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Antidiabetic and metabolic regulatory effects of *Bergenia stracheyi* and *Rheum spiciforme* mixtures via sirtuin modulation in STZ-induced diabetic rats

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Abstract: **Background**: Type 2 diabetes mellitus (T2DM) is characterized by hyperglycemia, dyslipidemia, altered adipokine secretion, and impaired sirtuin signaling, all of which contribute to disease progression. **Objectives**: This study evaluated the antidiabetic potential of botanical mixtures of *Bergenia stracheyi* (*B.s*) and *Rheum spiciforme* (*R.s*) in streptozotocin (STZ) induced diabetic rats. **Methods**: Acute (8 h), subacute (14 days), subchronic (21 days), and chronic (28 days) studies were conducted in diabetic rats treated with botanical mixtures A (75 *B.s*: 25 *R.s*), B (50 *B.s*: 50 *R.s*), C (25 *B.s*: 75 *R.s*) and compared with metformin and glibenclamide. Biochemical analyses included blood glucose, lipid profiles, adipokines and expression of sirtuin 1 (SIRT1) and sirtuin 2 (SIRT2). **Results**: Botanical mixture B significantly reduced blood glucose across all study phases, with a 53.78% ($p<0.001$) decline at day 28, comparable to metformin and glibenclamide. It also demonstrated a more effective restoration of SIRT1 ($p<0.01$) and SIRT2 ($p<0.05$) expression compared to standard drugs. Adiponectin levels increased while leptin decreased, indicating improved metabolic regulation. All botanical mixtures ameliorated dyslipidemia, with mixture B showing the most consistent effects ($p<0.0001$). **Conclusion**: It was concluded that botanical mixture B (50 *B.s*: 50 *R.s*) exerted potent antidiabetic effects through glycemic control, lipid regulation, adipokine modulation, and sirtuin activation, supporting its therapeutic potential for T2DM.

Keywords: Adipokines; Botanical mixture; Sirtuins; Streptozotocin; Type 2 diabetes

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INTRODUCTION

Diabetes mellitus (DM) is a major global health concern, affecting over 463 million people worldwide, with 90% of cases being type 2 diabetes (T2DM). Its prevalence in Pakistan is alarmingly high, ranging from 26.7% to 30.8%, with projections indicating further increases in the coming years. Its management strategy is essential, as if it is left untreated, it can lead to vital organs damage such as the kidneys, heart, eyes, and nerves (Azeem *et al.,* 2022; Galicia-Garcia *et al.,* 2020).

The management of T2DM has traditionally involved pharmaceutical interventions; However, the side effects associated with conventional therapies such as insulin, sulfonylureas, and biguanides underscore the need for safer alternatives. In this context, botanical medicine has gained significant attention, especially in developing countries, due to its affordability, cultural acceptance, and efficacy in managing chronic conditions like T2DM. Studies have shown that up to 86% of people in developing countries rely on herbal remedies (Hidayat and Wulandari, 2021; Pang *et al.,* 2019). In Pakistan, more than 800 plant species have been identified for their antidiabetic properties, with decoctions being the most common form of preparation. These decoctions are valued for extracting bioactive, water-soluble compounds such as phenols and flavonoids, which contribute to their therapeutic effects (Bitwell *et al.,* 2023; Jan *et al.,* 2020). The potential of plant-based therapies is central not only to diabetes management but also to the discovery of novel drugs (Hidayat and Wulandari, 2021).

A promising molecular target for managing T2DM is sirtuins (SIRTs) that regulate several key processes, including glucose and lipid metabolism, mitochondrial function, and insulin sensitivity, all of which are vital in the pathogenesis of T2DM. Reduced sirtuin 1 (SIRT1) and sirtuin 2 (SIRT2) activity is associated with insulin resistance and obesity, while activation of these enzymes, either through natural compounds or caloric restriction mimetics, has shown potential in improving metabolic regulation in diabetic models (Kitada *et al.,* 2019; Oza and Kulkarni, 2020; Song *et al.,* 2018). Sirtuin signaling is also closely linked to adipokines, such as adiponectin and leptin, which are important regulators of glucose and lipid metabolism. Adiponectin enhances insulin sensitivity and promotes lipid utilization through adenosine monophosphate-activated protein kinase (AMPK) activation, which in turn modulates SIRT1 and SIRT2, especially via its adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2). These mechanisms facilitate glucose uptake, fatty acid oxidation and improve

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lipid homeostasis. However, leptin, a hormone that regulates appetite and energy expenditure, is often resistant in diabetes, exacerbating insulin resistance and lipid imbalance (Kitada *et al.,* 2019; Tagieva, 2023; Zhuge *et al.,* 2021).

