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LC-MS PROFILING AND ANTIDIABETIC EVALUATION OF ETHANOLIC ROOT EXTRACT OF ASPARAGUS CURILLUS BUCH.-HAM. EX ROXB. IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Background: Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia due to impaired insulin secretion or action and represents a major global health concern, with high prevalence in India. Although several antidiabetic drugs are available, their side effects and limited efficacy highlight the need for safer alternatives, such as traditionally used medicinal plants, such as *Asparagus curillus*. **Methodology:** This work used LC-MS to create the phytochemical profile of the ERE of *A. curillus* and tested for its anti-diabetic potential in STZ-induced diabetic rats. FBG level and biochemical markers, including lipid profile, insulin, HOMA-IR, liver and kidney function, and HbA1c, were measured. Kidney, liver, and pancreatic tissues were examined microscopically. **Results & Discussion:** LC-MS analysis identified twelve major phytoconstituents, mainly steroidal saponins including Shatavarin I, Shatavarin IV, and sarsasapogenin. The ERE produced dose-dependent changes in the biological parameters. At the dose of 200 mg/kg, ERE reduced FBG by 37% and HbA1c by 22%, while at 400 mg/kg, reductions of 42% in FBG and 25% in HbA1c were observed compared to the diabetic control. Histopathological examination of organs showed corresponding microscopic changes consistent with the biochemical findings. **Conclusion:** The ERE showed significant antidiabetic activity, improving glycemic indices, insulin sensitivity, lipid profile, and renal parameters, as supported by histopathological findings. LC-MS confirmed the presence of steroidal saponins, suggesting that the extract may serve as a potential natural antidiabetic agent. However, further studies are required to elucidate the mechanism and to validate it in clinical trials.

INTRODUCTION

Diabetes is one of the most deadly metabolic disorders in modern society [1]. It's a chronic condition that arises when the pancreatic beta cells don't produce adequate insulin, or when the body can't use it properly. Insulin, a hormone synthesized by the pancreas, is a crucial mechanism that enables the movement of glucose (a simple sugar, monosaccharide) from the bloodstream

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into cells, where it is used to produce energy [2]. The body metabolizes all carbohydrate foods into glucose in the bloodstream, and insulin facilitates its transport into the cells. When insulin production or utilization is impaired, it results in higher levels of blood glucose - a state recognized as hyperglycemia. Persistent hyperglycemia is associated with adverse effects, including damage to multiple tissues and organs. The occurrence of diabetes is currently 1 in every 9 individuals [3, 4]. Diabetes mellitus (DM) is classified into three main categories: Type 1 diabetes (T1DM), Type 2 diabetes (T2DM), and gestational diabetes [5]. T1DM can be diagnosed at any age and requires constant insulin use. T2DM, which accounts for about 90% of all cases, is most commonly diagnosed in adults.

Gestational diabetes arises during pregnancy due to elevated blood glucose levels and may result in complications for both mother and child [4, 6]. Lifestyle factors like eating junk food, being inactive, and being under chronic stress make the global burden of diabetes much worse. Many prescription drugs help people with Type 2 diabetes control their blood sugar levels, but none of them cure the disease for good. These drugs do not restore pancreatic beta-cell function or normalize glucose metabolism. In addition, synthetic medications can cause side effects like heartburn, nausea, bloating, constipation, diarrhea & obesity [1, 7]. Recent research has linked many genes, hormones, proteins, and enzymes to the pathophysiology of diabetes, which worsens insulin resistance and kills pancreatic beta cells [8]. Because of this, diabetes has become a global health issue, with a steadily rising number of cases and catastrophic complications [9]. At present, approximately 537 million adults globally are impacted by diabetes. It is concerning that a substantial majority of these individuals, specifically 3 out of 4, live in low and middle-income countries, highlighting a notable inequality in access to effective healthcare. In 2021, diabetes accounted for 6,700,000 deaths worldwide, highlighting the critical necessity for enhanced approaches to prevention, diagnosis, and treatment. The International Diabetes Federation (IDF), Diabetes Atlas (2025), indicates that 11.1% (one in nine) of adults aged 20–79 are affected by diabetes, with more than 40% unaware of their condition. Estimates for 2050 suggest that approximately one in eight adults, totaling around 853 million individuals, will be affected by diabetes, indicating a 46% rise. T2DM accounts for more than 90% of these cases and is influenced by a combination of socioeconomic, demographic, environmental, and genetic factors [4]. T2DM continues to be the predominant type of diabetes, impacting almost 90% of individuals globally. It demonstrates an absence of bias concerning race, gender, or location. Factors that contribute to risk encompass obesity, insufficient physical activity, and inadequate nutrition. In 2013, India held the second position amongst the first ten nations with the highest occurrence of diabetes in adults aged 20–79 years, affecting 65.1 million individuals. Conversely, China reported 98.4 million cases, with diabetes-related deaths exceeding 1 million in each nation. The prevalence of diabetes in India was recorded at 8.56% [10]. The IDF's 2017 report indicates that around 425 million people worldwide were affected by diabetes, with 199 million of them being women. This represents 8.8% of the worldwide population. The South East Asian region (which includes India, Nepal, Bangladesh, the Maldives, Mauritius, and Sri Lanka) accounts for approximately one-fifth of the global population with diabetes. It is referred to as the "Home of Diabetes" [11, 12]. Among IDF regions, North America and the Caribbean report the highest diabetes prevalence (13%). In India, a key country in the IDF South-East Asia region, diabetes affected 74 million adults out of an adult population of 892 million in 2017, with a prevalence of 8.3%, resulting in over 1.12 million deaths and a total diabetes-related health expenditure of USD 8.7 billion [10, 11]. In terms of research and experimental models, Streptozotocin (STZ) has become a widely used agent for inducing diabetes in laboratory animals [13]. Originally isolated from *Streptomyces achromogenes* (in 1960), STZ was identified as diabetogenic in 1963 [14, 15]. Its effect is mediated through selective damage of β-cells in the pancreas, leading to deficiency of insulin, hyperglycemia, polydipsia, and polyuria- marks of diabetes mellitus. Various species, including mice, rats, and monkeys, are sensitive to STZ, although rabbits show reduced sensitivity. STZ is currently the most commonly used agent for inducing diabetes in rodent models (particularly rats and mice) [14]. *Asparagus curillus* Buch.-Ham. ex Roxb., is a medicinal plant native to India, Nepal, and the West Himalaya [16, 17]. This perennial species primarily resides in the subtropical Himalayan region, especially flourishing in open Oak–Rhododendron forests at elevations between 600 and 1300 m above sea level. The roots (tuberous) of *A. curillus* have traditionally been utilized in a range of ethnic medicinal practices. The ethnobotanical literature highlights various therapeutic properties of the root, noting its applications as a bitter tonic, aphrodisiac, appetite stimulant, energizer, anti-inflammatory agent, galactagogue, and diuretic. In the

Himalayan region (Uttarakhand), India, the roots are utilized in traditional medicine for addressing diabetes, either independently or alongside other herbal remedies. These are used to manage conditions such as dysuria, gonorrhoea, piles, cough, and dysentery, and serve as a general rejuvenator [18, 19]. Despite its rich ethnomedicinal background, *A. curillus* remains underexplored in terms of pharmacological validation, especially concerning its antidiabetic potential. Currently, there is a lack of comprehensive experimental evidence, particularly *in vivo* studies that scientifically substantiate its traditional use in the management of diabetes. Thus, the current investigation was considered to assess the antidiabetic efficiency of *A. curillus* (roots) in the STZ-induced diabetic animals (rats). The study includes biochemical assessments of key markers of glycemic control & histopatholo- gical analysis of tissues. Additionally, phytochemical profiling of the roots was conducted using Liquid Chromatography–Mass Spectrometry (LC-MS) to detect and identify potential bioactive constituents responsible for the observed effects. This approach aims to bridge the gap between traditional knowledge and scientific validation, thereby contributing to the pharmacological understanding and therapeutic potential of this Himalayan medicinal plant.

