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Antidiabetic Activity of Wogonin Isolated from *Tetracera indica* Merr. Leaves Extract in Streptozotocin-Nicotinamide Induced Diabetic Rats

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The leaves of *Tetracera indica* Merr. (Dilleniaceae) have been used to treat diabetes in Malaysia. However, the active principles responsible for the leaf's antidiabetic effect are yet to be confirmed and evaluated through an in vivo investigation. Hence, a phenolic compound as a flavonoid (wogonin) was isolated as a major compound from the leaves methanol extract and subjected to antidiabetic evaluation. Initially, the powdered leaves were macerated with methanol for 72 h. The resultant methanol extract was subjected to column chromatography to isolate the pure wogonin. Its structure was elucidated through 1H- & C-13 NMR spectral analysis. Finally, the wogonin was orally given to streptozotocin-nicotinamide (STZ-NA) induced diabetic rats at three different doses (25, 40, 80 mg/kg b.w.). The histological alteration of vital organs, insulin release, biochemical assays, and blood glucose serum were evaluated and compared to standard hypoglycaemic drug i.e., metformin (0.5 mg/kg b.w.). Data of the blood glucose serum of rats treated with wogonin at 40 and 80 mg/kg b.w. revealed a significant decrease in blood glucose when compared to the diabetic control ($p<0.05$). Biochemical analyses for all doses displayed no significant difference when compared to the normal group and positive control ($p>0.05$) for triglyceride, total cholesterol, high-density, and low-density lipoprotein. However, the results were significantly different for triglyceride, total cholesterol, high-density, and low-density lipoprotein when compared to diabetic control ($p<0.05$). In addition, wogonin at 40 mg/kg b.w. was found to enhance insulin secretion by day 30. The histopathology data of the pancreas showed that wogonin at 40 and 80 mg/kg b.w. was observed to regenerate pancreatic β-cells in diabetic rats without demonstrating any liver and kidney toxicities. The results indicated that wogonin possesses an in vivo antidiabetic property and therefore might provide a lead for the synthesis of a safe natural product-based antidiabetic agent.

Key words: Wogonin; Streptozotocin-Nicotinamide induced rat; antihyperglycemic; antidiabetic; insulin secretion

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Diabetes mellitus (DM) is a chronic condition that has become more common in recent years because of the aging population and lifestyle changes that leads to obesity. The International Diabetes Federation (IDF) forecasts that the global population of individuals with diabetes (aged 18–99 years) would rise to 783 million by 2045, based on current statistics from worldwide studies [1]. Meanwhile, IDF also stated that DM in Malaysia will increase from 11.6% in 2010 to 13.8% in 2030 [2]. According to the World Health Organization (WHO), the disease has become a priority health problem in the 21st century [1].

Diabetes affected 8.5 percent of people aged 18 and above in 2014. Diabetes was the direct cause of 1.5 million fatalities in 2019, with 48 percent of all diabetes-related deaths occurring before the age of 70. Diabetes is implicated in a 5% increase in premature mortality rates (death before the age of 70) between 2000 and 2016 [3]. According to the WHO Global

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Report on Diabetes, being overweight or obese is the most important risk factor for type 2 diabetes mellitus (T2DM), and T2DM and prediabetes are becoming more common in children, adolescents, and young adults [4].

The prevalence and severity of DM and the resultant metabolic syndrome are rapidly increasing. Therefore, there is always continuous research for alternative drugs related to plant-active compounds. In Malaysia, indigenous people have traditionally used medicinal plants to manage diabetes, but not all plants have been scientifically verified or widely commercialized. One of the ethnomedicinal plants that can be explored to tackle the DM issue is *Tetracera indica* Merr. (Dilleniaceae). It is a woody, Malaysian rainforest climber which is commonly known as "Mempelas paya" or sandpaper plant. It has berry-like fruits which have been described as sour in taste [5].

5,7-dihydroxy-8-methoxyflavone also known as wogonin isolated from the leaves of *T. indica* Merr. has been reported for an in vitro antidiabetic potential [5,6,7,8]. It is worth noting that 5,7-dihydroxy-8-methoxyflavone has been shown to lower fasting blood glucose levels [9]. In accordance with the antidiabetic potential of 5,7-dihydroxy-8-methoxy-flavone isolated from the *T. indica* Merr. leaves extract, this study was conducted to investigate the antihyperglycemic potential through evaluation of the blood glucose serum, biochemical assays, insulin release, and histological alteration. This in vivo study provides additional alternative data for traditional medicinal therapy against type 2 diabetes mellitus.

EXPERIMENTAL

Plant Materials

Fresh leaves of *T. indica* Merr. were collected from the local garden of Taman Pertanian, Indera Mahkota, Pahang Darul Makmur, Malaysia in the year 2016. The plant was identified and authenticated by the botanist, Assoc. Prof. Dr. Norazian Binti Mohd. Hassan from Kulliyyah of Pharmacy, IIUM, Kuantan. The plant sample (voucher specimen number: QPC-017) was deposited in the herbarium of Kulliyyah of Pharmacy, IIUM, Kuantan. Fresh leaves of the plant were dried in the laboratory dryer for about 5 days under the dark condition at 40 °C. After that, the dried plant material was pulverized and finally, the powdered sample was kept in the desiccator at 4 °C until further experiments.

