ORAL CELLGEVITY® IMPROVES ANTIOXIDANT PARAMETERS AND STALLS DAMAGES IN STZ-DIABETIC RAT PANCREAS

Supported by tetfund TERTIARY EDUCATION TRUST FUND

Bukunola Oluyemisi Adegbesan[1]*, Olugbenga Owolabi Ogunlabi[1], Olatunbosun Oladipupo Olawale[2], Adeleye Adegboyega Edema[1] and Oladayo Olaoluwa Onasanya[1]

[1]Department of Biochemistry, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Ogun State, Nigeria

[2]Department of Chemical Pathology and Immunology, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Ogun State, Nigeria

*Corresponding author: adegbesan.bukunola@oouagoiwoye.edu.ng, bukrol02@yahoo.com

Received: September 13, 2020 **Accepted:** February 19, 2021

Abstract: Oxidative stress-induced pancreas damage is a key event in beta cell impairment and diabetes development. Catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) are three important cellular antioxidant enzymes protecting the pancreas against oxidant insult. The present study examines the protective capabilities of Cellgevity® (a popular poly-antioxidant supplement) on pancreas oxidative stress parameters. Diabetes was induced by streptozotocin (STZ) intraperitoneal intoxication (55 mg/kg) in adult male Wistar rats. Control and diabetic untreated (DM-untreated) groups received distilled water, diabetic treated 1 group (DM-treated 1) received Cellgevity® (25 mg/kg body weight) and Diabetic treated 2 (DM-treated 2) group received Cellgevity® (40 mg/kg body weight) for twenty-eight days, respectively. The results show that diabetes in DM-untreated significantly increased MDA compared to non-diabetic control group while Cellgevity® significantly ($p<0.001$) reduce level of pancreas MDA (40 and 45%) in DM-treated 1 & 2, respectively compared to DM-untreated animals. Serum total antioxidant status (TAS) was also significantly increased (55 and 80%, respectively) in the DM-treated 1&2 groups compared to DM-untreated. The activities of pancreatic superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were significantly reduced in DM-untreated compared to control. However, treatment with Cellgevity®in DM-treated 1 & 2 groups improved the activities of the antioxidant enzymes when compared to DM-untreated group. These results show the protective role of Cellgevity® against streptozotocin-induced pancreatic damage; which may be due to the support of endogenous glutathione system by Cellgevity®.

Keywords: Antioxidant enzymes, β-cells, diabetes, lipid peroxidation, total antioxidant status

Introduction

Diabetes is a multifactorial clinical disorder characterized by hyperglycemia, resulting from defects in insulin sensitivity of peripheral tissues and or insulin secretion (Brownlee, 2001; Negre-Salvayre *et al.*, 2009). Chronic hyperglycemia leads to oxidative stress which is caused by excess free radicals produced through glucose autoxidation and macromolecule's glycation reactions (Negre-Salvayre *et al.*, 2009). Hyperglycemia induces the non-enzymatic glycation of macromolecules such as proteins, lipids, and nucleic acids thereby altering their functions, increasing lipid peroxidation, and cells damage (Ceriello, 1999; Gugliucci, 2000; Henning, 2018). The excess free radicals negatively impact the delicate physiological balance between free radical production and endogenous antioxidant defence mechanism; causing exacerbation of oxidative stress, metabolic distress and cell damages. This leads to various cellular dysfunctions and physiological complications that could trigger an autocatalytic cycle of further metabolic disturbances, cell death and several health complications (King and Loeken, 2004; Asmat *et al.*, 2016). Oxidative stress in diabetes persists due toloss of insulin action and impaired glucose uptake which causes blood glucose concentrations to remain high; this leads to increased glucose flux, excessive production of reactive species (ROS and RNS) and exhaustion of the natural antioxidant defence system.

