Antidiabetic Effects and Mechanisms of Action of *Uncaria gambir* Roxb. in Diabetic Sprague–Dawley Rats

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The antidiabetic properties of *Uncaria gambir* are not yet fully understood, particularly concerning how it affects diabetic animal models. Further investigation in this aspect is pivotal before initiating clinical evaluations. This study aimed to investigate the antidiabetic activity of *U. gambir* and how it influences blood glucose levels in diabetic Sprague–Dawley rats. In this study, 28 rats were divided into 7 groups. The groups were as follows: a nondiabetic rat group, a nondiabetic rat group given *U. gambir*, a diabetic rat group, a diabetic rat group given glibenclamide, and 3 diabetic rat groups given *U. gambir* at 3 doses (200, 300, and 400 mg/kg). Diabetes was induced using streptozotocin (50 mg/kg) given by intraperitoneal injection. Blood glucose levels were measured weekly, and the animals were euthanized at the end of the experiment. Intracardiac blood and tissues such as the pancreas, liver, and skeletal muscle were collected for further analysis. The results showed that administering *U. gambir* to diabetic rats resulted in significantly lower blood glucose levels than untreated diabetic rats. *U. gambir* has a complex mechanism to reduce blood glucose levels. including increase of insulin production, preservation of the islets and pancreatic β cells, and optimization of glycogenesis, as reflected in a significant increase in liver glycogen levels. These findings suggest that *U. gambir*'s multicompound and multitarget capabilities in controlling blood glucose levels may have utility for treatment of diabetes.

Abbreviations and Acronyms: DM, diabetes mellitus; Glib, glibenclamide; PAS, periodic acid–Schiff; UG, *Uncaria gambir*

Introduction

Diabetes, marked by elevated blood glucose levels or hyperglycemia,[9] stands as the most prevalent endocrine disorder. Globally, diabetes remains a formidable challenge for the healthcare system,[41] ranking among the top 10 causes of death with an estimated 6.7 million deaths in 2021.[38,41] Notably, it emerges as the second most common comorbidity after hypertension for COVID-19 patients in several nations, such as China, South Korea, and Indonesia.[8,36]

Diabetes consists of 2 main types: type 1 diabetes, the result of autoimmunity toward the pancreatic β cells; and type 2 diabetes, mostly linked to insufficient secretion of glucose-specific insulin.[56] Despite their distinct etiologies, both types share overlapping pathophysiology. For instance, both types can lead to a reduction in β-cell mass.[13,48] They are both characterized by persistent hyperglycemia, which ultimately leads to the death of β cells.[17,37] This is also causatively connected to dysfunction in pancreatic β cells and the decline of insulin levels.[55] Given the complex pathophysiology, it has been speculated that certain antidiabetic medications approved for type 2 diabetes treatments might confer benefits for type 1 diabetes individuals.[14]

Combination antihyperglycemic drugs could be of value for patients struggling to attain clinical moderation with first-line oral antidiabetic agents as monotherapy.[47] This complexity underscores the necessity for multifaceted approaches in diabetes treatment. In the United States, only 36% of individuals achieve glycemic control despite many medications available.[47] Patient nonadherence is the main contributing factor to its ineffectiveness. On the other hand, a study has reported that the efficacy of antidiabetic drugs is only 41%.[1] Hence, there is an urgent need for new treatment alternatives. Identifying novel antidiabetic agents with potent multimechanistic actions could offer a promising approach. Such medications would likely target multiple pathways and mechanisms involved in the development of diabetes, thereby maximizing effectiveness and potentially improving patient outcomes.

Plants have emerged as sources of herbal medicines and often serve as precursors for synthetic drugs.[59] Approximately half of today's medicines derive from natural sources, including plants.[54] Various mechanisms offered by medicinal plants contribute to lowering blood glucose levels. Several plant species exhibit a capacity to diminish blood glucose levels by inhibiting the α-glycosidase enzyme.[20,63] Others are known to rejuvenate pancreatic β cells, enhance insulin secretion and sensitivity, accumulate antioxidants, impede hepatic gluconeogenesis, and alleviate metabolic syndrome in diabetic patients.[16,43,53,58]

Uncaria gambir, native to Southeast Asia and classified under the Rubiaceae family, is commonly known as 'Gambir' or 'Gambier' in Indonesia and Malaysia.[49] Locally, its leaves and twigs undergo traditional processing to produce an extract used in diverse practices, such as 'menyirih', where it is combined with other ingredients, wrapped in betel leaves, and chewed, as well as in remedies for ailments like diarrhea, dysentery, and premature ejaculation.[44] Numerous studies have elucidated the antidiabetic potential of *Uncaria gambir*. In vitro experiments have indicated its capability to inhibit the α-glucosidase enzyme.[6,39,70] Furthermore, various studies have corroborated its possession of antioxidant properties.[4,34] Nevertheless, despite these findings, a fundamental understanding of *U. Gambir*'s antidiabetic activity in animal models of diabetes remains limited.

While many studies have used in vitro and in silico approaches, animal models are still crucial for investigating different aspects of diabetes, including its underlying causes, progression, and response to treatment.[40] Streptozotocin is widely used to induce experimental diabetes in animals.[25] It has been found to be more effective than other chemicals, like alloxan, due to its superior diabetogenic properties, and works well across different species and produces more consistent experimental outcomes.[3] The diabetogenic mechanism of streptozotocin primarily revolves around the DNA methylating activity of its methyl nitrosourea component. It leads to DNA damage with resultant necrosis of the pancreatic β cells.[24] In addition, streptozotocin generates superoxide dismutase anions that act on the mitochondria and cause diabetic complications.[52] Given the above, the present study was conducted to investigate the potential of *Uncaria gambir* as an antidiabetic agent and elucidate the underlying mechanisms that contribute to its efficacy in this diabetic model.

