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Hispidulin: A potential antihyperglycemic and anti-oxidant agent-mechanistic insights into its modulatory effects on PI3K, AKT, mTOR, IRS1, GSK-3β, and GLUT-4 pathways through *in vitro* and *in vivo* studies

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ABSTRACT

Objective(s): Hispidulin, a naturally occurring flavonoid with neuroprotective and anticancer properties, has shown therapeutic potential for the treatment of metabolic disorders, including diabetes mellitus. This study was designed to explore the anti-diabetic efficacy of hispidulin by examining its role in regulating glycemic markers, insulin responsiveness, oxidative stress, and the inflammatory response, and by evaluating transcriptional profiling of pivotal genes involved in the PI3K/AKT signaling cascade.

Materials and Methods: Experimental induction of type 2 diabetes was achieved using a high-fat diet regimen, followed by intraperitoneal administration of nicotinamide 110 mg/kg and streptozotocin 55 mg/kg. Following diabetes induction, rats were treated with hispidulin (10–20 mg/kg orally/day). Over 28 days, various parameters were assessed, including Fasting blood glucose, serum glucose, serum insulin, HOMA-IR, HOMA-β, QUICKI, CISI, oxidative stress markers (TAC, TOS, TBRAS, SOD, CAT, NO), and inflammatory cytokines (IL-6, TNF-α, CRP, NFkB). Gene expression levels of PI3K, AKT, mTOR, IRS-1, GSK-3β, and GLUT-4 were determined via qRT-PCR.

Results: Hispidulin treatment significantly ($P<0.001$) enhanced glycemic regulation and insulin sensitivity, as reflected by decreased fasting blood glucose levels and improved insulin indices. It favorably modulated oxidative stress markers and reduced the pro-inflammatory cytokines. Gene expression analysis indicated up-regulation of PI3K, AKT, mTOR, IRS-1, and GLUT-4, with down-regulation of GSK-3β, suggesting up-regulation of the PI3K/AKT signaling cascade.

Conclusion: Hispidulin exhibits potent antidiabetic properties by improving insulin sensitivity, reducing oxidative stress and inflammation, and modulating key genes in the PI3K/AKT pathway. These findings suggest hispidulin as a therapeutic agent for managing type 2 diabetes mellitus.

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Introduction

The rising global incidence of type 2 diabetes mellitus (T2DM) has become a significant public health concern, currently affecting more than 460 million individuals worldwide (1, 2). T2DM is a complex, multifactorial metabolic disorder marked by persistent hyperglycemia, insulin resistance (IR), and progressive decline in pancreatic β-cell functional capacity (3). In addition to impaired glucose metabolism, oxidative stress and a persistent low-grade inflammatory response play pivotal roles in the onset and progression of T2DM, which is marked by a gradual decline in pancreatic β-cell function and exacerbates peripheral insulin resistance, ultimately leading to multi-organ dysfunction (4).

One of the critical contributors to diabetic pathogenesis is oxidative stress, arising from the unnecessary generation of reactive oxygen species (ROS) and the correct bonding decline of the anti-oxidant defense mechanism (5, 6). This redox imbalance impairs insulin signaling pathways, disrupts mitochondrial function, and contributes to beta cell apoptosis (7). At the same time, pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), exacerbate insulin resistance and impaired glucose homeostasis, forming a vicious cycle that sustains the diabetic state (8, 9). Together, these alterations accumulate in a cycle of metabolic impairment, and searching for effective anti-oxidant and anti-inflammatory interactions is critically important (10, 11).

Despite the availability of several anti-diabetic drugs, current treatment regimens often fail to address the multifaceted nature of T2DM comprehensively and are frequently associated with undesirable side effects (12,

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13). This has prompted a growing interest in identifying safer, naturally derived compounds with multi-targeted therapeutic potential (14). Recent therapeutic strategies have focused on natural compounds with multi-targeted actions, especially those capable of mitigating oxidative and inflammatory stress while improving glycemic parameters (15). These natural compounds and plant-derived bioactives have attracted considerable attention for their potential in motivating key metabolic pathways with minimal side effects (16, 17). Hispidulin, naturally occurring as a bioactive flavonoid, found in diverse medicinal plants in the Mediterranean diet such as *Salvia officinalis*, *Artemisia* species, and *Eriocaulon buergerianum*, has shown promising pharmacological properties (18). It possesses notable anti-inflammatory (19), neuroprotective (20), and anticancer activities (21).

