



Antioxidant Enzymes and Lipid Peroxidation in Alloxan-Induced Diabetic Rabbits

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Abstract: The study was carried out to evaluate the effect of alloxan-induced diabetes on some oxidative stress markers in rabbits. Ten rabbits weighing between 1.0-1.5kg were used and divided into two groups of five rabbits each. Diabetes was induced in one of the groups, by intraperitoneal administration of alloxan at a dosage of 150mg/kg body weight and the control rabbits were administered normal saline (0.9% NaCl). The animals were monitored for 14days after confirmed diabetes in the test group. The fasting plasma glucose, plasma insulin, plasma lipids, the antioxidant enzymes: Superoxide dismutase (SOD), catalase, and lipid peroxidation were assayed for, in the plasma and the brain of the diabetic and non-diabetic rabbits. The results showed a significant decrease in mean body weight of the alloxan-induced diabetic and control rabbits pre and 14days post alloxan-induction. There was a significant ($p < 0.01$) increase in fasting plasma glucose, a significant ($p < 0.01$) decrease in the activity of SOD and a significant ($p < 0.01$) increase in the activity of catalase in the plasma and brain; there was also a significant ($p < 0.01$) increase in the level of lipid peroxidation, plasma cholesterol and triglycerides in the diabetic rabbits. Culminating a depletion of SOD activity, the altered activity of plasma (or brain) catalase and increased lipid peroxidation is due to the oxidative stress induced by the diabetic condition, hence the present study indicate that alloxan – induced diabetic potentiated oxidative stress in a short term duration. This shows the importance of antioxidant mimetics to be administered at early stage of diabetics in other to increase the antioxidant capacity thus preventing oxidative stress which may lead to complication in diabetes.

Keywords: Oxidative Stress, Alloxan, Superoxide Dismutase, Catalase, Lipid Peroxidation, Antioxidant

1. Introduction

Diabetes Mellitus (DM) has been classified as a metabolic disorder primarily characterized by elevated blood glucose level (hyperglycemia) and this may be due to lack of insulin or significantly reduced effectiveness of insulin. Symptoms associated with hyperglycemia include fatigue, weight loss, polyphagia and polyuria. [1]. The world health organization reported that the number of people with diabetes will increase by 150% in the next 25 years, with an increasing proportion of affected people in the younger age groups [2, 3]. Hyperglycemia increases the risk of developing acute as well as the long-term complications of diabetes. The most common long-term complications of diabetes include

cardiovascular disease (CVD), nephropathy, retinopathy and neuropathy. Diabetes is the leading cause of adult blindness, non-traumatic lower limb amputation and end-stage renal disease. [4, 5]. In healthy individuals, cells utilize oxygen in the making of energy but the process produces free radicals which are toxic by-products that damage the DNA and proteins. The body produces natural antioxidants to sop up those free radicals and reduce the oxidative damage to tissues. The oxidative damage is reduced or prevented by a system of defences which include antioxidant enzymes (SOD, catalase, glutathione peroxidase) and small molecules with scavenging ability such as antioxidant vitamins (A, C and E) [6].

An imbalance between formation and removal of free radicals can lead to a pathological condition called oxidative

stress. It is of noteworthy that if the generation of free radicals exceeds the protective effects of antioxidants, this can cause oxidative damage which accumulates during the life cycle, and this has been implicated in aging and age-dependent diseases such as cardiovascular disease, cancer, neurodegenerative disorders, and other chronic conditions. [6]

In diabetes, there is an altered balance between reactive oxygen species production and total antioxidant status in prolonged diabetes and this has been reported [7]. However much is still yet to be done on the actual status of the individual antioxidant enzymes in diabetes with regards to the early stages of diabetes. Thus the study was designed to ascertain the effect of oxidative stress via the actual status of the antioxidant enzymes in plasma and brain of alloxan-induced diabetes in short term duration.

