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Comparative Biochemistry and Physiology Part A 135 (2003) 539–547

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# Oxidative stress in streptozotocin-induced diabetic rats: effects of garlic oil and melatonin

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Received 30 August 2002; received in revised form 19 March 2003; accepted 20 March 2003

**Abstract**

In the present study, oxidative stress in diabetic model and the effect of garlic oil or melatonin treatment were examined. Streptozotocin (60 mg/kg body weight, i.p.)-induced diabetic rats, showed a significant increase of plasma glucose, total lipids, triglyceride, cholesterol, lipid peroxides, nitric oxide and uric acid. Concomitantly, significant decreases in the levels of antioxidants ceruloplasmin, albumin and total thiols were found in the plasma of diabetic rats. Lipid peroxide levels were significantly increased in erythrocyte lysate and in homogenates of liver and kidney, while superoxide dismutase (SOD) activities were decreased in tissue homogenates of liver and kidney. Treatment of diabetic rats with garlic oil (10 mg/kg i.p.) or melatonin (200 μg/kg i.p.) for 15 days significantly increased plasma levels of total thiol, ceruloplasmin activities, albumin. Lipid peroxides, uric acid, blood glucose, total lipid, triglyceride and cholesterol were decreased significantly after treatment with garlic oil or melatonin. Nitric oxide levels were decreased significantly in rats treated with melatonin only. In erythrocytes lysate, glutathione *S*-transferase (GST) activities were increased significantly in rats treated with garlic oil or melatonin, while lipid peroxides decreased significantly and total thiol increased significantly in melatonin or garlic oil treatment, respectively. In liver homogenates of rats treated with garlic or melatonin, lipid peroxides were decreased significantly, and GST activities increased significantly, while SOD activities were increased significantly in liver and kidney after garlic or melatonin treatment. The results suggest that garlic oil or melatonin may effectively normalize the impaired antioxidants status in streptozotocin induced-diabetes. The effects of these antioxidants of both agents may be useful in delaying the complicated effects of diabetes as retinopathy, nephropathy and neuropathy due to imbalance between free radicals and antioxidant systems. Moreover, melatonin may be more powerful free radical scavenger than garlic oil.


*Keywords:* Streptozotocin; Diabetic rats; Oxidative stress; Free radicals; Antioxidants; Garlic oil; Melatonin; Cholesterol; Triglyceride; Superoxide dismutase; Glutathione *S*-transferase

## 1. Introduction

Recently, attention has been focused on the relationship between 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

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1095-6433/03/$ - see front matter © 2003 Elsevier Science Inc. All rights reserved.
doi:10.1016/S1095-6433(03)00114-4

radical production and scavenger systems somehow become unbalanced, cells are exposed to oxidative damage resulting in cell injury (Parinandi et al., 1990; Griesmacher et al., 1995).

Diabetes has been shown to be a state of increased free radical production (Baynes, 1991). Mechanisms that contribute to the formation of free radicals in diabetes may include not only increased non-enzymatic and auto-oxidative glycosylation, but also metabolic stress resulting from changes in energy metabolism, levels of inflammatory mediators, and the status of antioxidant defense (Griesmacher et al., 1995).

Streptozotocin as an antibiotic and anticancer agent has been widely used for inducing type 1 diabetes in a variety of animals by affecting degeneration and necrosis of pancreatic β-cells (Merzouk et al., 2000). Evidence is accumulating suggestion that free radicals play a crucial role in the streptozotocin-induced diabetes. Damasceno et al. (2002) and Gul et al. (2002) reported that streptozotocin produced oxidative stress and depletion of antioxidant systems in both blood and tissues particularly, liver. Moreover, Kedziora et al. (2002) found a reduction in antioxidant systems and elevation in lipid peroxidation in kidney of streptozotocin-induced diabetic rats.

