Antioxidant Effect of Ethanol Extract of *C. sativus* on Streptozotocin-Induced Diabetic Rat Liver

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ABSTRACT

The present study investigated the antioxidant effect of ethanol extract of *Cucumis sativus* on streptozotocin (STZ)-induced diabetic rat liver**.** Adult male Wistar albino rats (n = 25, mean weight = 215 ± 15 g) were randomly assigned to five groups of 5 rats each: normal control, diabetic control, metformin, 200 mg/kg body weight (bwt) extract and 300 mg/kg bwt extract groups. Diabetes mellitus was induced in the rats via intraperitoneal injection of 50 mg/kg bwt STZ. The diabetic rats were then 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 molecules like retinoic acid, ascorbic acid and α-tocopherol were evaluated in 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 FBG concentration and body weights of rats ($p < 0.05$). Treatment of diabetic Wistar rats with ethanol extract of *C. sativus* significantly increased 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 suggest that ethanol extract of the medicinal plant can enhance antioxidant defense in the liver of STZ-induced diabetic rats.

Keywords: Antioxidants, Hepatocytes, Lipid peroxidation, Oxidative stress, Reactive oxygen species.

INTRODUCTION

Oxidative stress is defined as an imbalance in the oxidant-to-antioxidant ratio, causing the generation of free radicals [1]. The liver is the main detoxification organ of the body and plays an important role in controlling normal glucose homeostasis [2]. The production of oxidants such as reactive oxygen species (ROS)-like superoxide anions, hydrogen peroxide and hydroxyl radicals by activated Kupffer cells has been identified as central to hepatic injuries [3]. Kupffer cells, also known as hepatic macrophages, are one type of non-parenchymal cell that help maintain the integrity of hepatocytes. However, these phagocytic cells are also susceptible to the effects of oxidative stress produced by the surrounding cells and its own immune reactions

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[4, 5].

Excessive ROS production results in several deleterious events, including an irreversible oxidative modification of lipids, proteins and carbohydrates [6, 7]. In addition, it will induce apoptosis in hepatocytes and the release of inflammatory cytokines, thereby increasing the expression of adhesion molecules and the infiltration of leukocytes. A combination of all of these processes causes massive tissue destruction in the liver [8, 9]. However, the liver is equipped with potent antioxidants such as SOD, catalase and the glutathione enzyme family, including glutathione-S-transferases (GSTs) and glutathione peroxidases (GPXs)—so as not only to neutralize free radicals but also to protect liver cells from oxidative damage [7, 10]. Studies have demonstrated that a decrease in SOD and catalase activities within a hyperglycaemic state leads to an increase in ROS, which eventually contributes to oxidation-induced liver damage [10, 11].

The liver also plays a pivotal role in the homeostasis of the glutathione enzyme family. Different cell components such as the endoplasmic reticulum (ER), mitochondria and nucleus consist of separate pools of GSH. Among these, mitochondrial GSH is more essential than cytoplasmic GSH in maintaining cell viability [10]. Acting as the first line of defense as an endogenous antioxidant, the oxidation process takes place in the thiol group of GSH [10]. Reduced levels of GSH in diabetic rat livers have been associated with depletion of decreased GST, GPx and GR activity and with the accumulation of oxidative stress products, such as advanced glycation end-products (AGEs), protein oxidation products (POPs) and lipid peroxidation (LPO) [12 – 14]. However, measuring certain redox couples in cells such as oxidized nicotinamide adenine dinucleotide (NAD)/reduced NAD and oxidized NAD phosphate (NADP)/reduced NADP—is difficult; as a result, the GSH-to-glutathione disulphide ratio in the liver is considered representative of a redox state [10]. This study investigated the antioxidant effect of ethanol extract of *C. sativus* on diabetic rat liver.

MATERIALS AND METHODS

Chemicals

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

Plant Extraction

Freshly harvested *Cucumis sativus* fruits were purchased from a major fruit/vegetable market in Benin City, Nigeria and identified by Dr. Henry Akinnibosun of Plant Biology and Biotechnology Department, University of Benin. They were thereafter washed, and air-dried for about 4 weeks at the Department of Biochemistry. The dry plant was ground with a mechanical blender. The pulverized sample was cold macerated in absolute ethanol for three days (72 h) in a bell jar and filtered using Whatmann filter paper No. 42 (125 mm). The ethanol extract was thereafter concentrated using rotary evaporator and freeze-dried using a lyophilizer [15, 16].

Experimental Animals

Mature male Wistar albino rats (n = 25) weighing 200 to 230 g (mean weight = 215 ± 15 g) were bought from the Department of Anatomy, University of Benin and housed in wooden cages. They were acclimatized for two weeks 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/group): normal control, diabetic control, metformin, 200 mg/kg bwt extract and 300 mg/kg bwt extract groups. Diabetes mellitus was induced in the rats via intraperitoneal injection of 50 mg/kg bwt STZ. The diabetic rats were then treated with either metformin (50

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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 anaesthesia after an overnight fast. Their liver were excised, and used to prepare 20 % tissue homogenate. The homogenate was centrifuged at 2000 rpm for 10 min to obtain supernatant which was used for biochemical analysis.

