Some Oxidative Biomarkers and Antioxidant Genes Detection in Diabetic Male Rats

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors ODC and SCM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ODC and TU managed the analyses of the study. Authors ISI, ICM and BNE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Diabetes mellitus is a group of metabolic disorder in which there are high blood sugar levels over a prolonged period. This study assessed the effects of diabetes on levels of some oxidative biomarkers and pattern of antioxidant genes expression in the peripheral blood cell of diabetic male rats.

Methods: This is an experimental study that involved 40 apparently healthy adult male albino rats (Wistar strain) which were randomly assigned to five groups (A, B, C, D and E) of eight (8) animals each. Group A (Normal Control of 72 hours post diabetes induction), Group B (Diabetic rats of 72 hours post diabetes induction), Group C (metformin treated diabetic rats), Group D (Diabetic Control untreated) and Group E (Normal Control of 3 weeks post diabetes induction). Ten milliliters

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of fasting blood sample was collected from all the subjects. Serum levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (Gpx), vitamin C and vitamin E as well as peripheral blood antioxidant genes (CAT and CU-ZnSOD) were analyzed using standard methods. It was analyzed statistically using SPSS version 23.0.

**Results:** The mRNA of CAT and Cu–ZnSOD genes were up-regulated at 72 hour post diabetes induction and down regulated 3 weeks after confirmation of diabetes (P<0.05). The mean values of GPx, CAT, SOD, VIT C and VIT E were significantly higher in the treated diabetic group when compared with untreated diabetic control (P<0.05) while MDA was significantly lower in treated diabetics when compared with the untreated diabetic control (P<0.05). Also, blood mean levels of GPx, CAT, VIT C and VIT E were significantly lower in the diabetic groups (treated and untreated) when compared with non diabetic control (P<0.05) while MDA was significantly higher in the diabetic groups (treated and untreated) when compared with non diabetic control (P<0.05). Additionally, there was significant negative relationship of blood glucose with GPx in the untreated group (P<0.05).

**Conclusion:** The study suggests that hyperglycemia can cause expression of mRNA of CAT and Cu–ZnSOD genes on the peripheral blood cell in acute condition and significant alterations of oxidative stress biomarkers; however metformin treatment has showed not only hypoglycemic effect, but also anti-oxidant properties.

**Keywords:** Diabetes mellitus; oxidative biomarkers; antioxidant genes; rats.

**1. INTRODUCTION**

Diabetes mellitus (DM) also simply known as diabetes, is a group of metabolic disorder in which there are high blood sugar levels over a prolonged period [1]. Diabetes is a chronic condition that arises when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin produced. In other words, diabetes has been characterized as a chronic metabolic disorder of multiple aetiologies characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action or both [2,3]. This condition induce production of free radicals that result in oxidative stress over a prolong period of time.

Free radicals and related reactive species are ubiquitous molecules taking part in essential biological processes at the cellular level. They are highly reactive and unstable species capable of generating new radicals via chain-reactions with consequences to cause injuries and damages to healthier cells and cellular responses. Human cells have mechanisms to maintain the production and clearance of this reactive species in a homeostatic state, and abnormalities leading to the imbalance of this equilibrium, may cause a condition termed “Oxidative stress”.

Excess cellular radical generation can be harmful; however, if there is a significant increase in radical generation, or a decrease in radical elimination from the cell, oxidative cellular stress ensues [4]. Because of these changes in the generation of free radicals and its elimination in the peripheral blood cell, this process may exhibit changes in expression of some oxidative stress biomarker gene in the peripheral blood.