2. Materials and Methods

2.1. Animal Preparation and Protocol

Ten Rabbits (10) weighing between 1.0 – 1.5kg were obtained from the laboratory animal Centre, College of Medicine, University of Lagos, Idiaraba. They were housed in stainless steel cages in a well-ventilated room, fed with standard commercial rabbit pellets and water *ad libitum*. The Rabbits were divided into two groups of five each (Diabetic and Non diabetic). They were fasted for 18hours and the plasma glucose level for the two groups was determined (baseline glucose level).

2.2. Induction of Diabetes

The rabbits were then fasted for 24hours before injection of a freshly prepared solution of alloxan monohydrate (Span Chemical CO, Mumbai), intraperitoneally at a dosage of 150mg/kg body weight. The single dose of alloxan produced type 1 diabetes having fasting blood glucose of $\geq 180 \pm 1.58$ mg/dl. Diabetes was also confirmed 48 hours after administration by symptoms of polyuria and glucosuria using uristic test strip (Bayer Health Care, USA) in the diabetic group. The diabetes was maintained throughout the duration of the experiment. The Non-Diabetic rabbits were administered 2ml of normal saline (0.9% NaCl). The blood glucose level was monitored and recorded on days 1, 5, 10 and 14 from the date of confirmed diabetes (48hours after induction with glucose level of 180mg/dl, using a Finetest glucometer). At the end of the Experiment, On the 14th day, the rabbits were all sacrificed, blood samples were collected using EDTA and plain bottles and centrifuged for assay of plasma insulin, Superoxide dismutase, catalase, lipid peroxidation, cholesterol and triglyceride estimation. The laboratory animals were handled in accordance with laboratory animal practice regulation and the principle of laboratory animal care as documented by [8].

2.3. Preparation of Brain Tissue Extract

The brain tissue homogenate was prepared by the method

of [9]. 1g of Brain tissue was homogenized with 10ml of ice cold 0.05ml phosphate buffer pH 7.0 using a mortar and pestle; centrifuge at 7,000g for 20minutes and the supernatant removed for the assay.

2.4. Biochemical Assay

2.4.1. Estimation of Fasting Plasma Glucose

Plasma glucose concentration was assayed for by the Glucose oxidase method as described by [10] in the diabetic and Non – diabetic rabbits.

2.4.2. Estimation of Plasma Insulin

The method of [11]; an automated Radioimmunoassay technique was adopted to estimate plasma insulin levels.

2.4.3. Estimation of Total Superoxide Dismutase (SOD) Activity

The Plasma and brain Total Superoxide Dismutase (SOD) was assayed according to the scheme proposed by [12] which is based on epinephrine autoxidation.

2.4.4. Estimation of Catalase Activity

The plasma/brain Catalase activity was assayed for by the method of [13], which is based on the Hydrogen peroxide-oxidoreductase activity.

2.4.5. Estimation of Plasma Total Cholesterol

Plasma cholesterol was assayed for by the method of [14], (colorimetric Kit)

2.4.6. Estimation of Plasma Triglycerides

The Plasma Triglycerides was assayed for by the method of [15]. (Colorimetric Kit)

2.4.7. Estimation of Plasma/Brain Lipid Peroxidation

Plasma/Brain lipid peroxidation based on the thiobarbituric acid assay according to method of [16].

2.4.8. Measurement of Body Weight

The body weight of the animals was determined by using a weighing balance. The initial body weight of each animal was recorded before induction of diabetes. The rabbits were subsequently weighed on days 1, 5, 10 and 14 after confirmed diabetes. The mean body weight was recorded the mean body weight pre and post alloxan was statistically analyzed.

2.4.9. Statistical Analysis

Results are presented as mean \pm standard deviation. Data was analyzed statistically using SPSS to test any significant differences between the two groups. A one way analysis of variance (ANOVA) post hoc test was used for comparison of means. Mean values of $p < 0.01$ were regarded as statistically significantly.

