RESEARCH PAPER

In-Vivo Pharmacological Evaluation of an Optimized Antidiabetic Herbal Transdermal Patch Containing Gymnema sylvestre and Tinospora cordifolia in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Background: Diabetes mellitus remains one of the most pressing global health challenges, with over 537 million adults affected worldwide and projections reaching 783 million by 2045 [1]. Conventional oral antidiabetic agents suffer from hepatic first-pass metabolism, gastrointestinal adverse effects, and poor patient adherence due to frequent dosing. Transdermal drug delivery offers a compelling alternative by bypassing presystemic metabolism and maintaining steady plasma drug concentrations.

Objective: The present study was designed to evaluate the in vivo antidiabetic efficacy, pharmacokinetic behaviour, and dermal safety of a previously optimized polymeric matrix transdermal patch co-loaded with extracts of Gymnema sylvestre and Tinospora cordifolia in streptozotocin (STZ)-induced diabetic Wistar rats.

Methods: Diabetes was induced through a single intraperitoneal injection of STZ (55 mg/kg) dissolved in citrate buffer (pH 4.5). Animals exhibiting fasting blood glucose above 250 mg/dL after 72 hours were enrolled and randomly assigned to five groups (n = 6 each): normal control, diabetic control, glibenclamide standard (10 mg/kg orally), optimized herbal transdermal patch, and placebo patch. Treatment continued for 28 days, with fasting blood glucose monitored on days 0, 7, 14, 21, and 28. Terminal biochemical assessment included serum insulin, HbA1c, lipid profile, hepatic enzymes, and renal function markers. A parallel pharmacokinetic investigation compared transdermal and oral delivery profiles using validated HPLC quantification of gymnemic acid and berberine. Skin irritation was evaluated through the Draize test in New Zealand white rabbits following OECD Guideline 404.

Results: The optimized herbal patch produced a sustained 52.9% reduction in fasting blood glucose (from 302.4 ± 5.2 to 142.3 ± 3.5 mg/dL), statistically comparable to glibenclamide (56.6% reduction). Serum insulin was restored to 13.6 ± 0.9 µU/mL, and HbA1c declined to 6.7 ± 0.3%. Lipid parameters, liver enzymes, and kidney markers were significantly normalized relative to diabetic controls ($p < 0.001$). Pharmacokinetic analysis revealed a relative bioavailability of 181.6% versus oral administration (AUC_{0-24}: 5842.3 vs 3216.8 ng·h/mL), with prolonged half-life (9.6 h) and mean residence time (14.2 h). The Draize test yielded a Primary Irritation Index of 0.00 at 72 hours, confirming non-irritant classification.

Conclusion: The optimized herbal transdermal patch demonstrated robust antidiabetic efficacy, superior pharmacokinetic performance, and excellent cutaneous biocompatibility, supporting its potential as a sustained, patient-friendly alternative to conventional oral antidiabetic therapy.....

Keywords: Gymnema sylvestre; Tinospora cordifolia; transdermal patch; streptozotocin; antidiabetic; pharmacokinetics; bioavailability; skin irritation; Draize test..

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INTRODUCTION

Diabetes mellitus has emerged as one of the most formidable non-communicable diseases of the twenty-first century. The International Diabetes Federation estimated that 537 million adults were living with diabetes globally in 2021, a number projected to swell to 783 million by 2045 [1]. India alone harbours over 101 million affected individuals, ranking second worldwide [2]. Type 2 diabetes mellitus (T2DM) accounts for approximately 90–95% of all cases and drives a cascade of microvascular and macrovascular complications including neuropathy, nephropathy, retinopathy, and cardiovascular disease [3, 4]. Persistent hyperglycaemia arising from defective insulin

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secretion, insulin resistance, or a combination of both underlies the progressive organ damage characteristic of this disorder [5, 6].

Although several classes of oral antidiabetic agents—biguanides, sulfonylureas, thiazolidinediones, DPP-4 inhibitors, and SGLT2 blockers—are clinically available, their therapeutic utility is constrained by significant pharmacokinetic and patient-related drawbacks. Oral antidiabetic drugs undergo substantial hepatic first-pass metabolism, reducing systemic bioavailability to 30–60% and necessitating multiple daily doses to maintain glycaemic control [7, 8]. Frequent dosing schedules, gastrointestinal intolerance, and the risk of hypoglycaemia collectively erode patient adherence, with studies indicating that compliance rates fall below 50% in chronic therapy [9]. These limitations underscore the need for alternative delivery platforms that can sustain therapeutic plasma levels while minimising dosing frequency.

Transdermal drug delivery systems (TDDS) circumvent hepatic first-pass metabolism by transporting therapeutic agents directly into the dermal microcirculation, thereby maintaining steady-state plasma concentrations and improving bioavailability [10]. Matrix-type transdermal patches offer the additional advantages of straightforward manufacturing, uniform drug release, and non-invasive application [11]. The stratum corneum, while presenting a formidable barrier to drug permeation, can be overcome through the strategic use of chemical penetration enhancers such as dimethyl sulphoxide (DMSO) and Tween 80, which transiently disrupt intercellular lipid lamellae without causing irreversible damage [12, 13, 26, 27].

Two medicinal plants form the pharmacological foundation of the present formulation. *Gymnema sylvestre* (Retz.) R.Br. ex Schult., traditionally known as 'Gurmar' or 'sugar destroyer,' harbours gymnemic acids and gurmarin that exert hypoglycaemic effects through stimulation of pancreatic β-cell insulin secretion, regeneration of islet tissue, and competitive inhibition of intestinal glucose absorption [14, 15]. Clinical investigations have demonstrated significant reductions in blood glucose and glycosylated haemoglobin following *G. sylvestre* supplementation in both type 1 and type 2 diabetic patients [17, 18]. *Tinospora cordifolia* (Willd.) Miers, known as 'Guduchi' or 'Amrita,' contains berberine, palmatine, and tinocordiside that exhibit insulin-mimetic activity, enhance insulin receptor sensitivity, suppress hepatic gluconeogenesis, and provide robust antioxidant defence against diabetes-induced oxidative stress [19, 20, 21, 22]. The combination of these two botanicals addresses multiple pathophysiological pathways simultaneously, offering a multi-targeted therapeutic strategy analogous to combination pharmacotherapy in conventional diabetology [24, 29, 30, 31].