Considering these molecular insights, it is clear that there is an increasing need for more integrated, multi-targeted approaches in the management of T2DM. Botanical mixtures, which contain a diverse array of bioactive compounds, may offer a synergistic solution to address the multifaceted nature of the disease. Thus, the present study aimed to investigate the antidiabetic potential of *Bergenia stracheyi* and *Rheum spiciforme* mixtures in streptozotocin (STZ)-induced diabetic rats, with a particular focus on glycemic control, lipid metabolism, adipokine modulation and sirtuin (SIRT1 and SIRT2) signaling. The study also compared these effects with those of standard antidiabetic treatments. The selection of *Bergenia stracheyi (B.s)* and *Rheum spiciforme (R.s)* for this study was influenced by their long-established ethnomedicinal use in the Himalayan region, particularly in Gilgit (Pakistan), where both plants are traditionally utilized to treat diabetes and associated metabolic disorders. *Bergenia stracheyi* has demonstrated antioxidant, antilipidemic and hypoglycemic effects (Hou *et al.,* 2021; Koul *et al.,* 2020) while *Rheum spiciforme* is known for its antidiabetic, laxative, hepatoprotective and metabolic regulatory properties (Bhat *et al.,* 2019). Given their complementary pharmacological activities and shared traditional uses, their combination was anticipated to produce synergistic antidiabetic effects by targeting multiple metabolic pathways, thus justifying their inclusion in a combined botanical decoction. By exploring both metabolic and molecular outcomes, this study sought to establish the therapeutic value of botanical mixtures as safer, multi-targeted alternatives for managing type 2 diabetes mellitus.

MATERIALS AND METHODS

Drugs and chemicals used

Streptozotocin was purchased from Sigma-Aldrich. Glibenclamide and metformin (standard drugs) were kindly provided by Martin Dow and PharmEvo, respectively. All other commercial reagents used were of analytical grade**.**

Plant collection and authentication

Roots of *Rheum spiciforme* and *Bergenia stracheyi* were collected in June–July from local markets and Gilgit Baltistan, respectively. Both were authenticated by Dr. Amin Shah (Associate Professor, Head of Herbarium, Botany Dept., University of Sargodha) and voucher specimens (KC-755, KC-756) were deposited in the SARGU Herbarium for future reference.

Preparation of botanical mixtures

Roots of both plants were shade-dried (8–10 days), cleaned, powdered and stored. Decoctions were prepared following a modified method (Ennaifer *et al.,* 2018; Pepato *et al.,* 2002) using a total of 1 g of plant material per preparation, combined according to the specified ratios. Specifically, 0.75 g *B. s*+ 0.25 g *R. s* for Mixture A (75:25), 0.50 g *B. s* + 0.50 g *R. s* for Mixture B (50:50) and 0.25 g *B. s* + 0.75 g *R. s* for Mixture C (25:75). Each mixture was boiled in 16 mL distilled water at 90 °C for 10 min with constant stirring, then left to stand for 30 min, filtered and stored at 4°C.

Experimental animals and induction of diabetes

Sprague-Dawley rats (150–200g, 6 weeks old) were obtained from the experimental animal unit, College of Pharmacy, University of Sargodha, and housed under standard conditions (23 ± 2°C; 12 h light/dark cycle) with free access to pellets and water. After 2 weeks of acclimatization, diabetes was induced by intraperitoneal injection of STZ (45 mg/kg, freshly prepared in normal saline). The diabetic state was confirmed when fasting blood glucose (FBG) levels exceeded 200 mg/dL, which is a widely accepted threshold for hyperglycemia in STZ-induced diabetic rat models. This criterion is consistent with previously published studies, including (Ahmad and Ahmad, 2018), who used a similar cutoff to define diabetes in STZ-induced rats. Animals were randomly divided into seven groups (n=6): normal control, diabetic control, three botanical mixture groups (A, B, and C*)*; 10 mL/kg p.o. for 28 days (Min *et al.,* 2024) and two standard drug groups (glibenclamide 10 mg/kg; metformin 500 mg/kg) (Irfan *et al.,* 2017). All procedures were approved by the Institutional Biosafety and Ethical Review Committee (SU/ORIC/2081).

In-vivo antidiabetic effect

The *in-vivo* antidiabetic effect was evaluated through acute, subacute, sub-chronic and chronic studies. Blood glucose levels were measured using a glucometer (On-Call Plus). In the acute test, blood glucose levels were measured at 0, 2, 4, 6, and 8 h post-treatment (Pandey *et al.,* 2014), while in the subacute study, fasting blood glucose (FBG) was assessed on days 0, 3, 5, 7, and 14 (Kumar *et al.,* 2010). Sub-chronic evaluation involved daily administration for 21 days with FBG recorded on days 0, 7, 14, and 21, whereas in the chronic study, treatment was extended to 28 days with FBG measured weekly days (Kumar *et al.,* 2012; Pandey *et al.,* 2014).

Measurements of animal body weight and sample collection

Body weight was recorded at baseline and sacrifice, while food and water intake were monitored throughout the study. Body mass index (BMI) was calculated as body weight (g)/length² (cm²) (Novelli *et al*., 2007), with length measured from nose to anus. Percentage change in body weight was determined as [(final–initial weight)/initial weight] × 100 (Rahman *et al.,* 2017). The Lee index, calculated as naso-anal length (mm)/cube root of body weight (g), was used to assess obesity (Bastías-Pérez *et al.,*

2020). At the end of the experiment, animals were anesthetized with ketamine (60 mg/kg, i.p.) and blood samples were collected in EDTA, gel and serum separator tubes (Ergün *et al.,* 2010). The latter were allowed to clot for 30 min at room temperature, centrifuged at 2000 rpm for 10 min and the separated serum was stored at −20 °C for further analysis.