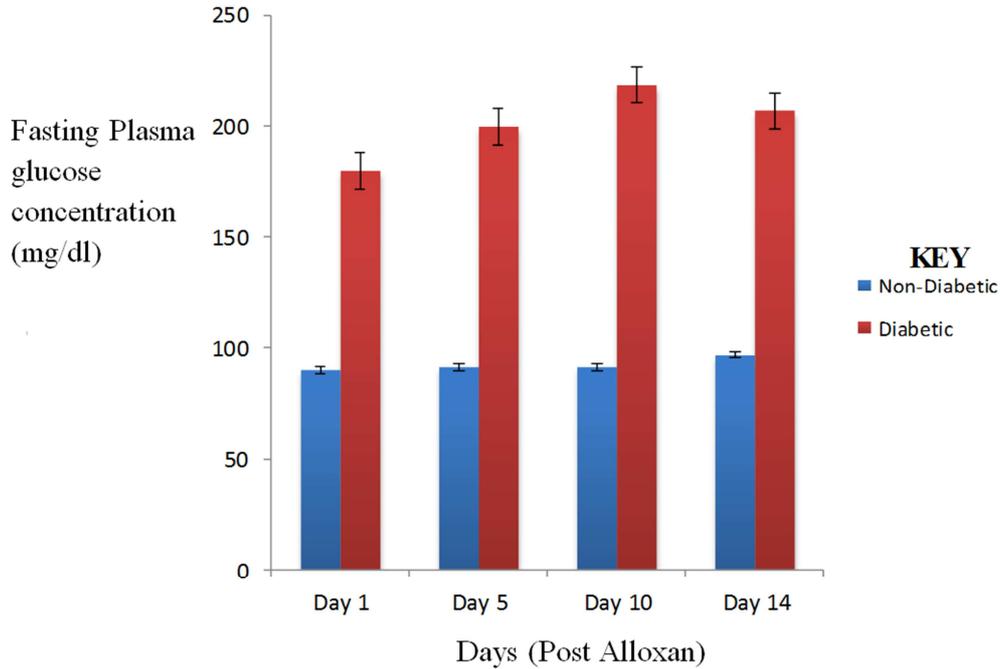
3. Results

3.1. Fasting Blood Glucose Level in the Diabetic and Non-Diabetic Groups

Figure 1 show that the rabbits injected with alloxan

monohydrate had a two fold increase in the level of fasting plasma glucose and the values consistently increased after administration of alloxan, the plasma glucose levels were

significantly ($p < 0.01$) higher than the non-diabetic group. (Figure 1).



Day 1 = 1st Day after confirmed diabetes

Figure 1. Plasma Glucose Concentration (mg/dl).

3.2. Plasma Insulin Level in the Diabetic and Non-Diabetic Groups

There was a significant reduction in the plasma insulin levels of the diabetic group as compared with the non-diabetic group ($p < 0.01$). Figure 2

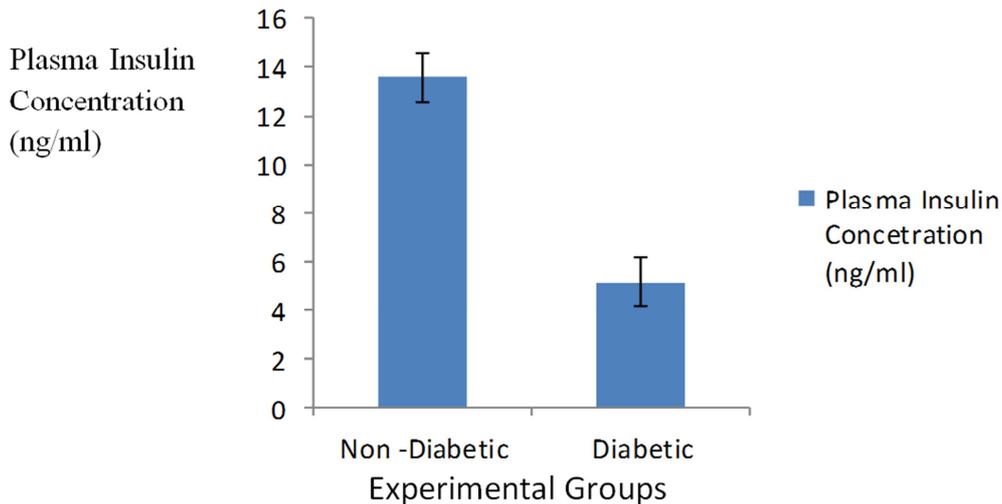


Figure 2. Plasma Insulin Concentration.

