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Hypoglycemic Effect of Methanolic Extract of *Annona Squmosa L.* (Seeds) on Streptozotocin-Induced Diabetes in Rats

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Abstract:

We thoroughly assessed the purity, safety, and long-term therapeutic efficacy of Annona squamosa L. seed extract in treating diabetes. Petroleum ether defatting and Soxhlet methanolic extraction were used to establish physicochemical standards (ash values). Quantitative phytochemical screening showed high total phenolic content (TPC) and total flavonoid content (TFC), which provided notable in vitro antioxidant and anti-glycation properties. According to OECD Guideline 420 (Fixed Dose Procedure), acute oral toxicity testing verified that the extract has a broad margin of safety, showing no evidence of acute toxicity or fatality up to the highest suggested limit dose. Oral administration of *Annona squamosa L.* seed extract (200 mg/kg and 400 mg/kg) significantly decreased fasting blood glucose levels and reversed progressive body weight loss in an 8-week in vivo study on streptozotocin (STZ, 50–55 mg/kg)-induced diabetic Wistar rats when compared to untreated diabetic controls. The therapeutic efficacy of the 400 mg/kg dose was similar to that of normal Glibenclamide (10 mg/kg). In summary, Annona squamosa L. seed methanolic extract is a safe, flavonoid- and antioxidant-rich substance that reduces glycation, hyperglycemia, and body weight in people with chronic diabetes.

Key Words: *Annona squamosa L.*, Methanolic Extraction, TPC, TFC, Antioxidant Activity, In-Vitro Antiglycation Activity, OECD Guideline 420, In-Vivo Antidiabetic Activity.

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1. Introduction

A metabolic disorder known as chronic hyperglycemia results from insufficient insulin production, decreased insulin action, poor glucose utilization, and increased glucose synthesis. Insulin is an anabolic hormone that primarily affects the liver, skeletal muscles, and adipose tissue and influences how proteins, lipids, and carbohydrates are metabolized. Nearly 90% of instances of Type 2 diabetes (T2DM) are caused by insulin resistance associated with aging

populations, high-calorie diets, and sedentary lifestyles, whereas Type 1 diabetes (T1DM) is characterized by absolute insulin insufficiency brought on by the autoimmune death of beta cells. Uncontrolled diabetes can lead to problems like coma, cognitive impairment, hyperosmolar hyperglycemic condition, or ketoacidosis, while severe progression can result in symptoms like polydipsia, dysuria, weight loss, and visual changes[1]. Additionally, prediabetes raises the risk of cardiovascular disease (CVD), and diabetes increases all-cause mortality mostly through kidney and cardiovascular disease[2,3]. Beyond glucose absorption, impaired insulin signaling cascades affect biomolecule synthesis and cell proliferation, leading to vascular disease, neuropathy, myopathy, and dysregulated immune responses. Diabetes patients are therefore more susceptible to infections due to complex and multidimensional causes[4]. Additionally, oxidative stress plays a crucial pathophysiological role by reducing beta-cell insulin production and increasing insulin resistance. Because pancreatic beta cells have low quantities of antioxidant enzymes including SOD, catalase, and GSH-Px, they are especially vulnerable to oxidative damage. Persistent ROS generation and continuous glycolytic flux drive chronic oxidative stress, systemic inflammation, and cellular damage, which eventually lead to beta cell failure and dysfunction[5].

2. Diabetes Mellitus Global Prevalence and Epidemiological Perspective

The prevalence of diabetes mellitus is expected to increase from 536.6 million in 2024 to 783.2 million by 2045, making it a rapidly growing global health issue. Together, India and China account for over 40% of all diagnosed cases, significant mortality, and Disability-Adjusted Life Years (DALYs), making them the countries with the biggest global burden. Ninety to ninety-five percent of cases are type 2 diabetes mellitus (T2DM), which is mostly caused by obesity, poor eating habits, and sedentary lifestyles. Epidemiological data show that T1DM incidence peaks between ages 40 and 44, while T2DM incidence peaks between ages 65 and 74. An alarming increase in young-onset T2DM accelerates long-term consequences. The critical need for early detection, prevention, and comprehensive illness management is highlighted by the fact that over 75% of cases worldwide occur in low- and middle-income countries (LMICs), where therapeutic and diagnostic gaps increase health risks[6,7].

3. Types of Diabetes mellitus

Although diabetes mellitus is clinically divided into discrete etio-pathophysiological subgroups, categorization schemes are constantly changing in response to new metabolic, genetic, and pathophysiological discoveries[8].

3.1 Type 1 Diabetes Mellitus (T1DM)

In type 1 diabetes (T1DM), the immune system gradually destroys pancreatic beta-cells, causing complete insulin insufficiency. Circulating islet autoantibodies, namely insulin autoantibodies (IAA), glutamic acid decarboxylase antibodies (GADA), insulinoma-associated protein-2 antibodies (IA-2A), and zinc transporter 8 antibodies (ZnT8A), indicate the disease's extended asymptomatic preclinical phase[9]. Reduced C-peptide secretion, significant glycemic fluctuation, overt hyperglycemia, and a lifelong reliance on exogenous insulin therapy are all consequences of progressive loss of beta-cell mass[9].

3.2 Type 2 Diabetes Mellitus (T2DM)

Peripheral insulin resistance and increasing beta-cell dysfunction involving multi-organ cross-talk across the liver, skeletal muscle, adipose tissue, kidneys, intestines, and brain are the main causes of type 2 diabetes, which accounts for more than 90% of cases worldwide[9,10]. Persistent hyperglycemia results from impaired peripheral glucose uptake and unsuppressed hepatic glucose production[9,10]. Long-term glycemic dysregulation accelerates microvascular and macrovascular consequences by causing endothelial dysfunction and the buildup of advanced glycation end products (AGE)[9,10].

3.3 Gestational Diabetes Mellitus (GDM)

GDM is glucose intolerance that usually resolves after birth and is initially identified in the second or third trimester of pregnancy[11]. Intrauterine exposure to maternal hyperglycemia, although temporary, results in unfavorable fetal metabolic programming that greatly increases the long-term risk of obesity and polygenic metabolic problems in the offspring[11].

3.4 Other Specific Types of Diabetes

About 2% of diabetes cases fall into alternative categories with etiologies different from the usual categorization. These include monogenic types like neonatal diabetes and Maturity Onset Diabetes of the Young (MODY), caused by single-gene abnormalities that affect insulin action or pancreatic beta cell function. Other specialized forms include secondary diabetes brought on by diseases like cystic fibrosis, pancreatic illness, or drug side effects (especially steroids and antipsychotics), as well as Latent Autoimmune Diabetes in Adults (LADA), which has characteristics of both type 1 and type 2 diabetes. Diabetes is a common characteristic of rare genetic diseases, including Wolfram Syndrome and Alström Syndrome[12].

4. Pathogenesis of Diabetes mellitus

Diabetes mellitus is caused by an endocrine imbalance in the islets of Langerhans, which is typified by paradoxical alpha-cell glucagon hypersecretion and insufficient beta-cell insulin[15].

While high glycemic spikes (>250 mg/dL) cause conventional osmotic symptoms, such as polyuria and polydipsia, sustained hyperglycemia (>180 mg/dL) creates a self-sustaining vicious loop that gradually impairs beta-cell function[13]. Pro-inflammatory cytokines and excess free fatty acids, which limit peripheral glucose uptake and speed up lipolysis, are the biological causes of insulin resistance[15]. Notably, overt diabetes only appears after beta-cell capacity is exceeded; clinical symptoms are latent during the early stages of the disease as the pancreas first increases insulin output to adapt[15]. Furthermore, oxidative stress and non-enzymatic glycation brought on by long-term hyperglycemia, which are measured by HbA1c, cause extensive microvascular damage that results in retinopathy, nephropathy, and neuropathy[13,14]. Clinical evaluation is still largely dependent on the patient's current metabolic and physiological state during assessment because of these dynamic pathophysiological mechanisms[13,14].

5. Limitation of the current therapy

Achieving durable, individualized glycemic control in Type 2 Diabetes Mellitus (T2DM) remains a major clinical challenge. Progressive beta-cell dysfunction inevitably leads to monotherapy failure, necessitating therapeutic escalation and complex multi-drug regimens. However, conventional antidiabetic agents are limited by significant class-specific adverse effects—such as hypoglycemia (insulin, sulfonylureas), weight gain (insulin, sulfonylureas,

TZDs), fluid retention, fractures, and heart failure risks (TZDs), alongside gastrointestinal intolerance—which adversely impact patient compliance. Furthermore, managing T2DM is complicated by patient heterogeneity and comorbidities including obesity, cardiovascular disease, and chronic kidney disease. This escalating treatment complexity, coupled with financial burden and safety trade-offs, underscores an urgent need for safer, durable, and targeted therapeutic strategies[15,16].

