

Aminat Omolola Imam-Fulani*, Kamaldeen Olalekan Sanusi and Bamidele Victor Owoyele

Effects of acetone extract of *Cola nitida* on brain sodium-potassium adenosine triphosphatase activity and spatial memory in healthy and streptozotocin-induced diabetic female Wistar rats

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Abstract

Background: This study was carried out to investigate the effects of acetone extract of *Cola nitida* on brain Na^+/K^+ -ATPase activity and spatial memory of healthy and streptozotocin (STZ)-induced diabetic female Wistar rats.

Methods: Forty-two female Wistar rats were used for this study and were randomly distributed into six groups ($n=7$). Rats in group 1 were used as control and were administered normal saline; group 2 rats were healthy rats administered 50 mg/kg of kola nut extract per day; group 3 rats were healthy rats administered 100 mg/kg of kola nut extract per day; group 4 rats were a diabetic group also administered normal saline; group 5 rats were diabetic rats administered 50 mg/kg of kola nut extract per day; and group 6 rats were diabetic rats administered 100 mg/kg of kola nut extract per day. Diabetes was induced with 50 mg/kg of STZ. After 3 weeks of administration, the spatial memories of the rats were tested using the Y-maze, followed by assay of Na^+/K^+ -ATPase activity.

Results: The result shows a significant increase in Na^+/K^+ -ATPase activity of diabetic treated groups (5 and 6) when compared with the diabetic group (4) and a significant increase in Na^+/K^+ -ATPase activity of healthy treated groups (2 and 3) when compared with control. Also, there was a significant increase in spatial memory of the diabetic treated groups when compared with diabetic group.

Conclusions: This study revealed that kola nut extract has restorative effect on brain Na^+/K^+ -ATPase activities and spatial memory of STZ-induced diabetic female Wistar rats.

Keywords: acetone extract; cognition; diabetes; kola nut; sodium-potassium ATPase.

Introduction

Diabetes mellitus (DM) is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secreted insulin [1]. Such deficiency results in increased blood glucose level, which in turn can damage many of the body's systems, including blood vessels and nerves [1]. One therapeutic approach for treating diabetes is to decrease post-prandial hyperglycemia [2]. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolyzing enzymes α -glucosidase and α -amylase in the digestive tract. Inhibition of these enzymes delays carbohydrate digestion and prolongs overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise [2, 3]. Many natural resources have been investigated with respect to the suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine [3, 4]. DM is associated with slowly progressive end-organ damage in the brain. Mild to moderate impairment of cognitive function has been reported in both type I and type II DM patients [5, 6]. More so, from studies performed in adults with type I diabetes, there is also observed reduction in motor speed and psychomotor efficiency [7].

Kola nut belongs to the plant family *Sterculiaceae*, having about 125 species of trees native to the tropical rainforests of Africa. Of these species, the most common in Nigeria are *Cola nitida* with two phenotypic varieties, the white and red cultivars, and *Cola acuminata*.

In West Africa and Sudan, kola nuts are popular masticatory agents, eaten as a principal stimulant to keep awake and withstand fatigue. Besides the fact that they contain caffeine and act as a stimulant and anti-depressant, they are also thought to reduce hunger, aid digestion, and work as an aphrodisiac. In some parts of Africa, kola nuts are given as gifts to visitors entering a home, usually with some formal ceremony [8]. Besides the ceremonial uses, kola nut extracts have become popular in Europe and North America as a natural or alternative medicine for the treatment of diabetes [9].

*Corresponding author: Aminat Omolola Imam-Fulani, Department of Physiology, College of Health Sciences, University of Ilorin, P. M. B. 1515, Ilorin 23401, Kwara, Nigeria, Phone: +2348032145355, E-mail: faminat2@gmail.com

Kamaldeen Olalekan Sanusi and Bamidele Victor Owoyele:
Department of Physiology, College of Health Sciences, University of Ilorin, Ilorin, Kwara, Nigeria

