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STUDY OF THE GENDER RELATED COMPLICATIONS OF EXPERIMENTALLY INDUCED HYPERGLYCEMIA ON SOME VITAL PARAMETERS IN LOCAL RABBITS

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Abstract

The impacts of hyperglycemia on some hematological and serum lipid parameters in rabbits during the seventy days were explored. The parameters assessed incorporate serum lipids, red and white platelet files. Fifty two local breed rabbits, weighing 1.7-2.2 kg was utilized in this study. The rabbits were divided equally into, diabetic group (26 rabbits) and a control group (26 rabbits). Each group in turn was subdivided equally according to gender into, male and female groups. The hyperglycemia was induced by a single injection of alloxan monohydrate at dose 100 mg /kg via the marginal ear vein. The blood samples were collected to assert fasting serum glucose and serum lipids, red and white platelet files in periods of 10, 30, 50 and 70 days. The outcomes display that the hyperglycemia affects both genders as the difference was mild in some parameters, especially with regard to lipid variables. A fundamentally diminished packed cell volume, RBCs, and WBCs count in diabetic groups. However, the hyperglycemia had a less considerable influence on MCH, MCHC and MCV. Moreover, the hyperglycemia extensively elevated total cholesterol and serum triacylglycerol levels in the serum whilst it possessed a less significant effect on serum HDL-cholesterol concentration when compared with control groups. In conclusion, the hyperglycemia may also have an impact on serum cholesterol concentration, although it adversely affects some hematological indices.

***Keywords*:** Hyperglycemia, Hematological parameters, Serum lipid, Rabbits

Introduction

Diabetes mellitus, a main non-transferable illness with different etiological agents, influences million of individuals worldwide and according to the World Health Organization (WHO) around 300 million person would encounter the ill effects of diabetes mellitus continuously 2025 and along these lines it considered as one of the five driving purposes behind death on the world (Zimmet 1999; Zheng *et al*., 2018; Organization, Canada, and Canada 2005; WHO, 2006). Diabetes mellitus is depicted by hyperglycemia in view of unsettling influence in carbohydrates metabolism and defective secretion of insulin, that outcomes in acute and long-term diabetic confusions, which are liable for premature death and disability (Paolisso *et al*., 1993; Southerland *et al*., 2006; Pavana *et al*., 2007).

The hyperglycemia impact the structure and function of all tissues, just as hemopoietic tissue. One of the most clear adverse effect of hyperglycemia is the defect in the ratio of low density lipoprotein LDL/LDL receptor. Anthor evident case of the functional disorder of the diabetes progresses is the chronic kidney disease (CKD), decreased production of erythropoietin, and anemia (Collaboration 2010; Inzucchi *et al*., 2011; Association 2014; Reiniger *et al*., 2010; Collaboration 2010; Webster *et al*., 2017; Loutradis *et al*., 2016).

In patients with diabetic nephropathy, the beginning of anemia can manifest from the get-go at some phase in CKD (Hassan 2012). As CKD progresses, anemia typically worsens (Webster *et al*., 2017). In diabetic patients, the serum creatinine, and albumin excretion rate are the controllers of hemoglobin concentration, proposing that the erythropoietin response in patients with diabetes may be due to early renal interstitial damage or the glycosylation mechanism (Artunc and Risler, 2007).

Anemia is one of the most common complications among individual with type 1 diabetes. People with diabetes who do not follow an appropriate diet are at risk of developing anemia, due to a lack of nutrition or eating inappropriate foods, as they are more likely to have iron and folate deficiency. The state of nephropathy associated with diabetes is one of the most important reasons that necessarily lead to anemia in patients (Shu *et al*., 2006; Mehdi and Toto 2009; Pyram *et al*., 2012). The current study aimed to investigate the effect of gender related complications in experimentally induced diabetes mellitus on some hematological parameters in the experimental rabbits.

Materials and Methods

1. The Experimental Animal:

Fifty two local breed rabbits, weighing 1.7-2.2 kg was utilized. Rabbits were housed in confines with suitable dimension (150×100×90), temperature 25±2 ºC and humidity 60%, with standard granulated nourishment, and freely accessible water. The experimental animals were left for two weeks for the purpose of adapting before starting the experiment.