Materials and Methods

Plant material. Fresh leaves and young twigs of *Uncaria gambir* were obtained from a local farm in Surantih district, Pesisir Selatan regency, West Sumatera province, Indonesia. The herbarium of Andalas University in West Sumatra, Indonesia confirmed the material as being from *U. gambir*.

Preparation of *Uncaria gambir* extract. In total, 36.5 kg of fresh leaves and twigs were boiled for 2 h and then pressed using a jack press to obtain the liquid extract. Liquid extract was filtered and deposited in a wooden container called *paraku* for 24 h. In the *paraku*, the extract was allowed to cool into a paste. The extract was drained and pressed again with stone ballast to make the paste denser and immediately printable. The printed extract was then sun dried for 3 to 7 d.

Phytochemical profile. Phytochemical examinations were conducted using both qualitative and quantitative approaches. Following standard procedures, qualitative methods identified alkaloids, flavonoids, saponins, quinones, tannins, triterpenoids, and steroids.[32]

The quantitative analysis involved the measurement of catechin referring to the Indonesian National Standard (SNI 01 to 3391-2000). Briefly, catechin measurement was performed using a spectrophotometric analysis, which involved comparing the absorption of the sample solution with that of a standard solution. The standard solution, containing catechin with a purity level of over 98% and sourced from Sigma-Aldrich (St. Louis, MO), was used as a benchmark. The analysis was carried out spectrophotometrically using a wavelength of 279 nm (U-2010 spectrophotometer; Hitachi, Tokyo, Japan).

Quercetin was quantitatively assessed using HPLC (HPLC-UV-VIS; Hitachi, Tokyo, Japan), following the protocols outlined in a previous study.[33] In summary, 1 gm of the sample was dissolved in 10 mL of MeOH tert-butylhydroquinone. The solution was sonicated for 20 min and then filtered using a 0.45-µM filter paper. The dissolved sample was then injected into the HPLC and evaluated using a wavelength of 370 nm.

Animals and housing. This investigation used 28 male Sprague-Dawley rats (*Rattus norvegicus)*. The rats were obtained from the Indonesian Food and Drug Authority (BPOM) Jakarta, with the breeding stock originating from Charles River Laboratories (Wilmington, MA) and supplied by the Japan International Cooperation Agency (JICA). The rats were housed in a controlled environment with the temperature maintained at 22 to 24 °C, following a 12-h dark/12-h light cycle, and a relative humidity ranging from 50% to 60%. Two rats per cage were kept in nontransparent polypropylene cages, with their tails marked using a permanent marker for identification.[12] The size of cage was 40 cm in length, 31 cm in width, and 18 cm in height. The cages were equipped with solid floors covered with medium to large wood shavings sourced from *Guazuma ulmifolia* Lamk. The placement of cages on the rack was randomized to minimize the effects of environmental variables. The cage was equipped with a wire mesh top, allowing rats to observe external activities and interact with neighboring rats while ensuring optimal ventilation inside the cage. Miniplastic cups were provided for playing and gnawing in each cage for enrichment. The rats were fed commercial pellets explicitly formulated for laboratory rats, including white rats. The food had a maximum water content of 12%, a minimum protein content of 20%, a maximum fat content of 4%, a maximum crude fiber content of 4%, and a calcium content of 12% with a phosphorus content of 0.7% (Rat Bio; Citra Ina Feedmill, Jakarta, Indonesia). The water provided was bottled drinking water that met the standard quality requirements for human consumption. Food and water were provided ad libitum. The rats, aged 8 wk and weighing 230 to 250 g, were in good health and underwent a 14-d acclimation before any treatment was administered. The rats were determined to be free from internal and external parasites. Veterinary examinations were regularly conducted throughout the study period. The Committee Responsible for Animal Welfare at the Veterinary Teaching Hospital, IPB University, approved the rat handling and the study protocol under No. 29-2016 ACUC RSHP FKH-IPB. Importantly, all procedures and the utilization of animals strictly adhered to the *Guide for the Care and Use of Laboratory Animals*.[35]

Sample size determinations. The sample size calculation for the study was done using G*Power software version 3.1.9.7.[28] The *F* test family through ANOVA (fixed effect, omnibus, one-way model, with a *P* value of 0.05 and a power of 0.8) was considered for this calculation. The chosen effect size value was 0.840, obtained from the η value resulting from evaluating the AUC value against blood glucose levels. A total sample size of 28 rats was determined to be required for the study, with 4 rats in each experimental group.

Experimental design. In this study, 28 rats were randomly divided into 7 experimental groups, each containing 4 animals. The groups were as follows: (1) Non-DM group, in which rats were administered a vehicle solution by injection and daily oral placebo administration via gavage needle; (2) DM group, in which rats received streptozotocin injection and daily oral placebo administration; (3) Non-DM + UG 400 group, where rats received a vehicle solution (sterile distilled water) by subcutaneous injection and daily oral administration of 400 mg/kg of *U. gambir*; (4) DM + Glib 2.5 group, where rats received

streptozotocin injection and daily oral administration of 2.5 mg/kg of glibenclamide; (5) DM + UG 200 group, where rats received streptozotocin injection and daily oral administration of 200 mg/kg of *U. gambir*; (6) DM + UG 300 group, where rats received streptozotocin injection and daily oral administration of 300 mg/kg of *U. gambir*; and (7) DM + UG 400 group, where rats received streptozotocin injection and daily oral given of 400 mg/kg of *U. gambir*. The dry *U. gambir* extract is dissolved in distilled water before use. The *U. gambir* solution, which tends to be bitter, was administered orally using a feeding needle. All treatments were administered daily for 28 d.