A complex crosstalk exists between multiple physiological pathways which are involved in the activation and inhibition of ROS generation; this is well reviewed elsewhere (He and King, 2004; King and Loeken, 2004; Hurrle and Hsu, 2017a). Pancreatic beta cells are the site of insulin production and their loss of function is a key event in the pathogenesis of diabetes (Drews *et al.*, 2010). Beta cells are highly sensitive towards oxidative stress because they have diminished endogenous antioxidant enzymes' expression (Lei and Vatamaniuk, 2011); therefore, oxidative stress induced loss of function is a key factor in beta cell's impairment and diabetes development (Kajimoto and Kaneto, 2004; Drews *et al.*, 2010; Lei and Vatamaniuk, 2011; Hurrle and Hsu, 2017b).

When the generation of free radicals rises beyond the inhibitory capacities of endogenous antioxidant defence system, it results in oxidative stress and cellular dysfunction (Bajaj and Khan, 2012). Diabetes-induced free radicals are important in the pathogenesis of diabetes and antioxidants have important roles in curbing these radicals (Bajaj and Khan, 2012). Antioxidants could act by; free radical scavenging, inhibition of ROS formation and support of natural antioxidant enzymes (Bajaj and Khan, 2012). Glutathione, vitamins C & E, copper, zinc, manganese, Superoxide Dismutase (SOD), Glutathione peroxidase, Glutathione reductase, Catalase, Glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGDH) are the key enzymes and molecules of the endogenous antioxidant defence system, and they have many essential biochemical, metabolic and physiological roles (Ulusu *et al.*, 2019).

In this study, we induced diabetes in male rats through high caloric feeding and drug (streptozotocin) intoxication. High sucrose feeding was to stimulates insulin resistance (Santuré *et al.*, 2002) while streptozotocin intoxication produces reactive oxygen species, which destructs pancreatic beta cells causing disruption in insulin production, thereby mediating the development of diabetes (Konrad *et al.*, 2001; Ulusu *et al.*, 2019). Many diabetes drugs are formulated to achieve hypoglycaemia but they show limited efficacy at ameliorating diabetes complications (resulting from ROS mediated tissue damages). Antioxidant pharmacotherapy aimed at boosting natural endogenous defence system could achieve broader therapeutic targets in diabetes due to antioxidants' numerous modulatory roles in the cell; antioxidants could therefore

function to enhance greater all-round cell rejuvenation and system repairs in diabetogenic management. This study was designed to evaluate the effect of Cellgevity® treatment (marketed for glutathione enhancement) on serum total antioxidant status, pancreas antioxidant enzymes (SOD, CAT and GPx) and pancreas lipid peroxidation (TBARS level) in streptozotocin induced diabetic male rats.

Materials and Methods

Chemicals

Cellgevity® was purchased from Max international, (USA). Streptozotocin (STZ) was purchased from Sigma Aldrich; table sugar (sucrose) was obtained at the local market and assay kit for total antioxidant status and GPx activity were purchased from Abcam (UK). All other chemicals and reagents used were of analytical grade.

Animals care

The study protocol was approved by the human and animal's ethics committee of Olabisi Onabanjo University, Ago Iwoye, Nigeria and followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Adult male Wistar rats (180 – 210 g) were maintained under standard laboratory conditions and fed with normal rat chow and clean water *ad libitum* to habituate.

Induction of diabetes and experimental design

After habituation, seven (7) rats were randomly selected into the control group while the remaining animals (designated experimental diabetes group) were maintained on high sucrose (HS) feed (60% w/w) for 3 weeks after which diabetes was induced by a single intraperitoneal injection of 55 mg/kg Streptozotocin (in 0.1M citrate buffer, pH 4.5). The FBG of the animals were confirmed after 72 h and rats with fasting blood glucose (FBG)> 250 mg/dL were considered diabetic. The diabetic rats were then randomised into three groups of seven rats each: DM-untreated (received 2 ml distilled water), DM-treated 1 (received Cellgevity® 25 mg/kg bw/day) and DM-treated 2 (received Cellgevity® 40 mg/kg bw/day). The animals were thereafter treated and fed according to their grouping for 28 days.