6. Role of Medicinal Plant

Medicinal plants serve as viable reservoirs for novel antidiabetic therapeutics owing to their rich composition of bioactive phytochemicals—including flavonoids, phenolics, alkaloids, tannins, terpenoids, and saponins[17,18]. These compounds maintain glycemic homeostasis through pleiotropic mechanisms, such as inhibiting carbohydrate-hydrolyzing enzymes, stimulating insulin secretion, augmenting peripheral insulin sensitivity, accelerating glucose clearance, and suppressing oxidative stress[17,18]. Among promising botanical candidates, *Annona squamosa L.* (Annonaceae) has demonstrated marked antidiabetic efficacy, driven by its phytochemical repertoire and specialized annonaceous acetogenins[19]. In streptozotocin–nicotinamide-induced Type 2 diabetic rat models, A. squamosa leaf extracts significantly restore fasting plasma glucose, serum insulin levels, and lipid profiles[20],[21] by modulating starch digestion, glucose absorption, DPP-IV enzymatic activity, and protein glycation[22]. While recent profiling (Hussain et al., 2025) using GC-MS, in vitro, and in silico approaches confirmed the antioxidant and antidiabetic properties of both leaf and seed matrixes, in vivo scientific validation of A. squamosa seed extract remains underrepresented compared to leaf tissues. To address this specific literature gap, the present study investigates the hypoglycemic and therapeutic potential of methanolic A. squamosa seed extract in an experimentally induced Type 2 diabetic rat model[23].

7. PLANT PROFILE

Annona squamosa L. (family Annonaceae), commonly designated as custard apple, sugar apple, or sweetsop, is a tropical tree species with an extensive ethnobotanical heritage[24,25]. Beyond the dietary consumption of its fruit (srikaya) in fresh and processed foods, *A. squamosa* exhibits a broad pharmacological profile, including antioxidant, antidiabetic, cytotoxic, antihypertensive, hepatoprotective, antimicrobial, antiparasitic, antimalarial, insecticidal, and molluscicidal activities[24.-29]. Phytochemical and pharmacological investigations primarily focus on the leaves, seeds, and agro-industrial fruit residues (peel and pericarp), with comprehensive literature reviews confirming the therapeutic efficacy of the species and broader genus[30-33].

7.1 Plant Characteristics

According to recent field evaluations, the species is a tiny, warm-climate tree characterized by its segmented/scaly aggregate fruit, creamy sweet pulp, many firm dark seeds, and significant genotype-to-genotype variation in fruit size, seed number, and mineral content[34].



Figure 1. Plant of *Annona Squamosa L.*

7.2 Geographical Distribution

A. squamosa is grown in the West Indies, South and Central America (including Ecuador, Peru, and Brazil), India, Mexico, the Bahamas, Bermuda, and Egypt[27]. In a broader sense, it is said to be found in Asia, Africa, and North and South America[25]. A comparable cultivation range was reported in an earlier update review that covered the pharmacology and agronomy of the species[31] [39]. It is widely farmed in India, particularly in Gujarat, Maharashtra, and other areas with adequate tropical conditions.

Table 1. Taxonomy & Classification

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Magnoliales
Family	*Annonaceae*
Genus	*Annona L.*
Species	*Annona squamosa L.*
Common names	Sitaphal, Sharifa (Hindi); Sugar apple, Sweetsop, Custard apple (English)[25,28,30]

7.3 Phytochemical Constituents

Annonaceous Acetogenins: Cytotoxic polyketides concentrated in seeds, leaves, and bark (e.g., squamocin, annonacin, bullatacin)[35].

Alkaloids: Bioactive foliar alkaloids exhibiting neuropharmacological, antimicrobial, and antidiabetic effects (e.g., annonaine, reticuline)[34,36].

Flavonoids & Phenolics: Leaf and pericarp antioxidants mediating radical-scavenging and anti-inflammatory activities (e.g., quercetin, rutin, gallic acid)[27,34,37].

Terpenoids & Phytosterols: Triterpene constituents including \beta-sitosterol, lupeol, and ursolic acid[34,37].

Cyclopeptides & Minor Bioactives: Seed-derived cyclic peptides, essential oils, tannins, and saponins contributing to overall plant bioactivity[26,36].

Additional Bioactives: Essential and fixed oils, tannins, saponins, ascorbic acid, primary amino acids, and reducing sugars present across fruit, seed, and foliar organs[26,34,36].

7.4 Pharmacological Activities

7.4.1 Antioxidant Activity

Annona squamosa L. exhibits potent radical-quenching capacity, primarily mediated by phenolic and flavonoid metabolites through single-electron and hydrogen-atom transfer mechanisms. Foliar extracts display superior DPPH free-radical scavenging capacity and elevated total phenolic content compared to other plant parts, alongside substantial antioxidant activity reported in fruit-derived fractions[28],[36].

7.4.2 Anti-inflammatory Activity

The anti-inflammatory properties of A. squamosa are attributed to the synergistic action of flavonoids, phenolics, and acetogenins. Mechanistically, plant extracts suppress pro-inflammatory pathways by significantly reducing interleukin-6 (IL-6) secretion in cell-based models, with validated efficacy demonstrated in both foliar and seed extract preparations[24,25].

7.4.3 Anticancer Activity

Preclinical in vitro and in vivo studies demonstrate notable antineoplastic potential, including anti-hepatoma efficacy observed with fruit pericarp extracts. Annonaceous acetogenins act as the primary bioactive drivers by selectively disrupting mitochondrial energy metabolism and cellular ATP synthesis[24,25,28].

7.4.4 Antidiabetic Activity

In vivo preclinical models demonstrate significant antihyperglycemic potential, particularly in foliar preparations. Proposed mechanisms include blood glucose attenuation, modulation of lipid homeostasis, and reduction of systemic oxidative stress; however, clinical translational studies are warranted to validate these therapeutic outcomes[25,28].

7.4.5 Antimicrobial Activity

Extracts derived from foliar, seed, cortical, and pericarp tissues exhibit distinct antibacterial efficacy. Bioactivity is heavily influenced by the plant matrix, solvent polarity, extraction

parameters, and target microbial strains, mediated primarily by phytoconstituents such as phenolics, flavonoids, alkaloids, terpenoids, and acetogenins[28].

7.4.6 Antimalarial and Antiparasitic Activity

Crude extracts and isolated fractions show notable antiparasitic and antimalarial efficacy, supporting empirical ethnobotanical applications. Further comprehensive pharmacological profiling and toxicological evaluation are required to establish clinical safety and efficacy thresholds[24,25].

7.4.7 Insecticidal Activity

Seed-derived preparations exhibit pronounced biopesticidal properties, primarily driven by annonaceous acetogenins that disrupt cellular bioenergetics and mitochondrial respiration in susceptible target pests, highlighting their utility in eco-friendly pest management[26].

7.4.8 Antihypertensive Activity

Validating its traditional ethnobotanical applications in cardiovascular healthcare, A. squamosa demonstrates significant antihypertensive, hypotensive, and vasorelaxant efficacy in experimental models, mediated predominantly by bioactive seed peptides[26,27,34,36].

7.4.9 Hepatoprotective Activity

Foliar and seed extracts display notable hepatoprotective activity by mitigating hepatic oxidative stress and cellular injury through intrinsic antioxidant and anti-inflammatory pathways[26,27,34,36].

7.4.10 Analgesic and Antipyretic Activity

Preclinical investigations indicate that *A. squamosa* extracts possess distinct antinociceptive and antipyretic properties, supporting its empirical use in alleviating pain and febrile conditions[26,28,34,36].

7.4.11 Antimalarial and Molluscicidal Activity

Annona squamosa exhibits notable antimalarial and molluscicidal properties, highlighting its potential in biological vector control and pest management. These biocidal activities are driven by its rich spectrum of secondary metabolites[28,36].

7.4.12 Anti-Acetylcholinesterase Activity

Extracts demonstrate significant acetylcholinesterase (AChE) inhibitory activity, enhancing cholinergic neurotransmission; however, inhibitory potency is contingent upon phytochemical profiles and specific experimental conditions[24].

8. Methodology

8.1 Chemicals:

Streptozotocin (STZ; HiMedia Laboratori, Mumbai, India), Glibenclamide (SM Pharma & Chemicals, Amravati, India), and all other analytical-grade reagent and chemical required for the study were procured from the Department's Central Store.

