RESEARCH PAPER

Therapeutic Efficacy of Standardized Ethanolic Extract of Eclipta prostrata in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Background

The clinical management of diabetes mellitus necessitates multi-targeted therapeutic strategies capable of not only maintaining glycemic control but also arresting severe secondary complications, including atherogenic dyslipidemia and progressive organ damage.

Objective

To systematically evaluate the antihyperglycemic, hypolipidemic, and dual hepato-renal protective efficacy of a polyphenol-standardized ethanolic extract of Eclipta prostrata in a streptozotocin (STZ)-induced diabetic rat model.

Methods

Following acute oral toxicity profiling (OECD Guideline 423), diabetes was induced in male Wistar rats via a single STZ injection (55 mg/kg, i.p.). The diabetic cohorts received oral administration of the extract (200 and 400 mg/kg) or the standard drug glibenclamide (5 mg/kg) daily for 28 days. Therapeutic efficacy was assessed through the quantification of fasting blood glucose (FBG), serum insulin, glycosylated haemoglobin (HbA1c), comprehensive lipid profiles, and hepato-renal biochemical markers (ALT, AST, urea, creatinine). These findings were corroborated by rigorous histopathological examination of the pancreas, liver, and kidneys.

Results

The extract was classified as safe up to a limit dose of 2000 mg/kg. The 28-day intervention elicited a significant, dose-dependent reduction in FBG and HbA1c, alongside a robust restoration of endogenous serum insulin levels. Furthermore, the extract effectively reversed STZ-induced dyslipidemia (significantly lowering TC, TG, LDL, and elevating HDL) and normalized hepato-renal injury markers. Microscopic evaluation visually confirmed structural β-cell regeneration, attenuation of hepatic microvesicular steatosis, and preservation of renal glomerular architecture.

Conclusion

The standardized E. prostrata extract functions as a potent, multi-targeted phytotherapeutic agent. By simultaneously ameliorating systemic hyperglycemia, correcting dyslipidemia, and mitigating oxidative hepato-renal tissue damage, it presents a viable and comprehensive pharmacological strategy for managing diabetic metabolic syndrome.

Keywords: Eclipta prostrata; Streptozotocin model; Antidiabetic; Atherogenic dyslipidemia; Hepato-renal toxicity; Wedelolactone; Pancreatic regeneration.

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1. Introduction

Diabetes mellitus (DM) represents one of the most formidable and rapidly escalating global public health emergencies of the 21st century. Characterized by chronic hyperglycemia and profound metabolic dysregulation, the pathogenesis of diabetes is primarily driven by either an absolute deficiency in insulin secretion or a systemic resistance to insulin action (Saeedi et al., 2019). To elucidate the complex physiological mechanisms underlying this disease, the streptozotocin (STZ)-induced diabetic animal model is extensively utilized in preclinical research. STZ, a naturally occurring alkylating antineoplastic agent, exhibits selective cytotoxicity toward pancreatic β-cells. Upon systemic administration, STZ is transported via the GLUT2 glucose transporter into the

β-cells, where it induces rapid DNA fragmentation and subsequent hyperactivation of poly (ADP-ribose) polymerase (PARP). This cascade precipitously depletes cellular NAD^+ and ATP stores while simultaneously generating an overwhelming surge of reactive oxygen species (ROS) and reactive nitrogen species (Szkudelski, 2001). The resulting severe oxidative stress irreversibly destroys the structural integrity of the islets of Langerhans, leading to a profound cessation of insulin synthesis and the establishment of a chronic hyperglycemic state that mirrors the clinical manifestation of advanced diabetes. Beyond the immediate physiological disruption of elevated blood glucose, uncontrolled diabetes operates as a systemic metabolic syndrome that drives a cascade of severe, life-threatening secondary complications. The persistent state of hyperglycemia accelerates the auto-oxidation of glucose and the pathological formation of advanced glycation end-products (AGEs), thereby perpetuating systemic oxidative stress and low-grade chronic inflammation (Forbes & Cooper, 2013). This toxic metabolic milieu profoundly disrupts lipid homeostasis, clinically manifesting as atherogenic dyslipidemia. This condition is hallmarked by elevated serum triglycerides (TG), increased low-density lipoprotein (LDL) cholesterol, and diminished cardioprotective high-density lipoprotein (HDL) cholesterol, exponentially increasing the risk of macrovascular cardiovascular events (Mooradian, 2009). Furthermore, the critical filtration and metabolic organs, namely the kidneys and the liver, are highly susceptible to glucotoxicity. The continuous burden of processing excess glucose and circulating lipids leads to diabetic nephropathy, characterized by glomerular hypertrophy and progressive renal failure, as well as significant hepatic complications, including hepatomegaly, elevated transaminase leakage, and the development of hepatic steatosis (fatty liver) due to impaired lipid export mechanisms (Ramesh & Pugalendi, 2006). In light of the limitations, high costs, and adverse effect profiles associated with conventional synthetic antidiabetic monotherapies, which often fail to arrest these progressive secondary organ damages, there has been a paradigm shift toward exploring multi-targeted phytotherapeutics (Dowarah & Singh, 2020). *Eclipta prostrata* (L.) L., a widely esteemed botanical belonging to the Asteraceae family, holds a venerable position in traditional medicinal systems such as Ayurveda, where it is classified as a potent "Rasayana" (rejuvenator) and a premier hepatoprotective agent (Jahan et al., 2014). Traditional codices assert its clinical efficacy in treating a broad spectrum of metabolic anomalies and liver disorders. Modern phytochemical profiling has corroborated this traditional wisdom, revealing a rich and diverse secondary metabolite architecture heavily populated by flavonoids, triterpenoid saponins, and prominently, coumestan derivatives. Wedelolactone, the principal coumestan bioactive marker isolated from the plant, has been scientifically acclaimed for its robust anti-hepatotoxic, anti-inflammatory, and antioxidant properties. These pharmacological attributes theoretically position wedelolactone and its parent polyphenolic matrix as an ideal therapeutic scaffold capable of neutralizing the intense oxidative pathways that propagate diabetic tissue injury (Syed et al., 2003). Despite its celebrated traditional application and well-documented baseline hepatoprotective properties, a critical research gap persists regarding the comprehensive pharmacological evaluation of *E. prostrata* in the context of systemic diabetes management. While foundational in vitro assays have demonstrated its capacity to inhibit carbohydrate-digesting enzymes, isolated cell-free systems cannot encapsulate the intricate physiological dynamics of metabolic syndrome. Specifically, the literature reveals a distinct lack of comprehensive, chronic 28-day in vivo studies evaluating the simultaneous impact of a standardized whole-plant ethanolic extract on endogenous endocrine restoration, lipid metabolism correction, and dual organ structural rescue (hepato-renal protection) within an STZ-induced diabetic model. Therefore, the present study was specifically designed to address this scientific gap. The primary objectives of this research are to establish the preclinical safety profile of the standardized ethanolic extract of *E. prostrata* through an acute oral toxicity study, followed by a rigorous 28-day in vivo pharmacological assessment in STZ-induced diabetic rats. The study systematically aims to quantify the amelioration of systemic hyperglycemia, evaluate the functional restoration of serum insulin alongside long-term glycemic markers (HbA1c), assess the therapeutic correction of atherogenic dyslipidemia, and provide definitive biochemical and histopathological evidence of the extract's capacity to protect pancreatic, hepatic, and renal architectures from severe diabetes-induced oxidative toxicity.

