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Antidiabetic effects of *Tetracarpidium conophorum* seed on biomarkers of diabetes− induced nephropathy in rats

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ABSTRACT

Objective: To investigate antidiabetic effects of *Tetracarpidium conophorum* seed (TECOSE) against biomarkers of diabetes-induced nephropathy in rats. **Methods:** Powdered seed (500 g) of TECOSE was extracted with 5 L of 100% methanol for 72 h followed by concentration of filtrate. Diabetes was induced in rats with 75 mg/kg body weight intraperitoneal streptozotocin. The rats were randomly divided into five groups (n=5 in each group) namely group A- normal control, group B- diabetic control, groups C, D and E were diabetic rats treated with 500 mg/kg body weight TECOSE orally, 7 mg/kg body weight metformin orally and subcutaneous 0.3 IU/kg body weight Humulin[R], respectively. All rats were treated once daily for 2 weeks. Blood samples and urine were collected for biochemical estimations. Kidney was harvested for histomorphological studies. **Results:** TECOSE (500 mg/kg body weight) significantly (P<0.05) reduced blood sugar levels as effective as metformin and insulin. The plant extract also significantly (P<0.05) reduced levels of serum urea, creatinine and uric acid, proteinuria, ketonuria, hematuria and glycosuria while it significantly (P<0.05) increased glomerular filtration rate. Histomorphological study of the kidney of untreated diabetic rats showed features suggestive of glomerulosclerosis and tubular necrosis while that of treatments with TECOSE extract, metformin and insulin showed near normal histoarchitectures. **Conclusions:** Streptozotocin at 75 mg/kg body weight induces diabetic nephropathy in rats and TECOSE possesses potentials to prevent diabetic renal damage.

1. Introduction

Diabetes mellitus (DM) is a disorder of carbohydrate metabolism. DM is characterized by increase in blood sugar above normal level due to absolute or relative insulin deficiency. It is associated with diabetic eye, kidney, heart, and liver diseases[1,2]. Diabetic kidney disease is characterized by reduction in the rate of glomerular filtration, increase in the thickness of glomerular basement membrane[3,4], reduced glomerulus, tubulo-interstitial fibrosis[5], reduced excretion of albumin[6] and decrease in creatinine clearance[7]. The major cause of chronic renal failure is DM. Oxidative stress induced by hyperglycemia is the main pathway for the development of pathological changes in the kidney[7–9].

Tetracarpidium conophorum (*T. conophorum*) known as African walnut is called "asala" by Yoruba people in South-Western Nigeria and "ukpa" by Igbo ethnic group in South-Eastern Nigeria. The nuts are eaten either raw or cooked[10]. Diabetic patients are hindered

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from accessing effective medications due to financial constraints. Therefore, there is a need for an affordable and accessible medicinal plant with little or no side effects to manage the disease. The aim of this research was to investigate glucose-lowering effects of *T. conophorum* seed (TECOSE) in normalizing markers of diabetic kidney damage in rats.

2. Materials and methods

2.1. Reagents and chemicals

Methanol, streptozotocin, citric acid, sodium citrate, normal saline, Humulin[R] and metformin were obtained from either Sigma chemical company, St. Lious, Mo, U.S.A., or British Drug House (BDH) chemical Ltd., Poole, England. The diagnostic kits were obtained from Randox Laboratories Ltd., Crumlin, Co. Antrim, U.K., or Agappe Diagnostics, Switzerland.

2.2. Preparation of T. conophorum

T. conophorum nuts were bought from a local market in Osogbo, Osun State, Nigeria. The plant was identified and authenticated at IFE Herbarium, Obafemi Awolowo University, Ile-ife, Nigeria. The herbarium identification number was 17713. The shells were removed and seeds were shade dried for 4 weeks.

2.3. Extraction of T. conophorum

T. conophorum seeds were pulverized, and dried powder (500 g) was subjected to cold extraction using 5 L of methanol for 72 h. The filtrate was concentrated using standard procedure. Crude methanolic extract of the seed was stored at -20 ℃ until further use.

2.4. Acute toxicity testing

Acute toxicity of the methanol extract was performed according to standard procedures[11].