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). Garlic (*Allium sativum*) has been used in herbal medicine for centuries for various ailments such as cardiovascular risk factors and diabetes (Jain et al., 1973). Melatonin is a powerful antioxidant has been secreted from the pineal gland. Anwar and Moustafa (2001) and Reiter and Tan (2002) reported that melatonin has a powerful antioxidant effects and reduced lipid peroxidation. Melatonin itself is an oxyradical scavenger and stimulates the endogenous antioxidant systems; superoxide dismutase (SOD), glutathione oxidase, glutathione *S*-transferase (GST) and total thiol in blood and in liver (Reiter and Tan, 2002; Martinez-Cruz et al., 2002).

This work was conducted to elucidate probable changes in free radicals (lipid peroxides and nitric oxide) and antioxidants (total thiols; albumin; uric acid; catalase; ceruloplasmin; SOD and GST in either plasma or erythrocyte lysate or liver and kidney homogenates of diabetic rats induced by streptozotocin. We analyzed the effect of garlic oil or melatonin treatment on oxidative stress and antioxidant balance in streptozotocin-induced diabetic rats.

## 2. Materials and methods

### *2.1. Chemicals*

All chemicals including melatonin were all obtained from Sigma (St. Louis, MO).

### *2.2. Induction of diabetes*

Fifty seven (57) adult healthy male Sprague–Dawley rats with body mass of approximately 200–225 g were used. Rats were purchased from Animal house of faculty of medicine, Assiut University. The animals were conditioned at room temperature and at natural photoperiods for 1 week before study. A commercial balanced diet and tap water ad libitum were provided. The animals were divided into two groups, the first group received saline solution (i.p.) and it was kept as control. The second group (45 rats) were injected with a single dose of streptozotocin (60 mg/kg, i.p.) dissolved in 0.01 M citrate buffer, pH 4.5, immediately before use. Three days later blood glucose levels were determined in this group in whole blood samples collected from the tip of the tail. The rats injected with streptozotocin were considered as diabetics if the fasting blood glucose levels was $>5.0$ mmol/l. Diabetic animals were further divided into three groups of 12 rats each. The second group was kept as diabetic group, the third group was diabetic and injected daily with garlic oil (10 mg/kg, i.p. for 15 successive days). The fourth group was diabetic and injected with melatonin (200 μg/kg i.p. for 15 successive days). Rats in all groups were sacrificed after 15 days. Blood samples were drown by cardiac puncture into tubes containing EDTA. Liver and kidney were excised immediately and homogenized in volume/tissue ratio of 100 mM phosphate buffer (pH 7.4), containing 22 mg% EDTA. The tissue homogenate was then stored at $-20$ °C for determination of lipid peroxides, total thiols, total protein and activities of catalase, SOD and GST. Blood sample was centrifuged to separate plasma and RBCs. Erythrocyte lysates was prepared after washing the RBCs with saline several times. RBCs were hemolyzed by addition of distilled water. The

content of hemoglobin was determined by a commercial kit (Boehringer Mannheim, Germany).

### 2.3. Lipid peroxides and nitric oxide

Lipid peroxide levels were measured in plasma, hemolysate and tissue homogenates as thiobarbituric acid reactivity (TBARS). The product of the reaction between malondialdehyde and thiobarbituric acid was measured as described by Thayer (1984). Nitric oxide was determined in plasma as nitrite concentration after reduction of nitrate to nitrite. The reaction was performed at 22 °C for 20 min and the absorbance at 546 nm was measured using $NaNO_3$ solution as standard (Ding et al., 1988).

### 2.4. SOD, Catalase, GST and ceruloplasmin activities

SOD activity in plasma, hemolysates and tissue homogenates was determined according to its ability to inhibit the autooxidation of epinephrine at alkaline medium (Misra and Fridovich, 1972). GST activity in hemolysates and tissue homogenates was chemically determined using 1-chloro-2,4-dinitrobenzene as substrate (Habig et al., 1974). Catalase activity in hemolysate and tissue homogenates was determined based on its ability to decompose $H_2O_2$ and measured at 240 nm (Luck, 1963). Ceruloplasmin activity was determined using a para-phenylenediamine dihydrochloride method (Houchin, 1959).

