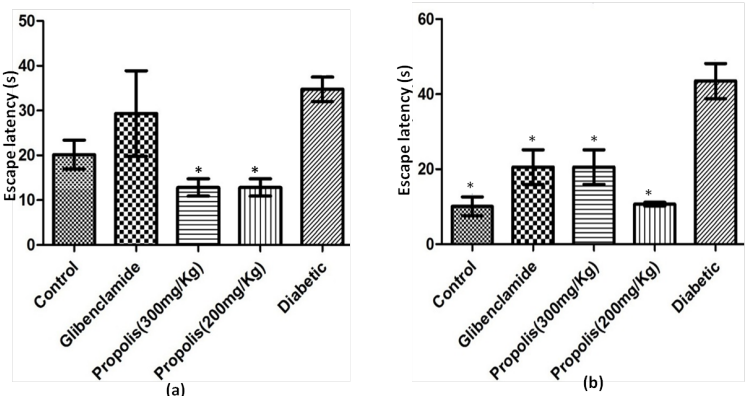

Fig. 1: Showing the effect of nicotinamide-streptozotocin induced DM and treatment with glibenclamide and *propolis*, (a) long term spatial memory (b) short term spatial memory. *P < 0.05 in comparison to diabetic untreated group.

Effect of Glibenclamide and *Propolis* on Plasma Insulin Levels and HOMA-IR

Diabetes produced a significant (P < 0.05) increase in the levels of plasma insulin of diabetic untreated rats in relation to the non-diabetic group as shown in Figure 2a. Significant (P < 0.05) decrease was recorded in plasma insulin after treatment with glibenclamide and *propolis* (200 mg/kg and 300 mg/kg). There was significant (P < 0.05) rise in HOMA-IR in the diabetic untreated group in relation to the non-diabetic group. HOMA-IR (Figure 2b) was decreased significantly (P < 0.05) in relation to the diabetic untreated group in groups that received *propolis* (200 mg/kg & 300 mg/kg) and glibenclamide.

Effect of Glibenclamide and *Propolis* on Some Markers of Oxidative Stress in Brain Homogenate

The effect of glibenclamide and *propolis* on oxidative stress markers in brain homogenate is presented in Figure 3.

Significant (P < 0.05) decrease in the level of catalase was observed in the diabetic untreated group in relation to the non-diabetic group (Figure 3a). Treatment with glibenclamide and 300 mg/kg *propolis* significantly (P < 0.05) increased the level of catalase in relation to the diabetic untreated group. The decrease level of catalase produced by administration of 200 mg/kg *propolis* was not statistically significant compared to the diabetic untreated.

Like catalase, significant decrease in SOD level was observed in the diabetic untreated group compared to non-diabetic group. Treatment with the two doses of *propolis* and glibenclamide significantly (P < 0.05) increased the level of SOD.

GSH level was reduced in the diabetic group in relation to the non-diabetic group, though this decrease was not significant (P > 0.05). Treatment with *propolis* significantly (P < 0.05) increased GSH level in relation to the diabetic untreated. GSH level in the groups that received *propolis* was higher than that in the non-diabetic group, though not significant. Increase in the level of GSH in the group treated with glibenclamide did not produce significant rise in relation to the diabetic untreated.

A significant (P < 0.05) rise in MDA level was observed in the diabetic untreated group in relation to the non-diabetic group. Treatment with glibenclamide and *propolis* significantly decreased MDA level. Decrease in MDA in the glibenclamide group was significantly lower than those in the *propolis* groups.

Table 1: The Effect of Glibenclamide and *Propolis* (200 mg/kg and 300 mg/kg) on Blood Glucose Level in Diabetic Wistar Rats

Group	Blood Glucose (mg/dl)					
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
Control	78.7 ± 4.7	77.0 ± 3.7*	84.7 ± 3.5*	77.2 ± 1.9*	79.3 ± 4.8*	79.5 ± 3.9*
Glibenclamide	83.7 ± 3.8	383.8 ± 11.4	308.4 ± 24.3	255.5 ± 23.5*	198.3 ± 15.4*	167.3 ± 5.9*
Propolis (200mg/kg)	80.5 ± 5.5	339.5 ± 11.4	242.5 ± 66.5*	218.5 ± 65.5*	160.1 ± 40.2*	130.7 ± 15.1*
Propolis (300mg/kg)	81.5 ± 3.5	357.2 ± 24.1	259.3 ± 12.7*	237.4 ± 28.2*	139.5 ± 2.7*	124.3 ± 13.4*
Diabetic	90.1 ± 5.2	331.3 ± 11.1	355.5 ± 4.5	388.6 ± 32.5	411.3 ± 24.7	429.5 ± 31.2