T2DM involves complex disruptions in insulin signaling, oxidative stress, and inflammation. The PI3K/AKT signaling pathway is essential for controlling glucose uptake and supporting cell survival (22). Dysregulation of the PI3K/AKT signaling pathway contributes to IR in hepatic and skeletal muscle tissues and facilitates β-cell apoptosis (23). Hispidulin, a flavone with known anti-inflammatory and anti-oxidant properties, has been shown to influence upstream modulators like SIRT1 (9). This study investigates the potential of hispidulin to restore PI3K/AKT signaling in an HFD-STZ-induced diabetic rat model, aiming to improve glycemic control and mitigate oxidative and inflammatory damage.

Enzymatic assays

The inhibitory effects of hispidulin on α-amylase and glucosidase activities were assessed using modified dinitrosalicylic acid (DNS) and p-nitrophenyl α-D-glucopyranoside (pNPG) assays. For the alpha-amylase assay, the enzyme (0.26 µM) mixtures were pre-incubated with different concentrations of hispidulin in phosphate-buffered saline (PBS) at pH 6.9, maintained at 37 °C for 15 min to allow adequate interaction, followed by the addition of soluble starch and further incubation for 10 min. The reaction was terminated with the DNS reagent, heated to 100 °C for 10 min, cooled, and the absorbance was measured at 530 nm (24). For the alpha-glucosidase assay, the enzyme (0.26 µm) was incubated with various concentrations of hispidulin in 0.1 M sodium phosphate buffer (SPB) (pH 6.8) at 37 °C for 2 hr, followed by the addition of 0.30 µmole pNPG, and absorbance was recorded at 405 nm (25). In both assays, the IC50 values were determined, with acarbose used as positive control.

Materials and Methods

Chemicals

Hispidulin (≥98% purity), metformin hydrochloride, streptozotocin (STZ), and nicotinamide were procured from Sigma-Aldrich (USA). All chemicals used were of analytical grade, and freshly prepared solutions were used as required.

Experimental animal

The experimental study involved 40 male Wistar rats, housed in the animal facility. Male rats were selected for their greater tendency to develop insulin resistance, a condition typically characterized by reduced insulin secretion and diminished pancreatic beta cell mass, compared to females. The rats were maintained in a regulated research lab with a 20 to 26 °C temperature range, relative humidity of 50 to 55% and a 12-hr light/dark cycle. Before the trial began, all animals underwent a 2-week acclimatization period to ensure proper adaptation to the laboratory environment.

Diabetes induction and experimental design

To induce T2DM, all rats, except the normal control group, were initially fed an HFD for 4 weeks to promote insulin resistance. Following the HFD regimen, animals received a single oral dose of nicotinamide (NA) at 110 mg/kg body weight, administered 15 min before streptozotocin. To induce partial pancreatic β-cell dysfunction, streptozotocin (55 mg/kg) was administered in 0.1 M sodium citrate buffer (pH 4.5) immediately before administration as described by (26). This NA-STZ combination effectively replicates the key pathophysiological features of T2DM, encompassing both IR and impaired insulin secretion.

Seven days after STZ administration, fasting blood glucose levels were assessed via tail vein sampling. Rats with FBG levels exceeding 300 mg/dL were classified as diabetic and subsequently included in the experimental protocol. These diabetic rats were allocated into five groups (n=8), ensuring unbiased distribution and experimental consistency. The study design consisted of the following experimental groups.

- Group I (Normal Control, NC): Healthy rats receiving a standard diet and no treatment.
- Group II (Diabetic Control, DC): Diabetic rats received streptozotocin (STZ) alone.
- Group III (Metformin-treated group, Met.): HFD/STZ-N treated rats with metformin at 250 mg/kg body weight, orally.
- Group IV (Hispidulin-treated group I, LD): HFD/STZ-N treated rats with hispidulin at 10 mg/kg body weight, orally.
- Group V (Hispidulin-treated group II, HD): HFD/STZ-N treated rats with hispidulin at 20 mg/kg body weight, orally.

Serum sampling and tissue sampling

Following the completion of the experimental period, the rats were placed on an overnight fast and lightly anesthetized for blood collection from the retro-orbital plexus. The collected samples were left to clot at ambient room temperature and centrifuged at 3000 RPM for 15 min to obtain serum, which was stored at -80 °C for subsequent biochemical analysis. Following blood collections, animals were sacrificed, and the pancreas was carefully excised, rinsed with ice-cold saline, and divided into portions. One Part of the pancreas was fixed in 10% neutral buffered formalin for histological analysis, while the remaining tissue was stored in tizzle reagent at -80 °C for gene expression analysis.