3.3. SOD and Catalase Activities in the Plasma and Brain of Diabetic and Non-Diabetic Groups

Figures 3 and 4 show a significant ($p < 0.01$) decrease in the activity of SOD and a significant ($p < 0.01$) increase in the activity of catalase in both the brain and plasma of the diabetic group when compared to the non-diabetic group.

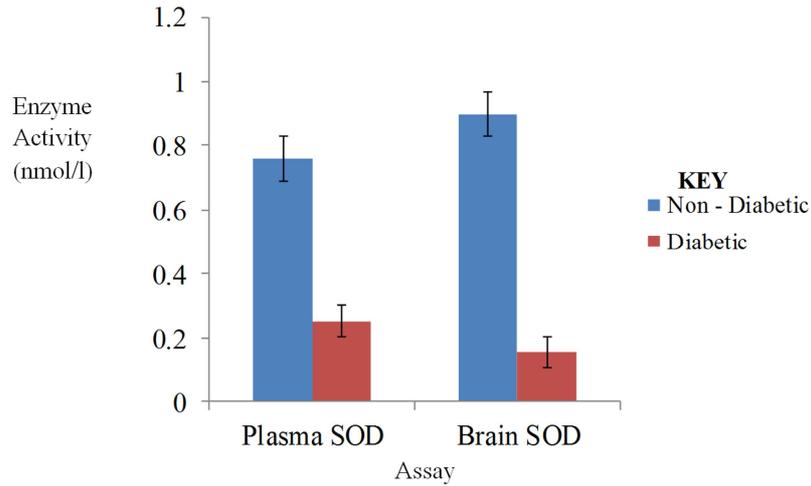


Figure 3. Superoxide Dismutase (SOD).

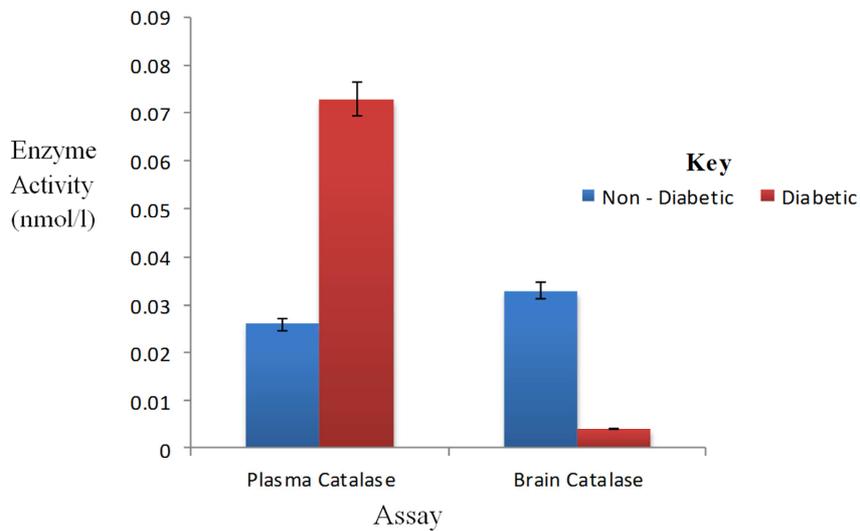


Figure 4. Catalase Activity.

