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Antioxidant and Radical Scavenging Properties of Garlic Oil in Streptozotocin Induced Diabetic Rats

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Abstract: Until now diabetes is a world wide problem, still rising and has no definite cure. The aim of this study was to investigate the short term effect of garlic oil on the antioxidant status as well as insulin level following induced diabetes in rats using streptozotocin (STZ). In the present experiment, sixty male rats were included and divided into four groups (n = 15male rats / group) as followe: control; control + galic oil; diabetic and diabetic + garlic oil. In diabetic rats (two groups), one treated by garlic oil (200 mg/kg b.wt) and the other group treated by vehicle (corn oil; 2 ml / kg b. wt,) for 8 weeks. The two groups of control rats were treated with the same dose of garlic oil as well as vehicle also for 8 weeks. Glucose, C - peptide, Insulin, superoxide dismutase (SOD), catalase and advanced oxidation protein products (AOPP) were measured in serum, while glutathione peroxidase activity was measured in the whole blood. Levels of F2 isoprostanes and 8-hydroxydeoxyguanosine (8-OHdG) were measured in urine. Levels of glucose, F2 isoprostanes, 8-OHdG and AOPP were significantly decreased, while levels of SOD, catalase, GPx, C-peptide and insulin were significantly increased on oral administrations of the garlic oil in the diabetic rats. Therefore, it could be concluded that garlic oil must be considered as an excellent candidate for future studies on diabetes mellitus.

Key words: Diabetes mellitus, antioxidants, lipid oxidation, protein oxidation, Garlic.

INTRODUCTION

It is reported that the incidence of diabetes is escalating at an alarming rate and becoming a serious health and cost issue to the point of being labeled an epidemic (Wild *et al*., 2004). Present number of diabetics worldwide is 150 million and this is likely to increase to 300 or more by the year 2025. Regions with greatest potential are Asia and Africa, where diabetes mellitus (DM) rates could rise to 2-3 folds than the preset rates (IDF Diabetes Atlas, 2009). Reasons for this rise include increase in sedentary lifestyle, consumption of energy rich diet, obesity, higher life span, etc. (Yajnik, 2001). In Egypt, rural populations with more traditional lifestyles exhibit lower rates of diabetes risk factors and diabetes, whereas urban populations, and particularly those of a higher socioeconomic status, have higher rates of both risk factors and diabetes. Left unchecked, further sociodemographic transformation of this population will be associated with a growing epidemic of diabetes mellitus. It was estimated that by the year 2025, nearly 9 million Egyptians will have diabetes (Herman *et al*., 1997).

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia and hyperlipidaemia that predisposes affected individuals to long-term micro- and macrovascular complications (Ahmed *et al*., 2007). Attention has been focused on the relationship between the production of activated oxygen species and diabetes. Activated oxygen species such as hydrogen peroxide, superoxide anions, singlet oxygen and hydroxyl radicals can be formed in cells not only during ionizing radiation but also during aerobic metabolism of either endogenous or exogenous substances. Cells have enzymatic and non-enzymatic scavenger systems against these free radicals. Nevertheless, if free radical production and scavenger systems somehow become unbalanced, cells are exposed to oxidative damage resulting in cell injury (Anwar and Meki, 2003). The free radical permanent damage to tissue structures results from an irreversible alteration in the molecular configuration of carbohydrates, lipids, proteins and nucleic acid bases (Winrow *et al*., 1993).

Attention has been developed to the protective biochemical function of natural antioxidants contained in dietary plants that are candidates for prevention or protection of oxidative damage caused by free radical species (Stavic, 1994). Many herbal medicines have been recommended for the treatment of diabetes (Alarcon-Aguilara *et al*., 1998). Plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones (Pari and Umamaheswari, 2000). Garlic have been shown to have diverse biological activities, including antitumorigenetic, anticarcinogenic, antiatherosclerotic, antithrombotic, antidiabetic and various other biological actions (Augusti, 1996). According to the report by Ryan *et al*., (2001), one third of diabetic patients take alternative medications that they consider efficacious, of which garlic is the most commonly used. The present work was conducted to elucidate the probable changes of oxidant/antioxidant status in diabetic rats induced by streptozotocin as well as to clarify the radical scavenging properties of garlic oil.

