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Evaluation of the Modulatory Effect of Aqueous Extract of *Dialium guineense* Stem Bark on Oxidative Status of Diabetic Rats Liver

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ABSTRACT: Oxidative stress is implicated in the pathogenesis of diseases, such that drugs with strong antioxidant property are promoted for health management. The present study evaluated the modulatory effect of aqueous extract of *Dialium guineense* (AEDG) stem bark on oxidative status of diabetic rat liver. Adult male rats (Wistar strain, n = 20, mean weight = 165 ± 15 g) were randomly assigned to four groups (5 rats/group): control, diabetic, metformin, and extract groups. Diabetes mellitus (DM) was induced in the rats via intraperitoneal injection of streptozotocin (STZ, 50 mg/kg body weight, bwt). The diabetic rats were thereafter treated for 21 days with either metformin (50 mg/kg bwt) or extract (1000 mg/kg bwt). Body weight and blood glucose data were recorded on weekly basis. Markers of oxidative stress were measured in rat hepatic tissue using standard methods. The results showed that induction of DM with STZ significantly increased plasma blood glucose concentration, while reducing antioxidant status of the rats. However, treatment of the diabetic rats with 1000 mg/kg bwt aqueous extract of *D. guineense* stem bark significantly reduced their fasting blood glucose, malondialdehyde (MDA) and nitric oxide (NO) concentrations, but it significantly increased activities of the antioxidant enzymes, and concentrations of total protein, reduced glutathione (GSH), and redox status (GSH/GSSG) ($p < 0.05$). These results suggest that AEDG stem bark possesses an ameliorative potential against STZ-induced oxidative stress in rat liver.

Keywords: Glutathione, Hepatic tissue, Lipid peroxidation index, Oxidative stress, Redox status.

Introduction

Diabetes mellitus (DM), a common health problem with multiple etiology, is characterized by hyperglycemia resulting from insulin secretion and/or functional abnormalities (Ruch and Zumsteg, 1988). The disease is also associated with major derangements in lipid and protein metabolism, thus leading to severe and life-threatening complications: neuropathy, nephropathy, retinopathy, vasculopathy and hepatopathy (Amos *et al*., 1997). Fibrosis, abnormal fat and glycogen deposition, cirrhosis and increased hepatic enzyme activities are some of liver abnormalities associated with DM (Levinthal and Tavill, 1999). Similarly, alterations in hepatic cell growth and cell number alter liver size during DM. Studies have shown that free radicals generation caused by glucose auto-oxidation and protein glycation as well as defects in antioxidant defense systems play a key role in the pathogenesis of type-I and type-II DM (Jiang *et al.*, 1990; Ahmad *et al*., 2005). It has been hypothesised that the most important cause of liver damage in diabetic patients is hyperglycemia-induced oxidative stress and subsequent disturbance in carbohydrate, protein and lipid metabolism (Mohamed *et al.*, 2016). These events, in turn, lead to further oxidative stress and activation of inflammatory cascades (Mohamed *et al.*, 2016). Reduction of hyperglycemia with drugs and insulin injection constitute the major strategies for treatment of DM. However, gradual resistance to these agents, in addition to their various adverse effects, has necessitated the need for

alternative therapies with less or even no side effects for diabetic patients. In recent years, the use of herbal medications/formulations has helped to minimize hyperglycemia and other DM associated complications (Balasubramanian *et al*., 2013; Alam *et al*., 2014). Several studies have demonstrated the pharmacological action of herbal antioxidants in scavenging free radicals and improving liver dysfunction in diabetic experimental models (Hassani *et al.*, 2017; Okpiabhele *et al*., 2018; Abu *et al*., 2020a; Abu *et al.,* 2020b).

