A Comparative Study between Antioxidant Activity of Medical Ozone and Selenium on Experimentally-Induced Diabetes in Male Rats

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Abstract

Introduction: Ozone therapy has been used in recent decades and has been found useful in treatment of diabetes. It improves glycemia, prevents oxidative stress, and normalizes level of organic peroxides. Selenium is an essential dietary trace element that has an antioxidant effect. It functions as an integral part of glutathione peroxidase in addition to its important role in vitamin E metabolism and normal pancreatic functions.

Aim of Work: To determine the roles of ozone administration in amelioration of oxidative stress in STZ-induced diabetes so as to establish its potential use in the strategy for treatment of diabetes.

Material and Methods: This study was applied on 50 male albino rats that were divided into 5 equal groups: Control group, diabetic group, ozone-treated diabetic group (received three IP injections/week on alternating days of medical ozone at a dose of 1.2mg/kg BW), selenium-treated diabetic group (received oral selenium as sodium selenite at a dose of 1.5mg/kg BW) and ozone plus selenium-treated diabetic group (received both ozone and selenium in the same previous schedule). The study proceeded for 4 weeks then fasting blood glucose and albumin concentrations were measured. MDA, glutathione, SOD, catalase, glutathione peroxidase, and glutathione reductase were measured in liver tissue homogenate. In addition, a histopathological study of the pancreas was performed.

Results: There were a significant increase in liver catalase, SOD, glutathione, glutathione peroxidase, glutathione reductase and a significant decrease in blood glucose and liver MDA in rats received medical ozone and/or selenium when compared to the diabetic group. The study also demonstrated that medical ozone and selenium had a synergistic antioxidant action. The histopathological study of the pancreas showed less necrosis and vaculations in rats received medical ozone and/or selenium when compared to the diabetic group.

Conclusion: The study confirmed the involvement of oxidative stress in DM and the ability of medical ozone similarly like selenium to up-regulate the activities of antioxidant enzymes. Therefore, they could offer protection against oxidative damage in DM. Medical ozone and/or selenium could be used as an adjuvant therapy in control of diabetes and its complications.

Key Words: Medical ozone – Selenium – Antioxidant – Diabetes mellitus – Oxidative stress.

Introduction

DIABETES mellitus is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. In addition to hyperglycemia, several other factors like hyperlipidemia and enhanced oxidative stress play a major role in diabetic pathogenesis [2].

Oxidative stress results from an imbalance between radical generating and radical scavenging systems i.e. increased free radical production, reduced activity of antioxidant, defensive system, or both [2]. Implication of oxidative stress in the pathogenesis of diabetes is suggested not only by oxygen free radical generation but also due to non enzymatic protein glycosylation, autoxidation of glucose, impaired glutathione metabolism, alteration in antioxidant enzymes, formation of lipid peroxides, and decreased ascorbic acid level [3]. In humans some antioxidants are produced in the body and others are obtained from diet [4].

Considered a complementary therapy, ozone therapy has been used in recent decades and has been found useful in treatment of diabetes [5]. It improves glycemic control, prevents oxidative stress, and normalizes level of organic peroxides [6]. Ozone also stimulates production of superoxide dismutase, catalase, and glutathione peroxidase.
which are the enzymes that protect the cells from damaging effect of free radicals [8].

Selenium is an essential dietary trace element that has an antioxidant effect [7]. It functions as an integral part of glutathione peroxidase in addition to its important role in vitamin E metabolism and normal pancreatic functions [8].

The main purpose of this work is to determine the roles of ozone and/or combination of ozone and selenium administrations in amelioration of oxidative stress in STZ-induced diabetes to establish their potential use in the strategy for treatment of diabetes.

Material and Methods

Experimental animals:

Fifty adult male albino rats of a local strain were used in this study. They were 10 to 12 weeks old, weighed about 140-182g, and obtained from the Animal House of Faculty of Medicine, Cairo University, Egypt. They were housed in plastic well aerated cages with meshed floor at room temperature with the natural dark and light cycle, provided with commercial pelleted rodent food, and drunk water ad libitum. They were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. All animals received care according to ethical guidelines of Al-Azhar University. The experiment was done during the year 2015.

