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Evaluation of Anti-Diabetic, Antioxidant and Anti-Inflammatory Activity of *Gossypium herbaceum* and *Grewia asiatica* in Alloxan-Induced Diabetic Rabbits

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ABSTRACT

OBJECTIVE: To evaluate the dose-dependent antidiabetic, antioxidant, and anti-inflammatory properties of methanolic extracts of *Gossypium herbaceum* and *Grewia asiatica* against standard drugs (Metformin and Gliclazide) using an alloxan-induced diabetic rabbit model.

METHODOLOGY: All animal experiments for this prospective study were conducted from October to November 2024, at the Department of Pharmacology, Jinnah Postgraduate Medical Centre, Karachi, Pakistan, using an alloxan-induced diabetic rabbit model. Sixty male Albino rabbits were divided randomly into 10 groups (n=6). Diabetes was induced using alloxan monohydrate. Over 10 days, test groups received daily oral treatments of methanolic extracts *of* varying doses of *G. herbaceum* or *G. asiatica,* respectively. In contrast, standard groups received Metformin or Gliclazide: serum glucose, antioxidant enzymes, superoxide dismutase, glutathione peroxidase.

RESULTS: Baseline characteristics were comparable across various groups ($p>0.05$). Diabetes induction significantly reduced antioxidant enzyme activity in untreated diabetic controls compared with normal controls. Treatment with plant extracts and standard drugs significantly improved these parameters, with a dose-dependent increase in GPx and SOD levels following administration of Grewia asiatica and Gossypium herbaceum. Higher doses of plant extracts showed significant restoration of enzyme activity, similar to that of standard drugs. TNF-α levels in the diabetic control group were significantly elevated and reduced dose-dependently by plant extracts, with higher doses achieving levels similar to those of standard antidiabetic therapy.

CONCLUSION: *Gossypium herbaceum* and *Grewia asiatica* have enhanced glycemic regulation, oxidative stress reduction, and systemic inflammation, using methanolic extracts of the plants. They have efficacy similar to that of conventional oral antidiabetic medications.

KEYWORDS: Diabetes Mellitus, *Gossypium herbaceum*, *Grewia asiatica*, Antioxidative Enzymes, TNF-α, Metformin

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic condition characterized by hyperglycemia, impaired insulin secretion, reduced insulin sensitivity, or a combination of these[1]. Recent estimates published by the International Diabetes Federation (IDF) in 2021 reported a rapidly growing global health crisis, with the disease affecting approximately 537 million adults, or 10.5% of this demographic.[2] Epidemiological models predict a concerning upward trend, forecasting the global caseload to reach 783 million by the year 2045. This health burden is unevenly distributed, overwhelmingly impacting low and middle-income groups. Within these regions, a staggering 50% of cases remain undetected. It could be considered a diagnostic failure that significantly drives avoidable morbidity and premature mortality[2]. The global health care cost of diabetes as of 2021 was 966 billion dollars, which may exceed 1,054 billion dollars by 2045, highlighting the necessity of developing accessible and cost-effective approaches of diabetes management[2]. More than 90% of type 2 diabetic cases are strongly linked to sedentary lifestyle, adiposity, and a state of chronic, low-grade systemic inflammation[3].

A primary pathological hallmark of diabetes mellitus is oxidative stress, a state in which the accumulation of reactive oxygen species (ROS) exceeds the body's endogenous antioxidant capacity, ultimately leading to cellular injury. The rapid progression of tissue damage in diabetes is further exacerbated by a marked reduction in vital defensive enzymes, such as glutathione peroxidase (GPx) and superoxide dismutase (SOD)[4]. At the same time, particularly TNF-α and other pro-inflammatory cytokines are sufficiently increased in diabetic patients, which further enhances insulin resistance and systemic inflammation[5]. Current pharmacological management of type 2 diabetes includes biguanides such as Metformin and sulfonylureas such as Gliclazide, which effectively lower blood glucose through complementary mechanisms[6].

However, the chronic use of such traditional antidiabetic medications tends to cause significant adverse drug reactions, including hypoglycemia, liver toxicity, and gastrointestinal side effects.