Biochemical Analyses

The activities of catalase, SOD and GPx were determined [17 – 19]. Hepatic levels of TP, MDA, GSH, NO as well as vitamins A, E and C were also measured [20 – 26]. The activity of GR was determined using a previously described method [27].

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

RESULTS

Effect of Ethanol Extract of *C. sativus* on Weight and Blood Glucose of Rats

As shown in Tables 1 and 2, induction of diabetes mellitus using STZ significantly increased the blood glucose concentrations of the rats ($p < 0.05$). However, treatment of the diabetic rats with the extract markedly reduced their FBG concentration and body weights ($p < 0.05$).

Table 1: Effect of Ethanol Extract of *C. sativus* on Weight and Blood Glucose Parameters

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
Extract (200 mg/kg bwt)	12.26	7.87	> 800	421	52.63
Extract (300 mg/kg bwt)	29.08	17.02	364	227	62.36

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

Table 2: Comparison of the Weights of Rat Liver Among the Groups

Group	Liver Weight (g)	Liver/Body Weight Ratio (x 10^{-2})
Normal Control	9.08 ± 0.81	4.91 ± 0.71
Diabetic Control	5.17 ± 0.17[a]	3.18 ± 0.06[a]
Metformin	5.75 ± 0.40[a]	3.91 ± 0.07[a]
Extract (200 mg/kg bwt)	5.34 ± 0.09[a]	3.18 ± 0.11[a]
Extract (300 mg/kg bwt)	5.59 ± 0.42a	3.94 ± 0.09a

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Data are relative liver weights and are expressed as mean ± SEM (n = 5).

Values with superscript "a" are significantly different from the diabetic control group.

Oxidative Status in Diabetic Rats Treated with the Medicinal Plant Extract

Treatment of diabetic Wistar rats with ethanol extract of *Cucumis sativus* significantly increased the activities of the antioxidant enzymes and molecules as well as concentrations of NO, but it markedly reduced the concentrations of hepatic TP and MDA ($p < 0.05$). These results are shown in Tables 3 to 6.

Table 3: Effect of Ethanol Extract of *C. sativus* on Oxidative Status in Rat Liver

Group	**Catalase (unit/min) x 10^{-2}**	**SOD (unit/min) x 10^{-4}**	**MDA (mole/mg tissue) x 10^{-3}**
Normal Control	18.64 ± 0.06	5.67 ± 0.29	3.89 ± 0.00
Diabetic Control	9.54 ± 0.00	3.18 ± 0.00	6.66 ± 0.20
Metformin	15.85 ± 3.97[a]	11.07 ± 0.00[a]	5.79 ± 0.17
Extract (200 mg/kg bwt)	9.17 ± 0.00	11.89 ± 0.00[a]	6.00 ± 0.00
Extract (300 mg/kg bwt)	12.14 ± 0.00[a]	15.95 ± 0.00[a]	3.77 ± 0.00[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.

Table 4: Effect of Ethanol Extract of *C. sativus* on Hepatic TP and Glutathione Level

Group	**Total Protein (g/dL)**	**GSH (mg/mL)**	**% GSH**
Normal Control	11.19 ± 0.00	64.25 ± 0.00	84.45 ± 2.22
Diabetic Control	24.24 ± 0.00	64.25 ± 0.00	46.88 ±1.46
Metformin	13.40 ± 7.11[a]	66.33 ± 0.00	66.04 ± 16.46[a]
Extract (200 mg/kg bwt)	17.71 ± 0.00[a]	62.70 ± 0.00	92.92 ± 0.00[a]
Extract (300 mg/kg bwt)	18.65 ± 0.00[a]	88.10 ± 0.00[a]	85.84 ± 9.59[a]

Data are hepatic TP and GSH levels and are expressed as mean ± SEM (n = 5).

Values with superscript "a" are significantly different from the diabetic control group.

Table 5: Effect of Ethanol Extract of *C. sativus* on Hepatic Glutathione Peroxidase Activity and NO Level

Group	**GPx (unit/min) x 10^{-4}**	**NO (µmole/L)**	**% NO**
Normal Control	6.86 ± 0.05	172.33 ± 28.39	22.36 ± 1.68
Diabetic Control	6.39 ± 0.00	91.38 ± 7.88	11.67 ± 1.19
Metformin	11.39 ± 1.62[a]	229.50 ± 0.00[a]	26.08 ± 1.49[a]
Extract (200 mg/kg bwt)	4.36 ± 0.00	112.25 ± 0.00[a]	13.36 ± 0.00[a]
Extract (300 mg/kg bwt)	10.92 ± 0.00[a]	193.58 ± 35.63[a]	46.63 ± 10.60[a]

Data are hepatic glutathione peroxidase activity and NO level, and are expressed as mean ± SEM (n = 5).

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Values with superscript "a" are significantly different from the diabetic control group.