Diabetes induction. After an overnight fasting period, each rat was administered 50 mg/kg of streptozotocin intraperitoneally. The streptozotocin was prepared for injection by dissolving in a 0.1 M citrate buffer with a pH of 4.5. Following injection, the rats consumed a 10% sucrose solution for 24 h and were later provided with standard drinking water ad libitum.[25] Seven days later, fasting blood glucose levels were evaluated using samples obtained from the tail tip, and glucose was measured with a glucometer (Accu-Check Active; Roche, Mannheim, Germany) to confirm diabetes induction. Rats having a fasting blood glucose of greater than 200 mg/dL were considered to be diabetic.[26] Treatment of the diabetic rats commenced after 7 d of streptozotocin induction.

Euthanasia and sample collection. The rats were humanely euthanized by cardiac exsanguination following deep anesthesia with ketamine (80 mg/kg) and xylazine (20 mg/kg) administered intraperitoneally. The blood was collected and deposited into vacuum serum tube tubes without any added anticoagulants. Afterward, these blood samples were moved to Eppendorf tubes and centrifuged at 1,300 RCF for 10 min to separate the serum which was then collected. Immediately following euthanasia, the pancreas was collected and preserved in Bouin solution; and liver and skeltal muscle samples were collected and fixed in paraformaldehyde. The organs were dehydrated in ethanol solutions, cleared using xylene, and subsequently embedded in paraffin wax for sectioning. Three-micrometer sections of the tissues were then produced using a rotary microtome.

Insulin level. Serum samples were used to determine the insulin level of rats. Insulin levels were assessed using The Rat Insulin ELISA Kit (catalog no. 90010, Crystal Chem, Inc., Elk Grove Village, IL). The assay was conducted in adherence to the manufacturer's guidelines and protocols.

Immunohistochemistry staining. After dewaxing and rehydrating, the tissue sections of the pancreas were incubated in 0.03% hydrogen peroxide, washed using 10% PBS, and incubated in 5% normal serum for 50 min and background sniper for 15 min. Subsequently, the samples were incubated with rat anti-insulin antibody (Genetex GTX28304, Genetex, Inc., Irvine, CA) (diluted in PBS 1:500) for 1 d. The samples underwent 3 washes in a PBS solution, followed by a 20-min incubation with Trekkie Universal Link (Biocare Medical, Inc., Pacheco, CA). Subsequently, the samples were rinsed again in PBS and incubated for 20 min with Trek-Avidin HRP (Biocare Medical). The sections were visualized with DAB (40%), rinsed, stained with hematoxylin, dehydrated, cleared, and well coverslipped. The tissues were examined under light microscopy using an Olympus CX31 Microscope with Indomicro Camera (Indomicro, Jakarta, Indonesia). In total, 15 visual fields per group were randomly chosen for evaluation.

Periodic acid–Schiff staining. Periodic acid–Schiff (PAS) staining was employed to visualize liver and skeletal muscle glycogen deposits. Paraffin sections of the organs were deparaffinized and incubated in 0.5% periodic acid (Sigma, Deisenhofen, Germany) for 5 min. Following these steps, the sections were rinsed in distilled water for 5 min, immersed in Schiff reagent (Sigma-Aldrich, St. Louis, MO) for 15 min, and then subjected to another 5-min wash in distilled water. Subsequently, they were counterstained with hematoxylin for 1 min, rinsed again in distilled water for 5 min, dehydrated, and finally mounted for observation. The staining results were observed and documented using a light microscope equipped with a camera and analyzed using the ImageJ application. Analysis was performed on 30 visual fields per group.

Statistical analysis. Before further analysis, testing the data for normality and homogeneity was crucial. If the data adhered to those criteria, the ANOVA test was initially performed, and the Duncan multiple range test was applied to explore and compare the variations among the groups in the study. Specifically for the AUC of glucose values, a post hoc test was performed using the Dunnett test, with the DM group as the control. In cases where the dataset deviates from normality and homogeneity, the Kruskal-Wallis test is used, followed by the Mann-Whitney test. Significant differences were indicated as $P \leq 0.05$. The outcomes are depicted as mean ± SEM. Statistical analyses were conducted using version 21.0 of SPSS (Chicago, IL).

Results

Phytochemical profile of *Uncaria gambir* extract. The analysis of the bioactive aqueous extract of *Uncaria gambir* indicated the presence of flavonoids, tannins, saponins, and triterpenoids while absent for alkaloids, quinones, and steroids. The HPLC analysis revealed the presence of quercetin, as shown in the chromatogram (Figure 1), indicating 2.65 mg/g of quercetin. In addition, as the marker for assessing the quality of the *U. gambir* extracts, the total catechin content was 80.56% ± 0.57, which agreed with National Standard Indonesia (SNI) No. 01 to 3391-2000 and was categorized as grade 1 in the trade market.

The impact of *Uncaria gambir* extract on blood glucose level. Blood glucose levels in diabetic rats treated with *U. gambir* at a dose of 200 mg/kg body weight returned to normal from severe (Figure 2A). By day 16, this group's average blood glucose level had decreased to 105 mg/dL from an initial level of 338 mg/dL. In contrast, rats in other treatment groups did not demonstrate similar reductions in blood gluose. The administration of *U. gambir* at doses of 300 and 400 mg/kg BW, as well as administration of glibenclamide, only managed to lower blood glucose levels to the intermediate level.

