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Therapeutic potential of *Tithonia diversifolia* extract: Modulating IL-35, TNF-α, and hematology profile in streptozotocin-induced rat model

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ABSTRACT

Background: Diabetes mellitus is a significant global health issue with increasing prevalence worldwide.

Aim: This study aims to investigate the potential of *Tithonia diversifolia* extract (TE) in lowering interleukin-35 (IL-35), tumor necrosis factor-alpha (TNF-α), and hematological profile in streptozotocin (STZ)-induced rats.

Methods: A total of 24 rats were divided into four treatment groups: control (P0), diabetic induction (P1), diabetic induction + TE (P2), and diabetic induction + quercetin (P3). Diabetes mellitus was induced by a single-dose injection of stz (60 mg/kg). TE treatment was administered orally for 7 days. On the 8th day post-treatment, all animals were euthanized, and blood samples were collected to assess inflammatory parameters, including IL-35, TNF-α, GPx, and hematological profiles. Kidney organs were fixed in 10% buffered neutral formalin for histopathological analysis. Data were analyzed using ANOVA followed by Duncan's test ($p < 0.05$).

Results: Evaluation of the hematological profile revealed significant improvements in the P2 and P3 groups, with decreased leukocytes, hemoglobin, lymphocytes, and neutrophils, as well as significantly lower IL-35 and TNF-α levels observed in diabetic rats following TE treatment.

Conclusion: TE treatment exhibited promising effects in reducing inflammatory markers and restoring hematological parameters in diabetic rats, indicating its potential as a therapeutic agent in diabetic rats.

Keywords: *Tithonia diversifolia*, Diabetes melitus, Interleukin-35, Tumor necrosis factor-alpha, Hematology.

Introduction

Diabetes mellitus represents a significant global health challenge, with its prevalence steadily increasing worldwide. For instance, Indonesia ranks sixth among the top ten countries with the highest number of diabetes cases, totaling 10.7 million in 2019 (Roglic, 2016). The prevalence of type 2 diabetes mellitus (T2DM) in Indonesia itself reaches 8.6% of the total population, placing the country fourth in terms of this specific diabetes type's prevalence (Arifin *et al*., 2022). Previous studies also project a drastic increase from 8.4 million cases in 2000 to 21.3 million cases by 2030. With this rapid growth, the primary challenge for healthcare systems is to provide effective care and management for diabetes sufferers worldwide (Saeedi *et al*., 2019; IDF, 2021).

T2DM poses a dual threat of microvascular complications (including kidney, nerve, and eye damage) and macrovascular complications (such as heart disease, stroke, and peripheral vascular disease). Among these, diabetic nephropathy, affecting 35%–45% of patients, stands as a significant contributor to kidney failure and holds the highest mortality rate among diabetic complications (ADA, 2020). Furthermore, insulin resistance, primarily stemming from a deficiency of insulin receptors compounded by increased fat accumulation, particularly in the abdominal region, impedes glucose transportation into cells, thus exacerbating hyperglycemia (Faselis *et al*., 2020).

Diabetes complications encompass both microvascular problems, such as kidney, nerve, and eye damage, and macrovascular issues, including heart disease, stroke, and peripheral vascular disease. Diabetic nephropathy, affecting 35%–45% of cases, stands as a primary cause of kidney failure and mortality. The fundamental pathogenesis of T2DM lies in insulin resistance, characterized by a deficiency of insulin receptors compounded by abdominal adiposity, ultimately resulting in hyperglycemia (Wu *et al*., 2015).

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Streptozotocin (STZ) is commonly employed to induce diabetes mellitus in rats due to its ability to elevate blood sugar levels. Compared to alloxan, STZ boasts a longer half-life and greater stability. STZ operates by generating reactive free radicals, which inflict damage upon cellular components such as membranes, proteins, and DNA, consequently disrupting insulin production by pancreatic beta cells (Furman, 2021).