Tissue harvest

At the end of treatment regime, animals were fasted overnight, anaesthetised and blood obtained through cardiac bleeding into plane bottles. Serum was separated at 4000 rpm for 10 minutes and stored at -20°C. The pancreas was quickly excised, perfused with ice-cold phosphate buffer saline (0.1M pH 7.4) and homogenised in Tris-HCl (0.1M, pH 7.4) 10% (w/v). Pancreas homogenate was there after centrifuged for 20 min at 20,000 rpm 4°C to obtain the post mitochondria supernatant. The supernatant was kept at -20°C and used for the antioxidant enzymes activity, lipid peroxidation and protein concentration assays.

Analytical procedures

Determination of blood glucose level

The blood glucose level of the rats was measured weekly after a 12 h fasting period using AccuChek Active© glucometer (Accu Check Roche, Germany) with blood obtained by tail vein puncture.

Measurement of serum total antioxidant status [TAS]

TAS in serum was determined using a commercial kit by Abcam Laboratories, USA following the manufacturer's protocol; the principle involves the suppression of coloured (dark blue green colour) 2,2'-azino-di-(3-ethylbenzthiazolinesulphonate) radical (ABTS*) with antioxidants (present in the sample or standard). The change in absorbance is measured at 660 nm and compared with Trolox (a vitamin E analogue) calibration. The assay results are expressed as Trolox equivalent (mmol Trolox/L).

Estimation of lipid peroxidation (MDA level)

The extent of pancreas lipid peroxidation (LPO) (concentration of thiobarbituric acid reactive substances - TBARS) was determined according to the method of Ohkawa (Ohkawa *et al.,* 1979). The reaction forms a stable pink/reddish chromophore (TBARS) which absorbs maximally at 532 nm. TBARS concentration was expressed as µmol of malondialdehyde (MDA) per mg protein.

Estimation of catalase (CAT) activity

Catalase (CAT) activity was measured as previously described (Cohen *et al.,* 1970; Sinha, 1972). Catalase preparation is allowed to split H_2O_2 for different periods of time. The reaction is stopped after a particular time by the addition of dichromate/acetic acid mixture and the remaining H_2O_2 is determined by measuring chromic acetate colorimetrically at 610 nm. CAT enzyme unit is defined as the amount of enzyme that catalyses the degradation of 1 mmol of hydrogen peroxide /min/mg protein.

Estimation of superoxide dismutase (SOD) activity

The level of SOD activity is determined as previously reported (Misra and Fridovich, 1972). The principle is based on the ability of superoxide dismutase to inhibit the auto-oxidation of epinephrine. 0.2 ml of enzyme preparation is added to 2.5 ml of 0.05 M Carbonate buffer (pH 10.2) to which 0.3 ml of freshly prepared 0.3 mM epinephrine is added and the increase in absorbance at 480 nm is monitored over a time course against a reference. 1 Unit of SOD activity is defined as the amount necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during an interval of one minute.

Estimation of glutathione peroxidase (GPx) activity

Glutathione peroxidase (GPx) activity was measured using commercial assay kit (Abcam, UK) according to manufacturer's instructions. The assay involves a coupling reaction in which reduced glutathione (GSH) is oxidised to GSSG (as GPx reduces cumene hydroperoxide). GSSG is reduced back to produce GSH by Glutathione reductase (GR), using NADPH. The decrease of NADPH is measured at OD 340 nm and is proportional to GPx activity.

Protein assay

Protein concentration was measured according to Bradford's method (Bradford, 1976) using bovine serum albumin as standard.

Statistical analysis

Results were expressed as mean ± standard error of mean (SEM). Data were subjected to one-way analysis of variance (ANOVA) followed by Turkey's multiple comparison test. All statistics were carried out using GraphPad prism 8.4.3 software. Values of $p < 0.05$ were considered statistically significant.