### 2.5. Total plasma thiols, uric acid and albumin

Total thiols was determined chemically as described by Ellman (1959). Albumin was determined colorimetrically using a commercial kit (Sclavo Diagnostics, Italy). Uric acid was determined by enzymatic colorimetric method using commercial kit (Biocon, Burbach/Germany).

### 2.6. Plasma cholesterol, triglycerides and total lipids

Cholesterol was measured by enzymatic colorimetric method using commercial kit (Biocon). Triglycerides were determined by enzymatic hydrolysis of triglycerides with subsequent determination of liberated glycerol by colorimetry (Boehringer). Total lipids were chemically determined by the phosphovanillin method (Knight et al., 1972).

### 2.7. Total protein and blood glucose

Total proteins in plasma and tissue homogenates of kidney and liver was determined by a Biurer-tartrate method using commercial kit (Sclavo Diagnostics). Blood glucose level was determined by commercial kit (Biocon).

### 2.8. Statistical analysis

Data are expressed as mean $\pm$ S.E.M. for all parameters in plasma, erythrocyte lysate and tissue homogenates. The data were analyzed by analysis of variance (ANOVA) with Bonferroni's post-test for multiple comparisons with confidence intervals at 90% as appropriate, using Graph pad prism 3.

## 3. Results

The characteristic abnormalities observed in the diabetic rats were shown in (Fig. 1). In diabetic rats blood glucose, total lipids, and cholesterol is increased significantly ($P<0.001$) while triglyceride is increased significantly ($P<0.01$) when compared with control animals. A reduction was observed in total lipids, and cholesterol ($P<0.001$) in diabetic rats treated with garlic oil or melatonin and blood glucose and triglyceride decreased significantly ($P<0.01$) when compared with diabetic group. No significant difference was seen between the treatment of rats with garlic oil or those treated with melatonin.

Table 1 shows that plasma levels of lipid peroxides and uric acid were significantly ($P<0.05$) increased in diabetic rats while albumin was reduced in diabetic rats ($P<0.05$). Nitric oxide was increased significantly ($P<0.001$) in diabetic rats, while total thiol and ceruloplasmin, were reduced significantly ($P<0.001$) when compared with control. Treatment with garlic oil or melatonin significantly increased total thiol and ceruloplasmin activity. Also treatment with garlic or melatonin significantly decreased ($P<0.05$, $P<0.001$) lipid peroxides and uric acid, respectively. Rats treated with melatonin only showed a significant decrease ($P<0.001$) in nitric oxide. Nitric oxide was significantly increased ($P<0.01$) in diabetic rats treated with melatonin when compared with those treated with garlic oil.