Estimation of hyperlipidemic parameters

Rat serum was used to quantify total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL), by conventional enzymatic kits (DiaSys diagnositic systems, Germany) in accordance with the manufacturer's protocol by an automatic Microlab 300 biochemistry analyzer (Ahmad *et al.,* 2020). Whereas very low-density lipoprotein (VLDL) was calculated by using the following Friedewald formula: VLDL= TG/5 (Divi *et al.,* 2012). The formula for calculating total cholesterol, based on the third report of the expert panel for the National Cholesterol Education Program (NCEP) 2002, was:

Total cholesterol HDL+LDL+20% triglycerides.

Serum adiponectin and leptin levels

Serum adiponectin (ADP) and leptin (LEP) levels were quantified using rat ELISA kits (ADP: CSB-E07271r; LEP: CSB-E07433r; Cusabio Biotech Co., Ltd., Wuhan, China) following the manufacturer's instructions (Sakr, 2013; Nishida *et al.,* 2003). The detection ranges were 0.156–10 ng/mL (sensitivity 0.039 ng/mL) for ADP and 0.78–50 ng/mL (sensitivity <0.195 ng/mL) for LEP. The assay employed a quantitative sandwich enzyme immunoassay, where target proteins bound to antibody-coated wells, followed by biotin-conjugated antibody, HRP-avidin, substrate addition and colorimetric detection. Absorbance was measured at 450 nm using an ELISA plate reader.

Gene expression analysis of sirtuins by quantitative, real- time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from whole blood using the HiPure Total RNA Kit (IVD4121, Magen Biotech, China) following the manufacturer's protocol. Purified RNA was quantified and stored at −20°C until use. cDNA was synthesized from 3 µg RNA using the Thermo First cDNA kit (Zokeyo, China) with oligo(dT) primers. qRT-PCR was performed in 15 µL reactions containing 300 ng cDNA, 10 µM primers (SIRT1, SIRT2; sequences listed in Table 1; Macrogen, Korea) and SYBR Select Master Mix (Zokeyo, China) on a SLAN-96P system (Sansure Biotech, China). Cycling conditions were 95 °C for 10 min, followed by 40 cycles of 95 °C for 30 s and 60 °C for 1 min. GAPDH was used as the internal control and relative expression levels were determined using the 2^−ΔΔCt method (Arab Sadeghabadi *et al.,* 2018).

Data analysis and statistical insights

Data were presented as Mean ± S.E.M. Body weight and blood glucose variations were analyzed using two-way ANOVA with Dunnett's post-test, while one-way ANOVA with Dunnett's test (GraphPad Prism 8.0) assessed adiponectin, leptin, sirtuins (SIRT1, SIRT2), and lipid profiles. Statistical significance was set at $p < 0.05$.

RESULTS

Evaluation of weight dynamics in STZ-administered diabetic rats

STZ-diabetic rats in the diseased control group showed progressive weight loss, confirming insulin resistance, while normal controls gained 19.07 ± 0.44% body weight. In contrast, groups treated with botanical mixtures A, B, and C showed reduced weight loss (−12.38%, −9.25% and −13.42%, respectively) compared with diseased controls (−25.10%). Standard drug groups also showed milder declines (metformin −11.06%; glibenclamide −7.54%). These results indicate that treatment partially prevented STZ-induced weight loss (Fig. 1).

Effects of botanical mixtures on blood glucose regulation in STZ-diabetic rats

Acute study (8 h)

In the acute phase, baseline blood glucose (BG) levels were elevated across all groups (~328 mg/dL). Within 2 h post-treatment, botanical mixture B (50 *B.s*: 50 *R.s*) showed the most pronounced decline (206.5 ± 10.08 mg/dL; −18.27%, $p<0.05$), comparable to metformin (−32.90%) and glibenclamide (−35.58%). At 4 h, mixture B maintained BG reduction ($p<0.01$), though less than standard drugs. By 6–8 h, BG levels plateaued or rebounded, indicating a transient effect as shown in fig. 2 (a).

Subacute study (14 days)

At day 3, mixtures A and B significantly reduced BG (209.17 ± 4.58 and 208.17 ± 5.35 mg/dL, $p<0.01$), comparable to metformin and glibenclamide, while mixture C showed only minor effects. Progressive improvement was observed, with mixture B achieving a 41.81% reduction ($p<0.01$) by day 14, nearly equivalent to metformin (42.03%) and glibenclamide (45.80%) as illustrated in Fig. 2 (b).

Subchronic study (21 days)

Fig. 2 (c) illustrates that all groups showed progressive BG reduction. On day 21, mixture B again demonstrated a marked effect (49.60% reduction; $p<0.05$), closely paralleling metformin (50.24%) and glibenclamide (53.08%). Mixture A showed moderate efficacy, while mixture C remained less effective.

