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ANTIOXIDANT, ANTIHYPERGLYCEMIC, AND RENAL PROTECTIVE EFFECTS OF *HIBISCUS TILIACEUS* L. LEAF EXTRACT ON STREPTOZOTOCIN-INDUCED HYPERGLYCEMIA

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Summary

Hibiscus tiliaceus L. (Malvaceae) plays a significant role in traditional medicine for treating various diseases. This study aimed to quantify the total polyphenolic and flavonoid contents and evaluate the antihyperglycemic and renal protective effects of *H. tiliaceus* leaf extracts using *in vitro* assays (DPPH and α-glucosidase inhibitory activity) and a streptozotocin (STZ)-induced hyperglycemic mouse model. The results showed that the total phenolic and flavonoid contents in the aqueous extract (27.67 mg GAE/g and 11.01 mg QE/g) were lower than those in the 45% ethanol extract (31.67 mg GAE/g and 15.64 mg QE/g). Furthermore, the ethanol extract demonstrated superior antioxidant activity (IC_{50} value of 5.75 µg/mL) and α-glucosidase inhibitory activity (IC_{50} value of 2.69 µg/mL) compared to the aqueous extract (IC_{50} value of 21.05 µg/mL and 7.71 µg/mL, respectively). Oral administration of the 45% ethanol extract at 1.33 g/kg for 14 days significantly reduced fasting plasma glucose and improved glucose tolerance in STZ-induced mice. This dose also attenuated the oxidative stress marker malondialdehyde (MDA) in the kidneys while significantly enhancing endogenous glutathione (GSH) levels. These findings suggest that the *H. tiliaceus* leaf 45% ethanol extract is a potential candidate for managing hyperglycemia and preventing diabetic kidney disease.

Keywords: *Antihyperglycemic effect; Antioxidant activity; α-Glucosidase inhibitory activity; Hibiscus tiliaceus leaves; Streptozotocin-induced oxidative damage.*

1. Introduction

Diabetes mellitus (DM) is the leading cause of kidney disease, known as diabetic nephropathy, which is characterized by the prevalence of micro- or macroalbuminuria and cardiovascular disease. Approximately 20% to 40% of type 2 diabetes patients with microalbuminuria progress to clinical diabetic kidney disease, a progression often linked to poor glycemic control or comorbid hypertension [1]. In Asia, research in Japan indicated that up to 56.7% of type 2 diabetic patients may have chronic kidney disease (CKD), while figures for China, South Korea, and Thailand were reported at 25.6%–49.3%, 24.4%–36.0%, and 25.4%, respectively [1]. Similarly, results in Vietnam are consistent with neighboring countries, ranging from 23.8% to 41.7% [2]. The normalization of both fasting plasma glucose (FPG) and postprandial plasma glucose (PPG) is essential for patients to achieve glycemic and HbA1C targets, thereby preventing diabetic nephropathy. Medicinal herbs and traditional medicines play a prominent role in maintaining long-term glycemic control due to their safety profiles and diverse bioactive compounds. However, further scientific data are required to validate the application of these medicinal herbs as alternative and complementary therapies.

Hibiscus tiliaceus L. (Malvaceae) is widely utilized in traditional medicine-primarily its bark and leaves-to treat fever, coughs, sore throat, infections, diarrhea, dysentery, and typhoid [3]. Various parts of the plant, including the bark, branches, flower buds, and leaves, have been reported to possess diverse pharmacological activities, such as antioxidant, antidiabetic, anti-inflammatory, analgesic, anthelmintic, antimicrobial, anticancer, and neuropharmacological effects [3]. In Indonesia, *H. tiliaceus* leaves (locally known as Waru leaves) are commonly used as natural food packaging due to their antioxidant and antimicrobial properties [4],[5]. Phytochemical screening of the leaves has identified several bioactive groups, including proteins, carbohydrates, phenols/tannins, flavonoids, terpenoids, glycosides, and steroids [4]. Sofia V. et al. (2024) demonstrated that a 70% ethanol extract of *H. tiliaceus* leaves attenuated blood glucose levels in glucose-loaded normoglycemic mice [5]. Furthermore, our previous study revealed that the 70% ethanol extract reduced blood glucose levels in high-fat diet-induced obese hyperglycemic

