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ARTOCARPUS COMMUNIS FORST. ROOT-BARK AQUEOUS EXTRACT-AND STREPTO-ZOTOCIN-INDUCED ULTRASTRUCTURAL AND METABOLIC CHANGES IN HEPATIC TISSUES OF WISTAR RATS

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Abstract

Decoctions and infusions of *Artocarpus communis* (Forst.) (family: Moraceae) root-bark are commonly used traditionally among the Yoruba-speaking people of Western Nigeria as folk remedies for the management, control and/or treatment of an array of human diseases, including type 2, adult-onset diabetes mellitus. Although numerous bioactive flavonoids have been isolated from the roots, stem-bark and leaves of *A. communis*, to the best of our knowledge, the effects of the plant's root-bark extract on animal model of diabetes mellitus and on liver tissues have hitherto, not been reported in the biomedical literature. In view of this, the present study was undertaken to investigate the glycaemic effect of, and hepatic tissue ultrastructural, morphological and metabolic changes induced by, *A. communis* root-bark aqueous extract (ACE) in Wistar rats. The ultrastructural, morphological and metabolic effects of ACE have been compared with those induced by streptozotocin (STZ) in rat experimental paradigms. Four groups (A, B, C and D) of Wistar rats, each group containing 10 rats, were used. Diabetes mellitus was induced in the diabetic groups B and C animals by intraperitoneal injections of STZ (75 mg/kg body weight), while group A rats received *A. communis* root-bark aqueous extract (ACE, 100 mg/kg body weight, i.p.) alone. Control group D rats received distilled water in quantities equivalent to the volume of ACE administered intraperitoneally. The rats in group C were additionally treated with ACE (100 mg/kg body weight i. p.) daily from day 3 to day 10 after STZ treatment. Hepatic glucokinase, hexokinase, glutamate dehydrogenase, succinate dehydrogenase, β-hydroxybutyrate dehydrogenase, serum insulin and blood glucose levels of the animals were measured and recorded before and after ACE, STZ and STZ+ACE treatments. Hepatic tissues were also processed for transmission electron microscopy. Electron microscopic examinations showed toxic, deleterious alterations in the ultrastructures of groups A, B and C hepatic cells, the most prominent deleterious effects being on the hepatocytes. Ultrastructural changes observed within the hepatocytes of groups A, B and C rats include disrupted mitochondria with increase in lipid droplets, extensive hepatocellular vacuolation, scanty rough endoplasmic reticulum (RER) and ribosomes. Large glycogen clusters were also noticed displacing the mitochondria and RER in group A rats. Group A rats also developed significant hyperglycemia ($p<0.05$) immediately after ACE administration, while groups B and C rats developed hyperglycemia 24 hours after STZ treatment. When compared with the control group D rats, the activities of all the three subsystems were disrupted, leading to overall inhibition of oxidative phosphorylation of the liver mitochondria in groups A, B and C rats, but remain normal in the untreated group D control rats. The findings of the present study indicate that *A. communis* root-bark aqueous extract induces hyperglycaemia in the experimental animal model used, and that the plant's extract disrupts the ultrastructural characteristics and architecture of hepatocytes as well as oxidative energy metabolism.

Key Words: *Artocarpus communis*; streptozotocin; hyperglycaemic effects; hepatic tissues; ultrastructural and metabolic changes

Introduction

Artocarpus communis (Forst.) is a perennial, evergreen, terrestrial, single-stemmed, erect flowering plant, popularly known in English as "breadfruit" tree because of the "bread-like texture" of its edible fruits. It is a member of the Moraceae family which consists of about 50 genera and over 1000 species (Tindal, 1965). The 'breadfruit' tree is a fast growing plant of up to 20–30 metres in height, with a trunk of up to 1–2 metres in diameter. All the morphological parts of the tree, including the unripe fruit, are rich in milky, gummy latex. The shoots, bark and latex of the plant have been reported to have many traditional, ethnomedical uses. For example, in the West Indies, a decoction of the plant's leaves is used to lower elevated blood pressure and to relieve asthma. In Taiwan, the leaves are used for fever and liver disorders, while the sap is used for thrush, stomach pain, and dysentery. Root extracts of the plant are used as antibacterial remedy against gram-positive bacteria, and as anticancer agents (Kasahara and Hemmi, 1986). In Nigeria, the root-bark of *A. communis* is traditionally used for a variety of human ailments, including management or treatment of diarrhea, dysentery as well as for diabetes mellitus. Natural compounds isolated from *A. communis* roots, stem and leaves include prenylflavonoids, dihydrocycloartomunin, dihydroisocyloartomunin, heteroflavanones, cylomorusin, artonins A and B, cycloheterhyllin, cyclocomunin, cycloartomunin artocarpanone A, and cudraflavone (Chan *et al*., 2003; Lin *et al*., 1991), and flavonoids, dihydroartomunoxanthone, artomunoisoxanthone, cyclocomunomethonol and artomunoflavanone (Wang *et al*., 2004). Some of these compounds possess significant anti-platelet (Lin *et al*., 1993) and anti-inflammatory (Wei *et al*., 2005) activities. Generally, flavonoids constitute a group of naturally-occurring chemical compounds widely distributed as secondary metabolites in the plant kingdom. They have been recognized for having interesting pharmacological properties, such as anti-inflammatory, antiallergic, antiviral, antibacterial, and antitumoral activities (Middleton, 1998). One of these flavonoids, quercetin (3,5,7,3´,4´-pentahydroxyflavone), prevents oxidantive injury and cell death by several mechanisms, including scavenging oxygen radicals (Inal *et al*., 2002), protecting cells and tissues against lipid peroxidation, and chelating metal ions (Afanas've *et al*., 1989).

It is known that streptozotocin (STZ)-induced diabetes mellitus causes functional and structural changes in some tissues and organs of animals. However, the mechanism of the cytotoxic action of STZ is not fully understood. Experimental evidence has also demonstrated that some of the toxic, deleterious effects of STZ are attributable to induction of metabolic processes, which lead on to an increase in the generation of reactive oxygen species (ROS) (Chen *et al*; 1990). Apart from production of ROS, STZ also inhibits free radical scavenger-enzymes (Kröncke *et al*; 1995). The superoxide radical has been implicated in lipid peroxidation, DNA damage, and sulphydryl oxidation (Tiedge *et al*; 1997; Matkovics *et al*; 1998).