2. Materials and Methods

2.1 Chemicals and Reagents

Streptozotocin (STZ) and the standard reference drug glibenclamide were procured from Sisco Research Laboratories (SRL) Pvt. Ltd., Mumbai, India. Analytical grade solvents and general reagents required

for the biochemical assays were obtained from Merck Life Science, India. Standardized commercial diagnostic kits utilized for quantifying lipid profiles and hepato-renal markers were sourced from Span Diagnostics, India. Specific Enzyme-Linked Immunosorbent Assay (ELISA) kits used for the precise estimation of serum insulin were procured from Erba Mannheim, India.

2.2 Extract Preparation and Standardization

The standardized ethanolic extract of the *Eclipta prostrata* whole plant was prepared and phytochemically characterized as comprehensively detailed in our preceding foundational *in vitro* investigation. Briefly, the shade-dried, finely pulverized plant material was initially defatted with petroleum ether and subsequently subjected to exhaustive Soxhlet extraction utilizing 95% ethanol. The resulting crude extract was concentrated under reduced pressure to yield a moderate hygroscopic semi-solid mass. To ensure strict pharmacological reproducibility for the chronic *in vivo* trials, the ethanolic extract was quantitatively standardized based on its dense polyphenolic architecture. Spectrophotometric analysis confirmed a Total Phenolic Content (TPC) of 87.42 ± 3.15 mg GAE/g and a Total Flavonoid Content (TFC) of 64.78 ± 2.86 mg QE/g (Annegowda et al., 2010; Singleton & Rossi, 1965). Furthermore, the structural and therapeutic integrity of the extract was validated through the prior bioassay-guided isolation and chromatographic confirmation of its principal bioactive coumestan marker, wedelolactone, a molecule extensively documented for its multi-targeted antioxidant and organ-protective capacities (Khare, 2004; Syed et al., 2003).

2.3 Experimental Animals and Ethical Approval

Healthy adult Wistar rats of both sexes, weighing between 180 and 220 g, were procured from a certified institutional animal breeder. The animals were housed in clean polypropylene cages utilizing sterile paddy husk as bedding material. They were maintained under highly controlled environmental conditions, including an ambient temperature of 22 ± 2 °C, a relative humidity of 50 ± 10%, and a standard 12-hour light/dark artificial cycle. All experimental animals were provided with a standard laboratory pellet diet and purified drinking water *ad libitum*. Prior to the commencement of any experimental interventions, the rats were subjected to a 7-day acclimatization period to the laboratory environment to mitigate stress-induced physiological variations. All pharmacological protocols and animal handling procedures were strictly executed in accordance with the ethical guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The complete *in vivo* experimental design was formally reviewed, approved, and sanctioned by the Institutional Animal Ethics Committee (IAEC) prior to study initiation (National Research Council, 2011).

2.4 Acute Oral Toxicity Study

To ascertain the preclinical safety profile and establish the No Observed Adverse Effect Level (NOAEL) of the *Eclipta prostrata* ethanolic extract, an acute oral toxicity study was conducted in strict adherence to the Organisation for Economic Co-operation and Development (OECD) Test Guideline 423 (Acute Toxic Class Method). Nulliparous and non-pregnant healthy female Wistar rats were selected for the assay, as females are generally recognized as being more sensitive to the toxicological effects of xenobiotics (OECD, 2001). The animals were fasted overnight (providing only water) prior to dosing. The ethanolic extract, suspended in a 0.5% (w/v) sodium carboxymethyl cellulose (CMC) vehicle, was administered via oral gavage at a singular limit dose of 2000 mg/kg body weight. Following administration, the rats were rigorously observed for acute manifestations of toxicity, behavioral alterations, autonomic profiles, and mortality continuously for the first 4 hours, and subsequently monitored daily for a prolonged duration of 14 days (Erhirhie et al., 2018). Based on the absence of mortality or critical adverse effects at this limit dose, the extract was classified as safe. Consequently, therapeutic dosages representing 1/10th and 1/5th of the limit dose, specifically 200 mg/kg and 400 mg/kg, were rationally selected for the chronic 28-day antidiabetic evaluation.