mice [6]. While the *in vitro* antioxidant and α-glucosidase inhibitory activities of isolated compounds from *H. tiliaceus* have been established [7], no studies have yet investigated the application of *H. tiliaceus* leaf extract in managing postprandial hyperglycemia or preventing oxidative stress-induced diabetic kidney disease in hyperglycemic models. Therefore, the present study aims to evaluate the potential of a 45% ethanol extract of *H. tiliaceus* leaves in monitoring plasma glucose after oral glucose overload and its anti-oxidative stress effects in the kidneys of streptozotocin (STZ)-induced hyperglycemic mice.

2. Materials and Methods

2.1. Plant Materials and Extraction

H. tiliaceus leaves were collected from Can Gio District, Ho Chi Minh City, Vietnam. The plant material was authenticated, and a voucher specimen was deposited at the Research Center of Ginseng and Medicinal Materials. The leaves were thoroughly washed, dried, and ground into a fine powder (passed through a 250 µm sieve). The powdered material was then subjected to extraction with either hot water or 45% ethanol using the percolation method at a solvent-to-solid ratio of 1:20 (w/v). The resulting *H. tiliaceus* leaf extracts were concentrated under reduced pressure to yield the aqueous extract (AE) (moisture content: 14.87%) and the 45% ethanol extract (EE) (moisture content: 8.79%), with extraction yields of 11.15% and 26.67%, respectively. The final extracts were stored at 2–8 °C and reconstituted in distilled water prior to administration in the mouse models.

2.2. Animals

Healthy male *Swiss albino* mice (4–5 weeks old, weighing 18–22 g) were obtained from the Institute of Vaccines and Medical Biologicals (IVAC) in Nha Trang, Vietnam. The mice were housed in polypropylene (PP) cages under controlled conditions, including a room temperature of 25 ± 2 °C and a 12 h light-dark cycle. They were provided with standard laboratory chow and tap water *ad libitum*. All animals were acclimatized for at least five days prior to the commencement of the experiments. The volume for oral (p.o.) and intraperitoneal (i.p.) administrations was maintained at 10 mL/kg of body weight. All animal procedures were conducted in strict accordance with the ethical guidelines of the Ministry of Health, Vietnam [8].

2.3. Determination of total phenolic content (TPC)

The total phenolic content of *H. tiliaceus* leaf extracts was determined spectrophotometrically using the Folin–Ciocalteu assay [9]. Extracts were prepared in methanol at various concentrations from a 1000 μg/mL stock solution. Gallic acid (Sigma-Aldrich, USA) served as the standard in the concentration range of 10–50 μg/mL. Briefly, the reaction mixture was prepared by combining 0.2 mL of extract or standard with 6 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent. After shaking, the mixture was incubated for 5 min in the dark, 1.5 mL of 20% Na_2CO_3 was added. The mixture was then adjusted to a final volume of 10 mL with distilled water and incubated at room temperature for 120 min in the dark. The absorbance was measured at 760 nm using a UV/Vis spectrophotometer (UV-VIS Heliosy, Unicam Limited, UK). TPC was expressed as milligrams of gallic acid equivalent (GAE) per gram of sample (mg GAE/g). All analyses were performed in triplicate.

2.4. Determination of total flavonoid content (TFC)

The total flavonoid content was measured using the aluminum chloride ($AlCl_3$) assay [10]. Quercetin (Sigma-Aldrich, USA) was used as the standard at concentrations ranging from 10 to 60 μg/mL. The reaction mixture was prepared by mixing 1.0 mL of extract or quercetin with 1.0 mL of 2% $AlCl_3$ and 8 mL of methanol. After a 10-min incubation, the absorbance was measured at 415 nm. TFC was expressed as milligrams of quercetin equivalent (QE) per gram of sample (mg QE/g). All measurements were conducted in triplicate.