* P < 0.05 vs. diabetic untreated group

DISCUSSION

The results of this study showed that streptozotocin-nicotinamide induced DM significantly increased plasma level of insulin (decreased insulin sensitivity) and induced oxidative stress in the brain by decreasing the levels of catalase, SOD, and GSH and promoting lipid peroxidation by increasing the level of MDA. Impairment in spatial memory was also evident in this model of DM. Treatment with Nigerian *propolis* significantly ameliorated cognitive impairment (spatial memory), decreased plasma insulin by improving insulin sensitivity, and improved the defence system of the brain against oxidative stress.

The idea of cognitive impairment in diabetes is gaining more attention and acceptance. Both type 1 and type 2 DM have been shown to be associated with reduction in performance on several domains of cognitive function (Xu et al. 2013). Young type 1 DM children when compared with normal children were reported to show lower general cognitive abilities, lower receptive language scores, and slower fine motor speed, effects that were associated with poor glycemic control as indicated by higher glycosylated haemoglobin (HbA1c) (Patino-Fernandez et al. 2010). This research finding has also been demonstrated in type 2 DM patients where cognitive dysfunction is associated with the duration of DM and elevated levels of HbA1c (Van Harten et al. 2007). Experimental DM studies in animals using streptozotocin, high-fat diet, glucocorticoids among other models have likewise demonstrated cognitive impairment and defect in hippocampal long-term potentiation (Biessels et al. 1996; Wasana et al. 2011; Amin et al. 2013). Though, the mechanism is yet to be completely understood, hyperglycemia and insulin resistance among other factors have been implicated as central to the pathogenesis of cognitive dysfunction in DM (Fukui et al. 2002; Wessels et al. 2008). In this study, streptozotocin-nicotinamide DM model produced a defect in long term and short term spatial memories evident with the increase time taken (escape latency) for the diabetic animals to find the platform submerged in opaque water providing evidence for memory impairment which is in consonance with a previous finding (Lee et al. 2013). From our findings, this cognitive impairment seems within the context of hyperglycemia and insulin resistance.

It has previously been suggested that though hyperglycemia may not lead to dementia, it can decrease the threshold for dementia (Wessels et al. 2008; Xu et al. 2013). Among the mechanisms via which hyperglycemia is thought to induce cognitive impairment in DM is via oxidative stress. Increase in the generation of reactive oxygen and nitrogen species and markers of oxidative stress such as increase lipid peroxidation and decreased level of antioxidants have been shown to result from hyperglycemia in

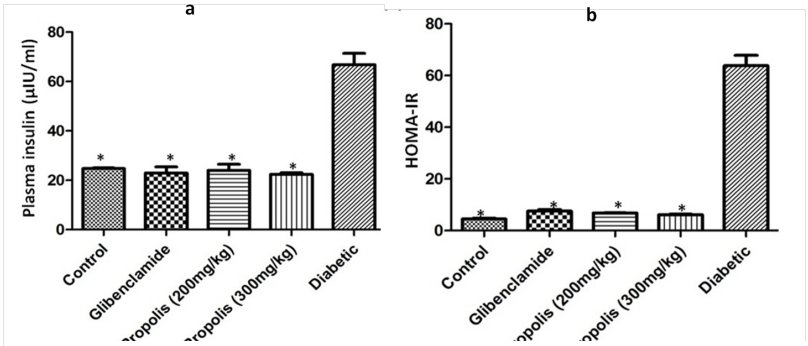

Fig. 2: The effect of nicotinamide-streptozotocin induced DM and treatment with glibenclamide and *propolis* on: (a) Plasma insulin and (b) HOMA-IR, a marker for insulin resistance. *P<0.05 in comparison to diabetic untreated group.