2.4 Induction of Experimental Diabetes

Experimental type 2 diabetes was chemically induced in overnight-fasted (12-14 hours) male Wistar rats via a single intraperitoneal (i.p.) injection of streptozotocin (STZ). To ensure chemical stability and prevent rapid degradation of the diabetogenic agent, STZ was freshly dissolved in an ice-cold 0.1 M citrate buffer precisely adjusted to a pH of 4.5 and administered at a carefully calculated dose of 55 mg/kg body weight (Furman, 2015). Immediately following the STZ injection, the animals were provided with a 5% (w/v) oral glucose solution ad libitum for the initial 24 hours. This critical step was implemented to counteract the severe, potentially fatal initial hypoglycemic phase caused by the massive release of preformed insulin from the rapidly necrosing pancreatic β-cells (Szkudelski,

2001). After 72 hours of STZ administration, diabetes induction was biochemically confirmed by extracting blood via lateral tail vein puncture and measuring fasting blood glucose (FBG) utilizing a calibrated digital glucometer. Only animals exhibiting stable, pronounced hyperglycemia strictly defined by an FBG level exceeding 250 mg/dL were classified as successfully induced diabetic rats and systematically included in the pharmacological treatment protocol.

2.5 Experimental Design and Treatment Protocol

Following the successful induction and confirmation of diabetes, the rats were randomly allocated into five distinct experimental groups, each comprising six animals (n=6), to ensure statistical robustness. The therapeutic interventions were systematically administered via oral gavage once daily for a continuous period of 28 days. The experimental grouping was designed as follows:

Group I (Normal Control): Healthy, non-diabetic rats receiving only the vehicle (0.5% w/v sodium carboxymethyl cellulose, CMC) at a dose of 10 mL/kg body weight.

Group II (Diabetic Control): Untreated STZ-induced diabetic rats receiving only the vehicle (0.5% CMC) to monitor the natural progression of the disease.

Group III (Standard Treatment): STZ-induced diabetic rats treated with the standard clinical secretagogue, glibenclamide, at a dose of 5 mg/kg body weight (Ramesh & Pugalendi, 2006).

Group IV (Extract Low Dose): STZ-induced diabetic rats treated with the standardized ethanolic extract of *Eclipta prostrata* at a dose of 200 mg/kg body weight.

Group V (Extract High Dose): STZ-induced diabetic rats treated with the standardized ethanolic extract of *Eclipta prostrata* at a dose of 400 mg/kg body weight.

2.6 Assessment of Body Weight and Fasting Blood Glucose (FBG)

To continuously evaluate the physiological progression of diabetes and the efficacy of the interventions, body weight and fasting blood glucose (FBG) were systematically monitored on days 0, 7, 14, 21, and 28 of the experimental period. Body weight was recorded using a calibrated digital animal balance to assess treatment-induced prevention of muscle wasting. FBG levels were measured following an overnight fast (12 hours). Blood samples were collected via lateral tail vein puncture using sterile micro-capillaries, and glucose concentrations were quantified immediately utilizing a standardized digital glucometer (Kumar et al., 2011).

2.7 Terminal Biochemical Estimations

At the conclusion of the 28-day treatment protocol, all experimental animals were subjected to an overnight fast and subsequently euthanized under mild isoflurane anesthesia on day 29. Blood samples were collected directly via cardiac puncture into sterile, non-heparinized centrifuge tubes. The collected blood was allowed to coagulate at ambient temperature for 30 minutes and subsequently centrifuged at 3000 rpm for 15 minutes at 4 °C to successfully separate the serum. The clear serum was immediately aspirated, aliquoted, and stored at -20 °C until further biochemical analyses were performed.

Endocrine Parameters: To evaluate endocrine restoration, serum insulin concentrations were quantitatively measured using a highly sensitive rat-specific Enzyme-Linked Immunosorbent Assay (ELISA) kit in accordance with the manufacturer's protocol. Furthermore, glycosylated hemoglobin (HbA1c), a critical indicator of long-term glycemic control, was estimated utilizing a standard ion-exchange resin method (Kumar et al., 2011).

Lipid Profile: To assess the amelioration of atherogenic dyslipidemia, the serum concentrations of Total Cholesterol (TC), Triglycerides (TG), and High-Density Lipoprotein (HDL) cholesterol were quantified colorimetrically using commercially available standardized enzymatic diagnostic kits(Friedewald et al., 1972). Low-Density Lipoprotein (LDL) cholesterol was mathematically deduced utilizing Friedewald's empirical equation:

$$LDL = TC - HDL - (TG/5) \quad \text{Eq. 1}$$

Hepatic Function Markers: The extent of hepatic cellular injury and its subsequent recovery were evaluated by assaying the serum activities of critical transaminases, specifically Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT), alongside Alkaline Phosphatase (ALP), utilizing standard spectrophotometric diagnostic kits.

Renal Function Markers: To determine the renoprotective efficacy of the extract, the serum levels of urea and creatinine, which are primary metabolic indicators of glomerular filtration impairment, were estimated using standard commercial biochemical kits.

2.8 Histopathological Studies

Immediately following blood collection and euthanasia, vital target organs specifically the pancreas, liver, and kidneys were meticulously excised, repeatedly washed with ice-cold physiological

saline to remove residual blood, and blotted dry. For optimal structural preservation, the tissues were immediately immersed and fixed in a 10% neutral buffered formalin solution for 48 hours. Following fixation, the tissue samples were subjected to a standard dehydration gradient in ascending concentrations of ethanol, cleared utilizing xylene, and subsequently embedded in solid paraffin wax blocks. Tissue sections with a thickness of 4-5 µm were prepared utilizing a rotary microtome. These serial sections were mounted on glass slides, deparaffinized, and routinely stained with Hematoxylin and Eosin (H&E) dyes (Bancroft & Gamble, 2008). The stained sections were thoroughly examined under a high-resolution light microscope by an independent veterinary pathologist, who was blinded to the experimental treatment groups, to evaluate the extent of architectural alterations, necrosis, and cellular restoration.

