DIABETES MELLLITUS AS AN OXIDATIVE STRESS

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Abstract. Diabetes Mellitus is one of the most costly burdensome chronic diseases of our time and is a condition that is increasing in epidemic population in the whole world. Diabetes mellitus is regarded as a syndrome, a collection of disorders that have hyperglycemia as the hallmark. Non-insulin dependent diabetes mellitus is now epidemic in many countries undergoing modernization and industrialization. High levels of glucose, which is the main indicator of diabetes mellitus, were attributed to oxidative processes. Therefore, this work interests in measuring the glutathione and lipid peroxidation levels. The present work also investigates the influence of some antioxidants on the glucose concentration. More than 30 persons who had a negative history of any diabetes mellitus were used as controls and more than 80 patients with different stages of diabetes mellitus were examined. Seven groups of Sprague Dawely rats (8 rats per group) were also used. The results showed that hydrogen peroxide (H_2O_2) is produced during the progress of the disease and increases with a percentage of about 27%. On the other hand, glutathione (GSH) content reduced with a percentage of about 20%. 200 mg/kg of body weight of vitamin E, vitamin A, vitamin C, ginseng extract and 50 μmole of nicotinamide adenine dinucleotide (NADH) showed good effects on both enhancement of glutathione content (up to 76.9%) in blood and decreasing lipid peroxides (to 19.4%) after 24 hours of administrating the rats with H_2O_2. Results also showed that diabetes is accompanied by an increase in electrical conductivity and a decrease in intrinsic viscosity.

Key words: diabetes, oxidative stress, antioxidants, conductivity.

INTRODUCTION

Diabetes is a serious disorder of pancreas glands. It is one of the most insidious disorders of the metabolism and, if left undiagnosed, may lead to rapid emaciation and ultimately to death. The pancreas consists of three types of cells (α, β and γ). Insulin is produced by β cells of Langerhans islets in the pancreas. β Cells deficiency may be an important factor in determining how diabetes develops. The presence of islet cell antibodies (ICA) or glutamic acid decarboxylase antibodies (GADA) and markers of autoimmune β-cell destruction are usually detected at onset [16, 22]. An early abnormality in β cells function may be loss of pulsatility in

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insulin secretion. Hyperglycemia, resulting from uncontrolled glucose regulation, is widely recognized as the causal link between diabetes and diabetic complications. Hyperglycemia-induced overproduction of superoxide is the causal link between high glucose and the pathways responsible for hyperglycemic damage [1]. Hyperglycemia may cause a further reduction in β cells function that may be related to glucose toxicity affecting β cells with increasing hyperglycemia and, therefore, the insulin concentration begins to fall. Diabetes mellitus is a syndrome initially characterized by a loss of glucose homeostasis [40].

Patients who have symptoms of uncontrolled diabetes, such as polyuria, polydipsia, nocturia, and weight loss, with a confirmatory random blood glucose level of more than 200 mg/dL, are easily to be diagnosed as having diabetes. Under further consideration by a number of international groups, including the WHO in 1985 and the American Diabetes Association [24], diabetes is classified into:

(I) Insulin dependent diabetes mellitus (IDDM) (or type 1 diabetes). It is an autoimmune disorder characterized by pancreatic β cell destruction. Individuals at risk developing IDDM can be identified by the presence of autoimmune serological marks, including islet cell antibodies, antibodies to insulin and glutamic acid decarboxylase (GAD) and a decline in β cell insulin secretory capacity. It is characterized by severe insulin deficiency, abrupt onset of severe symptoms, a tendency to ketosis and dependence on exogenous insulin to sustain life.

(II) Non-insulin dependent diabetes mellitus (NIDDM**)** (or type 2 diabetes)**.** It is characterized by presence of insulin, moderate symptoms or sometimes asymptomatic, no tendency to ketosis and no dependence on exogenous insulin. Patients require insulin to control hyperglycemia if this is not achieved with diet alone or with oral hypoglycemic agents.