Hyperglycemia generates reactive oxygen species (ROS), which in turn cause damage to the cells in many ways. Consequently, imbalance between cellular generation and scavenging capacity of free radicals elicits tissue damage associated with diabetes pathology [5,6]. Over time, convincing evidence has established the role of free radicals and oxidative stress in the pathogenesis and development of complications from diabetes [7,8]. Hyperglycemia increases oxidative stress, which contributes to the impairment of the main processes that fail during diabetes, insulin action and insulin secretion. In addition, antioxidant mechanisms are diminished in diabetic patients, which may further augment oxidative stress [9,10]. The production of free radicals is engendered by uncontrolled hyperglycemia, which may occur via several routes [5] such as increased glycolysis [11]; intercellular activation of sorbitol (polyol) pathway [12]; autoxidation of glucose; protein kinase C (PKC) dependent activation of NAD(P)H oxidase [13]; increased hexosamine pathway flux; increased intracellular formation of advanced glycation end products (AGEs) [14]; increased expression of receptor for AGEs and its activating ligands [14] and non-enzymatic protein glycation [14]. Damage to the cells ultimately
Regulation of the cellular redox status depends on the rate of reactive oxygen species counterbalance and elimination by the enzymatic and/or non-enzymatic antioxidants. Human antioxidant enzymes are mobilized during hyperglycemia, but they cannot meet the continued demand due to increased oxidative stress. This problem is compounded by a possible breakdown in the ability to produce these enzymes from a decreased intake of the needed precursors or an inability to synthesis them. Antioxidant supplementation may provide the only means in which to reverse this process. Superoxide is converted by superoxide dismutase (SOD) to hydrogen peroxide (H$_2$O$_2$) and oxygen (O$_2$) molecule. There are 3 isoforms of superoxide dismutase such as cytosolic Cu/Zn SOD (SOD1), mitochondrial Mn-SOD (SOD2) and extracellular SOD (SOD3). Catalase, the heme metalloenzyme is expressed in peroxisomes, mitochondria, cytoplasm and nucleus. H$_2$O$_2$ is catalyzed by catalase to oxygen (O$_2$) and water (H$_2$O) [16], while glutathione peroxidase the selenoprotein, is found in both intracellular and extracellular. Glutathione peroxidase has a highly sensitive function for lipid peroxides degradation, converses H$_2$O$_2$ to water by using the thiol group of glutathione. Their H$_2$O$_2$ detoxification plays the important roles to prevent lipid peroxidation and regulation of the cellular redox status [16]. The glutathione system, thioredoxin peroxidase is key enzyme to regulate the cellular levels of thiol/disulfide while the production of antioxidant enzymes is regulated by the redox-cellular transcription factors [17]. Vitamin C has the same transport mechanism as that of glucose in the body. When the plasma concentration of vitamin C is plentiful it tries to compete with glucose in binding with hemoglobin and protein amino groups. This action reduces or inhibits excessive glycosylation of red blood cells and proteins, which in turn will decrease the creation of free radicals. Also, vitamin C may function as a natural aldose reductase inhibitor preventing the conversion of sugars to their corresponding alcohols by not allowing cellular destabilization in susceptible sites. The prevalent long term depletion of vitamin C, common in diabetes, may contribute to depressed immune function, compromised wound healing ability and reduced blood vessel integrity. These and other related ailments can possibly be arrested and reversed by vitamin C supplementation. Vitamin E has been shown to reduce lipid peroxidation and to increase the cell viability. The supplementation of vitamin E to lead-treated erythrocytes has been shown to prevent the inhibition of δ-aminolevulinic dehydratase activity and lipid oxidation in vivo and in vitro [18]. Vitamin E could also be useful to protect membrane-lipids and proteins from oxidation due to lead intoxication.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Animals

Forty (40) adult male albino rats (wistar strain) weighing between 100 – 176 g, obtained from the animal breeding unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, were used as experimental animals. The experimental animals were between eight weeks to ten weeks old (8 – 10 weeks old). The rats were kept in cages for two weeks to allow acclimatization to Animal House of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus and were allowed free access to food and water. The animals were maintained under standard conditions (12 hours light /12 hours dark cycle, temperature of about 37±2°C). All the rats were fed a commercial diet (Vital feed, Nigeria) during the experiment. The protocol was in line with the guidelines of the National Institute of Health (NIH) (NIH Publication 85-23, 1985) for laboratory animal care and use.

### 2.2 Experimental Design

The Forty (40) adult male wistar rats were randomly (while controlling for weight differences) assigned to five groups (A, B, C, D and E) of eight (8) animals each.

