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Research Article

Renal Oxidative Status in Diabetic Wistar Rats Administered Methanol Fraction of Ethanol Extract of *Dialium guineense* Stem Bark

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A B S T R A C T

Survey has shown that the number of persons with diabetes mellitus (DM) would increase in the coming decade. Diabetes mellitus is primarily defined by the level of hyperglycemia which causes nephropathy. The present study investigated renal oxidative status of diabetic rats administered methanol fraction of ethanol extract of *Dialium Guineense* (MEDG) stem bark. Male Wistar rats (n = 25, mean weight = 215 ± 15 g) were randomly assigned to five groups (5 rats per group): normal control, diabetic control, metformin, and 200 mg/kg body weight (bwt) and 300 mg/kg bwt extract groups. Diabetes mellitus was induced via intraperitoneal injection of streptozotocin (STZ, 50 mg/kg bwt). The diabetic rats were then treated for 21 days with metformin (50 mg/kg bwt) or the extract at doses of 200 and 300 mg/kg bwt, respectively. The results showed that the activities of catalase, superoxide dismutase (SOD), glutathione peroxidase (GPX), and Glutathione Reductase (GR) as well as levels of reduced glutathione (GSH) and % GSH were significantly lower in diabetic control group than in the normal control group, but they were increased by MEDG treatment ($p < 0.05$). On the other hand, the levels of nitric oxide (NO) and malondialdehyde (MDA) elevated by STZ were markedly reduced after treatment with MEDG ($p < 0.05$). It can be concluded that MEDG stem bark possesses an ameliorative potential against STZ-induced oxidative stress in rats.

Keywords: Antioxidants, *Dialium guineense*, Kidney, Lipid peroxidation, Oxidative stress

Introduction

The metabolism of glucose is influenced by glucagon and insulin, secreted by the pancreas. Insulin acts as a doorway to drive glucose into cells, stimulating glucose utilization, while glucagon does the opposite, making glucose available for cells from glycogen. Following the consumption of carbohydrates, insulin levels quickly increase while glucagon levels drop. Hyperglycemia, a symptom of diabetes mellitus, occurs when there is insufficient or aberrant insulin production. If the blood glucose level remains high (hyperglycemia) over a long period of time, it results in long-term damage to organs such as kidney, liver, eye, nerves, heart and blood vessels [1,2] Oxidative stress plays a role in insulin resistance. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) affect insulin signaling cascade [3]. At lower doses, ROS play a physiological role in insulin signaling. After the stimulation of insulin receptor in adipocytes, hydrogen peroxide (H_2O_2) is produced by the catalytic action of NADPH oxidase, which inhibits PTP1B catalytic activity, thus increasing tyrosine phosphorylation [4]. Oxidative stress caused by hyperglycemia in diabetes mellitus may impair insulin signaling leading to insulin resistance. Hyperglycemia and insulin resistance may also lead to altered mitochondrial function and insulin action impairment by cytokines in response to metabolic stress [5,6].

Many medicinal plants have been advocated for the treatment of diabetes mellitus [7,8]. The leaves and other parts of *D. guineense* are used in Traditional Systems of Medicine to treat different diseases [9,10] This study was aimed to investigate renal oxidative status of diabetic rats administered MEDG stem bark**.**

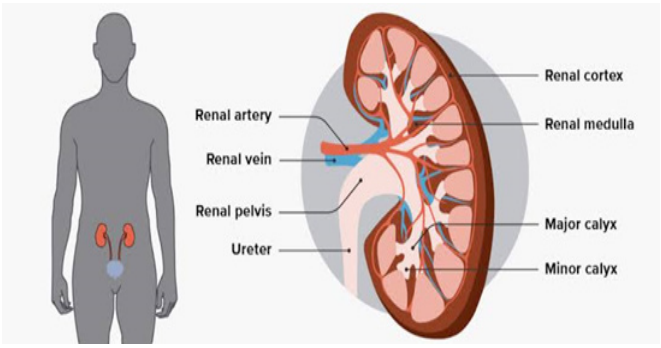

Figure 1: Location and Anatomy of the Kidney

Material and Methods

Chemicals

Every substance used in this study, including chemicals as well as reagents, was of analytical grade. The reagents were obtained from Sigma-Aldrich, Ltd. (USA).

Collection of Plant Material

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

Plant Preparation and 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 [11]. The ethanol extract was further fractionated with absolute methanol.

