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Full Length Research Paper

Effect of aqueous root extract of *Treculia africana* on haemoglobin glycosylation and plasma lipid per oxidation in streptozotocin-induced diabetic rabbits

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Measurement of glycosylated hemoglobin has been proposed to be a very sensitive index for glycemic control as various proteins, including hemoglobin, albumin, collagen, low-density lipoprotein cholesterol (LDL), or crystalline proteins has been reported to undergo non-enzymatic glycation in diabetes. In the present study an attempt was made to elucidate the effect of aqueous root extract of *Treculia africana* on levels of haemoglobin glycosylation and plasma lipid peroxidation in streptozotocin (STZ),-induced diabetic rabbits. Twenty (20) STZ-induced diabetic male rats divided into two groups (n = 10) designated: Control and Test were treated respectively with 20 ml/kg distilled water and 200 mg/kg aqueous root extract of *Treculia africana* for five weeks. Plasma total haemoglobin, glycosylated haemoglobin and thiobarbituric acid reactive species (TBARS), concentration were determined at pre- and post-treatment. Administration of *Treculia africana* root extract at 200 mg/kg to STZ-diabetic rabbits was observed to significantly decreased concentration of blood glucose, glycosylated haemoglobin and increased blood total haemoglobin. The elevated plasma levels of lipid peroxidation of diabetic rabbits were reverted back to near control levels following administration of *T. africana* extract. The results obtained in this study clearly indicate that aqueous root extract of *T. africana* diminishes the rate of haemoglobin glycosylation in diabetic animals and shows some levels of antioxidant principle.

Key words: Haemoglobin, glycosylation, diabetes, extract, glycemic control.

INTRODUCTION

In diabetes mellitus, chronic hyperglycemia produces multiple biochemical sequelae, and diabetes-induced oxidative stress could play a role in the symptoms and progression of the disease (Giugliano et al., 1996). Oxidative stress in cells and tissues results from the increased generation of reactive oxygen species and/or from diseases in antioxidant defense potential (Gumieniczek et al., 2002). Several hypotheses have been proposed to explain the genesis of free radicals in diabetes. These include autoxidation processes of glucose, the non-enzymatic and progressive glycation of proteins with the consequently increased formation of glucose-derived advanced glycosylation end products, and enhanced glucose flux through the polyol pathway (Tiwari and Rao, 2002). Elevated generation of free radicals resulting in the consumption of antioxidant defense components may lead to disruption of cellular functions and oxidative damage to membranes and may enhance susceptibility of lipid peroxidation. Under physiological conditions, a widespread antioxidant defense system protects the body against the adverse effects of free radical production. The efficiency of this defense mechanism is altered in diabetes and, therefore, the ineffective scavenging of free radicals may play a crucial role in determining tissue damage (Wohaieb and Godin, 1987).

Since ancient time phytotherapy has been used as folk

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medicine to treat various diseases, including diabetes mellitus. More than 400 plants treatments for diabetes mellitus have been recorded, but only small numbers of these have received scientific and medical evaluation to assess their efficacy (Oyeola et al., 2007). However, increased attention has focus on extraction, characterization and evaluation of antidiabetic principles of some of these plants.

Trecuia africana Decne. (Moraceae), commonly known as African bread fruit is a plant food native to tropical West and parts of East Africa. Ethnomedically, it is used as a verbrifuge, vermifuge, galactogogue and laxative (Ogbonnia et al., 2008). The plant is also an important component of some ancient anti-diabetic recipe used in Western and Middle-Belt of Nigeria. The pods of *T. africana* have been evaluated for its nutritive properties by some workers for its flavonoids, phenolic and poly-saccharide content (Akubor and Badifu, 2004; Chukwu et al., 1994). Our previous experimental results were highly encouraging as they revealed that the levels of blood glucose, triglycerides and LDL-cholesterol were lowered significantly after oral administration of aqueous root extract of *T. africana* in glucose load condition and in STZ-induced diabetes.

The present study was carried out to evaluate the effect of aqueous root extract of *T. africana* on the rate of plasma haemoglobin glycosylation in rabbits with STZ-induced diabetes and to correlate such with plasma lipid peroxidaion level.

MATERIALS AND METHODS

Plant materials

Dried roots of *T. africana* were obtained from herbal and medicine market in Lagos (Nigeria). The plant material was botanically authenticated by Dr. Obembe O. of Plant Science Department, Adekunle Ajasin University Akungba (AAUA), Nigeria and the samples (AU. 032) were stored in herbarium of Department of Plant Science, AAUA.