The influence of *U. gambir* on fluctuations in blood glucose levels over 28 d was further statistically evaluated by analyzing AUC (Figure 2B). The results showed that diabetic rats treated with 200 mg/kg of *U. gambir* had significantly lower blood glucose levels than untreated diabetic rats (DM group). This result suggests that administration 200 mg/kg of *U. gambir* to diabetic rats could reduce high blood glucose levels. However, diabetic rats treated with higher doses of *U. gambir* (300 and 400 mg/kg) and those treated with glibenclamide did not show significant differences in blood glucose levels compared with those of untreated diabetic rats.

The effect of *Uncaria gambir* extract on insulin level. The insulin levels were significantly lower in diabetic rats (DM) than in nondiabetic rats (Non-DM). After being treated with *U. gambir*, diabetic rats exhibited significantly higher insulin levels when compared with the untreated diabetic rat group. This increase was observed in all groups of rats treated with different doses
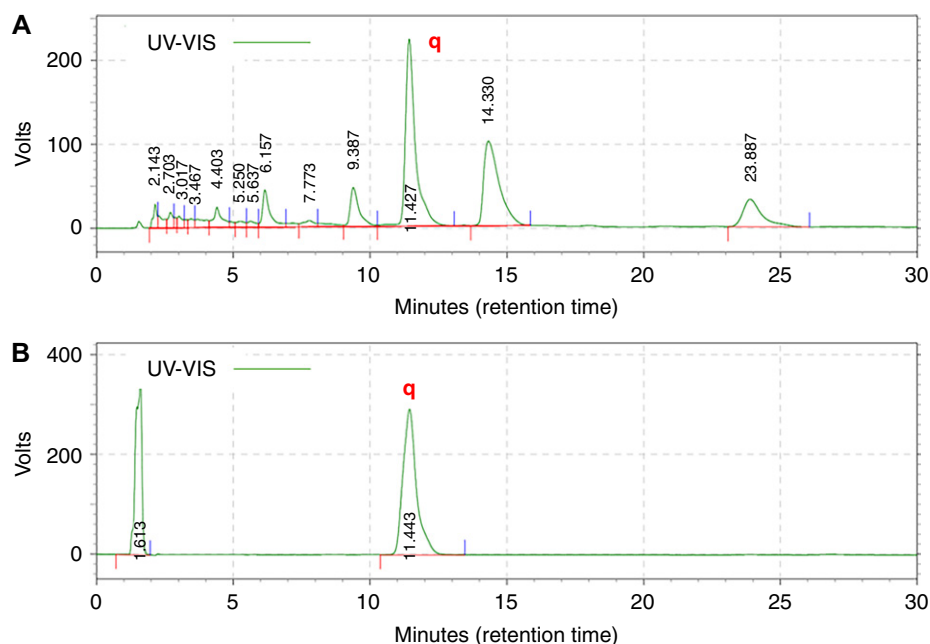
Figure 1. HPLC-UV-VIS quercetin profile of *Uncaria gambir*. (A) Quercetin in *Uncaria gambir* extract. (B) Quercetin standard. The red letter 'q' at the peak of the chromatogram indicates quercetin.

of *U. gambir* (DM + UG 200, DM + UG 300, and DM + UG 400). Furthermore, the insulin levels in these groups (DM + UG 200, DM + UG 300, and DM + UG 400) were not significantly different from nondiabetic rats (Non-DM). Conversely, diabetic rats treated with glibenclamide (DM + Glib 2.5) displayed no significant improvement, as their serum insulin levels were akin to those in the untreated diabetic rat group (DM) (Figure 3).

The effect of *Uncaria gambir* on the Langerhans β cells. Insulin-positive pancreatic β cells were cells were identified as those that stained a reddish-brown color (Figure 4A). In the diabetic rat group (DM), the β-cell number was reduced deficient. In contrast, those diabetic rats given *U. gambir* (DM + UG 200, DM + UG 300, and DM + UG 400) had more β cells than those of diabetic rats (DM). This result demonstrates that administration of *U. gambir* to diabetic rats could sustain the β cell population, although the quantity of β cells observed was not as extensive as those found in nondiabetic rats.

The diabetic rat groups receiving *U. gambir* extract (DM + UG 200, DM + UG 300, and DM + UG 400) quantitatively exhibited significantly higher β-cell percentages than the untreated

diabetic rat group (DM), although lower than the β-cell percentage observed in the nondiabetic group (Non-DM) (Figure 4B). Diabetic rats treated with glibenclamide (DM + Glib 2.5) did not display a notably higher β-cell percentage than the untreated diabetic rat group (DM). Furthermore, administration of *U. gambir* extract at 400 mg/kg to nondiabetic rats (Non-DM + G 400) did not impact the β-cell percentage, as evidenced by the equivalent percentage of β cells between this group (Non-DM + UG 400) and the nondiabetic rat group (Non-DM).