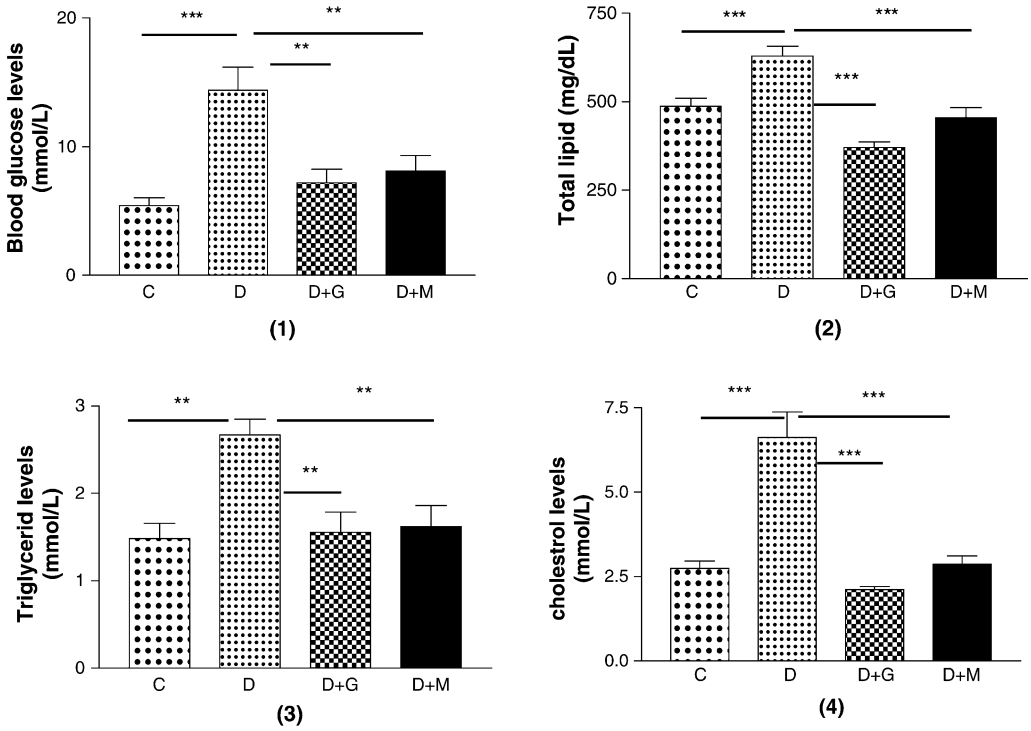


Fig. 1. Blood glucose levels (1), total lipid (2), triglyceride (3) and cholesterol (4) levels in control (C), diabetic (D), diabetic treated with garlic oil (D+G) and diabetic treated with melatonin (D+M) in different experimental groups. ANOVA test with Bonferroni's post-test, using Graph pad prism 3 were done. ***$P<0.001$, **$P<0.01$.

Erythrocyte lysates showed a significant increase in lipid peroxides in diabetic rats compared to the controls (Table 2). GST activities were increased significantly ($P<0.01$) in diabetic rats treated with garlic or melatonin. Total thiol was increased significantly ($P<0.05$) only in rats treated with garlic oil.

Streptozotocin treated rats showed a significant ($P<0.001$) increase in the lipid peroxides in liver compared with control (Table 3), while SOD activities were decreased significantly ($P<0.05$ and $P<0.001$) in liver and kidney, respectively in diabetic rats. In diabetic rats treated with garlic or melatonin, lipid peroxides were decreased signifi-

Table 1
Levels of lipid peroxides, nitric oxide and antioxidants in plasma of rats

| | Control rats | Diabetic rats | Diabetic rats treated with garlic oil | Diabetic rats treated with melatonin |
|---|---|---|---|---|
| LPO (nmol/ml) | 0.27±0.02 | 0.53±0.12* | 0.26±0.02* | 0.24±0.05* |
| Nitric oxide (ng/ml) | 144.6±11.35 | 254.5±16.58*** | 238.4±24.11 | 155.05±8.05***[a] |
| Total thiols (nmol/ml) | 1.09±0.11 | 0.46±0.10*** | 1.23±0.12*** | 1.20±0.09*** |
| SOD (ng/ml) | 136.9±11.2 | 94.13±13.12 | 128.9±13.9 | 132±9.05 |
| Ceruloplasmin (mg/l) | 285.4±14.8 | 124.6±12.2*** | 299.3±10.8*** | 349.1±20.1*** |
| Albumin (g/l) | 43.8±1.9 | 33.3±3.2* | 39.4±1.2 | 32.1±3.1 |
| Uric acid (mmol/l) | 0.37±0.03 | 0.50±0.4* | 0.32±0.01*** | 0.39±0.03* |

*$P<0.05$, **$P<0.01$ and ***$P<0.001$ between diabetic group and control one and between diabetic group vs. diabetic treated with garlic or melatonin.