Chronic study (28 days)

Weekly assessments confirmed sustained BG reduction in treated groups. On day 28, mixture B (53.78%, $p<0.001$) produced reductions comparable to metformin (53.77%) and glibenclamide (57.09%), exceeding 50% efficacy. Mixture A (41.16%) and C (43.09%) were less potent but still significantly improved glycemic status relative to diseased controls as shown in fig. 2 (d).

Table 1: Forward-reverse primer sequences used in real RT-PCR with respect to gene markers. SIRT1 = Sirtuin 1 and SIRT2 = Sirtuin 2.

Gene indicator	Sequence (5'->3')	Forward -Reverse	Base pair (Product length)	Gene accession number
SIRT1	AGGGAACCTCTGCCTCATCTAC	Forward	99	NM_001372090.1
	GGCATACTCGCCACCTAACCT	Reverse		
SIRT2	CGAAGGAGTGACACGCTACATG	Forward	143	NM_001399630.1
	GGTGGTACTTCTCCAGGTTTGC	Reverse		

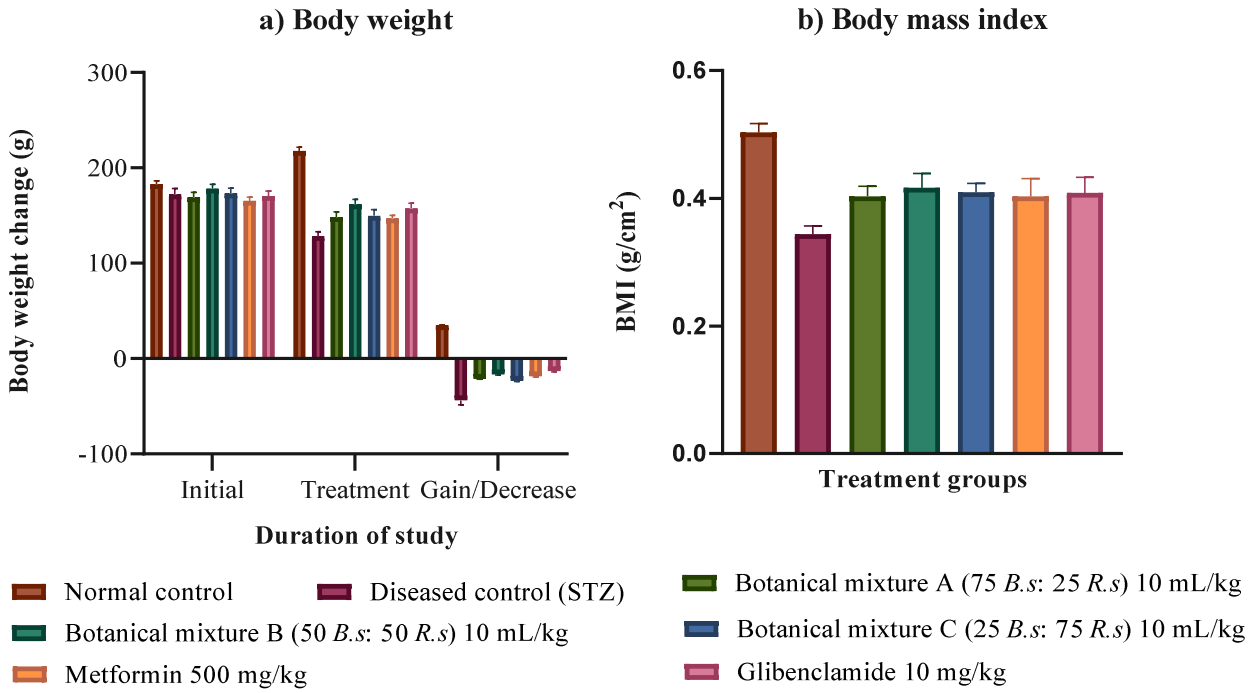

Fig. 1: Impact of treatment on a) body weight and b) body mass index variation in streptozotocin induced diabetic rats. Values articulated as Mean ± SEM (n=6). Note: - sign indicated the decrease in body weight. *B.s* = *Bergenia stracheyi* and *R.s* = *Rheum spiciforme.*

Botanical mixture B (50 *B.s*: 50 *R.s*) consistently demonstrated the most effective glucose-lowering potential across acute, subacute, subchronic, and chronic studies, showing effects comparable to standard antidiabetic drugs as evident in fig. 2. In the acute phase, only botanical mixture B showed significant glucose reduction at 2 h (*), 4 h (**), 6 h (***), and 8 h (**). In the subacute phase, both A and B showed significant glucose reduction at day 3 (**), day 5 (*), and day 7 (*), while at day 14, all three mixtures showed a significant difference. In the subchronic phase, * at day 7 and 21 indicated mixture A and B showed significant differences, while ** at day 14 the p-value for three mixtures was **($p<0.01$). In chronic phase: * (Day 7), ** (Day 14) indicated for all mixtures. The value was * for (A, C), ** for (B) at day 21, and indicated *** (at day 28) for mixtures A, B, and C. All treatment comparisons were against diseased control.