2.5. Grouping and treatment of experimental animals

Animals were used in accordance with the institution guidelines (Osun State University, College of Health Sciences Health Reseach and Ethics Committee, UNIOSUN/HREC/2014 Protocols) for Experimental and other Scientific Purposes. Twenty five female albino rats with average weight of 150 g were kept in clean plastic cages and fed with rat feeds and water *ad libitum*. The animals were acclimatized to standard laboratory conditions. Diabetes was induced in twenty five rats with a single dose of 75 mg/kg body weight streptozotocin in freshly prepared 0.1 M citrate buffer, pH

4.5 intraperitoneally following 12 h overnight fasting and after the baseline glucose concentrations had been measured. Seven days following induction of diabetes, fasting blood sugar estimations were done with the aid of glucometer using blood samples from tail veins of the rats, and blood sugar level >10 mmol/L was considered diabetic.

The rats were randomly divided into five groups (*n*=5 in each group) as follows: Group A: Normal control rats; Group B: Diabetic control rats; Group C: Diabetic rats treated with 500 mg/kg body weight of TECOSE extract orally; Group D: Diabetic rats treated orally with 7 mg/kg body weight metformin; Group E: Diabetic rats treated subcutaneous with 0.3 IU/kg body weight Humulin[R].

All the rats were treated once daily for 2 weeks. Blood sugar of each rat was recorded before induction of diabetes, after induction of diabetes, and at the end of treatment. Blood samples and urine were collected by standard procedures. Urinalysis was done using standard (DUS 10, DFI Co., Ltd.) reagent strips.

2.6. Preparation of blood sample

Fresh blood (5 mL) sample was collected by ocular puncture from each rat into a clean plain labeled tube, allowed to clot, and then centrifuged at 3 000 rpm for 10 min. The clear serum was separated and kept at -20 ℃ till assay.

2.7. Estimation of renal function indices

Serum urea was estimated as described by Fawcett and Scott[12] and uric acid was evaluated as described by Fossati *et al*[13] using standard diagnostic kits. Serum creatinine was determined using alkaline picrate method[14]. Renal clearance was estimated as a function of glomerular filtration rate (GFR) using modified diet for renal disease equation:

Estimated GFR (eGFR) (mL/min/1.73 m^2) = 186.3 × creatinine/88.4

2.8. Histological studies

At the end of 2nd week of treatment, kidney tissues were harvested from the sacrificed rats, immediately fixed in 10% formalin and used for histomorphological studies.

2.9. Statistical analysis

Data obtained were analyzed using one way analysis of variance (SPSS version 20.0). Levene statistic was used for tests of homogeneity of variance, and Tukey for multiple comparisons and homogenous subsets. Results were considered to be statistically significant with *P* values less than 0.05.

3. Results

3.1. Average blood glucose in control and treatment groups

Figure 1 showed average blood glucose with corresponding percentage blood glucose change. Seven days after induction of diabetes, blood glucose level was significantly ($P<0.05$) increased in all the treatment groups. At the end of 2nd week, there was significant ($P<0.05$) decrease in the blood glucose levels following treatments with TECOSE extract, metformin and insulin. The percentage reduction in blood glucose level was highest (38%) in rats treated with 500 mg/kg body weight methanol extract of TECOSE, followed by insulin (36%) and metformin (32%), respectively.
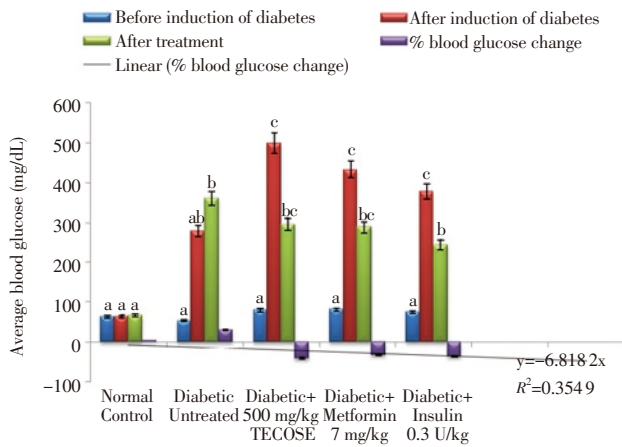
3.2. Serum renal function indices at the end of treatment