Plant Extraction and Isolation

All solvents were distilled before use. Dried powdered material (2 kg) was soaked in 95% methanol in a 20 L round bottom flask for 72 h. The extract was filtered and concentrated using a rotary evaporator at reduced pressure to recover methanol. Subsequently, the recovered methanol was poured again into the already extracted powdered sample of the leaves and then it was filtered and concentrated again by following the above procedure. The whole procedure was repeated several times until the plant sample stopped producing coloration to ensure the maximum yield of 95% methanol extract. The final concentrated extract upon freeze drying (methanol extract 380 g) was stored at 4 °C in the labeled sterile bottle until further use. The entire process was done based on the previous work with slight modifications [10].

Isolation and Purification of Wogonin from the Methanol Extract of *T. indica*

The concentrated methanol extract (380 g) was chromatographed over a silica gel column using a gradient solvent system of hexane, hexane-EtOAc, EtOAc-Methanol (1:0~0:1), and methanol only to yield thirteen combined fractions based on the TLC evaluation in different binary and tertiary solvent systems [(1-15), (16-38), (39-61), (62-82), (83-99), (100-121), (122-139), (140-160), (161-184), (185-205), (206-223), (224-245) and (246-370)]. Fractions 39-61 (4 g), 62-82 (5 g), 83-99 (4.1 g), and 100-121 (2.7 g) as greenish-yellowish solution were obtained from the column in a solvent system i.e., hexane-ethyl acetate (9:1, 8:2, 7:3, 6:4, 5:5), ethyl acetate-methanol (9:1, 8:2), respectively.

All fractions were further recrystallized up to three to four times to get a yellow crystallized compound in good yield. After getting the maximum quantity of yellow crystallized compound from all these fractions and checking their similarity on the TLC plate, they were mixed to get a crude form of yellow crystallized compound (6.5 g). Later, the fraction was further subjected to column chromatography packed with silica gel. This preparatory column was run using a gradient solvent system of hexane-acetone (9:1, 8:2, 7:3, 6:4), respectively to get fractions (1-9), (10-124), (125-156) and (157-189). Fractions 10-124 afforded a single yellow crystallized compound upon crystallization with EtOH. It was further recrystallized up to three to four times to give a yellow crystallized compound that was found to be sparingly soluble in chloroform and diethyl ether and highly soluble in ethyl acetate, acetone, ethanol, and methanol. It gave a single spot-on TLC plate in different solvent systems when TLC was run to check its purity.

The purity of this compound was further determined by a sharp melting point ranging from 207 – 210 °C. It gave a dark yellow spot with vanillin/ H_2SO_4 reagent on the TLC plate upon heating at 122 °C. Rf value of this compound in toluene-ethyl formate-formic acid (5:4:1) and pyridine-benzene-formic acid (BPF, 36:9:5) solvents system was calculated as 0.635 and 0.570, respectively. While with $FeCl_3$ reagent, a black spot was observed on the TLC plate indicating the presence of a pure phenolic compound. The compound's structure was characterized by spectroscopic analysis.

The spectral data were further compared with the previously reported spectral data of a similar compound already isolated from different plants to ensure the correct structure. It was found to be the major compound in these fractions and was coded as wogonin (5.9 g).

Experimental Animals

Thirty-six (36) Sprague Dawley male rats weighing about 200-300 g were procured from Sapphire Enterprise, Selangor. The animals were kept in cages (3 rats per cage) in an air-conditioned and environmentally controlled room with a 12h/12h dark and light cycle and the temperature was maintained at 25 °C along with relative humidity (46-79%). They were acclimatized for two weeks and given feed and water ad libitum. This experimental study, prior to commencement, was initially approved by the institutional animal ethical committee, IACUC-IIUM, with reference number: IIUM/519/14/4/IACUC (IIUM/IACUC Approval/2016/(9)(49)) prior to the experimental procedure.

The rats were divided into six groups consisting of six rats per group (n=6) viz., normal control, diabetic control, diabetic standard, and diabetic rats treated with 3 different doses of wogonin i.e., 25, 40, and 80 mg/kg per body weight (Table 1). These three doses were chosen below the LD50 of wogonin i.e., 286.15 mg/kg b.w., which was previously reported by Qi et al. [11].

Wogonin was found to be partially water insoluble and therefore it was prepared in the form of emulsion [ratio 6 (oil):4 (water)] to ensure the solubility of the test compound prior to administration. The vegetable-grade oil and double distilled water were used to prepare the emulsion of wogonin for an in vivo study. The emulsion was administered via force feeding using a force-feeding needle (size 19).

The chosen diabetic groups of rats fasted for 12 hours prior to the induction of diabetes. After 1 h, the basal weight (g) and the fasting blood glucose (mmol/L) of all the rats were recorded. Diabetes was induced in all fasted rats through intraperitoneal (IP) injection of 65 mg/kg body weight streptozotocin (STZ) (Sigma, Germany) and 100 mg/kg body weight of nicotinamide (NA) dissolved in 0.9% ice-cold sterile saline solution [12]. Diabetes-induced rats were given 20% glucose solution for 24 hours immediately after STZ-NA injection to prevent fatal acute hypoglycemic effects [13].

Diabetes was confirmed after five days in STZ-NA-induced rats through the manifestation of high fasting blood glucose level including other symptoms like polyuria, polydipsia, and weight loss. Rats with elevated fasting blood glucose level ≥ 14 mmol/L were considered diabetic and utilized for further experiments [14].

Treatment of Wogonin, Collection of Body Weight and Blood Glucose

The treatment of wogonin was started after the confirmation of the hyperglycemic condition of the rats. The first treatment day was considered day 0. Body weight (g) was taken on day 0 and day 29. Meanwhile, the blood glucose level of all rats was measured on every alternate day using an Accu Check Performa glucometer (Roche Diagnostic GmbH, Germany) and the results were compared with the normal control and the diabetic control groups.