The effect of *Uncaria gambir* on Langerhans islets. The diabetic rats (DM) had the lowest count of islets compared with all other groups (Figure 4C). However, when diabetic rats were given *U. gambir* at any dosage (DM + UG 200, DM + UG 300, and DM + UG 400), they had more islets than untreated diabetic rats. Even though improvement was evident in the diabetic rats that underwent treatment, the number of islets remained below that observed in the nondiabetic rat group (Non-DM). In addition, administration of glibenclamide to diabetic rats (DM + Glib 2.5) did not significantly affect the number of islets in diabetic rats.
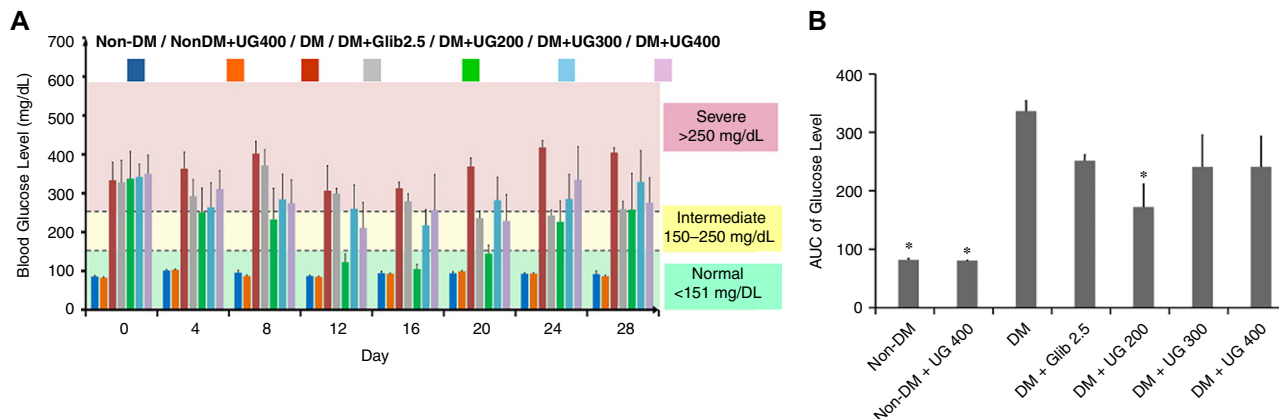

Figure 2. The effect of *Uncaria gambir* on blood glucose level. (A) Fluctuations in blood glucose levels during 28 d of observation. (B) AUC of glucose level. *Significant difference compared with the DM group according to the Dunnett test ($P \leq 0.05$). DM, diabetes mellitus (DM); Glib, glibenclamide; UG, *Uncaria gambir*.
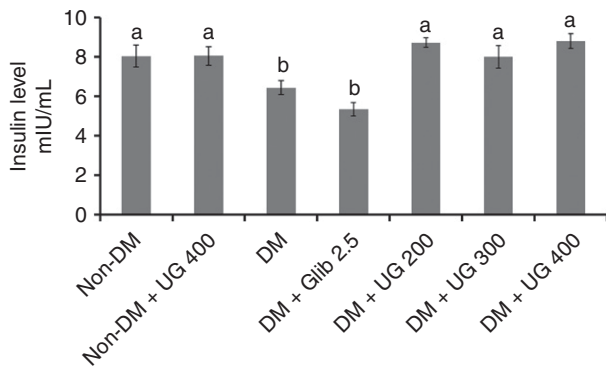
Figure 3. The effect of *Uncaria gambir* to insulin level. Different letters above the bar indicate significant differences according to the Duncan multiple range test (*P* ≤ 0.05). DM, diabetes mellitus; Glib, glibenclamide; UG, *Uncaria gambir*.

The effect of *Uncaria gambir* on liver glycogen. Staining of liver samples with PAS demonstrated (Figure 5A). The magenta color staining was more widespread in the group of nondiabetic rats (Non-DM) than in the group of diabetic rats (DM) due to a positive PAS reaction. This result was also observed in the groups that received *U. gambir* at any dosage (DM + UG 200, DM + UG 300, and DM + UG 400) and the diabetic rat group given glibenclamide (DM + Glib 2.5) stained more intensely for glycogen compared with the diabetic group (DM). PAS staining was even distributed across the liver in the nondiabetic rat group given

U. gambir 400 mg/kg (Non-DM + UG 400). Qualitatively similar results show that the diabetic rats had the lowest glycogen levels compared with other groups, while the treated diabetic groups demonstrated higher levels (Figure 5B).

The effect of *Uncaria gambir* on skeletal muscle glycogen. It was observed that the skeletal muscle cells of diabetic rats (DM) had less intense PAS staining for glycogen than the other groups (Figure 6A). However, there were no significant statistical differences between the groups (Figure 6B).

Discussion

This study aimed to determine whether *Uncaria gambir* could act as an antidiabetic agent in a rat model of diabetes. Our hypothesis is that *U. gambir* may exert antidiabetic effects via a multitarget mechanism of action. Prior studies have demonstrated the significant potential of the various components of *U. gambir*.[5,7,39,45,49] These findings suggest that *U. gambir* may have multiple target actions in regulating blood glucose levels, and its constituents may work synergistically.