Induction of diabetes mellitus:

After an overnight fast (16h), type 1 diabetes was induced in rats by a single IP injection of streptozotocin in a dose of 50mg/kg BW [9]. Before injections, STZ was freshly dissolved in 10mmol/l cold sodium citrate buffer at pH 4.5 [10]. Drinking water was supplemented with 10% glucose for the first 48h after injection for protection against the drug-induced hypoglycemia [11]. Development of diabetes was confirmed 1 week after injection of STZ by measuring fasting blood glucose levels [11]. Only rats with fasting blood glucose levels greater than 200mg/dl and less than 400mg/dl were included in the experiment [12].

Ozone injection:

Medical ozone (mixture of 5% O₃ and 95% O₂) was obtained from Ext 50 Ozone equipment (manufactured by Longevity Resources Inc., Canada) in Prof. Taymour,s Unit for Research of Medical Ozone at Al-Azhar Faculty of Medicine, Cairo. Ozone equipment Fig. (1) is composed of ozone generator by which ozone concentration is adjusted and oxygen tank by which gas flow rate is adjusted. A special table (Table 1) is used to calculate ozone dose. The total ozone dose is equivalent to the gas volume (ml) multiplied by ozone concentration (µg/ml).

Table (1): Which is used to calculate the ozone dose.

<table>
<thead>
<tr>
<th>FLOW FLOW [LPM] [ml/min]</th>
<th>Ozone concentration regulator setting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10</td>
</tr>
<tr>
<td>1/32</td>
<td>31 12 31 46 60 73 85 95 105 113 120</td>
</tr>
<tr>
<td>1/16</td>
<td>62 7 16 26 36 46 56 68 79 90 100</td>
</tr>
<tr>
<td>1/8</td>
<td>125 3 12 21 29 37 45 53 61 71 80</td>
</tr>
<tr>
<td>1/4</td>
<td>250 7 13 18 23 28 34 39 43 47 51</td>
</tr>
<tr>
<td>1/2</td>
<td>500 1 4 7 9 12 15 19 22 24 26 28</td>
</tr>
<tr>
<td>3/4</td>
<td>750 1 3 5 7 9 11 14 16 17 18 20</td>
</tr>
<tr>
<td>1</td>
<td>1000 1 2 4 5 7 8 10 12 14 15 17</td>
</tr>
</tbody>
</table>

The total ozone dose is equivalent to the gas volume (ml) multiplied by ozone conc.

(A) Ozone equipment composed of ozone generator (a) and oxygen tank.

(B)
Experimental design:

Rats were classified into 5 equal groups (n=10 per group) as follows:

- **Group I**: Control group (normal) was kept under the same laboratory conditions of other groups for 4 weeks and received no treatment.

- **Group II**: Diabetic group was kept under the same laboratory conditions of other groups for 4 weeks and received no further treatment after streptozotocin injection.

- **Group III**: Ozone-treated diabetic group received three IP injections/week (on alternating days) of medical ozone at a dose of 1.2mg/kg BW [13] for 4 weeks.

- **Group IV**: Selenium-treated diabetic group received oral selenium (as sodium selenite dissolved in distilled water) at a dose of 1.5mg/kg BW [14] daily for 4 weeks.

- **Group V**: Ozone plus selenium-treated diabetic group received both ozone and selenium in a similar schedule to groups III and IV respectively for 4 weeks.

At the end of the experimental period, rats were fasted overnight (10-12 hours), anesthetized by diethyl ether, weighed then retro-orbital blood samples were collected in clean and dry test tubes, left 10 minutes to clot and centrifuged at 3000rpm for serum separation. The separated sera were used for biochemical analysis of glucose and albumin concentrations.

After blood collection, rats were sacrificed by cervical dislocation. Then, the pancreas and the liver were excised quickly from each rat. The pancreas was used for histopathological examination, and the liver was used for assessment of lipid peroxidation products and hepatic antioxidant status.

Chemicals:

STZ, sodium selenite, and chemicals used in measurement of antioxidant enzymes were purchased from Sigma Chemical Company (USA). Glucose kits were purchased from BioMerieux Company (France), and albumin kits were purchased from Spinreact Company (Spain). All other chemicals were of analytical grade and obtained from standard commercial supplies.

Biochemical assays:

I- Estimation of FBG level:

FBG was determined in serum according to the method of Siest and Schielef [15], using reagent kits purchased from bio Merieux chemicals (France).