Results and Discussion

Cellgevity® significantly improved serum total antioxidant status (TAS) of diabetic rats

The level of TAS was determined in the serum and the result (Fig. 1) shows that there was a significant ($P < 0.005$) decrease in the serumtotal antioxidant status of diabetic untreated rats compared with control non-diabetic rats. Treatment with Cellgevity® (DM-treated 1 & 2) significantly improved TAS ($p< 0.05$ and $P < 0.005$) compared with the untreated animals

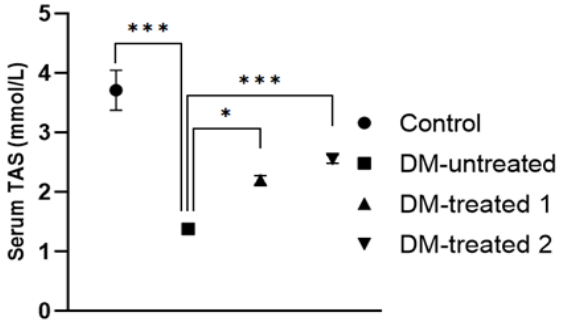

Fig. 1: Effect of Cellgevity® treatment on serum total antioxidant status

Chart shows the level of serum TAS in control and diabetic. The data are expressed as mean with error bars representing SEM. *indicates p< 0.05 and*** indicates p< 0.005. Value for the diabetic untreated group was compared with the corresponding value for normal control animals or diabetic treated animals.

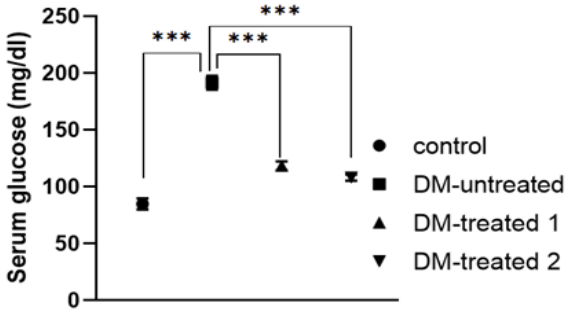

Fig. 2: Effect of Cellgevity® treatment on fasting serum glucose level

Chart shows the level of serum glucose in control and diabetic. The data are expressed as mean with error bars representing SEM. *** indicates p< 0.005 when value for the diabetic untreated group was compared with the corresponding value for normal control animals or diabetic treated animals.

Cellgevity® significantly improved blood fasting glucose of diabetic rats

The fasting blood glucose level was determined and result presented in Fig. 2. There was significant (p< 0.005) increase in the fasting blood glucose of diabetic untreated rats compared with control non-diabetic rats. However, chronic 28 days' treatment with Cellgevity® (DM-treated 1&2) significantly reduced FBG (p< 0.005) compared with the untreated animals.

Oxidative stress parameters

Cellgevity® stalls lipid peroxidation in diabetic rats' pancreas

Lipid peroxidation across the groups was estimated and results presented in Fig. 3A show a significant increase (P < 0.01) in tissue MDA of diabetic untreated versus control. However, this increase was significantly reversed (P <0.05 & P<0.005) with Cellgevity® treatment in DM-treated 1&2 respectively compared to DM-untreated group.

