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Research Article

Investigation of Renal Function in Diabetic Wistar Rats Treated with Methanol Fraction of Ethanol Extract of *Dialium Guineense* Stem Bark

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Abstract

Diabetes mellitus (DM) is one of the leading causes of death globally. Its incidence is expected to more than double in the coming decade. The present study was aimed to investigate renal function in diabetic rats treated with methanol fraction of ethanol extract of *Dialium guineense* (MEDG) stem bark. Male Wistar rats (n = 25, mean weight = 215 ± 15 g) were divided into five groups (5 rats per group): normal control, diabetic control, metformin, MEDG (200 mg/kg body weight, bwt) and MEDG (300 mg/kg bwt) groups. Diabetes mellitus was induced in the rats via intraperitoneal injection of streptozotocin (STZ) at a dose of 50 mg/kg bwt. The diabetic rats were then treated for 21 days with metformin (50 mg/kg bwt) or MEDG (200 and 300 mg/kg bwt, respectively), leaving the diabetic control group untreated. The results showed that induction of DM using STZ significantly increased plasma urease activity, and urea and chloride concentrations, but it reduced the weight of rat kidney and concentrations of sodium, potassium and bicarbonate ions significantly ($p < 0.05$). However, treatment of the diabetic rats with MEDG markedly reduced plasma urease, and urea and chloride ion concentrations, while increasing kidney weight, organ/body weight ratio as well as concentrations of sodium, potassium and bicarbonate ions ($p < 0.05$). The effect of the extract on potassium and chloride ions was dose dependent. These results indicate that MEDG stem bark can ameliorate kidney dysfunction caused by STZ-induced DM.

Keywords: Biocarbonate ion; chloride ion; electrolytes; medicinal plant; renal toxicity; urease

Introduction

The kidney is an organ that regulates acid-base balance, electrolyte concentrations, extracellular fluid volume, and blood pressure. These homeostatic functions are carried out by the kidney both independently and in concert with other organs, particularly those of the endocrine system [1]. Taking place in the nephrons, the different functions of the kidney are accomplished via filtration, reabsorption, and secretion [2]. Filtration is the process by which cells and large proteins are filtered from the blood to make an ultrafiltrate that eventually becomes urine. Although the kidney produces 180 L of filtrate per day, it reabsorbs a large percentage, thus generating only approximately 2 L of urine [3,4]. The transport of molecules from this ultrafiltrate into the blood is known as reabsorption. During secretion molecules are transported in the opposite direction, from the blood into urine.

The kidneys are tasked with the job of elimination of unmodified drugs and metabolites. Alterations in kidney ultrastructure and function are often found in severe liver disease and once liver function falls below a critical threshold, sodium retention occurs followed by ascites, accompanied by profound disturbances of splanchnic and systemic hemodynamics which in turn may affect renal function [5,6]. Defined as injury to kidneys or impairment of kidney function nephrotoxicity is caused by exposure to xenobiotics such as drugs, food additives, alcohol, chlorinated solvents, peroxidized fatty acids, fungal toxins, radioactive

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isotopes, environmental toxicants, and even some herbs [7,8]. Diabetes mellitus (DM) remains a serious health challenge, globally. Strategies currently employed for its treatment are targeted at ameliorating the different metabolic derangement associated with the disease [9,10]. Nephropathy (damage to kidney leading to renal failure) is a microvascular complication of the disease [11]. This study investigated renal function in diabetic rats treated with MEDG stem bark.

Materials and Methods

Chemicals and Kits

Reagents used in this study were of analytical grade. Kidney function tests kits were obtained from Randox Laboratories Limited (UK). All other chemicals and solvents were purchased from British Drug House (BDH) (England) and Sigma-Aldrich Ltd. (USA).

Collection of Plant Material

The authenticity of the "stem barks of D. guineense, which were obtained from Auchi, Edo State, Nigeria, was verified by Dr. Henry Akinnibosun" of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. The prepared plant specimen was deposited in the herbarium of the same department (No. UBHD330).

Plant Extraction

The plant's stem bark was" washed and shade-dried for 2 weeks at room temperature, and thereafter ground into powder using a blender. A portion (500 g) of powdered plant material was steeped in 5,000 mL of 100 % ethanol. The resulting extract was filtered through muslin cloth and freeze-dried with a lyophilizer. The ethanol extract was subsequently fractionated with absolute methanol [12-15].

Experimental Animals

Wistar albino rats (n = 25) weighing between 200 and 230 g were purchased from the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria. The rats were kept in metal cages under standard laboratory settings and had unrestricted access to feed (pelletized mash) and clean drinking water. Seven days were used to acclimate the rats to laboratory conditions just before commencement of the study. The investigation adopted a standard experimental protocol.