2. Study Design

The rabbits were divided equally into two groups: diabetic group (26 rabbits) and control group (26 rabbits). Each group in turn was subdivided equally according to gender into two groups**:** male and female groups.

3. Experimental Induction of Diabetes Mellitus

- **Preparation of Alloxan for venous infusion :** Alloxan (Sigma Pharmaceuticals Company) was prepared by dissolving 100 mg of alloxan in 1 ml of 0.9% normal saline (Bhimji, Godin, and McNeill 1985).
- **Injection of Alloxan and post animal care :** A sole injection dose of alloxan monohydrate 100 mg /kg via the marginal ear vein was used for induction of diabetes mellitus in overnight fasting rabbits. Immediately, after injection, 10 ml/20% and 2 ml/5% glucose were given intravenously and orally, respectively, in order to beat the sudden hypoglycemia. While, the control groups were injected intravenously with 1 ml of 0.9 % of normal slain. Moreover, all experimental rabbits was prevented from eating for 12 h, and drinking water was substituted by 5% glucose for 24 h. The procedures of alloxan infusion and blood collection done under kitamine 44 mg /kg-xylazine 5 mg/kg sedation.

4. Collection of Blood

According to the schedule set by the study 10, 30, 50, and 70 days, the blood samples were collected and placed in test tube free of anticoagulant. The blood samples were allowed to clot and the serums were obtained by centrifuging at 4000 rpm for 5 minutes (Parasuraman, Raveendran, & Kesavan, 2010). The clear serum was removed by pipetting and part of the blood serum was used directly for a blood glucose level examination and the other part was preserved at a – 20 ºC for the purpose of conducting the serum lipid variables at the end of the study.

5. Estimation of Fasting Serum Glucose

The evaluation of the fasting serum glucose (FSG) in the rabbits of the experiment was conducted three days after the introduction of hyperglycemia. The rabbits were starved for 12 hours before checking. Use the diagnostic kit supplied by the (Spinrecta, Spin), for this purpose. The evaluation of FSG was then carried out at different intervals 10, 30, 50 and 70 days after the introduction of hyperglycemia.

6. Determination of Serum Lipid Parameters

The total serum cholesterol and serum triacylglycerol concentration were estimated by using special kit prepared by (Spinrecta, Spin), while serum HDL -cholesterol concentration was determined by the method of (Assmann, Schriewer, Schmitz, & Hägele, 1983).

7. Determination of Hematological Parameters

The hematological parameters including the packed cell volume (PCV) checked by the method of Jain, (1986), red blood cell (RBC) and white blood cell (WBC) counts estimated by the routine haemocytometer method. The platelet count (PLC) was determined by the RBC/Platelet Ratio Method (International Council for Standardization in Haematology, 2001).

The red cell indices were calculated according to the method described by Jain (1986):

Mean Corpuscular Volume (MCV) = PCV * 10/RBC(fl)

Mean Corpuscular Haemoglobin (MCH) = Hb * 10/RBC (pg)

Mean Corpuscular Haemoglobin concentration = Hbc = 100/PCV

Statistical Analysis

The outputs presented as mean ±SD were analyzed using the Duncan Multiple Range test and differences at $P< 0.05$ were considered significant (Montgomery 2017). The SPSS software version 22 was used for the purpose of conducting various statistical analyzes (SPSS, 2012).

Results and Discussion

The effect of hyperglycemia on some serum lipid indices is presented in (Figures: 1, 2 and 3). Hyperglycemia significantly ($P<0.05$) caused increased serum total cholesterol concentration and posed a significant effect ($P<0.05$) on serum HDL-cholesterol concentration compared to controls. Furthermore, serum triacylglycerol concentration was notably elevated ($P<0.05$) in diabetic groups.

As per various investigations, high blood cholesterol concentration is one of the significant hazard factors for cardiovascular disease (Jackson *et al*., 2005). Al-Karagoly (2007) uncovered that the most significant gross appearance at necropsy after the experimental induction of hyperglycemia in local rabbits was cardiac hypertrophy. Hence the rise in serum total cholesterol fixation instigated by diabetes mellitus expanded the danger of cardiovascular disease (Lorenzo *et al*., 2007). The increment in serum total cholesterol concentration is because of increment in serum HDL-cholesterol concentration. It accordingly infers that height in different fractions of serum total cholesterol concentration other than HDL-cholesterol might be liable for the rise of serum total cholesterol concentration watched.

