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Chronopharmacology of the fisetin/glimepiride combination in the amelioration of diabetic retinopathy in rat model

Original Article

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Abstract:

Diabetic retinopathy is a complication of diabetes caused by high blood sugar levels that damage the back of the eye (retina), potentially leading to blindness. The aim of the present study is to investigate the efficacy of fisetin and glimepiride combination in the management of diabetic retinopathy through a chronopharmacological approach. For the *in vivo* chronopharmacological study, male Wistar rats were divided into six groups, each comprising six animals. The treatment groups received 100 mg/kg of fisetin and glimepiride at different circadian time points (8:00 h, 14:00 h, and 20:00 h) for 28 consecutive days. The blood glucose levels of the animals were monitored during the treatment. On day 29, rats were euthanized, and biochemical and histopathological analyses were performed using a section of the rat eye. The group that received fisetin and glimepiride at 20:00 h showed a significant reduction in the glucose levels (9.46±7.86 mmol/L, $p<0.05$), decreased cholesterol and triglyceride levels, as well as lower malondialdehyde level compared to other treated groups and the positive control group. It can be concluded that the administration of glimepiride with fisetin at 20:00 h was more effective than other groups and that this administration time will reduce diabetic retinopathy complications. However, further studies are needed to elucidate these effects before clinical translation for managing diabetic retinopathy.

Key words:
chronopharmacology, diabetic retinopathy, flavonoids, streptozotocin

Apstrakt:

Hronofarmakologija kombinacije fisetina i glimepirida u ublažavanju dijabetičke retinopatije kod pacova

Dijabetička retinopatija je komplikacija dijabetesa izazvana visokim nivoima šećera u krvi, koji oštećuju zadnji deo oka (retinu), što potencijalno može dovesti do slepila. Cilj ove studije je da ispita efikasnost kombinacije fisetina i glimepirida u lečenju dijabetičke retinopatije primenom hronofarmakološkog pristupa. Za *in vivo* hronofarmakološku studiju, mužjaci pacova soja Wistar podeljeni su u šest grupa, pri čemu je svaka grupa imala po šest jedinki. Grupe koje su dobijale tretman primale su 100 mg/kg fisetina i glimepirida u različitim tačkama cirkadijalnog ritma (u 8:00 h, 14:00 h i 20:00 h) tokom 28 uzastopnih dana. Tokom tretmana, praćen je nivo glukoze u krvi životinja. Na 29. dan, pacovi su eutanazirani, a biohemijske i histopatološke analize su izvršene na uzorcima očiju. Grupa koja je dobijala fisetin i glimepirid u 20:00 h pokazala je značajno smanjenje nivoa glukoze u krvi (9,46±7,86 mmol/L, $p<0,05$), smanjene nivoe holesterola i triglicerida, kao i niži nivo malondialdehida u poređenju sa ostalim tretiranim grupama i pozitivnom kontrolnom grupom. Može se zaključiti da je primena glimepirida sa fisetinom u 20:00 h bila efikasnija od ostalih režima, i da ovaj vremenski period primene može doprineti smanjenju komplikacija dijabetičke retinopatije. Međutim, potrebne su dodatne studije kako bi se ovi efekti razjasnili pre kliničke primene u lečenju dijabetičke retinopatije.

Ključne reči:
hronofarmakologija, dijabetička retinopatija, flavonoidi, streptozotocin

Introduction

Chronopharmacology is the field of study that focuses on maximizing the benefits of drugs and minimizing their side effects by adjusting dosage schedules to align with biological rhythms. The purpose is to enhance the comprehension of regular and predictable alterations in pharmaceutical

tolerance and intended effects (Erkekoglu & Baydar, 2012). The scientific field of chronopharmacological studies reveals how drugs interact with biological rhythm and affect pharmacotherapy. It is the area of pharmacology that examines the relationships between the time of drug administration and its therapeutic outcome (Dobrek, 2021). The significance of chronobiology and its important role in creating chronopharmacology were highlighted in the study conducted by Nobel laureates (Kim & Lee, 2020; Lee, 2022).

Diabetes is a disordered metabolism that typically results from a mix of inherited and environmental factors. It commonly results in abnormally high blood sugar levels (Chait & Hartigh, 2020). The pancreas secretes the hormone insulin, which allows body cells to absorb glucose and convert it into energy. When glucose is not absorbed by the body's cells, it builds up in the blood and can cause immediate metabolic problems such as ketoacidosis, as well as long-term chronic microvascular problems (Taylor et al., 2015).