II- Determination of serum albumin concentration:

Serum albumin binds selectively to the dye bromocresol green in an acidic medium. The increase in absorbance of the resulting albumin-dye complex, read at 630nm, is proportional to the albumin concentration.

III- Determination of liver lipid peroxidation:

Liver lipid peroxidation was determined according to the method of Preuss et al., [16].

IV- Determination of glutathione (GSH) content:

GSH was determined according to the procedure of Beutler et al., [17].

V- Determination of superoxide dismutase (SOD) activity:

Liver SOD activity was estimated according to the procedure of Marklund and Marklund [18].

VI- Determination of catalase (CAT) activity:

CAT (E.C.:1.11.1.6) activity in the liver was determined according to the method of Cohen et al., [19].

VII- Determination of glutathione peroxidase (GPx) activity:

GPx activity was determined according to the method of Kar and Mishra [20].

VIII- Determination of Glutathione Reductase (GR):

Glutathione reductase catalyses the reduction of oxidized glutathione (GSSG) by NADPH to GSH. The activity of the enzyme was measured by following the oxidation of NADPH spectrophotometrically at 340nm according to the method of Pinto and Bartley [21].

Preparation of liver tissue homogenate:

The liver was removed immediately after dissection from each animal, blotted between filter papers and weighed. About 0.5g of liver sample was homogenized in 4.5ml of ice-cold phosphate buffer saline (pH 7.5). Then, the homogenates were cold centrifuged for 10min at 3000rpm and the supernatant was stored at −20°C and used for determination of Lipid peroxidation products and antioxidant enzymes. The homogenization was carried out as described by Newsholme [22].
Histopathological study:

At the end of the experimental period, pancreas was immediately removed from each animal after being sacrificed, fixed in 10% neutral buffered formalin and transferred to the histopathology lab in Faculty of Veterinary Medicine, Beni-Suef University for preparation. The preparation of the samples for histopathological examination was performed as follows: The samples were washed in running water, then, dehydrated in graduated ethanol 50%, 70%, 95%, and 100% 2hrs for each. Then, the samples were cleared in 2 changes of xylene 2 hrs for each and embedded in paraffin wax at 70°C (3 changes 2hrs for each). The samples were blocked in paraffin wax and underwent microtomy. Five microns tissue sections were mounted on clean glass slides and stained with H & E stain [23]. Eventually, the slides were examined by a pathologist and the pictures were clicked with the help of a binocular microscope fixed with a camera.

Statistical analysis:

The results were analyzed using the the Statistical Package for the Social Sciences (SPSS for Windows, version 20.0; SPSS Inc., USA). Quantitative data were expressed as mean ± Standard Deviation (SD). Differences between groups means were analyzed by one way ANOVA test, and comparison between groups were analyzed by Tukey HSD test as a post hoc test. Values of $p<0.05$ were considered statistically significant.

Results

I- Study of FBG levels in the different animal groups:

The diabetic group (Group II) showed a significant increase in FBG when compared to the control group (Group I). Treatment of diabetic rats with either medical ozone or selenium (Groups III, IV respectively) exhibited a significant increase in FBG when compared to the control group but significantly alleviated FBG when compared to the diabetic group. Similarly, diabetic rats treated with a combination of medical ozone and selenium (Group V) showed a significant increase in FBG in comparison to the control group and a significant reduction when compared to the diabetic group. Diabetic rats treated with medical ozone had a significant reduction in FBG in comparison with diabetic rats treated with selenium. A combination of medical ozone and selenium therapy significantly decreased FBG when compared to mono-therapy by either medical ozone or selenium as shown in Fig. (2). One way ANOVA revealed that the general effect between groups on fasting blood glucose levels was significant throughout the experiment.

II- Study of % change of BW in the different animal groups:

Diabetic group showed a significant decrease in % change of BW when compared to the control group. On the other hand, treatment of diabetic rats with medical ozone and/or selenium for 4 weeks showed a significant decrease in the percentage change when compared to the control group but showed a significant increase in comparison to the diabetic group. Diabetic rats treated with medical ozone had a significant increase in % change of BW when compared to diabetic rats treated with selenium. A combination of medical ozone and selenium therapy significantly decreased the percentage change as compared to medical ozone monotherapy, while, and showed a non-significant change as compared to selenium monotherapy as illustrated in (Table 2). One-way ANOVA revealed that the general effect between groups on body weight changes was significant ($p<0.001$) throughout the experiment.

