Research Article

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The Effect of Diabetes on the Physiological Parameters in Male Rats

Ziadoon fawzi mukhlif [1], Saleh M Rahim[2] and Mustafa nuhad jumaa jamal[3]

[1]Dept. of biology, College of education for pure science, University of Anbar

[2]Dept. of Biology, College of Science, University of Kirkuk

[3]Dept. of Biology, College of Science, University of Anbar

Corresponding author; zaidon22@yahoo.com

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Abstract

The aim of this study is to assess the effects of high blood glucose levels in male rats with diabetic induced by alloxan on their physiological conditions. Diabetes mellitus is a category of metabolic hyperglycemia disorders caused by insufficient body production or pancreatic action. In addition to hyperglycemia, a number of other factors are also important for pathogenesis, such as Hyperlipidemics and cortisol oxidative. Forty five mature male rats used in this study, these were divided into 3 groups, Control group(G1) : gavage distilled water, Group2(G2): injection intraperitoneally with Alloxan150 mg/kg bw for two weeks and Group3(G3): injection intraperitoneally with Alloxan 150mg/kg bw for twenty four days. Blood samples taken from all groups estimation of Peroxynitrate, GSH, CAT, and SOD concentrations%. The findings revealed a significant improvement in diabetic classes in glucose ($p<0.05$) and peroxynitrate relative to control (G1). Diabetic concentrations of (G2, G3) relative to the control group (G1) were substantially reduced in SOD, GSH, CAT and diabetes mediated ($P < 0,05$). In conclusion, diabetes can cause significant changes in SOD, GSH, CAT levels in rats.

Keywards: Rats, Diabetes, Physiological parameters, SOD, GSH and CAT

تأثير السكري على الصفات الفسيولوجية في ذكور الجرذان

الخلاصة

داء السكري هو فئة من اضطرابات فرط سكر الدم الأيضية الناجمة عن عدم كفاية إنتاج الجسم أو عمل البنكرياس. بالإضافة إلى فرط سكر الدم ، هناك عدد من العوامل الأخرى مهمة أيضًا للإمراض ، مثل فرط شحميات الدم وأكسدة الكورتيزول. خمس واربعون ذكر من الجرذان قسمت الى ثلاثة مجاميع، مجموعة سيطرة اعطيت الماء المقطر، المجموعة 2 (G2): الحقن داخل البريتون مع Alloxan150 mg / kg bw لمدة أسبوعين و Group3 (G3): الحقن داخل البريتون مع Alloxan 150mg / kg bw لمدة أربعة وعشرين يومًا. اخت عينات الدم من جميع المجموعات لتقدير تركيزات البيروكسينترات ، GSH، CAT ، و SOD.أظهرت النتائج تحسنا كبيرا في فئات مرض السكري في الجلوكوز ($P <0.05$) وبيروكسين نترات بالنسبة للسيطرة (G1). تم تخفيض تركيزات السكري (G2 ، G3) بالنسبة للمجموعة الضابطة (G1) بشكل كبير في SOD ، GSH، CAT وداء السكري بوساطة ($P <0$،05). الهدف من هذه الدراسة هو تقييم آثار ارتفاع نسبة الجلوكوز في الدم على ذكور الفئران التي يسببها الالوكسان على المتغيرات الفسيولوجية الخاصة بهم.

Introduction

Diabetes mellitus is group of Metabolic disturbances triggered by an inadequate development or insulin activity of pancreas within the body (1). Associate Harm in the long term, failure and dysfunction of different functional organs, particularly the kidneys, heart, eyes, blood vessel and nerve (2). It can be classified by several ways: Type I Diabetes (IDDM) and Type II Diabetes (NIDDM). In Type I diabetes, an autoimmune destruction of pancreas (β-cells) results in insulin secretion deficiency (3). Medical disease beginning with the final stage of β-cells degradation ending in type I diabetes mellitus (4). The death of cell islets is responsible for hereditary make-up, the climate and autoimmunity (5).