2.5. DPPH radical scavenging activity

The antioxidant activity of *H. tiliaceus* leaf extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, as described by [11] with slight modifications. Standard ascorbic acid (Sigma-Aldrich, USA) at concentrations of 1.8, 8.8, 17.6, 44, and 88 μg/mL, and *H. tiliaceus* leaf extracts at concentrations of 10, 50, 100, 250, 500, and 1000 μg/mL, were prepared from stock solutions. Briefly, 0.5 mL of the extract or ascorbic acid was mixed with 0.5 mL of DPPH solution (0.6 mM in methanol). The mixture was shaken thoroughly, and the total volume was adjusted to 4 mL with methanol. The reaction tubes were then kept in the dark for 30 min. Finally, the

absorbance was measured at 515 nm using a spectrophotometer. The control was prepared by mixing 3.5 mL of methanol and 0.5 mL of DPPH solution. All analyses were performed in triplicate. The percentage of DPPH radical scavenging activity was calculated as follows:

$$I(\%) = \frac{(A_c - A_{0c}) - (A_t - A_{0t})}{(A_c - A_{0c})} \times 100$$

In which: Ac: The absorbance of control (DPPH + methanol), A0c: The absorbance of blank (only methanol), At: The absorbance of test sample (DPPH + *H. tiliaceus* leaf extracts or ascorbic acid), A0t: The absorbance of sample control (only test samples in methanol).

2.6. α-Glucosidase inhibitory activity

The α-glucosidase inhibitory activity was evaluated using α-glucosidase enzyme (EC 3.2.1.20, *Saccharomyces cerevisiae*, 20 U/mg) and *p*-nitrophenyl glucopyranoside (*p*NPG) as the substrate, both purchased from Sigma-Aldrich. Briefly, 50 μL of α-glucosidase (0.2 U/mL) in potassium phosphate buffer (0.1 M, pH 6.8) was pre-incubated at 37 °C with 60 μL of *H. tiliaceus* leaf extracts at various concentrations in DMSO. After a 20-min incubation, 50 μL of the substrate (final concentration of 4 mM) was added to the mixture and incubated at 37 °C for an additional 10 min. The reaction was terminated by adding 160 µL of 0.2 M Na_2CO_3. The absorbance was measured at 405 nm using a microplate reader (BioTek, USA). DMSO (10% final concentration) served as the control, while acarbose (Sigma-Aldrich, USA) was used as the standard inhibitor. All assays were performed in triplicate [12].

2.7. Streptozotocin-induced hyperglycemia

The hyperglycemic model was induced in overnight-fasting mice via a single intraperitoneal (i.p.) injection of streptozotocin (STZ, 170 mg/kg) prepared in sodium citrate buffer (0.1 M, pH 4.5). Seven days post-injection, mice with fasting plasma glucose levels > 200 mg/dL were selected and randomly assigned into the following groups (n = 8 per group): (1) Hyperglycemic control (STZ + distilled water); (2) Test groups (STZ + *H. tiliaceus* leaf ethanol extract at 0.67 or 1.33 g/kg); and (3) Reference group (STZ + glibenclamide at 5 mg/kg). Treatments were administered orally (p.o.) for 14 consecutive days. In parallel, normal control mice (n = 8) received only sodium citrate buffer (i.p.) and distilled water (p.o.). Additionally, separate normal groups were treated with the ethanol extract (0.67 or 1.33 g/kg) for 14 days to evaluate baseline effects. On day 14, 60 min after the final administration, tail vein blood was collected to determine fasting plasma glucose and glucose levels during the oral glucose tolerance test (OGTT). Plasma glucose concentrations were measured using a Glucose Liquicolor (GOD-PAP) kit (Human Diagnostic Ltd., Germany) [13].

