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Plasma Oxidative Status of Diabetic Rats Treated with Ethanol Extract of *C. sativus* Fruit

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Abstract

At present, diabetes mellitus remains a serious health challenge, worldwide. The current treatment strategies cannot sufficiently ameliorate the various complications caused by the disease. The aim of the present study was to investigate oxidative status of diabetic rats plasma administered ethanol extract of *C. sativus* fruit. Male albino rats (Wistar strain, n = 25) weighing between 200 and 230 g (mean weight = 215 ± 15 g) were randomly assigned to five groups (5 rats in a group): control, diabetic, metformin, and 200 mg/kg body weight (bwt) and 300 mg/kg bwt extract groups. Diabetes mellitus was induced in the rats via intraperitoneal injection of streptozotocin (STZ, 50 mg/kg bwt). The diabetic rats were then treated with metformin (50 mg/kg bwt) or the medicinal plant extract (200 and 300 mg/kg bwt, respectively), for 21 days. Indices of oxidative stress were measured in rat plasma. The results showed that the activities of all the antioxidant enzymes [catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR)], and concentrations of glutathione (GSH) as well as organ/body weight ratio were significantly lower in diabetic group than in the control group, but they were increased by extract 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 ($p < 0.05$). These results indicate that ethanol extract of *Cucumis sativus* fruit can enhance antioxidant defense system in rats exposed to STZ.

Keywords: *Cucumis sativus,* Free radicals, Glutathione, Glutathione reductase, Oxidative stress

Introduction

Diabetes mellitus is a heterogeneous group of syndromes characterized by an elevation of fasting blood glucose (FBG) caused by a relative or absolute deficiency of insulin.[1] It is a metabolic disorder that affects millions of people worldwide.[2]

Implicated in the pathogeneses of many diseases, oxidative stress contributes significantly to insulin resistance. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) impair insulin signaling pathway.[3] Due to low enzymatic antioxidant defenses in pancreatic β-cells, the tissue is highly sensitive to ROS and RNS. In addition, the cells lack the capacity to adapt their low enzyme activity to biological stress (high glucose or oxygen level).[4] Studies have demonstrated that hyperglycemia causes oxidative stress through glucose oxidation pathway in mitochondria, leading to increased mitochondrial superoxide anion production, which further activates uncoupling protein-2 (UCP-2).[5] The direct consequence of this is reduction in ATP/ADP, which in turns hampers insulin secretion.[6]

In pancreatic β-cells, NO regulation of glucokinase activity via S-nitrosilation reaction, may enhance insulin secretion.[7] However, excess NO and concomitant NRS could cause apoptosis through caspase-3 activation and decrease in ATP levels.[9] The aim of this study was to investigate oxidative status of diabetic rats plasma administered ethanol extract of *C. sativus* fruit.

Materials and Methods

Chemicals

Chemicals, reagents and solvents used in this study were of analytical grade, and they were products of Sigma-Aldrich, Ltd. (USA).

Collection of Plant Material

Whole fruits of *C. sativus* were bought from a reputable supplier in Benin City, Nigeria. The plant was identified and authenticated at the University of Benin herbarium domiciled in the Department of Plant Biology and Biotechnology. The prepared plant specimen was deposited in the herbarium after obtaining the voucher number.

Plant Preparation and Extraction

The fruits were washed and shade-dried at room temperature for 14 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.[9-12]

Experimental Rats

Male albino rats (Wistar strain, n = 25) weighing between 200 and 230 g (mean weight = 215 ± 15 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. 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 the extract (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.[13-16] Concentrations of plasma total protein, MDA and GSH were also measured.[17-19] Nitric oxide (NO) concentration was determined as described in literature.[20]

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 Weight and Relative Organ Weights

As shown in Figure 1, STZ-induced diabetes mellitus significantly reduced the organ/body weight ratio ($p < 0.05$). However, treatment of the diabetic rats with the extract markedly increased the organ/body weight ratio ($p < 0.05$).