The wide range of pharmacological uses and health-promoting properties of *Gossypium herbaceum*. L. (family Malvaceae) have proven its ancient eminence in the folkloric and Ayurvedic medicine. Recent *in vitro* studies revealed that ethanolic leaf extracts *G. herbaceum* possess substantial, concentration-dependent inhibitory effects against α-glucosidase and α-amylase, thereby targeting the key enzymes responsible for carbohydrate metabolism. HPLC analysis identified several bioactive phytoconstituents, including catechin, epicatechin, gossypol, and flavonoids, which likely underpin its antidiabetic and antioxidant mechanisms[7]. Similarly, *Grewia asiatica*, commonly called *phalsa*, is a medicinally valued plant across South Asia. In vivo studies conducted on rats, in which diabetes was induced by streptozotocin, have demonstrated that *G. asiatica* fruit extract at a 200 mg/kg dosage significantly reduced blood glucose levels; its effects were comparable to the standard drug glibenclamide, suggesting meaningful hypoglycemic potential warranting further investigation[8-10]. Both plants are rich in polyphenolic compounds known to modulate oxidative and inflammatory pathways implicated in diabetic pathophysiology[11].

The standard anti-diabetic drugs are often associated with undesirable adverse effects, and they even place a heavy financial burden on diabetic patients in the developing world. In addition, these antidiabetic drugs are costly, which restricts their clinical application[3]. These economic constraints have raised concerns about scientific investigations to develop more modern plant-based complementary therapies. This creates an urgent need to explore easily accessible, affordable, and well-tolerated therapeutic alternatives for clinical use derived from the herbal

wealth of this country. In recent years, individual experimental studies have reported positive antidiabetic properties of Gossypium herbaceum and Grewia asiatica; however, there is a notable lack of comparative literature evaluating their dose-dependent effects against standard drug treatments on parameters such as inflammatory cytokines (TNF-α) and antioxidant enzymes (SOD and GPx)[11]. Our study aims to bridge that gap by directly comparing the therapeutic potential of two botanical extracts *(Gossypium herbaceum and Grewia asiatica)* with conventional drugs (Metformin and Gliclazide) in an alloxan-induced diabetic rabbit model.

METHODOLOGY

All animal experiments for this prospective study were conducted from October to November, 2024, at the Department of Pharmacology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, Pakistan, using an alloxan-induced diabetic rabbit model. The total study duration was approximately four weeks, including acclimatization of animals, diabetes induction, and a 10-day therapeutic intervention phase.

Plant Material and Extract Preparation

Fresh plant material of *Gossypium herbaceum* (Cotton/Binola Seeds) and Grewia asiatica (Phalsa fruit) was procured from local markets and agricultural fields. A qualified taxonomist botanically authenticated these plants. The plant material was washed and dried in the shade at 20°C-25°C for 7 to 10 days, followed by grinding into a fine powder. A Soxhlet apparatus was used to prepare methanolic extracts using absolute methanol (99.8%) at 60°C-65°C for 8-10 hours. A rotary evaporator was used to evaporate the solvent. The plant extracts were then dried and stored separately in airtight glass containers at 4°C until their use. For administration, the methanolic extracts of the study plant were suspended in 1.5%. Carboxymethyl Cellulose (CMC) solution.

Experimental Subjects and Sampling Procedure

Sixty healthy adult male Albino Rabbits, weighing 1000-1700 grams, were locally purchased and supplied by BMSI, JPMC, Karachi. A non-probability purposive randomized sampling method was used. The randomization was achieved using a computer-generated random number sequence. This sample size was selected to provide adequate statistical power to detect meaningful biological effects while adhering to principles of animal research ethics and minimizing unnecessary animal use. The animals were used after 1 week of acclimatization. The selection criteria were limited to healthy male rabbits with a defined range of body weights. The subjects having a baseline systemic illness or any other physical abnormalities, or the inability to develop persistent hyperglycemia after induction of diabetes by alloxan administration were excluded. We have used only male rabbits to achieve endocrine homogeneity and reduce the confounding influence of sex-dependent variations of glucose metabolism. The selected animals were housed in standard laboratory cages in a climate-controlled facility, with a temperature maintained at 22 °C - 24 °C and a relative humidity of 45-55%. A normal 12/12-hour (light/dark) cycle was maintained in the animal house. Green fodder and fresh tap water were made freely available to all the animals during the study period. All experimental procedures were reviewed and approved by the Institutional Review Board of JPMC, Karachi, and were also conducted in accordance with international standards for the use of laboratory animals.