The basic defects in diabetes mellitus include impaired glucose metabolism and basement membrane alterations (Striker *et al*., 1993). Besides, mitochondrial oxidative energy metabolism also seems to be impaired in experimental diabetes (Brady *et al*., 1985). With regard to mitochondrial respiratory activity, the regulatory factors which are affected in diabetes are glutamate, succinate and β-hydroxybutyrate dehydrogenase (Park and Drake, 1982). Thus, mitochondria seem to be the direct targets of the diabetic state (Berdanier, 2001). There are several reasons why crude medicinal plant products used as herbal medicines should be studied. Although herbal remedies may have recognizable therapeutic effects (Bailey and Day, 1989), they may also have toxic side-effects (Keen *et al*; 1994). Not only is there an absence of documented toxicity profile for most herbs, but also there is no standard system for reporting adverse reactions to herbal medicines. To the lay public, 'natural' is equivalent and related to 'safety'. This is, however, a false assumption.

The present study was prompted by the claim of some traditional health practitioners in Western Nigeria that decoctions and infusions of *A. communis* root-bark are effective remedies for the management and/or control of type 2, adult-onset diabetes mellitus. The core aims of the present study were, therefore, to examine the (i) glycaemic effect of *A. communis* root-bark aqueous extract in rats, (ii) effects of the plant's root-bark extract on the morphology of hepatocytes, and (iii) effects of the plant's root-bark extract on the oxidative energy metabolism of liver mitochondria in ACE-, STZ-, STZ-+ACE-treated rats and various liver tissues of rats.

Materials Methods

The experimental protocol and procedures used in this study were approved by the Ethics Committee of the University of Durban-Westville, Durban 4000, South Africa; and conform with the *"Guide to the Care and Use of Animals in Research and Teaching"* [Published by the Ethics Committee of the University of Durban-Westville, Durban 4000, South Africa].

Animals

This study was carried out in healthy, male and female Wistar rats, weighing 250-300 g. The animals were housed under standard laboratory conditions of light, temperature and humidity. The animals were given standard rat pellets and tap water *ad libitum*. The rats were randomly divided into four experimental groups: Group A (*A. communis* root-bark extract-treated), Group B (STZ-treated), Group C (STZ-+ *A. communis* root-bark extract-treated), and Group D (distilled water-treated control) rats. Each group consisted of 10 rats. All the animals were fasted for 16 hours, but still allowed free access to water, before the commencement of our experiments.

Plant material

The root-bark of *Artocarpus communis* (Forst.) [family: Moraceae] (locally known by its common English name of "Breadfruit" or "Gbere" in Yoruba language of Western Nigeria) were collected in Ile-Ife, Western Nigeria, between April and May 2006. Pieces of the plant's root-bark were identified by the Taxonomist/Curator of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria, as those of *A. communis* Forst. (family, Moraceae). A voucher specimen of the plant has been deposited in the Herbarium of the University's Botany Department.

Preparation of *Artocarpus communis* root-bark aqueous extract

Pieces of *Artocarpus communis* fresh root-bark were air-dried at room temperature. One kilogram (1 kg) of the air-dried root bark of the plant was milled into fine powder in a Waring commercial blender. The powdered root-bark was macerated in distilled water and extracted twice, on each occasion with 2.5 1itres of distilled water at room temperature for 48 h. The combined aqueous extract solubles were concentrated to dryness under reduced pressure at $60^{o}C \pm 1^{o}C$ in a rotary evaporator. The resulting aqueous extract was freeze-dried, finally giving 56.23 g (i.e., 5.62% yields) of a clay-colour, powdery, crude aqueous root-bark extract of *A. communis* (ACE). Aliquot portions of the crude extract residue were weighed and dissolved in distilled water for use on each day of our experiment without any further purification.

Acute toxicity testing

The median lethal dose (LD_{50}) of *A. communis* root-bark aqueous extract (ACE) was determined in male and female rats (weighing 250–300 g), using a modified method of Lorke (1983). Rats fasted for 16 h were randomly divided into groups of 10 mice each. Graded doses of ACE (12.5, 25, 50, 100, 200, 400, 800 and 1600 mg/kg) were separately administered intraperitoneally to the rats in each of the 'test' groups. Each of the rats in the 'control' group was treated with distilled water (3 ml/kg, i.p.) only. The rats in both the 'test' and 'control' groups were then allowed free access to food and water, and observed over a period of 48 h for signs of acute toxicity. The number of deaths (produced by the extract) within this period of time was noted and recorded. Log dose-response plots were constructed for the plant's extract, from which the LD_{50} value of the root-bark aqueous extract was determined.

Induction of experimental diabetes mellitus

Diabetes mellitus was induced (in groups B and C 'test' rats) by intraperitoneal injections of STZ (75 mg/kg i. p.), freshly dissolved in 0.1mol/l citrate buffer (Rossini *et al*; 1978). Group D 'control' rats were injected with volumes of distilled water equivalent to the volume of administered ACE intraperitoneally. The 'test' animals in groups B and C became diabetic within 48 hours after STZ administration. Diabetes was allowed to develop and stabilize in these STZ-treated rats over a period of 3–5 days. Group C rats additionally received intraperitoneal injections of ACE (100 mg/kg/day i.p.) daily from the 3^{rd} to the 10^{th} day after STZ treatment. All the animals in groups A, B, C and D were kept and maintained under laboratory conditions of light, humidity and temperature. Before the commencement of our experiments, both the control normal (normoglycemic) and STZ-treated, diabetic (hyperglycemic) test rats were fasted for 16-h, but still allowed free access to water throughout. At the end of the 16-h fasting period – taken as 0 time (i.e., 0 h) – blood glucose levels (initial glycemia, G_0) of the fasted normal and STZ-treated, diabetic rats were determined and recorded. The test compound [i.e., *Artocarpus communis* root-bark aqueous extract (ACE, 100 mg/kg i.p.)] was administered to groups A and C fasted rats.