Type II includes several mechanical disorders that control the balance of insulin sensitivity in the body, which lead to impaired secretion and insulin resistance by pancreatic β cells (6).

Alloxan is a chemical compound also called 5dihydroxyl-pyrimidine-2,4,6-trione and it is commonly used in the research on diabetes (7). Permanent hyperglycemia during diabetes leading to free forming of radicals in all protein glycosylation and auto-oxidation of glucose, particularly reactive oxygen species (8). Chromically oxidative hyperspiration, an important link between diabetes development and serious diabetic complications such as myocardial infarction, strokes, and diabetic foot ulcers (9) .

Materials and methods

Animals and Housing:-

The current work took place in the animal room of the College of Veterinary Medicine, University of Fallujah in the period between October, 2017 and April, 2018.

Collection of the samples: -

Forty-five mature male rats (weighted 10-150g) and aged 13 weeks were used in this study, during the experimental periods male rats were monitored. At the close of each procedure, male rats were estheticized and blood samples from tail vein tubes free of Heparin were collected after injection of (0.1 ml of xylazine /kg + 0.3 ml of ketamine b.w. ip). Separation of the serum Blood samples were done for 5min at 3000rpm by centrifugation and freezed till estimate the concentrations of peroxynitrate, GSH, CAT, and SOD % .

Experimental design:-

45animals were collected and grouped into 3 classes, each contain 15 male rats as follow:

1. Control group(G1) : gavage distilled water.
2. Group2(G2): injection intraperitoneally with Alloxan150 mg/kg bw for two weeks.
3. Group3(G3): injection intraperitoneally with Alloxan 150mg/kg bw for twenty four days.

Preparation of alloxan solution :-

Alloxan was used in rats to cause diabetes from M/S Sigma Chemicals, St.Louis, USA. Citrate buffer 0.1M freshly prepared at the pH4.5 and held at 4-8°C. To dissolve the alloxan, a cold citrate buffer was used**.**

Experimental induction of diabetes :-

Diabetes in the group III and III overnight animals is caused by asingle dose of 150mg/kg b.w. intraperitonally administering freshly formulated Alloxan solution. PH buffer of cold citrate of 0,1M in 4.5. The monitoring community of animals alone gave water purified.

In order to avoid serious hypoglycemia, caused by pancreatic damaging to beta cells, the first day was a 5percent glucose solution was provided with drinking water. The food was then eaten by animals after the injection.

Confirmation of diabetes :-

The Glucochek glucometer (Aspen Diagnostic (P) Ltd) was used to estimate blood glucose levels following 72 hours of alloxan injections by using GO D-POD (Glucose Oxydase-Peroxidase Method) to measure blood glucose levels. Diabetic animals were considered high blood glucose (above 300mg/ dl).

Biochemical test of blood :-

At the end of experiment period blood collected from heart and after centrifugation plasma collected to measurement the following parameter (Serum Glucose concentration, total glutathione concetration, Superoxide dismutase determination) according company instructions (10) serum peroxy nitrite radical concentration (μmol/L)(12) and catalase was measured by assessing the degradation rate of H2O2 was used for an evaluation of the CAT activity and its rate of disappearance by spectrophotometer was monitored at 230 nm(11).