Evaluation of SIRT1 and SIRT2 expression

STZ-induced diabetic rats showed markedly reduced SIRT1 (1.01±0.06) and SIRT2 (1.03±0.12) compared with healthy controls (3.25±0.31 and 2.61±0.20, respectively). Botanical mixtures A, C, metformin and glibenclamide significantly restored ($p<0.05$) SIRT1 levels, while mixture B (50 *B.s*: 50 *R.s*) produced the highest increase (3.60±0.66, $p<0.01$), exceeding standard drugs as depicted in fig 3 (a). For SIRT2, only mixture B showed a significant rise (2.49±0.48, $p<0.05$), comparable to metformin (2.47±0.36) and glibenclamide (2.39±0.54) as shown in fig. 3 (b).

Leptin and adiponectin modulation

In STZ-diabetic rats, adiponectin decreased (0.78±0.27 vs. 4.41±0.27 ng/mL in healthy) while leptin increased (27.31±1.26 vs. 10.59±1.96 ng/mL). Botanical mixtures A, B and C significantly restored adiponectin, with mixture B (2.36±0.32 ng/mL, $p<0.01$) showing the strongest effect, comparable to metformin (2.63±0.36 ng/mL, $p<0.001$) as indicated in fig 4 (a). Leptin levels were markedly reduced in mixture B, metformin and glibenclamide groups (12.55±2.35, 11.14±1.05 and 21.28±1.00 ng/mL, respectively), indicating improved leptin sensitivity as demonstrated in fig. 4 (b).

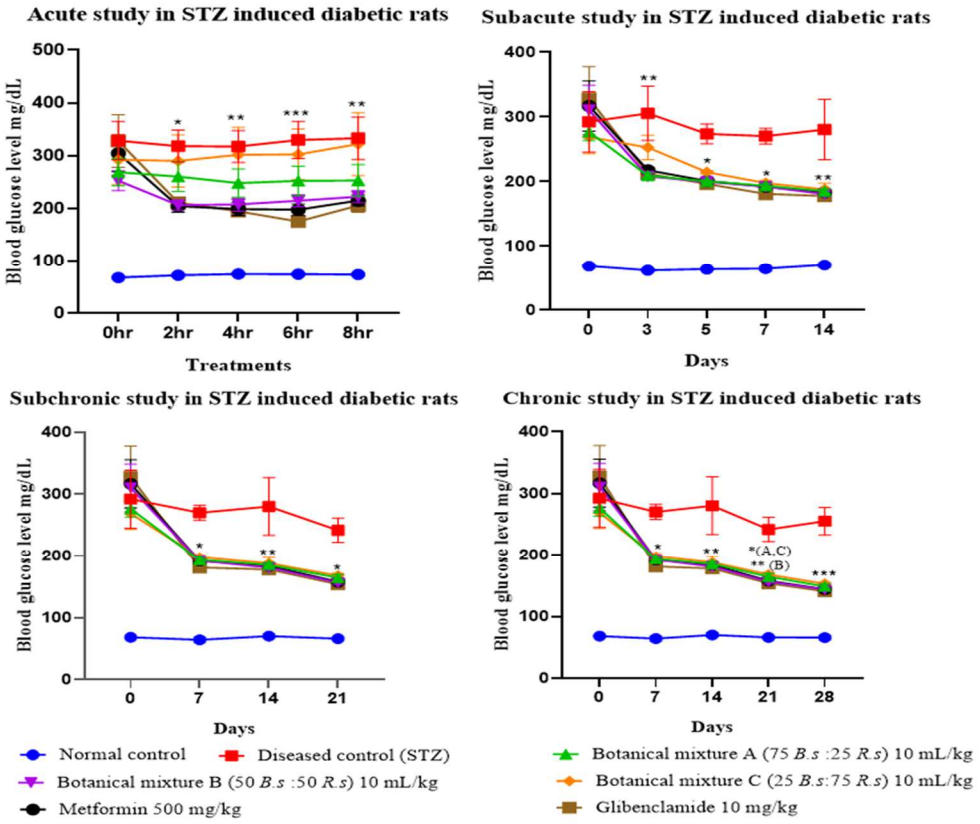

Fig. 2: Effect of botanical mixtures on blood glucose levels in STZ-induced diabetic rats at different treatment durations: (a) Acute, (b) Subacute, (c) Sub-chronic, and (d) Chronic study.
Asterisks denote significance vs. diseased control (STZ): **** ($p<0.0001$), ***($p<0.001$), **($p<0.01$) and * ($p<0.05$); Result articulated as Mean ± SEM followed by Two way ANOVA (Dunnett's test) where n= 6. *B.s = Bergenia stracheyi* and *R.s = Rheum spiciforme.*