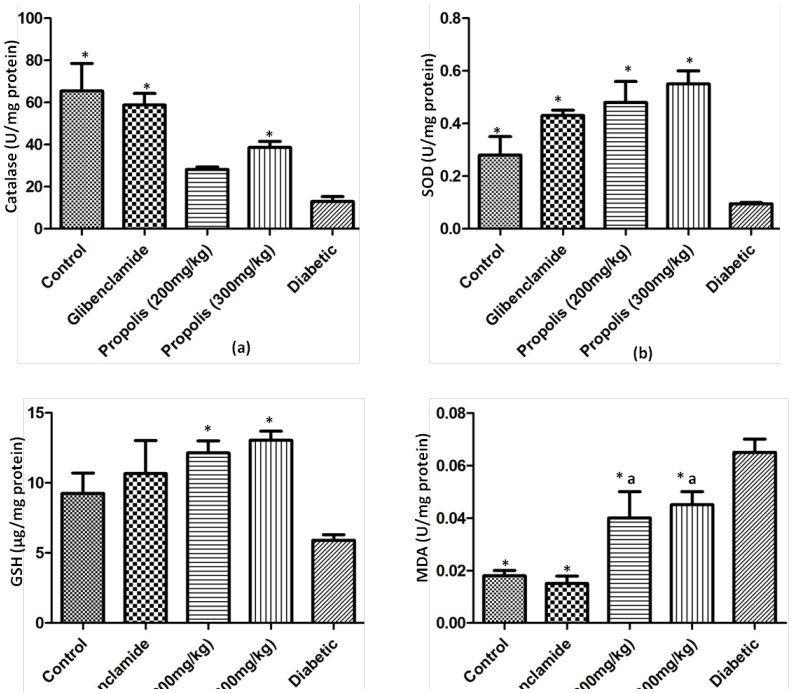

Fig. 3: The effect of nicotinamide-streptozotocin induced DM and treatment with glibenclamide and *propolis* on brain: (a) catalase (b) SOD; (c) GSH, and (d) MDA as markers of oxidative stress. *P<0.05 in comparison to diabetic untreated group; [a] P < 0.05 in comparison to glibenclamide

experimental models of DM (Wasana et al. 2011; Amin et al. 2013; Tamaddonfard et al. 2013; Ebuehi and Dibie 2015). In the present study, streptozotocin-nicotinamide administration led to sustained hyperglycemia which likely resulted in increased lipid peroxidation and decrease in the level of antioxidant and antioxidant enzymes. Other mechanisms via which hyperglycemia is thought to induce cognitive dysfunction in DM is by increasing polyol pathway flux, activation of the diacylglycerol (DAG) protein kinase C (PKC) pathway, and increase in glycated end products (AGEs) production (Brownlee 2001; Srivastava et al. 2005; Ahmad et al. 2005). But will pure antihyperglycemic drugs relieve cognitive impairment (acarbose SGLT2Is)?

The effects of oral hypoglycemic and insulin as therapy for cognitive impairment in diabetes have been tested with varying results. The primary draw back regarding insulin therapy is the increased risk of hypoglycemia. Metformin, a biguanide has shown promise in preventing cognitive impairment in experimental DM but varying results were observed in clinical studies with some studies conferring it with protective role while others point to the fact that the use of metformin may worsen cognitive function in DM (Gupta et al. 2011; Hu et al. 2011; Moore et al. 2013). Sulphonylureas, such as glibenclamide and glipizide also showed promise in experimental models of DM via their anti-inflammatory activity but offered little protection against cognitive impairment in clinical studies (Lamkanf et al. 2009; Imfeld et al. 2012). A combination of biguanides and sulphonylureas was found to decrease the risk of dementia in DM by about 35% (Hu et al. 2011). Hence, despite well recognized efficacy of clinically useful antidiabetic drugs, their therapeutic efficacy in correcting cognitive dysfunction is controvertible.

