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# Methanol Fraction of Ethanol Extract of *Dialium Guineense* Stem Bark Mitigates STZ-Induced Oxidative Stress in Rat Liver

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**ABSTRACT**

The present study investigated the effect of methanol fraction of ethanol extract of *Dialium guineense* (MEDG) stem bark on streptozotocin (STZ)-induced oxidative stress in rat liver. Male albino rats of Wistar strain (n = 25, mean weight = 215 ± 15 g) were randomly assigned to five groups (5 rats/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 50 mg/kg bwt STZ. The diabetic rats were treated for 21 days with either metformin (50 mg/kg bwt) or the extract at doses of 200 and 300 mg/kg bwt, respectively. Activities of antioxidant enzymes such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) as well as antioxidant vitamins (retinoic acid, ascorbic acid and α-tocopherol) were measured in 20 % liver homogenate. The results showed that induction of diabetes mellitus with STZ significantly increased the fasting blood glucose (FBG) concentrations of the rats ($p < 0.05$). However, treatment of the diabetic rats with the extract markedly reduced the blood glucose concentration and body weights of rats ($p < 0.05$). Treatment of diabetic Wistar rats with MEDG stem bark significantly increased the activities of the antioxidant enzymes and molecules as well as concentrations of nitric oxide (NO), but it markedly reduced the concentrations of hepatic total protein (TP) and malondialdehyde (MDA) ($p < 0.05$). These results indicate that MEDG stem bark can potentiate antioxidant defense in diabetic rat liver.

**Keywords:** Antioxidant Enzymes; Hepatocytes; Malondialdehyde; Oxidative Stress; Reactive Oxygen Species

## Introduction

The liver plays a vital role in the regulation of glucose levels in physiological and pathological states such as diabetes mellitus (DM). In type-2 DM (T2DM), insulin resistance in the liver causes hyperglycaemia and further distortion of glucose metabolism. Even under severe hyperglycemic conditions, the liver does not detect the availability of glucose and produces more via the combined action of a few enzymes such as glucose-6-phosphate dehydrogenase (G6PDH), fructose-1,6-bisphosphatase, hexokinase and glucokinase [1,2]. The liver also regulates glucose homeostasis via modulation of the expression of genes encoding secretory proteins (that is, hepatokines) [3,4]. Different types of hepatokines via different pathways activate either positive or negative feedback mechanisms to control liver metabolism [3]. Three dominant hepatokines exist: fetuin-A, betatrophin/angiopoietin-like protein 8 (ANGPTL8) and fibroblast growth factor 21 (FGF21). These hepatokines significantly exacerbate the diabetic condition, especially in T2DM, by regulating subclinical inflammation [3].

Both oxidative stress and inflammatory responses act as damaging agents in aggravating the pathological condition of DM [5,6]. In some cases, DM causes excessive accumulation of fat cells in the liver resulting in a fatty liver and, consequently, non-alcoholic fatty liver disease (NAFLD). Subsequently, 2 – 3 % of NAFLD patients experience hepatic inflammation, necrosis, and fibrosis, which are symptoms of non-alcoholic steatohepatitis (NASH) [1,6]. Injured or fibrotic liver then become cirrhotic, form hepatocellular carcinomas (HCCs) and, eventually, liver failure [7,8]. Antioxidants from plant-based and natural products with strong antidiabetic, anti-inflammatory and antiglycative properties are emerging as future therapy for DM. As opposed to conventional medications, antioxidants from plant sources may be an alternative and beneficial way to prevent and treat this life-threatening disease [9-16]. The aim of this study was to investigate the effect of MEDG stem bark on STZ-induced oxidative stress in rat liver.

## Materials and Methods

### Drugs and Chemicals

The standard antidiabetic drug, metformin, was purchased from Micronova Laboratories (India), and STZ was a product of British Drug House (BDH) Chemicals Ltd. (England). Absolute ethanol, chloroform and other solvents were obtained from Bell, Sons & Co. (England), while formaldehyde was purchased from Thermo Fisher Scientific Ltd. (USA). All the chemicals and solvents used in this study were of analytical grade.

### 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 further fractionated with absolute methanol [17,18].

### Animals

Male Wistar albino rats (n = 25, mean weight = 215±15 g) were bought from the Department of Anatomy, University of Benin, Nigeria and housed in wooden cages. They were acclimatized for fourteen days before commencement of the study and had free access to feed and water.