Experimental Rats

Twenty-five mature male Wistar rats weighing between 200 and 230 g were purchased from the Department of Anatomy at the University of Benin in Benin City, Nigeria. The rats were kept in metal cages under standard laboratory settings, which included a 12-h light/12-h dark cycle, room temperature, as well as relative humidity range of 55 to 65 %. They had unhindered access to both potable water and pelletized mash. One week was spent acclimating the rats to laboratory conditions prior to the start of the experiment. The investigation followed a standard experimental protocol.

Experimental Design

The rats (n = 25; mean weight = 215 ± 15 g) were randomly assigned to five groups (5 rats per group): normal control, diabetic control, metformin, and 200 mg/kg bwt and 300 mg/kg bwt extract groups. Diabetes mellitus was induced in the rats via intraperitoneal injection of STZ (50 mg/kg bwt). The diabetic rats were then treated for 21 days with metformin (50 mg/kg bwt) or the extract at doses of 200 and 300 mg/kg bwt, respectively, leaving the diabetic control group untreated.

Tissue Sample Collection

At the end of the treatment period, the rats were euthanized under mild anesthesia. Their kidneys were excised, washed in ice–cold saline, blotted dry and placed in plain containers. Weighted portions of the kidney were used to prepare 20 % tissue homogenate used for biochemical analyses.

Biochemical Analyses

The activities of catalase, SOD, GPx and GR were determined [12, 13, 14,15]. Levels of renal total protein, MDA and GSH were also measured [16,17,18]. The level of NO was determined using a previously described method [19].

Statistical Analysis

Data are presented as mean ± SEM (n = 5). Statistical analysis was performed using SPSS version 21. Statistical differences between means were compared using Duncan multiple range test. Values of $p < 0.05$ were considered statistically significant.

Results

Effect of MEDG Stem Bark on Kidney Weight is 5.1

Induction of diabetes mellitus with STZ significantly reduced the weights of rat kidney ($p < 0.05$). However, treatment of the diabetic rats with the extract markedly increased the weights of this organ as well as the corresponding kidney/body weight ratio ($p < 0.05$) (Table 1).

Table 1: Comparison of the Weights of Rat Kidney Among the Groups.

Group	Kidney Weight (g)	Relative Organ Weight x 10^{-3}
Normal Control	0.73 ± 0.05	3.95 ± 0.41
Diabetic Control	0.44 ± 0.02[b]	2.71 ± 0.17[b]
Metformin	0.60 ± 0.30[a]	4.08 ± 0.22[a]
200 mg/kg bwt MEDG	0.49 ± 0.04[a]	2.92 ± 0.08[a]
300 mg/kg bwt MEDG	0.63 ± 0.03[a]	4.44 ± 0.11[a]
Data are weights of rat kidneys and are expressed as mean ± SEM (n = 5).		
Values with superscript "a" are significantly different from the diabetic control group.		
Values with superscript "b" are significantly different from the normal control group.		

Effect of MEDG Stem Bark on Renal Oxidative Status in Diabetic Rats

The activities of all the antioxidant enzymes measured and levels of GSH were significantly lower in diabetic control group than in the normal control group, but they were increased by MEDG treatment ($p < 0.05$). On the other hand, the levels of NO and MDA elevated by STZ were markedly reduced after treatment with MEDG ($p < 0.05$). These results are shown in Tables 2 to 5.

Table 2: Levels of Tissue Total Protein and Lipid Peroxidation Index in the Different Groups.

Group	TP (mg/dL)	MDA (mole/mg tissue) x 10^{-6}
Normal Control	38.65 ± 1.30	10.05 ± 0.05
Diabetic Control	57.90 ± 0.52[b]	31.85 ± 1.65[b]
Metformin	39.48 ± 0.75[a]	13.05 ± 1.95[a]
200 mg/kg bwt MEDG	51.55 ± 2.62	18.25 ± 4.05[a]
300 mg/kg bwt MEDG	41.73 ± 1.83[a]	16.45 ± 8.25[a]
Data are renal total protein and MDA levels and are expressed as mean ± standard error of mean (SEM, n = 5).		
Values with superscript "a" are significantly different from the diabetic control group.		
Values with superscript "b" are significantly different from the normal control group.		

Table 3: Activities of Glutathione Reductase and Levels of GSH in the Different Groups.

Group	Catalase (U/mg protein) x 10^{-4}	SOD (U/mg protein) x 10^{-3}	GPx (U/mg protein) x 10^{-4}
Normal Control	14.62 ± 2.72	4.15 ± 0.15	2.04 ± 0.06
Diabetic Control	7.23 ± 0.80[b]	1.02 ± 0.07[b]	0.14 ± 0.02[b]
Metformin	12.90 ± 2.78[a]	3.97 ± 0.01[a]	1.31 ± 0.01[a]
200 mg/kg bwt MEDG	10.20 ± 5.39[a]	3.26 ± 0.04[a]	1.42 ± 0.02[a]
Data are renal total protein and MDA levels and are expressed as mean ± standard error of mean (SEM, n = 5).			
Values with superscript "a" are significantly different from the diabetic control group.			
Values with superscript "b" are significantly different from the normal control group.			