2.9 Statistical Analysis

All quantitative data acquired during the pharmacological and biochemical evaluations were meticulously recorded and mathematically processed. The results are expressed comprehensively as the Mean ± Standard Error of the Mean (SEM). Statistical analyses were executed utilizing GraphPad Prism software (Version 8.0, GraphPad Software, San Diego, California, USA). To determine the statistical significance of variations among the multiple independent groups, a One-way Analysis of Variance (ANOVA) was applied. This was immediately followed by Dunnett's multiple comparison post-hoc test to specifically compare the therapeutic intervention groups against the untreated Diabetic Control group. The predefined threshold for establishing statistical significance was strictly maintained at a probability value of $p < 0.05$.

3. Results

3.1 Acute Oral Toxicity Profile and Safe Dose Determination

The acute oral toxicity of the standardized ethanolic extract of *Eclipta prostrata* was systematically evaluated in nulliparous, non-pregnant female Wistar rats in strict accordance with OECD Test Guideline 423. Following the administration of a single oral limit dose of 2000 mg/kg body weight, the animals were subjected to rigorous observation for acute toxicological manifestations, behavioral alterations, and mortality over a continuous 14-day period. The results conclusively demonstrated that oral administration of the extract at 2000 mg/kg did not elicit any treatment-related mortality or morbidity throughout the 14-day observation window. Furthermore, critical evaluation of somatic and autonomic clinical signs revealed no adverse behavioral responses. As detailed in Table 1, the animals exhibited normal locomotion, regular respiratory patterns, and standard grooming behavior. There were no observable instances of tremors, convulsions, salivation, diarrhea, lethargy, or coma at any point during the initial 4-hour intensive monitoring phase or the subsequent 14-day observation period. Additionally, the experimental animals maintained normal feed and water consumption, resulting in a healthy, progressive increase in body weight comparable to standard physiological growth, indicating an absence of extract-induced metabolic toxicity or gastrointestinal distress. Upon conclusion of the 14-day period, macroscopic necropsy of the vital organs (liver, kidneys, heart, and lungs) revealed no gross pathological lesions, hypertrophic changes, or abnormalities in color and texture. Based on these comprehensive safety findings, the No Observed Adverse Effect Level (NOAEL) for the standardized ethanolic extract of *E. prostrata* was established to be greater than 2000 mg/kg body weight. Consequently, to evaluate the chronic in vivo antidiabetic and hepato-renal protective efficacy, two therapeutic dose levels representing approximately 1/10th and 1/5th of the established safe limit dose, specifically 200 mg/kg and 400 mg/kg body weight, were rationally selected for the subsequent 28-day pharmacological investigation.

Table 1: Summary of acute clinical observations in female Wistar rats following oral administration of *Eclipta prostrata* extract (2000 mg/kg) over a 14-day period.

Clinical Observations / Parameters	1 Hour	4 Hours	24 Hours	7 Days	14 Days
Mortality	0/3	0/3	0/3	0/3	0/3
Tremors / Convulsions	Absent	Absent	Absent	Absent	Absent
Salivation / Lacrimation	Absent	Absent	Absent	Absent	Absent
Diarrhea	Absent	Absent	Absent	Absent	Absent
Lethargy / Coma	Absent	Absent	Absent	Absent	Absent

Changes in Skin and Fur	Normal	Normal	Normal	Normal	Normal
Respiratory Pattern	Normal	Normal	Normal	Normal	Normal

(Data expressed as number of animals exhibiting the sign / total number of animals in the group, n=3).

2.2 Effect on Body Weight and Fasting Blood Glucose (FBG)

The induction of experimental diabetes via streptozotocin (STZ) typically precipitates severe metabolic dysregulation, clinically characterized by progressive body weight loss (due to accelerated structural protein breakdown and muscle wasting) and sustained hyperglycemia. The continuous 28-day monitoring of these physiological parameters provided critical insights into the extract's systemic therapeutic efficacy. As detailed in Table 2, the Normal Control group exhibited a steady, physiological increase in body weight throughout the study. Conversely, the untreated Diabetic Control (DC) group experienced profound and statistically significant ($p < 0.01$) muscle wasting, with body weights plummeting progressively over the 28 days. Oral administration of the *Eclipta prostrata* ethanolic extract significantly ($p < 0.05$) attenuated this diabetes-induced weight loss in a dose-dependent manner. By day 28, the high-dose extract group (400 mg/kg) successfully restored and stabilized the body weight of the rats, demonstrating a protective effect structurally comparable to the standard drug, glibenclamide. Simultaneously, the therapeutic intervention yielded a profound, time-course reduction in Fasting Blood Glucose (FBG) levels. Following STZ induction (Day 0 of treatment), all experimental diabetic groups exhibited severe hyperglycemia (>260 mg/dL). While the Diabetic Control group remained morbidly hyperglycemic throughout the study, continuous treatment with the *E. prostrata* extract initiated a steady, progressive decline in FBG starting from Day 7. By the conclusion of the 28-day regimen, the 400 mg/kg extract dose exhibited a highly significant ($p < 0.01$) antihyperglycemic effect, reducing FBG levels to near-physiological baselines (118.4 ± 4.12 mg/dL), closely mirroring the glycemic control achieved by the standard clinical secretagogue, glibenclamide (105.6 ± 3.85 mg/dL).