The present examination shows that the two genders are influenced by the hyperglycemia, the most elevated impact stays in females contrasted with the male, because of the system of lipid metabolism in females. Where, increasingly checked collection of fat in the intra- abdominal visceral fat depots of men. Besides, circling blood lipid concentrations likewise show gender-related contrasts on the grounds that the physiological properties of the female that identified with ovulation and pregnancy, though complete cholesterol, LDL-cholesterol and triacylglycerol focuses are lower and HDL-cholesterol focus is higher than in male (Williams, 2004).

As saw in (Figures: 4, and 5), hyperglycemia fundamentally ($P<0.05$) diminished RBC includes and PCV in diabetic gatherings, when compared to control groups. Be that as it may, MCH, MCHC and MCV were not fundamentally modified by diabetes mellitus when matched to control groups (Figures 6, 7, and 8).

Since MCHC, MCH and MCV relate to individual red blood cells, while RBC and PCV relate to the total population of red blood cells in the blood, it in this manner infer that the diabetes mellitus may neither influence the incorporation of hemoglobin into red platelets nor the morphology and osmotic fragility of red platelets produced (Blaslov *et al*., 2019). In any case, the decrease in RBC and PCV suggests that the hyperglycemia diminishes the number of population of red blood cells from the bone marrow (US, 2019). The hyperglycemia might impact the oxygen binding capacity of individual red blood cell, but may reduce the oxygen- carrying capacity of the whole blood due to his influenced on RBCs indicies, as well as population of red blood cells and anemia occur (Seeley *et al*., 2005; US, 2019; Barrett *et al*., 2010; Ali and Hassan 2019).

Hyperglycemia significantly reduced (P<0.05) WBC counts when compared with control (Figure 9). This indicates, that the hyperglycemia may prompt development of some bioactive agents that could cause decimation or impaired production of white platelets (Barrett *et al*., 2010 and Mishra 2013). It can be concluded that the direct effect of diabetes on white blood cells comes from its action on the granulocyte-macrophage colony stimulating factor, macrophage colony stimulating factor, interleukins IL-2, IL-4 and IL-5 that regulate responsible for the proliferation, differentiation of white blood cells (Grossmann *et al*., 1996, Pennig *et al*., 2019; Banerjee and Saxena 2012; Hall 2015). Thus hyperglycemia may incline to reduce immune response and thus, enhances the possibility of developing various infections (Weekers *et al*., 2003).

The reduction of WBC and at all periods of hyperglycemia and the RBC at the late period of experiment without corresponding reduction in platelet count, suggest that the hyperglycemia may possess the potential of causing a progressive but selective bone marrow depression with increasing time (Marles and Farnsworth 1995, Kornicka *et al*., 2018). Likewise the production of these components of blood might be vulnerable to regulation by the hyperglycemia in an order having white blood cell production as the most susceptible and platelet production as the least susceptible (Malomo *et al*., 2002; Carrizzo *et al*., 2018).

Conclusion

It was inferred that hyperglycemia, antagonistically influence some hematological indices, particularly those identifying with red platelets and white platelets, as well as special effect on serum cholesterol concentration.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Disclosure of potential conflicts of interest and current submission

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other peer-reviewed media.

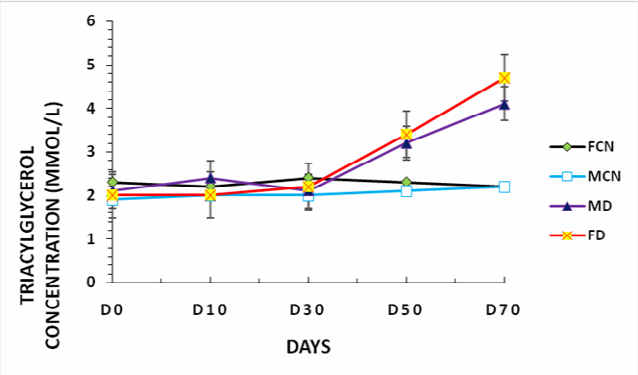

Fig. 1 : Effect of the induced hyperglycemia on Triacylglycerol concentration (mmol/L) in the control and treated groups of the female and male rabbits. (*n*=52)