[a] $P<0.05$ between diabetic rats treated with garlic oils and diabetic rats treated with melatonin.

Table 2
Levels of lipid peroxides (LPO), total thiols, and the activities of superoxide dismutase (SOD), catalase and glutathione *S*-transferase (GST) in the erythrocytes lysate of the different experimental groups

| | Control rats | Diabetic rats | Diabetic rats treated with garlic oil | Diabetic rats treated with melatonin |
|---|---|---|---|---|
| LPO (pmol/mg Hb) | 13.92±2.29 | 24.86±4.18* | 14.41±2.55 | 10.22±1.09** |
| Total thiols (nmol/mg Hb) | 21.18±2.10 | 15.24±1.62 | 24.57±2.52* | 22.01±2.84 |
| GST (nmol/min/mg Hb) | 214.9±37.2 | 179.0±20.8 | 377.8±50.8** | 390.98±30.98** |
| SOD (ng/mg Hb) | 5.50±0.65 | 3.34±0.74 | 5.78±0.96 | 5.99±1.09 |
| Catalase (U/mg Hb) | 0.61±0.09 | 0.32±0.06 | 0.57±0.12 | 0.51±0.08 |

*$P<0.05$ and **$P<0.01$ between diabetic group and control one and between diabetic group vs. diabetic treated with garlic or melatonin.

cantly ($P<0.001$) in liver and kidney homogenates. In the same groups, the activities of GST and SOD were increased significantly ($P<0.01$) in liver of rats treated with garlic. In melatonin treated rats, GST activities were increased significantly ($P<0.001$) in liver tissues, while SOD activities were increased significantly ($P<0.01$) in liver and kidney.

## 4. Discussion

Diabetes produces substantial changes in intracellular metabolism in many tissues including liver and kidney (Rifkin and Porte, 1990; Parinandi et al., 1990). The β-cell toxicity caused by streptozotocin is apparently due to injury in β-cell and elevation of local free radicals in β-cell after increasing free radicals in other body organs (Muruganandan et al., 2002; Gorgun et al., 2002). In the present study, diabetic rats induced by streptozotocin showed the expected elevation in plasma glucose, total lipids, triglycerides and cholesterol, confirming abnormalities of glucose and lipids in diabetes (Verges, 1991; Manzato et al., 1993; Merzouk et al., 2000). However, treatment of the diabetic rats with garlic oil decreased to some extent plasma glucose and lipids, but not to control levels. Kumar and Reddy (1999) found

Table 3
Levels of lipid peroxides (LPO), total thiol and activities of superoxide dismutase (SOD), glutathione *S*-transferase (GST) and catalase in tissue homogenates of liver and kidney in different experimental groups

| | Control rats | Diabetic rats | Diabetic rats treated with garlic oil | Diabetic rats treated with melatonin |
|---|---|---|---|---|
| LPO (nmol/mg proteins) | | | | |
| Liver | 1.26±0.08 | 2.08±0.19*** | 1.04±0.16*** | 1.00±0.09*** |
| Kidney | 1.12±0.09 | 1.29±0.23 | 0.97±0.08 | 0.95±0.04 |
| Total thiols (nmol/mg proteins) | | | | |
| Liver | 2.98±0.36 | 1.89±0.27 | 3.09±0.53 | 4.05±1.02 |
| Kidney | 7.75±0.75 | 4.04±0.87 | 5.81±1.03 | 6.55±1.59 |
| GST (nmol/min/mg proteins) | | | | |
| Liver | 603±60 | 510±48 | 752±45** | 850±40*** |
| Kidney | 548±79 | 420±50 | 520±43 | 550±43 |
| SOD (ng/mg proteins) | | | | |
| Liver | 1.34±0.10 | 0.50±0.10* | 1.47±0.26** | 1.55±0.25** |
| Kidney | 2.09±0.18 | 1.06±0.14*** | 1.55±0.11 | 1.98±0.21** |
| Catalase (U/mg proteins) | | | | |
| Liver | 0.80±0.11 | 0.44±0.06 | 0.38±0.05 | 0.76±0.08 |
| Kidney | 1.45±0.14 | 0.93±0.14 | 1.04±0.17 | 1.39±0.21 |