III- Study of hepatic lipid peroxidation in the different animal groups:

In the diabetic group, MDA levels were significantly increased when compared to the control group. Conversely, diabetic rats treated with medical ozone and/or selenium showed a significant decrease in MDA levels when compared to the diabetic group but showed a non-significant change when compared to the control group. No significant change in MDA levels were detected between groups III, IV, and V as shown in Fig. (3). One-way ANOVA revealed that the general effect between groups on liver MDA levels was significant ($p<0.001$) throughout the experiment.

IV- Study of liver GSH in the different animal groups:

Liver GSH content of the diabetic group showed a significant decline when compared to the control group. Treatment of diabetic rats with either medical ozone or selenium exhibited a significant decrease in GSH content as compared to the control group and a significant increase when compared to the diabetic group. On the other hand, GSH contents of the livers of diabetic rats were normalized by a combined therapy of medical ozone and selenium. In a comparison between the effects of medical ozone and selenium on liver GSH content of diabetic rats, medical ozone treatment showed a sig-
significant increase when compared to selenium treatment. A combination of medical ozone and selenium therapy significantly increased liver GSH content in diabetic rats when compared to monotherapy by either ozone or selenium Fig. (4). One-way ANOVA revealed that the general effect between groups on liver GSH content was significant (p<0.001) throughout the experiment.

V- Study of liver SOD activity in the different animal groups Fig. (5):

In the diabetic group, liver SOD activity was significantly decreased when compared to the control group. Medical ozone and/or selenium treatment to diabetic rats showed a significant decrease in the enzyme activity as compared to the control group; while, they increased SOD activity significantly when compared to the diabetic group. Diabetic rats received medical ozone treatment showed a significant increase in the enzyme activity as compared to selenium-treated group.

Diabetic rats received both medical ozone and selenium exhibited a significant increase in the enzyme activity as compared to selenium monotherapy but no significance demonstrated when compared to medical ozone monotherapy. One-way ANOVA revealed that the general effect between groups on liver SOD activity was significant (p<0.01) throughout the experiment.

VI- Study of liver CAT activity in the different animal groups:

CAT activity in the livers of diabetic group showed a significant decrease when compared to the control group. Treatment of diabetic rats with medical ozone and/or selenium showed a significant decrease in the enzyme activity as compared to the control group but increased the enzyme activity significantly when compared to the diabetic group. No significant change in CAT activity was detected between groups III, IV, V as shown in Fig. (6). One-way ANOVA revealed that the general effect between groups on liver CAT activity was significant (p<0.01) throughout the experiment.

VII- Study of liver GPx activity in the different animal groups:

Diabetic group exhibited a significant reduction in liver GPx activity when compared to the control group. Diabetic rats treated with medical ozone and/or selenium showed a significant decrease in GPx activity as compared to the control group but showed a significant increase in the enzyme activity when compared to the diabetic group. There was a significant change in the enzyme activity between ozone-treated and selenium-treated groups. However, diabetic rats treated with both medical ozone and selenium showed a significant amelioration as compared to ozone or selenium monotherapy Fig. (7). One way ANOVA revealed that the general effect between groups on liver GPx activity was significant (p<0.001) throughout the experiment.

VIII- Study of liver GR activity in the different animal groups Fig. (8):

Diabetic group showed a significant decrease in the activity of liver GR when compared to the control group. The enzyme activity was significantly decreased in diabetic rats treated with either medical ozone or selenium as compared to the control group but increased significantly as compared to the diabetic group. On the other hand, GR activity normalized by combined therapy of medical ozone and selenium. GR activity increased significantly in diabetic group treated with medical ozone as compared to diabetic rats treated with selenium. The enzyme activity increased significantly in diabetic rats treated with a combination of medical ozone and selenium as compared to either selenium or ozone monotherapy. One-way ANOVA revealed that the general effect between groups on liver GR activity was significant (p<0.01) throughout the experiment.