2.8. Oral glucose tolerance test (OGTT)

Normal and hyperglycemic mice were administered a repeated dose of *H. tiliaceus* leaf ethanol extract (0.67 or 1.33 g/kg) for 14 consecutive days. Following a 16-hour fast, the initial fasting plasma glucose concentration was determined. One hour after the final administration of the extract, the mice were orally challenged with a glucose solution (2 g/kg body weight). Subsequently, plasma glucose levels were measured at 30 and 120 minutes post-glucose load to evaluate glucose tolerance using the GOD-PAP kit [14].

2.9. Determination of oxidative stress markers

Oxidative stress markers, including malondialdehyde (MDA) and reduced glutathione (GSH), were estimated in kidney tissue homogenates following a previously described method [13]. Mouse kidneys were dissected, washed with ice-cold saline, and then homogenized (1:9 w/v) in a 1.15% KCl buffer for 2 min at 4 °C using a SpeedMill PLUS homogenizer (Analytik Jena, Germany). The resulting homogenate was centrifuged at 4 °C to obtain the supernatant. For the assays, 2 mL or 1 mL of the supernatant was used for MDA and GSH quantification, respectively. The final volume was adjusted to 3 mL with Tris-HCl (pH 7.4), followed by incubation at 37 °C for 60 min. The reaction was terminated by adding 10% trichloroacetic acid (TCA), and the mixture was centrifuged at 10,000 × g for 10 min at 4 °C.

To determine MDA levels, 2 mL of the resulting supernatant was mixed with 1 mL of 0.8% thiobarbituric acid (TBA). The mixture was heated at 90 °C for 15 min, cooled, and the absorbance was measured at λ = 532 nm. For GSH determination, 1 mL of the supernatant was reacted with 0.2 mL of Ellman's reagent and adjusted to 3 mL with phosphate-EDTA buffer. The reaction was incubated in the dark at room temperature for 3 min, and the absorbance was measured at λ = 412 nm. Total protein concentration in the samples was determined using the Bradford assay with bovine serum

albumin (BSA) as the standard. MDA (μM/mg protein) and GSH (mmol/mg protein) contents were calculated using linear regression equations derived from malondialdehyde, tetrabutylammonium, and L-glutathione standards (Sigma-Aldrich), respectively.

2.10. Statistical analysis

Experimental data were analyzed using SigmaStat software (version 3.5, USA) and are expressed as mean ± standard error of the mean (SEM). Statistical significance was determined using One-way analysis of variance (ANOVA), followed by the Student–Newman–Keuls post-hoc test for multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Total phenolic and total flavonoid contents in H. tiliaceus leaf extracts

Table 1. The contents of total polyphenols and total flavonoids in the *H. tiliaceus* leaf extracts

***H. tiliaceus* leaf extracts**	**TPC (mg GAE/g extract)**	**TFC (mg QE/g extract)**
Ethanol extract	31.67 ± 0.24	15.64 ± 0.36
Aqueous extract	27.67 ± 0.21	11.01 ± 0.11

Based on the linear regression equations for gallic acid ($y = 0.0109x + 0.0245$, $R^2=0.9934$) and quercetin ($y = 0.0067x + 0.006$, $R^2=0.9963$), the total phenolic and flavonoid contents of *H. Tiliaceus* leaf extracts were calculated and are presented in Table 1. The results indicated that the total phenolic and flavonoid contents in the aqueous extract (27.67 mg GAE/g and 11.01 mg QE/g, respectively) were significantly lower than those in the ethanol extract (31.67 mg GAE/g and 15.64 mg QE/g, respectively).

3.2. Antioxidant activity of H. tiliaceus leaf extracts

Table 2. DPPH radical scavenging activity of the *H. tiliaceus* leaf extracts

Test samples	**Linear/non-linear regression equation**	**R^2**	**IC_{50} (µg/mL)**
Ethanol extract	$y = 17.223\ln(x) + 19.873$	0.9894	5.75 ± 0.47
Aqueous extract	$y = 23.466\ln(x) – 21.496$	0.9891	21.05 ± 0.76
Ascorbic acid	$y = 8.0056x + 10.181$	0.9798	4.97 ± 0.12

As shown in Table 2, the *H. tiliaceus* ethanol extract exhibited potent DPPH radical scavenging activity with an IC_{50} of 5.75 µg/mL, which was significantly higher than that of the aqueous extract (IC_{50} of 21.05 µg/mL).