3.4. Mean Body Weight of the Diabetic and Non-Diabetic Rabbits

Figure 5 shows the change in the mean body weight of the alloxan-induced diabetic and non-diabetic rabbits; there was significant ($p < 0.01$) increase in the non-diabetic rabbits; while in the alloxan-induced diabetic rabbits there was a significant ($p < 0.01$) reduction in mean body weight 14 days post alloxan.

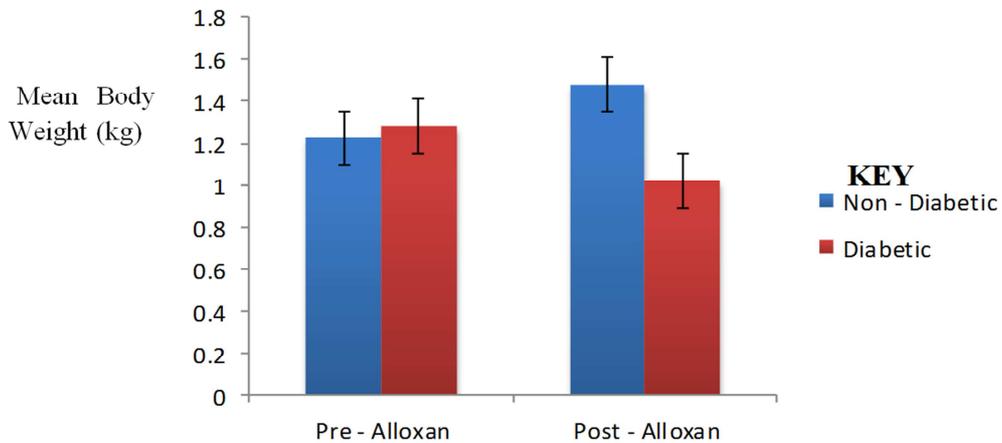


Figure 5. Mean Body weight.

3.5. Lipid Peroxidation in Plasma and Brain of the Diabetic and Non-Diabetic Groups

Figure 6 shows that there was also a significant increase ($p < 0.01$) in lipid peroxidation in the plasma and brain of the diabetic group when compared with the non-diabetic group.

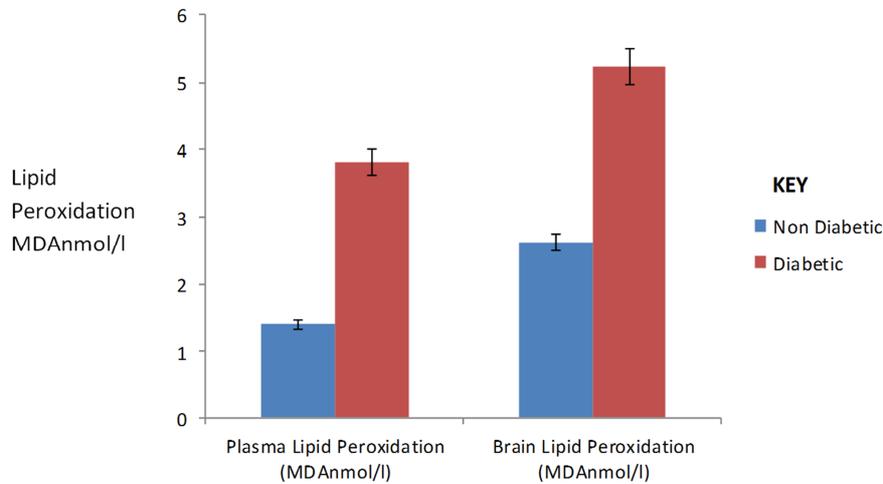


Figure 6. Lipid Peroxidation.

3.6. Plasma Cholesterol and Triglyceride Levels in the Diabetic and Non-Diabetic Groups

Figure 7 shows that there was a significant ($p < 0.01$) increase in the cholesterol and triglyceride levels in the diabetic rabbits compared with the non-diabetic rabbit.

