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ORIGINAL ARTICLE

THE GREEN TEA IMPROVE THE OXIDATIVE STATUS IN STEREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT: The present study was conducted to explore the effects of diabetes mellitus (DM) and green tea on some antioxidant enzymes in liver of rats. The obtained data showed that DM caused reduction of both glutathione S-transferase and glutathione peroxidase activities as well as reduced glutathione, while it significantly augment the lipid peroxidation in liver of rats. Furthermore, the green tea additions results in amelioration of the most mentioned adverse effects.
KEYWORDS: Green tea; Antioxidant enzymes; Glutathione; Rats.

INTRODUCTION

Diabetes Mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Bell, 2003). Clinically, many subtypes of diabetes mellitus, insulin dependent diabetes mellitus (IDDM), non-insulin dependent diabetes mellitus (NIDDM), gestational diabetes mellitus (GDM), mal-nutrition-related diabetes mellitus (MRDM) and other types were identified by World Health Organization (WHO, 1985). While, IDDM and NIDDM are the two most common forms of diabetes, IDDM is the result of an autoimmune process that specifically destroys the insulin producing pancreatic cells (beta cells), resulting in absolute insulin deficiency, NIDDM results from a combination of tissue insulin resistance and relative insulin deficiency; the insulin resistance of tissues in type II diabetes is attributed to abnormalities of insulin-signaling pathways (Davis *et al.,* 2005). Over the long-term, the chronic hyperglycemic microenvironment in both types of diabetes leads to damage, dysfunction and failure of multiple organs including eyes, kidneys, nerves, heart, and blood vessels (Olsen *et al.,* 2004). A new trend of studies is to use the active hypoglycemic constituents in different plants, which are used traditionally in folklore medicines to treat diabetes mellitus. Drinking of green tea can protect against such serious diseases as strokes, cancer and heart disease. Also it has antibacterial, antiviral, neuroprotective and cholesterol lowering effects (Weinreb *et al.,* 2004).

MATERIAL AND METHODS

2.1. Chemicals

2.1.1. Streptozotocin

(STZ) [2-deoxy-2- (3-methyl-3nitrosoureido)-D-glyco pyranoside], as a diabetogenic agent, was purchased from Sigma Company. This agent was injected intraperitoneally into 16 hours fasted rats at a single dosage of 45 mg/kg b. wt. dissolved in citrate buffer (pH 4.5; El-Seifi *et al.,* 1993). Ten days after streptozotocin injection, rats were screened for measuring blood glucose levels. Overnight fasted (12 hours) animals' blood samples were taken from orbital venous sinus and kept without anticoagulant at room temperature for one hour then centrifuged and serum glucose concentration was measured. Rats having serum glucose more than 300 mg/dl were considered as diabetic and included in the experiment.

2.2. Animals

The present study was carried out on 40 White male albino rats (*Rattus norvegicus*) weighing about 200-230g. The chosen animals were housed in metal (stainless steel) separate bottom cages at normal atmospheric temperature (25 ± 5°C) as well as under good ventilation and received water *adlibitum* and standard balanced diet for two weeks before the start of experiment for acclimatization and to ensure the normal growth and behaviour as well as exclude any intercurrent infection.
The animals were divided into 4 groups.
Group I: 10 rats were fed on basal diet and served as control.
Group II: 10 rats were injected intraperitoneally by Streptozotocin, (STZ) at a single dosage of 45 mg/kg b. wt. dissolved in citrate buffer (pH 4.5; El-Seifi *et al.,* 1993).

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Group III: 10 rats were given basal diet containing 10 g of green tea per kg diet (1%) daily through out the experiment (Swen et al., 2006).

Group IV: 10 rats were injected intraperitoneally by Streptozotocin, (STZ) at a single dosage of 45 mg/kg b. wt. dissolved in citrate buffer (pH 4.5) and given basal diet containing 10 g of green tea per kg diet (1%) after ten days of diabetes induction and given daily through out the experimental period.

All rats were housed in automatic boxes and kept under the same conditions of light and climate during the experimental period (60 days). At the end of 2nd month the sacrified rats were eviscerated and the liver was harvested from the carcass and washed by normal saline and blotted on filter paper. The collected tissue of each groups were kept frozen at -20°C for biochemical analysis of the following parameters: Lipid peroxidation (LP), Glutathione peroxidase activity (GPx), Glutathione S-transferase activity (GST) and Glutathione (G-SH).

RESULTS

Table (1) showed that, injection of STZ i.p. resulted in significant increase in lipid peroxidation of liver. While, administration of green tea in combination with STZ, resulted in significant decrease in lipid peroxidation in liver. GPx activity was significantly decreased up on STZ Injection. However, supplementation of green tea in combination with STZ resulted in significant increase in the activity of GPx in liver. Injection of STZ i.p. resulted in significant decrease in GST activity of liver. In the contrary, administration of green tea in combination with STZ resulted in significant increase in the activity of GST in liver. In addition, injection of STZ i.p. was resulted in sever decrease in GSH contents of liver. But, supplementation of green tea in combination with STZ resulted in significant increase in the level of GSH in liver.