Materials and methods

Chemicals and reagents: All chemicals and reagents used in this study were of analytical grade, and they were products of Sigma-Aldrich Ltd. (USA).
Plant sample collection and extract preparation: The stem barks of *D. guineense* were collected from Auchi, Edo State, Nigeria, and authenticated at the herbarium of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria (No. UBH_D330). The plant's stem bark was washed and shade-dried for 1 month at room temperature, and subsequently pulverized using an electrical blender. Exactly 500 g of the powdered plant material was steeped in 5 L of distilled water. The resulting extract was filtered through muslin cloth and freeze-dried via lyophilization (Abu *et al.*, 2017a; Omoregie *et al.*, 2017).
Experimental rats: Adult male albino rats (Wistar strain, n = 20, mean weight = 165 ± 15 g) were purchased from the Department of Anatomy, University of Benin, Nigeria and housed in wooden cages. They were acclimatized for 14 days just before commencement of the study, and had free access to feed (rat chow) and water.
Experimental design: The rats were divided into four groups (5 rats/group): control, diabetic, metformin, and extract groups. With the exception of control group, DM was induced in the rats using a single intraperitoneal injection of STZ (50 mg/kg bwt). Rats in the extract group received 1000 mg/kg bwt AEDG orally, for twenty-one days.
Blood sample collection and preparation: At the end of day 21 of treatment, the rats were euthanized under mild chloroform anaesthesia after an overnight fast. Blood samples collected via cardiac puncture in heparin containers were centrifuged at 2000 rpm for 10 min to obtain plasma.
Determination of oxidative status in rat hepatic tissues: The activities of catalase, SOD, GPx and GR were determined in hepatic tissue using standard methods (Cohen *et al*., 1970; Misra and Fridovich, 1972; Rotruck *et al*., 1973; Dubler and Anderson, 1981). Levels of hepatic TP, MDA, GSH, and NO were also measured ((Henry *et al*., 1957; Ellman, 1959; Guttridge and Wilkins, 1982; Marcocci *et al*., 1994).
Data analysis: Data are expressed as mean ± standard error of mean (SEM, n = 5). Statistical analysis was performed using SPSS version 21. Statistical differences between means of the different groups were compared using Duncan multiple range test. Statistical significance was assumed at $p < 0.05$.

Results

Effect of AEDG stem bark on body and organ weights: Induction of diabetes mellitus in the rats caused significant reductions in their body weights ($p < 0.05$), but extract treatment significantly and time-dependently increased the body weighs of rats ($p < 0.05$). There were no significant increases in the weights of the liver among the groups ($p > 0.05$), but there were significant reductions in the corresponding relative organ weights of diabetic rats treated with AEDG/metformin, when compared with diabetic group ($p < 0.05$). These results are presented in Figures 1 – 4.