### 2.3 Induction of Diabetes

In group B, C and D, wistar rats were fasted for about 12 – 15 hours, after which diabetes was induced by a single intraperitoneal injection of freshly dissolved Alloxan monohydrate (Sigma-Aldrich, USA) (130 mg/kg b.w) dissolved in 0.5 ml of 10 percent normal saline maintained at 37°C [19,20]. Normal control rats (Group A and E) received a similar volume of normal saline alone. After 72 hours of alloxan injection, the animals were fasted overnight and their fasting blood glucose was measured. The rats having fasting blood glucose level greater than 200 mg/dL were selected for the study. Fasting blood glucose level of all the diabetes induced rats were greater than 200 mg/dl and were all selected for the study.
2.4 Blood Sample Collection and Serum Preparation

The animals were fasted overnight and sacrificed using chloroform as an anesthetic agent. Blood samples of Groups A and B rats were collected 72 hours post diabetes induction, and that of groups C, D and E rats were collected 3 weeks after confirmation of diabetes. Blood sample (10 ml) was collected through cardiac puncture after 12 hours fasting from all the animal and divided into two well- labeled, sterile plain bottle, (6.5 ml and 0.5 ml), EDTA bottle (1.5 ml) and a fluoride oxalate bottle, (1.5 ml) under aseptic conditions. Five hundred (500) µl of the blood sample was in a plain bottle containing DNA/RNA shield for molecular analysis. The blood samples in the plain bottle without DNA/RNA shield were centrifuged at 4000 rpm for 5 minutes after allowing the blood to clot and retract from walls of the sample container at room temperature. The sera were separated from the whole blood into a new container and then stored frozen in a refrigerator at −20°C. Also blood samples for molecular analysis were stored at 4° C.

The sera were separated from whole blood samples of Groups A and B rats were collected 72 hours post diabetes induction, and of groups C, D and E rats were collected 3 weeks after confirmation of diabetes. Blood sample (10 ml) was collected through cardiac puncture after 12 hours fasting from all the animal and divided into two well- labeled, sterile plain bottle, (6.5 ml and 0.5 ml), EDTA bottle (1.5 ml) and a fluoride oxalate bottle, (1.5 ml) under aseptic conditions. Five hundred (500) µl of the blood sample was in a plain bottle containing DNA/RNA shield for molecular analysis. The blood samples in the plain bottle without DNA/RNA shield were centrifuged at 4000 rpm for 5 minutes after allowing the blood to clot and retract from walls of the sample container at room temperature. The sera were separated from the whole blood into a new container and then stored frozen in a refrigerator at −20°C. Also blood samples for molecular analysis were stored at 4° C.

The analysis was carried out within one week of sample collection. Haemolysed blood samples were not used to avoid error in the result. The blood sample was used for the analysis of glucose, malondialdehyde, superoxide dismutase, catalase, glutathione peroxidase, vitamin C and vitamin E as well as peripheral blood antioxidant genes detection (CAT and CU-ZnSOD). Malondialdehyde, superoxide dismutase, catalase, glutathione peroxidase, vitamin C and vitamin E were analyzed using colorimetric method while CAT and CU-ZnSOD were analyzed using real time polymerase chain reaction (qRT-PCR) [21]. Glucose was determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase with phenol and 4-aminophenazone to form a red violet quinoneimine dye as indicator [22]. Malondialdehyde which is a product of lipid peroxidation was determined when heated with 2-thiobarbituric acid (TBA) under alkaline condition; it formed a pink colored product. The absorbance was measured spectrophotometrically at a wave length of 532nm. The intensity of color generated is directly proportional to the concentration of MDA in the sample [23]. The principle method for measuring superoxide dismutase is that adrenaline undergoes auto oxidation at pH 10.2 to form adrenochrome which has an absorption maximum at 480 nm. The presence of superoxide dismutase in the reaction mixture inhibits the auto oxidation of adrenaline and the decrease in the formation of this adrenochrome is proportional to the rate of superoxide dismutase activity in the sample [24]. Catalase activity was assessed by incubating the enzyme sample in substrate, hydrogen peroxide in sodium–potassium phosphate buffer, (pH 7.4) at 37°C for three minutes. There action was stopped with ammoniummolybdate. Absorbance of the yellow complex of molybdate and hydrogen peroxide was measured at 374 nm against the blank [25]. Glutathione peroxidase in the presence of H_2O_2 oxidizes reduced glutathione (GSH) to form H_2O. The amount of GSH consumed is directly proportional to the activity of GPx and it is expressed as U/ml (µmol of GSH consumed/minute). The GSH remaining after the reaction is allowed to react with 5'-5' dithiobis-2-nitrobenzoic acid (DTNB) to form a yellow complex that absorbs maximally at 412 nm [26]. The principle of vitamin C measurement is that ascorbic acid is oxidized by copper to form dehydroascorbic acid. The product was treated with 2, 4 dinitrophenyl hydrazine to form tris 2,4 dinitrophenyl hydrazone which undergoes rearrangement to form a product with the absorption maximum at 520 nm [27].