Experimental Groups

Sixty rabbits were divided into 10 different experimental groups (n=6 animals per group):

- **Group I (Normal Control):** Received 20 mL of carboxymethyl cellulose (CMC) 1.5% as a vehicle.
- **Group II (Diabetic Control):** Group without treatment.
- **Groups III, IV, and V (Diabetic + *Grewia asiatica*):** Received methanolic extract of *Grewia asiatica* at 200, 300, and 400 mg/kg, respectively. [12]
- **Group VI, VII and VIII (Diabetic + *Gossypium herbaceum*):** Received methanolic extract of *Gossypium herbaceum* 200, 250 and 300 mg/kg, respectively.
- **Group IX (Diabetic + Metformin):** Received Metformin 150 mg/kg/day.
- **Group X (Diabetic + Gliclazide):** Received Gliclazide 4 mg/kg/day.

The experimental intervention was preceded by measuring baseline characteristics (body weight and serum blood glucose) of all the subjects, as shown in **Table I**. No significant physiological differences were found between the groups before drug treatment, as per the study protocol.

Induction of Experimental Diabetes

Rabbits in Groups II to X were starved overnight, and the experimental diabetes was induced by Alloxan monohydrate (110 mg/kg), which was prepared in normal saline and was given as a single intraperitoneal injection. Diabetes was confirmed 8 days after the injection; if fasting blood glucose levels exceeded 200 mg/dL, the animals were considered diabetic and included in the experimental phase.

Treatment Protocol

To identify the diabetes status, the corresponding daily doses of plant extracts and standard drugs were administered by oral gavage over the course of 10 consecutive days. The dosages of plant extracts and standard drugs were administered in the morning to reduce the circadian variation.

Collection of Blood Samples

Blood samples were drawn from the marginal ear vein of rabbits at baseline and on day 10 of the treatment regimen. Serum was separated by centrifuging at 3000 rpm for 12 minutes, after which it was stored at -20 degree centigrade until performance for biochemical analyses.

Biochemical Assessments

Blood Glucose

Serum glucose was analyzed using the GOD-PAP enzyme colourimetric method (Spinreact S.A., Spain; Catalogue No. 1002516).

Oxidative Stress Markers

Glutathione Peroxidase (GPx)

GPx activity was quantified through a glutathione reductase-coupled assay (Cayman Chemical, USA; Cat. No. 703102). In this method, the enzymatic activity is determined indirectly by monitoring the rate of NADPH oxidation to NADP+.

Superoxide Dismutase (SOD)

SOD enzyme activity was measured by inhibiting pyrogallol autoxidation using reagents from Cayman Chemical (Cat. No. 706002). The resultant specific activity was determined and expressed as units per milligram protein (U/mg protein).

Inflammatory Marker (TNF-α)

The TNF-α levels in serum were determined by rabbit-specific ELISA (CUSABIO, China; Cat. No. CSB-E06998Rb). The assay was performed according the manufacturer's instructions, and TNF-α concentrations were reported in pg/mL.

Statistical Analyses

The statistical analysis was done by SPSS version 26. The normality of the data was tested using skewness and kurtosis tests. The results were expressed as means and standard deviation (SD). A *p*-value <0.05 at a confidence interval of 95% was used as the criterion to be considered significant. Paired *t*-test was used to analyze intra-group variances, and a one-way analysis of variance (ANOVA) was used to compare groups.

RESULTS

Physical and Biochemical Baseline Characteristics

Group comparability was assessed by measuring physiological and biochemical parameters (fasting blood glucose levels (FBG) and body mass) before the therapeutic interventions. The mean body weights of the experimental animals ranged from 2.11±0.20 kg to 2.33±0.23 kg. The baseline fasting blood glucose levels ranged from 97.02 ± 10.31 mg/dl to 119.79 ± 13.80 mg/dl. The statistical test did not reveal any significant intergroup differences within each of the ten experimental groups, ensuring cohort homogeneity before treatment on any of the baseline parameters ($p>0.05$) **(Table I)**.