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MATERIALS AND METHODS

Animals:

Sixty adult male albino rats (Sprague Dawely Strain) weighing 200-250 g were randomly chosen from the animal house of the National Research Center, Cairo- Egypt. The animals were kept under a 12 hour light-dark cycle at an ambient temperature at 23°C and were given free access to water and standard rat feed. All animals were allowed to adapt to the environment for 1 week after their arrival before the start of experiment.

Induction of Diabetes:

Diabetes was induced by oral injection of streptozotocin (50 mg/kg body weight) dissolved in 0.05 M citrate buffer, pH4.5 according to Ketan *et al*., (2006). Three days after the injection, blood glucose level was estimated. The rats were considered diabetics when fasting blood glucose level was more than 140mg %.

Experimental Design:

Diabetic rats were assigned into two groups and received garlic oil (200 mg/kg b.wt. purchased from a commercial supplier) or the vehicle (corn oil; 2 ml/kg b.wt. according to Wu *et al*., (2001) every other day for 8 weeks. The control rats (thirty) were injected with the same volume of vehicle; 15 received corn oil; 2 ml/kg b.wt. and 15 received garlic oil 200 mg/kg b.wt. During the treatment, the animals were given free access to water and a powdered diet. All animals received professional human care in compliance with the guidelines of the Ethical Committee of Medical Research Division of the National Research Center, Cairo- Egypt.

Urine Collection:

The day preceding the end of the experiment, rats were housed individually in metabolic cages for 24 hours to collect urine then centrifuged at 2000 rpm for 10 min and supernatants were withdrawn. Each sample was aliquoted into two parts; one was adjusted at PH 4.5 and stored at - 20°C until assayed for the analysis of 8-hydroxydeoxyguanosine (8-OHdG) and the other was treated with 0.005% butylated hydroxy toluene (BHT) and stored at -80°C for the assessment of F2 isoprostane.

Blood Collection:

At the final day of the experiment, the rats were subjected to light ether anesthesia and blood was withdrawn from the optical vein .The blood was collected into two polypropylene tubes, one containing EDTA and the other left to clot and centrifuged at 3000 rpm for separation of sera. All samples were frozen at -20°C till assayed.

Biochemical Parameters:

Fasting blood sugar (FBS) was assessed immediately in sera (Passing and Bablok, 1983). C-peptide as well as Insulin was estimated in sera by ELISA kit (Kao *et al*., 1994 and Ashby & Frier, 1981) respectively.

Antioxidant Markers:

Serum superoxide-dismutase (SOD) and catalase were determined according to the method described by Suttle, (1984) and Aebi, (1986) respectively using the colorimetric method. Glutathione peroxidase (GPX) was assessed in the whole blood (Kraus and Ganthen, 1980).

Oxidant Markers:

Serum advanced oxidation protein products (AOPP) was measured according to the method described by Witko-Sarsat *et al*., (1996). Urinary F2 isoprostanes level was determined by enzyme immunoassay according to the method of Milne *et al*., (2007), using kit purchased from Cayman chemical company, AnnArbor,USA. Analysis of urinary 8-OHdG was modified from the method described by Kasai *et al*., (2001). Briefly, 8-OHdG was extracted from 1 ml urine. The eluents were dried under ultra-pure N2 stream and reconstituted in 5 ml de-ionized water for injection in HPLC.

HPLC condition: HPLC column for 8-OHdG was C18 (250 ×4.6, particle size 5μ). The mobile phase consists of acetonitrile / methanol / phosphate buffer (25/10/965). Phosphate buffer was prepared by dissolving 8.8 of potassium dihydrogen phosphate in 1000 ml de-ionized water and PH was adjusted to 3.5, the buffer then filtered 2 times before use. Flow rate was 1 ml/min and the detector was electrochemical with cell potential 600 mv.

Statistical Analysis:

The data analysis was carried out using the statistical package for social science (SPSS software version 12, Chicago, Illinois). All numeric variables were expressed as mean ± standard deviation (SD). Statistical

comparisons were performed using one way analysis of variance (ANOVA) test which was applied for multigroup comparison. Comparison of different variables in various groups was carried out using Post Hoc LSD multiple comparisons. Pearson's Correlation test was used for correlating parametric variables. For all tests a probability ($p < 0.05$) was considered significant.