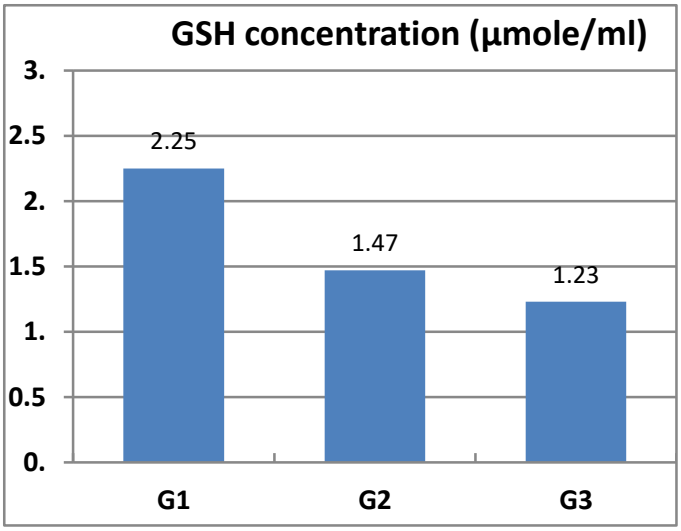
The quantity of the enzyme that cause approximately 90% of the substrate degeneration in 1 ml in 1 min is described as a unit of CAT efficacy. Behavior for hepatocyte CAT was seen asU/ L.

Results and discussion

Biochemical Results :

1.GSH Concentration :-

The findings from this study reveal that the GSH concentration in the diabetes G2, G3(1.23±0.02**)µ, has decreased significantly($P \leq 0.05$) compared to G1 (2.25±0.03)µ/ml controls respectively as shown in fig(1).

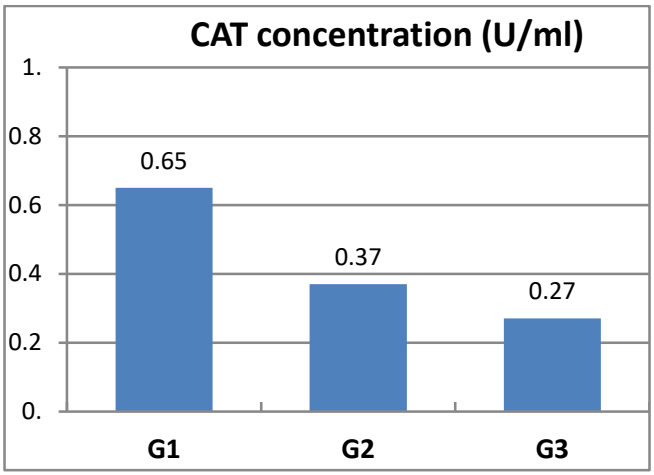


Fig(1): GSH concentration according to the groups:G1(D.W) ,G2 (Alloxan 150 mg/kg for two weeks),G3 (Alloxan 150 mg/kg for 24 days).

A variety of laboratory experiments have shown that GSH is a crucial protecting factor for species from disease and toxicity. The concentration of blood and GSH is an indicator for GSH and thus a risk of disease in people (13).

2.Catalase concentration :-

The finding from this study reveal that the concentration in diabetic G2, G3(0.37±0.07*), (0.27±0.02**)U/ml has decreased significantly (P≤ 0.05)compared with control group G1 (0.65±0.11) U/ml as shown in fig(2).

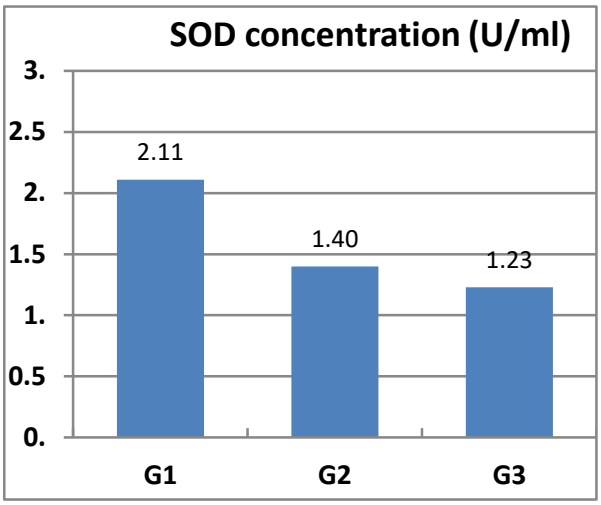


Fig(2) : CAT concentration according to the groups : G1 (D.W) , G2 (Alloxan 150 mg/kg for two weeks) , G3 (Alloxan 150mg/kg for24 days).