A rat model of diabetes was used in this study and produced by administration of streptozotocin at 50 mg/kg. The rats exhibited various pathophysiologies associated with diabetes, such as persistent hyperglycemia and diminished insulin levels, islets, β cells, and liver glycogen content. Intriguingly, treatment with *U. gambir* improved some of these pathophysiologies, thus supporting the hypothesis that *U. gambir* acts through various mechanisms to decrease blood glucose levels.
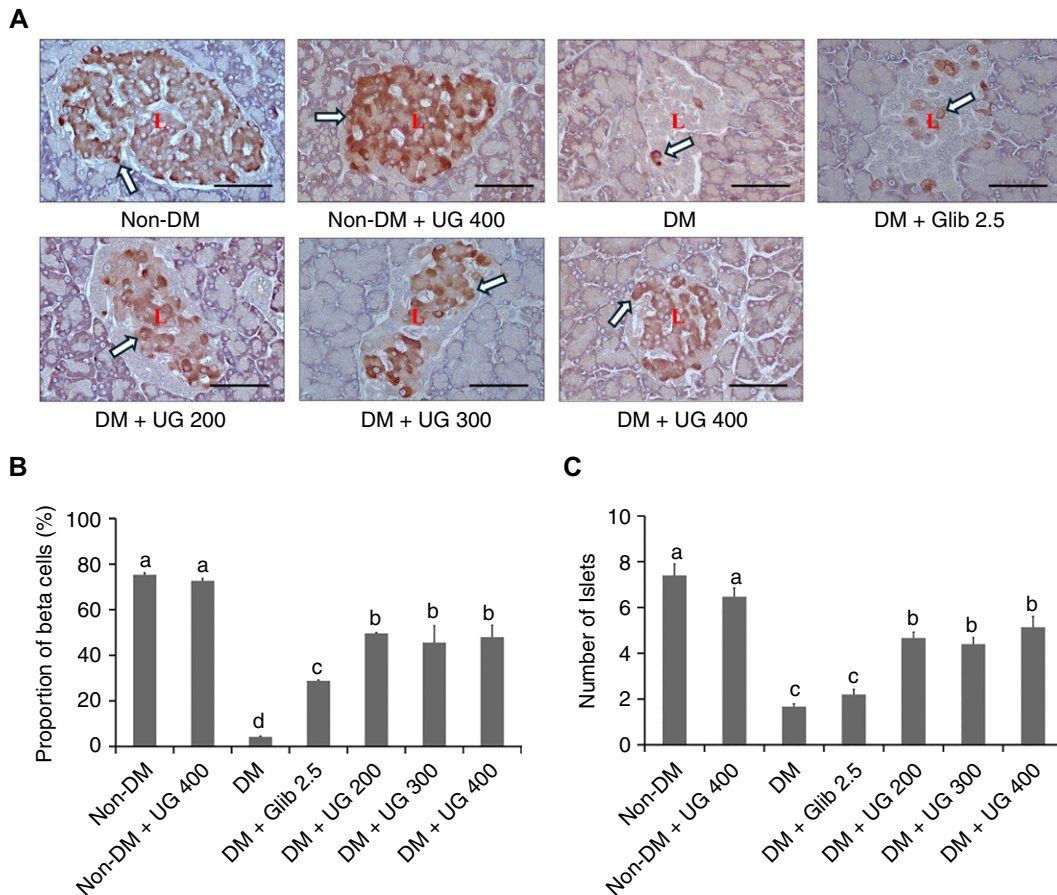

Figure 4. The effect of *Uncaria gambir* on islets of Langerhans and β cells. (A) Photomicrograph of immunohistochemical staining of insulin-activated pancreatic β cells; scale = 50 μm; 10 × 40 magnifications. (B) The proportion of β cells to the total number of cells. (C) The number of islets per high-powered field; 10 × 10 magnifications. Different letters above the bar indicate significant differences according to the Duncan multiple range test for the proportion of β cells or Mann-Whitney test for the number of islets (*P* ≤ 0.05). Arrows are pancreatic β-cell representation. DM, diabetes mellitus; Glib, glibenclamide; L, islet of Langerhans; UG, *Uncaria gambir*.
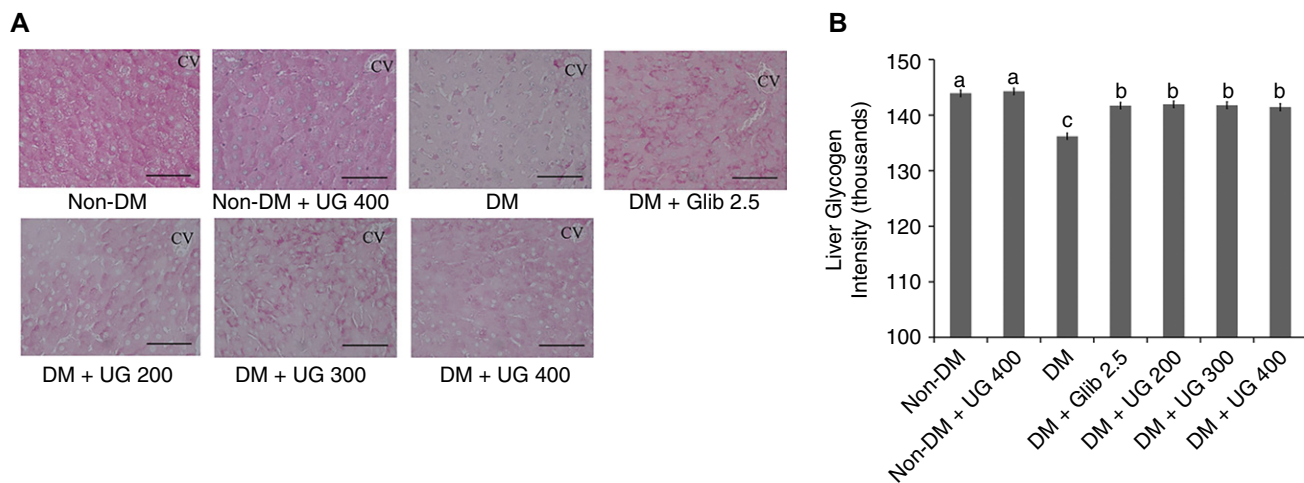
A

B

Figure 5. The effect of *Uncaria gambir* on liver glycogen. (A) Photomicrograph of PAS staining of liver glycogen; scale = 50 μm. (B) Quantitative liver glycogen intensity per high-powered field; 10×40 magnifications. Different letters above the bar indicate significant differences according to the Duncan multiple range test ($P \leq 0.05$). CV, central vein; DM, diabetes mellitus; Glib, glibenclamide; UG, *Uncaria gambir*.