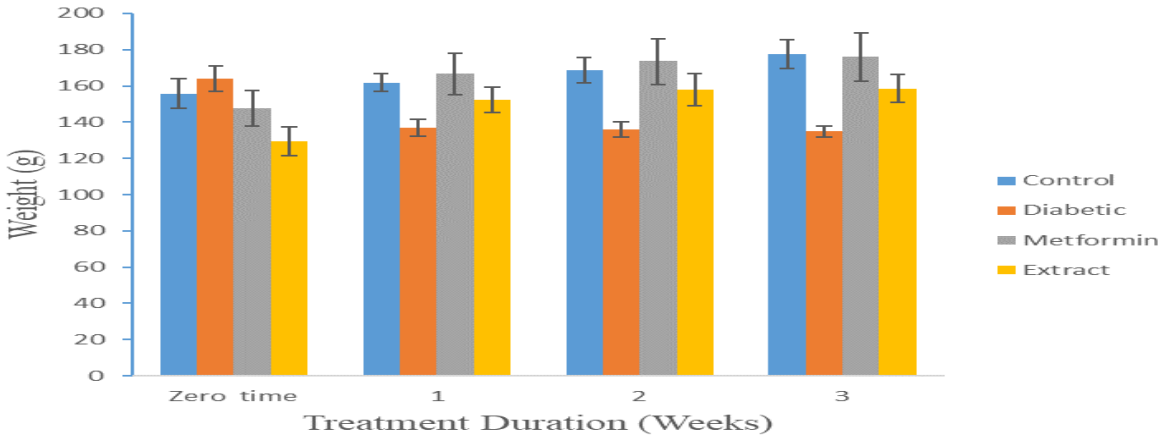

Figure 1: Weights of rats

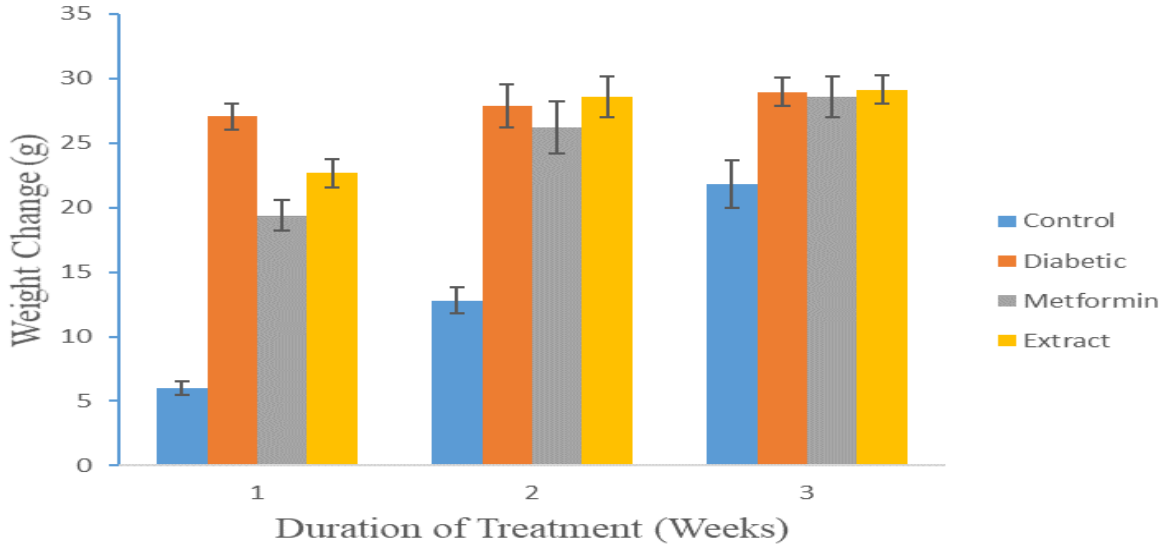

Figure 2: Weight changes

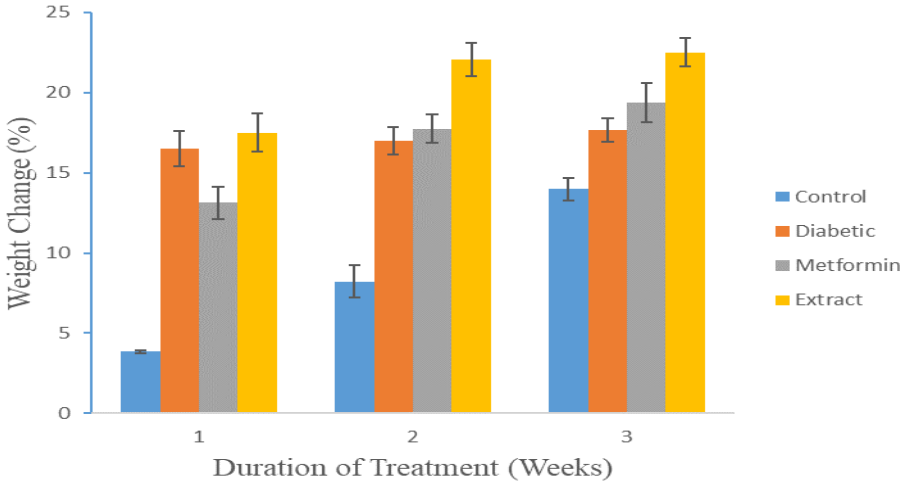

Figure 3: Percentage weight changes

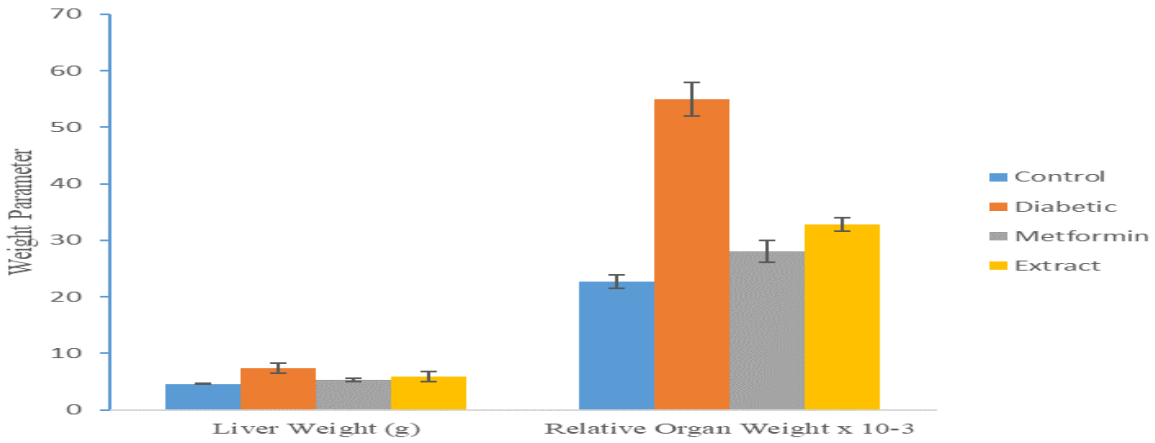

Figure 4: Weight of liver and relative organ weight

Effect of AEDG stem bark on blood glucose concentration: As shown in Figure 5, treatment of diabetic rats with 1000 mg/kg bwt aqueous extract of *D. guineense* stem bark significantly and time-dependently reduced their fasting blood glucose concentrations ($p < 0.05$).