Effects of treatments with TECOSE extract, metformin and insulin on renal function parameters (urea, uric acid, creatinine and glomerular filtration rate) were shown in Figure 2. There was significant ($P<0.05$) increase in the levels of urea, uric acid and creatinine in untreated diabetic rats while the levels were significantly ($P<0.05$) reduced in rats treated with TECOSE extract, metformin and insulin. Average eGFR was significantly ($P<0.05$) raised in rats treated with TECOSE extract, metformin and insulin while the level was significantly ($P<0.05$) reduced in diabetic untreated rats.
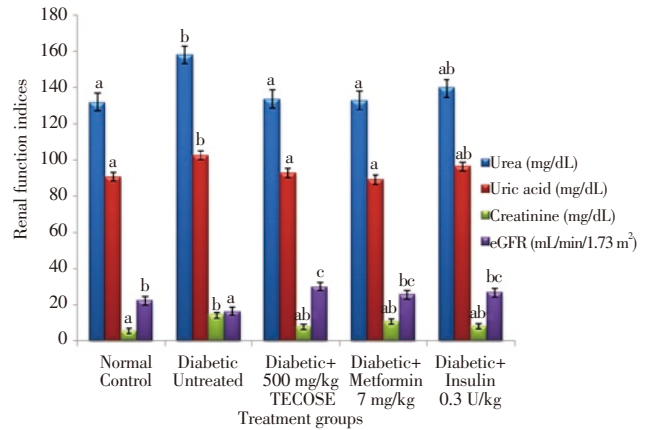

Figure 1. Average blood glucose in control and other treatment groups.

Values are expressed as mean ± SD (n=5). Means of bars of the same legend with different Tukey superscripts along the row are statistically significant at $P<0.05$.

Figure 2. Renal function indices in control and treatment groups.

Values are expressed as mean ± SD (n=5). Means of bars of the same legend with different Tukey superscripts along the row are statistically significant at $P<0.05$.

Table 1
Urinalysis profile in control and treatment groups.

Indices		Normal control	Diabetic untreated	Diabetic + TECOSE (500 mg/kg)	Diabetic + Metformin (7 mg/kg)	Diabetic + Humulin[R] (0.3 IU/kg)
1st week	Leucocyte	-ve	-ve	-ve	-ve	-ve
	Nitrite	-ve	-ve	-ve	-ve	-ve
	Urobilinogen (mg/dL)	0.1	0.1	0.1	0.1	0.1
	Protein (mg/dL)	-ve	30 (+)	trace	30 (+)	trace
	pH	7.5	6.0	6.5	6.5	6.5
	Blood (Rbc/μL)	-ve	25 (+)	trace	trace	25 (+)
	SG	1.015	1.030	1.030	1.030	1.030
	Ketones (mg/dL)	15 (+)	160 (++++)	40 (++)	40 (++)	80 (+++)
	Bilirubin	-ve	+ve	-ve	+ve	+ve
	Glucose (mmol/L)	5.5 (±)	111.0 (++++)	55.0 (+++)	55.0 (+++)	55.0 (+++)
2nd week	Leucocyte	-ve	-ve	-ve	-ve	-ve
	Nitrite	-ve	-ve	-ve	-ve	-ve
	Urobilinogen (mg/dL)	0.1	0.1	0.1	0.1	0.1
	Protein (mg/dL)	-ve	30 (+)	trace	30 (+)	trace
	pH	7.5	6.0	7.5	6.5	6.5
	Blood (Rbc/μL)	-ve	25 (+)	trace	trace	trace
	SG	1.015	1.030	1.015	1.015	1.020
	Ketones (mg/dL)	15 (+)	160 (++++)	15 (+)	40 (++)	40 (++)
	Bilirubin	-ve	+ve	-ve	+ve	+ve
	Glucose (mmol/L)	5.5 (±)	111.0 (++++)	28.0 (++)	55.0 (+++)	55.0 (+++)

3.3. Profile of urinalysis in control and treatment groups

Table 1 showed results of urinalysis done using reagent strips at the end of first and second week of treatments. In the diabetic untreated group, there was persistent proteinuria, ketonuria, hematuria and glycosuria throughout the course of treatment. In the first week of treatment, urine was denser with specific gravity (SG) of 1.030 in all the treatment groups when compared with SG of 1.015 observed in the control group. However, urine became lighter with SG of 1.015 in TECOSE and metformin groups and 1.020 in insulin group, with decrease in the levels of protein, ketone, red blood cells and glucose at the end of second week of treatment.