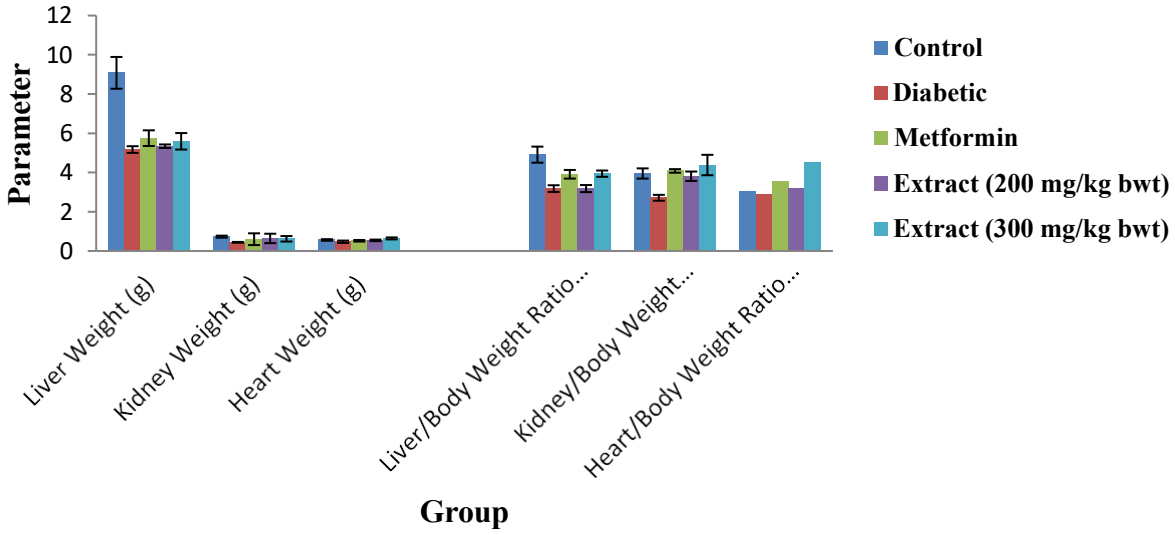

Figure 1. Comparison of the Effect of Weight and Relative Organ Weights.

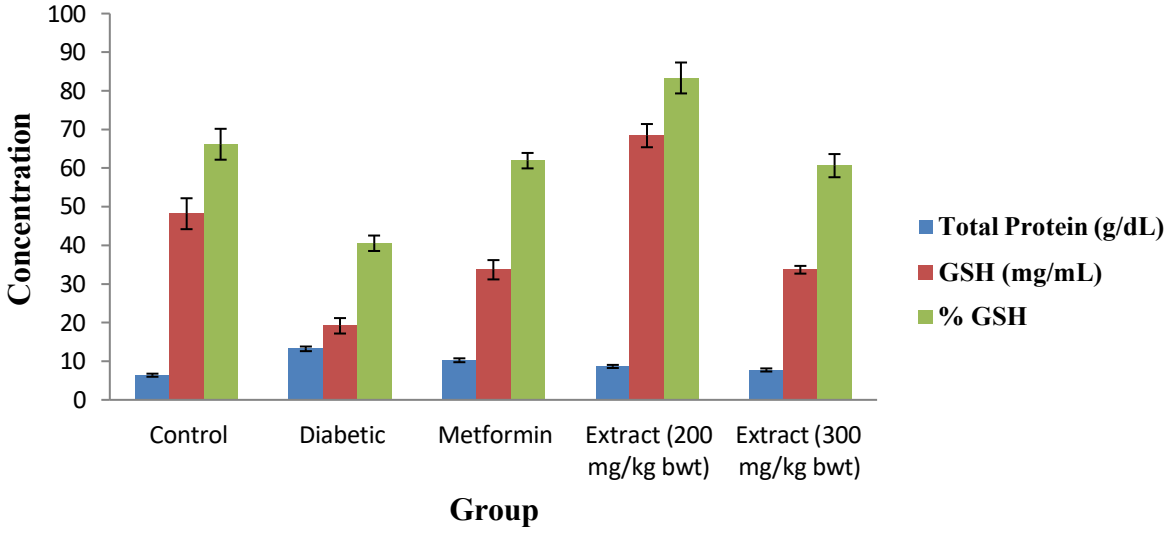

Figure 2. Effect of Ethanol Extract of *C. sativus* Fruit on Plasma Total Protein and Glutathione Levels. Data are plasma total protein and GSH levels and are expressed as mean ± SEM (n = 5).