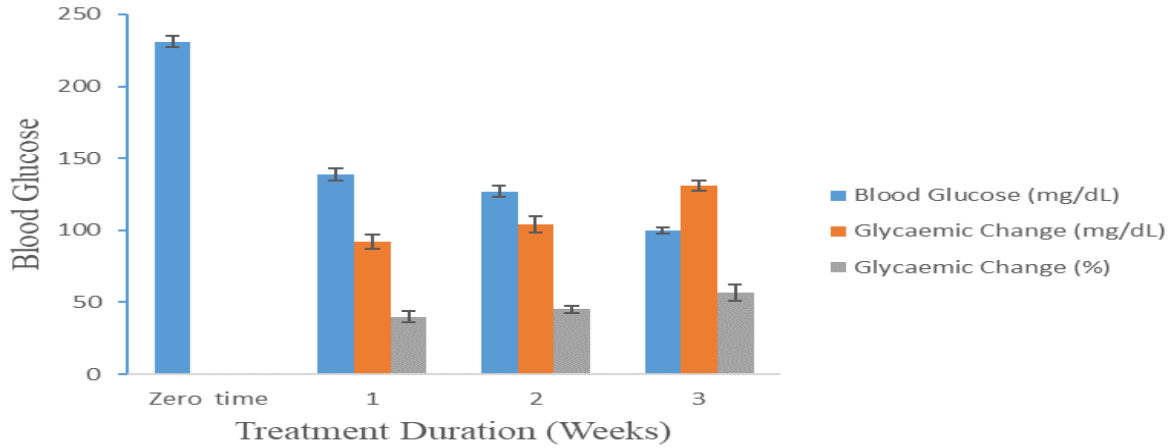

Figure 5: Blood glucose of rats

Effect of AEDG stem bark on hepatic activities/concentrations of antioxidant enzymes/molecules: Treatment of diabetic rats with 1000 mg/kg bwt aqueous extract of *D. guineense* stem bark significantly reduced the hepatic concentrations of MDA and nitric oxide, but significantly increased the activities of the antioxidant enzymes, and concentration of total protein, GSH, and redox status ($p < 0.05$). These results are shown in Figures 6 – 11.