Streptozotocin (STZ)-induced diabetes in rats is a widely validated experimental model that recapitulates key features of human diabetes, including progressive β-cell destruction, insulin deficiency, hyperglycaemia, and associated metabolic disturbances [36]. This model has been extensively employed for evaluating novel antidiabetic agents and delivery systems. In the preceding phase of this research, a polymeric matrix transdermal patch co-loaded with *G. sylvestre* and *T. cordifolia* extracts was formulated and optimised through Box-Behnken response surface methodology. The optimised formulation (Run 14: PVA 70%, ethanol 35%, drying time 36 h) demonstrated 96.2 ± 1.5% cumulative drug release over 24 hours via zero-order, non-Fickian kinetics, with excellent physicochemical stability under ICH-compliant storage conditions.

The present investigation was undertaken to evaluate the in-vivo pharmacological performance of this optimised formulation. Specifically, the study aimed to: (i) assess the antidiabetic efficacy through measurement of fasting blood glucose, body weight, and a comprehensive panel of biochemical parameters in STZ-induced diabetic rats over a 28-day treatment period; (ii) characterise the pharmacokinetic profile of the transdermal patch relative to oral extract administration; and (iii) evaluate dermal safety through the Draize skin irritation test in rabbits. The placebo-controlled, standard-drug-comparativedesign employed herein provides a rigorous framework for establishing both the therapeutic efficacy and safety of the herbal transdermal antidiabetic system.

2. MATERIALS AND METHODS:

2.1 Experimental Animals:

Adult male *Wistar albino* rats weighing 200–250 g were procured from a registered animal supplier and acclimatised to laboratory conditions for one week before experimentation. Animals were housed under standard environmental conditions (temperature 25 ± 2 °C, relative humidity 50–60%, 12-hour light/dark cycle) with unrestricted access to standard pellet diet and water ad libitum. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) from the Deshpande Lab, Bhopal, under protocol number CPCSEA/IAEC/05/2024/12, and all procedures were conducted in strict compliance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

2.2 Induction of Diabetes:

Diabetes was induced through a single intraperitoneal injection of freshly prepared *streptozotocin* (STZ, Sigma-Aldrich, USA) at a dose of 55 mg/kg body weight, dissolved in ice-cold 0.1 M citrate buffer (pH 4.5) immediately before administration [36]. Normal control animals received an equivalent volume of citrate buffer alone. Following a 72-hour stabilisation period, fasting blood glucose was measured from tail vein blood using an Accu-Check Active glucometer (Roche, Switzerland). Animals exhibiting fasting blood glucose consistently exceeding 250 mg/dL were confirmed as diabetic and enrolled in the study.

2.3 Experimental Design:

Confirmed diabetic animals were randomly allocated to five experimental groups (n = 6 per group): Group I — Normal Control (healthy, untreated rats); Group II — Diabetic Control (STZ-induced, no treatment); Group III — Standard Drug (STZ-induced diabetic rats treated with glibenclamide 10 mg/kg body weight orally once daily); Group IV — Herbal Transdermal Patch (STZ-induced diabetic rats treated with the optimised herbal patch applied

to shaved dorsal skin); Group V — Placebo Patch (STZ-induced diabetic rats treated with a patch containing all excipients but no herbal extract). The treatment duration was 28 days for all groups.

2.4 Transdermal Patch Application:

The dorsal thoracic region of Group IV and Group V animals was shaved with an electric clipper and depilatory cream 24 hours before the first patch application. The optimised herbal transdermal patch (2 cm × 2 cm, containing 20 mg equivalent herbal extract) was applied to the shaved skin of Group IV rats and secured with Micropore surgical tape. Placebo patches of identical dimensions were applied to Group V animals using the same protocol. Patches were replaced every 24 hours throughout the 28-day study to maintain continuous drug delivery. Glibenclamide (Group III) was administered daily by oral gavage at 10 mg/kg dissolved in distilled water.

2.5 Blood Glucose and Body Weight Monitoring:

Fasting blood glucose levels were measured on days 0 (baseline), 7, 14, 21, and 28 using tail vein blood samples and a calibrated glucometer. Body weight was recorded on day 0 and day 28 using an electronic balance. All measurements were performed between 9:00 and 11:00 AM to minimise circadian variability.

2.6 Biochemical Parameters:

On day 28, following an overnight fast, animals were anaesthetised with diethyl ether and blood was collected from the retro-orbital plexus. Serum was separated by centrifugation at 3000 rpm for 10 minutes and analysed using an automated biochemistry analyser. The following parameters were quantified: fasting blood glucose (FBG) by glucose oxidase-peroxidase method; serum insulin by enzyme-linked immunosorbent assay (ELISA); glycated haemoglobin (HbA1c) by ion-exchange chromatography; total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) by standard enzymatic methods; serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) by kinetic UV methods; serum creatinine by Jaffé reaction; and blood urea nitrogen (BUN) by urease-glutamate dehydrogenase method.

2.7 Pharmacokinetic Study:

A separate pharmacokinetic investigation was conducted in non-diabetic Wistar rats (200–250 g, n = 6 per group) to compare the systemic exposure profiles of the transdermal patch and oral extract administration. Group A received the optimised herbal transdermal patch (2 cm × 2 cm) applied to shaved dorsal skin; Group B received an equivalent dose of the herbal extract blend administered by oral gavage. Blood samples (0.3 mL) were collected via tail vein puncture at 0, 1, 2, 4, 6, 8, 12, and 24 hours post-application. Plasma was separated by centrifugation (3000 rpm, 10 minutes, and 4 °C) and stored at −80 °C pending analysis. Gymnemic acid and berberine plasma concentrations were quantified by a validated reversed-phase high-performance liquid chromatography (HPLC) method employing a C18 column with UV detection. Non-compartmental pharmacokinetic analysis was performed using PK Solver software to derive Cmax, Tmax, $AUC_{0–24}$, $AUC_{0–\infty}$, elimination half-life ($t_{1(2)}$), and mean residence time (MRT). Relative bioavailability was calculated as (AUC_patch / AUC_oral) × 100 [38, 39].