3.3. α-Glucosidase inhibitory activity of *H. tiliaceus* leaf extracts

Table 3. *α*-Glucosidase inhibitory activity of the *H. tiliaceus* leaf extracts

Test samples	**Linear/non-linear regression equation**	**R^2**	**IC_{50} (µg/mL)**
Ethanol extract	$y = 13.944x + 12.459$	0.9900	2.69 ± 0.13
Aqueous extract	$y = 7.1037x – 4.741$	0.9967	7.71 ± 0.27
Acarbose	$y = 14.974\ln(x) – 11.584$	0.9960	61.11 ± 0.58

The *α*-glucosidase inhibitory activity of the *H. tiliaceus* ethanol extract (IC_{50} of 2.69 µg/mL) was superior to that of the aqueous extract (IC_{50} of 7.71 µg/mL). Notably, both *H. tiliaceus* leaf extracts demonstrated IC_{50} values significantly lower than that of the positive control, acarbose (IC_{50} of 61.11 µg/mL).

3.4. Antihyperglycemic effect of H. Tiliaceus leaf 45% ethanol extract

The *H. tiliaceus* ethanol extract was selected for the *in vivo* study in hyperglycemic mice due to its higher total phenolic and flavonoid contents, as well as its superior antioxidant and *α*-glucosidase inhibitory activities compared to the aqueous extract. The experimental doses were determined based on the common traditional human dose (10–20 g of crude material per day). By applying the dose conversion factor between humans and mice, this range is equivalent to 2.5–5 g of crude material per kg of mouse body weight. Given the extraction yield, these values correspond to the administered test doses of 0.67 and 1.33 g/kg of the *H. tiliaceus* ethanol extract.

Table 4. Effect of *H. Tiliaceus* leaf 45% ethanol extract (EE) on fasting plasma glucose in normoglycemic (non-STZ) and hyperglycemic (STZ) mice

	Groups (n = 8)	Fasting plasma glucose (mg/dL)	
		Before treatment	After 14-day administration
STZ (-)	Normoglycemic control	90.00 ± 2.78	93.50 ± 2.68
	EE 0.67 g/kg	86.75 ± 3.21	98.63 ± 1.18
	EE 1.33 g/kg	87.13 ± 1.67	98.75 ± 2.17
STZ (+)	Hyperglycemic control	177.50±14.20* (↑ 97%)	163.50±8.81* (↑ 75%)
	EE 0.67 g/kg	169.75±14.31* (↑ 89%)	137.63±19.44 (↓ 16%)
	EE 1.33 g/kg	165.63±16.79* (↑ 84%)	120.50±8.52# (↓ 26%)
	Glibenclamide 5 mg/kg	172.63±16.79* (↑ 92%)	107.25±5.56# (↓ 34%)

*: $p < 0.05$ vs Normoglycemic control; #: $p < 0.05$ vs Hyperglycemic control; (...): The percentage of increase/decrease.

In normoglycemic mice, the results showed that the *H. tiliaceus* ethanol extract administered at the tested doses for 14 consecutive days had no significant effect on plasma glucose levels compared to the control group. However, in STZ-induced hyperglycemic mice, both the extract at a dose of 1.33 g/kg and glibenclamide (5 mg/kg) significantly reduced plasma glucose levels compared to the hyperglycemic control, with reductions of 26% and 34%, respectively (Table 4). In contrast, the *H. tiliaceus* ethanol extract at the lower dose of 0.67 g/kg did not exert a statistically significant hypoglycemic effect in the STZ-induced diabetic model.