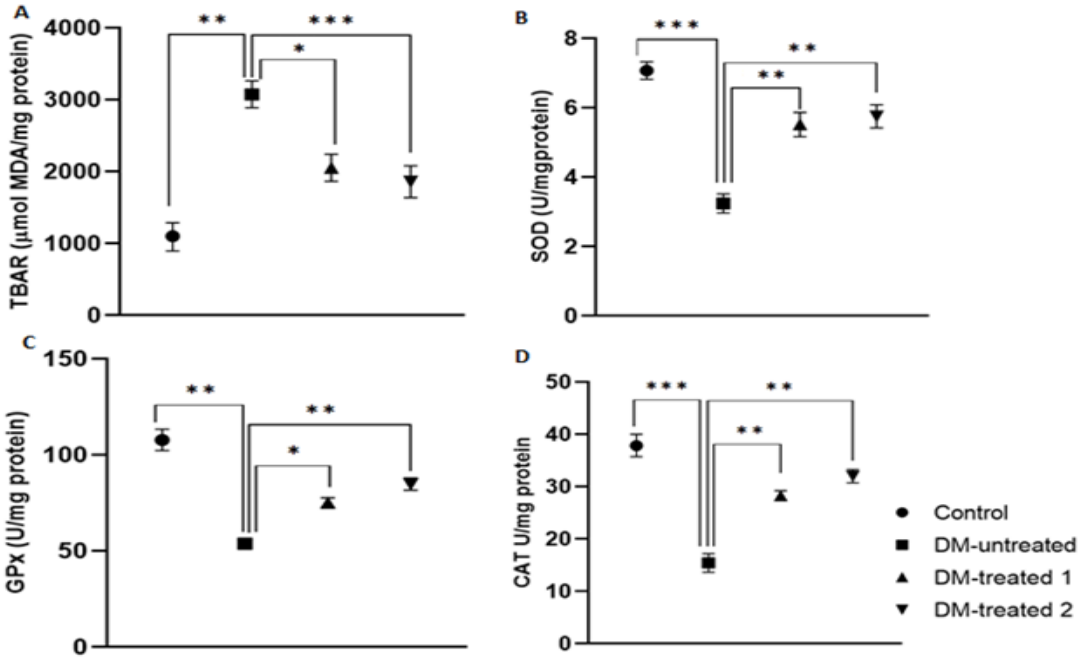

Fig. 3: Effect of Cellgevity® on pancreas LPO and antioxidant enzymes

Charts show; Level of lipid peroxidation in pancreas (3A) and the activities of pancreas antioxidant enzymes; SOD (3B), GPx (3C) and CAT (3D). The data are expressed as mean with error bars representing SEM. * indicates p< 0.05, ** indicates p< 0.01 and *** indicates p< 0.005. Value for the diabetic untreated group was compared with the corresponding value for normal control animals or diabetic treated animals.

Cellgevity® improved pancreas antioxidant enzymes in STZ-diabetic rats

The activities of pancreas cytosolic antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were determined in the pancreas homogenate post mitochondrial supernatant. Fig. 3B, 3C and 3D show significant decreases in the enzymes' activity in untreated diabetic (DM-untreated) rats compared with control while treatment with Cellgevity® in DM-treated 1&2 increased the enzyme's activity compared with DM-untreated. Supplementation with antioxidants as a complementary pharmacotherapy could exert beneficial effects in diabetes. This study investigated the therapeutic potential of Cellgevity® at preventing oxidative damage in pancreas of diabeticrats. The antioxidant enzyme system works to prevent oxidative stress by scavenging excess ROS thereby preventing its accumulation within the system (Asmat *et al.,* 2016). Similar to our current results, other workers have reported decreases in the levels of SOD, GPx and CAT in organs of diabetic rat's models (Ueno *et al.*, 2002; Kuhad and Chopra, 2008; Drews *et al.,* 2010; Kataya *et al.*, 2011) possibly by inhibiting mitochondrial free radical generation. The glutathione system (consisting of SOD, GPx, GR and CAT) is an important endogenous antioxidant enzyme mechanism that guards the cells against free radical-induced tissue insults (Lutchmansingh *et al.,* 2018). Disruption in oxidant-antioxidant balance would cause alterations in the antioxidant enzymes' activity, causing impaired glutathione metabolism (Maritim *et al*., 2003). Beta cells of the pancreas are highly vulnerable to ROS induced damages because the pancreas is relatively low on antioxidant enzymes' expressions. As a result, hyperglycaemia induced excess ROS production leads to pancreatic damages and beta cell disruption. Our current results show that STZ-induced diabetes impairs the activity of pancreatic antioxidant enzymes; which may be due to excessive production of free radicals such as superoxide (O^{-2}), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH*) that could reduce the activity of these enzymes (Onozato *et al.*, 2002). Increases in antioxidant enzymes expression and/or activities may occur in some organs in response to increased free radicals; however, excessive free radical increase would overwhelm the coping capacities of the enzyme system, causing impaired ROS signaling and oxidative distress (Ha and Lee, 2005).

Antioxidants may act at different levels; inhibiting ROS formation, ROS scavenging and supplementing the antioxidants defence system through enhanced enzyme expressions. Diabetes-induced overproduction of free radicals and protein glycation could cause reductions in the activity of the antioxidant enzymes (Ceriello, 1999).

Conclusion

The present study highlights that Cellgevity® treatment could reinforce the pancreas antioxidant defence system thereby inhibiting lipid peroxidation and tissue damage. Cellgevity® also improved the total antioxidant status in the STZ-diabetic rats. The effects of Cellgevity® could be due to glutathione enhancement (GPx, SOD and CAT are members of the glutathione enzyme system) in the animals as well as antioxidant actions of other compounds present in Cellgevity® (Awodele *et al.*, 2018).