Sodium-potassium adenosine triphosphatase (Na⁺/K⁺-ATPase), also known as Na⁺/K⁺ pump or sodium pump, is an antiporter enzyme (an electrogenic transmembrane ATPase) located in the plasma membrane of all animal cells. The Na⁺/K⁺-ATPase helps maintain resting potential, allows transport, and regulates cellular volume [10]. It also functions as signal transducer/integrator to regulate mitogen-activated protein kinases pathway, reactive oxygen species, and intracellular calcium. In most animal cells, the Na⁺/K⁺-ATPase is responsible for about one fifth of the cell's energy expenditure. For neurons, the Na⁺/K⁺-ATPase can be responsible for up to two thirds of the cell's energy expenditure [11]. Oxidative metabolism is very active in the brain, where large amounts of chemical energy as ATP molecules are consumed. This is mostly required to maintain cellular Na⁺/K⁺ gradients that underlie resting and action potentials, which are involved in nerve impulse propagation, neurotransmitter release, and cation homeostasis [12].

Brain Na⁺/K⁺-ATPase enhances neuroprotection and memory consolidation and diminishes cognitive deficits [13]. However, Na⁺/K⁺-ATPase activity was found to be decreased in diabetic male rat brains [14]. Also, according to an experiment carried out by Obochi et al. [15], kola nut increases sodium pump activity in healthy Wistar rats.

It has been reported [16] that female and male brains differ. The differences begin early during development due to the combination of genetic and hormonal events and continue throughout the life span. This should be considered when studying the pharmacological effects of drugs in the study of brain and behavior. Physiological sex differences account for marked differences in disease incidence, manifestation, prognosis, and treatment observed between the sexes [17].

The high prevalence of diabetes, coupled with the harsh side effects of some synthetic drugs, has led to increasing search for alternatives that are relatively cheap with minimal side effects.

Thus, previous studies have shown contrasting views on the effect of kola nut on blood glucose level [18–20]. However, there is paucity of information on kola nut effects on brain Na⁺/K⁺-ATPase activities and spatial memory in diabetic female Wistar rats.

This report highlights the need to conduct hypothesis-driven biomedical studies that take into account sex as a basic human variable. Therefore, the aim of this study is to investigate the effects of kola nut on blood glucose level, brain Na⁺/K⁺-ATPase activities, and spatial memory in healthy and diabetic female Wistar rats.

Materials and methods

Experimental animals

Forty-two female Wistar rats were procured from Biochemistry Department, University of Ilorin. They were kept in well-ventilated house conditions [temperature (28 ± 1) °C; photoperiod: 12 h light and 12 h dark cycle; humidity: 45–50%]. They were given access to feed and water (*ad libitum*) and were acclimatized for 2 weeks. The study was generally conducted in accordance with the care and the use of laboratory animals and approved by the Ethical Committee of the University of Ilorin, Ilorin, Nigeria.

Preparation of kola nut extract

Seeds of species *C. nitida* were used in this study. The seeds were purchased from Oja-Oba market in Ilorin, Kwara State, Nigeria.

The seeds were peeled and chopped into small chips and allowed to dry at room temperature on the laboratory bench. Dried chips were blended into powdery form. About 10 g of the powdered seed was extracted with 100 mL of 80% acetone (Sigma-Aldrich, St. Louis, MO, USA) and was filtered (Whatman No. 2) under vacuum [20]. The filtrate was then evaporated using a water bath (Joan Lab, Hangzhou, Zhejiang, China) at 45 °C until about 90% of the filtrate had been evaporated. The extract was frozen and stored for subsequent use.

Induction of diabetes

Diabetes was induced in overnight fasted female Wistar rats by a single intraperitoneal injection of freshly prepared solution of streptozotocin [(STZ) Sigma-Aldrich, St. Louis, MO, USA] at a dose of 50 mg/kg body weight in 0.1 M citrate (Sigma-Aldrich, St. Louis, MO, USA) buffer (pH 4.5). Dextrose saline (Sigma-Aldrich, St. Louis, MO, USA) was given after induction of diabetes for rehydration. Also, food and water intake were closely monitored. The animals were confirmed diabetic by the measurement of fasting blood glucose in the tail vein blood after 72 h of injection with a portable glucometer (on-call plus, ACON labs, San Diego, CA, USA). The animals with hyperglycemia (blood glucose >8.1 mmol/L) and stable symptoms of DM such as polyuria, polyphagia, and polydipsia were used for the study.