MATERIAL AND METHODS

Collection and identification of plant material

The *A. curillus* roots were collected from the Nainbagh area (Village Dhakrol) of Tehri District (Uttarakhand), India. The collected plant sample was certified by the Regional Government Institute, Botanical Survey of India (BSI) in Dehradun, and assigned accession number 753. A specimen of the collected plant has also been deposited at the BSI-herbarium for future reference.

Processing of plant material

The freshly harvested roots were thoroughly rinsed with ample tap water to remove impurities. After cleaning, the roots were placed in a Hot Air Oven and dried for 48 hours at 50°C, with periodic turning to ensure uniform drying. The roots (dried) were then coarsely powdered for further study.

Preparation of different extracts

The Soxhlet extraction method was employed on the dried, coarsely powdered material to obtain the extracts using the solvents. The powdered material is defatted with petroleum ether and subsequently extracted with ethanol to prepare the root extract. The ethanolic root extract (ERE) was dried using a vacuum rotary evaporator (Equitron, India) and stored at 4 °C for subsequent applications [18, 20].

Liquid Chromatography- Electrospray Ionization Tandem Mass Spectrometric (LC-ESI-MS/MS) analysis of *A. curillus* ERE

Liquid Chromatography–Mass Spectrometry (LC-MS) is a highly sensitive investigative method that combines liquid chromatography to separate complex mixtures based on polarity and hydrophobic interactions with mass spectrometry, which ionizes the analytes and measures their mass-to-charge ratios (m/z) for precise identification. In the present study, LC-ESI-MS/MS was employed to detect and identify bioactive constituents present in the ERE of *A. curillus*. The investigation was performed using a Waters SYNAPT-XS HDMS mass spectrometer (Model DBA064, UK), integrated with a UPLC ACQUITY H CLASS Series System, and operated through MassLynx Version 4.2 software. Separation was achieved on a Waters Acquity BEH C18 column (2.1 × 100 mm, 1.7 μm) using a mobile phase consisting of Solvent A- 0.1% formic acid (FA) in LC-MS grade water and Solvent B- 0.1% FA in acetonitrile, at a flow rate of 0.2mL/min over a 45-minute run time. The injection volume was 5 μL. The mass spectrometer was operated in positive ESI mode with a mass acquisition range of 100–1200 m/z. Key ESI parameters included a capillary voltage of 3.22 kV, cone voltage of 50 V, collision energy of 4 eV, source offset of 80 V, desolvation temperature of 550°C, desolvation gas flow of 950 L/h, cone gas flow of 50 L/h, and a source temperature of 120°C. Data acquisition was carried out in Multiple Reaction Monitoring (MRM) mode with unit resolution. The integrated system enabled the accurate identification of various phytochemicals present in the ERE under optimized chromatographic and spectrometric conditions [21, 22].

Animal and ethical approval

8-12-week-old male Wistar rats (225-350g) were used in the study. The rats were kept in standard polypropylene cages and had free access to food and water ad libitum. Animals were housed in groups of 4-5 per cage and kept at controlled room temperature (24 ±2°C) and relative humidity (50-70%) under a 12-h light-dark cycle. The experiment was performed in a noise-free environment. The guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA) of the Government of India were followed. Earlier approval was obtained from the Institutional Animal Ethical Committee (IAEC) (Reference no. 273/PO/Re/S/2000/CCSEA)

(Application No. CCSEA/IAEC/SBS/2024-01/01) to conduct animal experimentation.

Acute toxicity study

A study on acute oral toxicity was conducted to assess the safety profile of the crude ethanolic extract, following the guidelines set forth by the Organisation for Economic Co-operation and Development Test Guideline 423 (OECD TG 423) Acute Toxic Class Method. Healthy Wistar rats were chosen at random, regardless of sex, and acclimatized under standard laboratory conditions before the experiment commenced. The subjects were categorized into two distinct groups, each containing six rats (n=6). Group 1 served as the control and received only a standard diet. In contrast, Group 2 received a single oral dose of the Ethanolic Root Extract (ERE) at 5, 50, 300, and 2000 mg/kg body weight (b.w.) using a calibrated oral gavage. Both groups had open access to a standard diet and water throughout the study. Following treatment, a thorough observation of all animals was conducted to identify any clinical signs of toxicity. This included monitoring for changes in skin, eyes, fur, salivation, respiratory patterns, diarrhea, urination, ptosis, tremors, relaxation, posture, gait, lethargy, coma, sleep & variations in water & food intake. The observations were shown hourly for the first twenty hours; thereafter, regular assessment was made over a period of fourteen days. Moreover, the body weight of each rat was documented every four days to assess overall health and identify any potential delayed toxic effects. Through careful clinical observations and analysis of survival data, the median lethal dose (LD_{50}) was estimated, and a safe dose range was identified for subsequent pharmacological and sub-chronic toxicity studies [23, 24].

EVALUATION OF ANTI-DIABETIC ACTIVITY

Induction of diabetes

In the first two weeks, the experimental group of animals was given a 10% w/v fructose solution in their drinking water ad libitum, while the control group had access to regular drinking water ad libitum. STZ was formulated by dissolving it in the distilled water immediately before use. All animals underwent an overnight fast, and each fructose-fed subject was administered a lower single intraperitoneal (i.p.) dose of STZ at 40 mg/kg. In the first twenty-four hours following the onset of diabetes, animals received a glucose solution (10% w/v) to avert an acute hypoglycemic state. Control animals received 1ml/100 gm of distilled water. Three days after the STZ injection, the blood samples were obtained from the rats via the retro-orbital plexus by using microcapillary techniques to measure blood glucose levels. Animals showing fasting blood glucose levels above 250 mg/dl were categorized as diabetic [14, 25, 26].

Experimental design

The Wistar rats were grouped into 6 groups (n=6). The 1st group, serving as the normal control group (GINC), comprises non-diabetic rats, whereas all the remaining five groups comprise diabetic rats. The GINC and the 2nd group diabetic control (G2DC) didn't get any treatment during the experimental period, but were allowed free access to distilled water (DW). The 3rd group (G3S) was administered the standard drug (Glibenclamide- 5 mg/kg, b.w.) orally in DW. In contrast, the other remaining groups, 4th, 5th & 6th (G4T1, G5T2 & G6T3) received ERE of *A. curillus* orally in DW at different doses of 100, 200 & 400 mg/kg b.w. respectively. The ERE doses were established following an acute toxicity study. The study was designed to last 28 days (Table 1) [24].

Table 1: Treatment protocol for the experimental group

Group	Description	Treatment (for 28 days)
Group I (GINC**)**	**Normal control**	Normal Rats- not received any treatment, but allowed free access to DW
Group II (G2DC)	**Diabetic control**	Diabetic Rats- not received any treatment, but allowed free access to DW
Group III (G3S)	**Standard**	Diabetic Rats- received treatment as Glibenclamide 5mg/kg b.w.
Group IV (G4T1)	**Test-1**	Diabetic Rats- received treatment as ERE of *A. curillus* roots at a dose of 100mg/kg b.w.
Group V (G5T2)	**Test-2**	Diabetic Rats- received treatment as ERE of *A. curillus* roots at a dose of 200mg/kg b.w.
Group VI (G6T3)	**Test-3**	Diabetic Rats- received treatment as ERE of *A. curillus* roots at a dose of 400mg/kg b.w.