Table 1. The grouping of animals and treatment regime.

Group (n=6)	Treatment
Normal control	Dry rat pellet, oil, and water only
Diabetic	65 mg/kg b.w. STZ- 100 mg/kg b.w. NA + 25 mg/kg b.w. of wogonin+ rat pellet + water
Diabetic	65 mg/kg b.w. STZ- 100 mg/kg b.w. NA + 40 mg/kg b.w. of wogonin + rat pellet + water
Diabetic	65 mg/kg b.w. STZ- 100 mg/kg b.w. NA + 80 mg/kg b.w. of wogonin + rat pellet + water
Diabetic with drug standard	65 mg/kg b.w. STZ- 100 mg/kg b.w. NA + 0.5 mg/kg b.w. metformin + rat pellet + water
Diabetic group (control)	65 mg/kg b.w. STZ- 100 mg/kg b.w. NA + rat pellet + oil + water

*b.w.: body weight (mg/kg)

Collection of Blood

Overnight-fasted animals (16 h) were sacrificed by cervical decapitation on day 29 of the experiments following ethical norms provided by ethics committees. The blood was collected via cardiac puncture after sacrifice and kept in serum preparation tubes (BD Vacutainer® SST II). The blood clot was removed by centrifugation at 2000 x g for 10 min in refrigerated centrifuge CR4 33 (Jouan, France) at 4 °C. The resulting supernatant, designated as serum was transferred into clean polypropylene tube (Eppendorf, Germany) and maintained at -20 °C prior to any further analysis [15].

Serum Biochemical Analysis

The clinical biochemistry assay was evaluated by Omega Diagnostics Laboratory, Kuantan, Pahang, Malaysia using UV-Vis spectrophotometer, Konelab 20XTi (Thermo Fisher Scientific, USA). Among the assays which were evaluated include:

a. Lipid function test – triglyceride (Tg), total cholesterol (TC), high-density cholesterol (HDL), low-density cholesterol (LDL) (mmol/L).
b. Diabetic test – serum insulin (µU/L).

Tissue Sampling and Histopathological Assessment

The pancreas, kidney, and liver of the rats were harvested and soaked in 10% (v/v) formalin for at least 48 h, cut into several pieces, inserted into cassettes, and labeled prior to tissue processing. Then, the dehydrated tissues were transferred into 50%, 70%, 80%, and 95% (v/v) ethanol 3 times for 90 min each, to remove water from the tissues (dehydration). Next, tissues were soaked into a mixture of ethanol and toluene (1:1 ratio) followed by 100% toluene overnight (dealcoholisation). On the following day, tissues were transferred into paraffin wax 3 times, 2 h each. Subsequently, tissues in the cassette were melted in a wax bath at 60 °C in the embedding machine (embedding). The tissue parts were removed from the cassette and put into a moulder before mixing with paraffin wax. The moulder was immediately cooled down to -10 °C to embed the tissues with wax, forming tissue blocks. Later, the blocks were cut with a microtome blade at 5 µm, resulting in a thin layer of tissue-containing wax which was then left in water in the water bath at 50 °C before it could be fished using normal microscope slides. After that, slides that contained tissues were dried on a hot plate for 24 h and were kept at room temperature for further analysis.

Statistical Analysis

Data were collected and expressed as the mean ± standard error of mean (S.E.M.) of three independent experiments and analyzed for statistical significance from each control. The data were tested for statistical differences by one-way ANOVA followed by Tukey's and Dunnet's multiple comparison tests of MiniTab (version 18). The criterion for significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Dry powder leaves (2 kg) of *T. indica* Merr. were soaked in 95% of distilled methanol which afforded 380 g 95% methanol extract upon concentration through rotary evaporator and freeze-drying process to remove all traces of water in the crude methanol extract.

The chemical structure of wogonin (Figure 1) was characterized through spectral data of the isolated compound and comparison was made with the previously published spectral data of the same compound reported from the different plants [16]. Table 2 shows the physical properties of the isolated compound.

^{1}H-NMR [600 MHz, Acetone-d6, δ (ppm)]: 6.67 (s, 1H, H-3), 6.20 (s, 1H, H-6), 7.97 (m, 2H, H-2′/H-6′), 7.50 (m, 3H, H-3′/H4′/H5′), 3.84 (s, -OCH3, 3H, H-8a), 12.43 (s, 1H, OH-5).

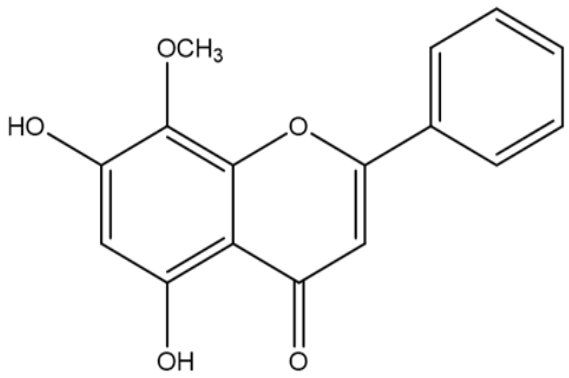

Figure 1. Chemical structure of wogonin (5,7-dihydroxy-8-methoxyflavone).

Table 2. Physical properties of wogonin (5,7-dihydroxy-8-methoxyflavone).