Preparation of extract

One kilogram of dried roots of *T. africana* was coarsely powdered and macerated in distilled water and extracted twice, on each occasion with 2.5 L of distilled water at room temperature for 48 h. The combined aqueous extracts soluble were concentrated to dryness under reduced pressure at 60°C in a rotary evaporator. Resulting aqueous extract was evaporated to dryness, finally giving 32.4 g (that is 3.24% yields) of a dark-brown, powdery crude root extract of *T. africana*. Aliquot portions of the crude plant extract residue were weighed and dissolved in distilled water for use on each day of experiments.

Experimental animals

Adult male rabbits (n = 20) weighing 950 - 1200 g and 5 - 6 months old obtained from Federal College of Agriculture, Akure (Nigeria) were selected as the experimental animal. They were housed individually in large clean metallic cages and kept in a well ventilated room with a 12 hour light/dark cycle and 50 - 60% relative humidity at a temperature of about 30°C and fed with standard growers mash supplied by Bendel Feed and Flour Mills Ltd, Ewu, Edo State (Nigeria), and tap water *ad libitum* throughout the experimental period. The animals were kept in the laboratory condition for two weeks before the commencement of the experiment for acclimatization. Maintenance and treatment of animals were in accordance with the principles of the "Guide for care and use of laboratory animals in research and teaching" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH) publication 86 - 23 revised in 1985.

Experimental induction of diabetes in rabbits

Diabetes was induced by a single intraperitoneal injection of 65 mg/kg of STZ Sigma® in physiological saline with pH adjusted to 4.5 using citric acid as described (Palanichamy et al., 1988). After one week of STZ administration and 18 h of fasting, blood glucose was measured by collecting 3 - 5 ml of blood from the marginal ear vein. The glucose levels that were above 250 mg/dl were included in the experiment.

Animal grouping

Rabbits were divided into two groups designated: Control and Test. Rabbits in test group received 200 mg/kg T. Africana aqueous root extract while animals in control group were administered 10 ml/kg distilled water. The water and extract were administered to each rabbit using a stomach tube attached to 20 ml standard syringe. The tube was inserted into stomach through the oesophagus, and the plunger of syringe was pressed slowly and steadily.

Blood collection and assays

Blood was collected into sodium oxalate tubes from each of the rabbits using 22 gauge sterile syringes from the marginal vein of the ear, before treatment and five weeks after treatment. Plasma was separated by centrifugation at 3000 g for 10 min. Plasma glucose levels were then measured by commercial kits (Boehringer Manheim, Germany) by using a glucose oxidase method with a HITACHI 917® auto analyzer. Serum total haemoglobin was determined Using BioAssay Systems' QuantiChrom™ hemoglobin assay kit which is based on an improve Triton/NaOH method, in which hemoglobin is converted into a uniform colored end product. The intensity of the color measured at 400 nm, is directly proportional to hemoglobin concentration. Glycosylated haemoglobin was assayed according to the method of Nayak and Pattabiraman (1981). Thiobarbituric acid reactive species (TBARS) were estimated by the method of Varshney and Kale (1990).

Statistical analysis

Data are mean ± SEM of five independent determinations. Statistical analysis was by student t-test using SPSS 10.0. $P < 0.05$ than control was considered significant.

RESULTS

As shown in Table 1, induction of diabetes resulted in a significant increase ($p < 0.05$) in blood glucose level of

Table 1. Changes in blood glucose concentrations (mg/dl) of STZ-induced diabetic rabbits treated with aqueous root extract of *T. africana*.

Time	Control	Test
Initial	310 .7 ± 28.3	315.9 ± 17.9
Final	342.1 ± 16.5	246.1 ± 32.3*

Values are mean ± SEM of five independent determinations. * Statistically different from control.

Table 2. Plasma total haemoglobin concentrations (g/dl) of STZ-induced diabetic rabbits treated with aqueous root extract of *T. africana*.

Time	Control	Test
Initial	17.3 ± 2.6	16.9 ± 2.2
Final	19.5 ± 1.3	28.5 ± 5.0*

Values are mean ± SEM of five independent determinations. *Statistically different from control.

Table 3. Changes in plasma glycosylated haemoglobin (% Hb) concentrations of STZ-induced diabetic rabbits treated with aqueous root extract of *T. africana*.