### Experimental Design

The rats were randomly assigned to five groups (5 rats per group): normal control, diabetic control, metformin, 200 mg/kg bwt MEDG and 300 mg/kg bwt MEDG groups. Diabetes mellitus was induced in the rats via intraperitoneal injection of STZ at a dose of 50 mg/kg bwt. The diabetic rats were then treated with either metformin (50 mg/kg bwt) or the extract at doses of 200 and 300 mg/kg bwt, respectively, for 21 days.

### Tissue Sample Collection and Preparation

At the end of day 21 of treatment, the rats were euthanized under mild chloroform anesthesia after an overnight fast. Their liver was excised, and used to prepare 20 % tissue homogenate. The homogenate was centrifuged at 2000 rpm for 10 min to obtain clear supernatant.

### Biochemical Analyses

The activities of catalase, SOD and GPx were determined [19-21]. Hepatic levels of TP, MDA, GSH, NO as well as vitamins A, E and C were also measured [22-28]. The activity of GR was determined using a previously described method [29].

### Data 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. Statistical significance was assumed at $p < 0.05$.

## Results

### Effect of MEDG Stem Bark on Weight and Blood Glucose of Rats

Induction of diabetes mellitus with STZ significantly increased the blood glucose concentrations of the rats ($p < 0.05$). However, treatment of the diabetic rats with MEDG stem bark markedly reduced the FBG concentration and body weights of rats ($p < 0.05$; Table 1).

**Table 1:** Weight and Blood Glucose Parameters. Data are weight and FBG parameters and are expressed as mean ± SEM (n = 5).

| Group | Weight Change (g) | Weight Change (%) | FBG (mg/dL) | Glycemic Change (mg/dL) | Glycemic Change (%) |
|---|---|---|---|---|---|
| Normal Control | _ | _ | _ | _ | _ |
| Diabetic Control | _ | _ | >800 | _ | _ |
| Metformin | 20.35 | 12.16 | >800 | 399 | 49.88 |
| MEDG (200 mg/kg bwt) | 16 | 11.19 | 427 | 311 | 71.61 |

### Hepatic Oxidative Status in Diabetic Rats

Induction of diabetes mellitus with STZ markedly reduced the activities of the markers of oxidative stress, % GSH, % NO as well as concentrations of retinoic acid, α-tocopherol, and ascorbic acid in hepatic tissue ($p < 0.05$). However, treatment of diabetic Wistar rats with MEDG stem bark significantly increased the activities of antioxidant enzymes and other parameters measured ($p < 0.05$; Figures 1-4).

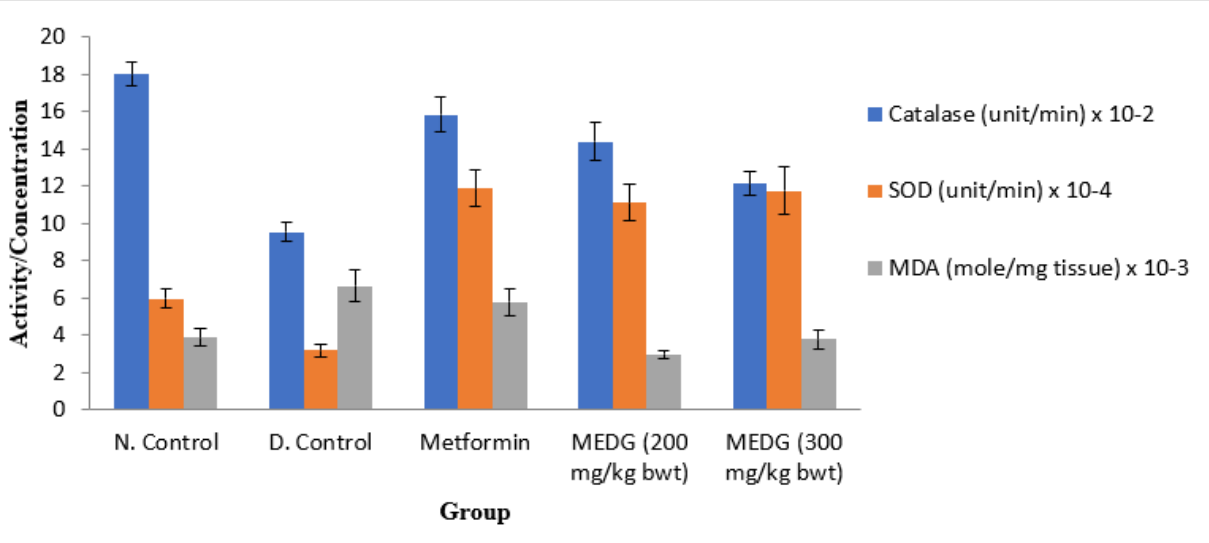


**Figure 1:** Effect of MEDG Stem Bark on Oxidative Status in Rat Liver. Data are markers of oxidative stress and are expressed as mean ± SEM (n = 5).