Measurement of fasting blood glucose (FBG) and oral glucose tolerance test (OGTT) in diabetic rats

All experimental animals were fasted for six hours in the morning, from 7:00 AM to 1:00 PM, prior to sample collection. FBG was monitored weekly via tail vein sampling throughout the study. To assess glucose tolerance, an OGTT was conducted following the method described by (27), with slight modifications. After a six-hour fast, rats received the hispidulin orally. 30 min later, a glucose solution (2 mg/kg BW.t.) was administered by oral gavage. Blood glucose levels were recorded at baseline (0 min) and subsequently

Table 1. Primer sequences used for quantitative real-time PCR (qRT-PCR) analysis of PI3K/AKT/mTOR pathway related genes in the rat model

Genes	Primer Type	Sequence (5′-3′)	NM number
PI3K (Phosphoinositide 3-kinase)	Forward	CGAGAGTACGCTGTAGGCTG	>NM_053481.2
	Reverse	AGAAACTGGCCAATCCTCCG	
AKT (Protein kinase B)	Forward	GAAGGAGGTCATCGTTGCCA	>NM_033230.3
	Reverse	GTTCTCCAGCTTGAGGTCCC	
mTOR (Mammalian target of rapamycin)	Forward	AATCGTGGTGGCTCTTGGAG	>NM_019906.2
	Reverse	TTTCACGATCGGAGGCAACA	
IRS-1 (Insulin receptor substrate-1)	Forward	TATCTGCATGGGTGGCAAGG	>NM_012969.2
	Reverse	GGTAGCACCTGGGATGTAGC	
GSK-3β (Glycogen synthase kinase-3 beta)	Forward	GGGACAGTGGTGTGGATCAG	>NM_032080.1
	Reverse	AAGCGGCGTTATTGGTCTGT	
GLUT-4 (Glucose Transporter-4)	Forward	CTCTCCGGTTCCTTGGGTTG	>NM_012751.2
	Reverse	CAAGGACCAGTGTCCCAGTC	
β-actin (Housekeeping gene)	Forward	CTTCCAGCCTTCCTTCCTGG	NM_031144.3
	Reverse	AATGCCTGGGTACATGGTGG	

at 30-, 60-, 120-, and 180-min post-glucose administration.

Serum biochemical analysis

Measurement of glycemic markers and insulin resistance

Serum glucose levels were assessed using an assay kit, and insulin levels were quantified using ELISA kits from Elabscience (USA) (28). IR was evaluated using the homeostasis model assessment of insulin resistance (HOMA-IR) (29), and β-cell function was assessed through the Homeostasis Model Assessment of β-cell function (HOMA-β) (30). Additionally, β-cell performance and insulin sensitivity were further analyzed using the Composite insulin sensitivity index (CISI) and the Quantitative insulin sensitivity check index (QUICKI), following established protocols (31–34).

Determination of inflammatory cytokines and mediators

The concentration TNF-α, IL-6, CRP, and NF-kB) were determined using ELISA kits procured from Elabscience (USA).

Oxidant and anti-oxidant markers

Total anti-oxidant capacity (TAC) and total oxidant status (TOS) were measured by using colorimetric assay kits (35). Thiobarbituric acid reactive substances (TBRAS) (36), superoxide dismutase (SOD), catalase (CAT), and nitric oxide (NO) levels were measured using spectrophotometric and ELISA kits from Elabscience (USA) (37).

Gene expression analysis (PI3K/AKT pathway)

Total RNA was isolated from the samples using the standard Trizol reagent extraction method. The integrity and purity of the extracted RNA were verified spectrophotometrically. Complementary DNA (cDNA) was then synthesized from the isolated total RNA using a commercial reverse transcription kit. qRT-PCR was then conducted employing SYBR Green master mix on an Applied Biosystems thermal cycler. The primer sequences used for the amplification of PI3K, AKT, mTOR, GLUT-4, IRS1, and GSK-3β are listed in Table 1. β-Actin was used as an internal control, and relative mRNA expression of target genes was quantified using the 2-ΔΔCt method (38, 39).

Statistical analysis

Data were presented as mean ± SEM and analyzed using one-way and two-way ANOVA, followed by Tukey's multiple comparison test in GraphPad Prism; differences were considered statistically significant at $P<0.01$ and $P<0.001$.

Results

Enzymatic assays

The inhibitory activity of hispidulin against carbohydrate-hydrolyzing enzymes was assessed using *in vitro* assays. Hispidulin demonstrated dose-dependent inhibition of both alpha-amylase and alpha-glucosidase enzymes. The IC_{50} value of hispidulin for alpha amylase was determined to be 4.44 µM, which was slightly higher than that of the reduction observed with the standard drug acarbose, 3.0 µM, indicating comparable potency. In the case of alpha-glucosidase, hispidulin exhibited an IC_{50} of 16.70 µM, which was notably lower than that of acarbose 25.0 µM, suggesting a strong inhibitory effect, as shown in Figure 1.