Biochemical analysis

The fasting blood sugar level was monitored every 7 days. Following a treatment duration of 28 days, the rats were subjected to Ether anesthesia [27, 28], and blood samples were collected from the retro-orbital plexus into Clot Activator tubes or Fluoride tubes (for glucose testing), which were then kept at 4°C for 3 hours. After that, the serum was obtained by centrifugation at three thousand revolutions per minute (rmp) for ten minutes using a cold centrifuge machine (Remi Centrifuge CE-412 LAG) [29]. The serum samples were isolated to evaluate several biochemical parameters to assess the therapeutic efficacy of the ERE of *A. curillus*. Fasting blood glucose level was

measured weekly. Plasma lipid profile parameters, including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), and atherogenic index (AI), were measured to determine the lipid-modulating effects of the extract. Insulin levels and the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) were assessed to evaluate insulin sensitivity. Liver function was monitored by measuring alanine aminotransferase (ALT) and aspartate aminotransferase (AST), while renal function was determined through serum creatinine and urea levels. Additionally, glycated hemoglobin (HbA1c) levels were measured to monitor long-term glycemic control. These parameters provided critical insights into the metabolic, hepatic, renal, and oxidative status of the experimental groups. Fasting blood glucose levels were systematically evaluated on the Zero, Seventh, Fourteenth, Twenty-first, and Twenty-eighth days (0th, 7th, 14th, 21st, and 28th days). In contrast, the remaining parameters were measured on the 0th and 28th day using Standard Assay Erba Diagnostic Kits (Erba Semi-Auto Chem 5x Biochemistry Analyzer, UK), excluding HbA1c (only on the 28th day). The Body weight and Body Mass Index (BMI) were also recorded weekly (0th, 7th, 14th, 21st, and 28th days) [30].

Isolation of organs and their histopathology

At the end of the study (on the 28th day), animals were humanely euthanized by cervical dislocation [31], as per the CCSEA/IAEC recommendations for the care and use of experimental laboratory animals [32]. The organs and tissues, including the liver, pancreas, and kidney, were taken for microscopic histo-pathological analysis. The organs and tissues were washed and cleaned with normal saline (NS), then preserved in 5% formalin (Rankem, Avantor Performance Materials India Ltd., Haryana) at room temperature (18°C to 25°C) for further histo-pathological investigation. The histo-pathological investigation of isolated organs and tissues was done by PATHVETS Lab (New Delhi), India, using a microscope at different magnification ranges (10x, 20x, 40x, and 100x) [30].

Statistical analysis

GraphPad Prism 10.0 software was used to analyze the data. All the observed values were denoted as Standard Error of the Mean (± SEM). Various assessments were conducted between groups; Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test. A p-value <0.001 was considered the most significant result. A p-value <0.01 was considered moderately significant.

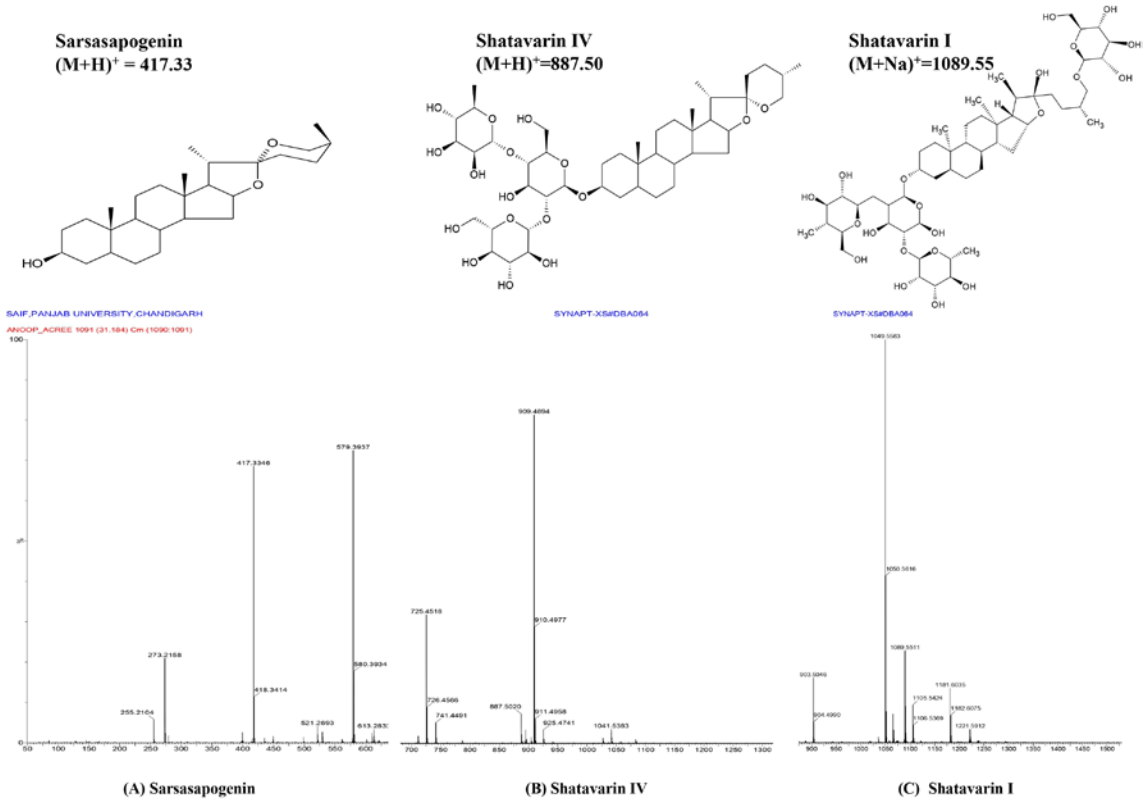

Figure 1: LC-ESI-MS/MS chromatogram of identified compounds (A) Sarsasapogenin, (B) Shatavarin IV, (C) Shatavarin I

Table 2: LC-ESI-MS/MS analysis of ERE of *A. curillus*

S. No	RT (min)	Compound Name	Chemical Formula	Mass (m/z)
1	28.44	Shatavarin I	$C_{51}H_{86}O_{23}$	1089.55
2	34.99	Shatavarin IV	$C_{45}H_{74}O_{17}$	887.50
3	31.84	Protodioscin	$C_{51}H_{84}O_{22}$	1071.55
4	32.28	Shatavarin IX	$C_{45}H_{74}O_{18}$	925.48
5	40.19	Asparagoside A	$C_{33}H_{54}O_{8}$	579.39
6	28.76	Officinalisnin I	$C_{45}H_{76}O_{19}$	943.49
7	35.99	Schidigera saponin D5	$C_{39}H_{64}O_{13}$	741.44
8	33.10	Aspoligonin A	$C_{39}H_{64}O_{14}$	779.42
9	31.67	Macranthoside I	$C_{45}H_{74}O_{19}$	941.47
10	34.99	Sarsasapogenin	$C_{27}H_{44}O_{3}$	417.33
11	38.62	Lantaiursolic acid	$C_{35}H_{56}O_{5}$	521.39
12	37.70	Steviol	$C_{20}H_{30}O_{3}$	319.22

RESULTS AND DISCUSSION

Identification of metabolites

LC-ESI-MS/MS analysis of A. curillus extract

The ERE of *A. curillus* was investigated. Identification of metabolites using LC-ESI-MS/MS, employing a Waters SYNAPT-XS HDMS mass spectrometer coupled with a UPLC system. The chromatographic separation was performed on a C18 column using a binary mobile phase of 0.1% FA in water (A) and acetonitrile (B) at a flow rate of 0.2 mL/min. The system functioned in positive ESI mode across a mass range of 100–1200 m/z, with data acquired in MRM mode. Twelve major phytoconstituents were identified, including Shatavarin I, Shatavarin IV, Protodioscin, Asparagoside A, Officinalisinin I, and Sarsasapogenin (Figure 1). These compounds are primarily steroidal saponins and glycosides, known for their anticancer, hypoglycemic, neuromodulatory, adaptogenic, immunomodulatory, and anti-inflammatory properties [33, 35]. The retention time, chemical formula, and molecular mass of each compound are presented in Table 2, confirming the rich phytochemical profile of *A. curillus*.