(III) Malnutrition-related diabetes mellitus. It was previously known as tropical diabetes. The individuals are characteristically under weight, and have clinical signs of present or past malnutrition and other dietary deficiency states.

(IV) Diabetes associated with other conditions or syndromes like specific endocrine diseases such as thyrotoxicosis and acromegaly.

Free radicals have been recognized as intermediates of some biological redox reactions essential for the maintenance of life. Biological materials, particularly membranes, contain high concentrations of unsaturated lipids. In the presence of a free radical initiator and oxygen they may be oxidized. This process is known as lipid peroxidation [26**]** and it has been implicated as a general biological degenerative reaction. It has been reported that glucose might be autooxidized generating free radicals [9]. Hydrogen peroxide (H_2O_2) is a product of a variety of cellular reactions such as amino acid oxidation, normal respiration and superoxide dismutation by superoxide dismutase. Although it is not a free radical and is more stable than free radicals, H_2O_2 possesses a serious threat to cells because it can react with O_2 or transition metals to form the serious hydroxyl radical [29].

Patients with type 2 diabetes, as reviewed, continually undergo oxidative stress that elevates glucose concentrations, increase levels of reactive oxygen species in ß-cells which have intrinsically low antioxidant enzyme defenses [23].

Living organisms usually possess defense systems such as antioxidants to eliminate the toxicity effect of oxygen free radicals. To oppose this potential toxicity, defenses have evolved help limiting the production of free radical damage to tolerable levels. In the cases of weak antioxidant defense or excess production of radicals, a state of oxidative stress occurs. Oxidative stress has been defined as the imbalance of pro-oxidant and antioxidant forces in favor of the former [35]. Overthreshold values of essential antioxidants in vegetarians imply a protective action against reactive metabolic oxygen products and toxic products of lipid peroxidation and may reduce the incidence of free radical diseases [6].

The present work interests in measuring both glutathione and lipid peroxidation concentrations in normal and diabetic samples which are taken from both individuals and rats. It also studies the influence of some antioxidants like vitamins (A, C and E), ginseng and NADH on glucose concentration.

MATERIALS AND METHODS

The investigated specimens in this work were classified into two populations:

The first population consisted of more than 80 samples from healthy and diabetic patients, not smokers. The age of them was within the range of 30–50 years. The samples were divided into 8 groups as Table 1 shows.

Table 1

The groups of first population

Group	G 1	G 2	G 3	G 4	G 5	G 6	G 7	G 8
Glucose conc. (mg/dL)	80–120	120–160	160–200	200–240	240–280	280–320	320–360	360–420

The persons of the first group (the control one) were selected carefully to be free from any disease that might affect the measurements. The medical states of the patients were reported in the Hematological Division of the Suez Canal University Hospital.

Each sample was divided into two parts, a part was used to determine the concentration of glutathione (GSH) and the other one was used to determine the concentration of lipid peroxidation.

The second population included the experimental animals. The experimental animals were male Sprague-Dawely rats (of weight of 130–155 g) obtained from the breeding unit of the National Research Center, Cairo. The rats were housed in a

well ventilated room of temperature 25+2 °C and 12 hour light and dark cycle at the animal house of Zoology Department, Suez Canal University. Animals were regularly fed on a standard diet *ad libitum* for at least one week before measurements.

The principles of laboratory concerning the ethical standards of animal care and protection were followed.

The rats were divided into 7 groups (8 rats per each). The 1st (control) group was administered with saline 0.9%, pH 7.2 and the other groups were administered with 25 mM H_2O_2. After 24 hours the 3rd group was readministered with 200 mg/kg of vitamin E, the 4th group was readministered with 200 mg/kg of vitamin A, the 5th group was readministered with 200 mg/kg of vitamin C, the 6th group was readministered with 200 mg/kg of Ginseng and the 7th group was readministered with 50 μM of NADH.

The blood samples were collected using the orbital sinus technique [31] and the whole blood was used to determine glutathione level [41]. To obtain serum, the collected blood was incubated at about 37 °C for an hour in clean dry test tubes, then centrifuged at 3000 rpm for 20 minutes by using MLWT 54 centrifugation apparatus (Germany). The sera were aspirated and stored at –20 °C until used. The measurements of lipid peroxidation and glucose level were determined in the serum.