2.8 Skin Irritation Study:

The dermal irritation potential of the optimised herbal transdermal patch was assessed using the Draize acute skin irritation test in New Zealand white rabbits (2–3 kg, n = 3), conducted in accordance with OECD Test Guideline 404 [39]. The dorsal region of each rabbit was clipped and divided into four treatment sites: (i) intact skin with the optimised herbal patch, (ii) abraded skin with the optimised herbal patch, (iii) intact skin with a placebo patch (negative control), and (iv) abraded skin with 10% sodium lauryl sulphate (positive control). Test patches (2.5 × 2.5 cm) were applied and secured with non-irritant tape for 4 hours. After removal, residual material was gently wiped with saline. Skin reactions (erythema and oedema) were graded at 1, 24, 48, and 72 hours post-removal using the Draize scoring scale (0–4 for each parameter). The Primary Irritation Index (PII) was calculated as the mean of erythema and oedema scores averaged across all time points and treatment sites. A PII below 2.0 was classified as non-irritant.

2.9 Statistical Analysis:

All quantitative data are expressed as mean ± standard deviation (SD). Statistical comparisons among multiple groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) post-hoc test for pairwise group comparisons. For pharmacokinetic parameters, the Student's t-test was employed to compare transdermal and oral groups. A probability value of $p < 0.05$ was considered statistically significant for all analyses.

3. RESULTS:

3.1 Fasting Blood Glucose Levels:

The effect of the optimised herbal transdermal patch on fasting blood glucose (FBG) across the 28-day treatment period is presented in Table 1 and Figure 1. *Streptozotocin* administration produced marked hyperglycaemia in all diabetic groups, with baseline FBG values ranging from 296.2 to 302.4 mg/dL, significantly elevated relative to the normal control group (89.4 ± 2.1 mg/dL, $p < 0.001$). The herbal transdermal patch group exhibited a progressive and sustained decline in FBG from 302.4 ± 5.2 mg/dL on day 0 to 142.3 ± 3.5 mg/dL by day 28, representing a 52.9% reduction. This hypoglycaemic response was statistically comparable to that achieved by the standard drug glibenclamide (296.2 ± 4.8 to 128.4 ± 3.1 mg/dL; 56.6% reduction; $p > 0.05$ between treatment groups). In contrast, both the diabetic control and placebo patch groups displayed progressive worsening of hyperglycaemia throughout the study, reaching 325.7 ± 7.1 and 318.4 ± 6.3 mg/dL respectively by day 28. The absence of any glycaemic improvement in the placebo group confirms that the observed antidiabetic effect was attributable to the herbal active constituents rather than the patch excipients or occlusive effects.

Table 1: Effect of Herbal Transdermal Patch on Fasting Blood Glucose (mg/dL) in STZ-Induced Diabetic Rats Over 28 Days:

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Normal Control	89.4 ± 2.1	88.6 ± 2.3	87.9 ± 1.8	88.2 ± 2.0	87.5 ± 1.9
Diabetic Control	298.6 ± 5.4	312.3 ± 6.1	321.5 ± 5.8	318.9 ± 6.2	325.7 ± 7.1
Glibenclamide (10 mg/kg)	296.2 ± 4.8	241.5 ± 4.2	198.3 ± 3.9	164.7 ± 3.4	128.4 ± 3.1
Herbal Patch (Opt.)	302.4 ± 5.2	258.7 ± 4.6	214.3 ± 4.1	178.6 ± 3.8	142.3 ± 3.5
Placebo Patch	299.1 ± 5.0	304.6 ± 5.5	309.2 ± 6.0	313.8 ± 5.9	318.4 ± 6.3

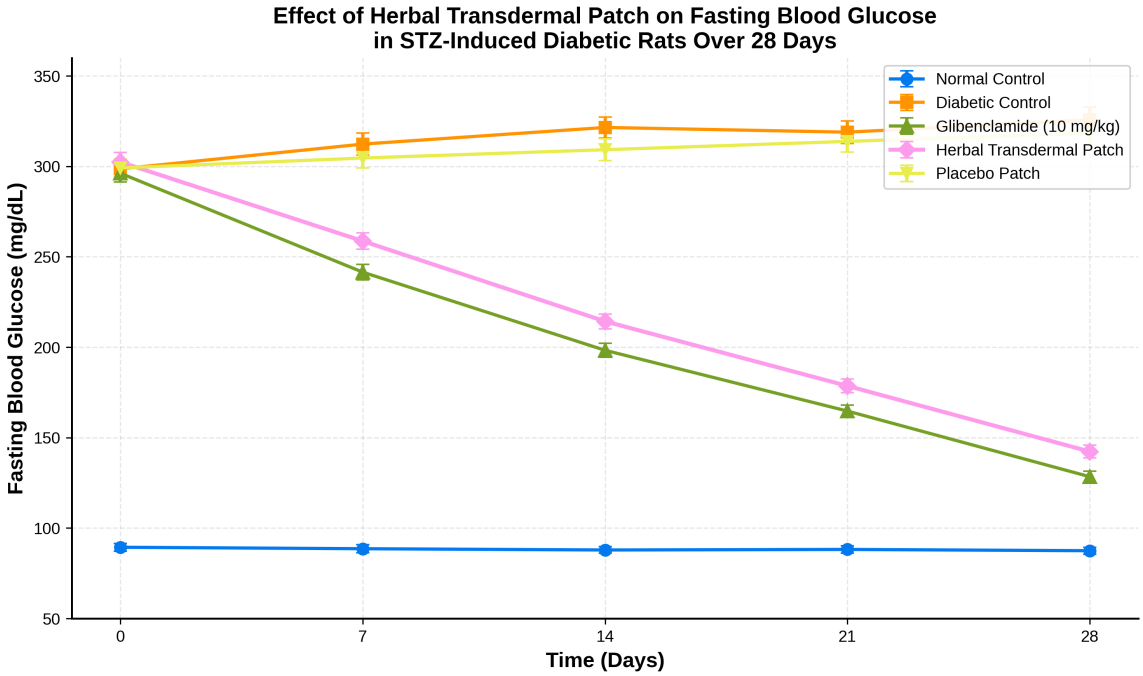

Figure 1: Effect of Herbal Transdermal Patch on Fasting Blood Glucose Levels in STZ-Induced Diabetic Rats Over 28 Days.