Blood Glucose and serum insulin estimations

Blood samples were obtained from each rat by repeated needle puncture of the same tail tip vein. Blood samples were taken 1 day before STZ-treatment, and also on various days after induction of diabetes mellitus. Blood glucose concentrations were determined by means of Bayer's Elite® Glucometer, and compatible blood glucose test strips (Henry, 1984). Fasted STZ–treated rats with blood glucose concentrations ≥18 mmol/L were considered to be diabetic, and used in this study. Serum insulin concentrations were determined by an enzyme-linked immunosorbent assay (ELISA), using a commercial kit (Crystal Chem, Illinois, Chicago, USA).

Ultrastructural examination

For electron microscopy, six biopsy tissues from each group of sacrificed animals were fixed overnight in formaldehyde-glutaraldehyde fixative at 4°C by the method of Karnovsky (1965). The tissues were subsequently post-fixed in 1% osmium tetroxide. After dehydration in graded concentrations of ethyl alcohol and propylene oxide, the tissues were embedded in spur medium. Tissue samples were sectioned with an ultramicrotome Reichert Ultracut S, using a glass knife. Semi-thin sections were stained with methylene blue, and ultra-thin sections with 8% uranyl acetate dissolved in 50% methanol, and thereafter, in lead citrate according to the method of Venable and Coggeshall (1966). All studies were performed under Transmission Electron Microscope (Jeol JEM 1011) operating at 80 kV, and Image Analysis System (Kontron 300 and AnalySIS). The primary magnification of the Electron Microscopic (EM) examination was x15, 000.

Hexokinase (HXK) and Glucokinase (GCK) Activities

Frozen liver tissues (1 g) were homogenized at 4°C in 9 ml of cold buffer solution (pH 7.4) containing Na-HEPES, 50 mM; KCl, 100 mM; EDTA, 1 mM; $MgCl_2$, 5 mM and dithiothreitol (DTE), 2.5 mM, using a glass-Teflon Potter Homogenizer. The suspension formed was centrifuged at 12000 x g for 1 h at 4°C. The clear supernatant formed was used for the measurement of HXK and GCK activities by coupled enzyme assay procedure of Davidson and Arion (1987). The incubation mixture contained the following ingredients in a final volume of 1 ml: HEPES, 50 µmol; KCl, 100 µmol; $MgCl_2$, 7.5 µmol; and DTE, 2.5 µmol; fatty acid free bovine serum albumin, 10 mg; NAD^+, 0.5 µmol; G-6-PD, 4 units; liver supernatant, 100 µl for HXK assay or 10 µl for total HXK and GCK assays; and D-glucose, 0.5 µmol for HXK and 10 µmol for total enzyme activities. Both 'control' and 'test' tubes were pre-incubated at 25°C for 5 min. 0.2 ml of distilled water was added to the control tubes, and to start the reactions in the test tubes, 0.2 ml of a solution containing 0.5 µmol of ATP was added. Control tubes were adjusted to zero absorbance in DU-7 spectrophotometer at 340 nm wavelength, and the increase in absorbance in the test tubes at this wavelength was plotted against time period of 15 min. Total enzyme activities (GCK + HXK and HXK activities) were calculated in terms of mU/ml of the liver supernatant. One milliunit of the enzyme corresponds to the amount of the enzyme producing 1 nmol of NADH per min under assay conditions at 25°C. Hexokinase activities were subtracted from the total HXK + GCK activities to obtain glucokinase activities. Protein content of liver homogenates was determined by using bicinchoninic acid (BCA) protein assay reagent (Pierce Chemical Company, Rockford, Chicago, USA) (Levinie *et al*., 1990).

Measurement of Oxidative Phosphorylation
Isolation of mitochondria

Mitochondria were isolated and purified using the method of Kristal *et al*., (1997). The harvested liver tissues were placed in an ice-cold solution containing 250 mM mannitol, 70 mM sucrose, 500 µM EDTA and 5 mM HEPES, all at pH 7.4. The liver tissues were homogenized with a motor-driven glass Telfon pestle. Following homogenization, samples were centrifuged at 1000 g for 10 min. Supernatants were removed and centrifuged at 10800 g for 15 min. Pellets were washed twice with the same solution but without EDTA, and re-spun at 10000 g for 5 min. Following the final wash, mitochondria were resuspended in a solution containing 120 mM KCl and 50mMTris-HCl.

Assay of Oxidative Phosphorylation

The respiration of the isolated mitochondria was measured polarographically by using a Gilson model K-

ICT-C Oxygraph fitted with a Clark-type electrode in a 2-ml stirred vessel at 30°C. The signal was recorded with a HealthKit strip Chart Recorder. The vessel contained a suspension of mitochondria (4–6 mg protein) in 0.25 M sucrose; 10 mM potassium phosphate; 5 mg/mL albumin; 5 mM $MgSO_4$, at pH 7.4, in a total volume of 2.2 mL. After 4–5 minutes temperature equilibration, the endogenous rate of oxygen uptake was recorded before adding exogenous substrate in 50 µL through the capillary stem of the oxygraph vessel. The substrate stock solutions used were 0.385 M glutamate/0.115 M malate, 0.5 M succinate, 0.385 M pyruvate/0.115 M malate; 0.385 M ornithine/0.115 M malate; and 0.385 M arginine/0.115 M malate. After recording oxygen uptake for another 4–5 minutes, 0.8 µmoles ADP, at pH 7.4, was added, and oxygen uptake was recorded for another 3–6 minutes. This measurement allowed calculation of the rate of state 3 (ADP-stimulated) respiration (V_3), the rate of state 4 (non-ADP-stimulated) respiration (V_4), the respiratory control index (RCI = V_3/V_4), and the phosphate to oxygen ratio (ATP generated divided by oxygen consumed in state 3).