Conventional diabetes treatments, such as insulin injections or synthetic drugs, frequently result in side effects and dependency. As a result, there is a burgeoning interest in natural alternatives like *Tithonia diversifolia* (moonflower), renowned for its antidiabetic properties attributed to its rich content of saponins, flavonoids, and polyphenols. Moonflower leaves harbor a plethora of terpenoid and flavonoid compounds, with flavonoids particularly demonstrating the potential to reduce blood glucose levels (Behl *et al*., 2022). This study aims to investigate the potential of *T. diversifolia* extract (TE) in lowering interleukin-35 (IL-35), tumor necrosis factor-alfa (TNF-α), and hematological profile on STZ-induced rats.

Materials and Methods

The study employed a Randomized Posttest Control Group Design, utilizing male Wistar rats aged 2–3 months and weighing between 200–250 g. Throughout the experiment, all rats were individually housed in plastic boxes with ad libitum access to distilled water. The *T. diversifolia* (TD) plants were sourced from the Traditional Flower Market in Magelang, Central Java, Indonesia. Upon acquisition, fresh TD plants were carefully separated from the roots, stems, and leaves, then thoroughly washed with tap water and weighed. The cleaned leaves were subsequently cut into small pieces and air-dried in a shaded area to avoid direct sunlight exposure. Once dried, the leaves were pulverized into a fine powder, followed by the maceration process utilizing 70% ethanol as a solvent. The resulting extract was then dissolved in a suspension containing 0.1% sodium carboxymethyl cellulose (CMC-Na). This CMC-Na suspension served as the treatment for the positive control group over a period of 7 days based on a previous study by Muniroh *et al*. (2022). Twenty-four rats were divided into four treatment groups: control (P0) receiving distilled water, diabetic-induced with 0.1% CMC-Na (P1), diabetic-induced with TE (P2), and diabetic-induced with quercetin (P3). Diabetes mellitus was induced by administering a single intraperitoneal dose of STZ at 60 mg/kg body weight. TE treatment was orally administered at a dose of 100 mg/kg body weight for 7 days. All rats were euthanized by cervical dislocation for the collection of blood serum and kidney tissue on the 8th day. Serum levels of blood glucose, serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT) were analyzed using the colorimetric method (Diasys diagnostic GmbG, Germany). The concentrations of TNF-α, and glutathione peroxidase (GPx) were determined utilizing rat-specific assay kits (Elabscience, China), while rat interleukin-35 levels were measured using an enzyme-linked immunosorbent assay kit (Bioassay Technology, China).

Hematological evaluation and HE staining

Blood samples were collected from the rats via intracardial puncture and placed in EDTA tubes for hematological profiling. Organ pathology examination involved macroscopic analysis for morphological diagnosis. The kidney specimens were preserved in 10% buffered neutral formalin for 48 hours before hematoxylin and eosin (HE) staining as per the protocol described by Hamid *et al*. (2023).

Statistical analysis

Hematological data were presented as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA) followed by the post hoc Duncan's test for multiple comparisons. Meanwhile, the serological parameters were evaluated using the Kruskal-Wallis and Mann-Whitney tests. Statistical significance was set at $p < 0.05$, as per the findings of a previous study by Solfaine *et al*. (2024).

Ethical approval

The research, conducted at the Experimental Animal Laboratory and Histopathology Laboratory of the Faculty of Veterinary Medicine, Universitas Wijaya Kusuma Surabaya, adhered to the guidelines set by the Animal Ethics and Care Committee (No: 15.KE.017.04.2023).

Results

Based on the results, significant differences were observed in treatment groups P2 and P3 compared to the induction group (P1). This indicates that the induction of diabetes mellitus can impact interleukin-35, TNF-α, and GPx concentrations among treatment groups compared to the control (P0). Additionally, blood glucose levels significantly increased, accompanied by elevated SGOT and SGPT concentrations in the treatment groups (Table 1). Hematological analysis revealed significant differences in fibrinogen and white blood cell (WBC) counts, particularly in lymphocytes and monocytes. However, no significant differences among the treatment and diabetic-induced groups were observed in red blood cell (RBC) count, hemoglobin, or eosinophil values (Table 2).