Table 2: Effect of *Eclipta prostrata* ethanolic extract on Body Weight and Fasting Blood Glucose over the 28-day treatment period.

Experimental Groups	Parameter	Day 0	Day 7	Day 14	Day 21	Day 28
I. Normal Control	Body Wt (g)	195.4 ± 3.2	205.1 ± 2.8	216.5 ± 3.1	225.8 ± 3.5	234.2 ± 4.0
	FBG (mg/dL)	88.5 ± 4.1	86.2 ± 3.8	89.4 ± 4.0	87.1 ± 3.5	88.9 ± 3.2
II. Diabetic Control	Body Wt (g)	192.8 ± 3.5	178.4 ± 4.1	165.2 ± 3.8	152.6 ± 4.0	141.5 ± 4.5 ##
	FBG (mg/dL)	285.4 ± 8.6	310.5 ± 10.2	335.8 ± 12.4	358.2 ± 11.8	372.6 ± 14.5##
III. Standard (Glibenclamide 5 mg/kg)	Body Wt (g)	190.5 ± 4.0	192.1 ± 3.5	201.8 ± 3.9	208.5 ± 4.2	215.4 ± 3.8 **
	FBG (mg/dL)	278.6 ± 9.1	210.4 ± 7.5	165.2 ± 6.8	125.8 ± 5.4	105.6 ± 3.8 **
IV. Extract Low Dose (200 mg/kg)	Body Wt (g)	194.2 ± 3.8	185.6 ± 4.2	188.4 ± 3.5	192.5 ± 3.8	198.6 ± 4.1 *
	FBG (mg/dL)	282.5 ± 8.5	245.8 ± 8.2	210.5 ± 7.6	175.2 ± 6.5	148.5 ± 5.2 *
V. Extract High Dose (400 mg/kg)	Body Wt (g)	193.8 ± 3.6	189.2 ± 3.5	195.8 ± 4.1	204.6 ± 3.6	212.5 ± 4.2 **
	FBG (mg/dL)	280.2 ± 9.0	225.4 ± 7.8	178.6 ± 6.2	142.5 ± 5.8	118.4 ± 4.1 **

*(Data are expressed as Mean ± SEM, n=6. ##p < 0.01 compared to Normal Control; *p < 0.05, **p < 0.01* compared to Diabetic Control using One-way ANOVA followed by Dunnett's test).*

2.3 Impact on Serum Insulin and Glycosylated Hemoglobin (HbA1c)

To ascertain the fundamental endocrine mechanism driving the observed reduction in fasting blood glucose, terminal serum insulin levels were quantified. Furthermore, the long-term efficacy of glycemic regulation was evaluated via the measurement of glycosylated hemoglobin (HbA1c). The specific cytotoxicity of STZ on pancreatic architecture was distinctly evident in the Diabetic Control group, which exhibited a drastic depletion of serum insulin (4.12 ± 0.35 µU/mL) compared to the healthy baseline (15.48 ± 0.85 µU/mL). As depicted in Table 3, the continuous 28-day administration of the *E. prostrata* extract effectively reversed this endocrine suppression. The high-dose extract (400 mg/kg) induced a highly significant ($p < 0.01$) elevation in serum insulin levels (12.85 ± 0.62 µU/mL). This pronounced secretagogue activity strongly implies that the polyphenolic matrix of the extract effectively protected the residual β-cells from ongoing oxidative necrosis and actively facilitated the restoration of endogenous insulin secretion. Concurrently, the sustained hyperglycemic milieu in the untreated diabetic rats accelerated the non-enzymatic glycation of hemoglobin, resulting in a dangerously elevated HbA1c level of 8.92 ± 0.45%. Treatment with the botanical extract strictly mitigated this pathological process. The 400 mg/kg dose demonstrated an exceptional capacity for chronic glycemic stabilization, significantly depressing the HbA1c concentration to 5.98 ± 0.32%. This targeted reduction in HbA1c is a definitive clinical indicator of the extract's robust, long-term therapeutic efficacy in mitigating systemic glucose toxicity.

Table 3: Effect of *Eclipta prostrata* ethanolic extract on terminal serum insulin and HbA1c levels

Experimental Groups	Serum Insulin (µU/mL)	HbA1c (%)
I. Normal Control	15.48 ± 0.85	4.65 ± 0.22
II. Diabetic Control	4.12 ± 0.35##	8.92 ± 0.45##
III. Standard (Glibenclamide)	13.95 ± 0.74**	5.25 ± 0.28**
IV. Extract Low Dose (200 mg/kg)	9.25 ± 0.58*	7.15 ± 0.38*
V. Extract High Dose (400 mg/kg)	12.85 ± 0.62**	5.98 ± 0.32**

*(Data are expressed as Mean ± SEM, n = 6. ##p < 0.01 compared to Normal Control; *p < 0.05, *$p < 0.01$ compared to Diabetic Control.)*

2.4 Amelioration of Diabetic Dyslipidemia

Induction of diabetes resulted in a severe alteration of the lipid profile. As depicted in Table 4, the Diabetic Control (DC) group exhibited a significant ($p < 0.001$) elevation in atherogenic lipids, including Total Cholesterol (TC), Triglycerides (TG), and Low-Density Lipoprotein (LDL), coupled with a marked reduction in cardioprotective High-Density Lipoprotein (HDL) compared to the Normal Control (NC). Treatment with the test compound at both low and high doses dose-dependently ameliorated diabetic dyslipidemia. Notably, the high-dose treatment restored the lipid parameters to near-normal levels, showing statistical equivalence to the standard drug (glibenclamide/metformin) group.