Oxidative phosphorylation was also monitored spectrophotometrically by the method described by Singer (1974). Phosphorylation coupled to the first and second sites was measured with ferricyanide as the electron acceptor. Phosphorylation coupled specifically to the third site was measured with ascorbate + N,N,N,´N´-tetramehtyl-p-phenylenediamine (TMPD). When succinate or ascorbate was used as electron donor, rotenone (10 µg) was added to the reaction system. Activities were expressed as a percentage of control (µmoles of ferricyanide reduced/min/mg protein) at 30°C. Protein was measured by the Bio-Rad™ Protein assay, using bovine serum albumin as standard (Lowry *et al*., 1951). The assay of oxidative phosphorylation was carried out soon after isolation of the mitochondria, and was completed within 1-2 h after mitochondrial isolation.

Enzyme assays

The activity of ATPase (ATP phosphohydrolase) was determined as described by Katyare and Satav (1986). Mitochondria concentration (300 µg of protein) and time of incubation (1 min or 4 min) were so adjusted as to ensure linearity in both the latent and the uncoupler-stimulated activity. Measurements of glutamate dehydrogenase (GDH), β-hydroxybutyrate dehydrogenase (BDH) and succinate dehydrogenase (SDH) activities were done according to the method of Katyatre and Satav (1986). In all determinations of the enzyme activities and rates of oxidative phosphorylation, an equal number of 'control' and experimental 'test' samples were used at any one time, to minimize errors due to variations.

Estimation of cytochrome content

The isolated mitochondria were washed repeatedly with acetone, chloroform/methanol (2:1, v/v) and acid/acetone as described by Bessman and Mohan (1997). Mitochondrial cytochrome contents were determined spectrophotometrically from the different mitochondrial spectra after solubilization (reduced–oxidized) of the pyridine haemochrome.

Statistical analysis

Results are expressed as means (±SEM). Statistical evaluation of the data obtained was done by Students' t-test. P values less than 0.05 were considered to be statistically significant.

Results

Acute toxicity testing

Intraperitoneal administrations of relatively low doses of *Artocarpus communis* (Forst.) root-bark aqueous extract (1–50 mg/kg) were found to be safe in rats. However, relatively moderate to high doses of the plant's extract (>100 mg/kg i. p.) were found to be toxic and/or lethal to the animals. The LD_{50} value of the plant's extract was found to be 135±16 mg/kg i. p. in rats. This relatively low LD_{50} value of 135±16 mg/kg probably suggests that *Artocarpus communis* (Forst.) root-bark aqueous extract is toxic to rats.

Blood glucose and serum insulin levels

The changes in blood glucose and serum insulin levels, and changes in body and liver weights of the four animal groups studied are shown in Table 1. The three treated rat groups, A (ACE-treated), B (STZ-treated) and C

(STZ-+ACE-treated) showed significant increase ($p<0.05$) in blood glucose levels when compared with the control group D rats. After 3 weeks, the blood glucose concentration of the ACE-treated group A rats started to drop, but it never attained group D rats normoglycemic level throughout the study period (Table 1). There was a progressive decrease in the body weights of the rats in groups A, B and C, whereas the body weights of the control group D rats showed moderate but insignificant ($p>0.05$) increase (Table 1). Furthermore, there was a significant decrease ($p<0.05$) in the serum insulin levels of the rats in the three treated groups A, B and C when compared with group D control rats.

Hexokinase and glucokinase activities

Table 2 shows the effects of *A. communis* root-bark aqueous extract on hepatic hexokinase and glucokinase activities. In groups A, B and C treated rats, both hexokinase and glucokinase activities significantly decreased ($p<0.05$) when compared with the group D control rats.

Table 1. Changes in blood glucose concentrations, serum insulin contents, body and liver weights of ACE-, STZ-, and STZ- + ACE-treated rats.

Blood glucose concentrations (mmol/L)					
	Control	←	Treated	→	
Days/Treatments	0	10	20	30	40
ACE-treated	4.1±0.1	22.7±0.4[a]	18.6±0.2[a]	14.2±0.1[a]	10.7±0.5[a]
STZ-treated	4.2±0.6	18.8±0.2[b]	20.2±0.4[b]	21.4±0.3[b]	21.2±0.1[b]
STZ + ACE-treated	4.0±0.2	22.6±0.4[c]	21.6±0.2[c]	20.5±0.3[c]	18.9±0.4[c]
Serum insulin concentrations (μU/ml)					
ACE-treated	13.0±1.1	11.5±1.3[a]	9.7±1.2[a]	9.0±1.7[a]	8.9±1.5[a]
STZ-treated	12.8±1.5	8.5±1.4[b]	6.3±1.4[b]	5.9±1.3[b]	5.7±2.3[b]
STZ + ACE-treated	12.2±1.2	10.3±1.2[c]	10.6±1.1[c]	10.9±1.7[c]	11.5±1.2[c]
Body weights (g)	ACE	STZ-treated	STZ + ACE-treated		Control
	212±11	217±14[b]	221±06[a]		242±10[a]
Liver weights (g)	7.79±0.2	8.27±0.5	8.56±0.4		9.46±0.8

Values are expressed as means (±SEM) of 10 rats for all groups. [a,b,c] Significant difference ($p<0.05$) in the same row between various treatments and control group D rats.

Table 2. Effects of *A. communis* root-bark aqueous extract on hepatic hexokinase and glucokinase activities of ACE-, STZ-, and STZ- + ACE-treated rats.

Parameters	ACE-treated	STZ-treated	STZ + ACE-treated	Control
Liver HXK	1.12±0.3[a]	1.32±0.7[b]	1.42±0.5[c]	2.66±0.3
Liver GCK	1.04±0.1[a]	1.18±0.6[b]	1.25±0.7[c]	5.21±0.4

Values are expressed as means (±SEM) of 10 rats for all groups. [a,b,c] Significant difference ($p<0.05$) in the same row between various treatments and control group D rats. Values are expressed as mU/mg protein.