3.2 Body Weight Changes:

Body weight monitoring over the 28-day study period revealed patterns consistent with the metabolic status of each group (Table 2, Figure 2). Diabetic control animals exhibited significant weight loss (−10.5%), declining from 221.5 ± 3.8 g to 198.2 ± 5.1 g, reflecting the catabolic consequences of uncontrolled hyperglycaemia—specifically enhanced proteolysis, lipolysis, and diminished anabolic activity secondary to insulin deficiency. The placebo patch group similarly lost weight (−9.8%), confirming that the patch vehicle itself exerted no metabolic benefit. In contrast, animals treated with the optimised herbal transdermal patch maintained near-normal body weight, recording a modest gain of +2.9% (220.4 ± 3.9 g to 226.7 ± 4.1 g), which was statistically comparable to the glibenclamide standard group (+3.9%) and significantly superior to both untreated diabetic groups ($p < 0.001$). This preservation of body mass indicates that the transdermally delivered herbal extracts effectively restored anabolic metabolism and curtailed the wasting associated with insulin-deficient diabetes.

Table 2: Effect of Herbal Transdermal Patch on Body Weight (g) in STZ-Induced Diabetic Rats:

Group	Initial Weight (Day 0)	Final Weight (Day 28)	% Change
Normal Control	218.3 ± 4.2	232.6 ± 4.5	+6.5% (gain)
Diabetic Control	221.5 ± 3.8	198.2 ± 5.1	−10.5% (loss)

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Glibenclamide (10 mg/kg)	219.8 ± 4.0	228.4 ± 4.3	+3.9% (gain)
Herbal Patch (Opt.)	220.4 ± 3.9	226.7 ± 4.1	+2.9% (gain)
Placebo Patch	218.9 ± 4.1	197.5 ± 5.0	−9.8% (loss)

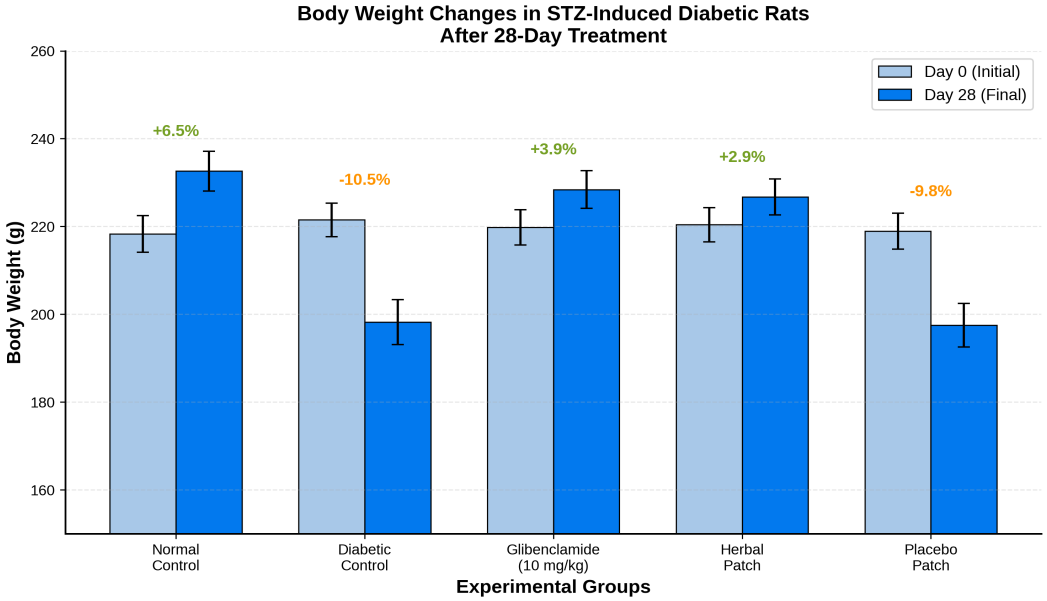

Figure 2: Body Weight Changes in STZ-Induced Diabetic Rats after 28-Day Treatment.

3.3 Biochemical Parameters:

Comprehensive biochemical profiling at day 28 provided compelling evidence of the systemic metabolic benefits conferred by the optimised herbal transdermal patch (Table 3, Figure 3). Serum insulin levels in the herbal patch group (13.6 ± 0.9 µU/mL) were significantly elevated relative to the diabetic control (4.2 ± 0.6 µU/mL, $p < 0.001$) and approached values observed in both the normal control (18.4 ± 1.2 µU/mL) and glibenclamide-treated groups (14.8 ± 1.0 µU/mL). This restoration of circulating insulin indicates enhanced pancreatic β-cell secretory function, plausibly mediated by the Insulinotropic actions of gymnemic acids and the β-cell regenerative properties attributed to *G. sylvestre* [15, 17, 37].

Glycated haemoglobin (HbA1c), a reliable indicator of long-term glycaemic control, was significantly reduced in the herbal patch group (6.7 ± 0.3%) compared with the diabetic control (9.8 ± 0.4%, $p < 0.001$) and fell within the American Diabetes Association target range for non-elderly adults (< 7%) [6]. The lipid profile exhibited substantial normalisation: total cholesterol decreased from 218.4 ± 5.1 mg/dL (diabetic control) to 163.5 ± 3.8 mg/dL, triglycerides from 186.2 ± 4.3 to 121.3 ± 3.0 mg/dL, and LDL-C from 152.3 ± 4.0 to 93.2 ± 2.6 mg/dL, while HDL-C increased from 28.4 ± 1.2 to 45.8 ± 1.5 mg/dL (all $p < 0.001$ versus diabetic control). These lipid-correcting effects reflect the combined actions of *G. sylvestre*, which attenuates intestinal cholesterol absorption, and *T. cordifolia*, which activates AMP-activated protein kinase (AMPK) to suppress hepatic lipogenesis and promote fatty acid oxidation [20, 22].