Time	Control	Test
Initial	57.2	42.6
Final	75.9	37.9*

Values are mean ± SEM of five independent determinations. *Statistically different from control.

Table 4. Levels of lipid peroxides (mM/100 ml plasma) of STZ-induced diabetic rabbits treated with aqueous root extract of *T. africana*.

Time	Control	Test
Initial	1.6 ± 1.0	1.4 ±1.0
Final	3.5 ± 1.0	1.9 ± 1.0*

Values are mean ± SEM of five independent determinations. * Statistically different from control.

rabbits. Treatment with 200 mg/kg aqueous root extract of *T. africana* for five weeks significantly lowered the rise in blood glucose concentration following STZ administration. This accounts for the decrease observed in plasma glucose concentration in group II animals as compared to the control.

Table 2 shows the effect of a five week treatment with aqueous root extract of *T. africana* on plasma total hae--moglobin concentration of STZ-diabetic rabbits. Plasma total haemoglobin concentration was significantly ($p < 0.05$) elevated in diabetic animals treated with *T. africana* aqueous root extract when compared to control.

The observed changes in percentage of total haemoglobin glycosylated in both test and control groups are as presented in Table 3. Following a five week treatment of diabetic rabbits with aqueous root extract of *T. africana*, rate of haemoglobin glycosylation was significantly lowered ($p < 0.05$) in test rabbits compared with the rate in control rabbits.

Plasma concentration of TBARS in both test and control rabbits is as shown in Table 4. As observed from the results of this study, plasma TBARS concentration, an index of plasma lipid peroxidation was depleted significantly ($p < 0.05$) in test animals compared with control rabbits.

DISCUSSION

STZ-induced experimental diabetes is a valuable model for induction of type I diabetes (Szkudelski, 2001; Oyeola et al., 2007; Ogbonnia et al., 2008). Further, the STZ-diabetes animals may exhibit most of the diabetic complications, namely, myocardial cardiovascular, gastrointestinal, nervous, vas deferens, kidney, and urinary urinary bladder dysfunctions through oxidative stress (Krishnamurti and Steffes, 2001). During diabetes, the excess glucose present in the blood reacts with haemoglobin to form glycosylated haemoglobin. It has been reported that various proteins, including haemoglobin, albumin, collagen, LDL, or crystalline proteins undergo non-enzymatic glycation in diabetes (Klein, 1995). The rate of glycation is proportional to the concentration of blood glucose. Glycosylated haemoglobin has been found to be increased over a long period of time in diabetes (Forbes et al., 2003). There is evidence that glycation itself may induce the formation of oxygen-derived free radicals in diabetic condition (Gupta *et al*., 1997). Therefore, the measurement of glycosylated haemoglobin is supposed to be a very sensitive index for glycemic control. In the present study, the diabetic untreated rabbits had shown higher rates of haemoglobin glycosylation compared with those treated with aqueous root extract of *T. africana* indicating their poor glycemic control. The ability of aqueous root extract of *T. africana* to bring about a significant decrease in plasma rate of haemoglobin glycosylation could be indicative of its potential ability to improve glycemic status. This suggests amelioration of oxidative stress due to hyperglycemia by treatment with aqueous root extract of *T. africana*. The most commonly used index of lipid peroxidation is TBARS. The increased lipid peroxidation in the plasma of diabetic animals may be due to the observed remarkable increase in TBARS concentration. The observed effect of aqueous root extract of *T. africana* on plasma lipid peroxidation may be due to its hypoglycemic effect on diabetic rabbits thus preventing excessive formation of free radicals through various biochemical pathways and also reduces the potential glycation of the enzymes. This is in agreement with the findings of Ziai et al. (2005) that *Psyllium* decreased serum glucose and glycosylated

haemoglobin significantly in diabetic outpatients. Similarly, a recent study by Shahid and Mahboob (2009) showed that serum levels of glycosylated haemoglobin (HbA1c) correlate positively with serum concentration of nitric oxide (NO), a potent vasodilator known to be depleted in advanced diabetic state, in both normotensive and hypertensive diabetic subjects.

Conclusion

The results generated in this study show that aqueous root extract of *Treculia africana* possesses antioxidative principles thus inhibiting the attendant haemoglobin glycosylation resulting from the formation of lipid peroxides in diabetes.

Conflict of interest

The authors declare that no competing financial interests exist.

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