Glucose level present in the sample was usually determined using the following reagents [37]: 100 mg/dL of standard glucose, 100 mM of phosphate buffer (pH 7.2), 10 mM of phenol, 0.7 mM/L of amino-4-antipyrine, 20 U/mL of glucose oxidase and 1 U/mL of peroxidase.

The standard glutathione was obtained commercially from Sigma Chemical Corporation. The concentration of glutathione for both control and patients groups was calculated as follows:

- 0.2 ml of blood was added to 1.8 ml of distilled water.
- 3.0 ml of precipitating solution was added and the samples were immediately vortexed.
- The mixture was centrifuged at 2200 rpm for 15 minute at room temperature.
- 1.0 ml of supernatant was added to 4 ml of 0.3 M phosphate solution.
- One half ml of DTNB (dithiobis nitro butyric acid) reagent was added and the absorption at 412 nm was measured by using Perkin Elmer 4B UV/visible spectrophotometer.

The determination of the lipid peroxidation of serum was based on the measurements of thiobarbutric acid (TBA) reactants [33] as follows:

- 0.5 ml of serum was mixed with 1.0% of phosphoric acid (3 ml, pH 2.0) and 0.6% TBA (1 ml) in airlight tubes which were kept in boiling water for 45 min.

- the samples were cooled in ice and butanol of 5 ml was then added.
- The butanol phase was separated by centrifugation (10000 rpm) for 10 minutes and transferred to glass cuvettes.
- The color of the TBA chromogen was measured at 520 nm and 532 nm by Perkin Elmer UV-visible spectrophotometer.
- The difference between absorbance at 520 nm and 532 nm gives rise to the TBA value, which primarily represents the malondialdehyde concentration and was taken therefore as a measure of the lipid peroxidation.

The determination of met-hemoglobin was done spectroscopically [38]. Electrical conductivity was carried out at a temperature of about 20 ± 1 °C by using two silver electrodes at frequency 1500 Hz immersed in an aqueous hemoglobin solution and connected to the conductivity meter (WISS Tech, D 812 Walhin, Germany) which was firstly calibrated by a standard solution [8]. The intrinsic viscosity η_{in} was carried by the following equations:

$$\eta_{in} = \lim \eta_{red}/C \quad (1)$$

$$\eta_{red} = \eta_{sp}/C \quad (2)$$

$$\eta_{sp} = \eta_{rel} - 1 \quad (3)$$

$$\eta_{rel} = \eta/\eta_0 \quad (4)$$

where η, η_o are the viscosities of Hb and distilled water as a function of density and flow time at room temperature; η_{sp}, η_{red} and η_{rel} are the specific, reduced and relative viscosities; *C* is the concentration of diluted hemoglobin [15, 27].

RESULTS

Glutathione is one of the important antioxidants which are found in biological systems. Therefore, the present work is interested in measuring glutathione content in both normal and diabetic cases. Table 2 and Fig. 1 show the mean values of glutathione content of the investigated samples which were taken from control and diabetic persons as the glucose level changes. It can be observed that glutathione content decreases as the glucose level increases.

The decrease in glutathione content as the glucose level increases makes it important to measure the lipid peroxidation of the samples taken from control and diabetic patients. Table 3 and Fig. 2 illustrate the relationship between the mean values of the concentrations of lipid peroxidation and glucose for control and

diabetic groups. It can be observed that the concentration of lipid peroxidation increases as the glucose level increases. At early stages of diabetes (120–160 mg/dL and 160–200 mg/dL), the lipid peroxidation increases with percentages of about 12% and 46% respectively. At advanced cases of diabetes (320–360 mg/dL and 360–430 mg/dL), the lipid peroxidation increases with about 92% and 103% respectively, i.e. the concentration of lipid peroxidation has been approximately doubled at advanced diabetic cases.