### Table 1. Showing the summary of experimental design

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (Normal Control of 72 hours)</td>
<td>Received 0.5 ml of 10 percent normal saline only + No intervention for 72 hours</td>
</tr>
<tr>
<td>Group B (Diabetic rats)</td>
<td>Induced diabetes + sacrificed 72 hours of post diabetes induction</td>
</tr>
<tr>
<td>Group C (Diabetic rats)</td>
<td>Induced diabetes + 500 mg/kg, p.o metformin drug treatment for 3 weeks after confirmation of diabetes</td>
</tr>
<tr>
<td>Group D (Diabetic Control)</td>
<td>Induced diabetes + without metformin drug treatment for 3 weeks after confirmation of diabetes</td>
</tr>
<tr>
<td>Group E (Normal Control of 3 weeks)</td>
<td>Received 0.5 ml of 10 percent normal saline only + NO intervention for three 3 weeks of study</td>
</tr>
</tbody>
</table>
vitamin E test is that after the proteins in the serum are precipitated by an equal volume of absolute ethanol, the whole mixture is subjected to extraction by xylene. The complex of ferrous ions generated in this reaction with 2, 2’-bipyridyl and alpha-tocopherol is determined using a plain enzyme-linked immunosorbent assay microplate (non-antibody coated) at 492 nm [28].

2.5 Method of Data Analysis

The Statistical Package for Social Sciences (SPSS) [29] version 23 was used for statistical analysis and the variables were expressed as mean ± standard deviation. Chi square was used to analyze non parametric data. Analysis of variance (ANOVA) was used to compare the mean difference among groups, and Post Hoc multiple comparison was used to assess inter group variability. Pearson’s correlation coefficient technique was used to measure the degree of relationship existing between variables. The level of significance was considered at P < 0.05.

3. RESULTS

In Table 3, the blood mean level of malondialdehyde was significantly higher in the diabetic rats (Group B) and (Groups C and D) when compared with non diabetic control Groups A and E respectively (P < 0.05). However, the mean level of malondialdehyde in Group B was significantly higher and lower when compared with Groups C and D respectively, while in Group C, it is significantly lower when compared with Group D (P < 0.05).

Blood mean levels of glutathione peroxidase and catalase were significantly lower in Groups C and D but higher in Group B when compared with control Groups E and A respectively (P < 0.05). However in Group D, mean level of glutathione peroxidase and catalase were also significantly lower when compared with Groups B and C, but higher in Group B when compared to Group C (P < 0.05).

Mean level of superoxide dismutase was significantly lower in Group D, but higher in Group B when compared with control Groups E and A, respectively (P < 0.05). Blood level of superoxide dismutase was significantly higher in Group B when compared with Groups C and D, also higher when Group C was compared to Group D. No significant difference exits in the mean level of superoxide dismutase in Group C when compared with the control Group E (P > 0.05).

Significant lower value was seen in blood mean level of vitamin C in Group B and Groups C and D when compared with control Groups A and E, respectively (P < 0.05). In Group D, mean level of vitamin C was also significantly lower when compared with Groups B and C (P < 0.05). There was no significant difference in the mean levels of vitamin C when Group B was compared with Group C (P > 0.05).

Significant lower value in the mean level of vitamin E was observed in Groups C and D when compared with the control Group E (P < 0.05). In Group D, mean level of vitamin E was significantly lower when compared with Groups B and C, also lower in Group C when compared with Group B (P < 0.05). There was no significant difference in the mean levels of vitamin E when Group B is compared with Group A (P > 0.05).