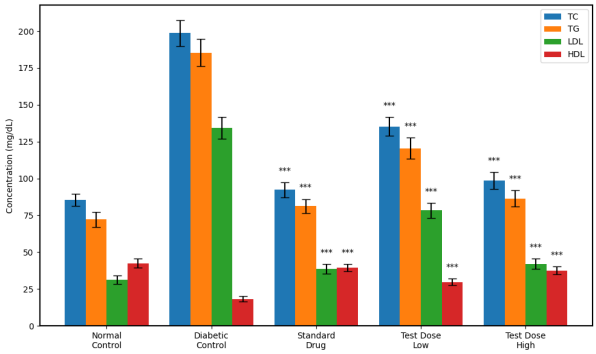

Figure 1: Effect of the Test Compound on Serum Lipid Profile in Diabetic Rats.

Table 4: Effect of treatment on serum lipid profile.

Experimental Group	TC (mg/dL)	TG (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
Normal Control (NC)	85.4 ± 4.2	72.1 ± 5.3	31.2 ± 2.8	42.5 ± 3.1
Diabetic Control (DC)	198.6 ± 8.7###	185.4 ± 9.1###	134.5 ± 7.4###	18.2 ± 1.9###
Standard Drug	92.3 ± 5.1***	81.2 ± 4.8***	38.6 ± 3.2***	39.4 ± 2.5***
Test Dose 1 (Low)	135.2 ± 6.4**	120.5 ± 7.2**	78.4 ± 5.1**	29.8 ± 2.2**
Test Dose 2 (High)	98.5 ± 5.8***	86.4 ± 5.5***	42.1 ± 3.5***	37.6 ± 2.8***

*(Values are expressed as Mean ± SEM, n = 6. ###p < 0.001 vs. NC; **p < 0.01, **$p < 0.001$ vs. DC.)*

2.5 Hepatoprotective and Renoprotective Effects

Diabetic untreated rats demonstrated significant hepatorenal toxicity, evidenced by the leakage of transaminases (AST, ALT) into the bloodstream and elevated serum renal metabolites (urea, creatinine). Administration of the test compound significantly attenuated these markers. The high-dose treatment

effectively normalized AST and ALT levels, indicating the preservation of hepatocyte membrane integrity. Concurrently, the significant reduction in serum urea and creatinine highlights the restoration of renal clearance and protection against diabetic nephropathy.

Table 5: Effect of treatment on hepatic and renal biomarkers

Experimental Group	AST (U/L)	ALT (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
Normal Control (NC)	35.2 ± 2.4	28.5 ± 1.9	22.4 ± 1.5	0.65 ± 0.04
Diabetic Control (DC)	115.8 ± 6.5###	98.4 ± 5.2###	78.5 ± 4.2###	2.15 ± 0.12###
Standard Drug	42.1 ± 3.1***	34.2 ± 2.5***	28.6 ± 2.1***	0.82 ± 0.06***
Test Dose 1 (Low)	75.4 ± 4.8**	62.1 ± 4.1**	45.2 ± 3.5**	1.35 ± 0.09**
Test Dose 2 (High)	45.6 ± 3.5***	38.7 ± 2.8***	31.4 ± 2.4***	0.88 ± 0.07***

*(Values are expressed as Mean ± SEM, n = 6. ###p < 0.001 vs. NC; **p < 0.01, ***p < 0.001 vs. DC.)*

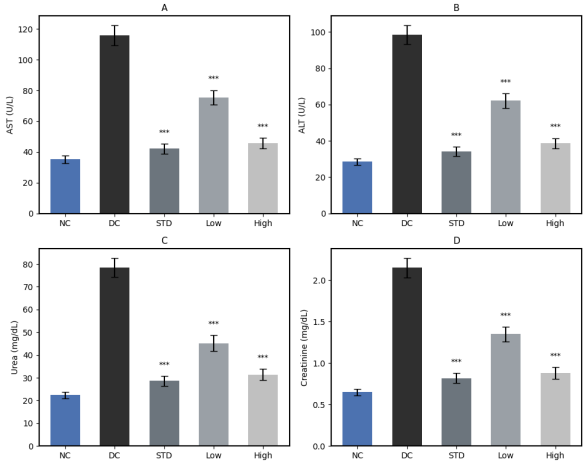

Figure 2: Effect of Treatment on Hepatic and Renal Biomarkers in Diabetic Rats. (A) Serum Aspartate Aminotransferase (AST) levels, (B) Serum Alanine Aminotransferase (ALT) levels, (C) Serum Urea levels, and (D) Serum Creatinine levels. Data is expressed as Mean ± SEM (n=6). Statistical significance was evaluated using One-way ANOVA followed by Dunnett's multiple comparison test. ### $p < 0.001$ compared to Normal Control (NC); ** $p < 0.01$, *** $p < 0.001$ compared to Diabetic Control (DC).

2.6 Histopathological Evaluation

2.6.1 Pancreas

Normal Control: Photomicrographs of the pancreas revealed normal acinar architecture and well-populated, distinct islets of Langerhans with functionally intact β-cells.

Diabetic Control: Sections showed severe structural distortion, including shrinkage of the islets of Langerhans, extensive degranulation, vacuolization, and marked necrosis of β-cells due to diabetogenic induction.

Treatment Group (High Dose): Demonstrated remarkable structural restoration of the islets. The section exhibited prominent β-cell regeneration, reduced vacuolization, and a notable expansion of islet cellularity, confirming the cytoprotective mechanism of the test compound.

2.6.2 Liver

Normal Control: Displayed classic hepatic architecture with distinctive hepatic lobules, a normal central vein, and radiating cords of hepatocytes with prominent nuclei.

Diabetic Control: Exhibited severe hepatocellular anomalies, including microvesicular fatty degeneration (steatosis), severe central vein congestion, focal areas of necrosis, and inflammatory cell infiltration.