Diabetes is becoming more prevalent in the world for a number of reasons, including altered dietary habits that lead to obesity (Reed et al., 2021). Diabetic retinopathy is the most prevalent cause of acquired blindness in working-age individuals worldwide (Ansari et al., 2022). The development of diabetic retinopathy is caused mainly by dysregulation in both local and systemic lipid metabolism (Ellis et al., 2019). Additionally, experimental models have shown that the coexistence of hyperglycemia and hyperlipidemia increases capillary cell apoptosis and the progression of diabetic retinopathy (Whitehead et al., 2018).

Flavonoids are plant secondary metabolites with a bioactive polyphenolic structure and belong to a significant family of low molecular weight chemicals (Roy et al., 2022). Literature data revealed that flavonoids exhibit anti-inflammatory, antiviral, and antibacterial activities (Alzand & Mohamed, 2012; Brodowska, 2017; Perez-Vizcaino & Fraga, 2018; Tajammal et al., 2022). Numerous studies have reported that flavonoids improve the markers of atherosclerosis, such as lipoprotein oxidation, vascular reactivity, and blood platelet aggregation (Brodowska, 2017). The aim of the present study is to investigate the efficacy of fisetin and glimepiride combination in the management of diabetic retinopathy through a chronopharmacological approach.

Materials and Methods

Chemicals used

Glimepiride, Fisetin, Tris-HCl buffer, streptozotocin (STZ), and other required chemicals were procured from Sigma Aldrich, USA, and Sisco Research Laboratories Pvt Ltd, Mumbai. All chemicals used for the present study were of analytical grade.

Experimental Animals

Male *Wistar* rats weighing 180-250 g were obtained from Karpagam Academy of Higher Education, Coimbatore, Tamil Nadu. The animals were placed under specific environmental conditions such as humidity (50±5%), temperature (25±2 °C), and 12-hour dark-light cycles. All the animals were housed for seven days before the start of the experiment. The animals were randomized to experimental groups and housed separately in sterile polypropylene cages with paddy husk used as bedding. The experimental animals had *ad libitum* access to food and water (Ghasemi et al., 2014). The experiments were carried out by the norms of the Committee for Purpose of Control and Supervision of Experiments on Animals after obtaining the Institutional Animal Ethics Committee approval (Approval No: KFMSR/KCOP/03/2023).

Experimental Procedure

A total of 36 *Wistar* male rats were separated into six groups, each comprising six animals. Group I animals received normal saline and served as a standard (normal) control group. Group II received only an inducing agent and acted as negative control. Group III to V animals were administered with 100 mg/kg of fisetin at different circadian time points: at 8:00 h (2 HALO), 14:00 h (8 HALO), and 20:00 h (14 HALO), along with glimepiride (10 mg/kg, p.o.). Group VI animals were treated with glimepiride (10 mg/kg, p.o.) and served as a positive control group. The treatment was performed for 28 consecutive days. Diabetic retinopathy was induced by a single intra-peritoneal injection at a dose of 65 mg/kg STZ in citrate buffer (0.1 M, pH 4.5) in all groups except the normal control group (Adekemi et al., 2024). Three days following the STZ administration, rats were allocated according to their fasting blood glucose levels which exhibited >300 mg/dl. The rats that did not show the above blood glucose range were omitted from the study. On day 29, the animals were sacrificed using excess anesthesia, and the rat's eyes were removed. While all eyes were stored in phosphate buffer solution for homogenization and the eventual identification of serum indicators, some were kept in formalin for histological studies (Wael et al., 2023).

Evaluation of glucose levels

During the treatment, the animals' fasting blood glucose was measured on days 0, 7, 14, 21, and

28, respectively, using a test strip attached to a glucometer and a drop of blood from the tail using the tail prick method (Kennard et al., 2021).

Evaluation of serum indicators

On the 29th day, the rats were treated with mild diethyl ether anesthesia, and blood was drawn from their jugular veins into simple sample containers. For ten minutes, blood samples were centrifuged at 2200 rpm. The acquired serum was examined on a spectrophotometer and utilized to determine serum indicators such as triglycerides and cholesterol using Randox diagnostic kits (Parasuraman et al., 2010).