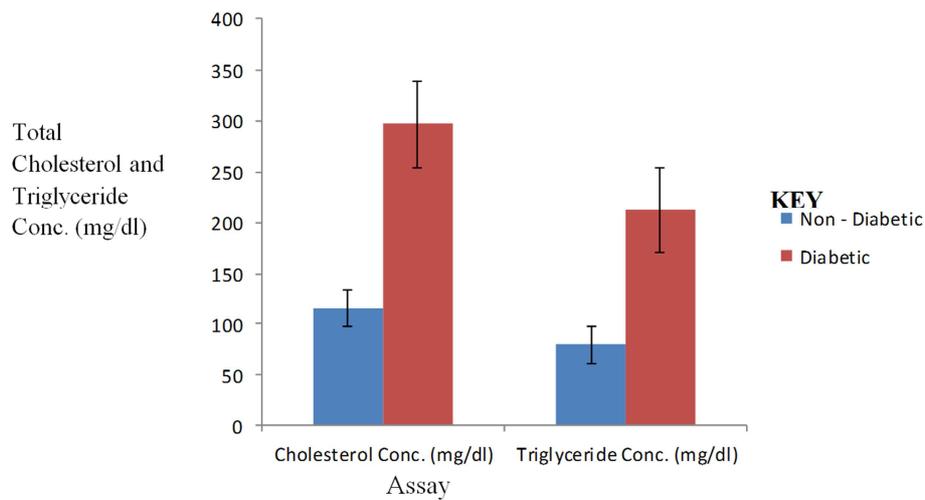


Figure 7. Plasma Total Cholesterol and Triglyceride.

4. Discussion

This study was designed to investigate the effect of oxidative stress via the actual status of the antioxidant enzymes in the plasma and brain of alloxan-induced diabetes in short term duration. The level of lipid peroxidation, cholesterol and triglyceride and the activities of two antioxidant enzymes; SOD and catalase were evaluated in order to examine the effect of oxidative stress in the early stage of diabetes.

Alloxan, a beta cytotoxin induces chemical diabetes in a wide variety of animal species through damage of the insulin secreting cells [17]. Diabetes Mellitus is a complex syndrome

affecting 3-5% of people; and long term complications are the main cause of morbidity and mortality. [2] While there are some evidences about the role of oxidative stress in the pathogenesis of diabetic complications but some relationship between hyperglycemia and generation of oxidative stress is still not very clear [5, 18].

Oxidative stress results from an imbalance between the generation of reactive oxygen species (ROS) and protective mechanisms. Free radicals, the main cause of oxidative stress, may react with a variety of biomolecules including lipids, carbohydrates proteins and DNA of connective tissues [19, 20]. Free radicals especially reactive oxygen species (ROS) had been implicated in a lot of degenerative diseases

such as Parkinsons diseases and diabetes. Overproduction of ROS can directly attack the polyunsaturated fatty acids of cell membranes and induce lipid peroxidation [21,]. Antioxidants are strong reducing agents and this is principally based on the redox relationships between different parts of their chemical structure. Antioxidants carry out their protective properties on cells either by preventing the production of free radicals or neutralizing /scavenging free radicals produced in the body. [22]

In the present study, the induction of diabetes in the diabetic group rabbits was confirmed by a significant rise in the fasting plasma glucose level, since fasting plasma glucose concentration in excess of 180mg/dl confirmed the diabetic state. There was a significant reduction in the plasma insulin levels of the diabetic group as compared with the non-diabetic group ($p < 0.01$). This is expected as a result of the effect alloxan that destroys the β cells that secret insulin. [23]

The activity of the antioxidant enzyme SOD in the alloxan – induced diabetic rabbits was significantly ($p < 0.01$) decreased when compared with the non-diabetic rabbits. This finding agrees with another study as carried out by [24, 25], however not compatible with others [26, 27]. In diabetes the auto-oxidation of glucose results in the formation of hydrogen peroxides and increase in lipid peroxidation which also produces free radicals, thus further depleting the antioxidant enzyme [28]; this may be the reason for the decreased activity of SOD in the diabetic rabbits in the present study.