The histopathological examination of kidney tissue revealed erythrocyte infiltration in both the glomerulus and kidney tubules, indicating hemorrhage within the tissue. Additionally, necrosis of cell nuclei was observed, characterized by pyknosis (condensed and darkened nuclei), karyorrhexis (fragmented nuclei), and karyolysis (lysed nuclei), alongside evidence of ballooning degeneration. These findings suggest that the administration of TE at a dosage of 100 mg/kg

Table 1. The concentration of interleukin-35 (IL-35), TNF-α, and glutation peroxidase (GPx) along with blood biochemical parameters, were measured in both the treatment and control group.

Group	Blood glucose (mg/dl)	SGOT (U/I)	SGPT (U/I)	GPx (pg/ml)	IL-35 (pg/ml)	TNF-α (pg/ml)
P0 (n = 6)	63.00 ± 48.90[a]	116.33 ± 35.07[a]	45.17 ± 18.32[a]	125.70 ± 37.83[a]	162.50 ± 33.80[a]	28.10 ± 4.51[a]
P1 (n = 6)	201.50 ± 50.26[b]	133.17 ± 34.45[b]	37.67 ± 8.52[b]	107.90 ± 24.57[b]	179.50 ± 27.30[b]	45.70 ± 3.94[b]
P2 (n = 6)	197.83 ± 97.04[c]	106.33 ± 15.33[c]	35.17 ± 8.52[b]	120.03 ± 23.82[c]	157.10 ± 38.40[c]	35.20 ± 4.53[c]
P3 (n = 6)	88.50 ± 10.23[d]	111.67 ± 12.59[c]	38.83 ± 7.16[b]	91.80 ± 20.96[d]	150.90 ± 27.10[c]	33.50 ± 5.73[c]

Values are expressed in mean ± SD. [a,b]different superscripts in the same row indicate significant differences ($p < 0.05$). P0 = control group. P1 = diabetic induced group. P2 = diabetic induced+TE group. P3 = diabetic induced+quercetin group. SGOT = Serum Glutamic Oxaloacetic Transaminase. SGPT = Serum Glutamic Pyruvic Transaminase. GPx = glutattion peroxidase, IL-35 = interleukin-35, TNF-α = tumor necrosis factor-alfa.

Table 2. The mean values of hematological and differential blood profiles were compared between both the treatment and control group.

Parameter/group	P0	P1	P2	P3
RBC (10^6/µl)	5.66 ± 1.37[a]	6.02 ± 1.99[b]	5.67 ± 1.30[a]	6.66 ± 1.82[b]
Hb (g/dl)	10.20 ± 1.08[a]	10.53 ± 1.81[a]	11.30 ± 1.82[b]	11.26 ± 1.47[b]
PCV (%)	37.00 ± 2.82[a]	35.33 ± 7.81[b]	38.50 ± 5.46[a]	37.17 ± 4.40[a]
Fibrinogen (g/dl)	0.26 ± 0.20[a]	0.13 ± 0.10[b]	0.23 ± 0.15[a]	0.13 ± 0.10[b]
WBC (10^3/µl)	21.51 ± 5.69[a]	17.70 ± 6.64[b]	13.45 ± 4.44[c]	15.26 ± 4.12[d]
Heterofil (10^3/µl)	9.40 ± 6.15[a]	8.68 ± 4.21[a]	7.88 ± 4.26[a]	6.30 ± 1.43[b]
Limfosit (10^3/µl)	5.28 ± 3.48[a]	2.40 ± 2.41[b]	1.36 ± 1.55[c]	3.40 ± 1.06[d]
Monosit (10^3/µl)	6.76 ± 2.11[a]	6.26 ± 3.24[a]	3.98 ± 1.64[b]	5.43 ± 2.04[c]
Eosinofil (10^3/µl)	0.45 ± 0.50[a]	0.30 ± 0.08[a]	0.067 ± 0.05[b]	0.167 ± 0.06[c]

Values are expressed in mean ± SD. Different superscripts in the same row indicate significant differences ($p < 0.05$). P0 = control group. P1 = diabetic induced group. P2 = diabetic induced+TE group. P3 = diabetic induced+quercetin group. RBCs = Red blood cells. Hb = *Hemoglobin. PCV* = packed cell volume. WBC = white blood cell.