IX- Study of serum albumin levels in different groups:

The effects of medical ozone and/or selenium on serum albumin levels are represented in Fig. (9). One way ANOVA revealed that the general effect between groups on serum albumin levels was non-significant (p>0.05) throughout the experiment.

Histopathological study:

Current study showed that, pancreatic samples obtained from normal rats showed no histopathological alteration in the structure of the exocrine or the endocrine portions Fig. (10). Following streptozotocin administration, there were marked vaculations and reduced numbers of islet cells Fig. (11). Pancreatic samples obtained from diabetic rats treated with medical ozone and/or selenium showed normal acini and increased numbers of islet cells with minimal vaculations Fig. (12).
**Fig. (2): Effect of medical ozone and/or selenium on FBG levels.**

Data are expressed as Mean ± SEM.

- *: p<0.05
- **: p<0.01
- ***: p<0.001 versus Group I.

**: p<0.05
- ###: p<0.001 versus Group II.

- ¥: p<0.05
- ¥¥: p<0.01 versus Group IV.

•: p<0.05 versus Group III.

**Fig. (3): Effect of medical ozone and/or selenium on liver MDA.**

Data are Mean ± SEM.

- ***: p<0.001 versus Group I.

- #: p<0.01.

**Fig. (4): Effect of medical ozone and/or selenium on liver GSH.**

Data are Mean ± SEM.

- **: p<0.01.
- ###: p<0.001 versus Group I.

**: p<0.01.

**: p<0.01 versus Group II.

¥¥: p<0.05 versus Group IV.

•: p<0.05 versus Group III.

**Fig. (5): Effect of medical ozone and/or selenium on liver SOD activity.**

Data are Mean ± SEM.

- *: p<0.05.

**: p<0.01.

***: p<0.001 versus Group I.

**: p<0.01.

###: p<0.001 versus Group II.

¥: p<0.05 versus Group IV.

**Fig. (6): Effect of medical ozone and/or selenium on liver CAT activity.**

Data are Mean ± SEM.

- **: p<0.01.

***: p<0.001 versus Group I.

**: p<0.01.

###: p<0.001.

**Fig. (7): Effect of medical ozone and/or selenium on liver GPx activity.**

Data are Mean ± SEM.

- *: p<0.05.

**: p<0.01.

***: p<0.001 versus Group I.

###: p<0.001 versus Group II.

¥: p<0.05.

•: p<0.05 versus Group III.
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Fig. (8): Effect of medical ozone and/or selenium on liver GR activity.

Data are Mean ± SEM. **: p<0.01. ***: p<0.001 versus Group I. ¥: p<0.05. ¥¥: p<0.01. ¥¥¥: p<0.001 versus Group II. ¥¥¥¥: p<0.01 versus Group IV. •: p<0.05 versus Group III.

Fig. (9): Effect of medical ozone and/or selenium on serum albumin con.

Data are Mean ± SEM. Number of animals in each group is 10.

Fig. (10): Photomicrograph of H & E-stained pancreas section from a normal rat showing normal exocrine portion (blue arrow) and normal endocrine portion (green arrow). Connective tissue also showed (yellow arrow).

Fig. (11): Photomicrograph of H & E-stained pancreas section from a diabetic rat showing reduction in numbers of islet cells (green arrow) and marked vacuolations (black arrow).

Fig. (12): Photomicrograph of H & E-stained pancreas section from a diabetic rat treated with medical ozone showed increased numbers of beta cells (green arrow) and minimal vacuolations (black arrow).

Table (2): Effect of medical ozone and/or selenium on BW.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial BW (g)</th>
<th>Final BW (g)</th>
<th>% change of BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (control)</td>
<td>158.35±4.71</td>
<td>194.06±5.29</td>
<td>25.8±2.1</td>
</tr>
<tr>
<td>Group II (diabetic)</td>
<td>168.25±6.34</td>
<td>120.30±3.71***</td>
<td>26.63±2.36***</td>
</tr>
<tr>
<td>Group III (diabetic + medical ozone)</td>
<td>167.30±4.12</td>
<td>183.20±4.70###¥</td>
<td>10.91±0.79###¥</td>
</tr>
<tr>
<td>Group IV (diabetic + selenium)</td>
<td>163.40±4.64</td>
<td>170.02±6.04###¥</td>
<td>4.88±0.44###¥</td>
</tr>
<tr>
<td>Group V (diabetic + medical ozone and selenium)</td>
<td>161.00±6.19</td>
<td>175.00±6.65###¥</td>
<td>5.82±0.54###¥</td>
</tr>
<tr>
<td>F-Prob.</td>
<td>p&gt;0.05</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as Mean ± SEM. ***: p<0.001 versus Group I. ¥: p<0.05 versus Group IV. ¥¥: p<0.001 versus Group II. **: p<0.01. ***: p<0.001 versus Group III. •: p<0.05 versus Group III.
**Discussion**