Oxidative phosphorylation
Early effects

The oxidative activities of hepatic mitochondria in the four groups (A, B, C and D) of rats are shown in

Tables 3 and 4. At one week of diabetic state, state 3 respiration rates (ADP present) of the rats in groups A, B and C did not change the rate of oxidative phosphorylation for glutamate, β-hydroxybutyrate or ascorbate + TMDP. However, there was a significant increase ($p<0.05$) in succinate, and decrease in pyruvate + malate. The changes in the state 4 respiration rate (after depletion of added ADP) were significantly lower ($p<0.05$) for GDH, BDH and ascorbate, and higher for SDH, but minimal for pyruvate. The respiratory control ratios (state 3 respiration rate/state 4 respiration rate) were generally high, and in the expected ranges for the substrates used. Diabetes affected only the oxidative activity of mitochondria, but the ability of mitochondria to phosphorylate (P/O ratio) remained unaffected. Thus, diabetic state did not cause any uncoupling of mitochondria at this early stage (Table 3). The primary dehydrogenases, especially GDH and BDH, showed significant increase ($p<0.05$), but the increase in SDH was dramatically high ($p<0.05$), despite the increase in state 3 respiratory activity (Table 3). Furthermore, in groups A, B and C animals, the contents of cytochromes b and $c + c_1$ significantly decreased ($p<0.05$), while those of aa_3 was only marginal when compared with those of the control group D rats (Table 6).

Late effects

At the end of 4 weeks when the diabetic state was fully established, the state 3 respiration rate was insignificantly decreased ($p>0.05$) for the different substrates used, and no change was observed for ascorbate + TMPD (Table 4). The state 4 respiration rates were elevated for NAD^+-linked substrates and succinate in the diabetic rats. The ADP/O ratios were also not affected; thus, even at the late stage of the disease, mitochondria were well coupled. ADP phosphorylation rates correlated well with the observed changes in the respiration rates (Table 4). The contents of cytochrome aa_3 significantly increased ($p<0.05$) in groups A, B and C diabetic rats, while those of b and $c + c_1$ significantly decreased ($p<0.05$) when compared with the control group D rats (Table 6).

ATPase activity

In the early diabetic stage (week 1), the basal and Mg-stimulated ATPase activities were insignificantly lower ($p>0.05$), while the +DNP and Mg^{2+} + DNP-stimulated ATPase activities were significantly decreased ($p<0.05$) in groups A, B and C rats compared with the control group D animals. At the end of 4 weeks, the basal and Mg-stimulated ATPase activities still remained lower, while the +DNP and Mg^{2+} + DNP-stimulated ATPase activities increased in groups A, B and C rats, compared with the control group D animals (Table 5).

Ultrastructural changes

Groups A, B and C diabetic rats showed similar ultrastructural changes and alterations. The most common findings in these animals as revealed by electron microscopy were the presence of lipid droplets and intracytoplasmic vacuoles (Fig. 1). Other remarkable changes within the cytoplasmic organelles of the animals included mitochondrial membranes that were poorly preserved, scanty rough endoplasmic reticulum and increased number of vesicular structures. Lysosome-like structures were seen in greater numbers among the group A rats. The control group D animals maintained normal architecture of the hepatocytes. Their organelles were viable, and they were in normal proportions.

Discussion

Artocarpus communis (Forst.) root-bark aqueous extract (≥135 mg/kg i. p.) is toxic to rats. This finding is quite intriguing because decoctions and infusions of *A. communis* root-bark are traditionally used by the Yoruba-speaking people of Western Nigeria as an effective folk remedy for the management, control and/or treatment of type 2, adult-onset diabetes mellitus (with no reported adverse effect to date). The 'toxicity' of *A. communis* root-bark aqueous extract observed in the present study confirms our earlier unpublished pilot study which showed that *A. communis* root-bark aqueous extract is not only lethal to rats at moderate to high doses, but also that it induces hyperglycaemia in Wistar rats at all dose levels. In view of the observed hyperglycaemic and toxic effects of *A. communis* root-bark aqueous extract in rats, the present study was undertaken to compare the glycaemic, metabolic and ultrastructural effects of *A. communis* root-bark aqueous extract (ACE) with those of streptozotocin (STZ) in rats and various hepatic tissues of rats.

It is an established fact that structural damage to organs and tissues, or complications of diabetes mellitus, may be due to oxidative stress. Oxidative stress may play an important role in the development of STZ-induced

Table 3: Early effects of ACE and STZ treatments on oxidative energy metabolism in rat liver mitochondria.

Substrate Treatment	ADP/O ratio	Respiration rate ($nmol\ O_2$/min/mg protein)		ADP phosphorylation rate (nmol/min/mg protein)
		+ ADP	- ADP	
Glutamate				
ACE	2.91±0.2	37.1±1.2	0.7±0.2*	229.7±6.9**
STZ	2.89±0.1	36.9±1.2	0.6±0.2*	231.9±8.4**
STZ + ACE	3.01±0.2	37.2±1.1	0.6±0.2*	234.8±9.3**
Control	2.81±0.1	36.7±1.2	1.0±0.1	213.6±7.5
β-hydroxybutyrate				
ACE	2.83±0.1	34.3±1.7	0.6±0.2*	195.7±7.8**
STZ	2.92±0.2	35.2±1.4	0.5±0.1*	198.9±8.4**
STZ + ACE	3.03±0.1	36.5±1.3	0.5±0.1*	203.6±5.7**
Control	2.82±0.2	32.7±1.8	1.2±0.2	180.9±6.5
Succinate				
ACE	1.93±0.3	92.4±1.3**	2.9±0.1**	295.4±7.7**
STZ	1.97±0.2	95.3±1.6**	3.1±0.2**	309.8±5.8**
STZ + ACE	2.01±0.1	91.5±1.7**	3.5±0.2**	312.6±7.9**
Control	1.82±0.5	72.4±1.5	1.9±0.3	280.4±9.7
Pyruvate + Malate				
ACE	2.96±0.2	26.5±1.1	0.7±0.2*	155.7±6.9*
STZ	3.03±0.1	24.9±1.0	0.8±0.3*	158.6±8.9*
STZ + ACE	3.05±0.2	23.8±1.3	0.7±0.2*	156.4±7.9*
Control	2.95±0.2	31.4±1.2	1.2±0.1	183.5±9.9
Ascorbate + TMPD				
ACE	0.65±0.1*	62.9±1.5*	19.3±1.5*	55.7±9.9*
STZ	0.72±0.2*	65.6±1.7*	20.5±1.9*	57.6±7.5*
STZ + ACE	0.70±0.2*	63.7±1.8*	18.9±1.8*	53.9±6.7*
Control	0.81±0.2	76.5±1.4	26.8±1.6	68.4±7.8