Table 4: Activities of Glutathione Reductase and Levels of GSH in the Different Groups.

Group	GSH (mg/dL)	% GSH	GR (U/mg protein) x 10^{-2}
Normal Control	1.53 ± 0.09	75.85 ± 3.01	7.65 ± 0.46
Diabetic Control	0.36 ± 0.04[b]	21.70 ± 1.40[b]	1.80 ± 0.08[b]
Metformin	1.25 ± 0.08[a]	44.17 ± 2.07[a]	6.25 ± 0.52[a]
200 mg/kg bwt MEDG	1.16 ± 0.04[a]	41.67 ± 1.22[a]	5.80 ± 0.31[a]
300 mg/kg bwt MEDG	1.27 ± 0.03[a]	46.70 ± 1.82[a]	6.35 ± 0.64[a]
Data are markers of oxidative stress and are expressed as mean ± SEM (n = 5).			
Values with superscript "a" are significantly different from the diabetic control group.			
Values with superscript "b" are significantly different from the normal control group.			

Table 5: Activities of Glutathione Reductase and Levels of GSH in the Different Groups.

Group	NO (µmole/L)	% NO
Normal Control	10.63 ± 0.00	53.24 ± 3.41
Diabetic Control	30.64 ± 0.03[b]	19.35 ± 1.80[b]
Metformin	15.82 ± 0.03[a]	29.64 ± 1.01[a]
200 mg/kg bwt MEDG	20.76 ± 0.05[a]	29.07 ± 2.44[a]
300 mg/kg bwt MEDG	17.26 ± 0.04[a]	37.41 ± 2.08[a]
Data are markers of oxidative stress and are expressed as mean ± SEM (n = 5).		
Values with superscript "a" are significantly different from the diabetic control group.		
Values with superscript "b" are significantly different from the normal control group.		

Discussion

Free radicals are involved in the pathogenesis of a number of diseases such as tumor, inflammation, hemorrhagic shock, atherosclerosis, diabetes mellitus, infertility, gastrointestinal ulcerogenesis, asthma, rheumatoid arthritis, cardiovascular disorders, cystic fibrosis, neurodegenerative diseases (for example, parkinsonism, Alzheimer's diseases), AIDS and even early senescence [20]. Although the human body produces insufficient number of antioxidants which are essential for preventing oxidative stress, free radicals generated within it are removed by natural antioxidant defenses such as glutathione or catalase [21]. Therefore, this deficiency is compensated for by making use of natural exogenous antioxidants, such as vitamin C, vitamin E, flavones, β-carotene and natural products in plants. Plants contain a wide variety of free radicals scavenging molecules such as phenols, flavonoids, vitamins and terpenoids [22]. Many plants, citrus fruits and leafy vegetables are the source of ascorbic acid, vitamin E, carotenoids, flavanols and phenolics. Antioxidants protect cells against the damaging effects of ROS such as singlet oxygen, superoxide anion (O_2^-), and peroxyl and hydroxyl radicals and peroxynitrite which results in oxidative stress leading to cellular damage [23]. Natural antioxidants play a key role in health maintenance and prevention of chronic and degenerative diseases. Antioxidants exert their effect by scavenging 'free-oxygen radicals' thereby giving rise to a fairly stable molecule. Free radicals are metastable chemical species, which tend to trap electrons from molecules in the immediate surroundings [24]. These radicals if not scavenged effectively in time, may damage crucial biomolecules like lipids, proteins (including those present in all membranes and mitochondria) and DNA resulting in abnormalities leading to disease conditions. Oxidative stress plays a role in insulin resistance since ROS and RNS affect insulin signaling cascade [3]. This study was aimed to investigate renal oxidative status of diabetic rats administered MEDG stem bark**.** The results indicate that the activities of all the antioxidant enzymes measured and levels of GSH were significantly lower in diabetic control group than in the normal control group, but they were increased by MEDG treatment. On the other hand, the levels of NO and MDA elevated by STZ were markedly reduced after treatment with MEDG. These results are in agreement with reports of previous studies [25-31].

Conclusion

It can be concluded that the methanol fraction of ethanol extract of *Dailium guineense* stem bark possesses an ameliorative potential against STZ induced oxidative stress in rats.

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