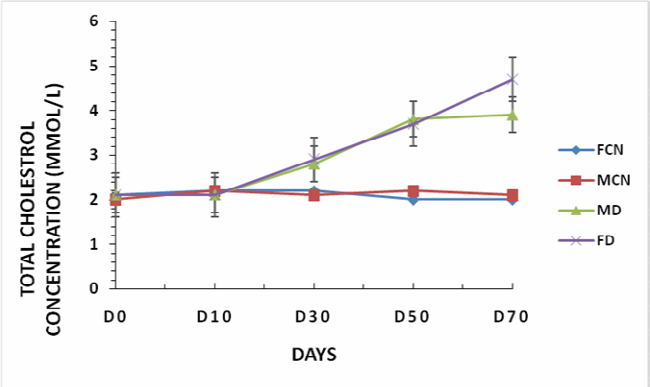

Fig. 2 : Effect of the induced hyperglycemia on total cholesterol concentration (mmol/L) in the control and treated groups of the female and male rabbits. (*n*=52)

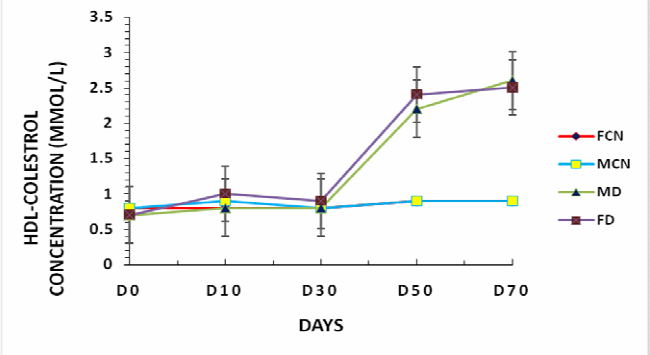

Fig. 3 : Effect of the induced hyperglycemia on HDL-Cholesterol concentration (mmol/L) in the control and treated groups of female and male rabbits. (*n*=52)

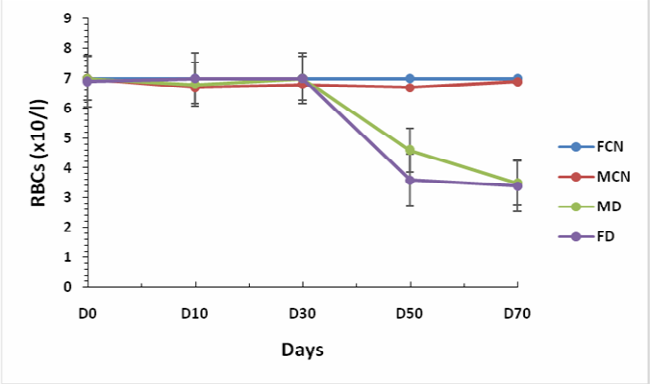

Fig. 4 : Effect of the hyperglycemia on RBCs count (x10/L) in the control and treated groups of the female and male rabbits. (*n*=52)

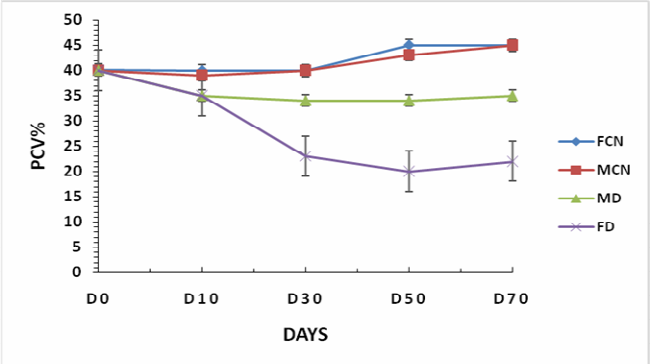

Fig. 5 : Effect of the induced hyperglycemia on PCV percent (%) in the control and treated groups of the female and male rabbits. (*n*=52)

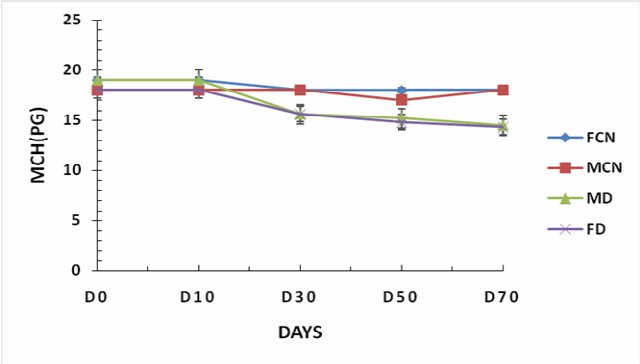

Fig. 6 : Effect of the induced hyperglycemia on MHC (Pg) in the control and treated groups of the female and male rabbits. (*n*=52)