Estimation of malondialdehyde (MDA) level

After treating 0.1 ml of the eye tissue homogenate with Tris-HCl buffer (pH 7.4) and 2 ml of TBA-TCA-HCl reagent (TBA 0.37%, 0.25 N HCl, and 15% TCA), the mixture was cooled and centrifuged at 1000 g for 10 minutes at room temperature. The supernatant's absorbance was measured at 535 nm using a reference blank, and the result was expressed as µM of MDA/mg of tissue protein (Kharoubi et al., 2011).

Histopathological studies

Hematoxylin and eosin were used to stain the tissues after they were divided into 6 µm sections. Tissue histoarchitecture from stained samples was examined under a bright-field microscope for histological analysis (Oraebosi et al., 2021).

Data analysis

For the experimental statistical analysis, one-way ANOVA was used to evaluate the data, and then Tukey's test was performed. GraphPad Prism 7.00 for Windows was used for statistical analysis and data visualization. The results were shown as mean ±standard deviation, with a $p<0.05$ being considered statistically significant (Cruz et al., 2021).

Results

Diabetes causes abnormally high blood sugar levels. In the present study, the diabetic control group animals exhibited a blood glucose level of 22.34±4.91 mmol/L. In contrast, the normal control group had a blood glucose level of 6.68±16.46 mmol/L on day 28, indicating STZ-induced diabetes mellitus in the negative control group. By the end of the treatment, the animals that received fisetin and glimepiride at 14 HALO showed a significant reduction ($p<0.05$) in glucose levels in comparison to other groups, with a value of 9.46±7.86 mmol/L. The groups that received therapy at 8 HALO and 2 HALO had glucose levels of 10.57±4.95 mmol/L and 11.23±4.95 mmol/L, respectively (**Tab. 1**). In the treatment groups that received glimepiride alone, the treated animals showed a significant decrease in blood glucose levels with a value of 14.45±7.78 mmol/L. It revealed that the fisetin and glimepiride combination-treated animals on 14 HALO potentially decreased the blood glucose level more than all other groups.

The animals in diabetes control showed an increase in cholesterol and triglyceride levels, which in turn resulted in the severity of diabetic retinopathy. The STZ-treated animals showed elevated cholesterol and triglyceride levels of 133±0.95 mg/dl and 177±1.30 mg/dl, while the normal control group had 53±0.24 mg/dl and 68±0.12 mg/dl, respectively. The animals treated with fisetin and glimepiride at 14 HALO showed significant reduction ($p<0.05$) in the cholesterol and triglyceride levels of 59±0.24 mg/dl and 85±0.12 mg/dl. The groups administered at 2 HALO had cholesterol and triglyceride levels of 75±0.26 mg/dl and 92±0.72 mg/dl, while the 8 HALO group showed levels of 77±0.76 mg/dl and 90±0.78 mg/dl, respectively (**Tab. 2**). The treatment groups that received glimepiride alone received animals resulted in a significant decrease

Table 1. Blood glucose levels measured weekly in treatment and control groups

Groups	Day 0 (mmol/L)	Day 7 (mmol/L)	Day14 (mmol/L)	Day 21 (mmol/L)	Day 28 (mmol/L)
Normal control	6.58±26.40	6.69±21.48	6.52±18.26	6.72±19.18	6.68±16.46
Diabetic control	6.13±37.27#	17.81±17.90#	20.58±12.68#	21.66±9.30#	22.34±4.91#
2 HALO	6.77±31.50*	16.76±17.96*	16.11±10.72*	14.56±12.68*	11.23±4.95*
8 HALO	7.14±31.50*	17.35±16.90*	15.03±9.30*	14.46±12.68*	10.57±4.95*
14 HALO	5.68±31.50*	18.92±10.72*	14.48±15.12*	13.35±4.15*	9.46±7.86*
Positive control	6.54±38.50*	17.24±17.74*	15.58±14.72*	15.29±34.50*	14.45±7.78*

Values expressed in mean±SEM, n=6. Data – one-way ANOVA followed by Tukey's test; #$p<0.01$ compared to standard in negative control group; *$p<0.01$ compared to negative control in treatment groups

