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Evaluation of the Effect of MEDG Stem Bark on Oxidative Stress in Rat Plasma Caused by Diabetes Mellitus

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ABSTRACT

Diabetes mellitus (DM) is a serious health condition which reduces the quality of life of sufferers. The different treatment options for DM cannot effectively ameliorate the various complications caused by the disease. The aim of the present study was to evaluate the effect of methanol fraction of ethanol extract of *Dialium guineense* (MEDG) stem bark on oxidative stress in rat plasma caused by DM. Male albino rats (Wistar strain; n = 25; mean weight = 215 ± 15 g) were divided into five groups (5 rats per group): control, diabetic, metformin, and 200 mg/kg body weight (bwt) and 300 mg/kg bwt MEDG groups. Diabetes mellitus was induced in the rats via intraperitoneal injection of streptozotocin (STZ, 50 mg/kg bwt). The diabetic rats were subsequently treated with metformin (50 mg/kg bwt) or the medicinal plant extract (200 and 300 mg/kg bwt, respectively), for 21 days. Markers of oxidative stress were measured in rat plasma. The results indicated that the blood glucose concentrations of the rats elevated by STZ-induced DM was significantly reduced after treatment with MEDG stem bark ($p < 0.05$). Similarly, activities of all the antioxidant enzymes [catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR)], and concentrations of glutathione (GSH) were significantly lower in diabetic group than in the control group, but they were increased by MEDG treatment ($p < 0.05$). However, the concentrations of nitric oxide (NO) and malondialdehyde (MDA) elevated by STZ were greatly reduced after treatment with the medicinal plant extract ($p < 0.05$). The results obtained in this study have shown that MEDG stem bark can stimulate antioxidant defense system in rats challenged with STZ.

Keywords: *Dialium Guineense*; Free Radicals; Glutathione Peroxidase; Nitric Oxide; Oxidative Stress

Abbreviations: ROS: Reactive Oxygen Species; RNS: Reactive Nitrogen Species; PKC: Protein Kinase C; DM: Diabetes Mellitus; GR: Glutathione Reductase

Introduction

Implicated in the pathogeneses of many diseases, oxidative stress contributes significantly to insulin resistance [1,2]. Impairment of insulin signaling pathway by reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been reported by different studies [3]. Oxidative stress plays a crucial role in the development of diabetic complications (micro- and macrovascular). The metabolic abnormalities of the disease cause mitochondrial overproduction of superoxide anion in endothelial cells of both large and small vessels, and also in the myocardium. This in turn causes the activation of five major pathways involved in the pathogenesis of diabetic complications: polyol pathway flux, increased formation of advanced glycation end-products (AGEs), increased expression of the receptor for AGEs and its activating ligands, activation of protein kinase C (PKC) isoforms, and overactivity of the hexosamine pathway. It also directly inactivates two important antiatherosclerotic enzymes, endothelial nitric oxide synthase (eNOS) and prostacyclin synthase. Through these pathways, increased intracellular ROS cause defective angiogenesis in response to ischemia, activates a number of pro-inflammatory pathways, and cause long-lasting epigenetic changes which drive persistent expression of pro-inflammatory genes after glycemia is normalized (hyperglycemia memory) [4-6]. In pancreatic β-cells, NO regulation of glucokinase activity via S-nitrosylation reaction, may enhance insulin secretion [7,8].

However, excess NO and concomitant NRS could cause apoptosis through caspase-3 activation and decrease in ATP levels [9]. Studies have speculated a direct relationship between the health benefits of a plant and its antioxidant content. *Dialium guineense* (Velvet Tamarind) is a medicinal plant used in Traditional Medicine for the treatment of infections [10]. It is a tall, tropical, fruit-bearing tree, belonging to the Leguminosae family, and has small, typically grape-sized edible fruits with brown hard inedible shells. In Africa, it is found in dense forests along the southern edge of the Sahel. The plant grows naturally in West African countries, Central African Republic, and Sudan [11]. In Nigeria, it is known by different local names: Icheku (Igbo), Awin (Yoruba), Tsamiyarkurm (Hausa) and Amughen (Bini) [12]. The aim of this study was to evaluate the effect of MEDG stem bark on oxidative stress in rat plasma caused by diabetes mellitus (DM).

Materials and Methods

Chemicals

Analytical grade reagents were used in this study. Kits used to carry out antioxidant assays were bought from Randox Laboratories Limited (United Kingdom). The other chemicals/reagents were products of Pyrex Scientific Limited (United Kingdom), Merck (Germany), British Drug House (BDH) (England), and Sigma-Aldrich Ltd. (USA).