Table 2

The mean values of glutathione content as glucose conc. changes

Group no.	Average conc. of GSH (mg/dL)	% decrease in GSH
1	4.843 ± 0.12	–
2	4.465 ± 0.16	7.8%
3	4.175 ± 0.07	13.8%
4	3.929 ± 0.06	18.9%
5	3.266 ± 0.05	32.6%
6	3.186 ± 0.09	34.2%
7	2.989 ± 0.06	38.3%
8	2.815 ± 0.03	41.9%

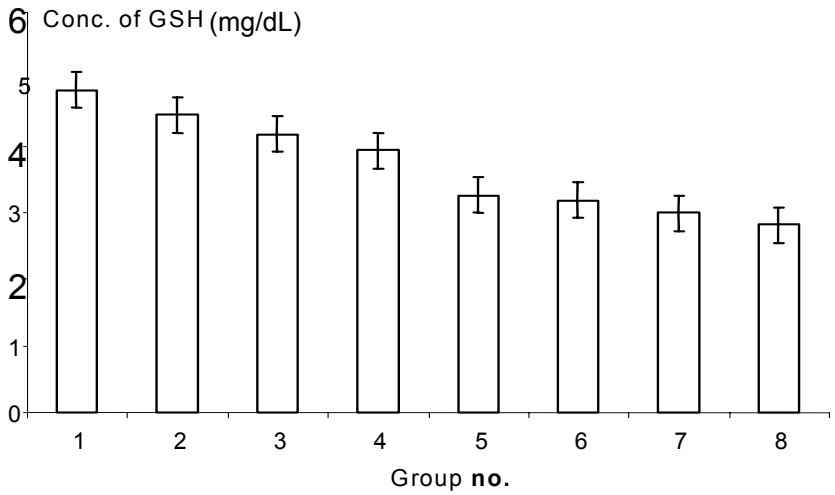

Fig. 1. Variation of GSH concentration as glucose level increases.

Table 3

The relationship between the mean values of lipid peroxidation and glucose concentration

Group no.	Lipid peroxidation Mean±SE (TBA units)
1	0.1782 ± 0.013
2	0.1987 ± 0.014
3	0.2598 ± 0.02
4	0.2684 ± 0.033
5	0.2945 ± 0.034
6	0.3184 ± 0.04
7	0.3417 ± 0.001
8	0.3615 ± 0.05

In the following part of research some antioxidants have been used to study their effects on the rats after they were orally administered with H_2O_2. The rats were classified into 7 groups.

The 1st group was left as a control group; the other groups were all administered with H_2O_2 and left for 24 hours and then administered with vitamin E, vitamin A, vitamin C, ginseng and NADH, respectively. Both glutathione and lipid peroxidation levels were measured.

Fig. 3 represents glutathione content for the normal and other groups of diabetic rats after administered with H_2O_2 and H_2O_2 in addition to vitamins E, A, C, ginseng and NADH. The seven groups of samples were administered with 25 mM of H_2O_2 and after 24 h, they were treated with the above mentioned antioxidants.

Group (1) represents the control group, Group (2) was orally administered with 25 mM of H_2O_2, Group (3) was orally administered with vitamin E (200 mg /kg body weight), Group (4) was orally administered with vitamin A (200 mg /kg body weight), Group (5) was orally administered with vitamin C (200 mg /kg body weight), Group (6) and Group (7) were orally administered with ginseng (200 mg /kg body weight) and 50 μM of NADH respectively.

From Fig. 3, it can be observed that when control rats in group 2 have been treated with H_2O_2, glutathione concentration decreased by about 58%. When diabetic rats in group 3 have been treated with vitamin E, glutathione content increased by about 57.5%. When diabetic rats in group 4 have been treated with vitamin A, glutathione content increased by about 63.7%. When diabetic rats in group 5 have been treated with vitamin C, glutathione content increased by about 64.4%. When diabetic rats in group 6 have been treated with ginseng, glutathione content increased by about 74.7%. When diabetic rats in group 7 have been treated

with NADH, glutathione content increased by about 76.9%. Figure 3 clarifies the role of antioxidants in the improvement of diabetic cases by increasing the glutathione content.