Nature	Color	Solubility	Melting Point (°C)
Crystal	Yellow	Acetone, MeOH, $CHCl_3$, insoluble in water	205-206

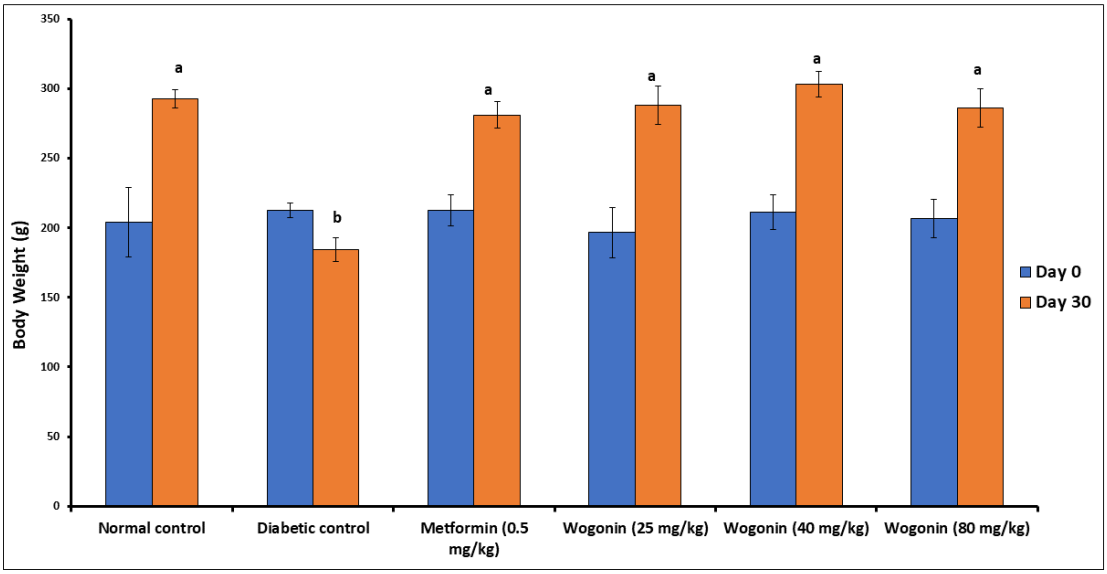

Figure 2. The effect of wogonin on body weight of rats. On day 30, diabetic control showed statistically significant ($p<0.05$) weight reduction as compared to all groups.

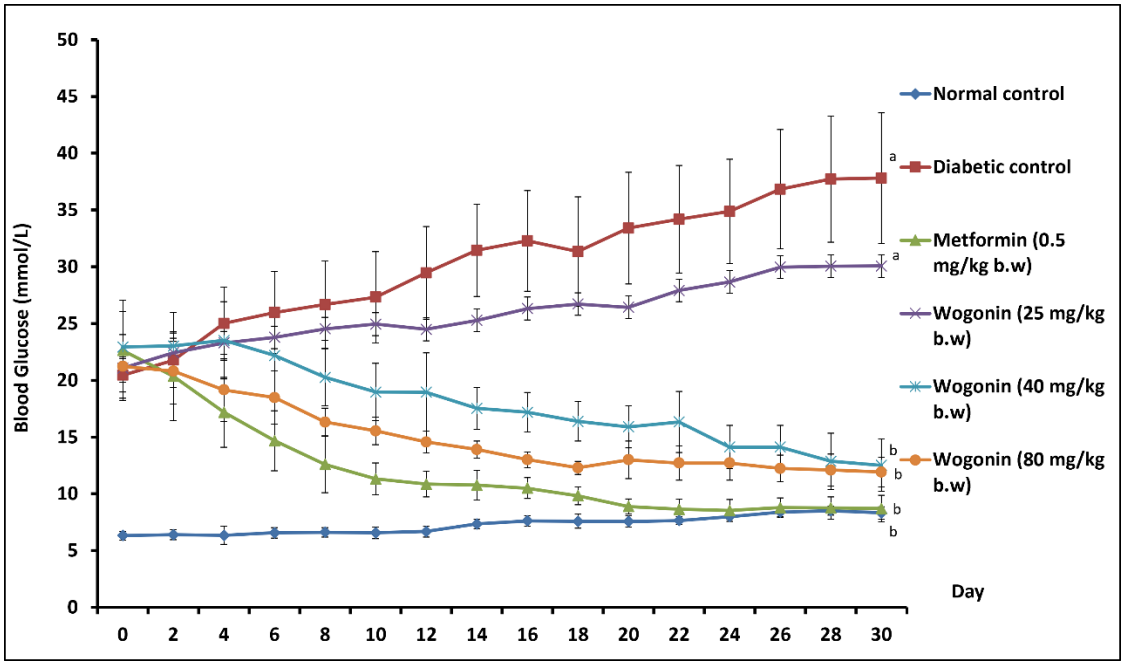

Figure 3. The effect of wogonin on blood glucose serum of rats. The blood glucose serum of rats treated with wogonin at 40 and 80 mg/kg b.w. showed significant blood glucose reduction as compared to diabetic control ($p<0.05$).