Table (1): Effect of STZ and green tea on lipid peroxidation, GPx activity, GST activity and G-SH of rats' liver

Groups	LP. (nmol MDA/g wet tissue)	GSH (µmol/g wet tissue)	GP_X (IU/g wet tissue)	GST (mol CDNB/min/g wet tissue)
Control	65.80±5.13cd	112.27±1.38b	87.06±1.71b	398.91±4.70c
Diabetes	183.15±20.12a	72.13±1.52e	38.72±0.47f	193.16±8.98e
Green tea	63.08±2.76cd	112.09±1.66b	86.58±1.42b	457.55±13.08b
Diabetes+Green tea	114.33±7.54b	89.63±1.84c	56.55±1.15d	254.81±10.05d

Means within the same column carrying different letters are significantly different.

DISCUSSION

The result postulated in Table (1) revealed that, the injection of STZ significantly increased lipid peroxidation as expressed by increased MDA level in liver. These results come in agreement with those obtained by Lei *et al.*, (2008) who concluded that, rats treated with STZ showed a significant increase in lipid peroxidation in liver and kidneys compared with controls. The same authors revealed that, the increased lipid peroxidation observed in DM returned to increased oxidative stress due either to (hyperglycemia or Streptozotocin which gives rise to oxygen free radicals) coupled with decreased antioxidant protective system. Similarly, increased plasma peroxide concentrations were reported in type 1 and type II DM patients (Walter *et al.*, 1991). Also, diabetic red blood cells were shown to be more susceptible to lipid peroxidation as measured by TBARS in rats and humans (Fujiwara *et al.*, 1989). On the other hand, the present results disagree with those obtained by MacRury *et al.*, (1993) who found that, no difference in serum conjugated diene levels between otherwise healthy diabetic patients and healthy control subjects. Also, TBARS levels in both poorly and well controlled type II DM patients did not differ from control subjects, whereas hydroxyl radical formation was elevated in DM patients (Ghiselli *et al.*, 1992). Moreover, plasma TBARS levels were similar in type 1 DM and type 2 DM patients as in control subjects (Zoppini *et al.*, 1996). Concerning the effect of green tea on lipid peroxidation, table (1) revealed a significant decrease in the level of MDA in liver during diabetes induction. These results come in accordance with those obtained by Haixia *et al.*, (2005) who found that, green tea decreased lipid peroxidation in diabetic mice injected with alloxan. The same authors reported that, green tea decrease lipid peroxidation by scavenge free radicals such as O_2 and H_2O_2 and chelate ions induced peroxidation as well as increased antioxidants enzyme activities. Furthermore, STZ induced diabetic rats significant increased lipid peroxidation in heart but green tea significant decreased it (Pon *et al.*, 2006).

Table (1) revealed that, the injection of STZ significantly reduced the activity of glutathione peroxidase in liver suggesting the increased utilization of this antioxidant enzyme to counter the increased level of free radicals induced by

STZ in this tissue. These results are in harmony with Walter *et al.*, (1991) who described that, red blood cell, whole blood and leukocyte glutathione peroxidase (GPx) activity was decreased in type 1 and type 2 DM patients compared to control groups. In addition, Carmen *et al.*, (1998) demonstrate that, erythrocyte GPx activity was significantly lower in diabetic children at the onset and in later stages of the disease compared with control subjects. The same authors revealed that, the low GPx activity could be directly explained by the low GSH content found in diabetic patients, since GSH is a substrate and cofactor of this enzyme, therefore, low GSH content implies low GPx activity, which may produce increased oxidative stress. Enzyme inactivation could also contribute to low GPx activity and this was proved by (Arai *et al.*, 1987) who showed that, enzymatic inactivation might occur through glycation governed by prevailing glucose concentrations, thus increased glycation in diabetic patients and the subsequent reactions of proteins might affect amino acids close to the active sites of the molecule or disturb the stereochemical configuration, thereby provoking structural and functional changes in proteins. On the other hand, there was no difference between GPx activity of type 1 or type 2 diabetic patients and control subjects (Jain and McVie, 1994). Moreover, high glucose concentrations exerted no significant effect on GPx expression and activity (Forsberg *et al.*, 1996).