Values are expressed as means (±SEM) of 10 rats for all groups. Oxygen uptake is expressed as ng-atomsO/min per mg of mitochondrial protein *Significant decrease (p<0.05) when compared with the control group D animals. **Significant increase (p<0.05) in the same column between treatment and control group D rats.

diabetes, and mitochondria are suggested to be one of the targets in STZ-induced toxicity (Bastar *et al*., 1998). Mitochondria are special organelles in eukaryotic cells that efficiently convert energy available in substrate molecules to the universal fuel, ATP, for cellular processes. Oxidative phosphorylation is a key pathway used by most aerobic cells to harvest energy, and about 40-50% of ATP in the liver is produced by mitochondria. Therefore, the normal function of all other processes within the liver cells is ultimately dependent on the energy production in mitochondria. Disturbance of mitochondrial function underlies many metabolic diseases (Hall *et al.*,1960). For this reason, evaluation of dysfunction of oxidative phosphorylation is often given crucial importance in biomedical and toxicological investigations.

From the data obtained in this study the animals treated with *A. communis* root-bark aqueous extract alone developed acute and severe hyperglycemia, faster than the STZ- and STZ- + ACE-treated groups of animals. The plant's extract also produced moderate body and liver weight losses. Serum insulin levels of the plant's extract-treated rats were significantly reduced (p<0.05), but still slightly higher than those of the STZ-treated group B rats. However, there was a decrease in blood glucose concentrations of the ACE-treated rats as from the 3rd week of our study, but the blood glucose levels of the animals failed to reach the normoglycemic levels of the control, group D rats.

Table 4. Late effects of ACE and STZ treatments on oxidative energy metabolism in rat liver mitochondria.

Substrate Treatment	ADP/O ratio	Respiration rate ($nmol\ O_2$/min/mg protein) + ADP	Respiration rate ($nmol\ O_2$/min/mg protein) - ADP	ADP phosphorylation rate (nmol/min/mg protein)
Glutamate				
ACE	2.52±0.1*	28.3±1.3*	1.4±0.2**	147.5±6.5*
STZ	2.43±0.1*	26.9±1.5*	1.6±0.2**	149.7±5.9*
STZ + ACE	2.39±0.2*	27.3±1.4*	1.5±0.2**	151.8±7.4*
Control	3.05±0.2	38.5±1.2	1.1±0.1	212.4±6.5
β-hydroxybutyrate				
ACE	2.59±0.3*	23.5±1.7*	1.6±0.1**	129.5±9.4*
STZ	2.62±0.2*	26.7±1.6*	1.4±0.2**	125.9±8.6*
STZ + ACE	2.56±0.2*	24.6±1.4*	1.5±0.1**	127.7±7.7*
Control	2.92±0.2	36.9±1.3	0.6±0.2	197.8±6.8
Succinate				
ACE	2.03±0.2	59.1±1.1*	4.3±0.3**	215.7±8.7*
STZ	2.01±0.1	56.3±1.4*	4.4±0.2**	209.8±9.8*
STZ + ACE	2.02±0.1	54.2±1.3*	4.5±0.5**	212.9±8.9*
Control	1.84±0.3	81.1±1.2	2.6±0.3	282.9±9.9
Pyruvate + Malate				
ACE	2.56±0.2	22.5±1.1*	1.0±0.3	115.7±8.3*
STZ	2.63±0.2	21.4±1.2*	1.1±0.2	112.6±7.5*
STZ + ACE	2.55±0.2	23.5±1.4*	1.2±0.2	116.7±7.8*
Control	2.95±0.2	34.6±1.2	0.8±0.1	187.9±9.7
Ascorbate + TMPD				
ACE	0.63±0.3*	67.5±1.5*	27.3±1.5*	52.4±6.2*
STZ	0.69±0.2*	65.3±1.7*	26.2±1.2*	54.3±5.1*
STZ + ACE	0.68±0.2*	66.5±1.8*	28.0±1.3*	51.2±6.0*
Control	0.82±0.2	79.2±1.4	36.2±1.4	62.4±7.0

Values are expressed as means (±SEM) of 10 rats for all groups. Oxygen uptake is expressed as ng-atomsO/min per mg of mitochondrial protein. *Significant decrease (p<0.05) when compared with the control group D animals. **Significant increase (p<0.05) in the same column between treatment and control group D rats.

Table 5: Effects of ACE and STZ treatments on ATPase activities in rat liver mitochondria.

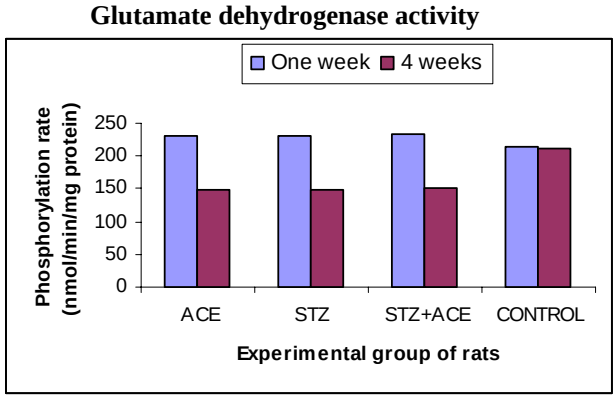
Duration	Animals	ATPase activity (nmol of Pi liberated/min/mg protein)			
		$-Mg^{2+}$ -DNP	$+Mg^{2+}$	+DNP	$+Mg^{2+}$ +DNP
One week	ACE	1.5±0.2*	1.4±0.3*	7.2±0.3*	7.0±0.4*
	STZ	1.4±0.3*	1.6±0.2*	7.1±0.5*	7.3±0.5*
	STZ + ACE	1.6±0.2*	1.7±0.3*	7.6±0.2*	7.1±0.3*
	Control	1.9±0.1	1.9±0.1	12.1±0.1	9.7±0.2
Four weeks	ACE	1.0±0.1*	1.4±0.2*	12.0±0.3**	11.5±0.3**
	STZ	1.2±0.2*	1.3±0.1*	12.3±0.5**	12.0±0.4**
	STZ + ACE	1.4±0.2*	1.6±0.2*	13.4±0.2**	12.6±0.5**
	Control	2.0±0.1	1.9±0.3	10.2±0.1	10.2±0.1