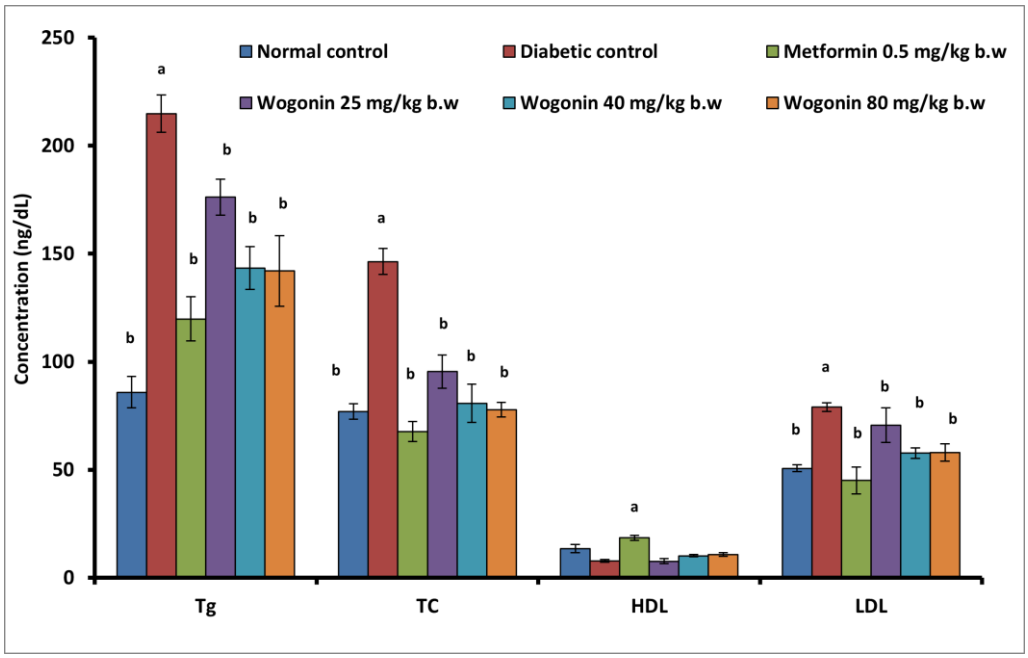

Figure 4. The concentration of serum lipid profiles after 30 days of wogonin) treatment. a=p<0.05 when compared with normal control values. Small letters represent Tukey's test. Each value is expressed in mean ± SEM). The result of serum lipid profiles i.e., triglyceride, total cholesterol, high density, and low-density lipoprotein for wogonin (25, 40, 80 mg/kg b.w.), metformin (0.5 mg/kg b.w) and normal control was significantly reduced as compared to diabetic control (p<0.05).
*TG: triglyceride, TC: total cholesterol, HDL: high-density lipoprotein, LDL: low-density lipoprotein.

The present study has successfully highlighted the antihyperglycemic effect of wogonin isolated from the leaves of *T. indica* on STZ-NA induced rats compared with the oral hyperglycemic agent, metformin which is the most widely prescribed antihyperglycemic agent. Metformin has been reported to show an anti-diabetic effect by enhancing the insulin-stimulated glucose uptake thereby increasing the cell receptor (GLUT4) surface [17]. Figure 2 shows the effect of wogonin on the body weight of rats at day 0 and day 30. At day 30, diabetic control showed statistically significant (p<0.05) weight reduction as compared to all groups. Figure 3 exhibits the effect of wogonin on the blood glucose serum of rats for every alternate day from day 0 to day 30. The blood glucose serum of rats treated with wogonin at 40 and 80 mg/kg b.w. showed significant blood glucose reduction as compared to diabetic control (p<0.05). Metformin, as the control group showed statistically significant blood glucose reduction in comparison to the diabetic control and has restored it back to normal level. Meanwhile, at 25 mg/kg b.w., wogonin showed no significant difference as compared to diabetic control (p>0.05).

The result of serum lipid profiles depicted the concentration of serum lipid profile after 30 days of wogonin treatment (Figure 4). It shows that wogonin at all concentrations (25, 40, 80 mg/kg b.w.) exhibited no significant difference as compared to the normal group and positive control (p>0.05) for triglyceride, total cholesterol, high density, and low-density lipo-protein. However, significantly different results were observed for triglyceride, total cholesterol, high-density, and low-density lipoprotein when compared with diabetic control (p<0.05).

The effect of wogonin on insulin release after the administration of wogonin on day 0 and day 30 was assessed (Figure 5). The results showed that wogonin at 40 mg/kg b.w. had significantly improved insulin secretion by day 30 as compared with diabetic control (p<0.05).

The result of the histopathology assessment of the pancreas, liver, and kidney of the rats showed in Figures 6, 7, and 8. Figure 6 displays the cross-sectional of the pancreas for all groups namely (a) normal control, (b) diabetic control, (c) metformin (0.5 mg/kg b.w), (d) wogonin 25 mg/kg b.w, (e) wogonin 40 mg/kg b.w and (f) wogonin 80 mg/kg b.w. STZ-NA usually caused impairment to β-islet cells in the pancreas and plays a key role in the development of T2DM in the animal model. However, in this study, wogonin at 40 and 80 mg/kg b.w were found to regenerate pancreatic β- cells in diabetic. For liver histology (Figure 7), it was revealed that administration of wogonin at all concentrations (25, 40, and 80 mg/kg b.w) caused no sign of toxicity and no fatty acid accumulation was formed. As for the kidney cross-section (Figure 8), the administration of wogonin at 40 and 80 mg/kg b.w. caused thickening of glomerular basement membrane and mesangial expansion of the surrounding of bowman capsule.