Acute toxicity study

Throughout the observation period, no mortality or significant toxicological signs (changes in skin, eyes, fur, salivation, respiratory patterns, diarrhea, tremors, urination, ptosis, relaxation, posture, gait, lethargy, coma, sleep, and variations in water and food intake) were recorded, even at the higher dose level of 2000 mg/kg p.o. B.W., suggesting that the extract is relatively non-toxic. According to the OECD TG-423 classification system, the absence of toxicity at this dose indicates that the Globally Harmonized System (GHS) hazard classification for the ERE falls into Category 5, which denotes a low acute toxicity potential [23].

These findings are consistent with previous studies on related species, such as *Asparagus racemosus*, in which high doses of root extract showed no mortality, no significant clinical signs, and no histopathological alterations in vital organs, confirming its safety for pharmacological evaluations [36, 37]. Based on the results of this acute toxicity assessment, the dose levels selected for further pharmacological evaluation of the ERE of *A. curillus* were 100, 200, and 400 mg/kg p.o. B.W. These dose levels were chosen to ensure a margin of safety while assessing the therapeutic potential of the extract in subsequent experiments.

ANTI-DIABETIC ACTIVITY

Effect on body weight

Body weight is an important indicator in antidiabetic studies, reflecting metabolic status and nutritional health. Diabetes often causes weight loss due to catabolism of muscle and fat [38]**.** Body weights of all investigational groups were measured over 28 days to assess the impact of ERE on diabetic rats. The G1NC exhibited gradual, steady body weight gain from 285.5 ± 1.89 g on day 0 to 293.5 ± 1.19 g on day 28, consistent with normal physiological growth. In contrast, the G2DC presented a progressive drop in body weight from 379.33 ± 0.80 g at baseline to 368.5 ± 0.42 g by day 28, which was statistically significant ($P < 0.001$) when compared to the G1NC. This weight loss is a typical feature of uncontrolled diabetes due to muscle wasting and enhanced catabolism of fat and protein. The standard group received Glibenclamide (5 mg/kg), which demonstrated no significant improvement in body weight, rising from 385.83 ± 1.37 g at day 0 to 392 ± 1.15 g on day 28. Group IV, administered 100 mg/kg, showed a slight decline in weight over the study, ending at 371.33 ± 1.02 g, indicating no significant difference. Group V, administered 200 mg/kg, exhibited a more regulated decrease, arriving at 376.33 ± 1.54 g ($P < 0.05$),

indicating enhanced metabolic support. Group VI, which received the highest dose of 400 mg/kg, showed a marked drop in body weight, dropping from 387.5 ± 0.84 g to 336.83 ± 2.02 g ($P < 0.001$). Rather than toxicological consequences, pharmacological mechanisms are examined in relation to the weight change reported at 400 mg/kg. The observed decrease in body weight at 400 mg/kg is due to AMPK activation and overexpression, which suppresses lipogenesis and adipogenesis by regulating PPARγ and C/EBPα expression. Major phytoconstituents found in the ERE of *A. curillus* suppress the production of proteins involved in lipogenesis and adipogenesis, thereby preventing adipocytes from accumulating fat and downregulating obesity and body weight (Table 3). This result indicates a strong anti-obesity effect in diabetic rats; the ERE of A. curillus exhibited dose-dependent effects on body weight.

Effect on Body Mass Index (BMI)

The effect on BMI is an important parameter in antidiabetic studies, as it reflects changes in overall body composition, including fat and lean muscle mass, which are often disrupted in diabetes. Uncontrolled diabetes typically leads to muscle wasting and fat catabolism, resulting in decreased BMI. Monitoring BMI allows evaluation not only of the extract's therapeutic efficacy in controlling hyperglycemia but also of its impact on maintaining healthy body composition [39, 40]. The present investigation assessed the impact of ERE of *A. curillus* on BMI over 28 days across all experimental groups. The G1NC showed a steady decline in BMI from 0.62 ± 0.001 to 0.54 ± 0.001 over 28 days, in line with physiological limits and expected as part of normal metabolic adaptation. Conversely, the G1DC exhibited a significant decrease in BMI from 0.90 ± 0.002 to 0.83 ± 0.002 ($P < 0.001$), indicating the progression of diabetes-related weight and muscle loss. The standard drug group (Group III), treated with Glibenclamide (5 mg/kg), showed no significant change in BMI, from 0.88 ± 0.003 to 0.94 ± 0.001. Group IV administered 100 mg/kg showed a moderate decline in BMI from 0.88 ± 0.001 to 0.77 ± 0.002 ($P < 0.05$), indicating partial protective efficacy. Group V, treated with 200 mg/kg, exhibited a more substantial reduction in BMI from 0.90 ± 0.004 to 0.71 ± 0.002 ($P < 0.01$), suggesting an enhanced antihyperglycemic effect compared to the lower dose. However, Group VI, which received the highest dose of 400 mg/kg, showed a marked decrease in BMI from 0.90±0.003 to 0.67±0.001 ($P<0.001$), indicating strong antiobesity action (Table 4). The findings indicate the promising role of *A. curillus* ERE in enhancing outcomes for diabetes management.

Table 3: Effect of 28-Day Treatment on Body Weight of Experimental Animals

GROUPS	Body Weight (g)				
	0 Day	7th Day	14th Day	21 Day	28 Day
Group I (GINC**)**	285.5±1.89	286.5±1.89	288.66±1.97	290.66±2.04	293.5±1.19
Group II (G2DC)	379.33±0.80	377.33±1.14	374.5±1.33	371.83±1.44	368.5±0.42[a]
Group III (G3S)	385.83±1.37	386.66±1.17	388.33±1.11	390±1.15	392±1.15
Group IV (G4T1)	377.83± 1.81	376±1.67	374.33±1.14	373±1.12	371.33±1.02
Group V (G5T2)	384.83±1.60	382±1.65	380.5±1.66	378±1.63	376.33±1.54*
Group VI (G6T3)	387.5±0.84	384.16±1.16	382±1.15	360.33±1.40**	336.83±2.02***

Note: *All values are expressed as mean ± standard error of the mean (SEM); n = 6 animals per group. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. P-values: *P < 0.05, **P < 0.01, ***P < 0.001 indicate statistically significant differences when values on Days 7, 14, 21, and 28 were compared to Day 0 within the same group. [a]P < 0.001 denotes a significant difference between the diabetic control group and the normal control group.*

Table 4: Effect of 28-Day Treatment on Body Mass Index (BMI) of Experimental Animals

GROUPS	BMI (g/cm^2)				
	0 Day	7th Day	14th Day	21 Day	28 Day
Group I (GINC**)**	0.62±0.001	0.60±0.004	0.58±0.002	0.56±0.002	0.54±0.001
Group II (G2DC)	0.90±0.002	0.89±0.003	0.87±0.002	0.85±0.001	0.83±0.002[a]
Group III (G3S)	0.88±0.003	0.89±0.002	0.90±0.002	0.92±0.008	0.94±0.001
Group IV (G4T1)	0.88±0.001	0.86±0.002	0.84±0.002	0.81±0.002	0.77±0.002*
Group V (G5T2)	0.90±0.004	0.88±0.002	0.82±0.001	0.79±0.001	0.71±0.002*
Group VI (G6T3)	0.90±0.003	0.86±0.001	0.80±0.001	0.76±0.001	0.67±0.001***

Note: *All values are expressed as mean ± standard error of the mean (SEM); n = 6 animals per group. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. P-values: *P < 0.05, **P < 0.01, ***P < 0.001 indicate statistically significant differences when values on Days 7, 14, 21, and 28 were compared to Day 0 within the same group. [a]P < 0.001 denotes a significant difference between the diabetic control group and the normal control group.*