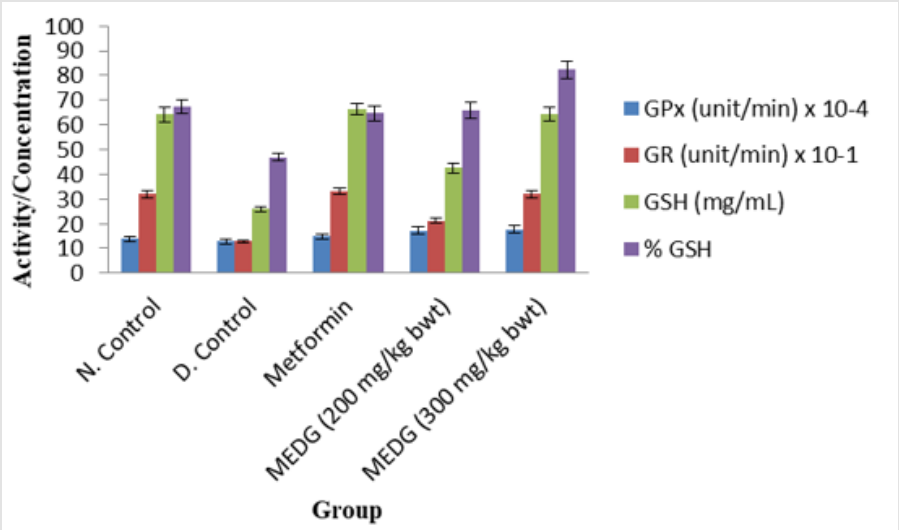


**Figure 2:** Effect of MEDG Stem Bark on Glutathione Enzyme System and GSH Level. Data are activity of GPx and GR, and GSH concentration, and are expressed as mean ± SEM (n = 5).

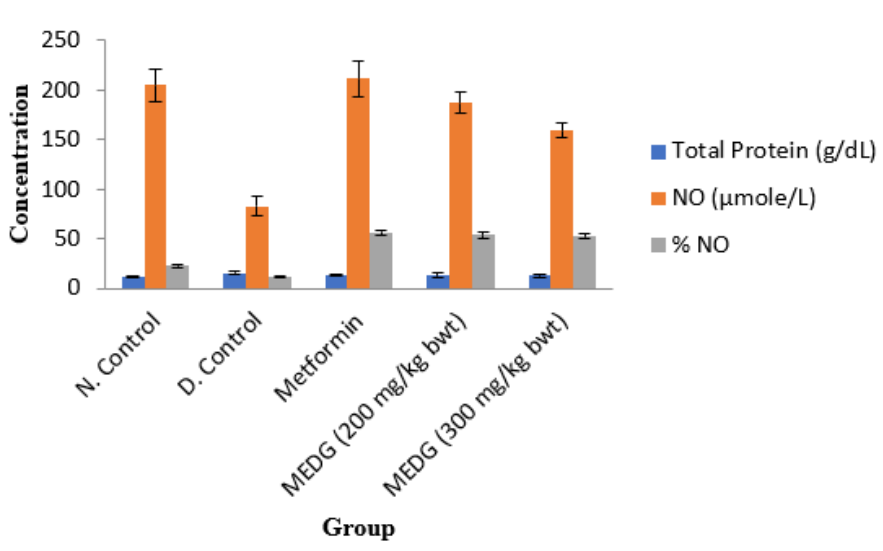


**Figure 3:** Effect of MEDG Stem Bark on Hepatic TP and NO Levels. Data are levels of Hepatic TP and NO and are expressed as mean ± SEM (n = 5).