Table 2. Evaluation of serum indicators

Groups	Cholestrol (mg/dl)	Triglyceride (mg/dl)
Normal control	53±0.24	68 ± 0.12
Diabetic control	133±0.95#	177 ± 1.30#
2 HALO	75±0.26*	92 ± 0.72*
8 HALO	77±0.76*	90 ± 0.78*
14 HALO	59±0.24*	85 ± 0.12*
Positive control	97±0.96*	99 ± 1.03*

Values expressed in mean±SEM, n=6. Data – one-way ANOVA followed by Tukey's test; #p<0.01 compared to standard in negative control group; *p<0.01 compared to negative control in treatment groups

Table 3. Estimation of malondialdehyde level

Groups	MDA (nmol/mg protein)
Normal control	82.65±1.19
Diabetic control	199.66±0.18#
2 HALO	88.90±1.34*
8 HALO	92.48±2.05*
14 HALO	78.65±1.19*
Positive control	112.71±0.86*

Values expressed in mean±SEM, n=6. Data – one-way ANOVA followed by Tukey's test; #p<0.01 compared to standard in negative control group; *p<0.01 compared to negative control in treatment groups

in cholesterol and triglyceride levels of 97±0.96 mg/dl and 99±1.03 mg/dl. The results showed that the fisetin and glimepiride administered to animals on 14 HALO effectively decreased cholesterol and triglyceride levels more than all other groups, which in turn resulted in decreased diabetic retinopathy progression.

Malondialdehyde levels were significantly elevated in the diabetes control group with a value of 199.66±0.18 nmol/mg protein because of free radical production, while the normal control group showed MDA level of 82.65±1.19 nmol/mg protein. The positive control group treated with glimepiride alone markedly reduced the MDA levels to 112.71±0.86 nmol/mg protein. The animals treated with glimepiride and fisetin at 14 HALO, 2 HALO, and 8 HALO showed significant reduction in the MDA levels with 78.65±1.19 nmol/mg protein, 88.90±1.34 nmol/mg protein and 92.48±2.05 nmol/mg protein, respectively. A noteworthy reduction in the MDA levels was observed, especially in the 14 HALO-treated group. Compared to the diabetic control group, this further emphasizes the potential role of combination therapy (**Tab. 3**).

Histopathological studies showed that the retinal layers of the diabetic control (group II) resulted in deformation, along with pronounced necrosis of the rods, cones, and outer nuclear layer cells. This indicates the formation of diabetic retinopathy due to the administration of STZ in the negative (diabetic) control group. The Group III and IV retina photomicrographs indicated that rats treated with glimepiride and fisetin at 2 HALO and 8 HALO showed moderate necrosis and deformation of cells, revealing mild diabetic retinopathy. In Group V, the rats administered with glimepiride and fisetin at 14 HALO showed the absence of cell necrosis and normal retinal layers with rods and cones (**Fig. 1**). The rats administered with glimepiride alone in Group VI exhibited lower necrosis of the outer layer of cells. The decreased necrosis level results from the administration of the positive control drug glimepiride. The animals treated with glimepiride and fisetin at 14 HALO effectively prevented the streptozotocin-induced cell necrosis in the retina, which further emphasizes that the combination at 14 HALO would be more helpful in the management of diabetic retinopathy.

Discussion

Diabetic retinopathy is a consequence of diabetes with many structural and functional abnormalities implicated in its development. The STZ-induced diabetic model is the most commonly used model for many aspects of diabetes mellitus research (Naderi et al., 2019). The time of drug administration, especially with reference to the rhythm of disease, has a profound effect on drug efficacy (Duh et al., 2017).

Reactive oxygen species resulting from uncontrolled hyperglycemia are the primary cause of retinopathy. Hence, therapy that maintains blood glucose or free radical levels should offer protection (Du et al., 2013). In the current study, a comparison of blood glucose levels across the groups treated with fisetin demonstrated that time of administration is a tool for effective therapy. The administration of fisetin and glimepiride at 20:00 h (14 HALO) resulted in a beneficial interaction by targeting both the peak of oxidative stress and blood glucose level.