Impact on Antioxidant Biomarkers (GPx and SOD):

Diabetes induction has resulted in severe oxidative stress, which is characterized by a strong loss of endogenous antioxidant enzymes. **Table II** showed that the untreated diabetic group II had a decline in glutathione peroxidase (GPx) activity (4.28 ± 0.97 U/mg protein) as compared to that of the normal control group I (10.87±2.22 U/mg protein; $p<0.001$). We have observed a significant dose-dependent increase in GPx concentrations with doses of *Gossypium herbaceum* extract 200, 250, and 300 mg/kg body weight (7.25±0.45, 8.76±0.44, and 9.15 ± 0.51 U/mg protein), respectively ($p<0.001$). We have also observed an increasing trend in GPx concentrations with standard pharmacological interventions: metformin 150 mg/kg (9.47±0.23 U/mg protein) and Gliclazide 4 mg/kg (8.93±0.41 U/mg protein), respectively. At the same time, administering Grewia asiatica at doses of 200, 300, and 400 mg/kg also caused a progressive recovery in GPx levels to 6.86±0.46, 8.54±0.38, and 9.80±0.55 U/mg protein, respectively. A similar trend has also been noted in superoxide dismutase (SOD) enzyme activity. The untreated diabetic group II exhibited a significant decrease in SOD enzyme levels (2.64±0.61 U/mg protein) as compared with the normal control group I (5.64±1.35 U/mg protein). We have also seen decreasing levels of SOD enzyme using maximum doses of *Grewia asiatica* (400 mg/kg) and *Gossypium herbaceum* (300 mg/kg) restored SOD activity to (4.92±0.33 and 4.58±0.28 U/mg protein), respectively ($p<0.001$).

The antioxidant enzyme SOD recovery was also similar to that of the reference drugs, Metformin 150 mg/kg (4.84 ± 0.27 U/mg protein) and gliclazide 4 mg/kg (4.70 ± 0.16 U/mg protein), respectively ($P<0.001$). **(Table II)**.

Impact on Systemic Inflammation (TNF-α)

The inflammatory profile was assessed by measuring serum tumor necrosis factor-alpha (TNF-α).

The induction of diabetes led to a significant increase of cytokine (TNF-α), as we have observed that the untreated group II subjects had a high level of TNF-α (67.43±9.81 pg/mL) as compared to the normal control group I (8.70±2.78 pg/mL respectively ($P<0.001$). Whereby, the use of the methanolic extracts of Grewia asiatica and Gossypium herbaceum resulted in significant, dose-proportional inhibition of TNF-α release. Specifically, *Grewia asiatica* administered at 200, 300, and 400 mg/kg lowered these concentrations to 46.08 ± 0.90, 35.45 ± 1.45, and 24.55 ± 1.63 pg/mL, respectively ($P < 0.001$). A similar dampening effect was observed for Gossypium herbaceum with dosages of 200, 250, and 300 mg/kg, reducing TNF-α levels to 44.50±1.20, 32.40±1.52, and 29.85±1.66 pg/mL, respectively ($P<0.001$).

It is important to note that the anti-inflammatory effects of varying dosages of plant extracts were comparable to those of standard drug therapy with Metformin (150 mg/kg) and Gliclazide (4 mg/kg), which reduced TNF-α level (27.80±0.87 and 28.95+1.78 pg/mL, respectively ($P<0.001$). **(Table III)**.

Table I: Baseline Physical and Biochemical Characteristics of Experimental Groups Before Treatment (n = 6 per group)

Group	Experimental group	Body weight (kg)	Fasting blood glucose (mg/dL)
I	Normal control (1.5% CMC, 20 mL)	2.25±0.23	97.02±10.31
II	Diabetic control (untreated)	2.11±0.20	114.11±13.90
III	Diabetic + *Grewia asiatica* (200 mg/kg)	2.30±0.22	98.66±9.63
IV	Diabetic + *Grewia asiatica* (300 mg/kg)	2.28±0.17	109.04±12.97
V	Diabetic + *Grewia asiatica* (400 mg/kg)	2.13±0.21	105.51±14.35
VI	Diabetic + *Gossypium herbaceum* (200 mg/kg)	2.19±0.23	119.79±13.80
VII	Diabetic + *Gossypium herbaceum* (250 mg/kg)	2.24±0.21	103.88±11.42
VIII	Diabetic + *Gossypium herbaceum* (300 mg/kg)	2.18±0.19	107.36±12.11
IX	Diabetic + Metformin (150 mg/kg/day)	2.33±0.23	104.04±9.53
X	Diabetic + Gliclazide (4 mg/kg/day)	2.19±0.24	99.16±10.79
	***P*-value**	**> 0.05**	**> 0.05**