In this study, there was a significant increase in fasting blood glucose level in diabetic group when compared to the control group which is in agreement with the results of Nagarchi et al. [24] who reported a significant increase in FBG after 1 week of a single dose of STZ (50mg/kg b.w.) in albino rats. These results could arise from a reduced uptake of glucose to peripheral tissues, increased glycogenolysis, and increased gluconeogenesis as a result of insulin deficiency due to destruction of β-cells of the islets of Langerhans in the pancreas by streptozotocin [25].

From our data, IP medical ozone administration at a very low dose (1.2mg/kg b.w.) to diabetic rats improved fasting blood glucose level significantly when compared to the diabetic group, but still increased significantly as compared to the control group. This result is in agreement with that of Saleh et al. [26]. This result may be attributed to the antioxidant activity of medical ozone which has been shown to reduce hyperglycemia induced by streptozotocin [27].

Also, selenium administration also diminished the hyperglycemia induced by STZ significantly when compared to the diabetic group. However, FBG level increased significantly when compared to the control group. This result is compatible with Zou et al. [28]. This may be explained, in part, through the integrity preservation of pancreatic β-cells and possibly by eliciting insulin-mimetic activity of selenium. Selenium has also been shown to accelerate renal glucose excretion in rats and stimulated glucose uptake in isolated rat adipocytes by translocating glucose transporters to the plasma membrane [29].

In addition, the current study showed a significant decrease in the percentage change of body weight of diabetic group as compared to the control group. This finding is in agreement with the reports of Nagarchi et al. [24] who attributed this decrease to loss of tissue proteins and muscle mass in diabetes. This result is also in accordance with that of Umran et al. [30] who attributed this decrease to the depressed synthesis of DNA and RNA in diabetic animals.

Treatment of diabetic rats in the present work with medical ozone induced a significant increase in percentage change of body weight as compared to diabetic group, and showed no significant difference as compared to the control group. These results are in agreement with the findings of Saleh et al. [26] who attributed the increase in BW of diabetic rats treated with medical ozone to the improvement of glycemic state.

The percentage change in body weight also increased significantly in diabetic rats supplemented with selenium when compared to diabetic group but decreased significantly in comparison to the control group. This result is compatible with Naziroglu and Cay [31] but disagrees with that of Al-Quraishy et al. [29] who stated that treatment of diabetic rats with selenium in a dose of 1mg/kg BW for 28 days failed to prevent reduction of body weight, and they attributed this decrease to a reduction in food intake, similar to the anorexigenic effect experienced by diabetic rats treated with vanadate, a well-established insulin-like agent. Our results regarding the significant reduction in body weight in relation to the control group could also be explained by the potential anorexigenic effect of selenium. The increase in percentage change of body weight of diabetic rats treated with selenium could be also ascribed to its ameliorative effect on the glycemic state [29].

In addition, the present study reported a significant increase in lipid peroxidation products, MDA in the livers of diabetic group in comparison to the control group. This observation agrees with that of Ghosh et al. [32] who suggested that peroxidative injury may be involved in the development of diabetes and its complications. The result could be explained in part by the high blood glucose level in diabetes because of accelerated metabolism by the thermic effect of food and increased mitochondrial respiration with a resultant increase in the production of superoxide anions [33] which leads to lipid peroxidation in cell membrane. Glucose autoxidation and protein glycation are important additional sources of free radicals during hyperglycemia [34].

Medical ozone treatment of diabetic rats in the current study revealed a significant reduction in hepatic MDA to a normal value. This is in accordance with Safwat et al. [35] who reported that ozone may have a potential to inhibit the oxidative damage of liver tissue. A similar result of normalization of MDA in liver tissue of diabetic rats treated with selenium has been shown. This result is in consistence with Ukperoro et al. [36] who demonstrated that the reduction of lipid peroxidation associated with selenium supplementation in diabetic rats could be due to a decreased level of oxygen free radicals.