Our results show that administration of *U. gambir* is associated with lower blood glucose levels in a diabetic rat model. The most effective dosage of *U. gambir* is 200 mg/kg, and we showed that this dosage can return blood glucose back to normal levels. According to a previous study, Sprague–Dawley rats have a fasting normal blood glucose level below 151 mg/dL.[26] In this study, diabetic rats treated with *U. gambir* at a dosage of 200 mg/kg achieved normal blood glucose levels from week 12 to week 20, with the best average blood glucose level (105 mg/dL) reached at week 16. Interestingly, extending the administration time and *U. gambir* dosage did not result in further reductions of blood glucose levels. This finding may be related to U-shaped dose-response relationships (often termed hormesis). Hormesis is a phenomenon where a substance exhibits a dual response, stimulating beneficial effects at low doses or concentrations and causing inhibitory or detrimental effects at higher doses or concentrations.[15,61] Hormesis has been documented in numerous biologic, toxicological, and pharmacological agents, including the effects of metformin as an antidiabetic drug,[51] as well as the influence of alcohol on blood sugar levels.[57] Perhaps, a hormetic response affects *U. gambir*'s ability to moderate blood glucose levels, with efficacy demonstrated at one dosage but not at greater dosages. However, further research is required to confirm this effect and elucidate the underlying mechanisms.

When administered to diabetic rats, *U. gambir* increased insulin secretion. Remarkably, the diabetic rats treated with *U. gambir* had insulin levels similar to those of nondiabetic rats, even though the number of Langerhans islets and β cells between the 2 groups was significantly different. The ability of *U. gambir* to increase insulin secretion could be attributed to the quercetin and catechins component of the preparation. The phytochemical test results indicate that the gambir used in the study contained a significant amount of catechin, up to 80%, while the quercetin content is measured at 2.65 mg/g. Quercetin could enhance insulin release by increasing Ca^{2+} uptake.[10] Catechin has several mechanisms to regulate diabetes. Indeed, a significant aspect involves enhancing mitochondrial function by maintaining oxidative phosphorylation and ATP production, encouraging mitochondrial biogenesis, and facilitating cellular protection within mitochondria.[64] Mitochondria play a vital role in cell viability, and the insulin secretion triggered by glucose is contingent upon mitochondrial respiration through the electron transport chain. In addition, epicatechin could act as a signaling molecule to stimulate insulin secretion by activating calmodulin-dependent protein kinase II (CaMKII).[67] These available studies provide evidence supporting the role of catechin and quercetin in increasing insulin secretion. However, this does not eliminate the possibility of other phytochemicals contributing to this action. As a result, further exploration is essential to determine the involvement of other phytochemicals in insulin secretion.

In this study, we show that *U. gambir* can help to maintain the number of Langerhans' islets and β cells in diabetic animals.
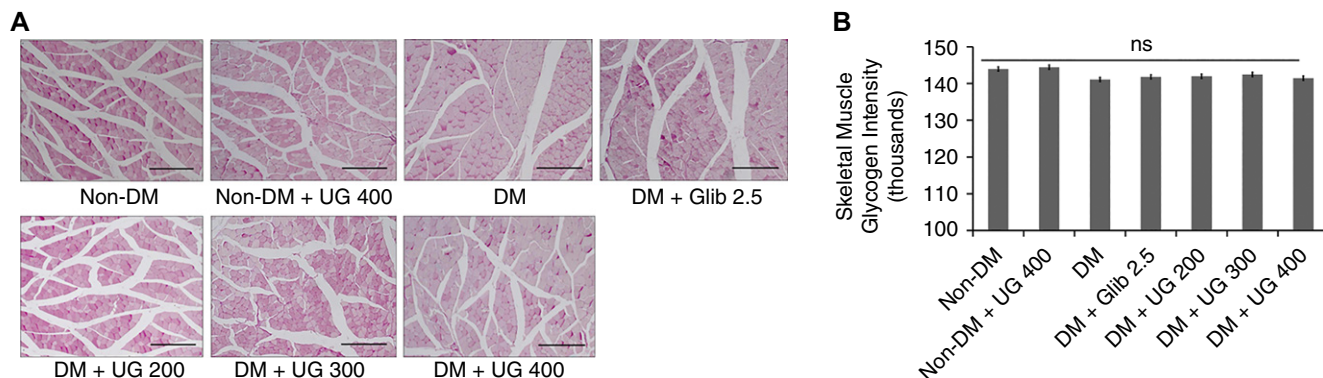
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Figure 6. The effect of *Uncaria gambir* on skeletal muscle glycogen. (A) Photomicrograph of PAS staining of skeletal muscle glycogen; scale = 50 μm. (B) Quantitative skeletal muscle glycogen intensity. DM, diabetes mellitus; Glib, glibenclamide; ns, not significant; UG, *Uncaria gambir*.

