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The effect of hyperglycemia on catalase activity and glutathione levels of liver in streptozotocin-induced diabetes mellitus in rat

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ABSTRACT

Introduction: The antioxidant enzymes and antioxidant capacity are affected by hyperglycemia in the different tissues of human body during diabetes mellitus (DM). Hence, in the present study we measured the activity of catalase and glutathione content in the livers of streptozotocin-induced diabetic rats.

Methods: Male Wistar rats were randomly divided into normal and diabetic groups (n=6). To induce DM, a single intravenous injection of streptozotocin was used (45 mg/kg). Blood glucose of rats was measured at the beginning and termination of study. Likewise, the activity of catalase and the content of glutathione were determined in the livers at termination of the study.

Results: Induction of DM increased blood glucose of the diabetic rats to 559 ± 35 mg/dL. This value did not change during the test (610 ± 17 mg/dL) in diabetic rats. Diabetes also increased the catalase activity in the livers of diabetic rats compared to normal group. Likewise, glutathione content increased in the livers of diabetic animals compared to normal rats.

Conclusion: Our findings revealed that the activity of antioxidant enzymes as well as antioxidant capacity of liver may be increased as a compensatory response to confront the tissue oxidative stress for the determined time during diabetes. It is suggested, if the period of diabetes be prolonged, this compensatory response may be weakened.

Keywords:

Glutathione, Catalase, Hyperglycemia, Diabetes mellitus, Liver antioxidant.

INTRODUCTION

Diabetes mellitus (DM) is a common metabolic disease with the major complications, which is mainly characterized by hyperglycemia (1). Defect in insulin synthesis or function affects the metabolism of lipids, carbohydrates, and proteins, which can result in nonalcoholic fatty liver disease (NAFLD) (2, 3). Overproduction of free radicals due to reducing the antioxidant capacity of liver plays a crucial role in the pathogenesis of diabetes complications (4, 5). In fact, ROS accumulation changes the structure and function of the main macromolecules, including lipids, proteins and nucleic acids (6, 7). During diabetes, the balance between the hepatic production of pro-oxidants and their elimination by the hepatic antioxidants is interrupted, and the vulnerability of liver to oxidative stress is enhanced by chronic hyperglycemia (8). It has been reported that pro-oxidant enzymes like NAD(P)H oxidase activity is increased in the liver of diabetic people (9, 10). Additionally, the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase as well as the levels of non-enzymatic antioxidants like glutathione is decreased in the liver of diabetic patients (11, 12). On the other hand, increasing antioxidants enzymes as well as antioxidant capacity of liver has been demonstrated in the diabetic patients (13). Based on the previous reports, the activity of antioxidant enzymes is sensitive to oxidative stress, and both enhancement and reduction have been demonstrated in several pathological situations (14, 15). Therefore, in the present study we assessed the catalase activity and glutathione

content of liver during diabetic states in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Animals

Male Wistar rats, weighing approximately 210-230 g, obtained from the animal house facility center of Baqiyatallah University of Medical Sciences. All procedures of the study were approved by the Institutional Care and Use of Animals Committee of the University of Baqiyatallah Medical Sciences, and were performed in accordance with accepted standards of animals use and care. The ethical code for present study was IR.BMSU.AEC.1402.034. Before to procedures, we allowed the rats to acclimatize to the new situation with controlled temperature (22-24°C), humidity (40-60%), light period (07.00-19.00), and also free access to the rat chow and water.

Induction of Diabetes

Induction of diabetes mellitus (DM) was performed by an intravenous injection of streptozotocin (STZ, Sigma Aldrich) In brief, under light anesthesia by using ethyl ether, diabetes was induced by an intravenous injection of 45 mg streptozotocin per kg body weight of rats (45 mg/kg) in lateral tail vein. Five days after injection of streptozotocin, blood glucose levels were tested to confirm diabetes and the rats with blood glucose levels between 500 to 600 mg/dL were considered as the diabetic animals.

Experimental protocols

To perform the study, the rats were randomly divided into two groups of equal numbers (six rats in each group) as follows: normal group (normal healthy rats that used as normal control) and diabetic group (diabetic rats that used as diabetic control). The rats of normal and diabetic groups were kept in the standard situation with free access to the rat chow and water during study. Blood glucose levels at the beginning (day 5) and termination (day 60) of the experiment were measured using a commercial kit (Pars Azmoon Company, Tehran, Iran) by an enzymatic colorimetric method according to the manufacture protocol.