In the present study, we observed a significant decrease in glutathione in liver tissue of diabetic
rats when compared to the control group. This result is in accordance with that of Oyenihi et al., [37] who attributed this decrease to the depletion of GSH, since NADPH, necessary for GSH regeneration, is constantly utilized by the polyol pathway that is prominent in chronic hyperglycemic conditions.

In diabetic rats treated with medical ozone, hepatic GSH content showed a significant reduction when compared to the control group. On the other hand, it increased significantly as compared to the diabetic group. This finding is supported by Husamettin [38] who attributed this increase to the hypoglycemic effect of ozone with resultant availability of NADPH which is needed for GSH regeneration.

In addition, there was a significant decrease in hepatic GSH content in diabetic rats received selenium treatment as compared to the normal rats but this value increased significantly in comparison to the diabetic group. This result is compatible with that of Zou et al., [28] who ascribed this result to a reduction in GSH depletion by oxidative stress which has been decreased by selenium administration.

The present study revealed a significant decrease in the activity of liver SOD in the diabetic group as compared to the control group. This result is supported by Amalan et al., [39] who reported that hyperglycemia can inactivate the antioxidant enzymes SOD, CAT and GPx by glycating their protein structure. The result is also concomitant with that of Sadi et al., [40] who reported a significant decrease in liver SOD resulted from a decrease in mRNA expression in hepatic cells of streptozotocin induced diabetic rats. On contrary, Oyenihi et al., [37] reported that streptozotocin injection in a dose of 50mg/kg had no effect on SOD activity in the liver of diabetic rats.

The results from this study showed that medical ozone treatment showed a significant decrease in the activity of liver SOD as compared to the control group and was able to significantly increase the enzyme activity in diabetic rats as compared to the diabetic group. This finding is supported by Lamberto et al., [41]. Who reported that this increase may result from stimulation of SOD gene expression by medical ozone. On contrary to our result, Yannis et al., [42] observed that treatment of diabetic rats with medical ozone had no effect on hepatic SOD.

The present study revealed a significant decrease in hepatic SOD activity in selenium-treated rats as compared to the control group but in comparison to the diabetic group, the enzyme activity increased significantly. This result is in accordance with that of Zou et al., [28]. This could be a result of decreased consumption of antioxidant enzymes that detoxify oxygen radicals generated by hyperglycemia as a consequence of improved glycemic state by selenium.

Our experimental results have shown that, a significant decrease in the activity of hepatic CAT in the diabetic group was observed when compared to the control group. This finding is in agreement with the reports of Ghosh et al., [32] but disagrees with Rakesh et al., [43] who reported an increase in liver CAT one week after streptozotocin injection, this could be due to the difference in the experimental duration of diabetes between their study and ours and or due to the differences in the methods of measurements.

Diabetic rats treated with medical ozone exhibited a significant decrease in catalase activity when compared to the control group. While, a significant increase was observed in the enzyme activity as compared to the diabetic group. This suggests that medical ozone promoted capture of $\text{H}_2\text{O}_2$, a precursor of hydroxyl radical, being the last one, capable of producing the peroxidation of unsaturated fatty acids, with resultant damage of the cell membrane functions [44].

Our results are similar to that of Helal et al., [45] who reported that medical ozone therapy reversed oxidative stress induced by hyperglycemia with resultant increase in the activity of catalase and other antioxidant enzymes in the livers of diabetic rats. The present study showed that catalase activity also increased significantly by selenium administration to diabetic rats in comparison to the diabetic group, but it still reduced in comparison to the control group. This is in concomitant with the results of Zou et al., [28] who attributed this increase to the antioxidant effect of selenium which improved redox status in hepatic cells.

GPx is a selenium dependent enzyme (selenoprotein). It helps to protect the cell from damage by free radicals like hydrogen and lipid peroxides and its action takes place in the presence of glutathione, the master antioxidant. GPx metabolizes hydrogen peroxide to water with the usage of reduced glutathione as a hydrogen donor [46].