Experimental Design

The rats were divided into five groups of 5 rats each: normal control, diabetic control, metformin, and 200 and 300 mg/kg bwt MEDG groups. Diabetes mellitus was induced in the rats via intraperitoneal injection of STZ (50 mg/kg bwt). The diabetic rats were subsequently treated for 21 days with metformin (50 mg/kg bwt) or MEDG (200 and 300 mg/kg bwt, respectively), leaving the diabetic control group untreated.

Kidney Function Tests

Kidney function tests (KFTs) such as urea and sodium, potassium, chloride and bicarbonate ions as well as urease activity were performed in plasma [16-20].

Statistical Analysis

Data are expressed as mean ± SEM (n = 5). The statistical analysis was performed using SPSS (version 20). Groups were compared using Duncan multiple range test. Statistical significance was assumed at $p < 0.05$.

Results

Effect of MEDG Stem Bark on Weight and Renal Function

Induction of DM using STZ significantly increased plasma urease activity, and urea and chloride concentrations, but it reduced the weight of rat kidney and concentrations of sodium, potassium, and bicarbonate ions significantly ($p < 0.05$). However, treatment of the diabetic rats with MEDG markedly reduced plasma urease, and urea and chloride ion concentrations, while increasing kidney weight, organ/body weight ratio as well as concentrations of sodium, potassium and bicarbonate ions ($p < 0.05$; Table 1 and Figures 1-3). The effect of MEDG on potassium and chloride ions was dose dependent.

Table 1: Comparison of the Effect of MEDG on Weight of Rat Kidneys.

Group	Kidney Weight (g)	Organ/Body Weight Ratio x 10^{-3}
Normal Control	0.73 ± 0.05	3.95 ± 0.41
Diabetic Control	0.44 ± 0.02	2.71 ± 0.17
Metformin	0.60 ± 0.30	4.08 ± 0.22
MEDG (200 mg/kg bwt)	0.64 ± 0.24	3.81 ± 0.18
MEDG (300 mg/kg bwt)	0.62 ± 0.14	4.38 ± 0.16

Data are kidney weight and relative organ weight and are expressed as mean ± standard error of mean (SEM, n = 5).

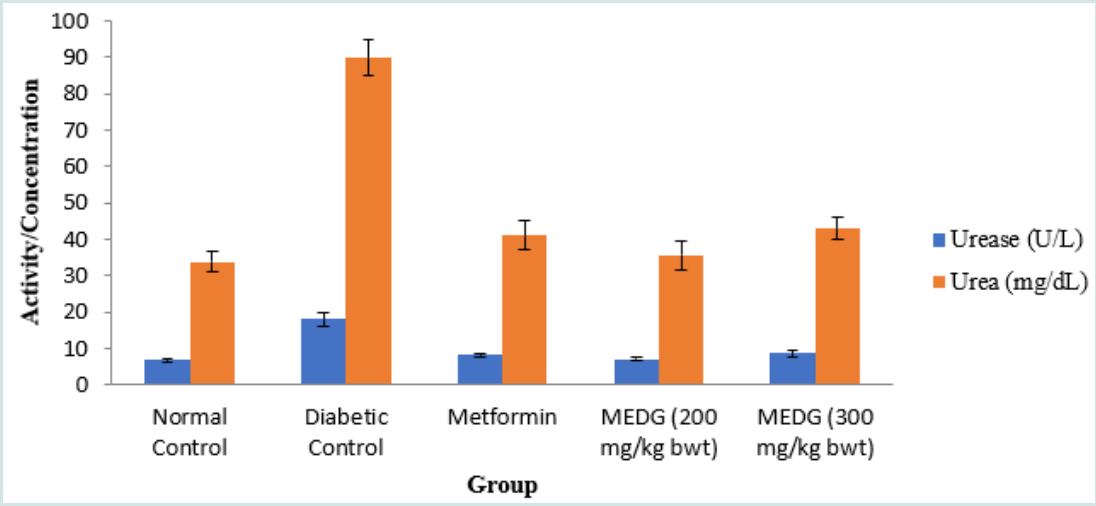

Figure 1: Effect of MEDG Stem Bark on Activity of Urease and Concentration of Urea.