*$P<0.05$, **$P<0.01$ and ***$P<0.001$ between diabetic group and control and between diabetic group vs. diabetic treated with garlic or melatonin.

that garlic extract reduced blood glucose levels in alloxan-induced diabetes. Moreover, garlic oil in diabetes not only corrected hyperglycemia but also reduced cholesterol level in blood (Duncan, 1999).

Treatment of diabetic rats with melatonin decreased blood glucose, triglyceride, total lipids and cholesterol levels. Also, melatonin reduced blood glucose levels, although some hyperglycemia remained. These results are in agreement with Montilla et al. (1998) and Gorgun et al. (2002), they found that melatonin treatment reduced blood glucose levels to control levels and reduced hyperlipidemia in diabetic rats. Some authors have tried to explain the possible mechanism of hypoglycemic effects of melatonin. Shima et al. (1997) reported that melatonin suppressed hyperglycemia caused by intracerebroventricular injection of 2-deoxy-D-glucose in rats. They reported that melatonin injection suppressed blood glucose levels possibly through a brain site. Maitra et al. (2000) reported that melatonin may decrease blood glucose levels through its role on catecholaminergic responses. Moreover, Nishida et al. (2002) reported that subcutaneous implantation of a melatonin-releasing pellet resulted in improved lipid metabolism in diabetic rats.

There is an evidence that streptozotocin-induced diabetes releases free radicals (Muruganandan et al., 2002). As glutathione is closely linked to glucose metabolism via NADPH of hexose monophosphate shunt, it is logical that free radical metabolism is altered in diabetes. Changes in SOD and catalase activity, glutathione and vitamin E levels have been reported (Karpen et al., 1982; Loven and Oberley, 1985; Oberley, 1988). In the present experiment, streptozotocin treatment caused a significant increase in the lipid peroxidation in plasma, erythrocytes lysate, and liver and kidney. Basically, in diabetic rats increased lipid peroxidation was associated with hypertriglyceridemia (Morel and Chisolm, 1989). Moreover, the lipid content of cell membranes seems to be disrupted by diabetes as proved by increased non-enzymatic glycation, lipid peroxidation and cholesterol/phospholipid ratio (Watala and Winocour, 1992). Increased glycation of collagen and plasma proteins in diabetes may stimulate the oxidation of lipids, which in turn may stimulate auto-oxidative reactions of sugars enhancing damage to both lipids and proteins in the circulation and continuing the cycle of oxidative stress (Baynes, 1991). Matkovics et al. (1982) found that lipid peroxides were increased in liver, unchanged in kidney of diabetic rats. Unaltered lipid peroxides in kidney may be due to the increased resistance of kidney toward lipid peroxidation (Parinandi et al., 1990).

Nitric oxide synthase is present in pancreatic β-cells and may be involved in the release of insulin under normal physiological conditions (Moncada et al., 1991). However, findings suggest that induction of nitric oxide formation may play a role in the destruction of the β-cells during the development of type 1 diabetes (Corbbet et al., 1993). In the present study, plasma nitrite as end product of nitric oxide activity was elevated in the untreated diabetic rats. Similar results were obtained by Welsh et al. (1994) and Anggered (1994). Nitric oxide reacts with oxygen yielding nitrogen dioxide or peroxnitrities; both are strongly oxidative and more cytotoxic than nitric oxide itself (Neri et al., 1995).