In the current study, the activity of hepatic GPx significantly decreased in diabetic group as regard to the control group. This result is compatible with the findings of Ghosh et al., [32] but disagrees with...
Rakesh et al., [43] who reported an increase in the activity of GPx in the liver of diabetic rats one week after streptozotocin injection.

The reduction in GPx activity in our study could be due to increased glycation reactions [47], in addition to a decrease in the concentration of GSH that are associated with diabetes [48].

Our finding may also be due to deficiency of selenium in blood of diabetic group which is needed for synthesis of GPx. This explanation supported by Joshi et al., [49] who observed a significant decrease in serum selenium level of diabetic patients when compared to their control. The activity of hepatic GPx significantly decreased in medical ozone-treated rats as compared to the control group and improved significantly when compared to the diabetic group. This finding agrees with that of Yannis et al., [42] who attributed this improvement to the medical ozone antioxidant activity.

Similarly, selenium administration to diabetic rats significantly decreased hepatic GPx as compared to the control group, but improved significantly when compared to the diabetic group. This result is compatible with the results of Ukperoro et al., [56] who reported that PGx is a selenium dependent enzyme and so increased with selenium supplementation. Glutathione reductase is the enzyme responsible for GSH regeneration by reduction of oxidized glutathione at the expense of NADPH. Increase in GSSG during oxidative stress is generally transient as reduction by glutathione reductase is relatively rapid [50].

Following streptozotocin injection in the present study, there was a significant decrease in the activity of GR in the liver tissue of diabetic group in comparison to the control group. This finding is in consistence with the results reported by Ghosh et al., [32]. This may be explained by the increase in oxidative stress in hepatic tissue following streptozotocin injection which results in consumption of glutathione reductase and other antioxidant enzymes.

Diabetic rats treated with medical ozone exhibited a significant reduction in the activity of GR as compared to the control group. However, they showed a significant increase in the enzyme activity as compared to the diabetic group. This result agrees with Martinez et al., [6] who showed that ozone treatment for 10 consecutive days increased GR and other antioxidant enzymes significantly in diabetic rats when compared with the diabetic group. Similarly, GR was significantly decreased in diabetic rats treated with selenium as compared to the control group and significantly increased as compared to the diabetic one. This finding is supported by Can et al., [51] who found that treatment of diabetic rats by IP sodium selenite for 4 weeks increased GPx, GR, and GST significantly as compared to the diabetic group. On contrary, our results demonstrated no significant change in serum albumin level in all diabetic groups when compared to the control one. This result agrees with Nasrolahi et al., [52] and disagrees with Yokozawa et al., [53] who reported a significant decrease in serum albumin and total protein in diabetic rats as compared to the control group. Our results may be related to the duration of the experiment since blood albumin level may be normal in early stage of diabetes [54].

Our study showed a significant decrease in the percentage of damaged islets in diabetic rats treated with ozone when compared to the diabetic group, and they reported that ozone treatment could protect β-cells against STZ damage by reduction of oxidative stress. Regeneration of the pancreas follows STZ-induced damage may support the fact that pancreas contains stable (quiescent) cells which have the capacity of regeneration [56].

Treatment of diabetic rats with selenium in our study as well as with medical ozone treatment partially restored the pancreatic islets when compared to the diabetic group. Thus, in this aspect the effect of medical ozone was similar to that of selenium.

Treatment of diabetic rats with both medical ozone and selenium in this work also resulted in an increase in number of β-cells with less vacuolations and necrosis which denotes a partial regeneration in the islets owing to the combined antioxidant effect of both two agents.

In conclusion, the study confirmed the involvement of oxidative stress in diabetes and the ability of medical ozone, in combination with selenium to up-regulate the activities of some antioxidant enzymes. Therefore, depending on the results of our study which proved that medical ozone produced a protective effects against diabetes mellitus similar to or even better than that produced by selenium, but medical ozone is a natural product and more safe. Therefore, antioxidant therapy may be helpful in relieving many symptoms and complications observed in diabetic patients. Additional studies using medical ozone in combination with anti-diabetic drugs are needed to prove the role of ozone as a single or an adjuvant medication in the treatment of diabetes. In addition, further detailed investigations are in progress to elucidate the exact mechanisms by which medical ozone elicits its anti-diabetic properties.
A Comparative Study between Antioxidant Activity of Medical Ozone & Selenium


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