Assessment of fasting blood glucose (FBG) and oral glucose tolerance test (OGTT) in diabetic rats

Diabetic rats exhibited a persistent and significant elevation in FBG levels throughout the experimental

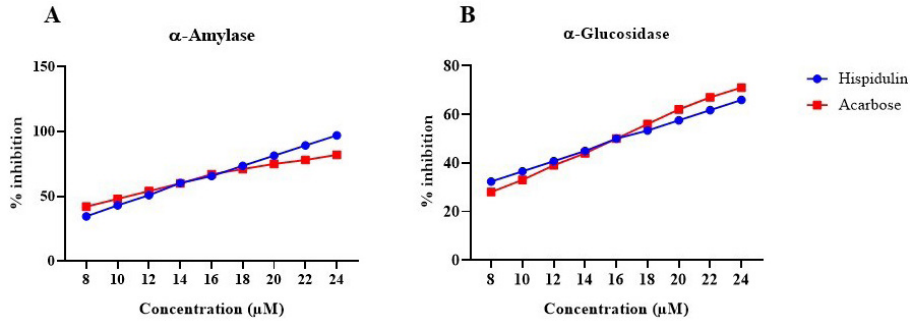

Figure 1. Inhibitory activity of hispidulin against (A) alpha-amylase and (B) alpha-glucosidase enzymes in mice

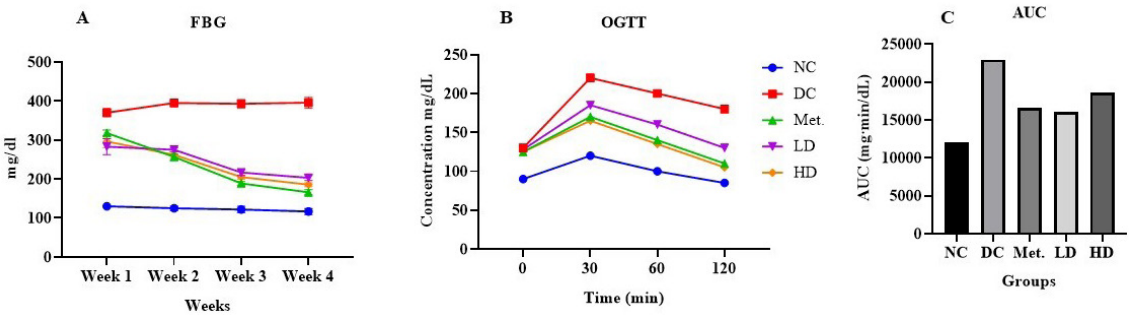

Figure 2. Measurement of (A) rat fasting blood glucose, (B) Oral glucose tolerance test, and (C) area under the curve (AUC)

period in contrast to NC (P<0.001). However, hispidulin treatment showed a marked (P<0.001) reduction in FBG levels starting from the second week, with highly significant differences observed by the end of the trial (P<0.001). During OGTT, diabetic control rats exhibited prolonged hyperglycemia, with significantly higher glucose levels at all time points compared with the NC group (P<0.001). In contrast, hispidulin-treated groups exhibited enhanced glucose tolerance, as indicated by a marked decrease in blood glucose level at 60, 120, and 180 min post-glucose load (P<0.001). The AUC was significantly reduced (P<0.001) in both hispidulin-treated groups in a dose-dependent manner, confirming hispidulin's glucose-lowering potential. Statistical analysis was performed using one-way ANOVA followed by Tukey's *post hoc* test (Figure 2).

Serum biochemical analysis

Measurement of glycemic markers and insulin resistance

Rats subjected to the high-fat diet/streptozotocin (HFD/STZ) protocol demonstrated a marked elevation in serum glucose concentration (P<0.001), along with a marked decline in serum insulin concentrations (P<0.001) in comparison with the NC group. Hispidulin administration at 10 and 20 mg/kg resulted in a significant (P<0.001) decrease in serum glucose levels, accompanied by a marked improvement in serum insulin concentrations. Evaluation of IR through the HOMA-IR demonstrated a significant (P<0.001) reduction in HOMA-IR values in the hispidulin-treated group in a dose-dependent pattern. Furthermore, the HOMA-β index, a representative marker of pancreatic β-cell functional activity, was significantly increased (P<0.01) following hispidulin administration, suggesting enhanced β-cell activity. In addition, QUICKI and CISI were notably improved (P<0.001) in the hispidulin-treated groups, as shown in Figure 3.