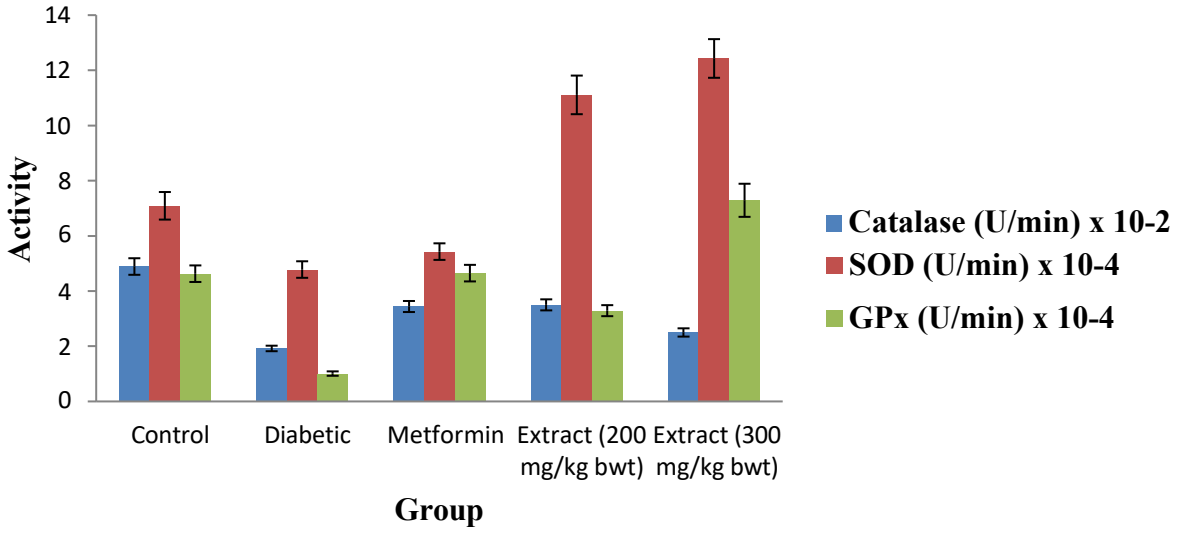

Figure 3. Effect of Ethanol Extract of *C. sativus* Fruit on Oxidative Status in Rat Plasma.
Data are markers of oxidative stress and are expressed as mean ± SEM (n = 5).

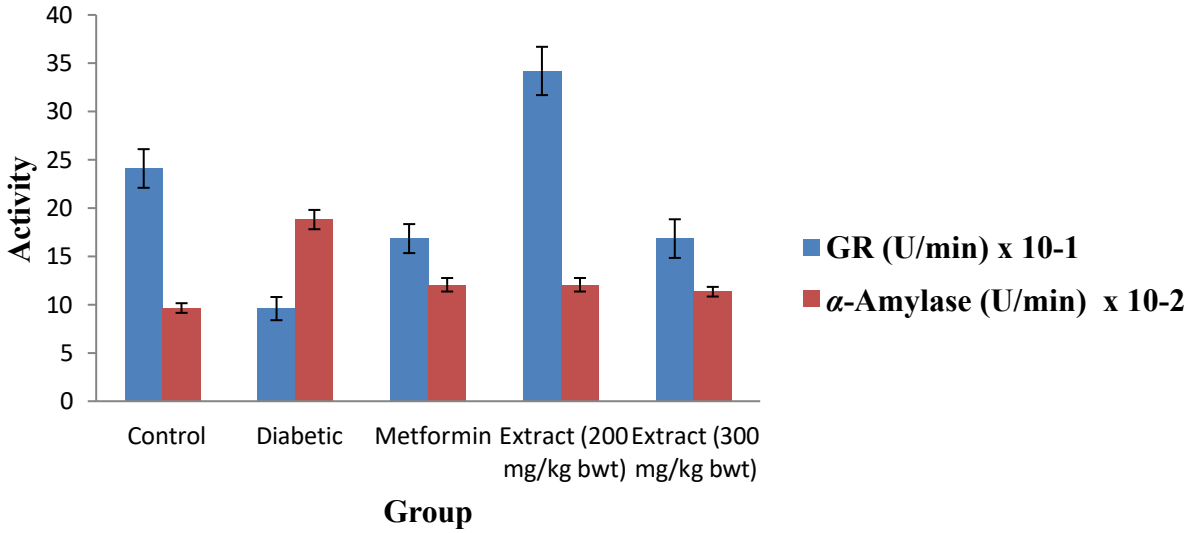

Figure 4. Effect of Ethanol Extract of *C. sativus* Fruit on Plasma Alpha-Amylase and Glutathione Reductase Activity.
Data are activities of plasma α-amylase and glutathione reductase and are expressed as mean ± SEM (n = 5).