Fisetin improves glycemic control by scavenging free radicals. In diabetes, levels of endogenous protective antioxidants are reduced with increased lipid peroxidation. Fisetin is postulated to be able

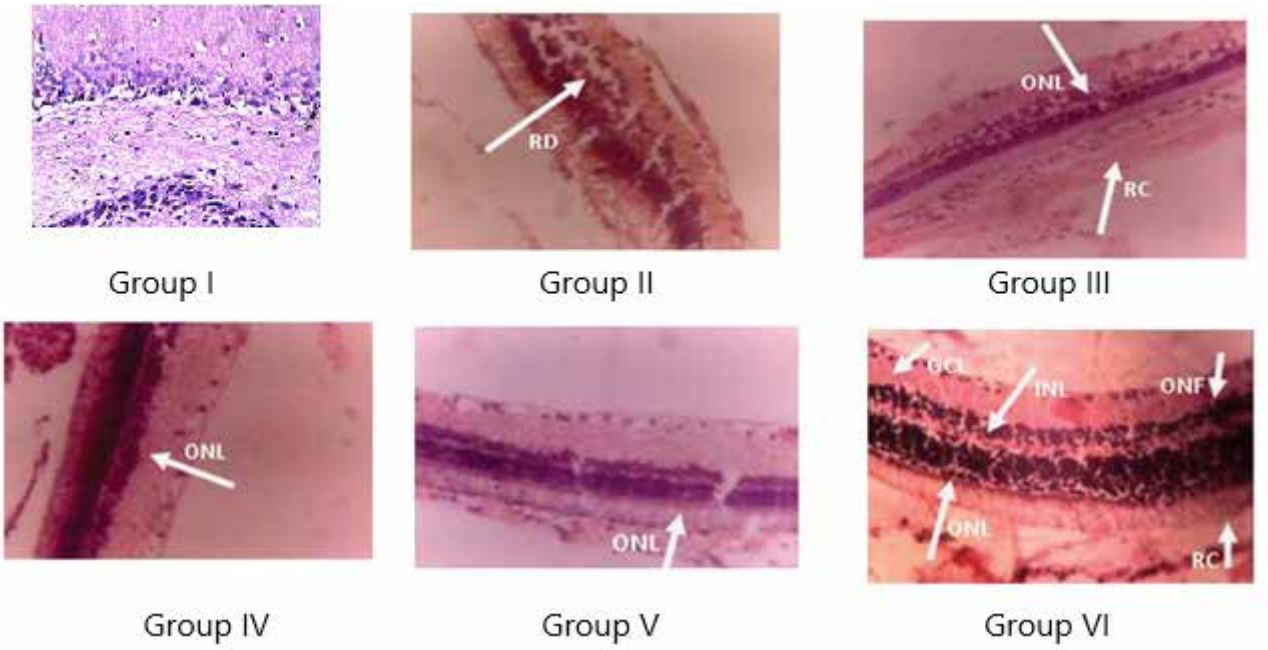

Fig. 1. Histopathology of retinal layers of rat eyes from different treatment and control groups. **RD** - Retinal detachment; **ONL** - Outer nuclear layer; **RC** - Retinal cells; **GCL** - Ganglion cell layer; **INL** - Inner nuclear layer; **ONF** - Optic nerve fiber

to combine effectively with the primary glucose-lowering effect of glimepiride to produce the observed effect on blood glucose and antioxidant profile (Prasath et al., 2014). Hence, in the present study, the combination of fisetin and glimepiride at 14 HALO may have ensured the prevention of oxidative stress by maintaining relatively high blood antioxidant levels.

Lai et al. (2023) investigated the protective role of fisetin in regulating diabetic retinopathy and discovered the involved mechanism. First, 30 mM glucose was used to create an in vitro DR cell model. Cell counting kit 8 (CCK8) assay was employed to study the effects of fisetin on cell viability by treating human retinal microvascular endothelial cells (HRMECs) with 25, 50, and 100 μM concentrations of fisetin. Transwell and wound healing assays were employed to detect the purpose of fisetin on the angiogenesis and migration of HG (high glucose)-induced HRMECs. Finally, OE-VEGF was shown to mimic VEGF, and western blotting (WB) was used to confirm the targeting genes of fisetin. HG provoked an elevation in cell viability, angiogenesis, and cell migration in HRMECs, whereas fisetin subdued these enhancements induced by HG by hindering VEGF.

Diabetic retinopathy is a serious microvascular complication that may lead to impaired vision or complete blindness with poor management. Furthermore, occlusion or permanent damage of the blood vessels supplying the retina is also implicated in diabetic retinopathy. In diabetes, accumulation of lipids due to increased fatty acid mobilization from fatty tissues may result in hypercholesterolemia, hypertriglyceridemia, and blockage of blood vessels (Zhong & Kowluru, 2011). Previous studies have shown that fisetin prevents dyslipidemia by decreasing cholesterol and triglyceride levels in diabetic rats (Prasath & Subramanian, 2011).