Measurement of inflammatory cytokines and mediators

Persistent low-grade inflammation is the key pathological factor in the onset and progression of DM. In the current study, rats with streptozotocin-induced diabetes demonstrated a highly significant increase (P<0.001) in circulating levels of pro-inflammatory markers including TNF-α, IL-6, CRP, and NF-κB, when compared to NC. Hispidulin administration at doses of 10 and 20 mg/kg markedly reduced these elevations in a dose-responsive reduction (P<0.001), suggesting its strong anti-inflammatory efficacy in T2DM (Figure 4).

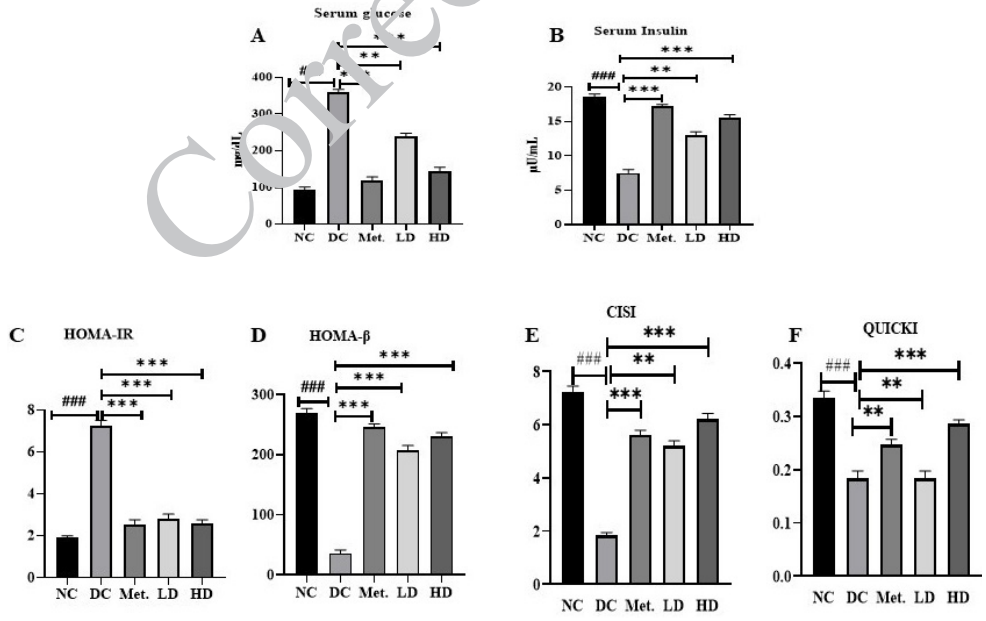

Figure 3. Bar graph showing serum levels of (A) glucose, (B) insulin, (C) Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), (D) Homeostatic Model Assessment of β-cell Function (HOMA-β), (E) Calculated Insulin Sensitivity Index (CISI), and (F) Quantitative Insulin Sensitivity Check Index (QUICKI) in Group I (Normal Control, NC), Group II (Diabetic Control, DC), Group III (Metformin-treated, Met.), Group IV (Hispidulin low dose, LD), and Group V (Hispidulin high dose, HD) rats

Data are expressed as mean ± SEM (n=8). Statistical analysis was performed by using one-way ANOVA followed by Tukey's *post hoc* test. ### P<0.001 vs normal control, ** P<0.01, ***P<0.001 vs diabetic control.

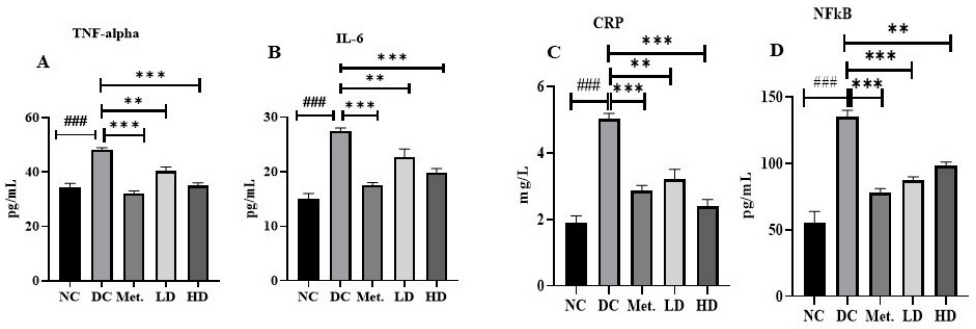

Figure 4. Bar graph showing serum levels of (A) Tumor Necrosis Factor-alpha (TNF-α), (B) Interleukin-6 (IL-6), (C) C-Reactive Protein (CRP), and (D) Nuclear Factor kappa B (NF-κB) in Group I (Normal Control, NC), Group II (Diabetic Control, DC), Group III (Metformin-treated, Met.), Group IV (Hispidulin low dose, LD), and Group V (Hispidulin high dose, HD) rats
Statistical analysis was performed by using one-way ANOVA followed by Tukey's *post hoc* test. ### P<0.001 vs normal control, ** P<0.01, ***P<0.001 vs diabetic control