RESULTS AND DISCUSSION

Data of the present study revealed that the induction of diabetes using streptozotocin caused a significant increase in blood glucose levels as compared to the control group. Inversely, significant decrease in serum insulin and C-peptide were clear in the same comparative groups. Oral administration of garlic oil clarified a significant reduction in blood glucose level reaching about 50%.

Evaluation of Oxidant/Antioxidant Status:

The induction of diabetes using Streptozotocin (STZ) developed a state of oxidative stress as denoted by a significant increase in protein oxidation, lipid peroxidation and DNA damage represented by AOPP, F2 isoprostane and 8-hydroxyguanosine respectively. On the other hand, a significant decrease in the level of glutathione peroxidase measured in the whole as well as in the levels catalase and superoxide dismutase measured in the serum. This oxidant/antioxidant status is represented in table 2.

The correlation analysis (Table 3) for the above studied parameters revealed a significant negative correlation between the antioxidant parameters (catalase, SOD and GPX) and each of AOPP, 8- OHdG and F2 isoprostane (P = - 0.78, - 0.73 and - 0.7, r = 0.01, 0.01 and 0.01 respectively). On the otherhand, these enzymatic antioxidants having significant positive correlation with insulin (p = 0.68, 0.76 and 0.83, r = 0.01, 0.01 and 0.01 respectively) and C-peptide (r = 0.685, 0.638, 0.743; r = = 0.01, 0.01, 0.01, respectively).

Table 1: Fasting blood sugar, insulin and C-peptide in the different studied groups.

	Control	Garlic	Diabetic	Diabetic+ Garlic
FBS (mg %)	92.1±11.1	92.9±9.2	276.6±31.0 [a]	134.7±21.3 [b]
C-peptide (ng/ml)	0.059±0.014	0.143±0.048	0.033±0.007 [a]	0.045±0.004 [b]
Insulin (µIU/ml)	1.84±0.24	2.64±0.34	0.98±0.19 [a]	1.34±0.2 [b]

Values are given as mean + SD. [a] = Statistical significant difference between diabetic and control rats at ($p < 0.01$). [b] = Statistical significant difference between diabetic rats treated with garlic and the diabetic one at ($p < 0.01$).

Table 2: Oxidant / antioxidant parameters in the different studied groups.

	Control	Garlic	Diabetic	Diabetic + Garlic
AOPP (µmol/L)	38.0±9.6	36.6±6.9	116.5±10.2 [a]	84.0±12.0 [b]
F2-Isoprostane (ng/mg creatinine)	2.38±0.39	1.27±0.37	4.61±0.52 [a]	2.81±0.47 [b]
8-OHdG	4.77±0.95	5.17±1.44	30.63±1.90 [a]	15.87±1.39 [b]
GPX (mU/ml)	422.6±31.6	613.0±79.2	236.9±33.8 [a]	307.1±37.8 [b]
SOD (U/ml)	258.3±18.8	268.5±27.8	161.7±21.2 [a]	192.7±18.6 [b]
Catalase (U/L)	310.8±34.1	402.9±48.4	180.6±14.1 [a]	302.5±21.6 [b]

Values are given as mean + SD. [a] = Statistical significant difference between diabetic and control rats at ($p < 0.01$). [b] = Statistical significant difference between diabetic rats treated with garlic and the diabetic one at ($p < 0.01$).

Table 3: General correlation coefficients between different biochemical parameters in the studied groups.