The highest reduction of serum indicators was observed when the fisetin and glimepiride were administered at 14 HALO rhythm. Understanding the chronopharmacological properties of fisetin and glimepiride in combination therapy for diabetic retinopathy in rat models may provide valuable insights for optimizing treatment strategies and improving outcomes in diabetic retinopathy. It is noteworthy that amelioration of retinopathy in this study follows a similar pattern with improved antioxidant profiles and glycemic control in a diurnal progression through fisetin and glimepiride administration.

Conclusion

Fisetin and glimepiride have individually shown potential in mitigating diabetic complications such as retinopathy, but their combined effects and the influence of timing on their administration remain largely unexplored in the context of circadian rhythms. It can be concluded that fisetin and glimepiride administered in 14 HALO significantly reduced the serum indicators, blood glucose level,

and malondialdehyde level compared to the other groups. This may be a consequence of the time of drug administration matching approximate endogenous biological rhythms involved in the expression of disease and biological time-dependent efficacy in drug actions. However, further research is needed to elucidate these effects before clinical translation can be considered.

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References

Adekemi, F.E., Folake, J.K. & Omowumi, F.P. (2024). Antidiabetic effects of aqueous leaf extract of *Vernonia amygdalina* on serum liver markers in streptozotocin-induced diabetic albino rats: a new data to support its anti-diabetic effect. *Clinical Phytoscience*, *10*, 13-18.

Alzand, K.I. & Mohamed, M.A. (2012). Flavonoids: chemistry, biochemistry, and antioxidant activity. *Journal of Pharmacy Research*, *5*(40134012), 37-42.

Ansari, P., Tabasumma, N., Snigdha, N.N., Siam, N.H., Panduru, R.V.N.R.S. Azam, S. & Hannan, J.M.A. (2022). Abdel-Wahab, Y.H.A. Diabetic retinopathy: An overview on mechanisms, pathophysiology and pharmacotherapy. *Diabetology*, *3*,159-175.

Brodowska, K.M. (2017). Natural flavonoids: classification, potential role, and application of flavonoid analogues. *European Journal of Biological Research*, *7*(2), 108-123.

Chait, A. & den Hartigh, L.J. (2020). Adipose tissue distribution, inflammation and its metabolic consequences, including diabetes and cardiovascular disease. *Frontiers in Cardiovascular Medicine*, *7*, 22-28.

Cruz, P.L., Moraes-Silva, I.C., Ribeiro, A.A., Machi, J.F., de Melo, M.D.T., Santos, F.D., da Silva, M.B., Strunz, C.M.C., Caldini, E.G. & Irigoyen, M.C. (2021). Nicotinamide attenuates streptozotocin-induced diabetes complications and increases survival rate in rats: role of autonomic nervous system. *BMC Endocrine Disorders*, *21*, 133-138.

Dobrek, L. (2021). Chronopharmacology in therapeutic drug monitoring-dependencies between the rhythmics of pharmacokinetic processes and drug concentration in blood. *Pharmaceutics*, *13*(11), 1915-1923.

Du, Y., Veenstra, A., Palczewski, K. & Kern, T.S. (2013). Photoreceptor cells are major contributors to diabetes-induced oxidative stress and local inflammation in the retina. *Proceedings of the National Academy of Sciences USA, 110*(41), 16586-16591.

Duh, E.J., Sun, J.K. & Stitt, A.W. (2017). Diabetic retinopathy: current understanding, mechanisms, and treatment strategies. *JCI Insight*, *2*(14), e93751-e93757.

Ellis, M.P., Lent-Schochet, D., Lo, T. & Yiu, G. (2019). Emerging concepts in the treatment of diabetic retinopathy. *Current Diabetes Reports*, *19*, 137-144.

Erkekoglu, P. & Baydar, T. (2012). Chronopharmacodynamics of drugs in toxicological aspects: A short review for clinical pharmacists and pharmacy practitioners. *Journal of Research in Pharmacy Practice*, *1*(2), 41-47.

Ghasemi, A., Khalifi, S. & Jedi, S. (2014). Streptozotocin-nicotinamide-induced rat model of type 2 diabetes. *Acta Physiologica Hungarica*, *101*, 408-420.