Plant Material and Authentication

Freshly harvested stem barks of D. guineense were collected from Auchi, Etsako West, Edo State, Nigeria. Their identification and authentication took place at the University of Benin herbarium in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences (No. UBHD330).

Plant Preparation and Extraction

The plant stem barks were washed and shade-dried at room temperature for 30 days, and thereafter ground into powder using an electric blender. A portion (500 g) of pulverized plant material was steeped in 5 L of absolute ethanol. The resultant extract was filtered through muslin cloth and freeze-dried with a lyophilizer [13-15].

Experimental Rats

Male rats (Wistar strain, n = 25) with weight ranging from 200 to 230 g (mean weight = 215 ± 15 g) were purchased from the Animal House of 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. They had unrestricted access to feed (pelletized mash) and potable drinking water. Seven days were allowed to acclimate the rats to laboratory conditions prior to commencement of the study. The investigation followed a standard experimental protocol.

Experimental Design

The rats were randomly assigned to five groups (5 rats in a group): control, diabetic, 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 a period of 21 days with metformin (50 mg/kg bwt) or MEDG (200 and 300 mg/kg bwt, respectively), leaving the diabetic group untreated.

Preparation of Plasma

At the end of the 21-day treatment, the rats were euthanized under mild anesthesia. Blood was collected through cardiac puncture into sterile heparin containers. The blood samples were centrifuged at 2000 rpm for 10 min to obtain clear plasma.

Biochemical Analyses

The activities of catalase, SOD, GPx and GR were determined [16-19]. Concentrations of plasma total protein, MDA and GSH were also measured [20-22]. Nitric oxide (NO) concentration was determined as described in literature [23].

Statistical Analysis

Data are expressed 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 Weight and Blood Glucose of Rats

As shown in Table 1, the blood glucose concentrations of the rats elevated by STZ-induced DM was significantly reduced after treatment with MEDG stem bark ($p < 0.05$).

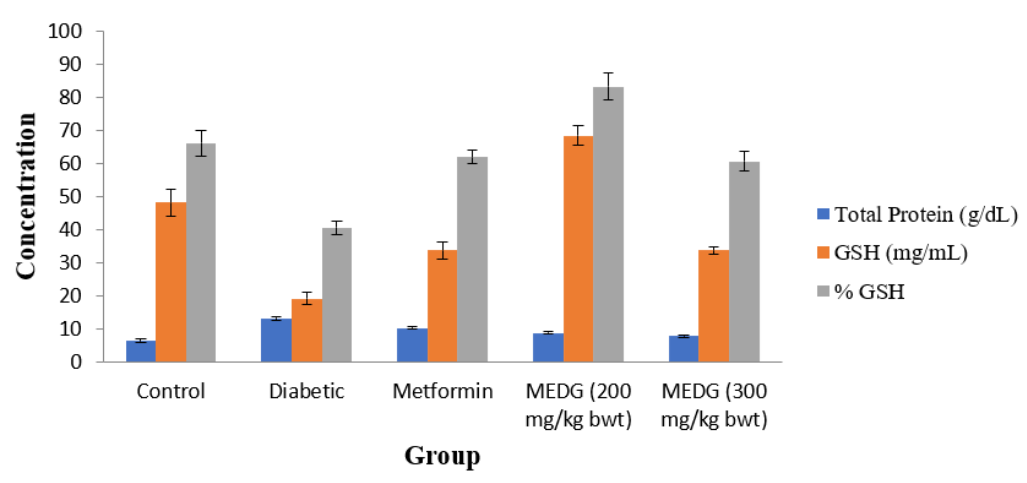
Table 1: Effect of MEDG Stem Bark on Weight and Blood Glucose of Rats.

Group	Weight Change (g)	% Weight Change	FBG (mg/dL)	Glycemic Change (mg/dL)	% Glycemic Change
Control	-	-	-	-	-
Diabetic	-	-	> 800	-	-
Metformin	20.35	12.16	> 800	399	49.88
MEDG (200 mg/kg bwt)	12.26	7.87	> 800	421	52.63
MEDG (300 mg/kg bwt)	29.08	17.02	364	227	62.36