	F2-Isoprostane	AOPP	8-OHdG	c- peptide	Insulin	Glucose
Catalase	r= - 0.87**	r= - 0.78**	R= - 0.74**	r = 0.685**	r = 0.685**	r = -0.820**
SOD	r= - 0.73**	r= - 0.73**	r= - 0.8**	r = 0.638**	r = 0.760**	r = -0.794**
GPX	r= - 0.87**	r= - 0.7**	r= - 0.8**	r = 0.743**	r = 0.839**	r = -0.558**

** Correlations are significant as the p value <0.05.

The importance of diabetes study and methods of its treatment is considerable. Diabetes mellitus is a severe health problem and the prevalence of diabetes keeps increasing markedly due to an aging population, increased urbanization and more sedentary lifestyles (King *et al*., 1998). Since perfect cure for diabetes is yet to be found and most of antidiabetic medications could have side effects (Cheng & Josse, 2004; Thomson *et al*., 2007), many studies have been conducted to identify natural substances that show potent hypoglycemic activity with fewer side effects (Youn *et al*., 2004; Park *et al*., 2006). Garlic could be one of the candidates for antidiabetic agents via antioxidant effects (El-Demerdash *et al*., 2005; Querioz *et al*., 2009).

Garlic (Allium sativum) is a member of the Liliaceae family and one of the most popular herbs used worldwide to reduce various risk factors associated with several diseases. Garlic and its abundant organosulphur molecules (S-allyl cysteine sulfoxide) have been shown to exert antioxidant (Anwar and Meki, 2003), hypocholesterolaemic (El-Sayyad *et al*., 2010) as well as hypoglycemic effects (Madkor *et al*., 2011) through its

ability to scavenge reactive oxygen species (ROS). It also inhibits lipid peroxidation as well as inhibits oxidative modification of LDL and preventing or even reducing oxidative stress resulting in protection of DNA against free radical-mediated damage and mutations (Shaarawy *et al*., 2009). Oxidative stress, an excessive production of ROS above the body's antioxidant capacity, has been implicated in the development of many pathophysiological conditions including hypertension, diabetes, atherosclerosis and cancer, as well as the process of aging (Betteridge, 2000 and Johansen *et al*., 2005). The present study shows that treatment of diabetic rats with garlic oil alleviates each of hyperglycemia, the biochemical marker of lipid peroxidation (F2-isoprostanes) and DNA damage (8-OHdG), and the enzymatic antioxidants. Our results are in agreement with the other studies that reported by Chang and Johnson, (1980); Augusti, (1996); Anwar and Meki, (2003) and Madkor *et al*., (2011).

Also, the present study shows an improvement in the insulin level in the diabetic rats when treated with garlic. In this regard, it has been reported that the garlic may affect the insulin secretion from β-cells, release of bound insulin or increase of insulin sensitivity (Thomson *et al*., 2007). In this regard, the previous author and his team work has been reported that (S-allyl cystein sulfoxide) allicin of garlic is the responsible component for enhancing serum insulin activity duo to its free SH group and also may be due to restoration of delayed insulin response or due to inhibition of intestinal absorption of glucose (Block, 1985). In addition, anti-oxidative property of allicin might be another reason of garlic beneficial effect on diabetes (Augusti, 1996). Therefore, treatment with garlic which contain compounds such as S-allyl cysteine and organosulfor can gradually normalize oxidative stress and causes an increase in serum insulin levels in diabetic rats, delaying the side effects of diabetes (Liu *et al*., 2005).

Selenium-dependent glutathione peroxidase (GPx), which works in parallel with SOD, protects cell proteins and cell membranes against oxidative damage. In the present study, the mean level of SOD and GPx were decreased significantly in the diabetic rats as compared to their control counterparts. Also, negative correlations coefficient between the activity of these antioxidant enzymes and the glucose concentrations were observed in these diabetic rats (Table 3). On the other hand, Anwar and Meki, (2003) and Madkor *et al*., (2011) have been reported that diabetic rats had an improvement in the antioxidant status when treated with garlic. Indeed, our results showing a great restoration in these enzymatic antioxidants as shown in table 2. The improvement in the antioxidant status may be occurred as garlic containing compounds such as allicin which has antioxidant effects and has the ability to stimulate GPx activity and preventing the increasing in hydrogen peroxide by increasing the activities of SOD as well as catalase (Borek, 2001).