Kennard, M.R., Daniels, G.L.F., Roberts, A.G., White, E.R.P., Nandi, M. & King, A.J.F. (2021). The use of mice in diabetes research: The impact of experimental protocols. *Diabetic Medicine*, *38*: e14705-e14711.

Kharoubi, O., Slimani, M. & Aoues, A. (2011). Neuroprotective effect of wormwood against lead exposure. *Journal of Emergencies, Trauma, and Shock*, *4*(1), 82-88.

Kim, S. & Lee, H.J. (2020). Sleep and circadian rhythm disturbances in eating disorders. *Chronobiology in Medicine*, *2*, 141-147.

Lee, H.J. (2022). Unraveling the significance of circadian rhythms for health. *Chronobiology in Medicine*, *4*, 93-94.

Naderi, A., Zahed, R., Aghajanpour, L., Amoli, F.A. & Lashay, A. (2019). Long term features of diabetic retinopathy in streptozotocin-induced diabetic Wistar rats. *Experimental Eye Research*, *184*, 213-220,

Oraebosi, M.I., Olurishe, T.O., Anafi, S.B. & Bisalla, M. (2021). Chronopharmacology of the alpha-lipoic acid/nifedipine/glimepiride combination in the amelioration of retinopathy in rats. *Chronobiology International*, *38*(3), 443-450.

Parasuraman, S., Raveendran, R. & Kesavan,

R. (2010). Blood sample collection in small laboratory animals. *Journal of Pharmacology and Pharmacotherapeutics*, *1*(2), 87-93.

Perez-Vizcaino, F. & Fraga, C.G. (2018). Research trends in flavonoids and health. *Archives of Biochemistry and Biophysics*, *646*, 107-112.

Prasath, G.S., Pillai, S.I. & Subramanian, S.P. (2014). Fisetin improves glucose homeostasis through the inhibition of gluconeogenic enzymes in hepatic tissues of streptozotocin induced diabetic rats. *European Journal of Pharmacology*, *740*, 248-254.

Prasath, G.S. & Subramanian, S.P. (2011). Modulatory effects of fisetin, a bioflavonoid, on hyperglycemia by attenuating the key enzymes of carbohydrate metabolism in hepatic and renal tissues in streptozotocin-induced diabetic rats. *European Journal of Pharmacology*, *668*(3), 492-496.

Reed, J., Bain, S. & Kanamarlapudi, V. (2021). A review of current trends with Type 2 diabetes epidemiology, aetiology, pathogenesis, treatments and future perspectives. *Diabetes, Metabolic Syndrome and Obesity*, *14*, 3567-3602.

Roy, A., Khan, A., Ahmad, I., Alghamdi, S., Rajab, B.S., Babalghith, A.O., Alshahrani, M.Y., Islam, S. & Islam, M.R. (2022). Flavonoids a bioactive compound from medicinal plants and its therapeutic applications. *BioMed Research International*, *2022*, 5445291-5445295.

Tajammal, A., Siddiqa, A. & Irfan, A. (2022). Antioxidant, molecular docking and computational investigation of new flavonoids. *Journal of Molecular Structure*, *1254*: 132189.

Taylor, S.I., Blau, J.E. & Rother, K.I. (2015). SGLT2 inhibitors may predispose to ketoacidosis. *The Journal of Clinical Endocrinology & Metabolism*, *100*(8), 2849-2852.

Wael, M.A., Noha, E.I., Amgad, K.H., Wagdy, K.B., Hassan, A., Hamdy, A.T. & Kawkab, A.A. (2023). The hepato- and neuroprotective effect of gold *Casuarina equisetifolia* bark nano-extract against Chlorpyrifos-induced toxicity in rats. *Journal of Genetic Engineering and Biotechnology*, *21*(1), 158-162.

Whitehead, M., Wickremasinghe, S., Osborne, A., Van Wijngaarden, P. & Martin, K.R. (2018). Diabetic retinopathy: A complex pathophysiology requiring novel therapeutic strategies. *Expert Opinion on Biological Therapy*, *18*, 1257-1270.

Zhong, Q. & Kowluru, R.A. (2011). Epigenetic changes in mitochondrial superoxide dismutase in the retina and the development of diabetic retinopathy. *Diabetes*, *60*(4), 1304-1313.