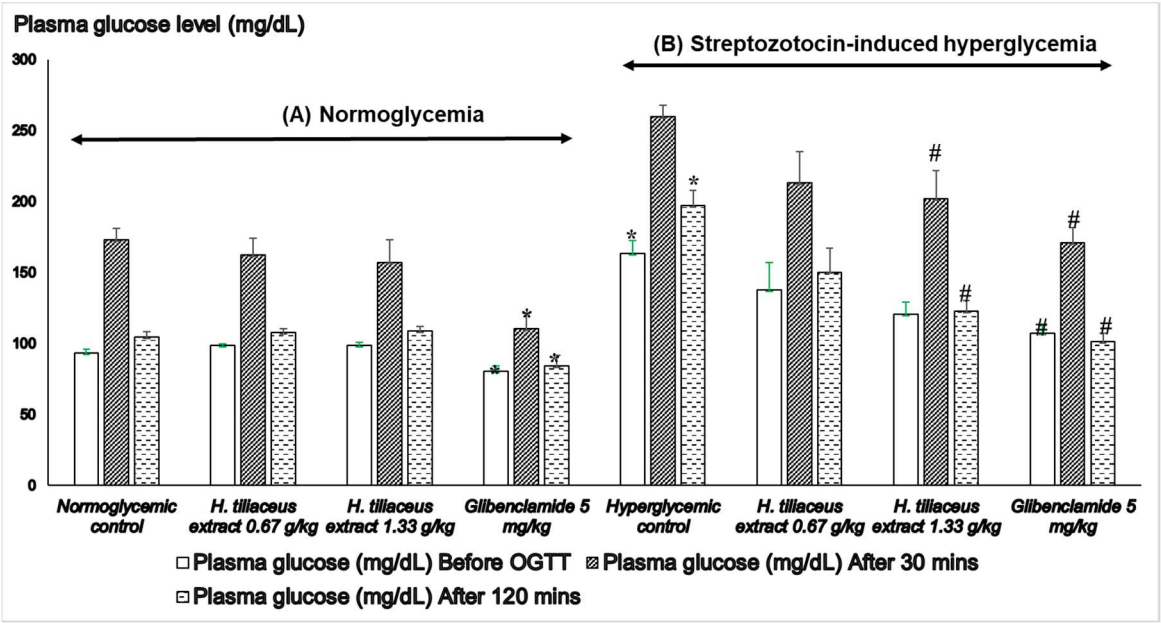

Fig. 1. Effect of *H. Tiliaceus* leaf 45% ethanol extract on plasma glucose in oral glucose tolerance test (OGTT). All bars are expressed as the mean ± SEM (n = 8). *: $p < 0.05$ vs Normoglycemic control; #: $p < 0.05$ vs Hyperglycemic control

In normoglycemic mice, plasma glucose levels in all groups rose markedly 30 min after the glucose challenge and returned to baseline within 120 min. Administration of the *H. tiliaceus* ethanol extract for 14 days at the tested doses did not significantly attenuate the glucose peak at 30 min compared to the control. In contrast, glibenclamide (5 mg/kg) significantly reduced plasma glucose levels at all time points (0, 30, and 120 min) with reductions of 14%, 36%, and 19%, respectively (Fig. 1A).

STZ-treated mice exhibited significantly

higher glucose levels at 30 and 120 min compared to their respective normoglycemic controls, confirming impaired glucose tolerance. Glibenclamide treatment (5 mg/kg) effectively lowered plasma glucose levels at 30 and 120 min by 34% and 49%, respectively, relative to the hyperglycemic control. Interestingly, the *H. tiliaceus* ethanol extract at 1.33 g/kg significantly blunted the rise in plasma glucose at both 30 and 120 min post-challenge, achieving reductions of 22% and 38%, respectively (Fig. 1B). However, the extract at 0.67 g/kg did not demonstrate a statistically significant hypoglycemic effect following the glucose bolus in the STZ-induced model.