8.2 Plant selection and Authentication

The seed of *Annona squamosa L.* was procured from Local Market, District-Balod (C.G.), where the authenticated by the Department of Botany, Govt. V.Y.T.P.G. Autonomous College, Durg (C.G.).

Seeds of *Annona squamosa L*. were collected during September-October, 2025. The collected seeds were washed thoroughly with distilled water to remove any remaining pulp and dust particles and other impurities. The seeds were cleaned properly and shade-dried at room temperature. The dried seeds were stored in a clean, dry container and used for further study.

8.3 Ash value:

Standard methods were used to determine the total ash value. Two to three grams of air-dried seed powder were precisely weighed into a silica crucible that had been previously tared. The crucible was then progressively heated to 550 ± 20 degrees Celsius until carbon-free, white or grayish ash was produced. The crucible was weighed after 30 minutes of cooling in a desiccator. Until the difference between two consecutive weigh-ins was insignificant, ignition, cooling, and weighing were repeated[37,38]. The value of total ash was computed as:

$$\text{Ash Value}(\%)=\left(\frac{Weight\ of\ ash}{Weight\ of\ crud\ drug}\right)\times 100$$

8.4 Acid-insoluble ash (%): The complete ash was filtered through ashless filter paper, boiled for five minutes with twenty-five milliliters of 2N HCL, and then cleaned with hot water to remove any remaining chloride. After igniting the residual at 550 ± 20 degrees Celsius, it was cooled and weighed to ensure it remained constant[37,38].

Formula of Acid-Insoluble Ash:

$$\%\text{Acid Insoluble Ash} = \left(\frac{\text{Weight of acid}-\text{insoluble residue}}{\text{Weight of sample}}\right)\times 100$$

8.5 Water-insoluble ash: The whole ash was heated for 5 min with 25 mL of distilled water and filtered. The insoluble residue was burned at 550 ± 20 degree Celsius for 15 min, cooled, and weighed. Water-soluble ash was derived by removing the water-insoluble ash from the total ash[37,38].

Formula of Water-Soluble Ash:

$$\text{Water-soluble ash }(\%)=\left(\frac{\boldsymbol{Total\ Ash}\ (\%)-\boldsymbol{Water\ Insoluble\ Ash}\ (\%)}{\boldsymbol{weight\ of\ crud\ drug}}\right)\times 100$$

8.6 Extraction of *Annona Squmosa* Seeds

Seeds from *Annona Squamosa L.* were thoroughly cleaned of pulp and debris by washing them with water. Seeds were then dryed under shade conditions and crushed into a coarse powder. Approximately 50 grams of powdered seed materials were extracted. First the powdered seeds

were defatted using petroleum ether in a Soxhlet apparatus to eliminate the oil/fatty components. Following defatting, the powdered seed was again dryed to eliminate any remaining petroleum ether[39-41].

Following defatting and drying the powdered seed was put into a Soxhlet thimble. Methanol was used at 400 ml as the extraction solvent to give a w/v ratio of 1:8. Methanol was gently warmed and allowed to vaporize/condensate and cycle back and forth through the powdered seed many times to remove soluble methanol fractions from the powdered seed. Extraction was performed close to the boiling point of methanol (approximately 65 degrees Celsius) until an adequate amount of extraction was completed[39].

Once extraction was complete the methanolic extract cooled and was filtered to eliminate particulate matter. Filtrate was removed of methanol using a rotary evaporator at vacuum conditions to create a crude methanolic seed extract. Crude methanolic seed extract was moved to a new labeled container and stored properly for future phytochemical/biological studies.

50gm of dried, powdered *Annona squamosal L*. seeds were extracted with 400 mL, of methanol (1:8) w/v) in a soxhlet apparatus for 24 hours. The methanolic extract was concentrated under reduced pressure using a rotary evaporator and then dried. The extractive yield was calculated as:

$$\text{Percentage Extractive Yield (\%)} = \left(\frac{Weight\ of\ extract\ (g)}{Weight\ of\ crude\ drug\ (g)}\right) \times 100$$



Figure 2: Hot continuous Soxhlet extraction method

8.7 Preliminary Phytochemical Screening:

Standard qualitative methods were used to screen the methanolic seed extract (1 mg/mL) for alkaloids, flavonoids, carbohydrates, proteins, saponins, tannins, phenolic compounds, glycosides, steroids, terpenoids, lipids, and oils. Alkaloids were tested using Dragendorff's, Mayer's, and Wagner's reagent; flavonoids using Shinoda and Lead Acetate; phenolics using Ferric Chloride (Fecl3); tannins using Lead Acetate; saponins using Foam; carbohydrates using Molish's and Fehling; glycosides using Keller Kiliani; steroids and terpenoids using Salkowski; proteins using Biuret; and fats and oils using the spot test. The outcomes were noted as either absent (-) or present (+)[42-44].

8.8 Total Phenolic Content (TPC): Gallic acid was used as the standard in the Folin-Ciocalteu colorimetric method[45,46] to calculate the total phenolic content of the methanolic seed extract. A stock solution (1000 µg/mL) in distilled water was used to create a working solution of gallic acid (20–100 µg/mL). The extrat was diluted (200-1000 µg/mL) in the same way. After adding 0.5 mL of Folin-Ciocalteu reagent (1:10, v/v, in distilled water), the mixture was left for five minutes. The mixture was then incubated in the dark for 30 minutes after 2 mL of sodium carbonate solution (7.5%, w/v) was added. Using a UV-visible spectrophotometer, the absorbance was measured at 760 nm in comparison to a reagent blank. Each measurement was done in triplicate. The TPC was determined using the regression equation and represented as mg gallic acid equivalents (GAE) per gram of dry extract after a calibration curve was produced using absorbance against gallic acid concentration.

8.9 Total Flavonoid Content (TFC): Using rutin as the reference standard, the aluminum chloride-potassium acetate colorimetric technique[45,46] was used to calculate the total flavonoid concentration. A 1000 µg/mL rutin stock solution was made and diluted to a working standard of 10–100 µg/mL. The same procedure was used to produce and dilute the extract (200–1000 µg/mL). 200 µL of 1 M potassium acetate was added to 1 mL of each standard or sample, and then 3 mL of distilled water was added. A UV-visible spectrophotometer was used to measure absorbance at 415 nm against a reagent blank after the reaction mixture had been thoroughly mixed and incubated for 30 minutes at room temperature. Every determination was carried out in duplicate. The rutin calibration curve was used to determine TFC, which was then represented as mg of rutin equivalents (RE) per gram of dry extract.

8.10 In Vitro Antioxidant Activity

8.10.1 DPPH Radical Scavenging Assay: With minor modifications to the Brand-Williams et al. reference, the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay was used to assess the antioxidant activity of the methanolic seed extract[46,47]. A 0.1 mM DPPH solution was made fresh in methanol and shielded from light. The concentrations of 1000, 500, 250, 125, and 62.5 µg/mL were obtained by serially diluting the extract stock solution (1000 µg/mL) twice in methanol. After adding 3 mL of DPPH solution to 1 mL of each dilution, the solutions were allowed to sit at room temperature in the dark for 30 minutes. A UV-visible spectrophotometer was used to measure absorbance at 517 nm against a methanol blank. There was also a control that included a DPPH solution devoid of extract. The percentage of DPPH radical scavenging activity was calculated as:

$$\text{DPPH Scavenging Activity (\%)} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

8.11 In Vitro Antiglycation activity

Using an in vitro BSA-glucose model system, the antiglycation activity of the methanolic seed extract of *Annona squamosa L.* was assessed using a slightly modified version of the Brownlee et al. method[48-50]. Working dilutions (20, 40, 60, 80, and 100 µg/mL) were made from a stock solution of the extract (1 mg/mL) made by dissolving 10 mg of the extract in 10 mL of distilled water. Background fluorescence was eliminated by using distilled water as the blank. Without the test extract, the control reaction mixture was made up of 4 mL of phosphate buffer (pH 7.4), 2 mL of BSA (10 mg/mL), 2 mL of glucose (500 mM), and 1 mL of sodium azide (0.5 mM). One milliliter of the extract at the appropriate quantities was added to test reaction mixtures in a similar manner. After properly mixing each combination and letting it stand for five minutes, it was incubated for seven days at 37°C in the dark. A photofluorometer with an excitation wavelength of 370 nm and an emission wavelength of 440 nm was used to measure the fluorescence intensity of the blank, control, and test samples. After deducting blank data, the percentage inhibition of glycation was computed with relation to the control. The regression equation of percentage inhibition versus concentration was used to derive the IC_{50} value:

$$\text{\% Inhibition of Protein glycation} = \left(\frac{Fluorescence\ intesity\ of\ control - Fluorescence\ intesity\ of\ test}{Fluorescence\ intesity\ of\ control}\right) \times 100$$

8.12 Experimental Animal

Healthy albino rats of either sex (male and female), weighing between 150–200 g, were used in the study. The animals were procured from an approved breeder and housed in polypropylene cages under standard laboratory conditions, with controlled temperature (25 ± 2°C), relative humidity (60 ± 5%), and a 12-hour light/dark cycle. Animals were provided free access to a standard commercial pellet diet and water ad libitum throughout the experimental period. All experimental protocols were conducted in strict accordance with the guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was reviewed and approved by the Institutional Animal Ethic Committee IAEC) (Registration No. 2312/PO/Rebi/S/2024/CCSEA, Proposal/Protocol No. SSPU/KIPS/IAEC/2026/PN08/056).