Acknowledgement

The authors wish to acknowledge Mr P.O. Oshineye and Mrs T.F. Ashidi (Laboratory Technologists) at the department of Biochemistry, Olabisi Onabanjo University, Nigeria for their technical assistance.

Conflict of Interest

All the authors declare that there is no conflict of interest whatsoever regarding this paper.

References

Asmat U, Abad K & Ismail K 2016. Diabetes mellitus and oxidative stress - A concise review. *Saudi Pharmaceutical Journal*, Elsevier B.V., 547–553. doi: 10.1016/j.jsps.2015.03.013.

Awodele O, Badru W, Busari A, Kale O, Ajayi T, Udeh R & Emeka P 2018. Toxicological evaluation of therapeutic and supra-therapeutic doses of Cellgevity® on reproductive function and biochemical indices in Wistar rats. *BMC Pharmacology and Toxicology., BioMed Central Ltd*., 19(1): 68. doi.org/10.1186/s40360-018-0253-y.

Bajaj S & Khan A 2012. Antioxidants and diabetes. *Indian Journal of Endocrinology and Metabolism*., 16(Suppl 2): S267. doi: 10.4103/2230-8210.104057.

Bradford MM 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochem., Academic Press*, 72(1–2): 248–254. doi: 10.1016/0003-2697(76)90527-3.

Brownlee M 2001. Biochemistry and molecular cell biology of diabetic complications. *Nature.*, 414(6865): 813–820. doi: 10.1038/414813a.

Ceriello A 1999. Hyperglycaemia: The bridge between non-enzymatic glycation and oxidative stress in the pathogenesis of diabetic complications.*Diabetes, Nutrition and Metabolism - Clinical and Experim.*, 12(1): 42–46.

Cohen G, Dembiec D & Marcus J 1970. Measurement of catalase activity in tissue extracts. *Analytical Biochemistry*, 34(1): 30–38. doi: 10.1016/0003-2697(70)90083-7.

Drews G, Krippeit-Drews P & Duïfer M 2010. Oxidative stress and beta-cell dysfunction. *Pflugers Archiv Euro. J. Physiol*., Springer., 460(4): 703–718. doi: 10.1007/s00424-010-0862-9.

Gugliucci A 2000. Glycation as the glucose link to diabetic complications. *J. Amer. Osteopathic Assoc*., 100(10): 621-634. doi: 10.7556/jaoa.2000.100.10.621.

Ha H & Lee HB 2005. Reactive oxygen species amplify glucose signalling in renal cells cultured under high glucose and in diabetic kidney. *Nephrology*, 10: S7-S10. doi: 10.1111/j.1440-1797.2005.00448.x.

He Z & King GL 2004. Microvascular complications of diabetes. *Endocrinology and Metabolism Clinics of North America*, 33(1): 215–238. doi: 10.1016/j.ecl.2003.12.003.

Henning RJ 2018. Type-2 diabetes mellitus and cardiovascular disease. *Future Cardiology., Future Medicine Ltd*., 14(6): 491-509. doi: 10.2217/fca-2018-0045.

Hurrle S & Hsu WH 2017a. The etiology of oxidative stress in insulin resistance. *Biomedical Journal*., Elsevier B.V., 40(5): 257–262. doi: 10.1016/j.bj.2017.06.007.

Hurrle S & Hsu WH 2017b. The etiology of oxidative stress in insulin resistance. *Biomedical Journal*., Elsevier., 40(5): 257–262. doi: 10.1016/J.BJ.2017.06.007.

Institute of Laboratory Animal Resources (US) 1986. Committee on Care, & Use of Laboratory Animals 1986. *Guide for the care and use of laboratory animals* (No. 86), US Department of Health and Human Services, Public Health Service, National Institutes of Health. National Academies Press. doi: 10.17226/5140.

Kajimoto Y & Kaneto H 2004. Role of Oxidative Stress in Pancreatic β-Cell Dysfunction. In: *Mitochondrial Pathogenesis*. Springer Berlin Heidelberg, pp. 168–176. doi: 10.1007/978-3-662-41088-2_17.