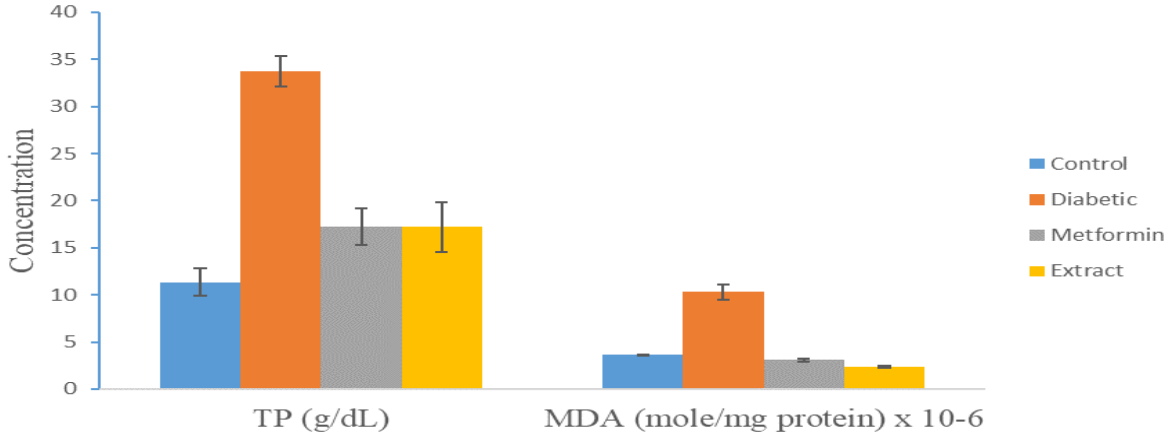

Figure 6: Hepatic concentrations of total protein and malondialdehyde

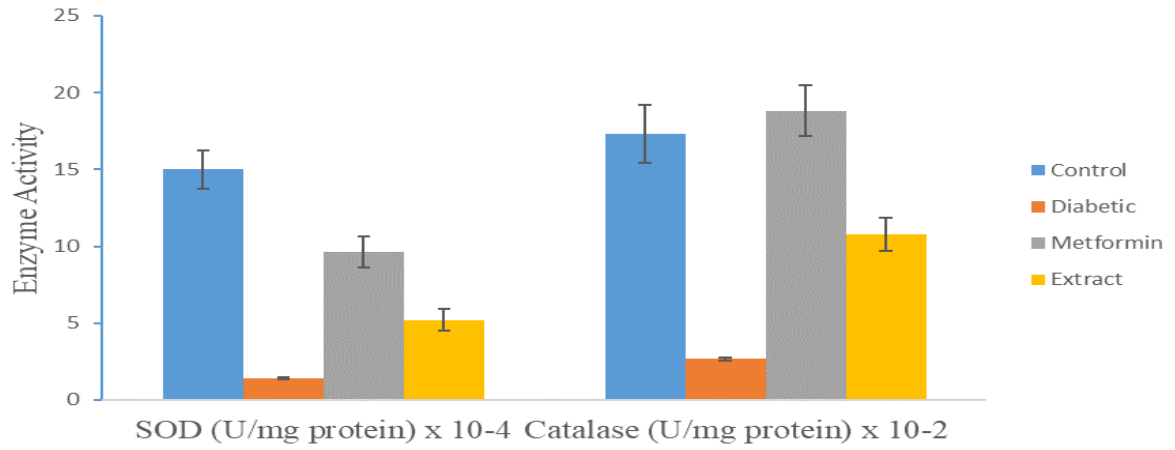

Figure 7: Hepatic activities of superoxide dismutase and catalase

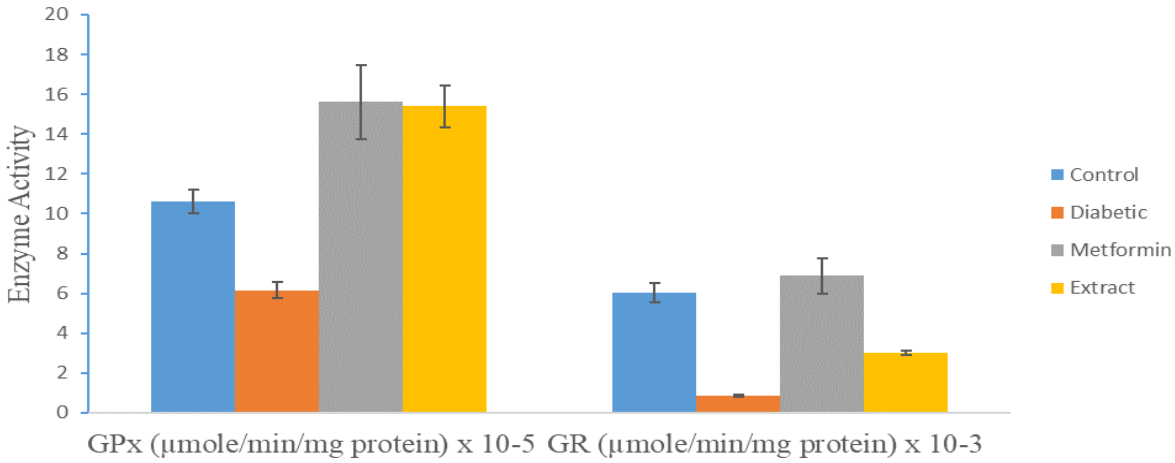

Figure 8: Activities of glutathione enzymes in hepatic tissue

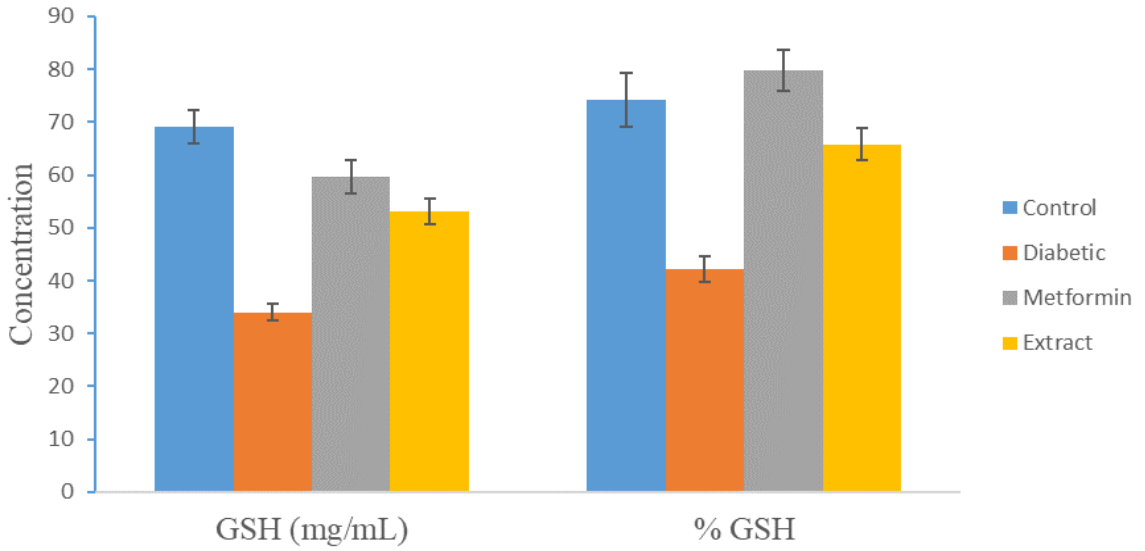

Figure 9: Concentration of glutathione in hepatic tissue

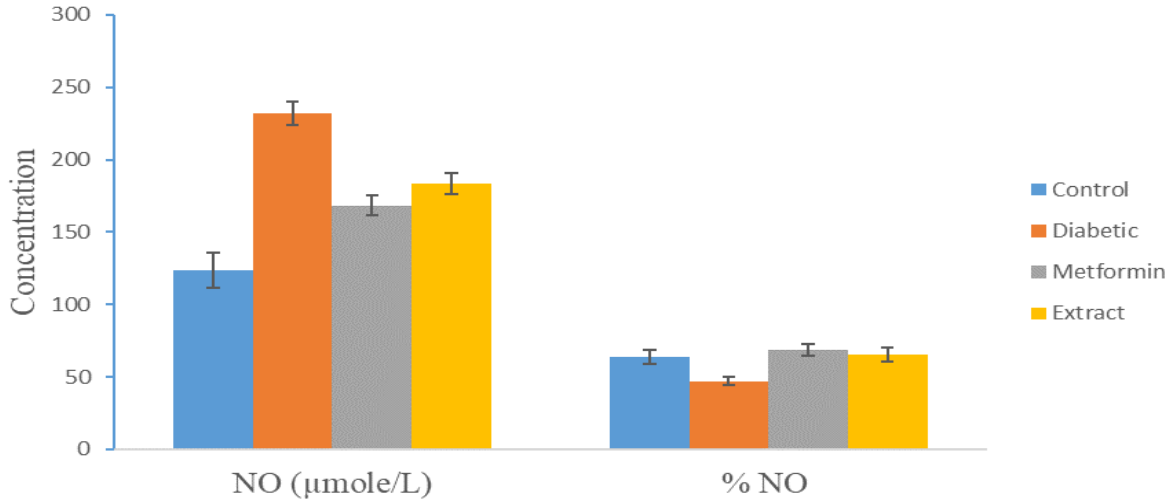

Figure 10: Concentration of nitric oxide in hepatic tissue

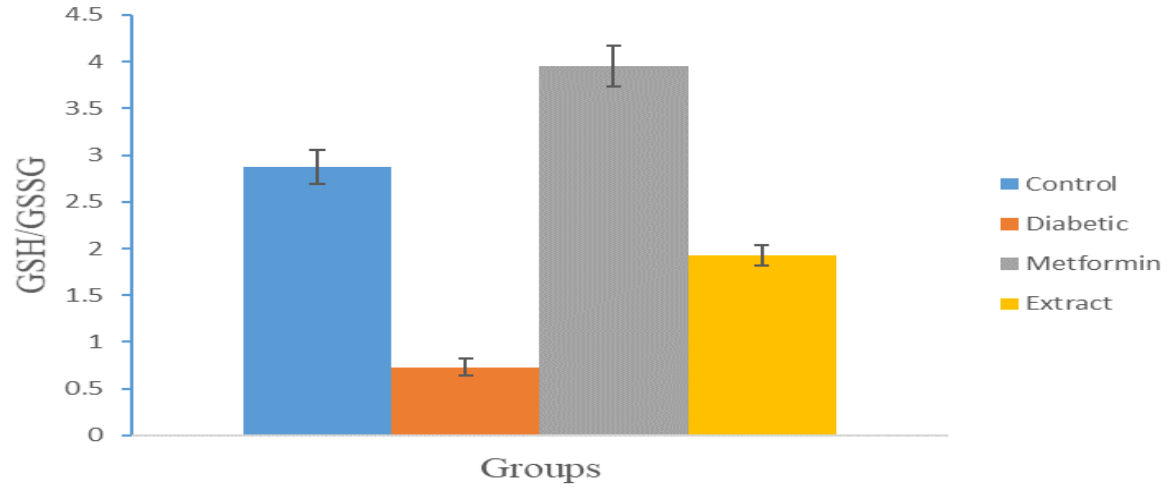

Figure 11: Effect of AEDG stem bark on hepatic redox status

Discussion

In the present study, treatment of diabetic rats with AEDG stem bark significantly improved alterations in hepatic oxidative damage induced by DM. The responses were comparable to those of the standard antidiabetic drug, metformin. The results are in agreement with those of previous studies **(**Chandrasekar *et al*., 1989; Minaiyan *et al*., 2011; Atta *et al*., 2020; Abu *et al*., 2023a; Abu *et al*., 2023b; Abu *et al*., 2023c; Abu *et al*., 2023d). In this study, STZ significantly elevated