8.13 In Vivo Acute Oral Toxicity Study

OECD Guideline 420 (Fixed Dose Procedure) was used to assess the acute oral toxicity of *Annona squamosa L.* methanolic seed extract[51]. Healthy female Wistar rats (6–8 weeks, 150–200 g) were acclimated for seven days and fasted all nite before treatment. The mice were randomly divided into four groups (n = 4): the extract-treated groups, which received oral gavages containing 300, 500, and 2000 mg/kg body weight, and the vehicle control group. The extract was freshly suspended in distilled water prior to administration. For four hours following the treatment and every day for fourteen days, the animals were continuously observed for signs of toxicity and mortality. Body weights were recorded on Days 0, 7, and 14. The previously

published LD[21] of 520 mg/kg for the ethanolic seed extract of *A. squamosa* served as a guide for dose selection[52].

8.14 In Vivo Antidiabetic Activity

8.14.1 Animal Preparation and Husbandry

To reduce physiological stress, male albino rats weighing between 150 and 200 grams were acclimated to conventional laboratory settings for seven days. The animals were kept in polypropylene cages with ad libitum access to normal chow and water under regulated climatic conditions (25 ± 2°C, 60 ± 5% relative humidity, and a 12-hour light/dark cycle). The picric acid tail-marking method was used to preserve individual identification[53,54].

8.14.2 Induction of Diabetes in Experimental Animals

A single intraperitoneal (i.p.) injection of streptozotocin (STZ; 45–55 mg/kg b.w.) freshly dissolved in cold 0.1 M citrate buffer (pH 4.5) was used to produce experimental diabetes in albino rats that had fasted for eight to twelve hours. Using a digital glucometer, lateral tail vein sample was used to measure fasting blood glucose (FBG) levels 72 hours after injection. After being diagnosed with diabetes, animals with persistent hyperglycemia (FBG > 200-250 mg/dL) were assigned at random to the appropriate therapy groups[53,54].

8.14.3 Experimental Design

To maintain inter-group homogeneity and reduce selection bias, five experimental cohorts (n = 4 per group) of healthy adult male albino rats (150–200g) were randomly assigned based on baseline body weight and fasting blood glucose levels. Animals with confirmed diabetes were assigned to the following treatment plans:

Group I (Normal Control): Healthy non-diabetic rats receiving normal saline/vehicle (10 mL/kg p.o.}).

Group II (Diabetic Control): Untreated STZ-induced diabetic rats receiving vehicle only.

Group III (Standard Group): STZ-induced diabetic rats treated with Glibenclamide (10 mg/kg b.w. p.o.).

Group IV (Low-Dose Test Group): STZ-induced diabetic rats treated with *Annona squamosa L.* seed extract (200 mg/kg b.w. p.o.).

Group V (High-Dose Test Group): STZ-induced diabetic rats treated with *Annona squamosa L.* seed extract (400 mg/kg b.w. p.o.).

Table 2. Groups of animals and Experimental Design

S.No.	Group Name	Treatment	Duration	No. of Animal uses
Group I	Normal	Saline	8 weeks	4
Group II	Diabetic	STZ/ High fat diet	8 weeks	4

	control			
Group III	STD Glibenclamide	STZ/ High fat diet +Glibenclamide	8 weeks	4
Group IV	AS-1 Extract A1	STZ/ High fat diet + Extract	8 weeks	4
Group V	AS-2 Extract A1	STZ/ High fat diet + Extract	8 weeks	4
Total				20

8.14.4 Blood Glucose and Body Weight Monitoring

After an 8–12 hour overnight fast, fasting blood glucose (FBG) levels and body weights were measured at predetermined intervals: Day 0 (baseline), Day 3 (post-induction confirmation), and every two weeks after that at Weeks 2, 4, 6, and 8. Lateral tail vein venipuncture was used to obtain blood samples, which were then measured using a digital glucometer (mg/dL). In order to evaluate glycemic control, metabolic recovery, and the treatment's long-term anti-diabetic effectiveness over the course of the eight-week trial, individual body weights were simultaneously measured using a calibrated digital balance.



a) **b)** **c)**

Figure 3. a) Blood collection from the tail of a laboratory rat, b) Blood glucose measurement on a laboratory rat using a digital glucometer, c) In vivo oral drug administration.

9. Result

9.1 Total Ash Value

The entire residual inorganic content after crude plant material has been completely burned is measured by total ash determination. It consists of non-physiological ash (produced by external pollutants like sand and dirt) and physiological ash (generated from intrinsic mineral components of plant tissue). This measure is an essential pharmacopeial index for determining the quality and purity of raw materials.

$$(\%)\ \text{Ash Value} = \left(\frac{Weight\ of\ ash\ (g)}{Weight\ of\ crude\ drug\ (g)}\right) \times 100$$

$$(\%)\ \text{Ash Value} = \left(\frac{0.40}{3}\right) \times 100 = 13\%$$

A total ash value of 13% (w/w) was obtained by burning 3 g of crude drug, which produced 0.40 g of total ash.

9.2 Acid-Insoluble Ash Value

The siliceous residue left over after total ash is broken down using diluted hydrochloric acid (HCl) is measured as acid-insoluble ash. As a crucial pharmacopeial indication of raw material purity, it finds extraneous environmental pollutants such sand, quartz, and soil silicates.

$$\text{Acid-Insoluble Ash}\ (\%) = \left(\frac{Weight\ of\ acid-insoluble\ ash\ (g)}{Weight\ of\ crude\ drug\ (g)}\right) \times 100$$

$$\text{Acid-Insoluble Ash}\ (\%) = \left(\frac{0.25}{3}\right) \times 100 = 8.33\%$$

An acid-insoluble ash value of 8.33% (w/w) was obtained from the digestion of total ash with diluted HCl, followed by filtering and igniting, which produced 0.25 g of insoluble residue from 3 g of crude medicine.

9.3 Water-Soluble Ash Value

The gravimetric differential between the total ash and its water-insoluble residue is known as water-soluble ash. In order to measure water-soluble inorganic components and evaluate the authenticity or previous exhaustion of the crude medication, this parameter is an important quality control metric.

$$\text{Water-Soluble Ash}\ (\%) = \left(\frac{Weight\ of\ Total\ ash - Weight\ of\ water-insoluble\ residue\ (g)}{Weight\ of\ crude\ drug\ (g)}\right) \times 100$$

$$\text{Water-Soluble Ash}\ (\%) = \left(\frac{0.40-0.06}{3}\right) \times 100 = \left(\frac{0.34}{3}\right) \times 100 = 11.33\%$$

0.06 g of water-insoluble residue was produced by boiling 0.40 g of total ash with water, filtering, and igniting the mixture. This residue was subtracted from the overall ash weight, yielding 0.34 g of soluble material and a water-soluble ash value of 11.33% (w/w) in relation to 3 g of crude medicine.

9.4 Percentage Yield Value

An initial estimate of the extractable phytochemical contents is provided by extraction yield, which also assesses solvent efficiency. Important operational factors that affect yield optimization include solvent polarity, temperature, extraction time, particle size, and plant matrix properties.

$$\text{Percentage Yield}\ (\%) = \left(\frac{Weight\ of\ extract\ (g)}{Weight\ of\ crude\ drug\ (g)}\right) \times 100$$

$$\text{Yield}\ (\%) = \left(\frac{2.37}{50}\right) \times 100 = 4.74\%$$

Desiccation of 50 g of crude botanical powder yielded 2.37 g of dried extract, corresponding to a 4.74% (w/w) yield for the methanolic seed extract of *Annona squamosa L.*

9.5 Phytochemical screening

Preliminary phytochemical screening of the methanolic seed extract of *Annona squamosa L.* revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, saponins, tannins, steroids, proteins, fixed oils, phenolic compounds, and reducing sugars, with alkaloids, carbohydrates, glycosides, flavonoids, saponins, fixed oils, and phenolic compounds present in moderate concentration.