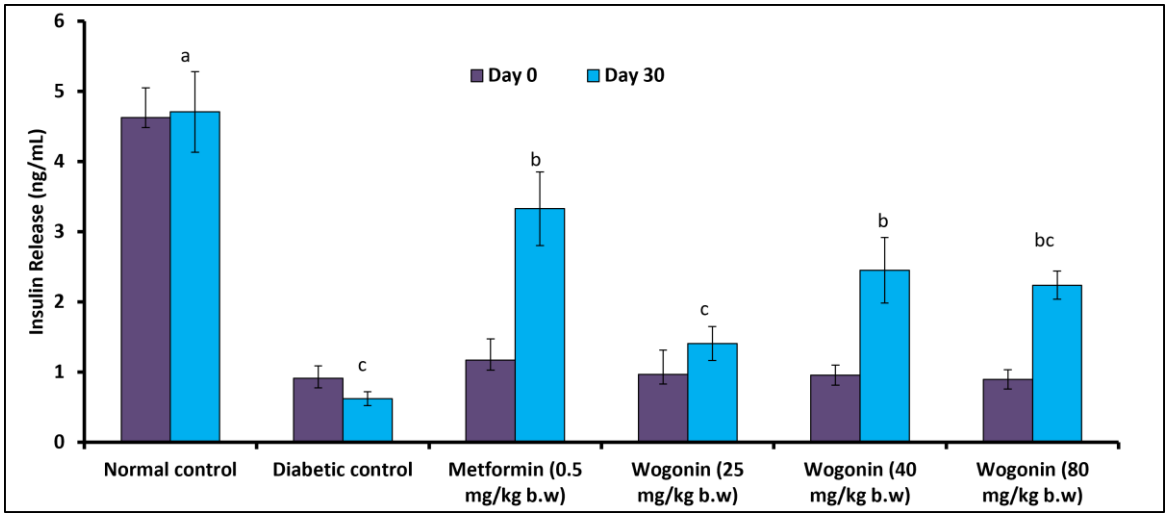

Figure 5. The effect of wogonin on rat's insulin release. Mean ± S.D, n=3, small letters represent Tukey's test. The means values that do not share a letter are significantly different ($p<0.05$). The result showed that at day 30, wogonin 40 mg/kg. b.w. has significantly increased the insulin released as compared to the diabetic control group.

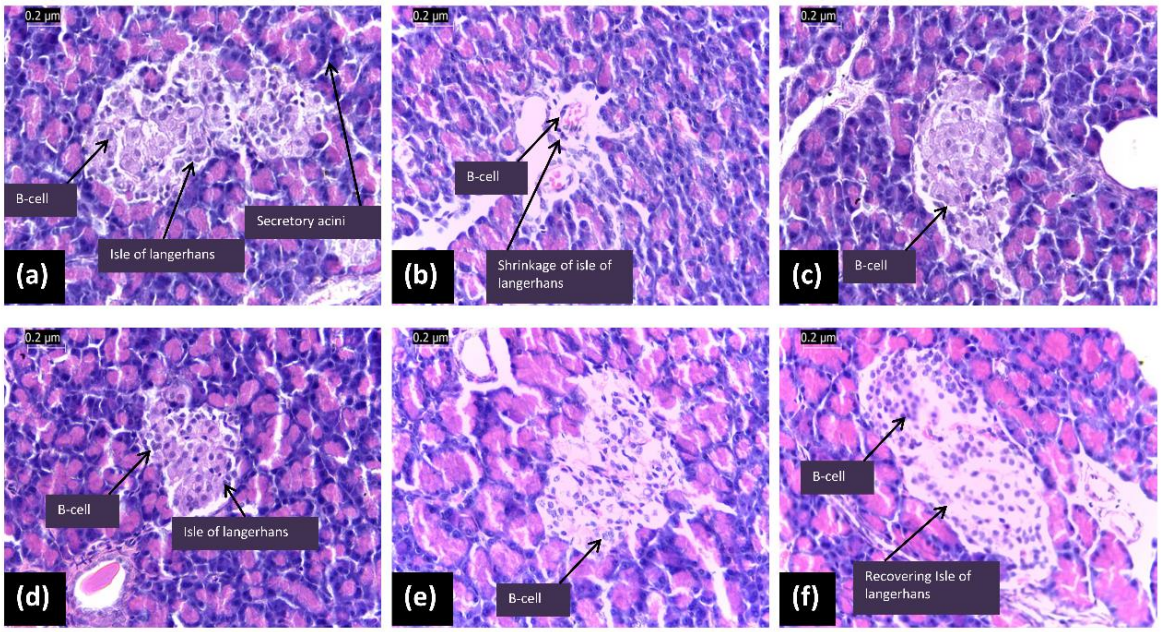

Figure 6. Pancreas histology (40x). (a) Normal control, (b) Diabetic control, (c) Metformin 0.5 mg/kg b.w., (d) Wogonin 25 mg/kg b.w., (e) Wogonin 40 mg/kg b.w., (f) Wogonin 80 mg/kg b.w. The result showed that wogonin at 40 and 80 mg/kg b.w. was found to regenerate pancreatic β- cells in diabetics. Scale bar: 0.2µm.