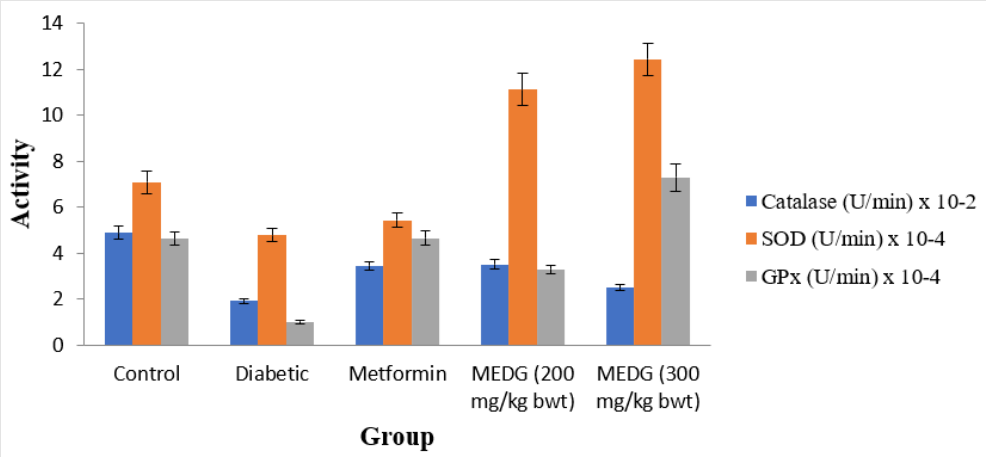
Note: Data are weight and FBG parameters and are expressed as mean ± SEM (n = 5).

Oxidative Status of Diabetic Rat Plasma

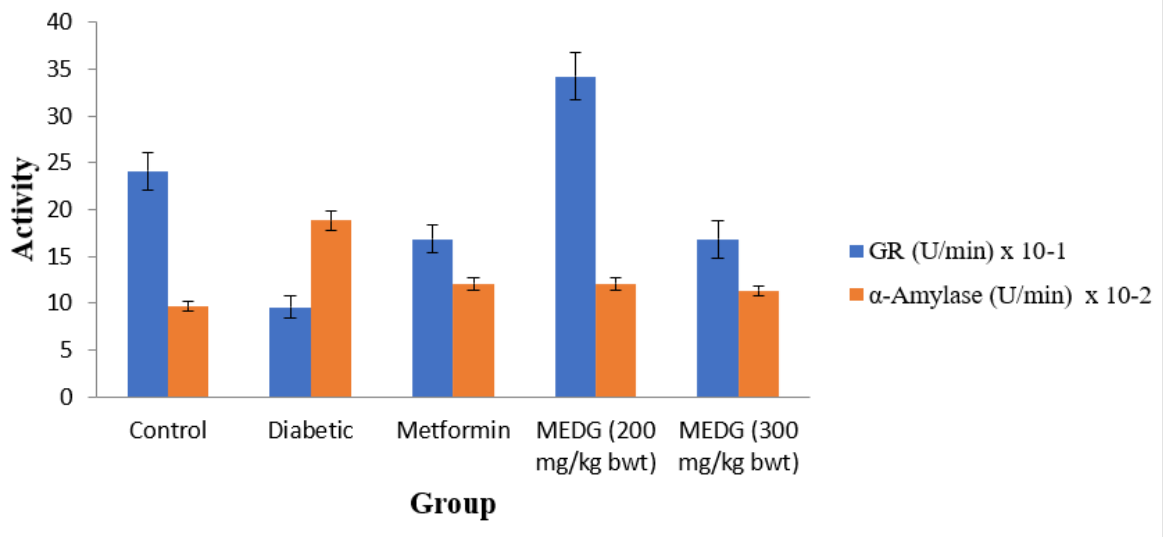
The activities of all the antioxidant enzymes and concentrations of GSH were significantly lower in diabetic group than in the control group, but they were increased by MEDG treatment ($p < 0.05$). However, the concentrations of NO and MDA elevated by STZ were greatly reduced after treatment with the medicinal plant extract ($p < 0.05$; Figures 1-4).



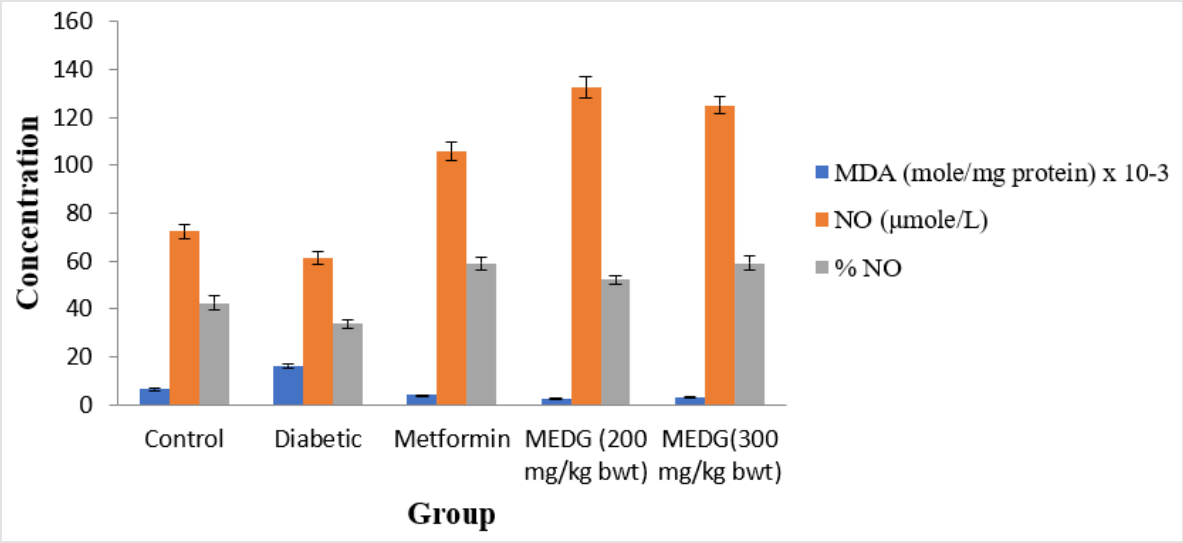
Note: Data are plasma total protein and GSH levels and are expressed as mean ± SEM (n = 5).
Figure 1: Effect of MEDG Stem Bark on Plasma Total Protein and Glutathione Levels.