Even though the group of diabetic rats given *U. gambir* had fewer Langerhans islets and β cells than the nondiabetic rat group, the number of islets and cells was still significantly greater than that in the diabetic rat group. Langerhans islets regulate glucose homeostasis and are vital components of the pancreas.[27] The number of Langerhans islets and pancreatic β cells could be maintained through protection, regeneration, or both. Antioxidant compounds could facilitate protection, preventing β cells from being damaged.[23] Beta cells have lower antioxidant defenses than others, making them more vulnerable to oxidative damage.[46] Beta cells have fewer prosurvival Bcl2 proteins, making them more susceptible to damage than others.[60]

U. gambir has been identified as a potential source of antioxidant activity.[34] Catechin, an abundant component of *U. gambir*, is a powerful antioxidant, and the Indonesian National Standard (SNI) has designated catechin as a biomarker compound for evaluating *U. gambir* quality. Others have suggested that catechin could significantly enhance the activity of antioxidant enzymes, lower the levels of reduced glutathione, and decrease malondialdehyde content in the livers and kidneys of streptozotocin-induced diabetic rats.[50] The high antioxidant activity in *U. gambir* could contribute to its antidiabetic effects, exceeding those of glibenclamide. Unlike glibenclamide, which does not contain antioxidants, antioxidants in *U. gambir* could protect β cells from damage. Several studies have reported that glibenclamide controls blood glucose levels by stimulating insulin secretion.[29] In contrast, another study suggested that glibenclamide administration can inhibit insulin secretion as part of its mechanism for repairing pancreatic β cells.[71]

Apart from protection, the number of Langerhans islets could also be maintained through regeneration. Recent studies have found that the pancreas has a significant self-renewal capacity.[27] Several pancreatic regeneration mechanisms have been proposed, including neogenesis of Langerhans islets through the proliferation and differentiation of duct cells,[62] endogenous progenitor cells,[66] preexisting β cells,[21] transdifferentiation of other endocrine cells,[11] and self-duplication.[22] Previous studies have reported that β-cell regeneration occurs 1 to 2 wk after pancreas ligation in adult *Mus musculus*.[62] Similarly, newly formed small Langerhans islets were found one week after pancreatic ligation in *Rattus norvegicus*.[62]

Various plants have also been reported to enhance β-cell function and regeneration, including *Centaurium erythraea*, *Cornus officinalis*, *Lactarius deterrimus*, *Panax ginseng*, and *Gardenia jasminoides*.[65] Saponin and quercetin in plants have specific effects on β cells that may be useful in treating diabetes.[65] Quercetin not only enhances insulin secretion but also prompts the regeneration of pancreatic β cells.[31] Saponins could facilitate β-cell regeneration by protecting cells from apoptosis and promoting cell proliferation, thereby safeguarding Langerhans islets from damage.[18] Saponins activate the Wnt/β-catenin/TCF7L2 pathway, initiating the release of GLP-1 and GIP, thereby fostering the proliferation of β cells.[18] The Wnt signaling pathway is pivotal in developing the pancreatic endocrine system and regulating β-cell functions such as insulin secretion, cell survival, and proliferation.[18] TCF7L2 influences the growth and differentiation of cells in Langerhans islets.[18] One study[68] suggested that saponin derived from *Astragalus membranaceus* notably boosted the expression of genes related to β-cell differentiation and also elevate the mRNA levels of Neurogenin 3 (Ngn3), recognized as a marker for endocrine progenitors. Hence, this study implies that *U. gambir* could potentially stimulate the regeneration of Langerhans islets and

pancreatic β cells. Further investigation is required to validate the molecular mechanisms through which *U. gambir* sustains pancreatic β cells.

U. Gambir's antidiabetic properties could be attributed to its ability to maintain liver glycogen content, which helps to reduce glucose output in the bloodstream. The present study found that diabetic rats had significantly lower glycogen content in hepatocytes compared with nondiabetic rats. Meanwhile, rats treated with *U. gambir* at varying dosages (200, 300, and 400 mg/kg) had higher glycogen content than diabetic rats, as did rats given glibenclamide. These findings indicate that *U. gambir* could effectively increase liver glycogen content in diabetic rats. The liver regulates blood glucose levels by balancing glycolysis, gluconeogenesis, and glycogen metabolism.[2] Therefore, it is one of the primary targets for diabetes treatment.[30] Inhibiting hepatic glucose release (gluconeogenesis) could be achieved by increasing glycogen storage.[2]

Metformin is an antidiabetic drug that also works in the liver by inhibiting gluconeogenesis.[42] The hepatic glycogen level can be a biomarker for evaluating the antihyperglycemic effects of various drugs.[69] Catechin is one of the phytochemicals in *U. gambir* that is believed to be responsible for maintaining hepatic glycogen content. Catechin increases glucokinase, glucose-6-phosphate, glycogen synthase, and glycogen phosphorylase to normal levels in diabetic rats.[19] Catechin also enhances the expression of GLUT4 protein and mRNA.[19]

This study also attempted to investigate the mechanism of *U. gambir* on glycogen content in skeletal muscles. Regrettably, the diabetes model used in this study was not accompanied by a decrease in skeletal muscle glycogen levels, posing a challenge to identifying the effect of *U. Gambir* on skeletal muscle glycogen.

Our results offer compelling evidence supporting the potential of *U. gambir* as a highly effective treatment for various pathophysiologies linked to diabetes. The results suggest that *U. gambir* holds promise in alleviating a spectrum of diabetes-related conditions, positioning it as a versatile and promising antidiabetic agent. By targeting multiple pathophysiologies simultaneously, *U. gambir* could provide an efficient and holistic approach to managing diabetes. Overall, these findings highlight the need for further research into the potential benefits of *U. gambir* for diabetes management.

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