Table 3. Phytochemical Screening of Methanolic Seed Extract of *Annona squamosa L.*

S.No	Test Performed	Phytoconstituent	Result
1.	Dragendroff's test Wagner's test Mayer's test	Alkaloids	++
2.	Shinoda test Lead acetate test	Flavonoids	++
3.	Ferric chloride test	Phenolic	++
4.	Ferric chloride test	Tannins	+
5.	Foam test	Saponins	+
6.	Fehling's test Molisch's test	Carbohydrates	++
7.	Salkowsski test	Steroids & Terpenoids	+
8.	Biuret test	Protein's	+
9.	Keller-Killiani test	Glycoside	++
10.	Spot test	Fixed Oil & Fat	++

Key: (-) Absent; (+) Slightly Present; (++) Morderately Present.

9.6 Total Phenolic Content (TPC)

The Folin-Ciocalteu colorimetric test was used to determine the total phenolic content (TPC) of *Annona squamosal L.* methanolic seed extract. The regression equation, $y = 0.0026x + 0.0618$ ($R^2 = 0.9919$), was obtained from the calibration curve for standard gallic acid, which showed excellent linearity over a concentration range of 20–100 µg/mL at 760 nm.

Over the tested range of 200–1000 µg/mL, the methanolic seed extract showed a consistent, concentration-dependent rise in phenolic content (Figure 4). At 200 µg/mL, the percentage of TPC was 6.19%, whereas at 1000 µg/mL, it was 9.05%. The extract's overall mean total phenolic content was determined to be 7.77 ± 1.02% w/w GAE using the standard gallic acid calibration curve.

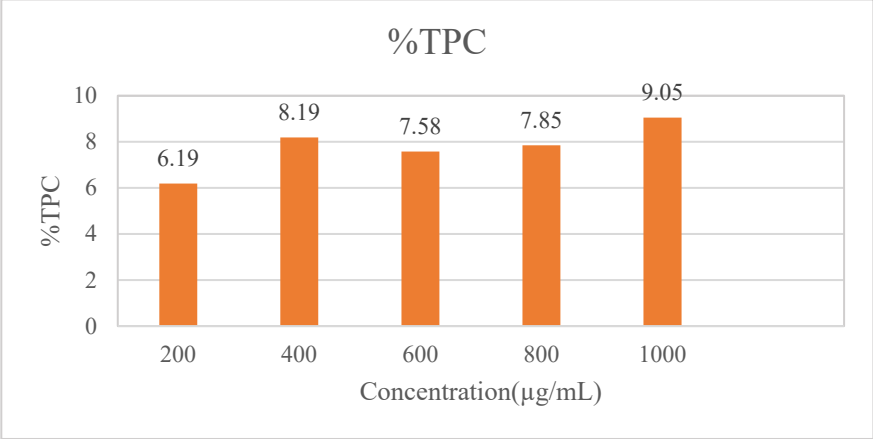


Figure 4: Total phenolic content (% TPC expressed as GAE) of *Annona squamosa L.* methanolic seed extract at various concentrations (200–1000 µg/mL).

9.7 Total Flavonoid Content (TFC)

The spectrophotometric aluminum chloride (AlCl3) colorimetric assay was used to quantify the total flavonoid content (TFC) of the methanolic seed extract of *Annona squamosa L.* Standard rutin was used as the reference compound to build a calibration curve across a concentration gradient of 10–100 µg/mL at a detection wavelength of 415 nm. Linear regression analysis produced the following equation: $y = 0.0037x + 0.1156$ ($R^2 = 0.9855$).

With absorbance values rising from 0.1240 ± 0.0066 at 200 µg/mL to 0.2890 ± 0.0046 at 1000 µg/mL, the methanolic seed extract showed a clear, concentration-dependent linear response in optical density. The relative yield of flavonoid components (% TFC) increased proportionately from 1.14% (2.27 µg/mL RE) to 4.69% (46.86 µg/mL RE) when measured in Rutin Equivalents (RE). Based on triplicate measurements (n = 3), the aggregate mean total flavonoid content was found to be 3.21 ± 1.32% w/w RE across the whole examined gradient range.

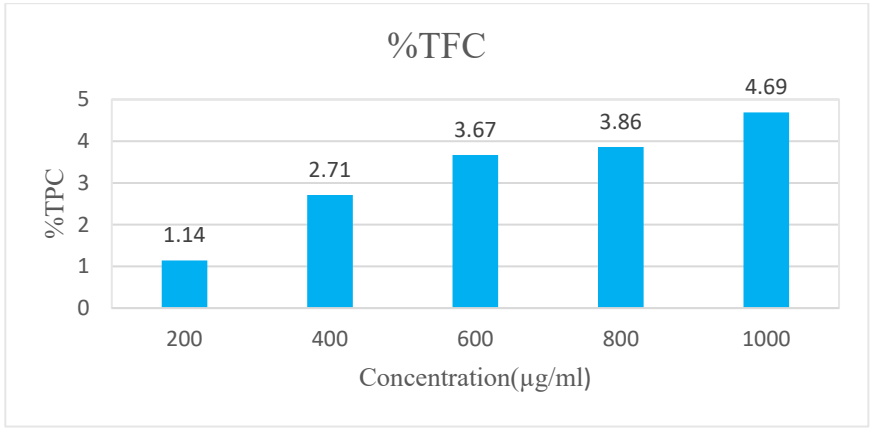


Figure 5: Concentration-dependent total flavonoid activity (% TFC expressed as Rutin Equivalents) of *Annona squamosa L.* methanolic seed extract evaluated across a range of 200–1000 µg/mL.

9.8 DPPH Radical Scavenging Assay

The DPPH radical scavenging experiment at 517 nm was used to assess the antioxidant ability of *Annona squamosa L.* seed extract. Over the concentration range of 62.5–1000 µg/mL, the extract demonstrated dose-dependent radical inhibition, with percentage inhibition rising from 10.61% to 85.61% (Figure 6). It was determined that the half-maximal inhibitory concentration (IC50) was 254.85 µg/mL.

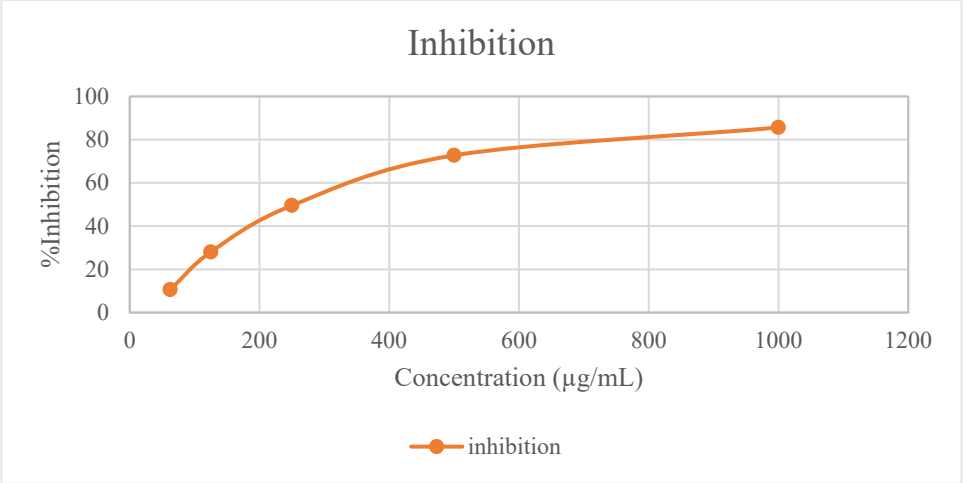


Figure 6: Concentration-dependent DPPH radical scavenging activity (% inhibition) of *Annona squamosa L.* methanolic seed extract (62.5–1000 µg/mL).

9.9 Antiglycation Activity

Using the bovine serum albumin (BSA)-glucose system, the antiglycation potential of *Annona squamosa L.* seed extract was assessed by measuring the fluorescence intensity of advanced glycation end-products (AGEs) at excitation (370 nm) and emission (440 nm) wavelengths. Over the measured concentration range of 20–100 µg/mL, the extract showed a substantial, dose-dependent suppression of AGEs production. In comparison to the glycated control (10.00), the average fluorescence intensity gradually dropped from 7.00 at 20 µg/mL to 3.00 at 100 µg/mL. Accordingly, the proportion of protein glycation inhibition rose from 30.00% at 20 µg/mL to 70.00% at 100 µg/mL (Figure 7). The extract's half-maximal inhibitory concentration (IC50) was determined to be 60 µg/mL.