Table II: Impact on Antioxidant Biomarkers (GPx and SOD) Levels

Group	Experimental group	GPx (U/mg protein)	*P* value	SOD (U/mg protein)	*P* value
I	Normal control (1.5% CMC, 20 mL)	10.87±2.22	<0.001	5.64±1.35	<0.001
II	Diabetic control (untreated)	4.28±0.97	NA	2.64±0.61	NA
III	Diabetic + *Grewia asiatica* (200 mg/kg)	6.86±0.46	<0.001	3.65±0.07	<0.01
IV	Diabetic + *Grewia asiatica* (300 mg/kg)	8.54±0.38	<0.001	4.17±0.20	<0.001
V	Diabetic + *Grewia asiatica* (400 mg/kg)	9.80±0.55	<0.001	4.92±0.33	<0.001
VI	Diabetic + *Gossypium herbaceum* (200 mg/kg)	7.25±0.45	<0.001	3.85±0.15	<0.001
VII	Diabetic + *Gossypium herbaceum* (250 mg/kg)	8.76±0.44	<0.001	4.35±0.25	<0.001
VIII	Diabetic + *Gossypium herbaceum* (300 mg/kg)	9.15±0.51	<0.001	4.58±0.28	<0.001
IX	Diabetic + Metformin (150 mg/kg/day)	9.47±0.23	<0.001	4.84±0.27	<0.001
X	Diabetic + Gliclazide (4 mg/kg/day)	8.93±0.41	<0.001	4.70±0.16	<0.001

Abbreviations: GPx, glutathione peroxidase; SOD, superoxide dismutase; NA, not applicable. Values are expressed as mean ± SD (n = 6). Comparisons were made against diabetic control (Group II).

Table III: Effect on Tumor Necrosis Factor (TNF-α) Level

Group	Experimental Group	TNF-α (pg/mL)	*P* value
I	Normal control 1.5% CMC (20 mL)	8.70±2.78	<0.001
II	Diabetic control (untreated)	67.43±9.81	NA
III	Diabetic + *Grewia asiatica* (200 mg/kg)	46.08±0.90	<0.001
IV	Diabetic + *Grewia asiatica* (300 mg/kg)	35.45±1.45	<0.001
V	Diabetic + *Grewia asiatica* (400 mg/kg)	24.55±1.63	<0.001
VI	Diabetic + *Gossypium herbaceum* (200 mg/kg)	44.50±1.20	<0.001
VII	Diabetic + *Gossypium herbaceum* (250 mg/kg)	32.40±1.52	<0.001
VIII	Diabetic + *Gossypium herbaceum* (300 mg/kg)	29.85±1.66	<0.001
IX	Diabetic + Metformin (150 mg/kg/day)	27.80±0.87	<0.001
X	Diabetic + Gliclazide (4 mg/kg/day)	28.95±1.78	<0.001

Abbreviations: TNF-α, tumor necrosis factor alpha; NA, not applicable.
Values are expressed as mean ± SD (n = 6). Comparisons were made against diabetic control (Group II).

DISCUSSION

This is a preclinical study that provides insight of supportive evidence of the therapeutic potential of extracts of *Grewia asiatica* and *Gossypium herbaceum* for the management of diabetes mellitus and associated systemic complications. This study explains the antidiabetic, antioxidant, and anti-inflammatory responses to these botanical extracts in an alloxan-induced diabetic rabbit model.

Baseline homogeneity was ensured to maintain the internal validity of these findings before the therapeutic interventions, as we did not observe any significant differences between the experimental groups in baseline body weight or fasting blood glucose (FBG) levels. Baseline homogeneity was observed, indicating that the subsequent pharmacological effects evaluated with the tested plant extracts were entirely attributable to the interventions in this study. Alloxan causes selective necrosis in pancreatic beta-cells, usually due to excess production of reactive oxygen species (ROS), which severely impairs glucose homeostasis [13,14]. Methanolic plant extracts showed remarkable, dose-dependent glycemic control recovery. This restoration of glycemic control was found to be statistically parallel to the efficacy of the reference pharmacologic agents, Metformin and Gliclazide. Long-term glycemic control is physiologically important since chronic hyperglycemia leads to the worsening of metabolism, which leads to body weight loss in untreated diabetic subjects. The hyperglycemic state is a chronic condition that inherently creates a pro-oxidative environment that exceeds host cellular defence mechanisms.

In this study, we observed that the diabetic control (untreated) group II exhibited a significant systemic inflammatory response, with TNF-α levels escalating to 67.43±9.81 pg/mL, compared with 8.70±2.78 pg/mL in normal controls (p<0.001). Both plants' methanolic extracts have significantly decreased the inflammatory spike in a dose-dependent fashion. The highest doses of tested plant methanolic extracts of *Grewia asiatica* (400 mg/kg) and *Gossypium herbaceum* (300 mg/kg) suppressed TNF-α levels to 24.55±1.63 and 29.85±1.66 pg/mL, respectively (p<0.001). This decrement was statistically comparable to that achieved by the reference standard drugs.