Effect on Fasting Blood Glucose (FBG) Level

FBG is a critical parameter for assessing glycemic control and the therapeutic efficacy of antidiabetic interventions [41]. In diabetes, elevated FBG levels result from impaired insulin secretion, insulin resistance, or both, leading to persistent hyperglycemia and associated metabolic complications [42]. Monitoring FBG provides a direct measure of how effectively a treatment, such as *Asparagus curillus* extract, regulates blood glucose and improves metabolic outcomes. The effect of *A. curillus* ERE on FBG levels was assessed over a 28-day treatment period in all investigational groups. The FBG levels were recorded on days 0, 7, 14, 21, and 28 for all investigational groups (Table 5 and Figure 2). In G1NC, blood glucose levels regularly remained within the biological range throughout the study (98.75 ± 0.22 mg/dl to 97.26 ± 0.07 mg/dl), showing normal glucose metabolism. The G2DC exhibited consistently high blood glucose levels, showing only a slight decrease from 275.61 ± 5.98 mg/dl on day 0 to 232.48 ± 5.75 mg/dl by day 28 ($P < 0.001$ compared to Group I), thereby confirming the presence of diabetes and the absence of recovery in the absence of treatment. The standard drug-treated group (Group III) that received Glibenclamide (5 mg/kg) exhibited a notable and continuous decrease in FBG levels, dropping from 264.58 ± 8.21 mg/dl to 107.48 ± 4.12 mg/dl by day 28 ($P < 0.001$), indicating strong antihyperglycemic efficacy. In the test groups, Group IV, which received 100 mg/kg of *A. curillus* ERE, showed a moderate decrease in fasting blood glucose levels, decreasing from 270.61 ± 4.39 mg/dl to 222.55 ± 7.40 mg/dl ($P < 0.01$). Group V, treated with 200 mg/kg, showed a marked decrease from 275.71 ± 3.22 mg/dl to 173.33 ± 7.67 mg/dl ($P < 0.001$). Group VI, which received 400 mg/kg, demonstrated the most significant reduction from 274.63 ± 4.95 mg/dl to 117.9 ± 4.32 mg/dl ($P < 0.001$), closely approximating the glucose-lowering effect of the standard drug. The results demonstrate a clear dose-dependent antihyperglycemic effect of *A. curillus* root ERE, with the highest dose (400 mg/kg) exhibiting efficacy nearly on par with Glibenclamide. The decrease in glucose levels was statistically significant across all treated groups, particularly at elevated doses, indicating the potential of *A. curillus* as a therapeutic option for managing Type II diabetes.

Table 5: Effect of 28-Day Treatment on Fasting Blood Glucose Level of Experimental Animals.

GROUPS	Fasting Blood Glucose (mg/dl)				
	0 Day	7th Day	14th Day	21 Day	28 Day
Group I (GINC**)**	98.75±0.22	101.05±0.22	102.06±0.22	99.4±0.13	97.26±0.07
Group II (G2DC)	275.61±5.98	277.33±5.71	267.80±5.01	244.83±4.83	232.48±5.75[a]
Group III (G3S)	264.58±8.21	211.03±6.31***	162.63±8.76***	130.65±5.08***	107.48±4.12***
Group IV (G4T1)	270.61±4.39	268.83±4.36	257.45±3.76	255.83±3.88	222.55±7.40**
Group V (G5T2)	275.71±3.22	273.1±2.90	265.13±4.54	212.76±7.36**	173.33±7.67***
Group VI (G6T3)	274.63±4.95	247.63±5.18*	224.42±4.03**	173.83±4.03***	117.9±4.32***

Note: *All values are expressed as mean ± standard error of the mean (SEM); n = 6 animals per group. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. P-values: *P < 0.05, **P < 0.01, ***P < 0.001 indicate statistically significant differences when values on Days 7, 14, 21, and 28 were compared to Day 0 within the same group. [a]P < 0.001 denotes a significant difference between the diabetic control group and the normal control group.*

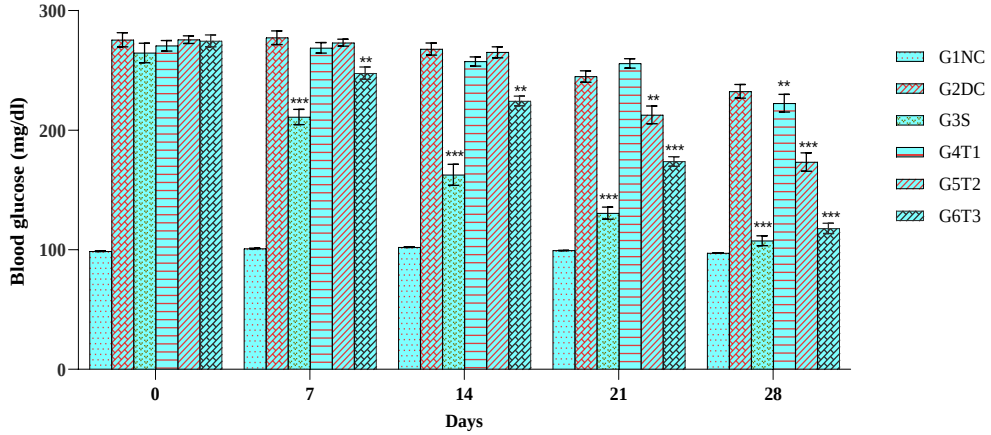

Figure 2: Effect of ethanolic root extract (ERE) of *A. curillus* on blood glucose levels in STZ-induced diabetic rats. Values are mean ± SEM (n = 6). Groups: (1) Normal control, (2) Diabetic control, (3) Standard (Glibenclamide, 10 mg/kg), (4) ERE 100 mg/kg, (5) ERE 200 mg/kg, (6) ERE 400 mg/kg. Statistical significance: p < 0.05 vs diabetic control

Effect on plasma lipid profile parameters: Plasma lipid profile, including triglycerides TG, total cholesterol (TC, high-density lipoprotein (HDL, and low-density lipoprotein (LDL, is an important marker to assess cardiometabolic risk in diabetes. Diabetes often induces dyslipidemia, characterized by elevated TC, TG, LDL, and reduced HDL, contributing to cardiovascular complications [43, 44]. The administration of ERE of *A. curillus* significantly influenced plasma lipid profile parameters, including TC, TG, HDL, LDL, VLDL, and AI in all experimental groups over a 28-day treatment period (Table 6 and Table 7). The G1NC exhibited no significant alterations in any lipid profile parameter from day 0 to day 28, with values remaining within normal limits. In contrast, the G2DC group exhibited markedly higher levels of TC (197.54 ± 0.55 mg/dl), TG (181.07 ± 1.32 mg/dl), LDL (140.04 ± 1.07 mg/dl), VLDL (36.21 ± 0.26 mg/dl), and AI (0.92 ± 0.02), alongside a reduced HDL level (21.28 ± 1.35 mg/dl) when compared to the G1NC group, emphasizing the dyslipidemia associated to diabetes (P<0.001). Treatment with Glibenclamide resulted in a significant (P < 0.001) improvement in lipid parameters. There was a marked reduction in TC (107.62 ± 0.82 mg/dl), TG (107.30 ± 1.07 mg/dl), LDL (39.13 ± 0.62 mg/dl), and VLDL (21.46 ± 0.21 mg/dl), alongside a significant increase in HDL (47.02 ± 1.24 mg/dl).