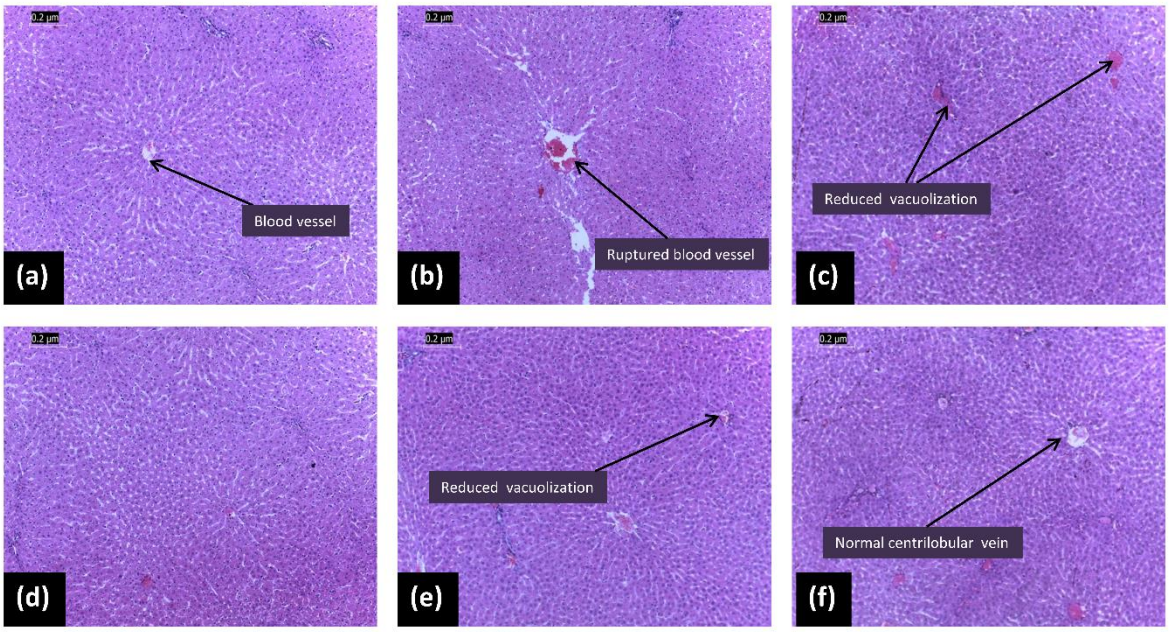

Figure 7. Liver histology (20x). (a) Normal control, (b) Diabetic control, (c) Metformin 0.5 mg/kg b.w., (d) wogonin 25 mg/kg b.w., (e) wogonin 40 mg/kg b.w., (f) wogonin 80 mg/kg b.w. No toxicity sign was manifested at the highest concentration of wogonin, 80 mg/kg b.w. Scale bar: 0.2µm.

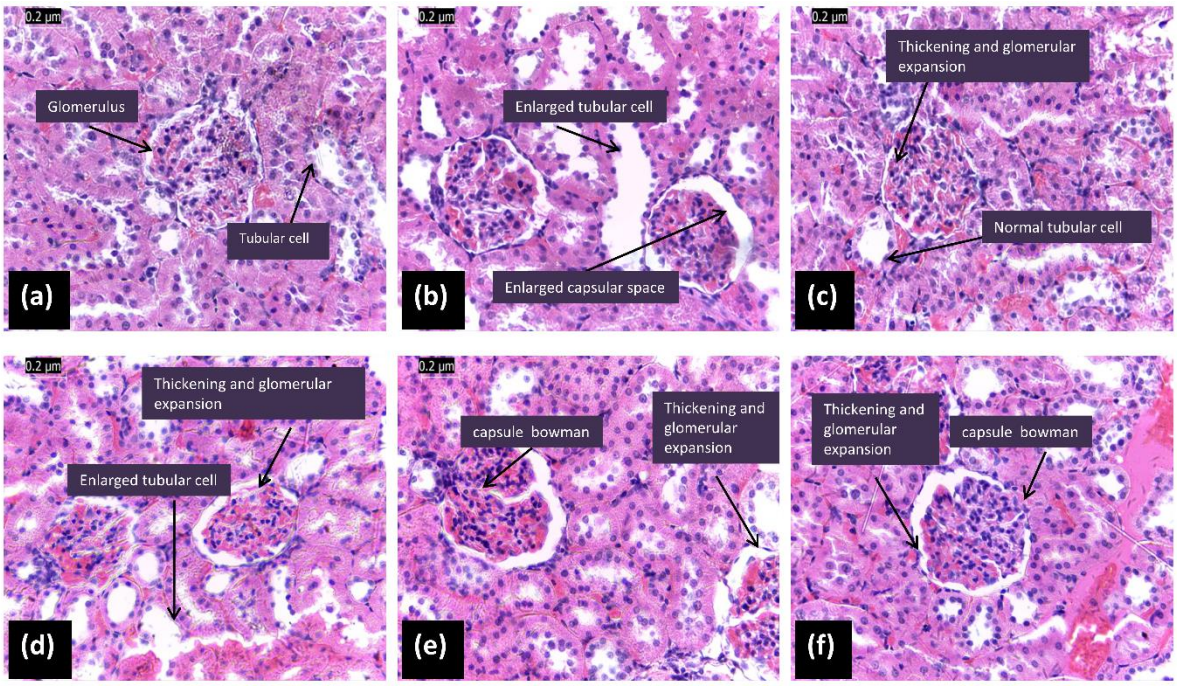

Figure 8. Kidney histology (20x). (a) Normal control, (b) Diabetic control, (c) Metformin 0.5 mg/kg b.w., (d) Wogonin 25 mg/kg b.w., (e) 5 Wogonin 40 mg/kg b.w., (f) Wogonin 80 mg/kg b.w. No toxicity sign was manifested at the highest concentration of Wogonin, 80 mg/kg b.w. However, the administration of wogonin at 40 and 80 mg/kg b.w. caused thickening of the glomerular basement membrane and mesangial expansion of the surrounding of the bowman capsule (figure d, e, f). Scale bar: 0.2µm.

CONCLUSION

Flavonoids are one of the most important groups of bioactive compounds among secondary metabolites. We have reported antidiabetic activity of a flavonoid i.e., wogonin, (5,7-dihydroxy-8-methoxyflavone) isolated from the leaves methanol extract of *T. indica* Merr. through in vivo perspectives. This study depicts the antidiabetic property of wogonin on STZ-NA-induced diabetic rats and is proven by the positive effects on blood glucose measurement, biochemical serum index, insulin release, and histopathological assessment. The results of this study have facilitated us to recognize the potential of wogonin and should be encouraged for further studies, which could ultimately lead to the development of nutritional products and semi-synthetic analogs that may retain substantial antidiabetic capacity with minimal adverse effects.