3.5. Renal protective effects of H. Tiliaceus leaf 45% ethanol extract

Table 5. Effect of *H. Tiliaceus* leaf 45% ethanol extract (EE) on MDA and GSH levels in the kidneys of normoglycemic and hyperglycemic mice

	Groups (n = 8)	MDA levels in kidneys (μM/g protein)	GSH levels in kidneys (nM/g protein)
STZ (-)	Normoglycemic control	205.85 ± 8.54	240.04 ± 21.65
STZ (+)	Hyperglycemic control	269.48 ± 8.81* (↑ 31%)	170.18 ± 12.90* (↓ 29%)
	EE 0.67 g/kg	243.54 ± 9.60	186.93 ± 9.95
	EE 1.33 g/kg	230.40 ± 6.21# (↓ 15%)	235.05 ± 9.19# (↑ 38%)
	Glibenclamide 5 mg/kg	204.63 ± 8.49# (↓ 24%)	238.80 ± 26.94# (↑ 40%)

**: $p < 0.05$ vs Normoglycemic control; # :$p < 0.001$ vs Hyperglycemic control; (...): The percentage of increase/decrease.*

The results presented in Table 5 show that the MDA content in the kidneys of the hyperglycemic control group significantly increased compared to the normoglycemic control, indicating lipid peroxidative damage induced by streptozotocin. Conversely, the renal GSH content in the hyperglycemic control group was markedly lower than that of the normoglycemic control group. Treatment with the *H. tiliaceus* ethanol extract (1.33 g/kg) or glibenclamide (5 mg/kg) for 14 consecutive days significantly reduced renal MDA levels and elevated GSH levels compared to the hyperglycemic control. Notably, the levels of MDA and GSH in these treatment groups showed no statistically significant difference when compared to the normal control group.

4. Discussion

The results demonstrated that the *H. tiliaceus* ethanol extract, characterized by higher total polyphenol and flavonoid contents, exerted more potent DPPH radical scavenging and α-glucosidase inhibitory activities compared to the aqueous extract. Furthermore, the ethanol extract exhibited significant antihyperglycemic effects by lowering both fasting plasma glucose and postprandial glucose levels during the OGTT. Notably, it simultaneously mitigated oxidative stress-induced kidney damage in STZ-induced hyperglycemic mice.

Postprandial blood glucose is a critical indicator of metabolic health and is a recommended glycemic target in diabetic management. In this study, the glucose-lowering effect of *H. tiliaceus* 45% ethanol extract was not observed in normoglycemic mice following glucose overload. This stands in contrast to the findings of Sofia V. et al. (2024), who reported that *H. tiliaceus* leaf extract reduced blood glucose levels during OGTT at doses of 200–800 mg/kg [5]. Notably, Sofia V. et al. employed a 70% ethanol solvent via maceration; this higher ethanol concentration may have resulted in a different phytochemical profile, potentially accounting for the varying pharmacological outcomes.

Our previous study revealed that the 70% ethanol *H. tiliaceus* leaf extract at 1.04 g/kg (equivalent to 5 g of crude material/kg) reduced blood glucose levels in high-fat diet-induced obese hyperglycemic mice [6]. Similarly, the present study demonstrated that the 45% ethanol *H. tiliaceus* leaf extract, administered at 1.33 g/kg (also equivalent to 5 g of crude material/kg), significantly reduced fasting plasma glucose levels in hyperglycemic mice. Furthermore, it effectively blunted the rise in plasma glucose at both 30 and 120 min post-glucose challenge in STZ-induced hyperglycemic mice.