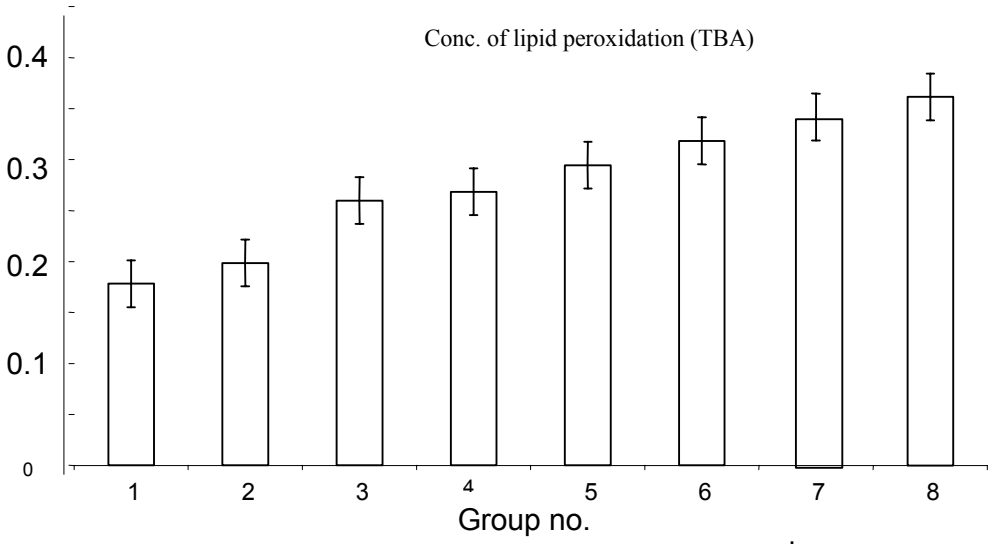

Fig. 2. Variation of lipid peroxidation as glucose level increases.

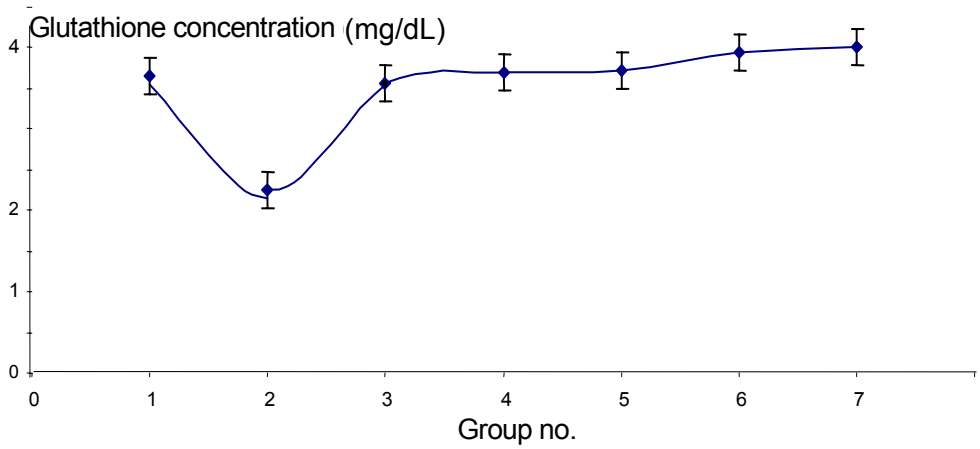

Fig. 3. Concentration of glutathione of control and treated groups.

Fig. 4 represents lipid peroxidation level for the normal and other diabetic groups after administration with H_2O_2 and H_2O_2 in addition to vitamins E, A, C, ginseng and NADH.