Blood mean level of glucose is significantly higher in diabetic rats (Group B) and (Groups C and D) when compared with control Groups A and E, respectively, and lower in Group C when compared with Groups B and D (P < 0.05). However, there is no significant difference in the blood mean level in Group B when compared with Group D (P > 0.05). Correlation analysis showed no significant relationship between blood glucose and malondialdehyde, catalase, superoxide dismutase, vitamin C and vitamin E (P > 0.05) in both diabetic and apparently healthy non diabetic male rats except negative significant relationship observed between blood

**Table 2. Assay conditions for each antioxidant genes primers from Inquaba Biotechnology Industries, Hartfield, South Africa**

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Primers 5'-3' (Forward and reverse)</th>
<th>Amplicon length, Bp</th>
<th>PCR efficiency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD</td>
<td>AGGCCTCGCACTTCTCGAA CTACAGGGTACTTTAAGCAACTCT</td>
<td>90</td>
<td>98</td>
</tr>
<tr>
<td>CAT</td>
<td>TTTCCCAGGAAAGATCTGAC ACCTTGTTGAGATCGAATGG</td>
<td>94</td>
<td>97.5</td>
</tr>
</tbody>
</table>
glucose and glutathione peroxidase, in the diabetic Group D of this study (P < 0.05) as shown in Table 4.

The reverse transcriptase polymerase chain reaction results for catalase (CAT) and superoxide dismutase (SOD) genes revealed that the genes were not detected on a 1% agarose gel electrophoresis stained with ethidium bromide in all the normal control rats (Group A) after 72 hours of receiving 0.5 ml of 10 percent normal saline and in all the non diabetic rats (Group E) and diabetic rats after 3 weeks of receiving metformin treatment (Group C) and 3 weeks without treatment (Group D) respectively as shown in Figs. 1 and 3.

4. DISCUSSION

Diabetes mellitus is a metabolic disorder with grievous pathophysiological complications which affects various part of the body. This condition induce production of free radicals that result in oxidative stress over a prolong period of time. The gradual increase in free radicals and
Table 3. Oxidative stress biomarkers and glucose in diabetic and apparently healthy non diabetic male rats (Group A-E)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A (n=8)</th>
<th>Group B (n=8)</th>
<th>Group C (n=8)</th>
<th>Group D (n=8)</th>
<th>Group E (n=8)</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/ml)</td>
<td>2.13 ±0.14</td>
<td>4.96 ±0.18</td>
<td>2.99 ±0.52</td>
<td>5.79 ±0.33</td>
<td>2.32</td>
<td>221.013</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>GPx (U/ml)</td>
<td>0.91 ±0.03</td>
<td>0.96 ±0.02</td>
<td>0.78 ±0.06</td>
<td>0.61 ±0.03</td>
<td>0.91</td>
<td>126.696</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CAT (kU/l)</td>
<td>28.72 ±0.71</td>
<td>39.92 ±1.79</td>
<td>26.24 ±0.63</td>
<td>21.37 ±1.58</td>
<td>28.21</td>
<td>239.981</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SOD (U/ml)</td>
<td>16.24 ±0.53</td>
<td>19.03 ±0.59</td>
<td>15.68 ±0.43</td>
<td>13.35 ±0.63</td>
<td>16.09</td>
<td>108.842</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>VIT C (mg/dl)</td>
<td>1.92 ±0.08</td>
<td>1.17 ±0.11</td>
<td>1.32 ±0.15</td>
<td>0.98 ±0.15</td>
<td>1.89 ±0.07</td>
<td>109.160</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>VIT E (µg/ml)</td>
<td>16.29 ±0.54</td>
<td>15.74 ±0.43</td>
<td>14.62 ±0.59</td>
<td>12.84 ±0.73</td>
<td>16.21 ±0.37</td>
<td>55.846</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FBS</td>
<td>4.58 ±0.60</td>
<td>17.33 ±4.37</td>
<td>9.02 ±1.70</td>
<td>18.85 ±2.81</td>
<td>4.61 ±0.51</td>
<td>61.419</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Key: a, Significant when compared with Group A (Normal Control of 72 hours). b, Significant when compared with Group B (Diabetic Rats). c, Significant when compared with Group C (Diabetic Rats). d, Significant when compared with Group D (Diabetic Control). e, Significant when compared with Group E (Normal Control of 3 weeks), Group A (Normal Control of 72 hours) - Received 0.5ml of 10 percent normal saline only, Group B (Diabetic Rats) - Induced diabetes + sacrificed 72hours of post diabetes induction Group C (Diabetic Rats) - Induced diabetes + 500 mg/kg, p.o metformin drug treatment for 3 weeks after confirmation of diabetes, Group D (Diabetic Control) - Induced diabetes + without metformin drug treatment for 3 weeks after confirmation of diabetes, Group E (Normal Control of 3 weeks) - Received 0.5ml of 10 percent normal saline only, MDA- Malondialdehyde, GPx- Glutathione Peroxidase, CAT- Catalase, SOD- Superoxide Dismutase, VIT C- Vitamin C and VIT E- Vitamin E