Testing of spatial memory

Three weeks after administration of extracts, the testing of spatial memory commenced in a Y-shaped maze with three opaque arms at a 120° angle from each other. After introduction of the animal to the center of the maze, the animal was allowed to freely explore the three arms. The number of arm entries and the number of triads were recorded in order to calculate the percentage of alternation. An entry occurs when all four limbs were within the arm. The percentage of their spatial memory is calculated as follows:

$$\% \text{ Spatial memory} = \frac{\text{Number of correct decisions}}{\text{Number of alternations}} \times 100.$$

Study design

Six groups of seven female Wistar rats (13–14 weeks old) weighing between 170 g and 200 g were used for the study. Administration of extract to the diabetic animals via oral gavage began 4 days after STZ induction, and this was considered as the first day of administration. Group 1 (control) received normal saline daily orally for 3 weeks; group 2 (healthy rats) received 50 mg/kg of kola nut extract daily orally for 3 weeks; group 3 (healthy) received 100 mg/kg of kola nut extract daily orally for 3 weeks; group 4 (diabetic group) also received normal saline daily orally for 3 weeks; group 5 (diabetic treated) received 50 mg/kg of kola nut extract daily orally for 3 weeks; and group 6 (diabetic treated) received 100 mg/kg of kola nut extract daily orally for 3 weeks. Their blood glucose levels were checked at the end of every week through tail pricking.

At the end of the experiment, the animals were fasted for 12 h and were sedated by intramuscular injection of ketamine (Sigma-Aldrich, St. Louis, MO, USA). Cervical dislocation was carried out to remove the brain.

Preparation of homogenate

A clean beaker was quarter filled with ice-cold sucrose buffer solution. The brain was removed immediately and put into the beaker containing ice-cold solution of 0.25 M sucrose (Sigma-Aldrich, St. Louis, MO, USA). The brain was then homogenized using a 50 mL Potter-Elvehjem glass homogenizer (ACON labs, San Diego, CA, USA) in ice-cold sucrose to avoid proteolytic hydrolysis or degradation of the enzyme of interest. The Teflon pestle (ACON labs, San Diego, CA, USA) was driven mechanically at approximately 1000 rpm. The supernatant therefore was centrifuged at 5000×g for 3 min to obtain the mitochondrial pellet. The pellet was washed three times by re-suspending it in the homogenizing medium. All the centrifugation steps were carried out at 4 °C in an H1850R tabletop high-speed refrigerated centrifuge (Selon Scientific Instrument, Shanghai, China). The mitochondria-containing solution was quickly transferred into a small storage container (dispensable) and stored in the freezer at below 4 °C until when they would be used.

Na⁺/K⁺-ATPase assay

The activity of Na⁺/K⁺-ATPase was investigated using a standard assay (Agappe Diagnostics, Switzerland). The reaction medium contained 200 mM NaCl, 40 mM KCl, 60 mM Tris (pH 7.4), 80 mM MgCl₂·6H₂O, 20 mM ethylene glycol-bis(2-aminoethylether) tetraacetic acid, enzyme source, and 8 mM ATP, after which it was incubated at 37 °C for 1 h. The reaction was stopped using 5% ice-cold sodium lauryl sulfate (Sigma-Aldrich, St. Louis, MO, USA). Reagent C (containing 2% ammonium molybdate and 9% ascorbic acid in the ratio 1:4) was added for color change, after which the inorganic phosphate was determined using the spectrophotometric method. Absorbance was read at 820 nm. The protein content of the homogenate was determined using the biuret method. Na⁺/K⁺-ATPase activities were expressed as micromoles of inorganic phosphate (Pi) ion released per milligram of protein per hour (i.e. μmol Pi mg⁻¹ protein h⁻¹ × 10⁻³).

Statistical analysis

The data obtained were expressed as mean and standard error of mean and analyzed using analysis of variance using Bonferroni *post hoc* test. Values were considered statistically significant at $p < 0.05$. Statistical analyses were performed using statistical packages for social sciences (SPSS) version 20.00.