BW to Wistar rats can influence the histopathological appearance of the kidneys (Fig. 1).

Discussion

In this study, TE was administered at a dosage of 100 mg/kg body weight to the subjects. The decision to use this specific dosage was based on previous research, which demonstrated that this concentration effectively reduced interleukin-1 levels in STZ-induced rats. Consequently, no additional dose variations were included in this study, as the primary aim was to replicate the previously observed anti-inflammatory effects of TE at the established effective dose. This approach ensured a focused evaluation of the extract's efficacy without the confounding variables introduced by varying dosage levels.

One of the microvascular complications associated with diabetes mellitus is nephropathy, leading to kidney damage. Hematological tests revealed an increase in leukocytes, attributed to the pro-thrombotic role of TNF-α, which stimulates adhesion molecules on leukocyte and endothelial cells and regulates macrophage activity and immune responses by stimulating growth factors and cytokines (Williams *et al*., 2023). The elevation in blood monocytes is a result of macrophage activation in tissues, leading to the production of TNF, IL-1, and IL-6, which induce various effects including hepatic acute phase response and leukocytosis (Kanter *et al*., 2008). Leukocytosis is closely linked to TNF-α production, which is generated by neutrophils, activated lymphocytes, NK cells, macrophages, astrocytes, endothelial cells, and smooth muscle cells, while TNF-β is primarily produced by T cells (Detrick *et al*., 2008).

Neutrophils, a type of leukocyte, play a crucial role in diabetes mellitus cases. Research by Mowat and Baum indicated that the chemotactic activity of neutrophils in diabetic patients is significantly lower compared to those in healthy controls. Reduced leukocyte phagocytosis and bactericidal activity are notably associated with elevated blood glucose levels. Similarly, findings by Alba-Loureiro *et al*. (2006)