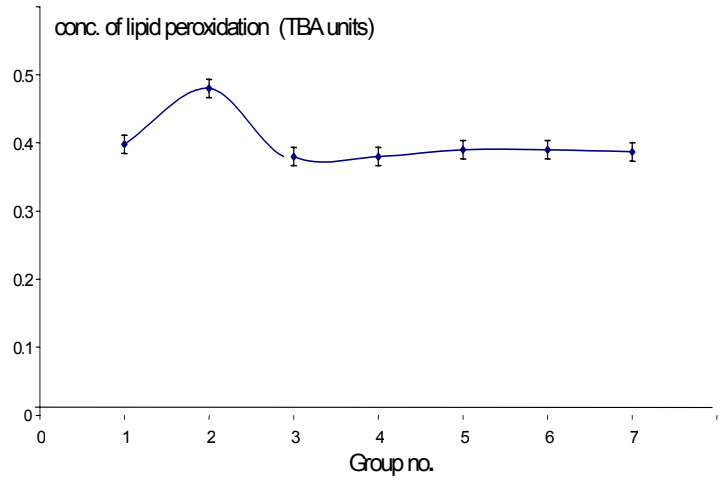

Fig. 4. Concentration of lipid peroxidation of control and treated groups.

It can be shown that the concentration of lipid peroxidation has been increased in (group 2) by about 27.1%. After administration with vitamins E, A, C, ginseng, and NADH the lipid peroxidation has been decreased by about 20.8%, 20.6%, 18.75%, 18.6% and 19.4%, respectively.

The measurements of electrical conductivity and intrinsic viscosity of diabetic and control subjects are illustrated in Table 4.

Table 4

The mean values of electrical conductivity and intrinsic viscosity of diabetic patients as compared to controls

	Conductivity (μS/cm)	Intrinsic viscosity (Poise)
Control	77.48 ±1.79	3.45 ±0.03
Subjects	57.06 ± 2.01	5.54 ±0.03

This table shows that the electrical conductivity of diabetic cases, group 5, decreased by about 26% while the intrinsic viscosity increased by about 61%.

The results also recorded an increase in met-hemoglobin concentration as a result of diabetes as Table 5 shows.

Table 5

The mean values of met-Hb concentration in diabetic patients as compared to control

	Met-Hb (mg/dL)
Control	0.38±0.01
Subjects	2.26 ±0.07

DISCUSSION

In addition to being a major cofactor for GSH-peroxidase, GSH, as has been reported, is necessary for the stability of sulfhydryl-containing enzymes and protects hemoglobin and many other cofactors from oxidation [17]. Tissue glutathione plays a central role in antioxidant defenses; it can detoxify reactive oxygen species such as hydrogen peroxide and lipid peroxides [32]. GSH was also reported to play a critical role in many cellular processes, including the metabolism and detoxification of oxidants, metal and other reactive electrophilic compounds of both endogenous and exogenous origin [17].

The decrease in glutathione content, which is shown in the results of the present work, seems to be due to its oxidation to GSSG (oxidized glutathione). The increase in GSSG concentrations as the glucose level increases in blood attributes the diabetic cases to some oxidative process. This indicates that GSH/GSSG redox system plays a significant role in diabetes. In other words, the GSH/GSSG ratio seems to play a major role in the modulations of glucose homeostasis in diabetes as it was also reported before [25]. It was also found that the levels of GSSG in plasma of diabetic subjects were higher than those of controls [7].

Dyslipidemia and possibly lipid peroxidation play important roles in the development of macro- and microvascular disease in type 1 diabetes mellitus [18]. The lipid peroxidation, as it was suggested, might be associated with a consumption of hydrogen peroxide (H_2O_2) yielding more reactive oxygen-derived species such as the hydroxyl radicals [20].

Glutathione depletion is relative to lipid peroxidation. Glutathione depletion usually renders the animal more susceptible to free radicals – mediated damages, especially the damage induced by cellular lipid peroxidation. Therefore, glutathione depletion, as it has been observed is accompanied by an increase in the amount of TBA reactants in experimental animals. This agrees with the results obtained in the present work. The results stress therefore that free radicals are involved in diabetes mellitus cases as it has been also suggested [4]. In other words, diabetes mellitus symptoms might be attributed to oxidative processes.