Tissue preparation

At termination of the study (day 60), the liver tissues were rapidly removed under deep anesthesia using ethyl ether. Tissues were washed in an ice-cold phosphate buffer saline (PBS), immersed in liquid nitrogen and finally kept on -80 °C until biochemical analysis. Then, the tissues were weighed and homogenized 1:10 in ice-cold PBS. The homogenates were centrifuged at 14000×g for 15 minutes at 4 °C. After centrifugation, the supernatants were removed and used for measurement of glutathione (GSH) and catalase (CAT) activity.

Determination of protein levels

The method of Bradford was used to quantify the protein levels of brains and livers for data calculation. To calculate the protein concentration, bovine serum albumin (BSA; Sigma, Germany) was used as a standard.

Determination of the catalase (CAT) activity at livers

The method of Aebi (1984) was used to determine the activity of CAT in tissue homogenates (16). First, the homogenate was incubated in the reaction mixture that contained 0.1 mL homogenate and 0.85 mL potassium phosphate buffer (50 mM and pH 7.0) at room temperature for 10 min. Then, the reaction was begun by adding 0.05 mL H_2O_2 (30 mM prepared in potassium phosphate buffer 50 mM and pH 7.0). A decrease in the absorbance was recorded by a spectrophotometer at an excitation of 240 nm for 3 min. The specific activity of CAT was calculated as 1 mmol H_2O_2 decomposed U/mg protein.

Determination of glutathione (GSH) content at livers

According to the method of Tietz (1969), the GSH levels of the liver tissues were assessed (17). First, by adding sulfosalicylic acid (5%), the cellular protein was precipitated. After centrifugation of the solution at 2000 g for 10 min, the supernatant was removed and glutathione level was assayed as follows: hundred microlitres of the protein-free supernatant of the cell lysate, 100 mL of 0.04% 5,50-dithiobis-(2-nitrobenzoic acid) (DTNB) in 0.1% sodium citrate and 800 mL of 0.3 mM Na2HPO4. After 5 min, the DTNB absorbance was recorded at 412 nm. Based on the measurement sensitivity, the standard curve for GSH was done between 1 and 100 mM. The GSH contents of the liver tissues were calculated as nmol/mg protein.

Statistical analysis

To perform the statistical analysis for data of

the current study, the SPSS (v.21, Chicago, IL, USA) was used. First, Kolmogorov-Smirnov test was applied to test for a normal distribution of data. The t-test was used to analyze the data between two groups (normal and diabetic groups). All data were expressed as mean±SEM. A $p<0.05$ was considered statistically significant.

RESULTS

Effect of DM induction on the blood glucose of diabetic rats

Table 1 shows the representative variations of blood glucose during the study in normal and diabetic rats. Blood glucose of the normal animals was 168 ± 24 mg/dL at beginning of the study. This value did not significantly change during the study at day 60 (175 ± 6 mg/dL). Likewise, blood glucose of the diabetic rats was 559 ± 35 mg/dL at beginning of the test (after diabetes induction). This value did not alter in diabetic rats during the study at day 60 (610 ± 17 mg/dL). Finally, there were no significant differences in the blood glucose levels of two groups at the different mentioned times (days 1 and 60) during the study.

Table 1: Blood glucose of diabetic rats (mg/dL) at the beginning and termination of the study.

	Normal group	Diabetic group
Beginning of the test	168 ± 24	559 ± 35 ***
End of the test	175 ± 6	610 ± 17 ***

All values are presented as mean ± SEM (n= 6). *** ($p<0.001$) as significant difference compared with normal group.

Effect of DM induction on the catalase activity of livers

Figure 1 shows the catalase activity of livers at termination of the study. The mean value of catalase activity in the livers of normal rats was 4.52 ±1.14 U/mg protein. Induction of chronic hyperglycemia (DM) significantly increased the catalase activity of livers in diabetic rats (9.32 ± 0.58 U/mg protein) compared to normal animals ($p< 0.01$).

Effect of DM induction on the glutathione levels of livers

As shown in Figure 2, the mean value of glutathione content in the livers of normal rats was 125 ± 11 nmol/mg protein at termination of the study. This value significantly increased in the livers of diabetic rats (175 ± 9 nmol/mg protein) compared to normal animals ($p<0.05$), at termination of the study.

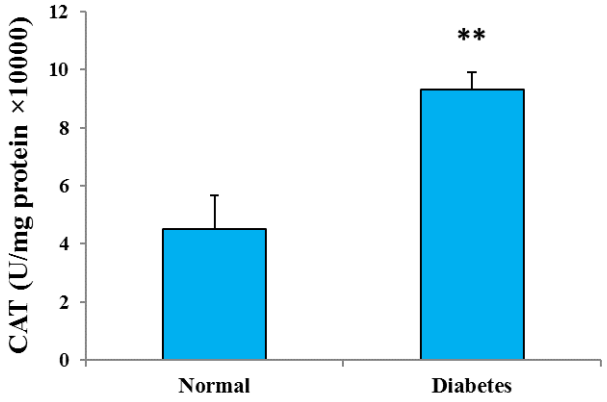

Figure 1: Effect of diabetes mellitus induction on the catalase (CAT) activity (U/mg protein × 10000), at termination of the study. All values are presented as mean ± SEM (n= 6).** ($p<0.01$) as significant difference compared with normal group.