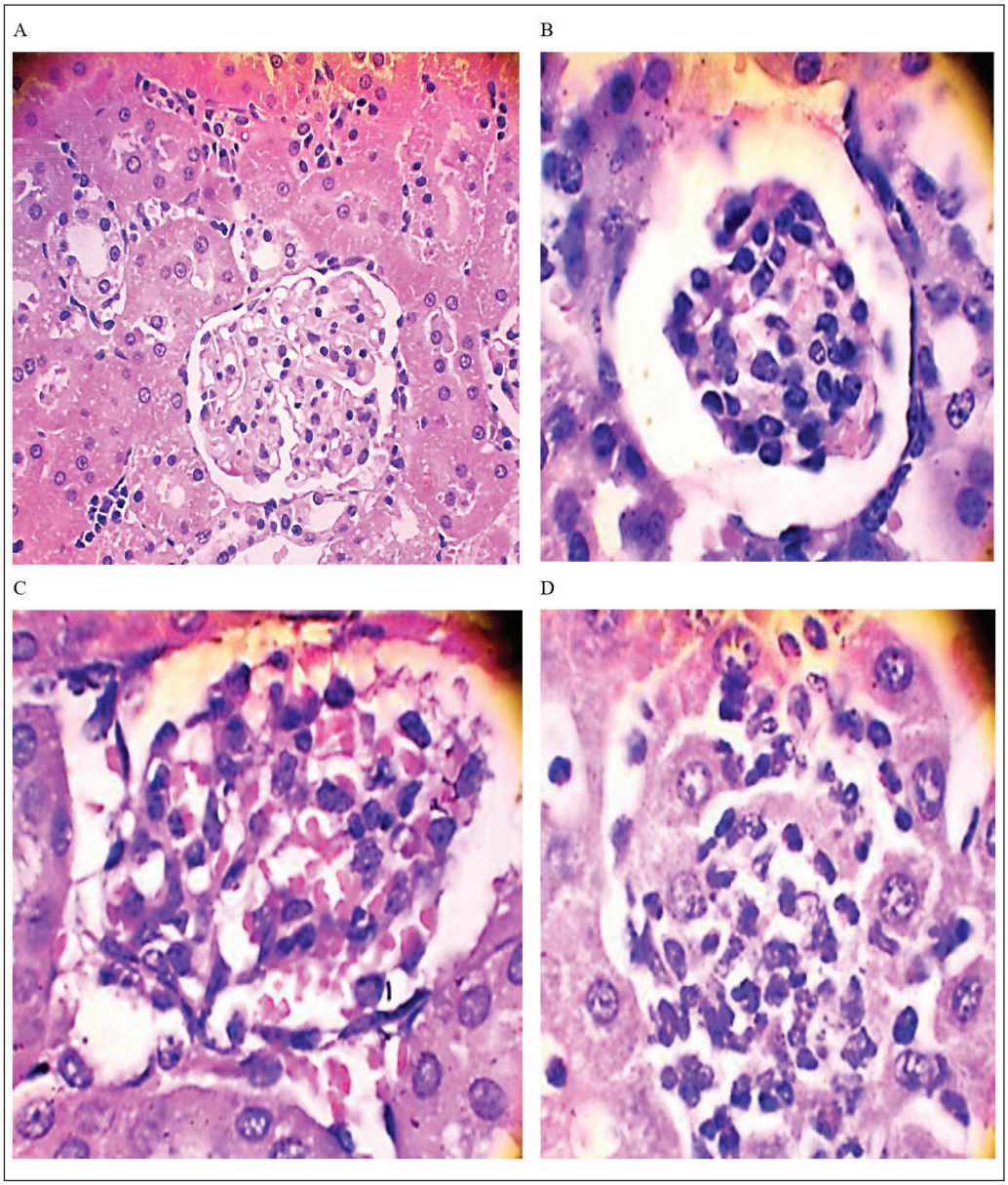

Fig. 1. The histomorphology of kidney tissues was evaluated in both control and treatment groups. Kidney sections from control rats (A) exhibited a normal structural glomerulus, while sections from Stz-Nam (Streptozotocin-Nicotinamide) induced rats (B) showed notable morphological alterations including enlargement of the glomerular regions, necrosis, and infiltration of mononuclear cells in mesangial cells. Kidney sections from Stz-Nam (Streptozotocin-Nicotinamide) induced rats treated with TE (C) displayed signs of improvement, with less severe alterations observed compared to the untreated group. Infiltration of mononuclear cells in the interstitial cells was evident in kidney sections from Stz-Nam (Streptozotocin-Nicotinamide) induced rats treated with quercetin (D). All samples were stained with hematoxylin-eosin and examined at 100x magnification.

demonstrated decreased neutrophil migration and phagocytic capacity in diabetic rats and mice, along with reduced hydrogen peroxide levels. Moreover, insulin treatment in diabetic mice resulted in lowered blood glucose levels, correlating with increased neutrophil phagocytic capacity.

Although no significant difference in erythrocyte values was observed between the control and treatment groups in the hematological test, histopathological findings revealed erythrocyte infiltration. Erythrocytes play a crucial role in oxygen transport, closely linked to hemoglobin. Wang *et al*. (2021) compared

erythrocyte morphology in normal individuals and diabetic patients, noting that diabetic patients exhibited "bowl-shaped" erythrocytes prone to shape changes. Patients with vasculopathy showed a significant increase in discocytes compared to normal individuals. Reported normocytic mild anemia in T2DM patients, particularly in older individuals. Babu and Singh (2004) found that increased glucose concentration led to changes in erythrocyte perimeter and area, causing irregularities in the erythrocyte membrane. Diabetic individuals exhibited more acanthocytes, distorted forms, and stomatocytes, but effective treatment restored erythrocyte morphology to normal (Barbieri *et al*., 2014). Changes in the internal environment of the body lead to a decrease in the number of normal, biconcave disc erythrocytes, accompanied by an increase in deformed erythrocytes, thereby elevating the risk of diabetic complications. Observing alterations in erythrocyte morphology and structure in diabetic individuals can provide valuable insights into the progression of diabetes (Gyawali *et al*., 2014).