Antioxidants were found to protect biological systems against the potential effects of processes that generate reactive oxygen species. They were also referred to be scavengers. The successful antioxidant was therefore defined to be that one which could prevent formation of free radicals and convert oxidants to less toxic species. Targeting antioxidants to mitochondria *in vivo* was suggested to be a promising new therapeutic strategy in a wide range of human diseases such as Parkinson's disease, diabetes, and Friedreich's ataxia where mitochondrial oxidative damage underlies the pathology [39].

Diabetes was observed to be accompanied by an increased production of free radicals and/or impaired antioxidant defense capabilities, indicating a central contribution for reactive oxygen species in the onset, progression, and pathological consequences of disease [1].

It has been suggested that hyperglycemia may contribute to the pathophysiology for hypertension in diabetes by generating an oxidative stress [5]. Generation of reactive oxygen species may play an important role in the etiology of diabetic complications. This hypothesis is supported by evidence that many biochemical pathways, strictly associated with hyperglycemia (glucose auto-oxidation, polyol pathway, prostanoid synthesis, protein glycation), can increase the production of free radicals. Many of the adverse effects of high glucose on endothelial functions, such as reduced endothelial-dependent relaxation and delayed cell replication, are reversed by antioxidants [13]. Analysis of blood GSH showed that its concentration level was significantly lower in diabetic cases [30]. Abnormalities of both free radical activity and ascorbic acid (vitamin C) metabolism have been documented in diabetes [34].

The pentose phosphate pathway was impaired in endothelial cells cultured under conditions of high-glucose and oxidative stress, resulting in a decreased supply of NADPH to various NADPH dependent pathways, including GSH redox cycle [2].

It has been reported that ascorbate (vitamin C) is a free radical scavenger against toxic effects of active oxygen, which can be generated by the action of xanthine oxidase on xanthine. L-Ascorbic acid, a water-soluble vitamin, is a modified hexose with strong acidic and reducing properties. Dehydro ascorbate also undergoes further oxidation leading to a loss of biological activities [34].

Vitamin E is located in biological membranes and plays an important role against oxidative damage of the membrane [10]. Supplementation of vitamin A, E and C was found to decrease lipid peroxidation to some extent in diabetic rats and they can be valuable candidates in the treatment of the complications of diabetes [11]. Fatty acids metabolism was observed to present alterations in the diabetes syndrome and that the status of the antioxidants E and A was affected [36].

Vitamin A was found to link the triggering of the replication mechanism, the stabilization of cellular and intracellular membranes and the glycoprotein synthesis [19]. The subjects with NIDDM, which is characterized by insulin resistance without insulin deficiency, were found to be associated with normal vitamin A status [3].

NADH exists in reduced form in biological systems. It can protect against oxidative stress. This leads to the appearance of NAD, the oxidized form of NADH. The possible abnormalities in NADH/NAD redox system were reported to be the most notable changes occurring in diabetes [42].

Ginseng also showed important implications for the treatment and prevention of diabetes mellitus [21] and a functional efficacy in consumers with diabetes [12] where panax quinquefolius was found to increase insulin production and reduce the death of pancreatic beta cells [43].

The increase in the viscosity which leads to a less quantity of water absorbed by blood cells was observed to reduce the electrical conductivity [14].

The results therefore suggest that diabetes mellitus can be attributed to an oxidative stress through which the redox potentials of vitamin C, NADH and GSH are disturbed. This interprets the enhancement in concentration of met-hemoglobin, shown in these results, as a result of disturbance in the redox potential of the met-hemoglobin reductase and its coupling enzymes resulting in a state of hypoxia, i.e. higher oxygen affinity of diabetic hemoglobin than normal [28].

CONCLUSION

The hydrogen peroxide (H_2O_2) is produced during the progress of diabetes, while glutathione (GSH) content is reduced. Each of vitamin E, vitamin A, vitamin C, ginseng extract and nicotinamide adenine dinucleotide (NADH) showed good effects on both enhancement of glutathione content (up to 76.9%) in the blood and decreasing lipid peroxides (to 19.4%) after 24 hours of administrating the rats with H_2O_2. Results therefore show that diabetes can be attributed to an oxidative stress.

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