Hepatic function markers were significantly improved in the herbal patch group: SGOT was reduced to 46.2 ± 1.9 IU/L and SGPT to 40.8 ± 1.7 IU/L, compared with 72.4 ± 2.8 and 68.2 ± 2.6 IU/L, respectively, in the diabetic control group ($p < 0.001$). Renal function markers were similarly normalised, with serum creatinine declining to 1.02 ± 0.06 mg/dL and BUN to 23.2 ± 1.2 mg/dL versus 1.68 ± 0.08 and 38.6 ± 1.8 mg/dL in untreated diabetic animals ($p < 0.001$). These hepatoprotective and nephroprotective effects, extending beyond simple glucose lowering, represent a particularly valuable attribute of the dual-herb formulation and are consistent with the antioxidant and anti-inflammatory properties of the flavonoids and polyphenolic compounds present in both botanicals [14, 19].

Table 3: Effect of Herbal Transdermal Patch on Biochemical Parameters in STZ-Induced Diabetic Rats after 28-Day Treatment:

Parameter	Normal Control	Diabetic Control	Glibenclamide (10 mg/kg)	Herbal Patch
FBG (mg/dL)	87.5 ± 1.9	325.7 ± 7.1***	128.4 ± 3.1	142.3 ± 3.5
Serum Insulin (µU/mL)	18.4 ± 1.2	4.2 ± 0.6***	14.8 ± 1.0	13.6 ± 0.9
HbA1c (%)	5.1 ± 0.2	9.8 ± 0.4***	6.2 ± 0.2	6.7 ± 0.3
Total Cholesterol (mg/dL)	142.6 ± 3.2	218.4 ± 5.1***	158.3 ± 3.5	163.5 ± 3.8

Triglycerides (mg/dL)	98.4 ± 2.1	186.2 ± 4.3***	114.6 ± 2.8	121.3 ± 3.0
HDL-C (mg/dL)	52.3 ± 1.8	28.4 ± 1.2***	48.2 ± 1.6	45.8 ± 1.5
LDL-C (mg/dL)	74.8 ± 2.2	152.3 ± 4.0***	87.6 ± 2.4	93.2 ± 2.6
SGOT (IU/L)	38.2 ± 1.5	72.4 ± 2.8***	43.6 ± 1.7	46.2 ± 1.9
SGPT (IU/L)	32.4 ± 1.3	68.2 ± 2.6***	38.4 ± 1.5	40.8 ± 1.7
Creatinine (mg/dL)	0.82 ± 0.04	1.68 ± 0.08***	0.96 ± 0.05	1.02 ± 0.06
BUN (mg/dL)	18.2 ± 1.0	38.6 ± 1.8***	21.4 ± 1.1	23.2 ± 1.2

**** = $p < 0.001$ vs Normal Control; all herbal patch vs diabetic control: $p < 0.05$ (ANOVA, Tukey's HSD).*

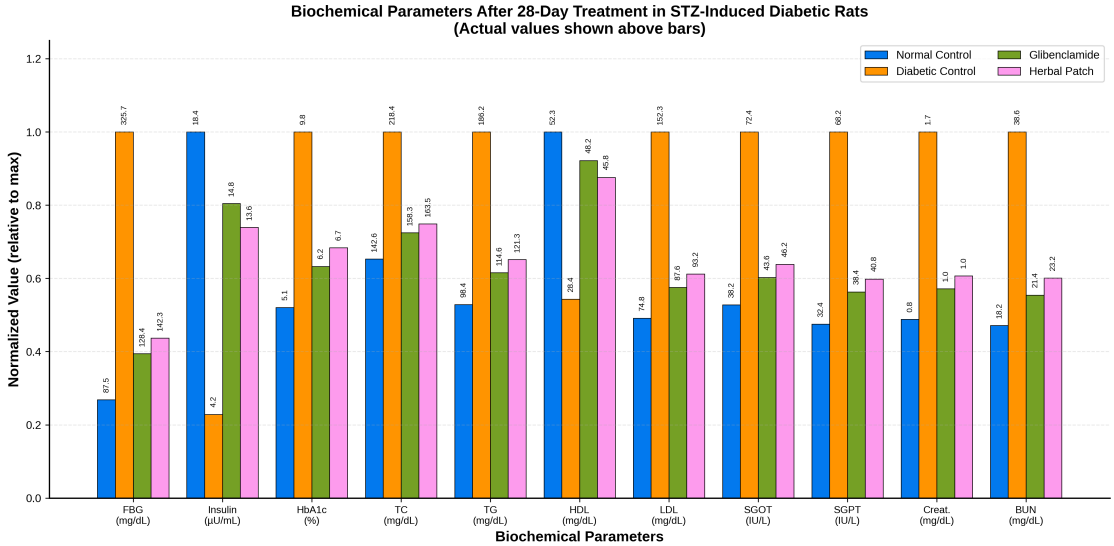

Figure 3: Biochemical Parameters after 28-Day Treatment in STZ-Induced Diabetic Rats.

3.4 Pharmacokinetic Study:

The pharmacokinetic investigation yielded definitive quantitative evidence for the superior systemic drug exposure achieved through transdermal delivery relative to oral administration (Table 4, Figures 4 and 5). The area under the plasma concentration-time curve from 0 to 24 hours ($AUC_{0–24}$) for the optimised herbal transdermal patch was 5842.3 ± 142.6 ng·h/mL, which was 81.6% greater than that of the oral extract (3216.8 ± 98.4 ng·h/mL), corresponding to a relative bioavailability of 181.6% ($p < 0.001$). The $AUC_{0–\infty}$ followed the same trend (6284.7 ± 168.3 vs 3402.1 ± 112.4 ng·h/mL, $p < 0.001$). This marked enhancement in systemic exposure is directly attributable to the circumvention of hepatic first-pass metabolism afforded by the transdermal route, combined with the sustained drug release from the optimised polymer matrix.

The time to maximum plasma concentration (Tmax) was significantly prolonged with the transdermal patch (8.0 ± 0.5 h) compared with oral gavage (2.0 ± 0.3 h, $p < 0.001$), reflecting the controlled and gradual permeation of herbal actives through the skin and into the systemic circulation. While the maximum plasma concentration (Cmax) was lower for the transdermal route (384.6 ± 12.4 vs 512.3 ± 18.6 ng/mL, $p < 0.05$), this represents a clinically favourable outcome, as it indicates avoidance of the supra-therapeutic peak plasma levels associated with oral administration that contribute to adverse effects. The elimination half-life ($t_{1/2}$ = 9.6 ± 0.8 h) and mean residence time (MRT = 14.2 ± 1.1 h) for the transdermal patch were approximately three-fold longer than those of the oral formulation ($t_{1/2}$ = 3.2 ± 0.4 h; MRT = 4.8 ± 0.5 h; both $p < 0.001$), confirming sustained drug absorption and prolonged systemic exposure consistent with the zero-order release kinetics demonstrated in the preceding in-vitro evaluation.