As stated earlier, results from the diabetic untreated group showed significant dysfunction in spatial memory (increased escape latency) with a significant dysfunction observed in short term spatial memory. Treatment with glibenclamide and *propolis* significantly decreased escape latency time in relation to the diabetic untreated group. This support the fact that *propolis* and glibenclamide protects against impairment in short term spatial memory in this model of DM. No significant increase was observed in escape latency between the diabetic untreated and non-diabetic group in long term spatial memory, though escape latency was higher in the diabetic untreated group. Treatment with glibenclamide provided no significant effect on long term spatial memory while treatment with *propolis* significantly decreased escape latency in relation to the diabetic untreated group. This suggests that *propolis*, aside from protecting against DM induced memory dysfunction in this model also has a ionotropic effect.

The protective effect of *propolis* against cognitive dysfunction in this model may partly be due to its hypoglycemic and antioxidant effects and its ability to increase the sensitivity of body tissues to insulin.

Hence, in this study, treatment with *propolis* significantly decreased blood glucose level after one week of treatment onwards in comparison to the diabetic untreated. This result conforms to the hypoglycemic property of *propolis* reported in previous studies in other models of DM (Yajing et al. 2012; Kitamura et al. 2013; Hung-Wei et al. 2015; Ibrahim et al. 2015). *Propolis* exacts its hypoglycaemic effect by increasing the synthesis of insulin from pancreatic cells as shown from previous studies, while this study also shows that *propolis* additionally improves sensitivity of body tissues to insulin. Administration of glibenclamide, a biguanide also decreased blood glucose level and decreased HOMA-IR level.

Oxidative stress, a result of sustained hyperglycemia in DM was ameliorated with *propolis* treatment. *Propolis* decreased lipid peroxidation (MDA), though not significantly in relation to that observed in diabetic untreated, which is in contrast to reports in alloxan model of DM where treatment with *propolis* significantly decreased lipid peroxidation (Amin et al. 2013; Oršolić et al. 2013). Significant increase was observed in the level of antioxidant (GSH) and antioxidant enzymes (catalase and SOD) following treatment with *propolis*. Treatment with glibenclamide significantly decreased lipid peroxidation and increased the synthesis of antioxidant and antioxidant enzymes.

Insulin defects have also been implicated in cognitive dysfunction in DM. Insulin receptors have been found to be unevenly distributed in the brain and have been found to be more abundant in hippocampus, olfactory bulb, hypothalamus, cerebellum, brainstem, and cerebral cortex (Schulingkamp et al. 2000). Insulin has been found to be involved in synaptic activity, plasticity and in memory formation and storage (Kremerskothen et al. 2002; Rahul et al. 2009). Hence, the defects in cognitive function occur both in hypoinsulinaemia (type 1 DM) or hyperinsulinaemia (insulin resistance). Brain insulin receptors unlike those in the peripheral system are not down-regulated on exposure to high insulin concentration suggesting roles probably different from those in the periphery (Zhao et al. 2004; Dou et al. 2005). Abnormality in the insulin/insulin receptor-mediated signals transduction as a result of the inactivation of glycogen kinase 3 (GSK3) is associated with hyperinsulinaemia in insulin resistance. Activation of GSK3 is associated with hyperphosphorylation of tau (a component of senile plaques) in addition to increased production of amyloid-β-peptide (Aβ) (component of neurofibrillary tangles) (Bhat et al. 2000; Farris et al. 2003). In the present study, plasma insulin was significantly elevated in the diabetic untreated animals in addition to hyperglycemia suggestive of decreased sensitivity of body tissues to insulin. The significant increase in HOMA-IR clearly indicates insulin resistance and abnormality in the insulin/insulin receptor mediated signal transduction. The observed resis-

tance is in agreement with a previous report (Yogendra et al. 2014).

Hyperinsulinaemia, a hallmark of type2 DM was observed in the diabetic untreated group evident in the increased HOMA-IR. Insulin level in plasma was also increased in this group signifying insulin resistance and abnormality in insulin/insulin receptor signal transduction. Treatment with both glibenclamide and *propolis* decreased plasma insulin with a concomitant decrease in HOMA-IR.

CONCLUSION

In conclusion, this study shows that Nigerian *propolis* can prevent cognitive dysfunction in experimental DM via its antihyperglycemic effects. In addition to this, its antioxidant activity may also be a contributory mechanism of action. Future studies would attempt to explain whether Nigerian *propolis* effects are directly acting on the brain or indirectly via peripheral mechanisms.

Conflict of Interest

None declared.

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