The histopathological image shows fatty degeneration, necrosis, and hemorrhage. Hemorrhage occurs due to erythrocyte infiltration, as explained previously. The onset of degeneration stems from damage to the kidney tubules initiated by the entry of toxic substances into tubular epithelial cells. Fatty degeneration indicates nephritis due to toxic substances in the tubules, leading to continued epithelial damage resulting in cell necrosis and desquamation. Desquamation of tubules leads to connective tissue formation and a decline in kidney function. A decrease in kidney function exceeding 25% can lead to kidney failure (Breshears *et al*., 2015). Elevated glucose levels are the primary cause of structural kidney changes. Mesangial cells produce TGFβ1 under hyperglycemic conditions, leading to increased glucose consumption and transport due to overexpression of GLUT-1 mRNA and protein, resulting in metabolic abnormalities in mesangial cells. Impaired kidney function in diabetes mellitus patients is indicated by elevated serum creatinine, uric acid, and blood urea nitrogen levels (Francesco and Loreto, 2005).

In diabetes mellitus, angiopathy causes the narrowing and obstruction of blood vessels, including those that supply the kidneys. This often leads to elevated blood plasma viscosity in diabetics, resulting in decreased blood flow. Consequently, oxygen and nutrient delivery to tissues diminishes, contributing to necrosis or cell death in various organs, notably the kidneys. While focal necrosis may be replaced by healthy cells through regeneration upon removal of the underlying cause, diffuse necrosis results in dead cells being substituted by connective tissue (Zakir *et al*., 2002). Treatment with TE has shown promise in restoring the hematological profile and reducing inflammatory cytokines such as IL-35 and TNF-α by inhibiting glomerular and tubular destruction in streptozotocin-induced rats.

Conclusion

In conclusion, administering TE at 100 mg/kg body weight effectively decreased blood glucose levels, reduced IL-35 and TNF-α concentrations, inhibited renal histomorphological damage, and restored hematological profiles in streptozotocin-induced rats.

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Conflict of interest

All authors declare no conflict of interest.

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Authors' contribution

The study was designed and executed by RS and ISH. The initial manuscript draft was prepared by KD and RS. KD collected the samples and conducted data analysis. The manuscript underwent revisions by ISH, RS, and KD. All authors reviewed and approved the final version of the manuscript.

Data availability

All data are provided in the manuscript.

References

Alba-Loureiro, T.C., Hirabara, S.M., Mendonca, J.R., Curi, R. and Pithon-Curi, T.C. 2006. Diabetes causes marked changes in function and metabolism of rat neutrophils. *J. Endocrinol.* 188, 295–303.

American Diabetes Association. 2020. Standards of Medical Care in Diabetes—2020 Abridged for Primary Care Providers. *Clin. Diabet.* 38(1), 10–38.

Arifin, H., Chou, K.R., Ibrahim, K., Fitri, S.U.R., Pradipta, R.O., Rias, Y.A., Sitorus, N., Wiratama, B.S., Setiawan, A., Setyowati, S., Kuswanto, H., Mediarti, D., Rosnani, R., Sulistini, R. and Pahria, T. 2022. Analysis of modifiable, non-modifiable, and physiological risk factors of non-communicable diseases in Indonesia: evidence from the 2018 Indonesian basic health research. *J. Multidiscip. Healthc.* 15, 2203–2221.

Babu, N. and Singh, M. 2004. Influence of hyperglycemia on aggregation, deformability and shape parameters of erythrocytes. *Clin. Hemorheol. Microcirc.* 31(4), 273–280.

Barbieri, J., Fontela, P.C., Winkelmann, E.R., Zimmermann, C.E., Sandri, Y.P., Mallet, E.K. and Frizzo, M.N. 2015. Anemia in patients with type 2 diabetes mellitus. *Anemia* 2015, 354737.

Behl, T., Gupta, A., Albratty, M., Najmi, A., Meraya, A.M., Alhazmi, H.A., Anwer, M.K., Bhatia, S. and Bungau, S.G. 2022. Alkaloidal phytoconstituents for diabetes management: exploring the unrevealed potential. *Molecules* 27(18), 5851.