Table 4: Pharmacokinetic Parameters of Optimized Herbal Transdermal Patch vs Oral Extract Administration:

Parameter	Transdermal Patch	Oral Extract	Significance
Cmax (ng/mL)	384.6 ± 12.4	512.3 ± 18.6	$p < 0.05$
Tmax (h)	8.0 ± 0.5	2.0 ± 0.3	$p < 0.001$
$AUC_{0–24}$ (ng·h/mL)	5842.3 ± 142.6	3216.8 ± 98.4	$p < 0.001$
$AUC_{0–\infty}$ (ng·h/mL)	6284.7 ± 168.3	3402.1 ± 112.4	$p < 0.001$
$t_{1/2}$ (h)	9.6 ± 0.8	3.2 ± 0.4	$p < 0.001$
MRT (h)	14.2 ± 1.1	4.8 ± 0.5	$p < 0.001$
Relative Bioavailability (%)	181.6%	100% (reference)	—

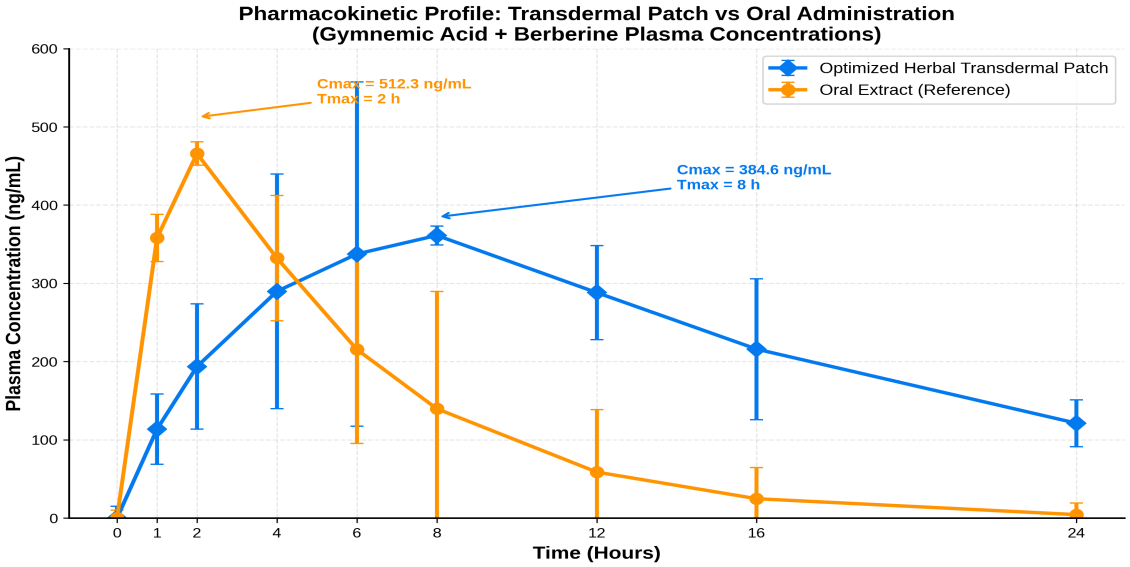

Figure 4: Pharmacokinetic Plasma Concentration-Time Profile of Herbal Transdermal Patch vs Oral Administration.

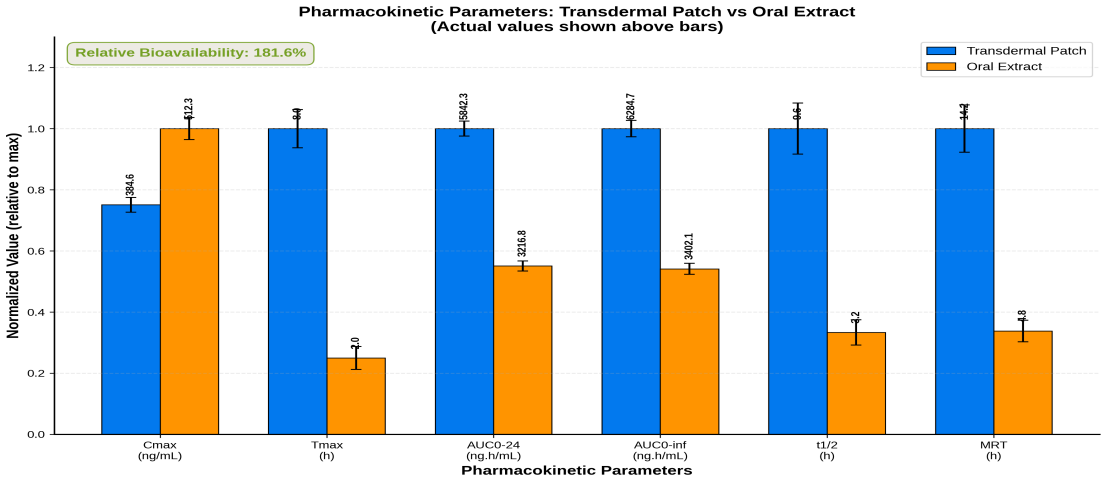

Figure 5: Comparative Pharmacokinetic Parameters of Transdermal Patch vs Oral Extract.

3.5 Skin Irritation Study:

The Draize skin irritation test demonstrated that the optimised herbal transdermal patch is dermally safe and well tolerated, with only negligible and transient responses observed (Table 5, Figure 6). At 1 hour post-removal, very slight erythema (score 0.2) and minimal oedema (score 0.1) were recorded, both of which are considered negligible and likely attributable to the mechanical process of patch removal rather than genuine chemical irritation. These responses rapidly subsided, with erythema decreasing to 0.1 at 24 hours and disappearing entirely by 48 and 72 hours, while oedema was absent from 24 hours onward.