Measurement of oxidant and anti-oxidant markers in diabetic rats

Induction with STZ caused a significant upsurge in oxidative stress markers, as evidenced by a significant elevation in lipid peroxidation levels, measured as thiobarbituric acid reactive substances (TBARS; 5.58±0.32 nmol/mg protein), compared with NC. This was accompanied by a significant (P<0.001) depletion of endogenous anti-oxidant defenses, including SOD, CAD, and NO levels, along with a reduced total anti-oxidant capacity (TAC; 1.1±0.10 mmol Trolox equi./l) and an increase in total oxidant status (TOS; 3.6±0.21 µmol H_2O_2 equi./l), compared to the NC group.

Hispidulin administration at doses of 10 and 20 mg/kg markedly (P<0.001) decreased MDA levels (3.06±0.19 and 2.66±0.30 nmol/mg protein), correspondingly, indicating attenuation of lipid peroxidation. Concurrently, a substantial restoration of anti-oxidant markers. Hispidulin significantly increases SOD (LD: 4.75±0.31; HD: 6.05±0.22 U/mg protein), CAT (LD: 37.6±2.09; HD: 47.01±2.10 U/mg protein), and NO levels (LD: 18.5 U/mg protein0.12; HD: 21.6±0.95 µmol/l) with reference to the DC group (P<0.001). Moreover, TAC levels were markedly increased in the HD group (2.41±0.1 mmol Trolox equi./l; P<0.001), approaching near-normal values, while TOS levels were significantly lowered (HD: 1.45±0.10 µmol H_2O_2 equi./l; P<0.001).

Gene expression analysis (PI3K/AKT pathway)

qRT-PCR analysis showed significant changes in PI3K/AKT signaling gene expression among the experimental groups. In the DC group, the expression of PI3K, AKT, mTOR, GLUT-4, and IRS1 was significantly down-regulated (P<0.005), whereas GSK-3β was up-regulated compared to the NC group. Treatment with hispidulin led to dose-dependent up-regulation of PI3K, AKT, mTOR, GLUT-4, and IRS1, and a down-regulation of GSK-3β (Figure 5).

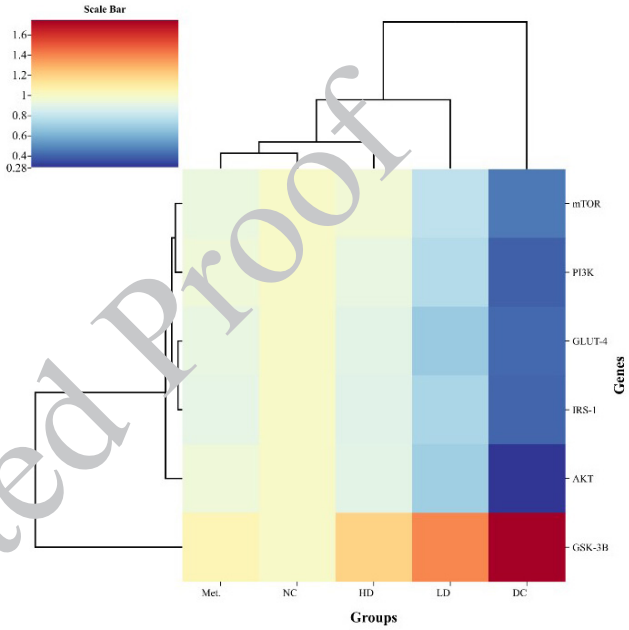

Figure 5. Heatmap showing the relative mRNA expression of PI3K, AKT, mTOR, GLUT-4, IRS1, and GSK-3β in pancreatic tissue of all experimental rat groups (Group I: Normal Control, NC; Group II: Diabetic Control, DC; Group III: Metformin-treated, Met.; Group IV: Hispidulin low dose, LD; Group V: Hispidulin high dose, HD)
PI3K: Phosphoinositide 3-kinase, AKT: Protein kinase B, mTOR: Mechanistic target of rapamycin, GLUT-4: Glucose transporter type 4, IRS1: Insulin receptor substrate 1, GSK-3β – Glycogen synthase kinase-3 beta