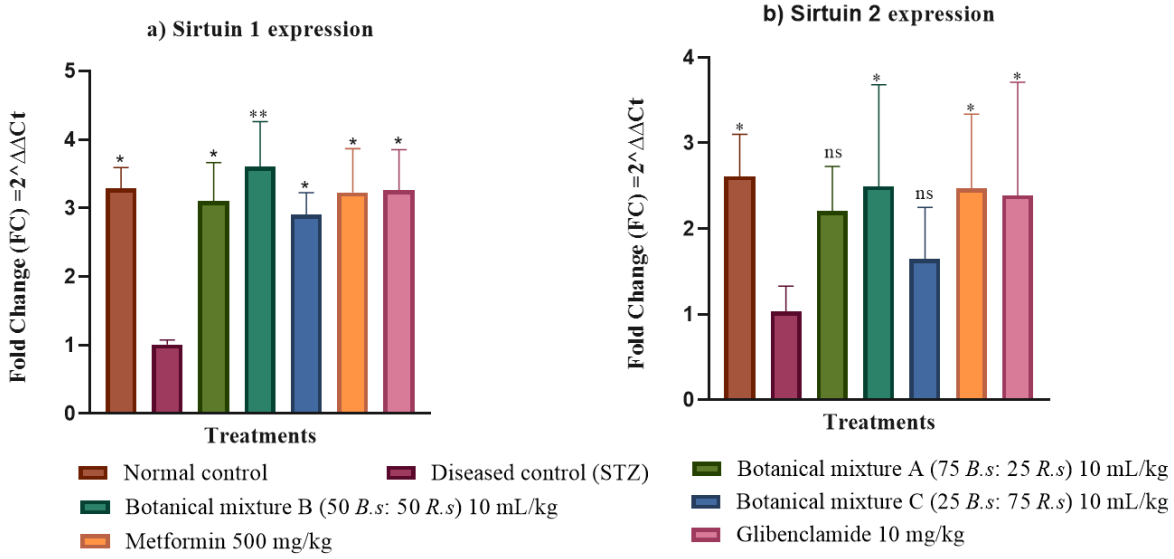

Fig. 3: Post treatment impact of botanical mixtures on a) Sirtuin 1 (SIRT1) and b) Sirtuin 2 (SIRT2) expression in STZ diabetic rats.
Result expressed as Mean ± SEM, followed by One way ANOVA (Dunnett's test). Where n= 6, ns= non-significant, **($p<0.01$) and * ($p<0.05$) vs. diseased control group. *B.s = Bergenia stracheyi* and *R.s = Rheum spiciforme*

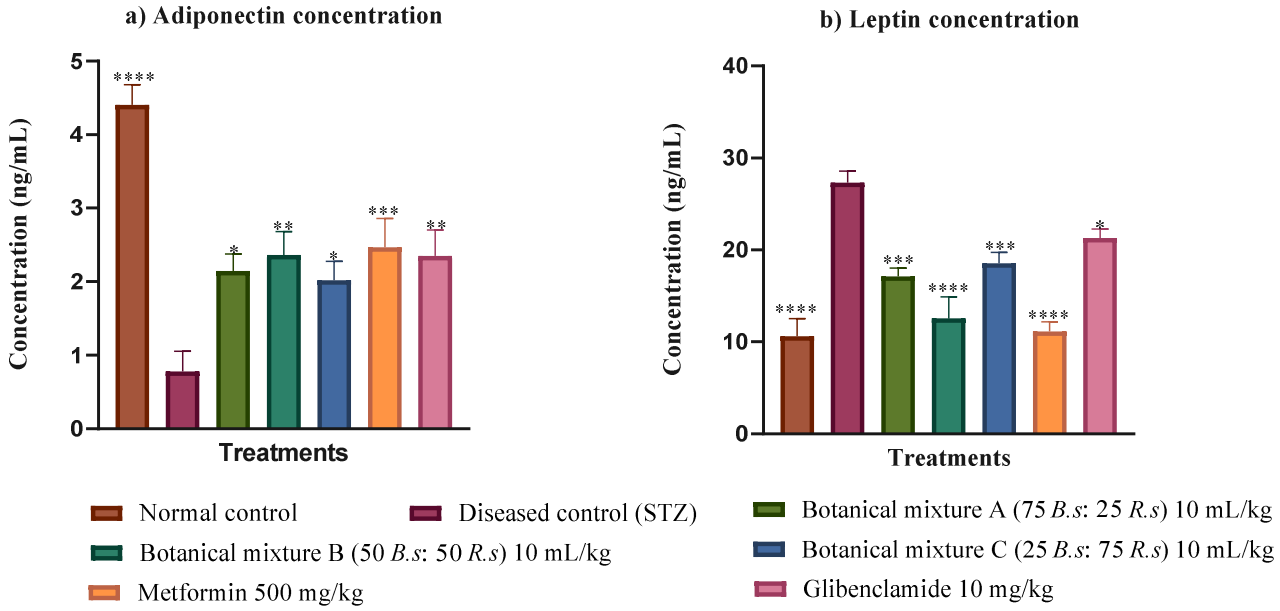

Fig. 4: Influences on a) adiponectin and b) leptin concentrations in STZ diabetic rats following botanical mixtures administration.
Result expressed as Mean ± SEM followed by One way ANOVA (Dunnett's test). Where n= 6, **** (p<0.0001), ***(p<0.001), **(p<0.01) and * (p<0.05) vs. diseased control group. *B.s = Bergenia stracheyi; R.s = Rheum spiciforme* and STZ= Streptozotocin.