The highest dosages of *Grewia asiatica* (400 mg/kg) and *Gossypium herbaceum* (300 mg/kg) have significantly restored the antioxidant enzyme activities, GPx (9.80±0.55 U/mg protein) and SOD (4.58±0.28 U/mg protein), respectively **(Table II)**. It could be attributed to the free-radical-scavenging effect of the tested plant extracts, which were further conferred by Metformin and Gliclazide treatment. This powerful restorative property can probably be related to the abundant concentration of electron-donating phytoconstituents inherent to these plants, including anthocyanins, tannins, and flavonoids, which physically protect pancreatic islets from free-radical-mediated lipid peroxidation[12,15-17]. By causing down-regulation of pro-inflammatory cytokine expression, these extracts most likely suppress downstream inflammatory signaling cascades, thereby they may promote β-cells survival and ameliorating peripheral resistance to insulin. It was also reported that chronic systemic inflammation is becoming a key determinant of β-cell apoptosis, as well as peripheral insulin resistance[18]. The findings of this current study have highlighted a remarkable pleiotropic (multi-targeting) pharmacological profile of the studied plant extracts.

Our study has certain limitations, as we used crude methanolic extracts that do not allow definitive identification of the specific isolated molecule(s) responsible for the evaluated effects. Further molecular research using techniques such as western blotting or RT-PCR would be required to elucidate the specific intracellular signalling cascades regulated by these therapeutic

agents. A profound decrease in fasting blood glucose levels in the plant extract-treated groups clearly shows that these interventions have actively regulated glucose metabolism.

Recently, a research study conducted by Oladokun O 2024[19] explained that the high phytonutrient concentrations of flavonoids and phenolic acids present in Gossypium herbaceum regulate carbohydrate metabolism at the gastrointestinal phase. The results of their study showed that these phytoconstituents are direct inhibitors of major carbohydrate-digestive enzymes, namely α-glucosidase and α-amylase, which effectively counter post-prandial blood glucose surges. Nonetheless, since the current study used an alloxan-induced diabetic rabbit model, typified by beta-cell necrosis and characterized by a consistent, stable decrease in fasting blood glucose, the therapeutic potential extends beyond an intestinal enzyme-inhibitory effect. It could be speculated that glycemic recovery is a strong indication of a systemic increase in peripheral insulin sensitivity. The findings of a recent study by Nogueira-Machado JA 2024[20] have supported the idea that natural polyphenols restore cellular metabolic signalling pathways disrupted by long-term hyperglycemia, thereby reversing systemic glucotoxicity. Consequently, the synergistic action of local gut enzyme inhibition and peripheral glucose disposal could be attributed to the mechanistic nature of the antihyperglycemic effects of our plant extracts, as evidenced by experiments. Such plant-based therapies have also been proposed to have translational potential based on these effects.

CONCLUSION

The current research indicates that *Gossypium herbaceum* and *Grewia asiatica* exhibit strong antidiabetic, antioxidant, and anti-inflammatory activity in an alloxan-induced diabetic rabbit model using methanolic extracts. Dose-dependent administration showed significant improvement in glycemic control and, at the same time, replaced lost endogenous antioxidant enzymes (GPx and SOD). Importantly, even the largest doses tested showed therapeutic effects equal to those of conventional anti-diabetic drugs (Metformin and Gliclazide), indicating a pronounced effect on inhibiting the pro-inflammatory cytokine TNF-α. Overall, these results highlight G. *herbaceum* and *G. asiatica* as promising, multi-targeted therapeutic agents for controlling diabetes and its complications.

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Ethical permission: Jinnah Postgraduate Medical Centre, Karachi, Pakistan, ERC approval letter No. F.2-81/2023-GENL/216/JPMC. All animal care and their handling procedures were conducted in strict accordance with the internationally accepted guidelines for the use of laboratory animals.

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Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publicly.

AUTHOR CONTRIBUTION

Mughal MA: Conceived study design, served as lead investigator, acquired and analyzed the data, interpreted the results, and authored the manuscript.

Alam SM: *C*onceived the study design, provided methodological guidance throughout the conductance of this study and has critically reviewed and edited the manuscript.

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