STZ injection induced diabetic symptoms similar to those observed in humans, particularly hyperglycemia, resulting from the partial or complete destruction of pancreatic β-cells in the islets of Langerhans [15]. Additionally, the OGTT is a standard procedure for diagnosing diabetes mellitus, insulin resistance, and impaired pancreatic β-cell function in clinical settings [14]. Collectively, these data suggest that *H. tiliaceus* leaf extract exerts its antihyperglycemic effect and improves oral glucose tolerance through multifaceted mechanisms. Based on the pathways of glucose uptake and metabolism, the antihyperglycemic effect of *H. tiliaceus* leaf extracts in STZ-induced hyperglycemic mice may involve: (1) inhibition of intestinal glucose absorption via transporter modulation (e.g., SGLT1/GLUT2); (2) enhancement of insulin secretion by improving pancreatic β-cell function; (3) protection or regeneration of β-cells; (4) augmentation of insulin sensitivity in target tissues; (5) suppression of gluconeogenesis and glycogenolysis; and (6) inhibition of carbohydrate-metabolizing enzymes such as α-glucosidase and α-amylase. Consequently, further studies are warranted to elucidate the specific molecular pathways underlying these effects.

Chronic hyperglycemia leads to severe complications, such as diabetic nephropathy, driven largely by the overproduction of reactive oxygen species (ROS). Pancreatic β-cells are particularly vulnerable to oxidative stress due to their inherently low antioxidant capacity, which is further compromised under hyperglycemic conditions [16]. STZ also promotes oxidative damage to other cells by generating ROS and nitrogen free radicals, leading to mitochondrial dysfunction and lipid peroxidation [15]. In this study, the *H. tiliaceus* ethanol extract effectively attenuated MDA levels, a key lipid peroxidation marker, and enhanced endogenous GSH levels in the kidneys of hyperglycemic mice. These findings are consistent with the extract's robust *in vitro* antioxidant activity and align with previous reports identifying high phenolic content and antioxidant potency in crude ethanol extract and ethyl acetate fractions of this species [17],[18],[19].

Phytochemical profiles have identified several bioactive compounds in *H. tiliaceus* leaves, including quercetin, kaempferol, β-sitosterol, catechin, rutin hydrate, and ellagic acid [17],[19]. Natural antioxidants, such as polyphenols and flavonoids, play a vital role in antidiabetic therapy by targeting mechanisms related to β-cell dysfunction, apoptosis, pro-inflammatory cytokines, mitochondrial dysfunction, and endoplasmic reticulum stress [20]. Specifically, polyphenols have been reported to enhance hepatic and pancreatic antioxidant enzyme activities, thereby reducing oxidative stress in organ-specific oxidative stress—an effect most pronounced in diabetic models when combined with standard antidiabetic drugs [20]. Similarly, flavonoids-a major class of polyphenols—exhibit potent inhibitory effects against α-glucosidase and α-amylase, contributing to both hypoglycemic and antioxidant outcomes in diabetic models [21]. Both quercetin and kaempferol have been documented as potent inhibitors of α-glucosidase and α-amylase, playing a crucial role in the management of postprandial hyperglycemia. This suggests a protective role for the extract in reducing oxidative tissue damage and mitigating the risk of diabetic kidney complications. However, as this study did not specifically investigate the protective mechanisms against pancreatic β-cell damage, further research is required to elucidate these specific protective mechanisms.

Additionally, both young and mature *H. tiliaceus* leaves contain essential oils, primarily linoleic acid, linolenic acid, and volatile compounds such as cis-vaccenic acid and glycidyl palmitate [18]. Essential oils have been reported to possess antidiabetic properties by modulating gluconeogenesis, upregulating insulin secretion, and enhancing insulin sensitivity [22]. Given these diverse bioactive components, further chemical and pharmacological investigations focusing on the essential oil fraction of *H. tiliaceus* are warranted to fully elucidate its therapeutic potential.

5. Conclusions

In conclusion, oral administration of *H. tiliaceus* leaf 45% ethanol extract (1.33 g/kg for 14 days) effectively reduced fasting plasma glucose and improved glucose tolerance in hyperglycemic mice. Furthermore, the extract

ameliorated hyperglycemia-induced oxidative damage by significantly attenuating malondialdehyde levels and elevating glutathione content in the kidneys. These findings suggest that the 45% ethanol extract of *H. tiliaceus* leaves is a promising natural candidate for glycemic management and the prevention of diabetic kidney complications.

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