3.4. Histomorphological study of kidneys

Photomicrographs of the renal cortex general histoarchitecture across the study groups were shown in Figure 3. The plates (A and B) showed renal corpuscles, renal glomeruli, macula densa, distal and proximal convoluted tubules and the Bowman's capsule. The collagen of the parietal layer of Bowman's capsule and the basal membrane of distal tubule were observable from the periodic acid–Schiff photomicrographs (Figure 3B). The glomerulus of the diabetic untreated group was completely collapsed. There was moderate shrinkage of the glomeruli of rats treated with metformin and insulin while that of the group treated with TECOSE appeared normal when compared with control.
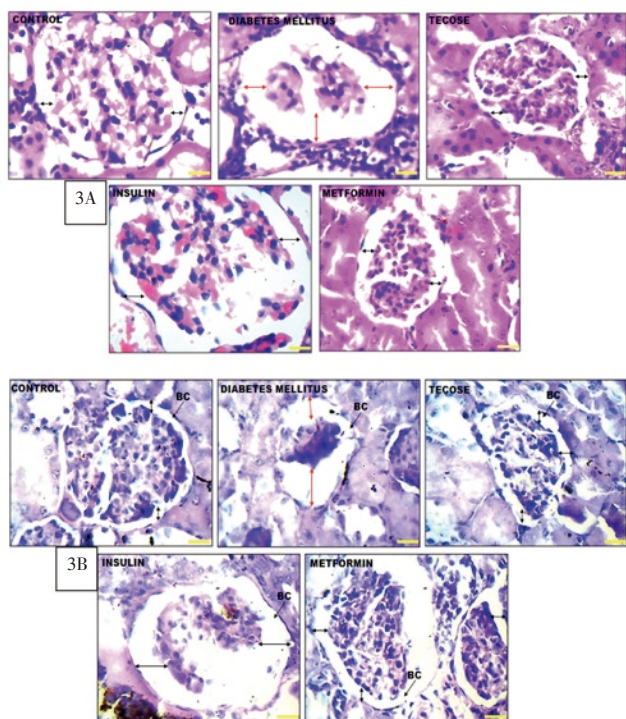

Figure 3. Photomicrographs of panoramic views of renal cortex general micromorphological across the study groups.
The plates showed renal corpuscles, renal glomeruli, macula densa, distal and proximal convoluted tubules and the Bowman's capsule were demonstrated across study groups. Stains: hematoxylin and eosin stain (A) and periodic acid schiff (B). Scale bar: 50 μm.

4. Discussion

This study investigated glucose-lowering potentials of TECOSE in normalizing biochemical and histomorphological markers of diabetic kidney damage in rats. Treatment of rats with streptozotocin causes significant increase in the blood glucose levels by destroying pancreatic beta-cells[15]. TECOSE (500 mg/kg body weight) significantly ($P<0.05$) reduced blood sugar levels as effective as reference drugs, metformin and insulin. The percentage reduction in blood glucose concentration was highest in rats treated with TECOSE, followed by insulin and metformin respectively. The mechanism of action of glucose-lowering property of TECOSE is not known, but some medicinal plants with hypoglycemic properties are known to increase circulating insulin level in normoglycemic rat[16].

Diabetes induced by administration of streptozotocin in this study significantly ($P<0.05$) raised serum urea, uric acid and creatinine, with significant reduction ($P<0.05$) in eGFR and characterized by proteinuria, ketonuria, hematuria and glycosuria in diabetic untreated rats. Estimation of kidney function indices in DM is important since DM is the major cause of chronic renal failure[6]. The biochemical markers which are used to determine renal functions in chronic kidney disease are proteinuria (mainly albumin), serum urea, uric acid, creatinine, and glomerular filteration rate. GFR was estimated in our study using modified diet for renal disease equation[17–19]. The significant decrease ($P<0.05$) in the levels of urea, uric acid and creatinine with subsequent improved eGFR in rats treated with TECOSE, insulin and metformin could be due to their blood glucose control potentials. This observation is in support of previous studies that high blood glucose determines progression of kidney disease from induced reactive oxygen species[9].

Histomorphological study of kidneys of diabetic untreated rats showed features of progressive glomerulo-nephritis and sclerosis[20]. These features are degenerative fibrosis and hemorrhage (red arrows), focal sclerosis of the glomerulus, widening of the Bowman's space, and shrinkage of glomerular architecture. Other features observed are hyaline arteriolosclerosis, interstitial fibrosis, poor staining intensity, which is indicative of low glycogen deposits, interstitial inflammation as well as acute tubular necrosis. Treatments with metformin and insulin showed mild insignificant histomorphological distortions. Administration of TECOSE significantly improved general histoarchitecture of renal cortex relative to control group.

In conclusion, diabetes nephropathy is induced in rats by 75 mg/kg body weight streptozotocin, and TECOSE possesses the potentials to prevent diabetic renal damage. The findings of this study contribute to knowledge about the use of the plant, and can provide a future template in the development of plant-based therapeutic compound.

Conflict of interest statement

The authors declare no conflict of interests.

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