Kataya HH, Hamza AA, Ramadan GA & Khasawneh MA 2011. Effect of licorice extract on the complications of diabetes nephropathy in rats. *Drug and Chemical Toxicology*, 34(2): 101–108. doi:10.3109/01480545.2010.510524.

King GL & Loeken MR 2004. Hyperglycemia-induced oxidative stress in diabetic complications. *Histochemistry and Cell Biology*, Springer, 122(4): 333–338. doi: 10.1007/s00418-004-0678-9.

Konrad RJ, Kolaenko I, Tolar JF, Liu K & Kudlow JE 2001. The potential mechanism of the diabetooenic action of streptozotocin: Inhibition of pancreatic β-cell O-GlcNAc-selective N-acetyl-β-D-glucosaminidase. *Biochemical Journal*, 356(1): 31–41. doi: 10.1042/0264-6021:3560031.

Kuhad A & Chopra K 2008. Effect of sesamol on diabetes-associated cognitive decline in rats. *Experimental Brain Research*, Springer, 185(3): 411–420. doi: 10.1007/s00221-007-1166-y.

Lei XG & Vatamaniuk MZ 2011. Two tales of antioxidant enzymes on β cells and diabetes. *Antioxidants and Redox Signaling*, 14(3): 489–503. doi: 10.1089/ars.2010.3416.

Lutchmansingh FK, Hsu JW, Bennett FI, Badaloo AV, Mcfarlane-Anderson N, Gordon-Strachan GM, Wright-Pascoe RA, JAHOOR F & BOYNE MS 2018. Glutathione metabolism in type 2 diabetes and its relationship with microvascular complications and glycemia. *PLoS ONE*. *Public Library of Science*, 13(6): e0198626. doi: 10.1371/journal.pone.0198626.

Misra HP & Fridovich I 1972.The Role of Superoxide Anion in the Autoxidation of Epinephrine and a Simple Assay for Superoxide Dismutase. *J. Bio. Chem.*, 247(10): 3170-3175, Available at: http://www.jbc.org/ (Accessed: 17 August, 2020).

Negre-Salvayre A, Robert S, Nathalie, Reinald P & Manuel P2009. Hyperglycemia and glycation in diabetic complications. *Antioxidants and Redox Signaling*, 11(12): 3071–3109. doi: 10.1089/ars.2009.2484.

Ohkawa H, Ohishi N& Yagi K1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95(2): 351–358. doi: 10.1016/0003-2697(79)90738-3.

Onozato ML Tojo A, Goto A, Fujita T & Wilcox CS 2002. Oxidative stress and nitric oxide synthase in rat diabetic nephropathy: Effects of ACEI and ARB. *Kidney International*, Elsevier, 61(1): 186–194. doi: 10.1046/j.1523-1755.2002.00123.x.

Santuré M, Pitre M, Marette A, Deshaies Y, Lemieux C, Lariviére R., Nadeau H & Bachelard H 2002. Induction of insulin resistance by high-sucrose feeding does not raise mean arterial blood pressure but impairs haemodynamic responses to insulin in rats. *British Journal of Pharmacology*, 137(2): 185–196. doi: 10.1038/sj.bjp.0704864.

Sinha AK 1972. Colorimetric assay of catalase. *Analytical Biochemistry*. *Anal Biochem*, 47(2): 389–394. doi: 10.1016/0003-2697(72)90132-7.

Ueno Y, Kizaki M, Nakagiri R, Kamiya T, Sumi H & Osawa T 2002. Dietary glutathione protects rats from diabetic nephropathy and neuropathy. *Journal of Nutrition*, *American Institute of Nutrition*, 132(5): 897–900. doi: 10.1093/jn/132.5.897.

Ulusu NN, Gok M, Erman B & Turan B2019. Effects of timolol treatment on pancreatic antioxidant enzymes instreptozotocin-induced diabetic rats: An experimental and computational study. *Journal of Medical Biochemistry*., 38(3): 306–316. doi: 10.2478/jomb-2018-0034.