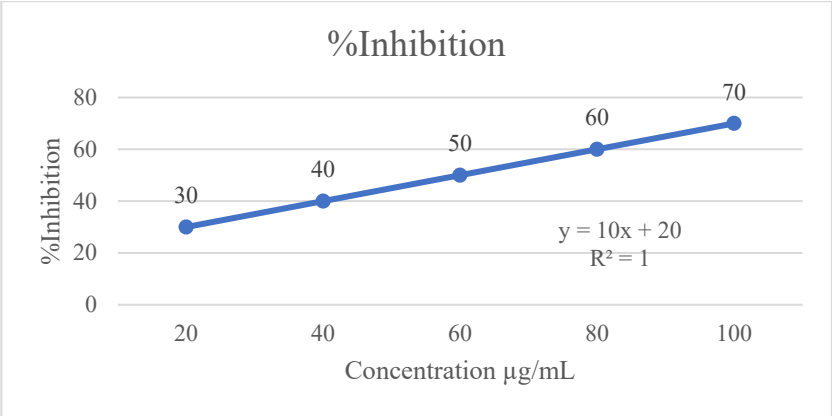


Figure 7: Concentration-dependent antiglycation activity (% inhibition) and IC_{50} value of *Annona squamosa L.* seed extract (20–100 µg/mL).

9.10 Acute Oral Toxicity Study

Using female Wistar rats, the acute oral toxicological profile of the methanolic seed extract of *Annona squamosa L.* was assessed during a 14-day post-administration period in compliance with OECD Guideline No. 420 (Fixed-Dose Procedure). Animals showed normal physiological behavior, unchanged feed consumption, and predicted body weight gain similar to controls at an oral dose of 300 mg/kg body weight (b.w.). No mortality or toxicological symptoms were noted. There was no mortality incidence at 500 mg/kg b.w., however there were moderate behavioral changes (mild dullness) along with slight decreases in feed intake and body weight. On the other hand, dosing at the 2000 mg/kg b.w. limit dose resulted in severe toxicological toxicity, which was distinguished by mortality, considerable body weight loss, marked reduction of feed intake, and marked lethargy. Oral dosages up to 500 mg/kg b.w. were therefore shown to be well-tolerated and non-lethal in test subjects.

9.11 In-Vivo Antidiabetic Activity

9.11.1 Body Weight Evaluation

The in-vivo antidiabetic effectiveness of Annona squamosa L. methanolic seed extract was assessed in an 8-week study using streptozotocin (STZ)-induced diabetic rats. Untreated diabetic rats experienced considerable weight loss (157.00 ± 1.22 g to 125.75 ± 2.17 g) as a result of severe metabolic catabolism and muscular atrophy, whereas normal control rats showed normal weight increase (160.25 ± 1.03 g to 205.50 ± 4.41 g). Weight improvement was dose-dependent when the extract (200 mg/kg and 400 mg/kg b.w.) was administered orally. By the conclusion of the eighth week, the higher dose of 400 mg/kg had successfully restored body weight to 200.25 ± 1.65 g, showing a therapeutic recovery similar to the reference standard drug group (205.00 ± 2.61 g).

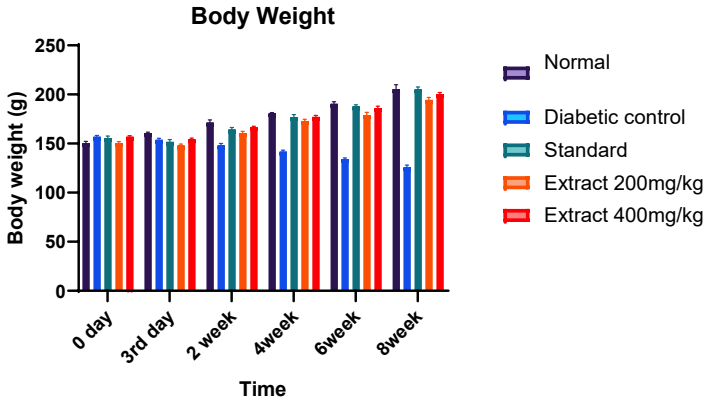


Figure 8: Two-way ANOVA and Tukey's multiple comparisons test were used to analyze the data, which are displayed as Mean ± SEM (n = 4). In comparison to the diabetic control group, the usual medication and both doses of *Annona squamosa L.* seed extract (200 and 400 mg/kg) markedly increased body weight starting in week two, and by week six, the values were comparable to those of the normal group. *$p < 0.05$ in relation to the diabetes.

9.11.2 Blood Glucose Evaluation

Over the course of an 8-week treatment period, the in-vivo antihyperglycemic effectiveness of *Annona squamosa L.* methanolic seed extract was assessed in STZ-induced diabetic rats. The hyperglycemia in the untreated diabetic controls was significant and persistent, reaching 314.75 ± 7.27 mg/dL by Week 8. When the extract (400 mg/kg b.w.) was taken orally, it considerably lowered fasting blood glucose levels to 103.25 ± 2.64 mg/dL and caused a noticeable, dose-dependent glycemic normalization. This reaction showed a strong antihyperglycemic impact that was on par with the standard reference medication (122.50 ± 13.74 mg/dL).

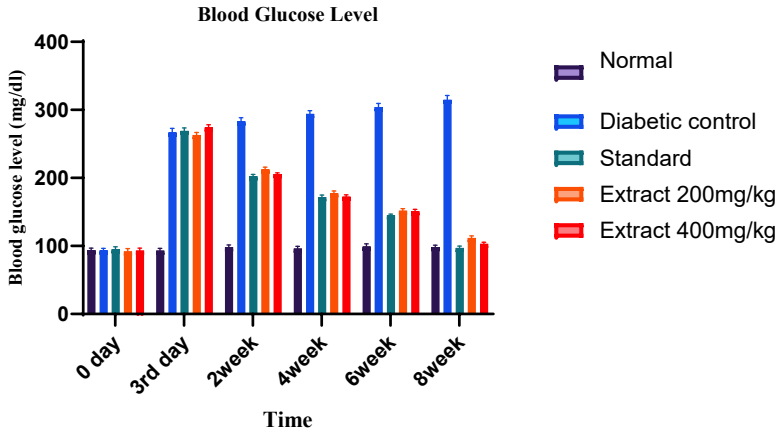


Figure 9: Tukey's test and two-way ANOVA are used to examine the results, which are expressed as Mean ± SEM (n = 4). By week eight, STZ dramatically raised the blood glucose levels of diabetic controls to 310.05 ± 6.68 mg/dL (216.3 mg/dL above normal; $p < 0.0001$). Starting in week two, treatment with *Annona squamosal L.* seed extract (200 and 400 mg/kg) dramatically decreased glucose levels ($p < 0.001$–0.0001). By week 8, glucose levels dropped to 111.75 ± 2.29 and 103.25 ± 2.36 mg/dL, respectively, which were similar to the normal control (97.25 ± 2.81 mg/dL; #$p > 0.05$) and conventional medication (119.50 ± 13.74 mg/dL), suggesting a dose-related antidiabetic effect.

10. Safety and Toxicological Consideration

The current findings offer crucial regulatory and physiological safety validation for the seed matrix of *Annona squamosal L.*, despite the plant's long history of ethnomedical usage and well-established safety profiles for its fruit pulp and leaves[53]. Toxicological data from other anatomical plant parts cannot be readily extrapolated because of basic changes in constituent ratios and toxicokinetics. This is because seed extracts naturally include complex secondary metabolites, such as cytotoxic annonaceous acetogenins[55]. While chemical characterization (TPC and TFC) in conjunction with in vitro DPPH radical scavenging and antiglycation activities confirmed a cytoprotective, non-toxic antioxidant profile[56], quality standardization via ash value determinations ensured the absence of inorganic contaminants[57]. Importantly, a broad therapeutic safety margin was confirmed by an acute oral toxicity test carried out in compliance with OECD Guideline 420 (Fixed dosage Procedure)[58], which established a cutoff median lethal dosage (LD_{50}) above 2000 mg/kg body weight. Additionally, in streptozotocin-induced diabetic rats, the progressive stabilization of body weight dynamics coupled with efficient blood glucose management[59] confirms that the observed antidiabetic efficacy of MEAS takes place within physiologically safe and non-toxic conditions.