Note: Data are markers of oxidative stress and are expressed as mean ± SEM (n = 5).
Figure 2: Effect of MEDG Stem Bark on Oxidative Status in Rat Plasma.



Note: Data are activities of plasma α-amylase and glutathione reductase and are expressed as mean ± SEM (n = 5).
Figure 3: Effect of MEDG Stem Bark on Plasma Alpha-Amylase and Glutathione Reductase Activity.



Note: Data are plasma MDA and NO concentrations and are expressed as mean ± SEM (n = 5).
Figure 4: Effect of MEDG Stem Bark on Plasma MDA and NO Levels.

Discussion

Diabetes mellitus (DM) is a group of metabolic diseases characterized by chronic hyperglycemia that results from disturbed insulin secretion or function or both. Currently, many countries are on the verge of a global "diabetes epidemic", which is rapidly spreading across the planet [1]. Chronic hyperglycemia in DM is accompanied by damage, dysfunction, and failure of various organs and tissues, development of micro- (retinopathy, nephropathy, and neuropathy) and macrovascular (cardiovascular disorders) complications [24]. Reactive oxygen species (ROS) are chemically active oxygen-containing molecules generated in living systems. They are natural by-products of oxygen metabolism in all aerobic organisms. The main ROS types include superoxide, hydroperoxyl radical, singlet radical, hydroxyl radical, nitric oxide, peroxynitrite, amongst others [25]. Although ROS are primarily generated in mitochondria, but other alternative mechanisms contribute to their formation: NADPH-oxidase (NOX), immune reactions, xanthine oxidase, arachidonic acid metabolism,

amongst others [26]. These molecules are widely involved in processes of intracellular signaling and regulation of cell activity— apoptosis induction, adaptation to the effects of various factors, and immune response [27]. Moreover, ROS can stimulate inflammatory responses through protein kinases, transcription factors, and pro-inflammatory factors genomic expression [28]. Increased ROS accumulation leads to oxidative stress, which contributes to major cellular components (lipids, proteins, and DNA) damage.

The antioxidant defense system provides critical defense for the biological system by limiting the damaging effects of ROS. There are many antioxidant enzymes, including SOD, GPx, GR, catalase, paraoxanase (PON), amongst others [29]. In addition to enzymatic antioxidants, non-enzymatic antioxidant defense (ascorbate, tocopherols, retinol, carotenoids, reduced glutathione (GSH), melatonin, polyphenols, ceruloplasmin, carnosine, amongst others) also play crucial role in maintaining normal ROS levels. Under different pathological conditions, including DM, the redox balance can be disturbed that leads to negative consequences for the cell [2,30]. Many phytochemicals including phenolics, flavonoids, tannins, proanthocyanidins, and various plant extracts have been reported as antioxidants [31-33]. The aim of this study was to evaluate the effect of MEDG stem bark on oxidative stress in rat plasma caused by DM. The results obtained indicated that the activities of all the antioxidant enzymes and concentrations of GSH were significantly lower in diabetic group than in the control group, but they were increased by MEDG treatment. However, the concentrations of NO and MDA elevated by STZ were greatly reduced after treatment with the medicinal plant extract. These results are in agreement with reports of previous studies [34-45]. Studies have shown that plants rich in important bioactive compounds/phytochemicals are very useful medicinally [46-69].

Conclusion

The results obtained in this study have shown that MEDG stem bark can stimulate antioxidant defense system in rats challenged with STZ. In addition, the extract may serve as natural source of antioxidants.

Competing Interests

The authors declare that they have no conflict of interest.

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