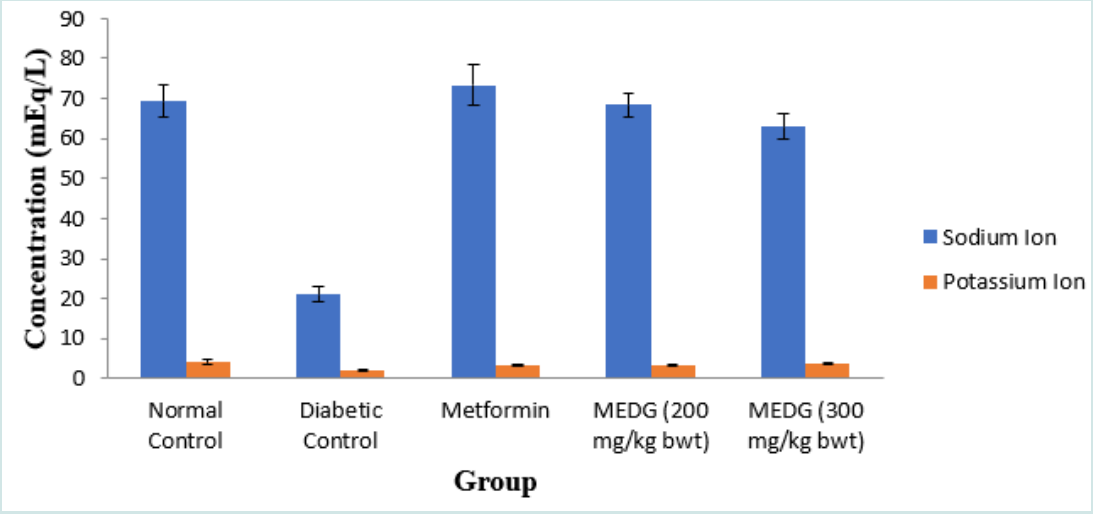

Figure 2: Effect of MEDG on Concentrations of Sodium and Potassium Ions.

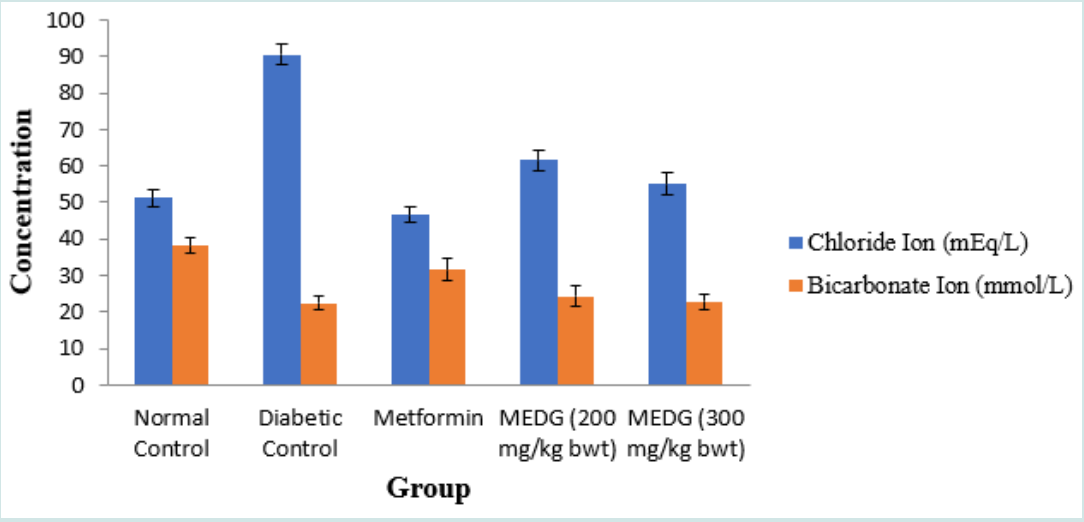

Figure 3: Effect of MEDG on Concentrations of Chloride and Bicarbonate Ions.

Discussion

Diabetes mellitus (DM) is a disease caused by derangements in carbohydrate, lipid and protein metabolism. Long-term complications of DM include retinopathy, nephropathy, and neuropathy [21-24]. As an organ that metabolizes harmful substances besides liver, kidney is constantly perfused with huge volumes of blood carrying different compounds, thus the organ is at increased risk of toxicity [25-27]. Measurement of KFTs aids the diagnosis of renal dysfunction or impairment. High levels of blood urea and creatinine are found in renal dysfunction or muscle injury. Blood urea and creatinine are considered traditional indices of kidney function. Urea is a by-product of protein catabolism. About 90 % of urea produced is excreted via the kidneys [28]. Creatinine, a waste product of muscle catabolism, is excreted exclusively through the kidneys [29].

Therefore, renal damage diminishes the kidney's capacity to excrete urea and creatinine, thereby making them accumulate in the blood. In this study, STZ caused significant damage to renal corpuscle as shown by elevated urea concentration and urease activity. Levels of specific ions such as sodium (Na+), potassium (K+), chloride (Cl−) and bicarbonate (HCO3−) are used as markers of electrolyte imbalance. Electrolytes promote fluid balance via maintenance of blood volume, fluid absorption and generation of impulses. In pathological conditions, electrolyte imbalance occurs with increased sodium and chloride, and decreased potassium levels. Nephrotoxicity is characterized by morphological destruction of intracellular organelles, necrosis, and functional alterations such as depletion of antioxidant defense system and mitochondrial damage. The results of this study are in agreement with those of previous studies [30-44]. The biological effect of extracts of D. guineense stem bark has been reported [45-72].

Conclusion

The results obtained in this study indicate that MEDG stem bark can ameliorate kidney dysfunction caused by STZ-induced DM. This study has provided first-time evidence of the nephroprotective effect of MEDG stem bark on diabetic Wistar albino rats.

Competing Interests

The authors declare that they have no conflict of interest.

Acknowledgment

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