Table 4. Correlation between blood glucose and oxidative stress biomarkers in diabetic and apparently healthy non diabetic male rats (Group A-E)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A (n=8)</th>
<th>Group B (n=8)</th>
<th>Group C (n=8)</th>
<th>Group D (n=8)</th>
<th>Group E (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS vs MDA</td>
<td>-0.392</td>
<td>0.692</td>
<td>-0.406</td>
<td>0.318</td>
<td>-0.296</td>
</tr>
<tr>
<td>FBS vs GPx</td>
<td>0.272</td>
<td>0.515</td>
<td>-0.109</td>
<td>0.797</td>
<td>0.204</td>
</tr>
<tr>
<td>FBS vs CAT</td>
<td>0.053</td>
<td>0.901</td>
<td>0.300</td>
<td>0.471</td>
<td>0.214</td>
</tr>
<tr>
<td>FBS vs SOD</td>
<td>-0.103</td>
<td>0.808</td>
<td>0.291</td>
<td>0.485</td>
<td>0.517</td>
</tr>
<tr>
<td>FBS vs VIT C</td>
<td>0.050</td>
<td>0.907</td>
<td>0.520</td>
<td>0.187</td>
<td>0.523</td>
</tr>
<tr>
<td>FBS vs VIT E</td>
<td>0.482</td>
<td>0.226</td>
<td>-0.036</td>
<td>0.932</td>
<td>-0.216</td>
</tr>
</tbody>
</table>

Key: a, Significant, Group A (Normal Control of 72 hours) - Received 0.5ml of 10 percent normal saline only, Group B (Diabetic Rats) - Induced diabetes + sacrificed 72hours of post diabetes induction Group C (Diabetic Rats) - Induced diabetes + 500 mg/kg, p.o metformin drug treatment for 3 weeks after confirmation of diabetes, Group D (Diabetic Control) - Induced diabetes + without metformin drug treatment for 3 weeks after confirmation of diabetes, Group E (Normal Control of 3 weeks) - Received 0.5ml of 10 percent normal saline only, MDA- Malondialdehyde, GPx- Glutathione Peroxidase, CAT- Catalase, SOD- Superoxide Dismutase, VIT C- Vitamin C, VIT E- Vitamin E and FBS- Fasting Blood Sugar
Fig. 3. Reverse transcriptase PCR results for CAT and SOD genes analyzed on a 1.0% agarose gel electrophoresis stained with ethidium bromide. L is a 100bp-1000bp DNA ladder (molecular marker). Samples 1A, 2A, 1B, 2B, 1C, 2C, 1D, 2D, 1E, 2E, 1F and 2F CAT and SOD genes are negative bands for the expressed from the non diabetic (Group E: Received 0.5ml of 10 percent normal saline only + NO intervention for three 3 weeks of study) and diabetic rats (Group C: Induced diabetes + 500 mg/kg, p.o metformin drug treatment for 3 weeks; Group D: Induced diabetes + without metformin drug treatment for 3 weeks). NC is a No template control, SOD- Superoxide Dismutase, CAT- Catalase, A, C & D-CAT gene, B, D & F-SOD gene, Group C (1A, 2A, 1B, and 2B), Group D (1C, 2C, 1D and 2D) and Group E (1E, 2E, 1F and 2F).