Table 6: Effect of 28-day treatment on plasma total cholesterol (TC) & plasma triglycerides (TG) of experimental animals

GROUPS	Plasma Total Cholesterol (mg/dl)		Plasma Triglycerides (mg/dl)	
	0 Day	28 Day	0 Day	28 Day
Group I (GINC**)**	101.66±1.14	102.78±0.65	105.17±1.47	103.52±1.57
Group II (G2DC)	202.95±1.23	197.54±0.55[a]	183.01±1.66	181.07±1.32[a]
Group III (G3S)	198.77±1.95	107.62±0.82***	184.27±1.67	107.30±1.07***
Group IV (G4T1)	202.71±1.45	195.11±1.33	181.49±1.89	172.16±2.49
Group V (G5T2)	200.38±1.93	145.33±1.77**	189.24±1.23	178.55±2.34*
Group VI (G6T3)	201.22±1.06	123.87±1.50***	187.09±1.32	125.06±1.66***

Note: *All values are expressed as mean ± standard error of the mean (SEM); n = 6 animals per group. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. P-values: *P < 0.05, **P < 0.01, ***P < 0.001 indicate statistically significant differences when values on Day 28 were compared to Day 0 within the same group. [a]P < 0.001 denotes a significant difference between the diabetic control group and the normal control group.*

Table 7: Effect of 28-Day Treatment on High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), Very-Low-Density Lipoprotein (VLDL), and Atherogenic Index (AI) of Experimental Animals

GROUPS	HDL (mg/dl)		LDL (mg/dl)		VLDL (mg/dl)		Atherogenic Index	
	0 Day	28 Day	0 Day	28 Day	0 Day	28 Day	0 Day	28 Day
Gr. I (GINC**)**	46.38±1.17	47.52±1.61	34.24±0.32	34.56±1.26	21.03±0.29	20.70±0.31	0.35±0.11	0.33±0.01
Gr. II (G2DC)	20.86±1.04	21.28±1.35[a]	145.49±0.14	140.04±1.07[a]	36.60±0.33	36.21±0.26[a]	0.94±0.02	0.92±0.02[a]
Gr.III (G3S)	21.98±1.14	47.02±1.24***	139.94±0.48	39.13±0.62***	36.85±0.33	21.46±0.21**	0.92±0.02	0.35±0.01***
Gr. IV (G4T1)	22.57±1.24	27.97±1.73	143.85±0.19	132.70±0.90*	36.29±0.37	34.43±0.49	0.90±0.02	0.78±0.03*
Gr. V (G5T2)	22.44±1.19	29.74±3.07*	140.1±0.49	79.87±1.75**	37.84±0.24	35.71±0.46	0.92±0.02	0.78±0.04*
Gr. VI (G6T3)	21.14±1.02	43.59±1.26***	142.67±0.22	55.27±0.09***	37.41±0.26	25.01±0.33**	0.94±0.01	0.45±0.01***

Note: *All values are expressed as mean ± standard error of the mean (SEM); n = 6 animals per group. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. P-values: *P < 0.05, **P < 0.01, ***P < 0.001 indicate statistically significant differences when values on Day 28 were compared to Day 0 within the same group. [a]P < 0.001 denotes a significant difference between the diabetic control group and the normal control group*

The atherogenic index decreased significantly, reaching 0.35 ± 0.01. The ERE at a dosage of 100 mg/kg demonstrated a modest enhancement in lipid parameters, evidenced by a slight decrease in TC to 195.11 ± 1.33 mg/dl & TG to 172.16 ± 2.49 mg/dl. Additionally, there was an increase in HDL to 27.97 ±1.73 mg/dl & a reduction in AI to 0.78 ± 0.03

(P<0.05). Group V (200 mg/kg) demonstrated a significantly enhanced hypolipidemic effect. TC decreased to 145.33 ± 1.77 mg/dl, TG to 178.55 ± 2.34 mg/dl, LDL to 79.87 ± 1.75 mg/dl, while HDL increased to 29.74 ± 3.07 mg/dl, and the AI dropped to 0.78 ± 0.04 (P<0.05). Group VI (400 mg/kg) showed the most significant effect, closely resembling that of the standard group. TC reduced to 123.87 ± 1.50 mg/dl, TG to 125.06 ± 1.66 mg/dl, LDL to 55.27 ± 0.09 mg/dl, and VLDL to 25.01 ± 0.33 mg/dl. The HDL levels increased notably to 43.59 ± 1.26 mg/dl, while the AI decreased to 0.45 ± 0.01 (P<0.001). The findings support the efficacy of *A. curillus* ERE in enhancing lipid metabolism and mitigating cardiovascular risks associated with diabetes in a dose-dependent manner.

Effect on Insulin and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR)

Insulin levels and HOMA-IR are critical parameters to assess insulin sensitivity and pancreatic β-cell function in diabetic models. Diabetes often results in a hyperglycemic state due to impaired insulin secretion and insulin resistance, as reflected by elevated HOMA-IR values [45]. The administration of ERE over a 28-day treatment period produced significant changes in insulin levels and insulin resistance (HOMA-IR) in all experimental groups (Table 8). In the G1NC group, insulin levels were stable at 24.01 ± 0.82 mIU/ml, with a consistent HOMA-IR of 5.76 ± 0.19, indicating normal metabolic function. The G2DC group showed a decline in insulin levels (13.37 ± 0.73 mIU/ml) and an increase in HOMA-IR (7.64 ± 0.42), indicating significant insulin resistance at this time compared to the G1NC group (P<0.05). The group receiving standard treatment with Glibenclamide (5 mg/kg) showed a notable increase in insulin levels, reaching 24.58 ± 1.05 mIU/ml (P<0.05), alongside a decrease in HOMA-IR to 6.51 ± 0.27. This indicates the effectiveness of the standard anti-diabetic medication in enhancing insulin function. Among the test groups, Group IV (100 mg/kg) showed a slight improvement in insulin levels (13.85 ± 1.35 mIU/ml) and a marginal decrease in HOMA-IR (7.19 ± 0.36), but the changes were not statistically significant. In Group V, there was a small rise in levels of insulin (18.77 ± 1.00 mIU/ml) & decrease in HOMA-IR to (7.10 ± 0.34). Still, the results presented were the same as those of Group IV, which were not statistically significant, indicating a moderate restoration of β-cell function, as evidenced by a slight increase in insulin levels. Group VI (400 mg/kg) demonstrated the most promising results among the test groups, with insulin levels rising significantly to 23.69 ± 0.90 mIU/ml and HOMA-IR decreasing to 6.85 ± 0.26 (P<0.05), closely mirroring the standard treatment group.

These findings indicate that *A. curillus* ERE improves insulin secretion and reduces insulin resistance in a dose-dependent manner, with the 400 mg/kg dose exhibiting the most pronounced glycemic and metabolic effects.

Table 8: Effect of 28-Day Treatment on Insulin & Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) of Experimental Animals

GROUPS	INSULIN (mIU/ml)		HOMA-IR	
	0 Day	28 Day	0 Day	28 Day
Group I (GINC**)**	24.14 ±0.84	24.01±0.82	5.87± 0.19	5.76±0.19
Group II (G2DC)	12.95±0.75	13.37±0.73[a]	8.8±0.51	7.64±0.42[a]
Group III (G3S)	11.94±0.58	24.58±1.05*	7.81±0.38	6.51±0.27
Group IV (G4T1)	10.94±0.41	13.85±1.35	7.31±0.35	7.19±0.36
Group V (G5T2)	11.74±0.82	18.77±1.00	8.00±0.60	7.10±0.34
Group VI (G6T3)	12.43±0.56	23.69±0.90*	8.44± 0.45	6.85±0.26*

Note: *All values are expressed as mean ± standard error of the mean (SEM); n = 6 animals per group. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. P-values: *P < 0.05, **P < 0.01, ***P < 0.001 indicate statistically significant differences when values on Day 28 were compared to Day 0 within the same group. [a]P < 0.05 denotes a significant difference between the diabetic control group and the normal control group.*

Effect on liver function markers

Liver function markers such as ALT, AST, and ALP are critical indicators of hepatic health and are often altered in diabetes due to hepatic stress, fat accumulation, and oxidative damage [46, 47]. The current investigation showed that diabetic control rats had much higher AST and ALT levels on Day 28, at 148.99 ± 2.80 IU/L and 76.87 ± 2.43 IU/L, respectively (Table 9). The G1NC, on the other hand, possessed levels of 95.51 ± 1.52 IU/L and 36.11 ± 2.17 IU/L.