Catalases occur mainly in the non-mitochondrial oxidized of fatty acids and amino acids in cells and peroxysomes. Therefore, CAT play a vital role in suppressing lipid peroxidation and preventing damage to RNA and DNA (14, 15).

3.Superoxide dismutase (SOD) :-

The finding of this study reveal that the concentration of SOD in diabetic G2, G3(1.40±0.07*), (1.23±0.03**) U/ml decrease significantly (P ≤ 0.05) compared with control group G1 (2.11±0.02) U/ml as show in fig(3)



Fig(3): SOD concentration according to the groups : G1(D.W), G2(Alloxan 150 mg/kg for two weeks) , G3(Alloxan 150mg/kg for 24 days).

SOD is considered as one of the major antioxidants in cells. It is a major super-oxide scavenger (O2-) is present in cell cytoplasm and mitochondria. Tissue degradation due to the development of lipid peroxides in diabetes because of the creation of free radicals (16).

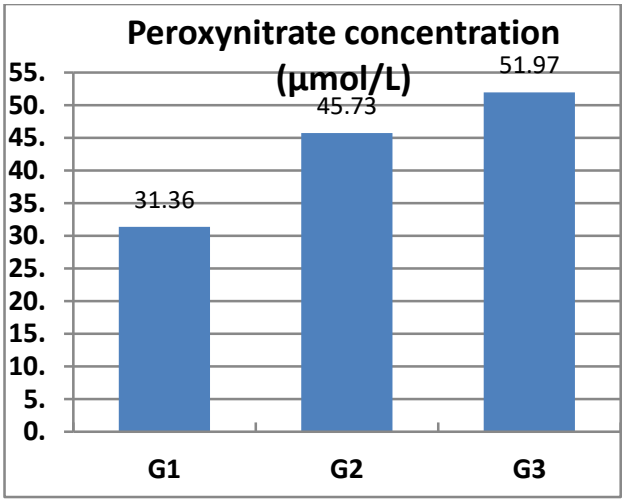
Diabetes-induced lipid peroxidization greatly decreases the amount of key antioxidant enzymes such as SOD and CAT.

The inactivation of reactive oxygen species may cause decreases in percentage of SOD and CAT in diabetic rats. The accumulation of H2O2 or glycation enzyme may contribute to a

substantial decline in SOD behavior, whereas prolonged exposure to H2O2 in vivo will lead to a decrease in CAT operation (17).

Peroxynitrite Concentration :-

The finding from this study reveal that the concentration of peroxynitrate in diabetic G2 , G3(45.73±5.16*), (51.97±3.51)m/L increase significantly (P ≤ 0.05) compared with control group G1(31.36±0.5) m/L.



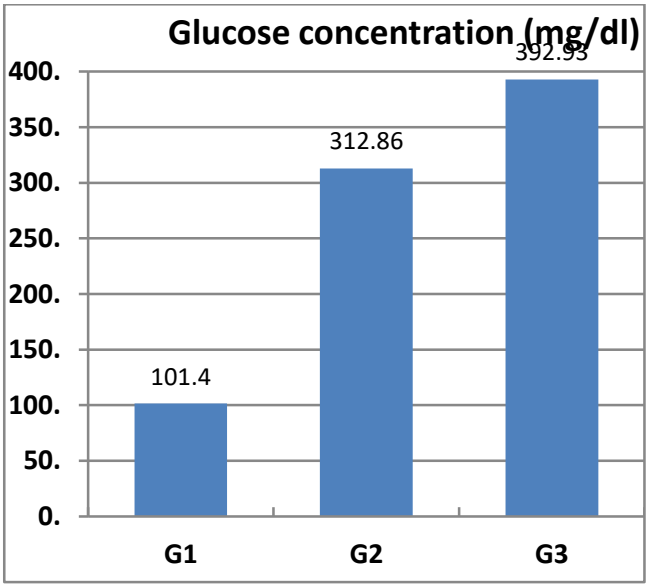
Fig(4): Peroxynitrate concentration according to groups: G1(D.W) , G2 (Alloxan 150 ml/kg for two weeks), G3 (Alloxan 150mg/kg for 24 days) .