11. Research Gap & Study Rationale

Annona squamosal L. pharmacological and ethnomedical properties are gaining attention[57,60], but scientific analysis is still mostly limited to the fruit pulp and leaves[59]. There are still significant gaps in our understanding of the seed matrix, which naturally contains complex secondary metabolites such as cytotoxic annonaceous acetogenins[61]. Because of different chemical ratios and possible toxicities, safety and pharmacological profiles from non-seed tissues cannot be directly applied to seeds. Additionally, standardized regulatory toxicity baselines, particularly acute oral toxicity profile carried out in compliance with OECD Guideline 420 (Fixed Dose Procedure) to identify a cut-off median lethal dose (LD50), are frequently absent from the literature currently available on seed extracts[58]. Importantly, there is a dearth of studies combining quantitative phytoconstituent mapping (TPC and TFC), in vitro cytoprotective profiling (DPPH radical scavenging and antiglycation), and in vivo physiological safety metrics (body weight dynamics along with glycemic control) in streptozotocin-induced diabetic rat models with raw material quality standardization through ash value determinations[62]. The current study creates a standardized, non-toxic, and therapeutically validated profile for the methanolic extract of *Annona squamosa L.* seeds (MEAS) in order to overcome these constraints.

12. Conclusion

In conclusion, the results of this study offer significant scientific backing for the customary usage of *Annona squamosa L.* seeds in the treatment of diabetes. In STZ-induced diabetic rats, the methanolic seed extract, which contains bioactive phytoconstituents like alkaloids, flavonoids, and phenolic compounds, shown encouraging antidiabetic actions by raising blood glucose levels and halting diabetes-related body weight loss. It's interesting to note that both tested doses had similar therapeutic effects within the toxicity study's acceptable dose range, indicating that raising the dose did not produce a commensurate increase in efficacy. These results offer positive evidence for more research on *Annona squamosal L.*, even though they are only a first step toward understanding its medicinal potential. Future research should concentrate on long-term safety, specific mechanisms of action, and identifying the active ingredients that give it its antidiabetic properties.

13. Acknowledgment

I would like to sincerely thank my great mentor, Dr. Arpan K. Tripathi, for his important supervision, wise counsel, and unwavering support during this research project. His extensive technical knowledge, insightful criticism, and unwavering support were crucial in developing and carrying out this investigation. I also want to express my gratitude to the Kamla Institute of Pharmaceutical Sciences' principal and distinguished faculty members for offering cutting-edge lab equipment, essential technical facilities, and a supportive learning atmosphere.

14. Ethical Approval

The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA/CCSEA), Government of India, established tight criteria for the animal experimental protocols employed in this work. The Kamla Institute of Pharmaceutical Sciences' Institutional Animal Ethics Committee (IAEC) examined and formally accepted the study protocol (Approval No.: SSPU/KIPS/IAEC/2026/PN08/056, dated 30/04/2026). Throughout the trial, every attempt

was taken to reduce animal suffering, use the fewest animals necessary to reach statistical significance, and closely follow humane goals.

Reference

1. Antar, S. A., Ashour, N. A., Sharaky, M., Khattab, M., Ashour, N. A., Zaid, R. T., Roh, E.J., Elkamhawy, A., & Al-Karmalawy, A. A. (2023). Diabetes mellitus: Classification, mediators, and complications; A gate to identify potential targets for the development of new effective treatments. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 168, 115734. https://doi.org/10.1016/j.biopha.2023.115734.
2. Abel, E. D., Gloyn, A. L., Evans-Molina, C., Joseph, J. J., Misra, S., Pajvani, U. B., Simcox, J., Susztak, K., & Drucker, D.J. (2024). Diabetes mellitus-Progress and opportunities in the evolving epidemic. Cell, 187(15), 3789–3820. https://doi.org/10.1016/j.cell.2024.06.029.
3. Kim, T., & Choi, S. H. (2025). Diabetes Mellitus and Infectious Diseases: Current Evidence and Clinical Implications. Diabetes & metabolism journal, 49(5), 915–933. https://doi.org/10.4093/dmj.2025.0508.
4. Chen, X., Xie, N., Feng, L., Huang, Y., Wu, Y., Zhu, H., Tang, J., & Zhang, Y. (2025). Oxidative stress in diabetes mellitus and its complications: From pathophysiology to therapeutic strategies. Chinese Medical Journal, 138 (1),1527. https://doi.org/10.1097/M9.0000000000003230.
5. Liu, J., Shen, M., Zhuang, G., & Zhang, L. (2025). Age-period-cohort analysis of diabetes incidence and mortality in China and globally, 1990–2019. Journal of Diabetes, 17(2), e70051. https://doi.org/10.1111/1753-0407.70051.
6. Rana, N. S., Vishvakarma, N. K., Sonkar, S. C., & Beg, M. M. A. (2026). Diabetes: A comprehensive review of the Indian landscape in contrast with global trends. World Journal of Diabetes, 17(2), 110701. https://doi.org/10.4239/wjd.v17.i2.110701.
7. American Diabetes Association Professional Practice Committee. (2024). 2. Diagnosis and classification of diabetes: Standards of care in diabetes—2024. Diabetes Care, 47(Supplement_1), S20–S42. https://doi.org/10.2337/dc24-S002.
8. Iheagwam, F. N., & Iheagwam, O. T. (2025). Diabetes mellitus: The pathophysiology as a canvas for management elucidation and strategies. Medicine in Novel Technology and Devices, 25, 100351. https://doi.org/10.1016/j.medntd.2025.100351.
9. Tegegne, B. A., Adugna, A., Yenet, A., Yihunie Belay, W., Yibeltal, Y., Dagne, A., Hibstu Teffera, Z., Amare, G. A., Abebaw, D., Tewabe, H., Abebe, R. B., & Zeleke, T. K. (2024). A critical review on diabetes mellitus type 1 and type 2 management approaches: From lifestyle modification to current and novel targets and therapeutic agents.FrontiersinEndocrinology,15,1440456.https://doi.org/10.3389/fendo.2024.1440456.
10. Kumar, R., Saha, P., Kumar, Y., Sahana, S., Dubey, A., & Prakash, O. (2020). A review on diabetes mellitus: Type 1 & type 2. World Journal of Pharmacy and Pharmaceutical Sciences, 9(10), 838–850. https://doi.org/10.20959/wjpps202010-17336.
11. Yameny, A. A. (2025). Diabetes mellitus: A comprehensive review of types, pathophysiology, complications, and standards of care in diabetes 2025. Journal of Medical and Life Science, 7(1), 134–141. https://doi.org/10.21608/jmals.2025.424001.

12. Kan, W., Qu, M., Wang, Y., Zhang, X., & Xu, L. (2025). A review of type 2 diabetes mellitus and cognitive impairment. Frontiers in Endocrinology, 16, 1624472. https://doi.org/10.3389/fendo.2025.1624472.
13. Tian, X., Wang, L., Zhong, L., Zhang, K., Ge, X., Luo, Z., Zhai, X., & Liu, S. (2025). The research progress and future directions in the pathophysiological mechanisms of type 2 diabetes mellitus from the perspective of precision medicine. Frontiers in Medicine, 12, 1555077. https://doi.org/10.3389/fmed.2025.1555077.
14. Li, S., Wang, J., Zhang, B., Li, X., & Liu, Y. (2019). Diabetes mellitus and cause-specific mortality: A population-based study. Diabetes & Metabolism Journal, 43(3), 319–341. https://doi.org/10.4093/dmj.2018.0060.
15. Taylor, S. I., Yazdi, Z. S., & Beitelshees, A. L. (2021). Pharmacological treatment of hyperglycemia in type 2 diabetes. The Journal of Clinical Investigation, 131(2), e142243. https://doi.org/10.1172/JCI142243.
16. American Diabetes Association Professional Practice Committee. (2022). 9. Pharmacologic approaches to glycemic treatment: Standards of medical care in diabetes—2022. Diabetes Care, 45(Supplement_1), S125–S143. https://doi.org/10.2337/dc22-S009.
17. Ardalani, H., Hejazi Amiri, F., Hadipanah, A., & Kongstad, K. T. (2021). Potential antidiabetic phytochemicals in plant roots: A review of in vivo studies. Journal of Diabetes & Metabolic Disorders, 20(2), 1837–1854. https://doi.org/10.1007/s40200-021-00853-9.
18. Mutha, R. E., Tatiya, A. U., & Surana, S. J. (2021). Flavonoids as natural phenolic compounds and their role in therapeutics: An overview. Future Journal of Pharmaceutical Sciences, 7(1), 25. https://doi.org/10.1186/s43094-020-00161-8.
19. Chowdhury, S. S., Tareq, A. M., Tareq, S. M., Farhad, S., & Sayeed, M. A. (2021). Screening of antidiabetic and antioxidant potential along with phytochemicals of Annona genus: A review. Future Journal of Pharmaceutical Sciences, 7(1), 144. https://doi.org/10.1186/s43094-021-00300-9.
20. Shirwaikar, A., Rajendran, K., Kumar, C. D., & Bodla, R. (2004). Antidiabetic activity of aqueous leaf extract of *Annona squamosa* in streptozotocin–nicotinamide type 2 diabetic rats. Journal of Ethnopharmacology, 91(1), 171–175. https://doi.org/10.1016/j.jep.2003.12.017.
21. Gupta, R. K., Kesari, A. N., Murthy, P. S., Chandra, R., Tandon, V., & Watal, G. (2005). Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of *Annona squamosa L.* in experimental animals. Journal of Ethnopharmacology, 99(1), 75–81. https://doi.org/10.1016/j.jep.2005.01.048.
22. Ansari, P., Flatt, P. R., Harriott, P., & Abdel-Wahab, Y. H. A. (2020). Evaluation of the antidiabetic and insulin releasing effects of *A. squamosa*, including isolation and characterization of active phytochemicals. Plants, 9(10), 1348. https://doi.org/10.3390/plants9101348.
23. Hussain, S. S., Pugalenthi, N., Rajendran, H., Narasimman, S., & Kingsley, J. D. (2025). Phytochemical analysis and bioactive potential of *Annona squamosa* extracts: Antioxidant and antidiabetic properties assessed through in vitro and in silico