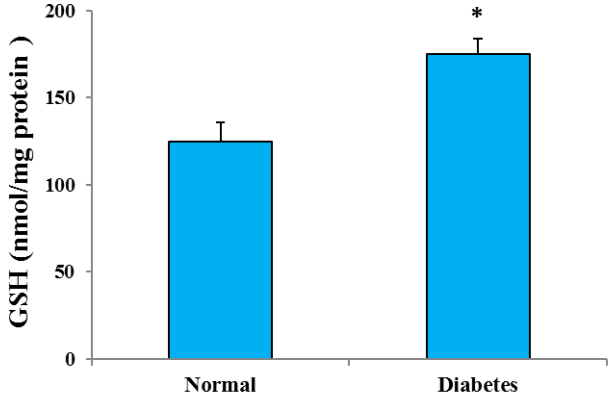

Figure 2: Effect of diabetes mellitus induction on the glutathione (GSH) levels of liver (nmol/mg protein) at termination of the study. All values are presented as mean ± SEM (n= 6). * ($p<0.05$) as significant difference compared with normal group.

DISCUSSION

The findings of the present study indicated that induction of chronic hyperglycemia mediated by DM in rats considerably enhanced the activity of antioxidant markers as well as antioxidant capacity of liver. In this regard, the activity of CAT increased in the livers of diabetic rats. The GSH content of livers also increased following diabetes induction as a prominent factor in the homeostasis of GSH-related enzymes family.

Based on previous studies, DM induces accumulation of ROS and oxidative stress in the different tissues of diabetes peoples (3, 7). In this regard, increasing the oxidative stress markers to the higher levels has been reported in the blood of diabetic patients (12, 18). The vulnerability of liver to ROS accumulation and happening the

oxidative stress also have been demonstrated in the diabetic patients (7, 10). For this purpose, diabetes interrupts the balance between the hepatic production of pro-oxidants and elimination of ROS by the hepatic antioxidant systems (7, 8). It has been reported that ROS generation is exacerbated by activation of pro-oxidant enzymes such as polyol pathway and NAD(P)H oxidase in the liver of diabetic patients (9, 19). On the other hand, the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase as well as the content of non-enzymatic antioxidants such as glutathione are changed in the liver of diabetes states (11). Increased and decreased as well as no changes of these stress oxidative markers during diabetes have been demonstrated by several previous reports (7, 12). Since, the activity of antioxidant enzymes is sensitive to oxidative stress, both enhancement and reduction of these variables have been demonstrated in various pathological conditions with ROS accumulation (7, 12, 14). Our findings showed that diabetes increased the catalase activity in the livers of diabetic rats compared to normal group. Similarly, glutathione content increased in the livers of diabetic animals compared to normal rats. Therefore, our results confirmed increasing the activity of antioxidant enzymes as well as antioxidant capacity of liver following eight weeks of diabetes. It seems that increased the antioxidant response be as a compensatory response to confront the tissue oxidative stress for the determined time during diabetes. Also it seems, by increasing the period of diabetes this compensatory response may be weakened by the chronic hyperglycemia. The liver has a potent antioxidant system, including SOD, catalase, and glutathione-related enzymes, such as glutathione S-transferase (GST) and glutathione peroxidase (GPX) (20). This potent antioxidant defense system protects the liver cells from oxidative stress by eliminating the free radicals and hydrolyzing hydrogen peroxides (15). In this regard, several studies have demonstrated that decreased SOD and catalase activities have increased oxidative stress and eventually liver damage during hyperglycemic situations (3). In addition to these enzymes, GSH is a prominent factor in the homeostasis of GSH-related enzymes family (7, 19). Reduction of its levels in the liver of diabetic animals correlate with decreased the activities of GST, GPX, and glutathione reductase (19).

CONCLUSIONS

It is concluded that chronic hyperglycemia in diabetic states might enhance the activity of antioxidant enzymes as well as antioxidant capacity of livers as a compensatory response to confront the tissue oxidative stress for the determined time during diabetes. It is suggested, if the period of diabetes be prolonged, this compensatory response may be weakened.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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There are no financial conflicts of interest to disclose.

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