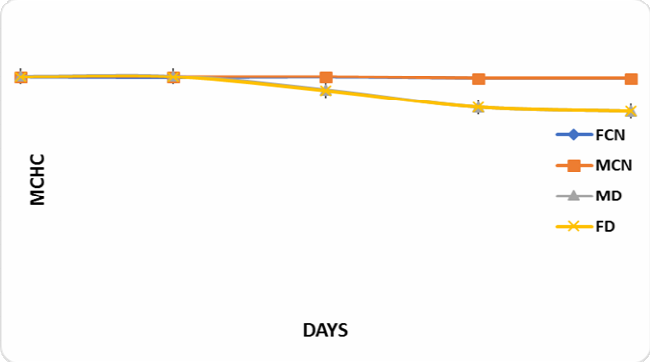

Fig. 7 : Effect of the induced of hyperglycemia on MCHCin the control and treated groups of the female and male rabbits. (*n*=52)

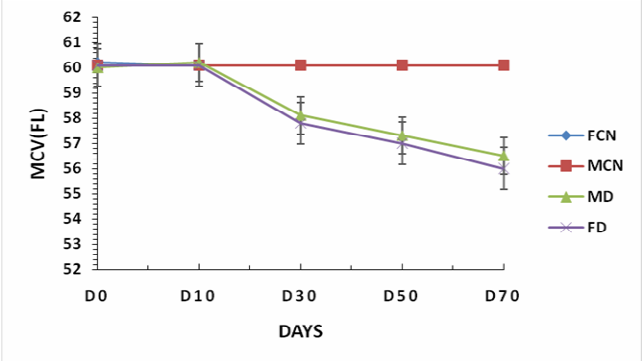

Fig. 8 : Effect of the induced hyperglycemia on MCV (fL) in the control and treated groups of the female and male rabbits. (*n*=52)

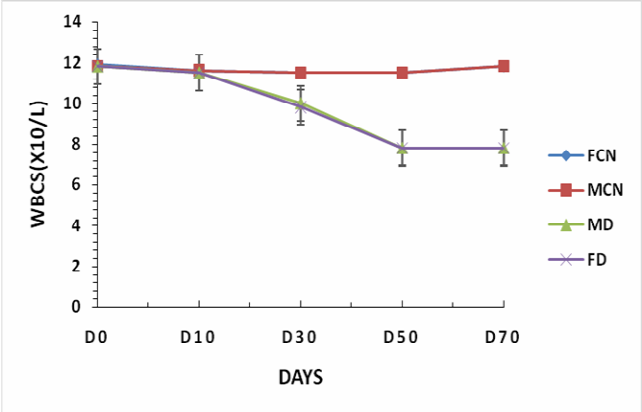

Fig. 9 : Effect of the induced hyperglycemia on WBCs (x10/L) in the control and treated groups of the female and male rabbits. (*n*=52)

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References

Al-Karagoly, HK. (2007). 'Clinlopathology study of Experimental induced diabetes mellitus domestic Rabbits', Master thesis, Collage of veterinary Medicine. University of Busrah. Iraq.

Ali, M.H. and Hassan, A.J. (2019). Assessment of the alteration of blood indices in patients with type 2 diabetic mellitus: A cross-sectional study. Mustansiriya Medical Journal, 18(1): 24.

Al-Karagoly, H.K. (2007). Clinlopathology study of Experimental induced diabetes mellitus domestic Rabbits (Doctoral dissertation, Master thesis, Collage of veterinary Medicine. University of Busrah. Iraq).

Artunc, F. and Teut, R. (2007). 'Serum erythropoietin concentrations and responses to anaemia in patients with or without chronic kidney disease', Nephrology Dialysis Transplantation, 22: 2900-08.

Quantification of high-density-lipoprotein cholesterol by precipitation with phosphotungstic acid/ $MgCl_2$. *Clinical chemistry*, 29(12): 2026-2030.