The data show that diabetes can cause liver problems, and they are statistically significant (P < 0.001). The conventional prescription led to a marked decrease in AST and ALT levels, measured at 95.20 ± 1.61 IU/L and 42.58 ± 1.49 IU/L, respectively (P < 0.001).

Table 9: Effect of 28-Day Treatment on AST and ALT of Experimental Animals

GROUPS	AST (IU/l)		ALT (IU/l)	
	0 Day	28 Day	0 Day	28 Day
Group I (GINC**)**	97.9±1.33	95.51±1.52	37.04±2.14	36.11±2.17
Group II (G2DC)	153.3±2.57	148.99±2.80[a]	78.98±2.52	76.87±2.43[a]
Group III (G3S)	151.75±2.83	95.20±1.61***	77.45±2.36	42.58±1.49***
Group IV (G4T1)	147.25±1.95	141.49±1.53	75.23±2.82	66.92±2.65
Group V (G5T2)	150.24±2.57	138.95±2.85*	74.84±2.21	61.99±3.00*
Group VI (G6T3)	149.08±2.92	114.11±1.91***	79.16±1.91	48.17±1.71***

Note: *All values are expressed as mean ± standard error of the mean (SEM); n = 6 animals per group. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. P-values: *P < 0.05, **P < 0.01, ***P < 0.001 indicate statistically significant differences when values on Day 28 were compared to Day 0 within the same group. [a]P < 0.001 denotes a significant difference between the diabetic control group and the normal control group.*

The test groups showed that Group VI, which received 400 mg/kg of *A. curillus* ERE, possessed the strongest hepatoprotective effect. This caused the level of AST to decline to 114.11 ± 1.91 IU/L, as well as the ALT level to drop to 48.17 ± 1.71 IU/L (P < 0.001). Group V (200 mg/kg) showed a statistically significant drop in both indicators (P < 0.05); Group IV (100 mg/kg) showed only a small, non-significant drop. The results imply that *A. curillus* ERE protects the liver in diabetic rats in a dose-dependent manner, perhaps due to its antioxidant and anti-inflammatory effects.

Effect on renal function markers

Renal function markers such as serum creatinine and urea are important indicators of kidney health, which is often compromised in diabetes due to hyperglycemia-induced nephropathy [48, 49]. The current investigation revealed that diabetic control rats (Group II) exhibited a notable growth in the level of serum creatinine and urea on day 28 (1.51 ± 0.43 mg/dL and 58.43 ± 6.56 mg/dL, respectively) when compared to normal control rats (0.70 ± 0.20 mg/dL and 21.52 ± 0.40 mg/dL, respectively; P < 0.001), signifying renal dysfunction as a concern of diabetes. Treatment with the standard drug in group G3S resulted in significant improvements, with creatinine and urea levels declining to 0.63 ± 0.56 mg/dL and 9.45 ± 2.06 mg/dL, respectively (P < 0.001). In the examination of the test groups, Group VI, administered 400 mg/kg ERE, revealed the most marked nephron-protective action, resulting in a discount of creatinine levels to 0.89 ± 0.46 mg/dL (P < 0.001) and urea levels to 12.68 ± 3.72 mg/dL (P < 0.01). Group V (200 mg/kg) demonstrated significant reductions in creatinine and urea levels (P < 0.05), whereas Group IV (100 mg/kg) displayed a non-significant trend toward improvement (Table 10). The results indicate that the ERE of *A. curillus* may protect renal function in diabetic rats, with the protective effects dependent on the administered dose.

Table 10: Effect of 28-Day Treatment on Creatinine and Urea of Experimental Animals

.GROUPS	Creatinine (mg/dl)		Urea (mg/dl)	
	0 Day	28 Day	0 Day	28 Day
Group I (GINC**)**	0.69±0.20	0.70±0.20	20.13±0.28	21.52±0.40
Group II (G2DC)	0.97±0.27	1.51±0.43[a]	35.09±5.60	58.43±6.56[a]
Group III (G3S)	1.25±0.19	0.63±0.56***	30.69±3.08	9.45±2.06***
Group IV (G4T1)	1.23±0.68	1.10±0.45	28.94±2.76	24.74±3.38
Group V (G5T2)	1.35±0.43	1.12±0.46*	29.33±2.65	15.31±3.67*
Group VI (G6T3)	1.27±0.51	0.89±0.46***	31.94±2.28	12.68±3.72**

Note: *All values are expressed as mean ± standard error of the mean (SEM); n = 6 animals per group. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. P-values: *P < 0.05, **P < 0.01, ***P < 0.001 indicate statistically significant differences when values on Day 28 were compared to Day 0 within the same group. [a]P < 0.001 denotes a significant difference between the diabetic control group and the normal control group.*

Effect on Glycated Hemoglobin (HbA1c) Levels

Glycated hemoglobin (HbA1c) serves as a key sign of long-term glycemic management [50, 51]. The present examination showed that the G2DC group exhibited a markedly higher HbA1c level ($10.95 \pm 0.72\%$) compared with the G1NC group ($4.87 \pm 0.24\%$) ($P < 0.01$), thereby validating the persistent hyperglycemia resulting from fructose and STZ-induced diabetes. The administration of the standard medication Glibenclamide (Group III) significantly lowered HbA1c levels to $5.20 \pm 0.30\%$ ($P < 0.05$), demonstrating effective glycemic control. A dose-dependent decrease in HbA1c was detected among the groups treated with *A. curillus* ERE. Group IV (100 mg/kg) and Group V (200 mg/kg) demonstrated no significant difference in HbA1c level, with values of $9.59 \pm 0.58\%$ $8.55 \pm 0.74\%$. Group VI (400-mg/kg), where HbA1c dropped significantly to $6.66 \pm 0.73\%$ ($P < 0.05$), approaching the level observed in the standard treatment group (Table 11). These findings suggest that the ERE of *A. curillus* improves glycemic control and reduces long-term glucose exposure, possibly through enhanced insulin sensitivity and antioxidant action.

Table 11: Effect of 28-Day treatment on glycated hemoglobin enzyme level of experimental animals

GROUPS	HbA1c (%)
Group I (GINC**)**	4.87±0.24
Group II (G2DC)	10.95±0.72[a]
Group III (G3S)	5.20±0.30*
Group IV (G4T1)	9.59±0.58
Group V (G5T2)	8.55±0.74
Group VI (G6T3)	6.66±0.73*

Note: *All values are expressed as mean ± standard error of the mean (SEM); n = 6 animals per group. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. P-values: *P < 0.05, **P < 0.01, indicate statistically significant differences when values on Day 28 were compared to the diabetic control group. [a]P < 0.01 denotes a significant difference between the diabetic control group and the normal control group.*

HISTOLOGY

Histopathological evaluation of the liver

Histological examination of liver sections across all groups showed well-preserved hepatic architecture in the G1NC group, with organized hepatic lobules, radially arranged hepatocytes around the central vein, and clear blood sinusoids. Hepatocytes exhibited normal polyhedral morphology with granular cytoplasm and vesicular nuclei. In the G2DC group, mild sinusoidal congestion and central and portal vein blockage were observed, indicating early hepatic stress induced by STZ and fructose, without severe necrotic or inflammatory lesions. The standard treatment group (Group III, glibenclamide) exhibited liver architecture comparable to that of the normal control, with an intact sinusoidal endothelium, preserved Kupffer cells, and occasional binucleated hepatocytes, without granulomatous infiltration, suggesting hepatoprotection. Group IV (100 mg/kg ERE) showed largely intact hepatic architecture with only mild sinusoidal congestion and no significant degenerative changes. Group V (200 mg/kg) exhibited mild degenerative alterations, including swollen hepatocytes, mild steatosis, and occasional karyolytic nuclei, indicating moderate hepatic stress. Group VI (400 mg/kg) showed occasional focal degenerative changes, including swollen hepatocytes and karyolytic nuclei, comparable to those in the normal control group. Importantly, no granulomatous inflammation or hepatocellular necrosis was observed. Preservation of hepatic cords and sinusoidal arrangement across all treated groups suggests that ERE did not induce significant hepatotoxicity (Figure 3). Overall, these findings confirm that *A. curillus* ERE is well tolerated at all tested doses with minimal hepatic alterations.