Table 6: Effect of Ethanol Extract of *C. sativus* on Hepatic Concentrations of Antioxidant Molecules

Group	**Retinoic Acid (mg/mL)**	**Ascorbic Acid (mg/mL)**	**α-Tocopherol (mg/mL)**
Normal Control	58.42 ± 9.43	12.06 ± 0.00	21.07 ± 5.11
Diabetic Control	42.59 ± 0.00	6.95 ± 0.00	15.49 ± 0.00
Metformin	65.66 ± 1.37[a]	14.19 ± 2.65[a]	15.14 ± 0.00
Extract (200 mg/kg bwt)	85.88 ± 0.00[a]	10.82 ± 0.00[a]	24.18 ± 0.00[a]
Extract (300 mg/kg bwt)	88.14 ± 0.36[a]	12.14 ± 0.60[a]	22.60 ± 0.30[a]

Data are hepatic levels of antioxidant molecules, and are expressed as mean ± SEM (n = 5).

Values with superscript "a" are significantly different from the diabetic control group.

DISCUSSION

The World Health Organization (WHO) describes diabetes mellitus as a chronic, progressive disease characterized by elevated levels of blood glucose which causes complications in many parts of the body and increases the overall risk of dying prematurely [28]. This chronic non-communicable disease of the endocrine system arises from multiple etiologies in the secretion of insulin [29, 30]. Diabetes mellitus describes a group of metabolic disorders characterized by a state of chronic hyperglycemia due to defects in insulin secretion, insulin action or both, which currently affects about 463 million people worldwide [31, 32]. Over time, elevated blood glucose, a common effect of uncontrolled diabetes mellitus, may lead to serious damage to the heart, blood vessels, eyes, kidneys and nerves [28]. This metabolic disorder is often described as "a silent killer" since it may be asymptomatic at onset. Therefore, the disease usually goes undiagnosed until major complications arise. The disease is associated with reduced life expectancy, significant morbidity as well as diminished quality of life [33 – 35]. It has been reported that 10 % of global health expenditure is spent on diabetes [36, 37]. There is profound reason to suggest that this figure might increase in the coming years giving the myriad of complications that result from diabetes mellitus. Unfortunately, four out of five people with diabetes are living in low and middle-income countries, where healthcare budgeting is not even prioritized [38]. The lack of prioritization of healthcare in these countries consequently creates a yawning gap in efforts aimed at possibly managing and tackling the disease scourge. Diabetes mellitus has ceased to be a disease of affluence and has become a disease of globalization. Currently, two out of three people with diabetes are living in urban areas of the world [38]. Therefore, it is a considered opinion that the enormous increase in prevalence of cases of diabetes mellitus may not be unrelated to factors such as increasing urbanization, increasing prevalence of overweight and obesity, lack of physical activity as well as changes in socio-demographic characteristics of the population [39, 40].

Many of the drugs currently used for the treatment of diabetes mellitus produce adverse effects: sulfonylureas stimulate pancreatic islet cells to secrete insulin, while metformin slows down hepatic glucose production [41]. All these therapies have limited effectiveness, thereby necessitating the search for novel plant-based compounds that can effectively reduce blood glucose. According to World Health Organization, about 80 % of the world's population rely essentially on plants for primary health care [42]. There is growing interest in the exploitation of plants for medicinal purposes, especially in Africa [43 – 53]. 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 [54- 57]. The aim of this study was to investigate the antioxidant effect of ethanol extract of *C. sativus* on STZ-induced diabetic rat liver. The antioxidants measured were catalase, SOD, GPx, GSH, ascorbic acid, retinoic acid

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and α-tocopherol. The concentrations of nitric oxide, TP and MDA were also determined.

Oxidative stress has mediatory role in the pathogenesis of diabetes mellitus and its related complications via promotion of free radicals production and impairment of antioxidant defense systems. The results of this study showed that induction of diabetes mellitus with STZ significantly increased the blood glucose concentrations of the rats. However, treatment of the diabetic rats with the extract markedly reduced the FBG concentration and body weights of rats. Similarly, treatment of the diabetic Wistar rats with the medicinal plant extract significantly increased the activities of the antioxidant enzymes and molecules as well as concentrations of NO, but it markedly reduced the concentrations of hepatic TP and MDA. The effect of the extract was comparable to that of metformin. The evaluation of plasma TP alone may not tell the actual picture of the metabolic state of an individual, since the concentration of the various proteins are not affected by each other. An elevated level of TP may be due to dehydration or infection. Plasma concentration may decrease due to impaired synthesis that can result from malnutrition, malabsorption, over-hydration and some forms of liver diseases [58]. The results of this study are consistent with those of earlier studies [59 – 61]. It is likely the medicinal plant extract contains important phytochemicals that can potentiate inherent antioxidant defense mechanism in rats [62 – 72].

CONCLUSION

Ethanol extract of *C. sativus* has protective effect in diabetes complications and can be considered a suitable drug candidate for reducing oxidative stress typically observed in diabetes mellitus.

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