Uric acid is considered as one of non-enzymatic antioxidant, but increased production of uric acid means increased free radical production due to activation of the xanthine oxidase enzyme system (Nemeth et al., 2002). In our experiment, uric acid levels were increased in diabetic rats. This may be due to metabolic disturbance in diabetes reflected in high activities of xanthine oxidase, lipid peroxidation and increased triglyceride and cholesterol (Madianov et al., 2000). Moreover, protein glycation in diabetes may lead to muscle wasting and increased release of purine, the main source of uric acid as well as in activity of xanthine oxidase.

Reduced antioxidant levels as a result of increased free radical production in experimental diabetes have been reported by many authors (Grankvist et al., 1981; Saxena et al., 1993; Giugliano et al., 1995). In the present study, streptozotocin treatment caused a significant depletion of both enzymatic and non-enzymatic antioxidants in plasma or erythrocyte lysates or the homogenates of both liver and kidney. SOD and catalase activities in streptozotocin induced diabetic rats were very reasonable (Muruganandan et al., 2002). Accordingly, SOD treatment can protect in vivo or in vitro against the high toxic potential of the superoxide radicals in alloxan-induced diabetic rats (Grankvist et al., 1981; Abdel-Rahman et al., 1992). It was also found a significant decrease was also found in plasma ceruloplasmin and albumin, however, uric acid was increased.

In our experiment garlic oil or melatonin treatment generally normalizes oxidative stress in strep-

tozotocin diabetic rats. The antioxidant potential of garlic oil is attributed to the presence of organo sulfur compounds that modulate glutathione and GST activity (Hori et al., 1992). Moreover, the normalization of lipid peroxides in the diabetic rats that was treated by garlic oil were in agreement with data obtained by Imai et al. (1994) who found that garlic extract inhibited the thiobarbituric reactive substances in liver tissues. Also, lipid peroxidation as malondialdehydes was prevented by garlic treatment (Singh and Rao, 1995). However, garlic oil treatment failed to restore the nitric oxide elevation in the plasma of the diabetic rats. In this aspect, Henry et al. (1993) suggested that extracellular and intracellular thiols may prolong the nitric oxide release. Ide and Lau (2001) and Banerjee et al. (2002) found that garlic had a powerful antioxidant system and minimized intracellular oxidative stress. The authors also reported that garlic increases glutathione content and SOD and GST activities in cardiac muscles.

Melatonin treatment reduced oxidative stress (lipid peroxidation, nitric oxide and uric acid) while elevating enzymatic and non-enzymatic antioxidant systems in blood, erythrocyte lysates and liver and kidney homogenates. Anwar et al. (1998) reported that melatonin had a potent reducing effect on the production of lipid peroxides in rats exposed to cytotoxic drugs. The reduction of nitric oxide levels may be due to inhibition of nitric oxide synthase enzyme activity by melatonin (Storr et al., 2002). Moreover, melatonin significantly inhibited the accumulation of cGMP levels induced by L-arginine or sodium nitroprusside and finally reduced nitric oxide production (Saenz et al., 2002). Nitric oxide, involved in the neuropathy which is one of the complication of diabetes as a result of oxidative stress, is reduced after melatonin treatment (Chang et al., 2002). Storr et al. (2002) reported that melatonin inhibited nitric oxide synthase enzyme and reduced nitric oxide production. The reduction in uric acid levels after melatonin treatment may be due to reduction of lipid peroxidation, triglyceride and cholesterol, while elevation of these substances may increase uric acid synthesis.

Melatonin is one of the most important pineal indols and it has a potent free radical scavenger. Melatonin treatment increased SOD and GST activities in plasma, erythrocyte lysate and liver and kidney tissues. Moreover, Reiter and Tan (2002) reported that melatonin produced a dramatic decrease in free radicals production.

It is evident from the present study that garlic oil and melatonin supplementation may help in the prevention or/and protection against the free radicals production in diabetes. The antioxidant effects of melatonin were better and melatonin caused dramatic elevation in the antioxidant systems activity and reduced oxidative stress. However, garlic is better than melatonin in lowering blood glucose levels, total lipid, triglyceride and cholesterol.

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