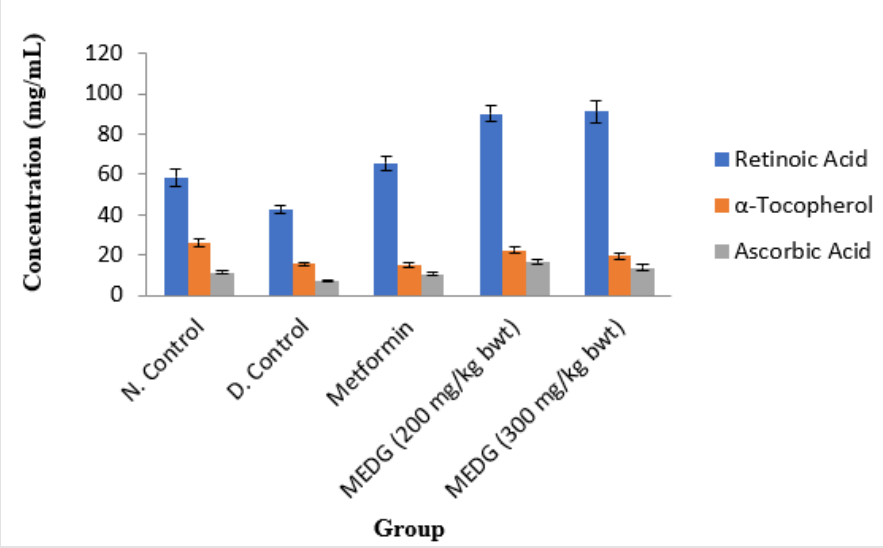


**Figure 4:** Effect of MEDG Stem Bark on the Concentration of Non-Enzyme Antioxidant Molecules in Rat Liver. Data are concentrations of antioxidant molecules and are expressed as mean ± SEM (n = 5).

## Discussion

Aggravated by oxidative stress and aberrant inflammatory signals, insulin resistance has become one of the main factors contributing to liver damage. The treatment of DM remains a challenging issue. Studies worldwide are exploring safe and effective medications to overcome the detrimental effects of insulin resistance-related metabolic derangement, including hyperglycaemia, hyperinsulinemia, hyperlipidemia, oxidative stress, inflammation, atherosclerosis, and other complications [30]. For patients with NAFLD and NASH, no specific treatments exist yet apart from diet and lifestyle modifications to reduce body weight and prevent further injury [2,31]. However, combined pharmacological therapy is recommended to improve insulin sensitivity in the liver (metformin and pioglitazone) and its periphery (thiazolidinediones), together with other drugs such as betaine, atorvastatin, losartan and orlistate [2,30,32].

Antioxidant therapy is a potential future therapeutic strategy. Increasing antioxidant levels in patients with DM-induced liver damage may hopefully counter the effects of oxidative stress, thereby reduc-

ing the severity of diabetic complications. A few plant-based products and vitamins have been investigated as ways of protecting against and possibly reversing liver damage believed to be caused by oxidative stress [33]. Vitamin E (α-tocopherol) and betaine are just a few of the antioxidants which have shown good clinical implications in the reduction of liver disease severity and the protection of the liver from DM-induced damage [34-36]. There is growing interest in the exploitation of plants for medicinal purposes, especially in Africa [37-46]. The antidiabetic effect of plant-derived compounds is due to their capacity to alter carbohydrate digestion/absorption, stimulate beta cell function, mimic insulin action, and mop up ROS [47-49]. The aim of this study was to investigate the effect of MEDG stem bark on STZ-induced oxidative stress in rat liver. The results obtained showed that induction of diabetes mellitus with STZ significantly increased the FBG concentrations of the rats. However, treatment of the diabetic rats with MEDG stem bark markedly reduced the blood glucose concentration and body weights of rats. Similarly, STZ-induced diabetes mellitus markedly reduced the activities of the markers of oxidative stress, % GSH, % NO as well as concentrations of retinoic acid, α-tocopherol and ascorbic acid in hepatic tissue. However, treatment of the diabetic Wistar rats with MEDG stem bark significantly increased the activities of antioxidant enzymes and other parameters measured. These results are consistent with reports of previous studies [50-52]. It is likely the medicinal plant extract contains important phytochemicals that can potentiate inherent antioxidant defense mechanism in rats.

## Conclusion

Streptozotocin-induced diabetes mellitus negatively impacts the natural antioxidant systems in rat liver, producing profound effect on liver function. *Dialium guineense* is demonstrated to have important biological activity including antihyperglycemic effect. The MEDG stem bark can potentiate antioxidant defense in diabetic Wistar rat.

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