serum glucose concentration, which confirmed successful induction of DM. Streptozotocin (STZ)-induced DM was also associated with significant alterations in liver oxidative stress markers. Hyperglycemia is the most common feature in both type-I and type-II DM. Previous studies suggest that free radicals generation and simultaneous defect in cells antioxidant capacity are the main mechanisms for DM complications (Giacco and Brownlee, 2010). Diabetes mellitus (DM) affects a number of organs, with liver being one of the most important organs (Ahmadieh *et al*., 2014). It has been reported that hyperglycemia-induced oxidative stress and subsequent disturbance in carbohydrate, protein and lipid metabolism are the most important causes of liver damage in diabetic patients (Mohamed *et al.*, 2016). The results of this study showed that treatment of STZ-induced diabetic rats with aqueous extract of the medicinal plant stem bark significantly decreased blood glucose level as compared to the untreated diabetic rats, and are consistent with those of previous reports (Abu *et al.*, 2023e; Abu *et al*., 2023f; Abu *et al*., 2023g; Abu *et al.*, 2023h; Abu *et al*., 2023i). The anti-hyperglycemic effect of aqueous extract of *D. guineense* stem bark might be

due to stimulation of insulin secretion from ß cells and reduction of tissue resistance to insulin, mainly by its bioactive compounds (Abu *et al.*, 2017b; Abu *et al*., 2020c; Obayuwana *et al.*, 2020). In the present study, MDA concentration was significantly decreased in the metformin and extract-treated groups, but the content of the total thiol groups and SOD activity significantly increased compared with the diabetic untreated rats. These results are in agreement with previous studies (Abu *et al*., 2022a; Abu *et al*., 2022b; Abu *et al.*, 2022c; Abu *et al.*, 2022d). Therefore, the beneficial action of aqueous extract of *D. guineense* stem bark on liver tissue oxidative damage in STZ-induced diabetic rats might be due to its antioxidant effects mainly by the bioactive compounds. As demonstrated by results of the present study, treatment of diabetic rats with AEDG stem bark conferred some protection on antioxidant enzyme systems in hepatic tissue.

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