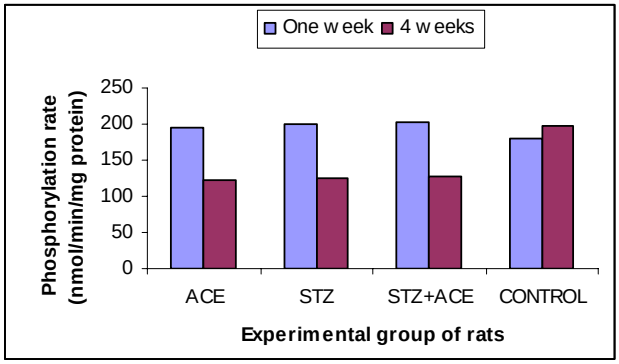
Values are expressed as means (±SEM) of 10 rats for all groups. *Significant decrease (p<0.05) when compared with the control group D animals. **Significant increase (p<0.05) in the same column between treatment and control group D rats.

Table 6: Effects of ACE and STZ treatments on cytochrome contents in rat liver mitochondria.

Duration	Animal Groups	Cytochrome contents (p mol/mg protein)		
		Aa_3	b	$c + c_1$
One week	ACE	126.0±8.0*	181.0±4.0*	329.0±7.0*
	STZ	125.0±5.0*	179.0±5.0*	331.0±5.0*
	STZ + ACE	123.0±6.0*	185.0±3.0*	336.0±7.0*
	Control	139.0±4.0	223.0±7.0	405.0±9.0
Four weeks	ACE	156.0±7.0**	182.0±6.0*	293.0±7.0*
	STZ	162.0±5.0**	187.0±7.0*	289.0±6.0*
	STZ + ACE	167.0±4.0**	191.0±4.0*	207.0±8.0*
	Control	142.0±6.0	221.0±5.0	397.0±9.0

Values are expressed as means (±SEM) of 10 rats for all groups. *Significant decrease (p<0.05) when compared with the control group D animals. **Significant increase (p<0.05) in the same column between treatment and control group D rats.





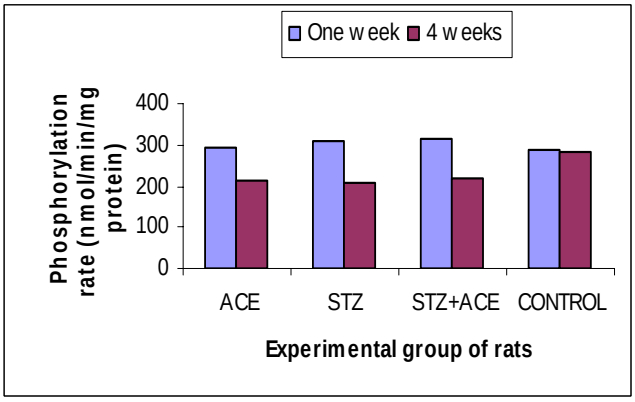


Figure1: Effects of ACE and STZ treatments on dehydrogenase activities in rat liver mitochondria. Values are expressed as means (±SEM) of 10 rats for all groups. Activities of all the substrates used were significantly decreased (p<0.05) 4 weeks post-treatment.