Breshears, M.A. and Confer, A.W. 2017. The urinary system. In *Pathologic basis of veterinary disease,* pp: 617–681.e1.

Detrick, B., Nagineni, C.N. and Hooks, J. 2008. Cytokines: regulators of immune responses and key therapeutic targets. In *Handbook of human immunology. Eds.,* Gorman, M.R.G. and Donnenberg, A.D., 2nd ed. Boca Raton, FL: CRC Press.

Faselis, C., Katsimardou, A., Imprialos, K., Deligkaris, P., Kallistratos, M. and Dimitriadis, K. 2020. Microvascular complications of type 2 diabetes mellitus. *Curr. Vasc. Pharmacol.* 18(2), 117–124.

Francesco, P.S. and Loreto, G. 2005. Pathogenetic mechanisms of diabetic nephropathy. *J. Am. Soc. Nephrol.* 16, S30–S33.

Furman, B.L. 2021. Streptozotocin-induced diabetic models in mice and rats. *Curr. Protoc.* 1, e78.

Gyawali, P., Richards, R.S. and Nwose, E.U. 2014. Erythrocyte morphology in metabolic syndrome. *Expert Rev. Hematol.* 5(5), 523–531.

Hamid, I.S., Fikri, F. and Purnama, M.T.E. 2023. Effects of *Tithonia diversifolia* on blood glucose levels, renal and pancreatic histopathology of wistar rats: a model of diabetic nephropathy. *Indian Vet. J.* 99(11), 37–39.

International Diabetes Federation. 2021. IDF Diabetes Atlas, 10th edn. Brussels, Belgium: International Diabetes Federation

Kanter, J.E., Hsu, C.C. and Bornfeldt, K.E. 2020. Monocytes and macrophages as protagonists in vascular complications of diabetes. *Front. Cardiovasc. Med.* 7, 10.

Muniroh, L., Mahmudah, M. and Solfaine, R. 2022. Effect of *Tithonia diversifolia* leaf extract on leptin, adiponectin, and insulin receptor levels in diabetic rats. *Prev. Nutr. Food Sci.* 27(1), 63–69.

Roglic, G. 2016. WHO Global report on diabetes: a summary. *Int. J. Noncommunicable Dis.* 1(1), 3–8.

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A.A., Ogurtsova, K., Shaw, J.E., Bright, D. and Williams, R. 2019. IDF diabetes atlas committee. global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the international diabetes federation diabetes atlas, 9th edition. *Diabetes Res. Clin. Pract.* 157, 107843.

Solfaine, R., Fikri, F., Maslamama, S.T. and Purnama, M.T.E. 2024. Exploring the protective efficacy of lactobacillus acidophilus against interleukin-1beta (IL-1β) expression in mice induced with canine coronavirus vaccine. *Adv. Anim. Vet. Sci.* 12(4), 673–678.

Wang, Y., Yang, P., Yan, Z., Liu, Z., Ma, Q., Zhang, Z. and Wang, Y., Su, Y. 2021. The relationship between erythrocytes and diabetes mellitus. *J. Diabetes Res.* 2021, 6656062.

Williams, A., Bissinger, R., Shamaa, H., Patel, S., Bourne, L., Artunc, F. and Qadri, S.M. 2023. Pathophysiology of red blood cell dysfunction in diabetes and its complications. *Pathophysiology* 30(3), 327–345.

Wu, J. and Yan, L.J. 2015. Streptozotocin-induced type 1 diabetes in rodents as a model for studying mitochondrial mechanisms of diabetic β cell glucotoxicity. *Diabetes Metab. Syndr. Obes.* 8, 181–188.

Zakir, M., Ahuja, N., Surksha, M.A., Sachdev, R., Kalariya, Y., Nasir, M., Kashif, M., Shahzeen, F., Tayyab, A., Khan, M.S.M., Junejo, M., Manoj Kumar, F., Varrassi, G., Kumar, S., Khatri, M. and Mohamad, T. 2023. Cardiovascular complications of diabetes: from microvascular to macrovascular pathways. *Cureus* 15(9), e45835.