According to the results of this study, 72 hours after induction of diabetes, antioxidant enzyme activities were high compared to control animals. There was significant increase in serum levels of catalase, superoxide dismutase, and glutathione peroxidase and molecular mRNA gene expression of CAT and Cu–ZnSOD at the peripheral blood cell of diabetic animals compared with non diabetic animal. It is clear that both the transcription and the translation of these enzymes were effected and hence the enzyme activities were found expressed. This is an indication of stress response in respect to changes in glycaemic level. Oxidative stress or changes in the balance between oxidation and reduction in a cell can also affect the translocation of redox-sensitive transcription factors into the nucleus [34]. Therefore, the elevation of this antioxidant and expression of mRNA of CAT and Cu–ZnSOD in diabetes could be due to rise in free radicals, which trigger the transcriptional factors responsible for the initiation machinery of antioxidant enzymes transcription process that will prevent oxidative damage. Furthermore, catalase, superoxide
diabetes induction and non detection of these antioxidants on peripheral blood cell. Thus, it is unknown the effect of oxidative stress on activities of these antioxidants in the diabetic animals without metformin treatment with metformin. The lower values observed in the activities of these antioxidants in the diabetic animals without metformin treatment suggest their excessive utilization in attenuating free radicals generated during the metabolism of alloxan. These findings have already been reported in diabetic animals [35,33,32,36]. However, reverse transcriptase polymerase chain reaction results for superoxide dismutase (SOD) and catalase (CAT) genes after 3 weeks of diabetes revealed that the genes were not detected on a 1% agarose gel electrophoresis stained with ethidium bromide in both metformin treated and untreated diabetic animal. Non detection observed in the expression of mRNAs could be due to the decrease in the half lives of mRNAs or chronic oxidative stress and therefore antioxidant defense may have been impaired which resulted to mRNAs not expressing on the peripheral blood cell. In the literature, there are articles stating that increased oxidative stress may lead to destabilization of mRNA [37,38].

Increased formation of ROS may occur in diabetes for reasons possibly related to an increase in glucose concentrations in plasma and tissues [7]. Maritim et al. [9] showed that increased oxidative stress and impaired antioxidant defense mechanisms are important factors in the pathogenesis and progression of the disease. Literally, there are varied reports on effect of oxidative stress on activities of antioxidant in peripheral blood cell. This may be due to experimental conditions such as the age of the animals and the duration of the diabetes. There are studies which reported that diabetic state caused the antioxidant activities to decrease in rats [32,36]. On the other hand, other researchers reported that antioxidant activities were increased [39,40,41], but none of these researchers have studied gene expression of these antioxidants on peripheral blood cell. Thus, it is unknown the effect of oxidative stress on the antioxidant enzymes at the level of mRNA or translation or activity or both. But expression of CAT and Cu–Zn SOD genes 72 hours post diabetes induction and non detection of these antioxidant genes after 3 weeks, both in treated and untreated diabetic animal was in parallel with the observations of decreased blood antioxidant levels. So, no detection of these antioxidant enzymes genes at the level of transcription indicates impaired antioxidant enzyme activity. It seems that actual damage caused by oxidative stress is most probably as a result of lowered transcription of the enzymes in the blood. Also in this study, some antioxidant genes were used to establish evidence of stress in type 2 diabetic subjects. In response to stressors, some genes are known to be either up-regulated while some are down-regulated. Some group of antioxidant genes have shown to be expressed in the peripheral blood cells in hyperglycemic condition [42]. There was a stress response of antioxidant in the peripheral blood cell. The stress response of these antioxidants effects both the expression of proteins (CAT and Cu–ZnSOD) and activities of the antioxidant in the blood 72 hour post diabetes induction.

5. CONCLUSION

In conclusion, hyperglycemia can cause expression of mRNA of CAT and Cu–ZnSOD genes on the peripheral blood cell in acute condition and significant alterations of oxidative stress biomarkers; however metformin treatment has showed not only hypoglycemic effect, but also anti-oxidant properties.

ETHICAL APPROVAL

Ethical approval was sought and obtained from the Research Ethics Committee of the Federal Medical Centre (FMC) Umuahia with reference FMC/QEH/G.596/VOL.10/282. The protocol was in line with the guidelines of the National Institute of Health (NIH) (NIH Publication 85-23, 1985) for laboratory animal care and use.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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