Table 2. Assessment of oxidative and anti-oxidant biomarkers in diabetic rats

Groups	TBRAS (nmol/mg protein)	CAT (U/mg protein)	SOD (U/mg protein)	NO (µmol/l)	TAC (mmol Trolox equi./l)	TOS (µmol H_2O_2 equi./l)
NC	2.42±0.20	54.2±0.80	7.01±0.39	25.2±0.60	2.4±0.12	1.01±0.06
DC	5.58±0.32 ***	28.01±1.28 ***	3.62±0.54 ***	11.4±0.10***	1.1±0.10 ***	3.6±0.21
Met.	3.19±0.28 ab	43.02±2.01 ab	5.80±0.30 ab	20.4±0.97 ab	2.02±0.11 ab	2.2±0.11 ab
LD	3.06±0.19 ab	37.6±2.09ab	4.75±0.31ab	18.5±0.12ab	2.10±0.10ab	1.82±0.12 ab
HD	2.66±0.30 ab	47.01±2.10ab	6.05±0.22 ab	21.6±0.95ab	2.41±0.14ab	1.45±0.10 ab

Oxidant and anti-oxidant defense markers in experimental rat groups (Group I: Normal Control, NC; Group II: Diabetic Control, DC; Group III: Metformin-treated, Met.; Group IV: Hispidulin low dose, LD; Group V: Hispidulin high dose, HD). Values are expressed as mean ± SEM (n = 8). Statistical analysis was performed using one-way ANOVA followed by Tukey's *post hoc* test. *** Indicates a statistically significant difference between the normal control and diabetic control groups. Symbols ab denote significant differences between the treatment groups and the diabetic control group.
TBRAS: Thiobarbituric acid reactive substances, CAT: Catalase, SOD: Superoxide dismutase, NO: Nitric oxide, TAC: Total anti-oxidant capacity, TOS: Total oxidant Status, SEM: Standard error of the mean, ANOVA: Analysis of variance

Discussion

Diabetes mellitus, particularly type II, is a long-term metabolic condition characterized by persistently elevated blood glucose levels and multiple metabolic abnormalities (40, 41). Therapeutic approaches often focus on modulating key enzymes involved in carbohydrate metabolism. Inhibitors of alpha-glucosidase and alpha amylase delay carbohydrate digestion and glucose absorption, thereby attenuating postprandial hyperglycemia (17, 42). Although conventional antidiabetic agents are effective, their use is often limited by side effects and high costs (43, 44). Consequently, there is growing interest in plant-derived alternatives, which are generally more affordable and associated with fewer adverse effects (44, 45). Nonetheless, most phytochemicals require rigorous validation through preclinical and clinical studies to establish their safety, efficacy, and therapeutic potential (46, 47). This study investigated the therapeutic efficacy of hispidulin in ameliorating HFD/STZ-induced type 2 diabetes in a rat model.

Hispidulin, naturally found in various medicinal plants, has attracted attention for its anti-inflammatory (19), neuroprotective (20), and anticancer (21) activities. Previous research has demonstrated that hispidulin regulates oxidative stress and inflammation in experimental models (48), enhances insulin sensitivity by activating AMPK, and protects pancreatic beta cells from apoptosis (49). However, the role of hispidulin in regulating PI3K/AKT signaling and glycemic enzymes, such as alpha-amylase and alpha-glucosidase, remains unexplored.

In this study, rats subjected to HFD/STZ developed significant hyperglycemia, confirmed by elevated FBG and impaired OGTT with a higher AUC. Treatment with hispidulin significantly (P<0.001) reduced FBG and ameliorated glucose tolerance as reflected in the lowered AUC. Improved glucose homeostasis may be attributed to hispidulin's ability to enhance insulin responsiveness and promote cellular glucose uptake by activating insulin-dependent signaling pathways.

Inhibiting α-amylase and α-glucosidase, the principal enzymes that catalyze carbohydrate digestion, represents a therapeutic strategy for attenuating postprandial hyperglycemia in diabetic conditions (50, 51). In the current study, hispidulin exhibited dose-dependent (P<0.001) inhibition of both enzymes, similar to the standard drug acarbose. This aligns with the findings of Visvanathan *et al.* and Gong *et al.* (52, 53), who reported that polyphenolic compounds can bind and inactivate these enzymes, thereby slowing glucose absorption. Hispidulin's interaction with digestive enzymes may thus contribute to its glycemic control effects.

Persistent hyperglycemia and insulin resistance promote chronic low-grade inflammation, which exacerbates pancreatic beta cell dysfunction (54, 55). In this study, diabetic rats showed significant elevations in TNF-alpha, IL-6, CRP, and NFkB, all of which were markedly reduced (P<0.001) following hispidulin treatment. These results are consistent with earlier findings that flavonoids suppress inflammatory pathways by inhibiting NFkB activation and downstream cytokines (56).