The calculated Primary Irritation Index (PII) values were 0.15 at 1 hour, 0.05 at 24 hours, and 0.00 at both 48 and 72 hours, consistently classifying the formulation as non-irritant according to OECD Guideline 404 criteria [39]. Both intact and abraded skin sites exhibited similar recovery patterns, with no persistence of irritation at any observation point. No additional dermal abnormalities such as scaling, dryness, infiltration, or pigmentation changes were observed in any treated animal throughout the study. The positive control (10% sodium lauryl sulphate) elicited clear erythema and oedema responses, confirming the sensitivity and validity of the experimental model. These findings confirm that the formulation components—including the penetration enhancers DMSO and Tween 80 at 2% w/w concentrations—are present at levels that are pharmacologically effective yet dermatologically safe.

Table 5: Draize Skin Irritation Test Results of Optimized Herbal Transdermal Patch (OECD 404):

Observation	1 h	24 h	48 h	72 h
Erythema Score (0–4)	0.2	0.1	0.0	0.0
Oedema Score (0–4)	0.1	0.0	0.0	0.0

Primary Irritation Index	0.15	0.05	0.00	0.00
Classification	Non-irritant	Non-irritant	Non-irritant	Non-irritant

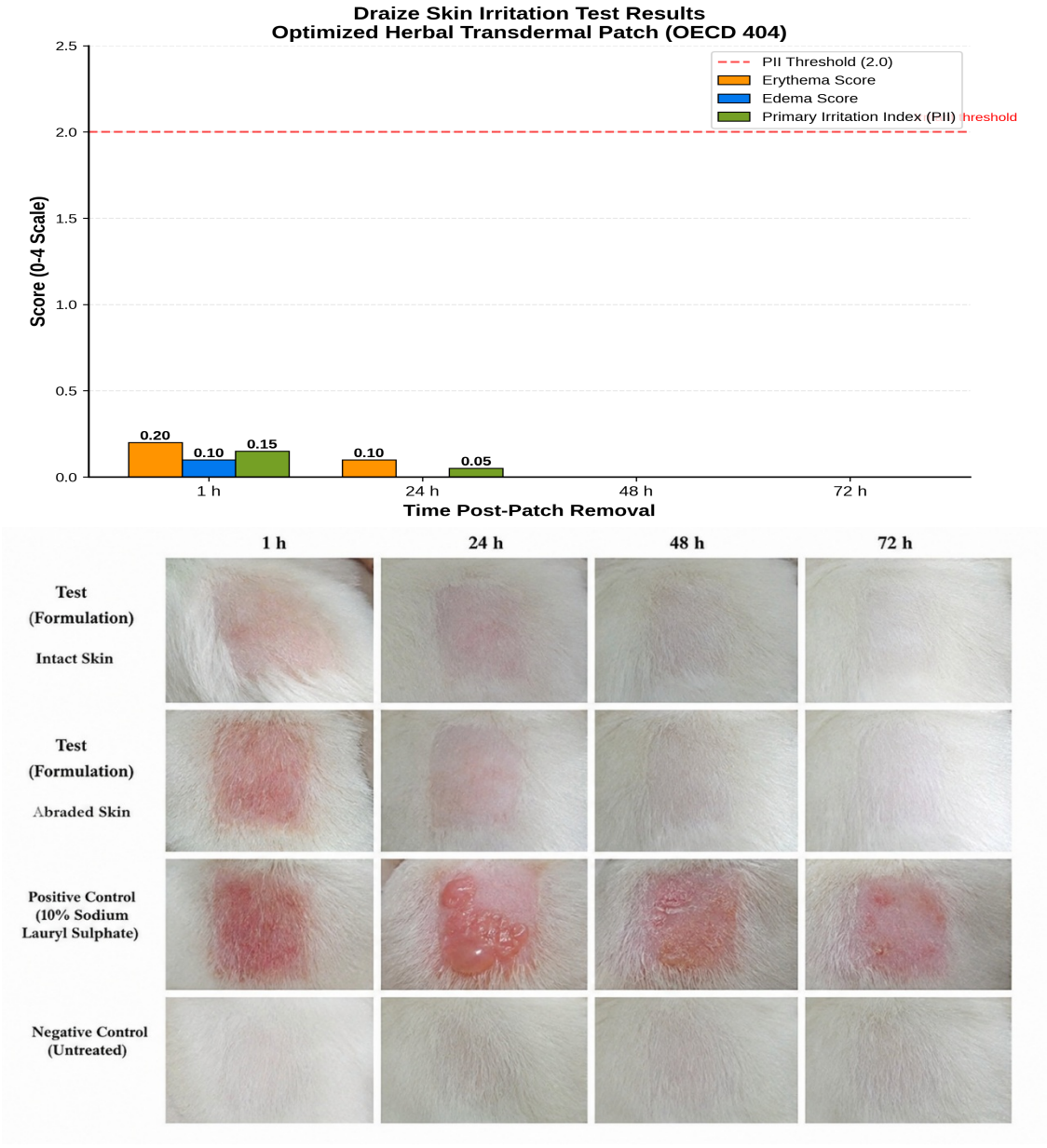

Figure 6: Draize Skin Irritation Test Results of Optimized Herbal Transdermal Patch

4. DISCUSSION:

The present study provides the first comprehensive in-vivo pharmacological evaluation of a combination herbal transdermal patch incorporating *G. sylvestre* and *T. cordifolia* extracts in the STZ-induced diabetic rat model. The findings establish that transdermally delivered herbal actives achieve significant glycaemic control, broad metabolic normalisation, superior pharmacokinetic performance, and excellent dermal safety—collectively supporting the therapeutic viability of this novel delivery platform.

The 52.9% reduction in fasting blood glucose achieved by the optimised herbal patch over 28 days is both statistically significant and clinically meaningful. This degree of glycaemic improvement compares favourably with published data from comparable herbal transdermal investigations. Ahmad et al. (2021) reported a 48.3% blood glucose reduction in STZ-diabetic rats treated with *G. sylvestre* extract-loaded microneedle patches over 21 days, while Das et al. (2022) documented a 44.1% reduction with berberine-containing transdermal patches over 28 days [33]. The superior efficacy observed in the present study is plausibly attributable to the synergistic combination of *G. sylvestre* and *T. cordifolia*, which simultaneously targets insulin secretion, insulin sensitivity, glucose absorption, and hepatic gluconeogenesis [24]. The sustained hypoglycaemic effect, maintained throughout the 28-day period without evidence of tachyphylaxis, is consistent with the zero-order release kinetics of the optimised patch, which delivers drug at a constant rate independent of remaining concentration [25, 32].