Peroxynitrite is a toxic portion produced by inactivation of the superoxide anion during the nitrient oxide (NO). Never in vivo has the physiological value of this process of NO metabolism been recorded. We believed that peroxynitrite interaction with physiological parameters was distinct from those linked to NO, which is the behavior of a vasodilator as the process for inactivating the NO (18).

The excessive development of anion-superoxide- and nitric oxide (NO) peroxynitrite, a cytotoxic composition that harm endothelium and jeopardizes its able to release NO, has been seen in animal models. The formation of peroxynitrite is increased presumably in diabetic human due to increase the content of nitrotyrosine (nTy) in plasma protein, however, whether peroxynitrite is formed enough for physiological importance in humans or not, it is unclear (19).

5.Blood glucose concentration: -

The finding from this study reveal that the concentration of glucose in the diabetic G2, G3(312.86±4.91*), (392.93±4.44**) mg/dl increase significantly (P ≤ 0.05) in compared with control group G1(101.4±2.16) mg/dl as shown in fig (5).



Fig(5) : glucose concentration according to the groups : G1 (D.W), G2(Alloxan 150 mg/kg for 14 days) , G3(Alloxan 150 mg/kg for twenty four days).

In this study, at varying intervals, all animals in the control group were healthy. All values were below the usual range of various parameters and indicated their health status. For the entire study period, the diabetic group of rats appeared hyperglycaemic and displayed numerous biochemical and pathomorphological modifications suggesting diabetes. The average body weight reduction significantly was observed in diabetic animals during the whole study. The weight decrease may be referred to hypo-insulin that occurs in diabetes (20).

Diabetic rats are reduced by body weight in relation to the control group in the current study. This can be caused by protein catabolism and dehydration in the diabetes mellitus environment. The elevated level of glucose serum may be induced by the insulin deficiency triggered by the specific disruption to the β cells of the Langerhans islets by alloxan and the lack of glucose homeostasis in effect. This is compatible with the findings from many staff that the cytotoxic activity of alloxan on pancreatic βcells is contributing to type I diabetes mellitus (21). Another research found that the cytotoxic process of alloxan activity on β-cello includes critical sulphydryl (-SH) oxidation in clusters, glucokinase inhibition, toxic free radicals and diseases of the gay calcium intracellular (22). The resulting difference in insulin discharge and associated hyperglycaemia with metabolic and other relevant diabetes complications are due to the subsequent β-cell injury, which is responsible for decreased secretion of insulin.

Conclusion

Results from this study suggest that a rise in serum glucose level may be attributed to an insulin deficiency induced by selective disruption of the β-cells of alloxan islets of Langerhans that in effect rise the Peroxynitrate concentrations as lipid peroxidation and free radical rates increase. Diabetes activation contributes to reduced GSH, CAT and SOD rates. These antioxidant enzymes play a significant function in defending against oxygen free radicals contributing to a rise in the degree of lipid peroxidation, the degradation of cellular organelles and enzymes, and the production of insulin-resistance in the antioxidant protection mechanism.

References

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes care*, *37*(Supplement 1), 2014; S81-S90.
2. Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress—A concise review. Saudi Pharmaceutical Journal. 2016 Sep 1;24(5):547-53.
3. Ozougwu, J.C., Obimba, K.C., Belonwu, C.D. and Unakalamba, C.B., 2013. The pathogenesis and pathophysiology of type 1 and type 2

diabetes mellitus. Journal of physiology and pathophysiology, 4(4), pp.46-57.