Oxidative stress is a major driver of IR and β-cell apoptosis (6). The diabetic rates in this study exhibited increased oxidative markers and decreased anti-oxidant enzymes, Indicative of a disrupted redox balance. Hispidulin significantly (P<0.001) restored anti-oxidant capacity, consistent with studies reporting its ROS-scavenging capacity and mitochondrial protective effects. The restoration of redox homeostasis contributes to beta cell preservation and improved insulin secretion (57, 58).

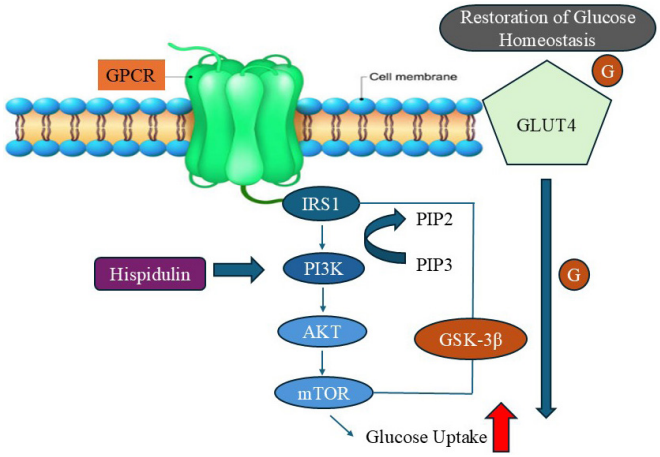

Figure 6. Hispidulin Modulation of PI3K/AKT pathway in diabetes

One of the key findings of this study is the significant (P<0.001) up-regulation of PI3K, AKT, mTOR, GLUT-4, and IRS alongside a significant (P<0.001) down-regulation of GSK-3β, following hispidulin treatment. This suggests that hispidulin restores insulin signaling, enhances glucose transport, and suppresses gluconeogenesis. The activation of this pathway promotes beta cell survival and insulin-stimulated glucose uptake, as reported in similar studies exploring the insulin-mimetic effects of phenols (59, 60). These molecular changes indicate that hispidulin's therapeutic effect may primarily be exerted through the reactivation of the PI3K/AKT axis.

Hispidulin exerts an antidiabetic effect primarily by regulating PI3K/AKT signaling and mitigating oxidative stress and inflammation through down-regulation of inflammatory cytokines, including TNF-α, CRP, IL-6, and NFkB, and by inhibiting ROS. This anti-inflammatory effect contributes to restoring insulin receptor sensitivity by up-regulating IRS-1, leading to PI3K activation and AKT phosphorylation. Activated AKT plays a dual role by inhibiting GSK-3β, thereby promoting glycogen synthesis, and by activating mTOR, which supports β-cell survival and regeneration. These molecular events collectively enhance GLUT-4 translocation and glucose uptake in peripheral tissues, ultimately restoring glucose homeostasis and improving insulin secretion, thereby highlighting its potential role in ameliorating metabolic dysfunction associated with diabetes.

Conclusion

The present study demonstrates that hispidulin significantly improves metabolic and molecular alterations linked with T2DM. Hispidulin treatment effectively lowered fasting blood glucose levels, enhanced serum insulin concentration, and improved insulin sensitivity indices, indicating restoration of glycemic markers. Furthermore, hispidulin significantly reduced levels of inflammatory cytokines, suggesting improved β-cell function and insulin dynamics. The compound also attenuated oxidative stress by increasing the anti-oxidative markers and reducing TOS, TBRAS, and NO, suggesting potent anti-oxidant potential.

At the genetic level, hispidulin significantly up-regulated the mRNA expression of PI3K, AKT, mTOR, IRS-1, and GLUT-4 while down-regulating GSK-3β, thereby indicating the PI3K/AKT signaling pathway and enhancing glucose uptake and insulin signaling.

Acknowledgment

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Ethical Approval

This study is a part of a Ph.D. research project and is original, unpublished, and not under consideration for publication elsewhere. All experimental procedures involving animals were conducted in accordance with the ethical standards for animal research and were approved by the Institutional Bioethical Committee of the University of Agriculture, Faisalabad, Pakistan (Approval No. 3278/ORIC).

Availability of Data and Materials

The data supporting this study's findings are available upon request from the corresponding author.

Authors' Contributions

MS T was responsible for preparing the original draft, conducting the research trial, collecting data, and performing statistical analysis. W M, B A, and M S contributed to the critical review, editing, and supervision of the study. All authors participated in the study's conception and design, reviewed and approved the final version of the manuscript for publication, and provided overall supervision throughout the research process.

Conflicts of Interest

The author declares that there are no conflicts of interest.

Declaration

The authors declare that they have not used any AI tools or technologies in the preparation of this manuscript.

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