Table (1) showed that, the injection of STZ resulted in significant decrease in the activity of GST in liver. These results come in agreement with those obtained by McDermott *et al.*, (1994) who demonstrated that, GST activity was decreased in heart and liver of diabetic patients. Also, Matkovics *et al.*, (1998) demonstrated a significant decrease of GST, SOD and CAT activity in erythrocyte hemolysates of streptozotocin diabetic rats. Furthermore, Jos *et al.* (1990) reported decreased GST and GPx in a group of type 1 diabetic adolescents with retinopathy. The low GST activity might be directly explained by the low GSH content found in diabetic patients, since GSH is a substrate and cofactor of this enzyme (Carmen *et al.*, 1998). In the contrary, the present results disagree with those obtained by Irina *et al.*, (2003) who elucidated that, glutathione S-transferase, and NADH oxidase but not catalase, were upregulated in diabetic rats liver vs. controls, concerning the effect of green tea in combination with STZ on the level of GPx and GST. Table (1) revealed highly significant increase in the activity of GPx and GST in liver. These results come in agreement with those obtained by Vanessa and Gary, (2004) who postulated that, intake of green tea extract was increased the activity of SOD,GST and GPx in serum and the expression of catalase in aorta of rats. The same authors reported that, green tea has both direct antioxidant effect (scavenge free radical as O_2 and H_2O_2, chelate metal ions as iron and copper to prevent their participation in fenton and haber-weiss reaction) and indirect antioxidant (increase expression of antioxidant enzymes). Simillary, green tea increased the activity of serum SOD and GPx, the antioxidant mechanism may be due to the supply of hydrogen by green tea components, which combines with radicals and itself forms a stable radical to terminate the radical chain reaction, the other possibility is that green tea can combine with the radical ions that are necessary for radical chain reaction; then the reaction is terminated (Haixia *et al.*, 2005).

Table (1) revealed that, the injection of STZ resulted in significant decrease in the level of glutathione in liver tissue. These results are parallel with those obtained by De Mattia *et al.*, (1994) who found that, Type 2 diabetic patients had decreased erythrocyte GSH and increased GSSG levels. Also, blood GSH was significantly decreased in different phases of type 2 DM such as: glucose intolerance, early hyperglycemia (within two years of diagnosis and before development of complications) and in poor glycemic control (Vijayalingam *et al.*, 1996). Moreover, red cells from type 2 DM patients had decreased GSH levels, impaired gammaglutamyl transferase activity and impaired thiol transport (Yoshida *et al.*, 1995). The same authors revealed that, treatment with an antidiabetic agent for 6 months corrected these changes. Also, a relative depletion of NADPH due to aldose reductase activation and secondary to reduced production through the pentose cycle impairs GSH regeneration and leads to depletion of this free radical scavenger in DM (Travis *et al.*, 1971). The same authors revealed that, the early decrease in GSH content detected at the onset of diabetes may disturb antioxidant defenses that together with increased oxygen free radical activity will result in an acceleration of the oxidative damage already present in initial stages of the disease. Furthermore, a decrease in the concentration of reduced glutathione (GSH) observed in erythrocytes from diabetic subjects, was resulted from decreases in activities of the enzymes involved in GSH synthesis (such as γ-glutamylcystein synthetase) or in the transport rate of oxidized glutathione (GSSG) from erythrocytes (Murakami *et al.*, 1989) and

enhanced sorbitol pathway (Ciuchi *et al.,* 1996). In addition, a decrease in the activity of glutathione reductase (GSSG-R) which acts to reduce GSSG to GSH, has also been reported (Tagami *et al.,* 1992). Also, Matkovics *et al.,* (1998) elucidated that, GSSG-R activity decreased in erythrocyte hemolysates of streptozotocin diabetic rats and attributed this decrease to the enzyme glycation by the uncontrolled hyperglycemia. Moreover, mean red cell GSH, NADPH levels, NADPH/NADP+ and GSH/GSSG ratios were decreased in 18 type II diabetic patients compared to16 non-diabetic control subjects (Bravi *et al.,* 1997). In the contrary to the present results, increased blood GSH levels in the DM men could represent an adaptive response to increased oxidative stress mediated possibly in part through increased red cell GRD activity (Mustafa and David, 2002). The data in Table (1) represented the extension of study to determine the effect of green tea in combination with STZ on the level of glutathione. The present findings revealed that, the supplementation of green tea resulted in significant increase in the content of glutathione in liver. These results come in agreement with those obtained by Pon *et al.,* (2006) who showed that, STZ induced diabetic rats characterized by significant decrease of GSH in heart but after taken green tea causing significant increase of GSH content of heart. The same authors revealed that, green tea act as scavenger of free radicals and so decrease utilization of GSH and subsequently increase its content in heart. Moreover, green tea was able to increase GSH content in liver of diabetic rats injected with alloxan (Sabu *et al.,* 2002).

CONCLUSION

Experimental diabetes mellitus induced oxidative stress. Green tea decreased oxidative stress reflected in decreased lipid peroxidation marker and increased the activities of antioxidant enzymes as GST and GPx as well as GSH.

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