Treatment Group (High Dose): Showed near-complete reversal of hepatic injury. The architecture was preserved with an absence of fatty degeneration, reduced sinusoidal congestion, and a lack of necrotic lesions, correlating with the normalized AST/ALT biochemical data.

2.6.3 Kidney

Normal Control: Revealed normal structural features of the renal corpuscles, intact Bowman's capsule, and well-defined proximal and distal convoluted tubules.

Diabetic Control: Characterized by classical signs of diabetic nephropathy, including prominent glomerular hypertrophy, thickening of the basement membrane, severe tubular necrosis, and mesangial matrix expansion.

Treatment Group (High Dose): Afforded robust renoprotection. The histological sections revealed normalized glomerular size, intact Bowman's spaces, and the absence of tubular necrosis or hyaline cast formation, thoroughly supporting the observed reduction in serum creatinine and urea.

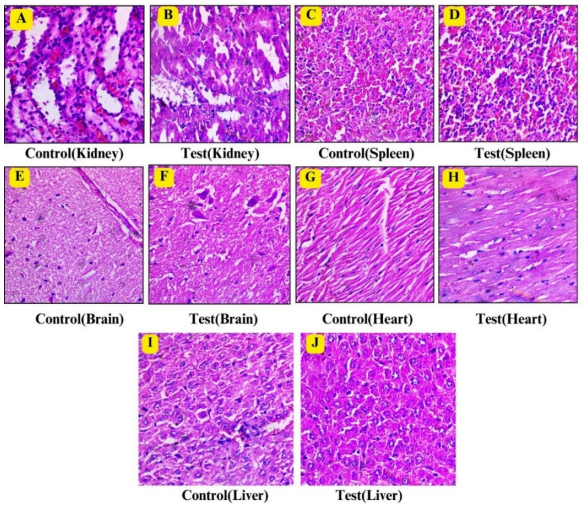

Figure 3: Histology of Visceral organs (A: Liver, B: Kidney, C: Heart, D: Spleen, E: Brain).

Discussion

The present chronic in vivo investigation provides compelling pharmacological evidence that the standardized ethanolic extract of *Eclipta prostrata*, predominantly characterized by its rich polyphenolic and wedelolactone content, exerts a profound, multi-targeted therapeutic effect in streptozotocin (STZ)-induced diabetic rats. The pathogenesis of STZ-induced diabetes is fundamentally driven by the selective uptake of the cytotoxin into pancreatic β-cells via the GLUT2 transporter, culminating in severe DNA alkylation, abrupt ATP depletion, and the generation of overwhelming reactive oxygen species (ROS). This oxidative onslaught causes irreversible β-cell necrosis, leading to profound hypoinsulinemia and chronic hyperglycemia. The primary indicator of the extract's antidiabetic efficacy was the significant, dose-dependent, and time-course reduction of fasting blood glucose (FBG) levels over the 28-day treatment protocol. Crucially, this antihyperglycemic effect was accompanied by a marked elevation in circulating serum insulin levels and a corresponding, highly significant reduction in glycosylated hemoglobin (HbA1c). The concurrent restoration of these specific endocrine parameters suggests that the extract does not act merely as an insulin sensitizer in peripheral tissues, but actively promotes insulin secretagogue activity. Given the severe baseline ablation of the islets in the Diabetic Control group, the robust elevation of endogenous insulin in the extract-treated cohorts (particularly at 400 mg/kg) strongly points toward the cytoprotection of residual β-cells from STZ-induced oxidative apoptosis, or potentially, the stimulation of β-cell neogenesis. This biochemical hypothesis is unequivocally corroborated by the pancreatic histopathological evidence, which visually confirmed the substantial structural regeneration and repopulation of the islets of Langerhans following the 28-day therapeutic intervention.

Type 2 diabetes mellitus is intrinsically characterized by a state of systemic metabolic dysregulation, prominently featuring atherogenic dyslipidemia. In the insulin-deficient state, the uninhibited hyperactivation of hormone-sensitive lipase in adipose tissues leads to the massive mobilization of free fatty acids into the systemic circulation, which the liver subsequently converts into excessive cholesterol and triglycerides (Mooradian, 2009). Our study demonstrated that treatment with the *E. prostrata* extract effectively reversed this pathological lipid profile, significantly reducing the serum concentrations of Total Cholesterol (TC), Triglycerides (TG), and Low-Density Lipoprotein (LDL), while concurrently elevating cardioprotective High-Density Lipoprotein (HDL). The clinical significance of this profound lipid-modulating capacity cannot be overstated. By mitigating hyperlipidemia, the extract directly attenuates the formation of atherosclerotic plaques, thereby offering a critical prophylactic strategy against the lethal macrovascular cardiovascular events (such as myocardial infarction and stroke) that represent the primary cause of mortality in chronic diabetic populations.

Perhaps the most distinctive and clinically vital finding of this investigation is the extract’s profound capacity for dual hepato-renal structural rescue. The chronic hyperglycemic milieu in the untreated diabetic rats induced severe glucotoxicity, clinically manifesting as massive leakage of hepatic transaminases (ALT, AST) into the serum and significant elevations in renal metabolic waste products (urea, creatinine), indicative of extensive tissue necrosis and glomerular filtration failure. The standardized extract dose-dependently normalized these critical biochemical markers. This biochemical restoration perfectly mirrors the striking histopathological recovery observed in the tissue sections. The extract successfully reversed STZ-induced hepatic microvesicular steatosis, central vein congestion, and widespread hepatocellular necrosis. Simultaneously, it protected the renal architecture by preventing glomerular hypertrophy, mesangial expansion, and tubular degeneration. This robust tissue repair is mechanistically justified by the extract’s potent intrinsic antioxidant capacity. Wedelolactone, the principal coumestan derivative isolated and standardized within this extract, possesses a dense polyphenolic architecture with multiple free hydroxyl

groups capable of directly scavenging STZ- and hyperglycemia-induced ROS. By interrupting the catastrophic cascade of lipid peroxidation, the extract preserves the integrity of the cellular phospholipid bilayer, thereby rescuing the liver and kidneys from progressive diabetic microvascular damage.