The beneficial effects of garlic and its constituents on neuronal physiology and brain function have begun to emerge in a wide range (Mathew and Biju, 2008). Diabetic polyneuropathy, the most common microvascular complication of diabetes mellitus, occurs in both type 1 and type 2 diabetes. In type 1, neuropathy tends to progress more rapidly and to result in a more severe disorder than in type 2 diabetes (Lee *et al*., 2010). The underlying pathogenetic mechanisms are multiple and thought to involve genetic predisposition as well as metabolic abnormalities consequent to hyperglycemia such as oxidative stress, accelerated polyol pathway metabolism and generation of advanced glycosylation end products (Shakher & Stevens, 2011). New data indicate that proinsulin C-peptide exerts important physiological effects and shows the characteristics of a bioactive peptide. It has been also reported that peripheral nerve function is improved by C-peptide replacement in diabetes type-1 patients with an early stage neuropathy (Kamiya *et al*., 2009). In present study, diabetic rats treated by garlic shows a significant increment in the serum concentration of C-peptide. Indeed, a significant positive correlation is observed between the level of C-peptide in serum and each of catalase ($r = 0.685$, $p = 0.01$), SOD ($r = 0.638$, $p = 0.01$) as well as GPx ($r = 0.743$, $p = 0.01$) as shown in table 3.

AOPP are the products of plasma protein oxidation, especially oxidation of albumin. Because of its rapid response to changes, it is thought to be suitable biochemical marker for measuring short-term changes in oxidative stress. This marker is increased in the inflammatory conditions such as diabetes, atherosclerosis and renal failure (Gelisgen *et al*., 2011). The present study shows a significant increase in the serum level of AOPP in the diabetic rats as compared to their control counterparts. In general, a significant negative correlations were observed between the levels of the enzymatic antioxidants such as catalase, SOD and GPx and the serum concentrations of the AOPP ($P = - 0.78, - 0.73$ and $- 0.7$, $r = 0.01, 0.01$ and 0.01 respectively) as shown in table 3. Although there is general agreement that insulin decrement affects fractional protein synthesis rates, the precise mechanism of impaired protein synthesis in diabetes is not fully understood (Farrel *et al*., 1999). In general, the presence of oxidative stress as a consequent of hyperglycemia is known to have a negative impact on the synthesis of different biochemical molecules. In the present study, a significant positive correlations between insulin concentration and the activity of catalase, SOD and GPx ($p = 0.68, 0.76$ and 0.83, $r = 0.01, 0.01$ and 0.01 respectively) as postulated in table 3.

A direct measurement of ROS production in target organs is not currently possible in vivo. However, it can be indirectly assessed by measurement of oxidative products because ROS, which is an inherently unstable molecule because of an unpaired electron, undergoes a series of interactions with biological macromolecules

such as lipids, proteins, and DNA (Hayakawa and Kuzuya, 1990 and Kennett *et al*., 2011). As a biomarker for oxidative DNA damage, 8-hydroxydeoxyguanosine (8-OHdG) in tissue or body fluid is known as a sensitive indicator (Ren *et al*., 2011). Previous studies have shown that diabetic patients have higher levels of 8-OHdG in the mononuclear cells and urine (Dandona *et al*., 1996 & Goodarzi *et al*., 2010). Indeed, the present study shows a significant increase in the 8-OHdG when measured in the urine of the diabetic rats. Furthermore, a significant decrease in the mean level of 8-OHdG is noticed when these diabetic rats were treated by garlic. In the diabetic state, there were many reasons for increased oxidative DNA damage. Hyperglycemia itself contributes to increased generation of ROS by producing reactive ketoimine and ketoamine adducts during oxidative glycosylation and glycoxidation, which further produce ROS such as hydrogen peroxide and superoxide anion in the presence of metal ions (Park *et al*., 2001). The restoration occurred in the diabetic rats treated by garlic may be attributable to the effectiveness of garlic in exerting anti-genotoxic, anti-clastogenic effects by modulating oxidative stress (Park *et al*., 2009). The present study revealed significant negative correlations between the 8-OHdG and the levels of the antioxidant enzymes as shown in table 3. However, the mechanism involved is unclear but garlic contains a number of organo sulfer compounds which are widely believed to be the active agent (Arnault *et al*., 2005). Finally, this study reinforces the important role of garlic as one of the antioxidants as well as natural remedy against hyperglycemia that can be used for prevention of diabetic vascular complications.

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