Association, American Diabetes (2014). 'Diagnosis and classification of diabetes mellitus', Diabetes care, 37: S81-S90.

Banerjee, M., and Saxena, M. (2012). Interleukin-1 (IL-1) family of cytokines: role in type 2 diabetes. Clinica chimica acta, 413(15-16): 1163-1170.

Barrett, K.E.; Susan, M.B.; Scott, B. and H, B. (2010). Ganong's review of medical physiology. 23 (USA: McGraw Hill).

Bhimji, S.; Godin, D.V. and McNeill, J.H. (1985). 'Biochemical and functional changes in hearts from rabbits with diabetes', Diabetologia, 28: 452-57.

Blaslov, K.; Kruljac, I.; Mirošević, G.; Gaćina, P., Kolonić, S.O. and Vrkljan, M. (2019). The prognostic value of red blood cell characteristics on diabetic retinopathy development and progression in type 2 diabetes mellitus. Clinical hemorheology and microcirculation, 71(4): 475-481.

Carrizzo, A.; Izzo, C.; Oliveti, M.; Alfano, A.; Virtuoso, N.; Capunzo, M. and Frati, G. (2018). The main determinants of diabetes mellitus vascular complications: endothelial dysfunction and platelet hyperaggregation. International journal of molecular sciences, 19(10): 2968.

Collaboration, E.R.F. (2010). 'Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies', The Lancet, 375: 2215-22.

Grossmann, A.; Lenox, J.; Hong Ping Ren, Humes, J.M.; Forstrom, J.W.; Kaushansky, K. and Sprugel, K.H. (1996). 'Thrombopoietin accelerates platelet, red blood cell, and neutrophil recovery in myelosuppressed mice', Experimental hematology, 24: 1238-46.

Hall, J.E. (2015). Guyton and Hall textbook of medical physiology e-Book (Elsevier Health Sciences).

Hassan, M.O. (2012). 'Prevalence and Pattern of Chronic kidney Disease in sickle cell patients', Faculty of Internal Medicine.

International Council for Standardization in Haematology Expert Panel on Cytometry International Society of Laboratory Hematology Task Force on Platelet Counting. (2001). Platelet counting by the RBC/platelet ratio method: a reference method. American Journal of Clinical Pathology, 115(3): 460-464.

Inzucchi, S.E. and Sherwin, R.S. (2011). Type 2 diabetes mellitus. Cecil Medicine. 24th ed. Philadelphia, Pa: Saunders Elsevier.

Jackson, R.; Lawes, C.M.; Bennett, D.A.; Milne, R.J. and Rodgers, A. (2005). Treatment with drugs to lower blood pressure and blood cholesterol based on an individual's absolute cardiovascular risk. The Lancet, 365(9457): 434-441.

Jain, N.C. (1986). Veterinary Haematology (Jain N C Ed) Lea and Ferbiger, Philadelphia.

Kornicka, K.; Houston, J. and Marycz, K. (2018). Dysfunction of mesenchymal stem cells isolated from metabolic syndrome and type 2 diabetic patients as result of oxidative stress and autophagy may limit their potential therapeutic use. Stem Cell Reviews and Reports, 14(3): 337-345.

Lorenzo, C.; Williams, K.; Hunt, K.J. and Haffner, S.M. (2007). The National Cholesterol Education Program–Adult Treatment Panel III, International Diabetes Federation, and World Health Organization definitions of the metabolic syndrome as predictors of incident cardiovascular disease and diabetes. Diabetes care, 30(1): 8-13.

Loutradis, Charalampos, Alexandra Skodra, Panagiotis Georgianos, Panagiota Tolika, Dimitris Alexandrou, Afroditi Avdelidou, and Pantelis A Sarafidis. 2016. 'Diabetes mellitus increases the prevalence of anemia in patients with chronic kidney disease: A nested case-control study', World journal of nephrology, 5: 358.

Malomo, S.O.; Adebayo, J.O. and Olorunniji, F.J. (2002). 'Modulatory effect of vitamin E on some haematological parameters in dihydroartemisinin-treated rats', The Tropical Journal of Health Sciences, 9: 15-20.

Marles, R.J. and Norman, R.F. (1995). 'Antidiabetic plants and their active constituents', Phytomedicine, 2: 137-89.