Results

Effects of kola nut extract on blood glucose level of healthy and diabetic female Wistar rats

Healthy rats administered low (50 mg/kg) and high (100 mg/kg) doses of kola nut extract showed no significant change in blood glucose level across the weeks, but there were significant decreases in the blood glucose levels of diabetic rats administered low (50 mg/kg) and high (100 mg/kg) doses of the extract in weeks 2 and 3 as shown in Table 1.

Effects of kola nut extract on brain Na⁺/K⁺-ATPase activity of healthy and diabetic female Wistar rats

The result of this experiment shows a dose-dependent significant ($p < 0.05$) increase in ATPase activity of diabetic rats administered 50 mg/kg and 100 mg/kg of the extract, compared with the diabetic group as shown in Figure 1. Also, in the healthy rats, those that were administered 50 mg/kg and 100 mg/kg of the extract show a significant increase in ATPase activities (555.00 ± 19.55

Table 1: Effects of kola nut extract on blood glucose level of healthy and diabetic female Wistar rats.

Groups	Blood Week 1	Glucose Week 2	Level, mmol/L Week 3
Control	4.16 ± 0.24	4.06 ± 0.3	4.4 ± 0.2
Low kola nut extract	5.23 ± 0.47	4.33 ± 0.53	4.15 ± 0.39
High kola nut extract	4.52 ± 0.52	4.28 ± 0.27	4.95 ± 0.37
Diabetic group	20.02 ± 1.07	23.62 ± 2.19	25.97 ± 1.61
Diabetic low kola nut extract	21.88 ± 3.02	11.00 ± 1.91 ^a	9.7 ± 1.91 ^a
Diabetic high kola nut extract	20.3 ± 2.06	13.18 ± 1.24 ^a	8.96 ± 1.79 ^a

Values are expressed as mean ± SEM. Low kola nut extract = 50 mg/kg extract. High kola nut extract = 100 mg/kg extract. ^aSignificantly ($p < 0.05$) different from week 1.

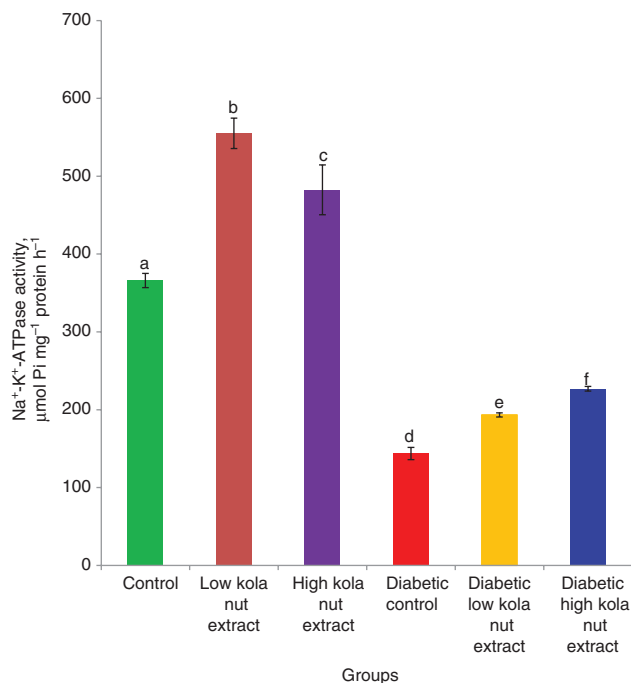


Figure 1: Effect of kola nut extract on brain Na⁺/K⁺-ATPase activity of healthy and diabetic female Wistar rats. Bars with different letters are significantly ($p < 0.05$) different.

and 482.50 ± 31.9) compared to control (366.00 ± 9.05) also shown in Figure 1.

Effect of diabetes and kola nut extract on spatial memory of the rats

There was a significant ($p < 0.05$) decrease in spatial memory of diabetic rats compared to healthy rats as indicated in Figure 2. However, there is no significant difference between the spatial memory of diabetic rats treated with low and high kola nut extracts, but they show a dose-dependent significant increase in mean of their spatial memory compared with the diabetic group as shown in Figure 2. Also, comparison within the healthy rats groups showed that there was no significant difference in the percentage of the spatial memory of treated rats compared to control as shown in Figure 2.