The result also showed that the enzyme catalase was significantly higher in the brain and plasma of the diabetic group than the non-diabetic group ($p < 0.01$). Studies carried out by [27], showed a significant increase in catalase and SOD activities on the 6th day of alloxan-induced diabetes; however, as the days progressed, there was a decrease in the level of SOD in both the brain and plasma. Previous study also reported by [29], showed a significant increase in catalase activity in the plasma and brain estimated at 3weeks of alloxan induced diabetes. This finding agrees with the result of the present study. The increase in catalase activity could be due to the increase in hydrogen peroxides and other peroxides; the body thus increases the activity of catalase in other to cope with the free radicals being produced. However when the level of free radicals due to oxidative stress, exceeds the production of antioxidant enzymes, its activity may progressively decrease as in prolonged diabetes. [29]

There was also a significant increase ($p < 0.01$) in lipid peroxidation in the plasma and brain of the diabetic group when compared with the non-diabetic group. An increase in lipid peroxidation was reported by [30]. Thus in the present study the increase could be due to the increased production of free radicals in the diabetic state which then resulted to an increased destruction of lipid membranes [20]

In the present study, there was a significant ($p < 0.01$) increase in the levels of cholesterol and triglycerides in the diabetic rabbits. This increase may be due to accelerated lipolysis in an insulin deficient condition [22, 31]; this

accelerated lipolysis results in elevated plasma fatty acids and triglycerides in the plasma. From literatures, it is also known that there is accelerated conversion of acetyl CoA to cholesterol resulting in an increase in plasma cholesterol in the diabetic. [32]. An increase in the cholesterol levels as observed in the present study, might be due to an increase in the transport of chylomicron cholesterol to the liver. Elevated blood lipid levels have been shown to be associated with increased incidence of cardiovascular diseases and this is a significant complication of diabetes [32].

The loss in weight in the diabetic animals as evidenced in figure 5 would be expected. This was also observed by [Nna 33, 34] This could be attributed to the alloxan which is used to induce type 1 diabetes in the diabetic animals. The destruction of the pancreas results in the utilization of non-carbohydrate moieties such as protein for the synthesis of glucose for energy. This accounts for the depletion of energy stores and weight loss in type 1 diabetes mellitus [22].

Japanese scientists have demonstrated that oxidative stress damages the insulin producing cells of the pancreas in rats, which can worsen diabetes. Their studies suggest that antioxidant supplements such as vitamin E in animals may reduce that damage [32].

Recent studies demonstrate that a single hyperglycemia-induced process of overproduction of superoxide by the mitochondria electron transport chain seems to be the first and key event in the activation of all other pathways involved in the pathogenesis of diabetes complications [18, 21].

5. Conclusion

In conclusion, data of the present study indicate that alloxan-induced diabetes potentiated oxidative stress. Antioxidant properties of brain and plasma SOD, catalase and lipid peroxidation are impaired by diabetes in the early stage in rabbits.

In view of the low activity of SOD and the altered levels of catalase and lipid peroxidation as observed in the diabetic rabbits, it is recommended that supplementary trace elements such as selenium, copper, zinc and manganese, the essential components of the enzyme SOD structures may be useful in preventing of diabetic complications. There are new antioxidant therapies, which consist of low molecular mass compound that act as SOD or catalase mimetics which work as superoxide scavengers being suggested for use. Since there is oxidative stress in a diabetic, due to reactive oxygen specie, these new drugs could be implemented at an early stage to increase the antioxidant capacity thus preventing oxidative stress which may lead to clinical complications in diabetes mellitus. These altered antioxidant defences may increase the susceptibility of diabetics to oxidative injury

6. Recommendations

The phytochemistry of antidiabetic and antioxidant plant

extracts which are natural sources of these elements, could be carried out, to ascertain the level of antioxidants for use in the treatment and management of Diabetes Mellitus. The intake of dietary antioxidants may also help to maintain an adequate antioxidant status in the body.

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