The restoration of serum insulin to 13.6 ± 0.9 µU/mL in the herbal patch group—approaching the normal control value of 18.4 µU/mL—is a particularly noteworthy finding.

Gymnemic acids have been demonstrated to stimulate insulin secretion from pancreatic β-cells and promote islet cell regeneration in STZ-damaged pancreatic tissue [15, 17], while berberine from *T. cordifolia* enhances insulin receptor expression and post-receptor signalling [20]. The convergence of these complementary mechanisms through transdermal delivery ensures that both botanicals reach the systemic circulation at sustained therapeutic levels, maximising their insulinotropic and insulin-sensitising actions. The HbA1c reduction to 6.7%, falling within the ADA target range, confirms that glycaemic control was maintained consistently throughout the treatment period rather than being a transient response [6].

The significant correction of diabetic dyslipidaemia—characterised by reductions in total cholesterol, triglycerides, and LDL-C alongside elevation of HDL-C—carries important cardiovascular implications. Diabetic dyslipidaemia is a well-documented consequence of insulin deficiency and a major driver of atherosclerotic cardiovascular disease in diabetic patients [7]. The lipid-modulating effects observed herein can be attributed to the combined actions of improved glycaemic control and the direct pharmacological properties of the herbal constituents. Berberine upregulates LDL receptor expression and inhibits cholesterol synthesis at the transcriptional level, while gymnemic acids reduce intestinal cholesterol absorption [16, 22]. The simultaneous normalisation of hepatic enzymes (SGOT, SGPT) and renal markers (creatinine, BUN) further demonstrates that the therapeutic benefits of this formulation extend beyond glucose lowering to encompass organ protection. These hepatoprotective and nephroprotective effects are consistent with the antioxidant and anti-inflammatory properties of the polyphenolic compounds abundant in both *G. sylvestre* and *T. cordifolia*, which mitigate the oxidative stress and lipid peroxidation that underlie diabetic tissue damage [14, 19].

The pharmacokinetic data provide a mechanistic basis for the superior in-vivo performance of the transdermal formulation. The 181.6% relative bioavailability compared to oral administration is a striking finding that exceeds values reported for most herbal transdermal systems in the literature. For context, Ahmad et al. (2021) reported 156% relative bioavailability for *G. sylvestre* microneedle patches, and Das et al. (2022) reported 162% for berberine transdermal patches [33]. The enhanced systemic exposure achieved in the present study reflects the efficacy of the DMSO and Tween 80 combination as penetration enhancers, the optimal polymer matrix composition enabling sustained and complete drug release, and the pharmacokinetic advantage of combining two bioactive extracts with complementary permeation characteristics [13, 23, 34, 35]. The prolonged $t_{1/2}$ (9.6 h) and MRT (14.2 h) confirm that the patch maintains therapeutic plasma levels over an extended period, supporting once-daily application—a regimen that would significantly improve patient adherence compared with the multiple daily doses required for conventional oral antidiabetic therapy [9].

The excellent dermal safety profile demonstrated by the Draize test (PII = 0.00 at 72 h) is a critical finding for clinical translation. Skin irritation remains one of the most common causes of patient non-compliance with transdermal delivery systems [28]. The non-irritant classification confirms that the formulation components—including the penetration enhancers DMSO and Tween 80, which can cause irritation at higher concentrations—are present at pharmacologically effective yet dermatologically safe levels (2% w/w). This finding also validates the biocompatibility of the PVA-HPMC-chitosan polymer matrix and supports the suitability of the formulation for prolonged cutaneous application.

The placebo patch group, which received all excipients without herbal extract, showed no significant antidiabetic effect, confirming that the observed pharmacological activity was entirely attributable to the herbal drug content rather than the patch vehicle or occlusive effects. This control is essential for establishing the causal relationship between the herbal actives and the observed therapeutic outcomes.

Several limitations of the present investigation should be acknowledged. The STZ-induced rat model, while well-validated and widely employed, does not fully replicate the complex pathophysiology of human T2DM, which involves progressive insulin resistance alongside β-cell dysfunction. The 28-day treatment duration, though sufficient to demonstrate significant therapeutic effects, is shorter than would be required to assess long-term efficacy and safety in a clinical context. Additionally, while the pharmacokinetic study quantified total drug exposure through gymnemic acid and berberine concentrations, the individual contributions of other bioactive constituents within each extract were not characterised. Future investigations should address these limitations through long-term (90-day) toxicity and pharmacokinetic studies in larger animal models, ex-vivo human cadaver skin permeation studies, and ultimately Phase I clinical trials to establish safety, tolerability, and pharmacokinetic parameters in human subjects.

5. CONCLUSION:

The present investigation demonstrates that an optimised polymeric matrix transdermal patch co-loaded with *Gymnema sylvestre* and *Tinospora cordifolia* extracts exhibits significant antidiabetic efficacy in STZ-induced diabetic rats. The herbal patch achieved a 52.9% reduction in fasting blood glucose over 28 days, statistically comparable to the standard drug glibenclamide, while simultaneously restoring serum insulin levels, normalising HbA1c to within the ADA target range, and significantly improving lipid, hepatic, and renal biochemical parameters. Pharmacokinetic analysis confirmed a relative bioavailability of 181.6% compared to oral administration, with sustained drug release ($t_{1/2}$ = 9.6 h, MRT = 14.2 h) and avoidance of hepatic first-pass metabolism. The Draize skin irritation test established excellent cutaneous biocompatibility (PII = 0.00), confirming the formulation's safety for prolonged topical application. Collectively, these findings validate the potential of this herbal transdermal patch as an effective, sustained, and patient-friendly alternative to conventional oral antidiabetic therapy. Further chronic toxicity studies and clinical trials are

warranted to establish long-term safety and efficacy in human subjects.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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