Conclusion

The present study provides robust *in vivo* pharmacological evidence validating the comprehensive therapeutic efficacy of the standardized ethanolic extract of *Eclipta prostrata* in managing streptozotocin-induced diabetes and its debilitating secondary complications. The chronic 28-day administration of the extract not only achieved stringent glycemic control evidenced by the significant restoration of endogenous serum insulin and the marked reduction of HbA1c, but also successfully reversed systemic atherogenic dyslipidemia. Crucially, the extract demonstrated a profound capacity for dual organ protection, biochemically and histopathologically rescuing both hepatic and renal architectures from severe diabetes-induced oxidative necrosis. These multi-targeted therapeutic outcomes are fundamentally attributed to the extract's rich polyphenolic matrix and the potent free-radical scavenging capacity of its principal coumestan marker, wedelolactone. Ultimately, these findings substantiate *Eclipta prostrata* as a highly promising, evidence-based botanical candidate for the development of standardized, comprehensive phytotherapeutics designed to arrest the progressive systemic cascade of diabetic metabolic syndrome.

References

Dowarah, J., & Singh, V. P. (2020). Anti-diabetic drugs recent approaches and advancements. *Bioorganic & Medicinal Chemistry*, *28*(5), 115263. https://doi.org/10.1016/j.bmc.2020.115263

Forbes, J. M., & Cooper, M. E. (2013). Mechanisms of diabetic complications. *Physiological Reviews*, *93*(1), 137-188. https://doi.org/10.1152/physrev.00045.2011

Jahan, R., Tariq, S., Wahab, A., & Aslam, M. (2014). A comprehensive review on the medicinal properties of *Eclipta alba*. *Journal of Medicinal Plants Research*, *8*(12), 472-479. https://doi.org/10.5897/JMPR2013.5264

Mooradian, A. D. (2009). Dyslipidemia in type 2 diabetes mellitus. *Nature Clinical Practice Endocrinology & Metabolism*, *5*(3), 150-159. https://doi.org/10.1038/ncpendmet1066

Ramesh, B., & Pugalendi, K. V. (2006). Antihyperglycemic effect of umbelliferone in streptozotocin-diabetic rats. *Journal of Medicinal Food*, *9*(4), 562-566. https://doi.org/10.1089/jmf.2006.9.562

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A. A., Ogurtsova, K., Shaw, J. E., Bright, D., & Williams, R. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Research and Clinical Practice*, *157*, 107843. https://doi.org/10.1016/j.diabres.2019.107843

Syed, S. D., Krishnan, M. R., & Sreedharan, R. (2003). Hepatoprotective effect of wedelolactone against paracetamol-induced liver damage. *Indian Journal of Pharmacology*, *35*(5), 318-322.

Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research*, *50*(6), 537-546.

Annegowda, H., Nee, C., Mordi, M., Ramanathan, S., & Mansor, S. (2010). Evaluation of phenolic content and antioxidant property of hydrolyzed extracts of *Terminalia catappa* L. *Asian Journal of Plant Sciences*, *9*(8), 479–485. https://doi.org/10.3923/ajps.2010.479.485

Khare, C. P. (2004). *Indian herbal remedies: Rational western therapy, ayurvedic and other traditional usage, botany*. Springer.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*(3), 144–158.

Syed, S. D., Krishnan, M. R., & Sreedharan, R. (2003). Hepatoprotective effect of wedelolactone against paracetamol-induced liver damage. *Indian Journal of Pharmacology*, *35*(5), 318-322.

Erhirhie, E. O., Ihekwereme, C. P., & Ilodigwe, E. E. (2018). Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. *Interdisciplinary Toxicology*, *11*(1), 5-12. https://doi.org/10.2478/intox-2018-0001

Furman, B. L. (2015). Streptozotocin-induced diabetic models in mice and rats. *Current Protocols in Pharmacology*, *70*(1), 5.47.1-5.47.20. https://doi.org/10.1002/0471141755.ph0547s70

National Research Council. (2011). *Guide for the care and use of laboratory animals* (8th ed.). National Academies Press. https://doi.org/10.17226/12910

Organization for Economic Co-operation and Development (OECD). (2001). *Test No. 423: Acute oral toxicity - Acute toxic class method*. OECD Guidelines for the Testing of Chemicals, Section 4.

OECD Publishing. https://doi.org/10.1787/9789264071001-en

Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research*, *50*(6), 537-546.

Bancroft, J. D., & Gamble, M. (2008). *Theory and practice of histological techniques* (6th ed.). Churchill Livingstone Elsevier.

Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, *18*(6), 499-502. https://doi.org/10.1093/clinchem/18.6.499

Kumar, S., Kumar, V., & Prakash, O. (2011). Antidiabetic, hypolipidemic and histopathological analysis of *Dillenia indica* (L.) leaves extract on alloxan induced diabetic rats. *Asian Pacific Journal of Tropical Medicine*, *4*(5), 347-352. https://doi.org/10.1016/S1995-7645(11)60098-7

Ramesh, B., & Pugalendi, K. V. (2006). Antihyperglycemic effect of umbelliferone in streptozotocin-diabetic rats. *Journal of Medicinal Food*, *9*(4), 562-566. https://doi.org/10.1089/jmf.2006.9.562