4. Yagi H, Matsumoto M, Kunimoto K, Kawaguchi J, Makino S, Harada M. Analysis of the roles of CD4+ and CD8+ T cells in autoimmune diabetes of NOD mice using transfer to NOD athymic nude mice. European journal of immunology. 1992 Sep;22(9):2387-93.
5. Cotran RS, Kumar V, Collins T. Robbins pathologic basis of disease. Noida. *The Pancreas*,2000: 902-929.
6. Wilcox G. Insulin and insulin resistance. Clinical biochemist reviews. 2005 May;26(2):19.
7. Macdonald Ighodaro O, Mohammed Adeosun A, Adeboye Akinloye O. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. Medicina. 2017 Dec;53(6):365-74.
8. Moussa SA. Oxidative stress in diabetes mellitus. Romanian J biophys. 2008;18(3):225-36.
9. Vanessa Fiorentino T, Prioletta A, Zuo P, Folli F. Hyperglycemia-induced oxidative stress and its role in diabetes mellitus related cardiovascular diseases. Current pharmaceutical design. 2013 Oct 1;19(32):5695-703.
10. Al–Zamely M Y.*"Toward understanding the ischemic heart disease via oxidative hypothesis "ph. D* (Doctoral dissertation, Thesis, college of science, AL-Mustansiriya university, 2001.
11. Aebi H. Catalase. Methods of enzymatic analysis. Bergmeyer HU, editor. 1974; 2:674-8.
12. Vanuffelen EB, Van Der ZEE J, De KOSTER MB, Vansteveninck J, Elferink GJ. Intracellular but not extracellular conversion of nitroxyl anion into nitric oxide leads to stimulation of human neutrophil migration. Biochemical Journal. 1998 Mar 1;330(2):719-22.
13. Kumar A, Pant MC, Singh HS, Khandelwal S. Assessment of the redox profile and oxidative DNA damage (8-OHdG) in squamous cell carcinoma of head and neck. Journal of cancer research and therapeutics. 2012 Apr 1;8(2):254.
14. Adams AK, Best TM. The role of antioxidants in exercise and disease prevention. The physician and sportsmedicine. 2002 May 1;30(5):37-44.
15. Jarad AS, Al-Kaisei BI. Effect in of L-Arginine on Some Biochemical and pathological parameter in diabetes mellitus induced by Alloxan-monohydrate in Rats. Al-Anbar Journal of Veterinary Sciences. 2013;6(1):89-100.

16. Rupeshkumar M, Kavitha K, Haldar PK. Hypoglycemic effect of Andrographis echioides on streptozotocin induced experimental rats. J App Pharm. 2015; 7:60-8.
17. Fathy SM, Drees EA. Protective effects of Egyptian cloudy apple juice and apple peel extract on lipid peroxidation, antioxidant enzymes and inflammatory status in diabetic rat pancreas. BMC complementary and alternative medicine. 2015 Dec 1;16(1):8.
18. Hoeldtke RD, Bryner KD, McNeill DR, Hobbs GR, Baylis C. Peroxynitrite versus nitric oxide in early diabetes. American journal of hypertension. 2003 Sep 1;16(9):761-6.
19. Coppey LJ, Gellett JS, Davidson EP, Dunlap JA, Lund DD, Yorek MA. Effect of antioxidant treatment of streptozotocin-induced diabetic rats on endoneurial blood flow, motor nerve conduction velocity, and vascular reactivity of epineurial arterioles of the sciatic nerve. Diabetes. 2001 Aug 1;50(8):1927-37.
20. Rang HP. Pharmacology. Churchill Livingstone; 2001.
21. Yin P, Wang Y, Yang L, Sui J, Liu Y. Hypoglycemic effects in alloxan-induced diabetic rats of the phenolic extract from Mongolian Oak Cups enriched in ellagic acid, kaempferol and their derivatives. Molecules. 2018 May;23(5):1046.
22. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiological research. 2001 Jan 1;50(6):537-46.