Table 2: Assessment of the botanical mixtures' treatment on the relationship between diabetes and dyslipidemia parameters in streptozotocin-induced diabetic rats.

Parameters (mg/dL)	Normal control	Diseased control (STZ)	Botanical mixture A (75 *B.s*: 25 *R.s*) 10 mL/kg	Botanical mixture B (50 *B.s*: 50 *R.s*) 10 mL/kg	Botanical mixture C (25 *B.s*: 75 *R.s*) 10 mL/kg	Metformin 500 mg/kg	Glibenclamide 10 mg/kg
HDL	46± 0.58****	26.83 ±0.60	44± 1.44****	48± 0.52****	37.50± 1.09**	47.50 ±1.26****	44.83 ±0.60****
LDL	35.83 ±1.72****	69.83 ±1.25	37.83 ±1.01****	36.50± 1.18****	39.50± 1.28****	37.67 ±1.76****	39.17 ±1.30****
VLDL	12.45 ±0.17****	40.08 ±0.27	15.50 ±0.20****	14.41± 0.17****	16.03± 0.32****	13.97 ±0.17****	15.83 ±0.14****
TG	61.17 ±1.08****	198.17 ±1.72	77.50 ±0.99****	72.05± 0.63****	80.17± 1.60****	69.83 ±0.83****	79.17 ±0.70****
Total cholesterol	73.67 ±1.12****	151 ±1.92	100.50 ±1.34****	93± 1.16****	101.17± 0.54****	97.96 ±1.52****	101.67 ±1.73****
Total lipids	320.31 ±5.36****	905.83 ±27.67	489.42 ±9.14****	414.2 ±8.31****	491.40± 18.55****	389.60 ±13.28****	425.93 ±15.48****

Result expressed as Mean ± SEM followed by One way ANOVA (Dunnett's test). Where n= 6, **** (p<0.0001) vs. diseased control group. *B.s = Bergenia stracheyi; R.s = Rheum spiciforme;* STZ = Streptozotocin; HDL= high density lipoproteins; LDL = Low density lipoproteins; VLDL = very low density lipproteins and TG = triglycerides.

Diabetes and dyslipidemia relationship

STZ-induced diabetes markedly increased TC, LDL, VLDL, TG, and total lipids while reducing HDL, confirming the strong association between diabetes and dyslipidemia.

Treatment with botanical mixtures A, B and C significantly (p<0.0001) improved lipid profiles, lowered harmful lipids and elevated HDL toward near-normal values. Mixtures A and B showed effects comparable to metformin and glibenclamide, highlighting their potential in restoring lipid homeostasis, as shown in Table 2.

DISCUSSION

Type 2 diabetes mellitus (T2DM) is a prevalent metabolic disorder characterized by hyperglycemia, dyslipidemia, and disrupted sirtuin signaling, all of which play crucial roles in its progression and complications (Galicia-Garcia *et al.,* 2020). The side effects associated with conventional

treatments have driven the search for safer, more effective alternatives. In this context, botanical mixtures have attracted significant attention for their potential to manage T2DM. This study evaluated the antidiabetic effects of *Bergenia stracheyi* and *Rheum spiciforme* in STZ-induced diabetic rats, focusing on glycemic control, lipid metabolism, sirtuin activation and modulation of adipokines such as adiponectin and leptin. The results suggest that these botanical mixtures offer promising therapeutic benefits in the management of T2DM, as evidenced by their impact on glycemic control, lipid profiles and the regulation of key adipokines, aligning with previous studies (Divi *et al.,* 2012; Sundaram, Nandhakumar and Haseena Banu, 2019).

STZ-diabetic rats exhibit reduced body weight, likely due to muscle deterioration and decreased tissue protein content from impaired carbohydrate utilization. Treatment with botanical mixtures of *Bergenia stracheyi* and *Rheum spiciforme* partly prevented this decline, similar to the previous plant-based studies, which demonstrates a reduction in STZ-induced weight loss (Divi *et al.,* 2012; Patel *et al.,* 2011; Sundaram *et al.,* 2019). The improvement in body weight in STZ-treated groups indicated that treatment might have a protective impact in reversing gluconeogenesis or muscle wasting.