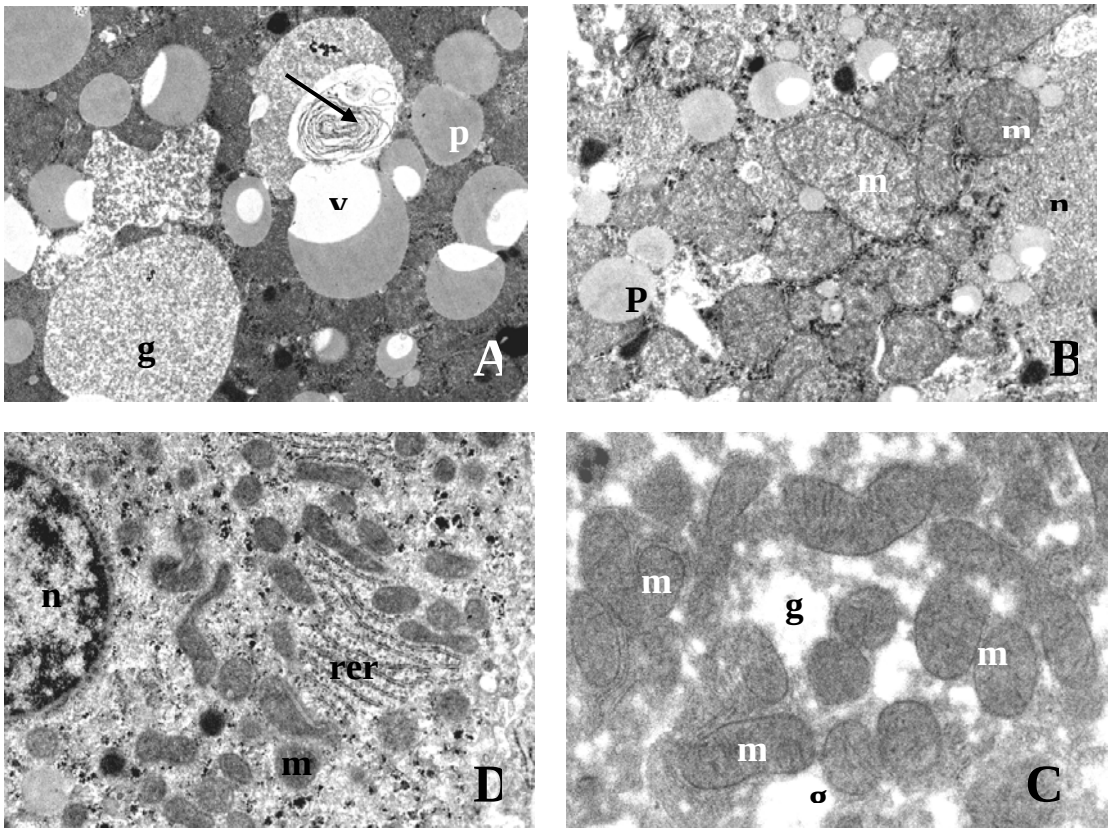


Figure 2: Micrographs illustrating hepatocyte contents of groups A, B, C and D animals used. Groups A, B and C rats showed poor organization of the organelles within the cytoplasm. (A) shows ACE-treated rat with giant liver glycogen (g), lipid droplets (p), multiple vacuoles and poor architecture of the cytoplasm. The arrow shows myelin formation within the glycogen. (B) shows STZ-treated rat with hepatic lipid droplets (p), mitochondria with poor inner membrane (m) and absence of rough endothelial reticulum. (C) shows STZ- + ACE-treated rat liver with multiple swollen mitochondria and patchy glycogen. (D) shows control rat's liver with normal organization of the organelles in relative proportions, intact rough endothelial reticulum (rer), nucleus (n) and mitochondria (m). Final magnification was at X15, 000.

Glucokinase (GCK) is the glucose sensing enzyme that is responsible for the phosphorylation of the majority of glucose in the liver and in the pancreas. GCK binds to and phosphorylates glucose when its levels are higher than normal, thus allowing it to maintain constant glucose levels (Kamata *et al*., 2004). In the present study, hepatic hexokinase (HXK) and GCK were observed to be lower. This observation is in agreement with the findings of Postic *et al*., (2001). GCK activity was lower in the ACE-, STZ-, and STZ- + ACE-treated diabetic rats as compared with the control group D rats. The decrease in hepatic GCK could result from hypoinsulinaemia, or decreased synthesis and/or increased degradation of GCK by oxidative stress in diabetes (Matschinsky and Magnuson, 2004).

In several mitochondrial studies, changes in separate functional parameters, such as rate of respiration in metabolic states 3 and 4, and/or uncoupled state, membrane potential and swelling, are measured with the aim of evaluating the influence of different effectors or damaging factors on oxidative phosphorylation. The present study has demonstrated that *A. communis* root-bark aqueous extract, concentrations, affected many sites in the machinery of mitochondrial energy transformation. It increased the membrane leak and inhibited both the respiratory and the phosphorylation subsystems, thus causing a slight (succinate oxidation) or substantial change (glutamate + malate oxidation) in mitochondrial membrane potential. The resulting change in mitochondrial membrane potential may serve as a sensitive measure to evaluate the degree to which different blocks are affected. This observation is in

agreement with the findings of Nishihara *et al*., (1986), and Kavanagh *et al*., (2000). Oxidative phosphorylation is the terminal process of cellular respiration in which ATP is formed. Thus, during oxidative phosphorylation, electrons are transferred from NADH or $FADH_2$, created in glucolysis, fatty acid metabolism and the Krebs circle, to molecular oxygen via a series of protein complexes located in the inner mitochondrial membrane (Leninger and Cox, 2001). The results of the presented study gave clear, discernible indications that diabetes at the early stage, causes a decline of respiration with pyruvate + malate. Our results also show that there was a transient increase in the rate of succinate oxidation, impairment of β-hydroxybutyrate and succinate dehydrogenases activities. These findings are probably due to a specific decrease in one or more of the electron-transport components. The liver mitochondria were well coupled with intact ADP/O ratios and the respiratory control ratios. Cytochromes b and c + c_1 contents were also decreased, but none of these changes altered respiration rate at this initial stage of the disease. Indeed, the ADP-phosphorylation rates were not significantly affected ($p>0.05$), but ATP-synthesizing potential, as judged by the ATPase activities, significantly decreased ($p<0.050$. Late diabetic state gave a clearer picture of oxidative stress, with the state 3 respiration rates decreased uniformly and significantly ($p<0.05$) with all the substrates, except ascorbate + TMPD. The decreased respiration rates in groups A, B and C diabetic rats were correlated with the decreased contents of cytochromes b and c + c_1, but increased aa_3 content. The increase in aa_3 content may, however, possibly be an attempt to increase oxidative potential. The ATP-synthesis rate also decreased in groups A, B and C diabetic rats, and this followed a pattern compatible with respiration rates, since any factor disturbing the activity of the respiratory subsystem via induced changes in mitochondrial membrane potential will elicit secondary effects on phosphorylation flux.

The β-hydroxybutyrate dehydrogenase activity serves as a clear pointer to diabetic state, and this is well recognized with ketoacidosis in diabetes (Rogers *et al*., 1986). It is thus clear from our results that the early and the late effects of diabetic state on hepatic mitochondrial oxidative energy metabolism differ. Also, the mitochondrial architecture differed significantly from the normal ones with respect to the contents of the dehydrgenases, cytochromes and the ATPase activities. Our study has shown that a disturbance in the activity of all the three subsystems contributes to the overall inhibition of oxidative phosphorylation. Our results have also revealed several individual components within the system that are sensitive to the toxic effects of *A. communis*.

We conclude that *A. communis* root-bark aqueous extract has a toxic, non-specific hepatic action, since it affects membrane leak, dehydrogenases, cytochromes b and c + c_1 in the respiratory chain, and ATPase. The extent to which these effects are caused by changes in the composition and properties of the mitochondrial membrane, and the lipid environment that is essential for the normal function of the membrane proteins, can only be speculated. Whatever may be the molecular details of *A. communis* root-bark aqueous extract, and its interaction with components of oxidative phosphorylation, it is obvious from the present study that by acting simultaneously on many steps and enzyme systems, the plant's extract efficiently inhibits the physiologically important features of respiration and ATP synthesis in liver mitochondria.

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