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REFERENCES

1. Urrutia, I., Martín-Nieto, A., Martínez, R., Casanovas-Marsal, J. O., Aguayo, A., Del Olmo, J. and Gaztambide, S. (2021) Incidence of diabetes mellitus and associated risk factors in the adult population of the Basque country, Spain. *Scientific Reports*, **11(1)**, 1–8.

2. Sekar, M., Zulhilmi, M., Hamdi, A. Y., Nabila, N., Zahida, Z. and Shafiq, M. (2014) Ten commonly available medicinal plants in Malaysia used for the treatment of diabetes-a review. *Asian Journal of Pharmaceutical and Clinical Research*, **7(1)**, 1–5.

3. World Health Organization (2021) Global Report on Diabetes.

4. World Health Organization (2016) Global Report on Diabetes.

5. Hasan, M. M., Ahmed, Q. U., Soad, S. Z. M., Latip, J., Taher, M., Syafiq, T. M. F., Sarian, M. N., Alhassan, A. M., and Zakaria, Z. A. (2017) Flavonoids from *Tetracera indica* Merr. induce adipogenesis and exert glucose uptake activities in 3t3-l1 adipocyte cells. *BMC Complement Alternative Medicine*, **17(1)**, 1–14.

6. Zhang, Y. M., Li, M. X., Tang, Z. and Wang, C. H. (2015) Wogonin suppresses osteopontin expression in adipocytes by activating PPARα. *Acta Pharmacologica Sinica*, **36(8)**, 987–997.

7. Bak, E. J., Kim, J., Choi. Y. H., Kim, J. H., Lee, D. E., Woo, G. W., Cha, H, H. and Yoo Y. J. (2014) Wogonin ameliorates hyperglycemia and dyslipidemia via PPARα activation in db/db mice. *Clinical Nutrition*, **33(1)**, 156–163.

8. Lei, L., Zhao, J., Liu, X. Q., Chen, J., Qi, X. M., Xia, L. L. and Wu, Y. G. (2021) Wogonin alleviates kidney tubular epithelial injury in diabetic nephropathy by inhibiting PI3K/Akt/NF-κB signaling pathways. *Drug Design, Development, and Therapy*, **15**, 3131–3150.

9. Liu, X. Q., Jiang, L., Li, Y. Y., Huang, Y. B., Hu, X. R., Zhu, W., Wang, X., Wu, Y. G., Meng, X. M. and Qi, X. M. (2022) Wogonin protects glomerular podocytes by targeting Bcl-2-mediated autophagy and apoptosis in diabetic kidney disease. *Acta Pharmacologica Sinica*, **43(1)**, 96–110.

10. Ahmed, Q. U., Dogarai, B. B. S., Amiroudine, M. Z. A. M, Taher, M., Latip, J., Umar, A. and Bala, Y. M. (2012) Antidiabetic activity of the leaves of *Tetracera indica* Merr. (Dilleniaceae) in vivo and in vitro. *Journal of Medicinal Plants Research*, **6(49)**, 5912–5922.

11. Qi, Q., Peng, J., Liu, W., You, Q., Yang, Y., Lu, N. and Guo, Q. (2008) Toxicological studies of 5,7-dihydroxy-8-methoxy flavone in experimental animals. *Phytotherapy Research*, **23(3)**, 417–422.

12. Ghasemi, A., Khalifi, S. & Jedi, S. (2014) Streptozotocin-nicotinamide-induced rat model of type 2 diabetes. *Acta Physiologica Hungarica*, **101(4)**, 408–420.

13. Alkan., E., Ugan, R. A., Basar, M. M., Halici, Z., Karakus, E., Balbay, M. D., and Un, H. (2017) Role of endothelin receptors and relationship with nitric oxide synthase in impaired erectile response in diabetic rats. *Journal of Andrologia*, **49(2)**, 1–8.

14. Bayrasheva, V. K., Babenko, A. Y., Dobronravov, V. A., Dmitriev, Y. V., Chefu, S. G., Pchelin, I. Y., Ivanova, A. N., Bairamov, A. A., Alexeyeva, N. P., Shatalov, I. S. and Grineva, E. N. (2016) Uninephrectomized high-fat-fed nicotinamide-streptozotocin-induced diabetic rats: a model for the investigation of diabetic nephropathy in type 2 diabetes. *Journal of Diabetes Research*, **2016**, 1–18.

15. Mahmoud, H. M., Zaki. H. F., El-Sherbiny, G. A. and Abdel-Latif, H. A. (2014) Modulatory role of chelating agents in diet-induced hyper-cholesterolemia in rats. *Bulletin of Faculty of Pharmacy, Cairo University*, **52(1)**, 27–35.

16. Sarian, M. N., Ahmed, Q. U., Mat So'ad, S. Z., Alhassan, A. M., Murugesu, S., Perumal, V., Mohammad. S. N. A. S., Khatib, A. and Latip, J. (2017) Antioxidant and antidiabetic effects of flavonoids: A structure-activity relationship-based study. *BioMed Research International*. **2017**, 1–14.

17. Herman, R., Kravos, N. A., Jensterle, M., Janež, A. and Dolžan, V. (2022) Metformin and insulin resistance: a review of the underlying mechanisms behind changes in GLUT4-mediated glucose transport. *International Journal of Molecular Sciences*, **23(3)**, 1–17.