Discussion

Management of the blood glucose level is a critical strategy in the control of diabetes complications. Some researchers have suggested that consumption of kola nut decreases insulin sensitivity and increases blood glucose level [18–20], while others reported its beneficial effect in

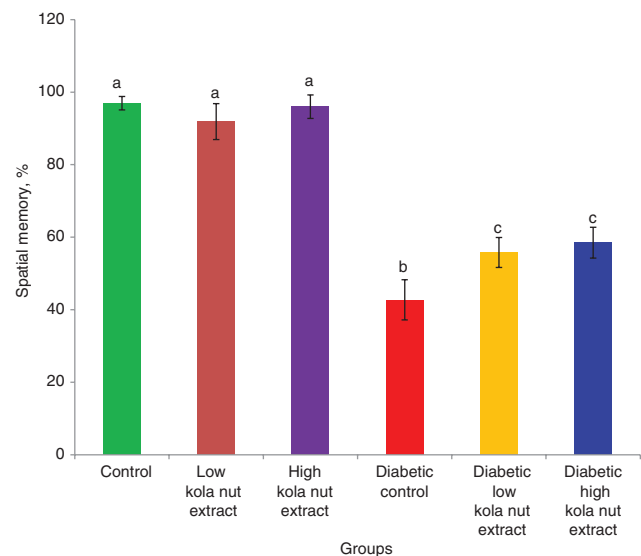


Figure 2: Effect of kola nut extract on spatial memory of healthy and diabetic female Wistar rats. Bars with different letters are significantly ($p < 0.05$) different.

diabetes through its antioxidant properties and the inhibition of α -amylase and α -glucosidase by its phenolic constituent [20]. However, from this study, no significant change was observed in the blood glucose level of normal rats administered kola nut extract, but the result from the diabetic rats administered the extract showed a significant decrease at the second and third weeks of administration (Figure 1), which seems to be in line with the report by Oboh et al. [20] in 2014.

The Na⁺/K⁺-ATPase or sodium pump is the membrane-bound enzyme that maintains the Na⁺ and K⁺ gradients across the plasma membrane of animal cells. Because of its importance in many basic and specialized cellular functions, this enzyme must be able to adapt to changing cellular and physiological stimuli [21].

Among the diabetes-induced metabolic changes, disturbances of Na⁺/K⁺-ATPase activity have been widely reported [22]. In line with Ver et al. [14], it was observed in this study that Na⁺/K⁺-ATPase activity decreased significantly in the diabetic control group of the rats when compared with the healthy groups. However, this study observed increase in the activity of Na⁺/K⁺-ATPase in the diabetic rats' brain following administration of kola nut extract. This indicates restorative effects of the extract on the diminished activities of the enzymes in diabetic state.

DM is also associated with slowly progressive end-organ damage in the brain. Mild to moderate impairment of cognitive functioning has been reported in both type I and type II DM patients [5, 6]. Interestingly, STZ-induced

diabetic rats of this study display a reduced spatial memory as opposed to the healthy rats. This may be due to the reduced density of synaptic proteins in diabetic rats previously reported [23, 24], which could be related to the observed spatial memory deficits [5, 24].

It was also observed in this study that the spatial memory of diabetic rats treated with kola nut extract increased significantly compared to untreated diabetic rats, which could be a result of the high content of caffeine present in kola nut. Hence, the study by Duarte et al. [25] indicates that chronic consumption of caffeine can prevent neurodegeneration and memory impairment.

Conclusions

This study showed that kola nut (*C. nitida*) extract exhibits a hypoglycemic effect in diabetic female Wistar rats but does not alter the blood glucose level of healthy rats. It also revealed a positive effect on the brain Na⁺/K⁺-ATPase activity of diabetic rat, as well as its restorative effect on spatial memory of the diabetic rats. The observed memory impairment in diabetes may be due to the reduced Na⁺/K⁺-ATPase activity, and the elevated enzyme activity following extract administration may be related to the improved memory. Although there is paucity of information on the effect of kola nut on memory, we therefore recommend that further research should be carried out in this field to determine the active constituents of kola nut responsible for the positive outcome of this study and should also be suggested for clinical experiment and evaluation.

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