The evaluation of the botanical mixtures across different time frames provided deeper insights into both their immediate and prolonged antidiabetic effects. In the acute studies (up to 8 hours), the 50:50 decoction (mixture B) was the only combination that led to a significant reduction in blood glucose ($p<0.01$), comparable to metformin, while mixtures A (75:25) and C (25:75) showed comparatively minimal effects. This rapid response likely stemmed from a synergistic interaction between *Bergenia stracheyi* and *Rheum spiciforme*, enhancing insulin secretion and glucose uptake. In the subacute phase (14 days), both mixtures A and B reduced blood glucose significantly ($p<0.01$), with responses aligning more closely with glibenclamide than metformin, suggesting improved insulin release and sensitivity. By day 14, blood glucose reductions with all mixtures were comparable to metformin (42.03%) and glibenclamide (45.80%). These complementary actions of the mixtures can be attributed to *R. spiciforme's* potential to enhance β-cell function, while *B. stracheyi* likely contributed to improving peripheral glucose uptake and overall metabolic regulation (Bhat *et al.,* 2019; Hou *et al.,* 2021). Subchronic and chronic studies (21–28 days) confirmed sustained efficacy, with mixture B showing results similar to metformin (50.24%) and glibenclamide (57.09%), indicating β-cell protection and enhanced insulin sensitivity, similar to effects reported for other plant-based therapies that showed a decline in BG level in the STZ diabetic group (Kumar *et al.,* 2012; Pandey *et al.,* 2014). Overall, mixture B consistently demonstrated the most robust antihyperglycemic effects across all study durations, likely due to synergistic actions between both species, improving β-cell function and peripheral glucose regulation in diabetic rats.

Beyond glycemic control, botanical mixture B significantly enhanced SIRT1 ($p<0.01$) and SIRT2 ($p<0.05$) expression, surpassing conventional drugs. This finding parallels with many other studies reporting lower SIRT1 expression in diabetic rats compared to healthy controls, with some also noting similar changes in SIRT2 expression (Abedimanesh *et al.,* 2021; Agrawal and Kulkarni, 2023; Oza and Kulkarni, 2020; Peredo-Escárcega *et al.,* 2015). Several plant-derived polyphenols are recognized as natural sirtuin activators. Both *Bergenia stracheyi* and *Rheum spiciforme* are rich in polyphenolic constituents (including gallic acid, catechins and anthraquinones), which are known to influence oxidative stress and metabolic regulation (Bhat *et al.,* 2018; Chauhan *et al.,* 2016; Hou *et al.,* 2021). Thus, the observed upregulation of SIRT1 and SIRT2 in our study may be attributed, at least in part, to the action of these bioactive polyphenols present in the combined decoction.

Adipose tissue secretes leptin, an insulin-sensitizing hormone that enhances peripheral insulin sensitivity, which eventually results in insulin resistance and disease progression (Ghanbari *et al.,* 2022) . In our study, the botanical mixture B significantly increased ($p<0.01$) adiponectin and decreased ($p<0.0001$) leptin, suggesting improved leptin sensitivity, comparable to metformin. Several studies have also demonstrated similar effects in STZ/HFD diabetic animals (Chandran *et al.,* 2019; Ghanbari *et al.,* 2022).

Cholesterol balance is disrupted in type 2 diabetes, with elevated LDL and triglycerides and reduced HDL, contributing to increased cardiovascular risk. Dyslipidemia accelerates atherosclerosis and heart disease, but correcting lipid imbalances by lowering LDL and triglycerides while increasing HDL can reduce cardiovascular complications, enhance insulin sensitivity, and stabilize atherosclerotic plaques, thus lowering CVD risk in diabetic patients (Aghajanyan *et al.,* 2018; Jayakumari *et al.,* 2020; Ngwen *et al.,* 2018). All mixtures improved dyslipidemia by reducing TC, LDL, VLDL, TG, and elevating HDL. This indicated that decoctions had played a significant antihyperlipidemic role in type 2 diabetic animals. *Jia-Wei-Jiao-Tai-Wan* (JWJTW) decoction demonstrated dose-dependent antihyperlipidemic and antidiabetic effects in STZ-induced diabetic rats, improving glucose tolerance, insulin sensitivity, and serum lipid levels. These effects are similar to those observed with our botanical mixture, highlighting the potential of multi-component herbal formulations in regulating glucose and lipid metabolism (Chen *et al.,* 2017).

Limitations and future directions

The study did not isolate or quantify individual bioactive compounds, and only one diabetic model was used. Future

work should identify active constituents, clarify underlying mechanisms-especially insulin signaling and β-cell regeneration, assess efficacy and safety in diet-induced and clinical settings.

CONCLUSION

Previous studies have reported the individual antidiabetic activities of *Rheum spiciforme* and *Bergenia stracheyi*, with the former enhancing insulin secretion and antioxidant defense (Bhat *et al.,* 2019) and the latter improves glucose uptake and lipid regulation (Hou *et al.,* 2021). However, no prior research has explored their combined or decocted form. The present study is therefore the first to evaluate the combined decoction of *Bergenia stracheyi* and *Rheum spiciforme*, demonstrating that the 50:50 mixture (B) effectively improved glycemic control, lipid profile, adipokine balance, and sirtuin expression in diabetic rats. These findings highlight its synergistic and multi-targeted potential as a natural adjunct for managing type 2 diabetes.

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Author's contributions
Hafiz Muhammad Irfan conceptualized and supervised the study. Komal Chaudhry designed the methodology and conducted the experimental work. Faiqa Chaudhry drafted and initially wrote the manuscript and Muhammad Omer performed the formal data analysis.

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Data availability statement
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical approval
The study procedure was approved by the Biosafety and Ethical Review Committees of the University of Sargodha (Approval No. SU/ORIC/2081) and all animal handling was conducted in accordance with the approved guidelines.

Conflict of interest
All the authors declare no conflict of interest

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