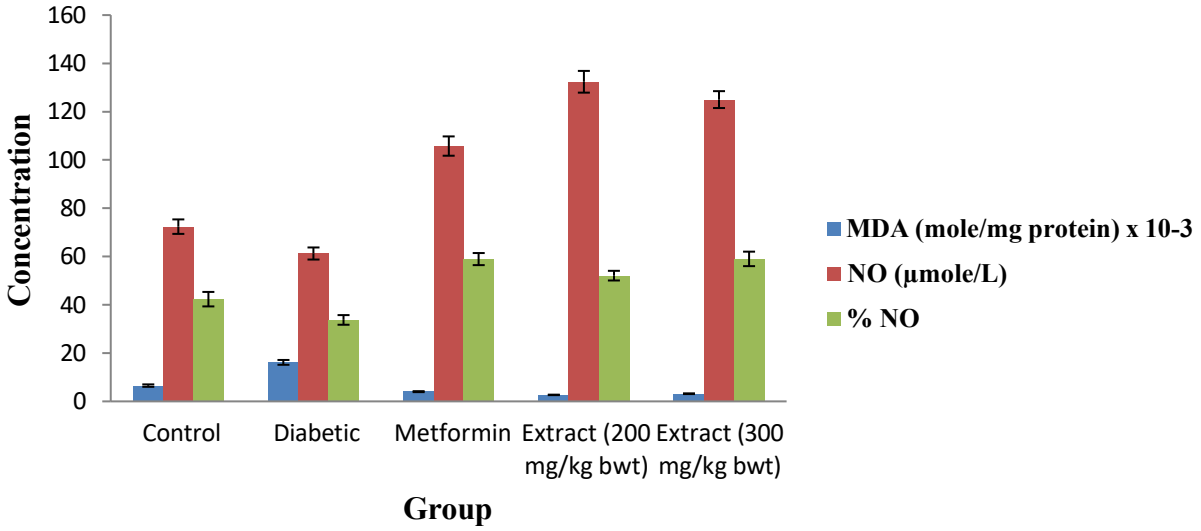

Figure 5. Effect of Ethanol Extract of *C. sativus* Fruit on Plasma MDA and NO Levels. Data are plasma MDA and NO concentrations and are expressed as mean ± SEM (n = 5).

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 extract treatment ($p < 0.05$). However, the concentrations of NO and MDA elevated by STZ were greatly reduced after treatment with the medicinal plant extract ($p < 0.05$; Figs. 2 to 5).

Discussion

One of the suggested mechanisms of STZ-induced toxicity is induction of oxidative stress.[6] Elevated blood glucose (hyperglycemia) promotes oxidative stress, which results to impairment of the main processes that fail during diabetes mellitus (insulin secretion and action). The observation that antioxidant mechanisms are diminished in diabetic patients, also supports the speculation that oxidative stress contributes significantly to the disease pathogenesis.[21,22] Hyperglycemia and free fatty acids are among the causes of oxidative stress.[23] Multicellular organisms convert 1 – 2 % of consumed oxygen into ROS, as byproducts of aerobic metabolism. The regulatory network comprising enzymatic and non-enzymatic antioxidant systems tends to keep the magnitude of ROS within cells to a non-damaging level. However, under stress conditions such as that induced by STZ, the production rate of ROS increases exponentially, exceeding the potential of antioxidant scavengers thereby instigating oxidative burst, which affects biomolecules, disturbing cellular redox homeostasis.[23] Reactive oxygen species (ROS) are similar to a double-edged sword; and, when present below the threshold level, mediate redox signaling pathways that actuate growth, development, and acclimatization against stresses.[24]

Natural antioxidants play a key role in health maintenance and prevention of chronic and degenerative diseases.

Medicinal plants contain a wide variety of free radicals scavenging molecules (phenols, flavonoids, vitamins and terpenoids).[25] 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.[26] Many plants, citrus fruits and leafy vegetables are the source of these antioxidant molecules.

Antioxidants are the agents that can interfere with oxidation process by various mechanisms, such as, reacting with free radicals, chelating free catalytic metals, and acting as oxygen scavengers.[27] Free radicals, with unpaired electrons, are produced in normal or pathological cell metabolism. Lipid peroxidation in cell membrane causes several types of biological damage. Interest in natural antioxidants, especially phytochemicals has greatly increased in recent years.[28-30] Many phytochemicals including phenolics, flavonoids, tannins, proanthocyanidins, and various plant extracts have been reported as antioxidants.[31-33] This study investigated oxidative status of diabetic rats plasma administered ethanol extract of *C. sativus* fruit. The results showed 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 extract 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.[34-39] It has been demonstrated that different parts of *C. sativus* contain bioactive compounds responsible for particular pharmacological activity.[40-45] Studies have shown that plants rich in important bioactive compounds/phytochemicals are very useful medicinally.[46-58]

Conclusion

The results obtained in this study indicate that ethanol extract of the medicinal plant fruit can enhance antioxidant defense system in rats exposed to the diabetogenic agent, STZ.

Acknowledgement

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Funding

None.

Conflicts of Interest

The author declares that there are no conflicts of interest.

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