Mehdi, U. and Robert, D.T. (2009). 'Anemia, diabetes, and chronic kidney disease', Diabetes care, 32: 1320-26.

Montgomery, D.C. (2017). Design and analysis of experiments (John wiley & sons).

Organization, World Health, Public Health Agency of Canada, and Canada. Public Health Agency of Canada. 2005. *Preventing chronic diseases: a vital investment* (World Health Organization).

Owoyele, B.V.; Alabi, O.T.; Adebayo, J.O.; Soladoye, A.O.; Abioye, A.I.R. and Jimoh, S.A. (2004). 'Haematological evaluation of ethanolic extract of Allium ascalonicum in male albino rats', Fitoterapia, 75: 322-26.

Paolisso, Giuseppe, Anna D'Amore, Giosuè Di Maro, Domenico Galzerano, Paola Tesauro, Michele Varricchio, and Felice D'Onofrio. 1993. 'Evidence for a relationship between free radicals and insulin action in the elderly', Metabolism-Clinical and Experimental, 42: 659-63.

Parasuraman, S.; Raveendran, R. and Kesavan, R. (2010). Blood sample collection in small laboratory animals. Journal of pharmacology & pharmacotherapeutics, 1(2): 87.

Pavana, P.; Sethupathy, S. and Manoharan, S. (2007). Antihyperglycemic and antilipidperoxidative effects ofTephrosia purpurea seed extract in streptozotocin induced diabetic rats. Indian Journal of Clinical Biochemistry, 22(1): 77.

Pennig, J.; Scherrer, P.; Gissler, M.C.; Anto-Michel, N.; Hoppe, N.; Füner, L. and Mullick, A. (2019). Glucose lowering by SGLT2-inhibitor empagliflozin accelerates atherosclerosis regression in hyperglycemic STZ-diabetic mice. Scientific Reports, 9(1): 1-12.

Pyram, R.; Abhishek, K.; Mary, A.B. and Lisel, L.H. (2012). 'Chronic kidney disease and diabetes', Maturitas, 71: 94-103.

Reiniger, N.; Lau, K.; McCalla, D.; Eby, B.; Cheng, B.; Lu, Y. and Rosario, R. (2010). Deletion of the receptor for advanced glycation end products reduces glomerulosclerosis and preserves renal function in the diabetic OVE26 mouse. Diabetes, 59(8): 2043-2054.

Seeley, R.R.; Trent, D.S. and Philip, T. (2005). Essentials of anatomy and physiology (McGraw-Hill).

Shu, D.H.; Thomas, P.P.R.; O'Connell, C.M.; Jafna, L.C.; Aiser, M.K.; Shirl, A.G.; Richard, C.R.; Ehud, U.R. and Syed, A.I. 2006. 'Anemia is an independent risk for mortality after acute myocardial infarction in patients with and without diabetes', Cardiovascular diabetology, 5: 8.

SM, S. and Mishra, S.S. (2013). Evaluation of hypoglycemic effect of Lagerstroemia Speciosa (Banaba) leaf extract in alloxan induced diabetic Rabbits. International Journal of Medical Research & Health Sciences, 2(2): 217-222.

Southerland, J.H.; George, W.T.; Kevin, M.; James, D.B. and Steven, O. (2006). 'Commonality in chronic inflammatory diseases: periodontitis, diabetes, and coronary artery disease', Periodontology 2000, 40: 130-43.

SPSS. (2012). Statistical Package for Social Science (SPSS) software version 22.

US, M.R. (2019). Effects of Quercitrin on Diabetic Physiological Criterions and Hematological Parameters Studied in Diabetic Rats. International Medical Journal, 26(4).

Webster, A.C.; Evi, V.N.; Rachael, L.M. and Philip, M. (2017). 'Chronic kidney disease', The lancet, 389: 1238-52.

Williams, C.M. (2004). Lipid metabolism in women. Proceedings of the Nutrition Society, 63(1): 153-160.

World Health Organization. (2006). Guidelines for the prevention, management and care of diabetes mellitus.

Zheng, Y.; Ley, S.H. and Hu, F.B. (2018). Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nature Reviews Endocrinology, 14(2): 88.

Zimmet, P.Z. (1999). 'Diabetes epidemiology as a tool to trigger diabetes research and care', Diabetologia, 42: 499-518.