Histopathological evaluation of the pancreas

Histological examination of pancreatic sections showed that the normal control group (Group I) exhibited typical pancreatic architecture with well-organized exocrine and endocrine components. Closely packed acinar cells formed small lobules separated by intact connective tissue septa, while the islets of Langerhans appeared lightly stained and evenly distributed, indicating normal morphology. In contrast, the diabetic control group (Group II) showed moderate to severe degenerative changes affecting both exocrine and endocrine regions, including swollen acinar cells with vacuolization, flattened ductal epithelium, and marked depletion of islet β-cells, reflecting diabetes-induced pancreatic damage. The standard treatment group (Group III) demonstrated pancreatic architecture comparable to that of the normal control, with only mild degenerative changes, including occasional acinar cell swelling and a slight reduction in islet β-cells, which were considerably less pronounced than in the diabetic control. Groups IV and VI treated with ERE (200 and 400 mg/kg) exhibited pancreatic histology largely similar to that of the normal control, with only mild degenerative alterations, occasional vacuolated acinar cells, and minimal depletion of islet β-cells. Overall, the preservation of pancreatic architecture in ERE-treated groups indicates a protective effect against

diabetes-induced pancreatic damage, supporting the role of ERE in maintaining both exocrine and endocrine integrity (Figure 3).

Histopathological evaluation of the kidney

Microscopic examination of kidney sections revealed mild hemorrhages in the intertubular spaces and mild vascular congestion across all groups. The diabetic control group (Group II) showed mild to moderate degenerative changes in the renal tubular epithelium, with epithelial desquamation in some tubules, indicating cellular injury. Focal interstitial inflammatory infiltrates predominantly composed of mononuclear cells were also observed, suggesting an ongoing inflammatory response. The standard treatment group (Group III) exhibited slight degenerative changes comparable to the normal control, with occasional epithelial desquamation but no interstitial inflammatory infiltrates. Groups treated with ERE at doses of 200 mg/kg and 400 mg/kg demonstrated renal histology similar to that of the normal control group, with only minimal tubular epithelial degeneration and no granulomatous lesions. No focal inflammatory infiltrates were observed in these groups, indicating attenuation of inflammation (Figure 3). Overall, these findings suggest that while diabetes induces tubular degeneration and inflammation, treatment with the standard drug and ERE effectively mitigates these changes and preserves renal architecture.

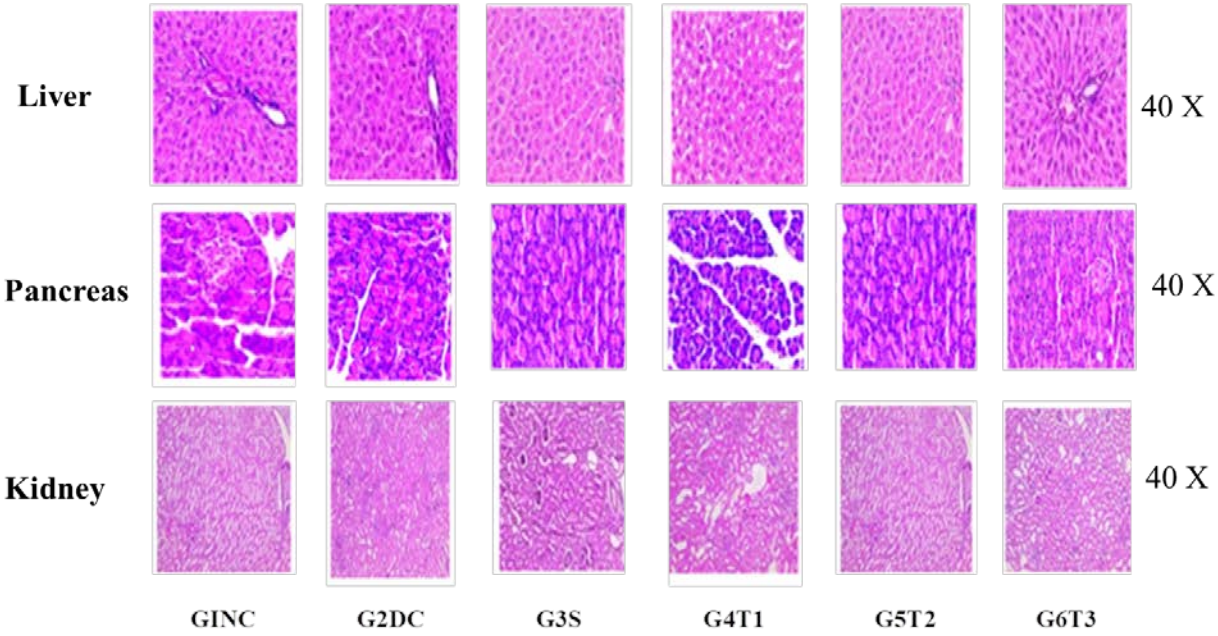

Figure 3: Histopathological examination of pancreas, liver, and kidney tissues of experimental rats at 40 X. (G1NC) Normal control group showing normal architecture, (G2DC) Diabetic control group showing cellular damage and necrosis, (G3S) Standard group (Glibenclamide 10 mg/kg) showing restoration of tissue architecture, (G4T1, G4GT2, G4T3) Treatment groups with ethanolic root extract (ERE) of *Asparagus curillus* at doses of 100, 200, and 400 mg/kg showing dose-dependent protective effects. Sections were stained with Hematoxylin and Eosin (H&E), observed under a light microscope at 40× magnification. Scale bar = 50 µm.

CONCLUSIONS

The present investigation provides a comprehensive evaluation of the ERE of *A. curillus* in streptozotocin-induced diabetic rats using biochemical, histopathological, and phytochemical approaches. LC-MS analysis identified twelve major phytoconstituents, predominantly steroidal saponins, which formed the phytochemical basis of the extract. Acute oral toxicity evaluation, performed in accordance with OECD TG-423 recommendations, confirmed the safety of ERE up to 2000 mg/kg, thereby enlisting it in GHS Category 5 for low acute toxicity. Administration of ERE resulted in dose-dependent quantitative changes in glycemic parameters, including fasting blood glucose and HbA1c, as well as measurable alterations in serum insulin levels and HOMA-IR values, reflecting changes in insulin sensitivity. Additionally, variations in lipid profile parameters such as total cholesterol, triglycerides, and other lipid functions were observed following treatment. Renal biochemical markers, including serum urea and creatinine, also showed dose-related changes, which were supported by histopathological examination of kidney tissue. Microscopic evaluation of the

pancreas, liver, and kidney revealed corresponding structural alterations that aligned with the observed biochemical data, indicating attenuation of diabetes-associated tissue damage at the cellular level. Overall, the findings of this study establish a clear relationship between phytochemical composition, biochemical parameters, and histopathological observations following ERE administration, providing experimental evidence for its biological influence on metabolic and organ-specific parameters in diabetic conditions.

FINANCIAL ASSISTANCE
NIL

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTION
Anoop Singh Negi conducted the research, prepared the experimental studies, and drafted the initial manuscript. Ankit Kumar edited, revised, and finalized the manuscript, while Veerma Ram provided supervision, identified the research problems, and approved the final version. All authors have reviewed and approved the final draft of the manuscript.

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