approaches. Medicinal Plants—International Journal of Phytomedicines and Related Industries, 17(3), 546–558. https://doi.org/10.5958/0975-6892.2025.00056.0.

24. Ma, C., Chen, Y., Chen, J., Li, X., & Chen, Y. (2017). A review on *Annona squamosa L.*: Phytochemicals and biological activities. The American Journal of Chinese Medicine, 45(5), 933–964. https://doi.org/10.1142/S0192415X17500501.
25. Ahmed, R. H. A., & Mariod, A. A. (2019). *Annona squamosa*: Phytochemical constituents, bioactive compounds, traditional and medicinal uses. In A. A. Mariod (Ed.), Wild fruits: Composition, nutritional value and products (pp. 143–155). Springer. https://doi.org/10.1007/978-3-030-31885-7_12.
26. Shehata, M. G., Abu-Serie, M. M., Abd El-Aziz, N. M., & El-Sohaimy, S. A. (2021). Nutritional, phytochemical, and in vitro anticancer potential of sugar apple (*Annona squamosa*) fruits. Scientific Reports, 11, 6224. https://doi.org/10.1038/s41598-021-85772-8.
27. Kumar, M., Changan, S., Tomar, M., Prajapati, U., Saurabh, V., Hasan, M., Sasi, M., Maheshwari, C., Singh, S., Dhumal, S., Radha, Thakur, M., Punia, S., Satankar, V., Amarowicz, R., & Mekhemar, M. (2021). Custard apple (*Annona squamosa L.*) leaves: Nutritional composition, phytochemical profile, and health-promoting biological activities. Biomolecules, 11(5), 614. https://doi.org/10.3390/biom11050614.
28. Leite, D. O. D., Camilo, C. J., Nonato, C. F. A., Carvalho, N. K. G. de, Salazar, G. J. T., de Morais, S. M., & da Costa, J. G. M. (2021). Chemical profile and evaluation of the antioxidant and anti-acetylcholinesterase activities of *Annona squamosa L.* (*Annonaceae*) extracts. Foods, 10(10), 2343. https://doi.org/10.3390/foods10102343.
29. Ridho, M. A. R., Indriani, R. D., Wulansari, A. T., Purwani, K. I., Nurhidayati, T., Trisnawati, I., Aprilia, V., Pradani, A. N., Putra, R. A., & Lekatompessy, V. C. (2025). Exploring the phytochemistry and pharmacological potentials of *Annona squamosa L.*: A systematic review of traditional uses and modern applications. Journal of Medicine and Health Technology, 2(2), 56–67. https://doi.org/10.12962/j30466865.v2i2.4287
30. Vyas, K., Manda, H., Sharma, R. K., & Singhal, G. (2012). An update review on *Annona squamosa.* International Journal of Pharmacy & Therapeutics, 3(2), 107–118.
31. Safira, A., Widayani, P., An-Najaaty, D., Rani, C. A. M., Septiani, M., Putra, Y. A. S., Solikhah, T. I., Khairullah, A. R., & Raharjo, H. M. (2022). A review of an important plants: *Annona squamosa* leaf. Pharmacognosy Journal, 14(2), 456–463. https://doi.org/10.5530/pj.2022.14.58.
32. Singh, R. P., Pattnaik, A. K., Rudrapal, M., & Bhattacharya, S. (2025). *Annona squamosa Linn.:* A review of its ethnobotany, pharmacology, phytochemistry, toxicity, and conservation needs. Pharmacognosy Magazine, 21(2), 403–412. https://doi.org/10.1177/09731296241281422.
33. Moussa, A. Y., Siddiqui, S. A., Elhawary, E. A., Guo, K., Anwar, S., & Xu, B. (2024). Phytochemical constituents, bioactivities, and applications of custard apple *(Annona squamosa L.*): A narrative review. Food Chemistry, 459, 140363. https://doi.org/10.1016/j.foodchem.2024.140363.
34. Kumari, N., Prakash, S., Kumar, M., Radha, Zhang, B., Sheri, V., Rais, N., Chandran, D., Dey, A., Sarkar, T., Dhumal, S., Kumar, S., Mahato, D. K., Vishvanathan, M.,

Mohankumar, P., Pateiro, M., & Lorenzo, J. M. (2022). Seed waste from custard apple (*Annona squamosa L.*): A comprehensive insight on bioactive compounds, health promoting activity and safety profile. Processes, 10(10), 2119. https://doi.org/10.3390/pr10102119.

35. Ghasemi, A., Khalifi, S., & Jedi, S. (2014). Streptozotocin-nicotinamide-induced rat model of type 2 diabetes (review). Acta Physiologica Hungarica, 101(4), 408–420. https://doi.org/10.1556/APhysiol.101.2014.4.2.
36. Sharma, A., Sharma, A. K., Chand, T., Khardiya, M., & Agarwal, S. (2013). Preliminary phytochemical screening of fruit peel extracts of *Annona squamosa Linn.* Journal of Current Pharma Research, 4(1), 1038–1043. https://doi.org/10.33786/jcpr.2013.v04i01.001.
37. Hasan, M. M., Hossain, M. M., Haque, T., Basunia, A. K., & Howlader, N. C. (2025). Morpho-biochemical evaluation of three sugar apple (*Annona squamosa L.*) genotypes. International Journal of Horticultural Science and Technology, 12(2), 189–198. https://doi.org/10.22059/ijhst.2024.369325.744.
38. Kulkarni, S., Joshi, S., Kamthe, P., Tekale, P., Pathare, S., & Rajale, A. (2013). Proximate analysis of peel and seed of *Annona squamosa* (custard apple) fruit. Research Journal of Chemical Sciences, 3(2), 92–94.
39. Biba, V. S., Lakshmi, S., & Sunil, K. (2017). Extraction and phytochemical screening of *Annona squamosa* seeds. Journal of Pharmacognosy and Phytochemistry, 6(4), 112–117.
40. Biba, V. S., Thomas, A., & Mathew, B. (2019). Evaluation of extraction solvents for seed matrix compounds. Asian Journal of Pharmaceutical and Clinical Research, 12(2), 45–50.
41. Soni, K., Sharma, R., & Patel, P. (2023). Methodological advancements in Soxhlet extraction of medicinal plants. Fitoterapia, 164, 105320.
42. Sawant, S. S., Gabhe, S. Y., & Singh, K. K. (2023). In vitro effect on Plasmodium falciparum and in vivo effect on Plasmodium berghei of Annomaal, an oily fraction obtained from the seeds of *Annona squamosa*. Molecules, 28(14), 5472. https://doi.org/10.3390/molecules28145472.
43. Bhattacharya, A., Pal, A., Datta, S., Biswas, S., Das, A., Chanda, P., Roy, D., Roy, S., Nag, M., Chattopadhyay, S., & Sen, A. (2023). Antimicrobial activity in methanolic seed extract of *Annona squamosa*. International Journal of Pharmaceutical Sciences and Research, 14(11), 5340–5348. https://doi.org/10.13040/IJPSR.0975-8232.14(11).5340-48.
44. Biba, V. S., Lakshmi, S., Dhanya, G. S., & Remani, P. (2013). Phytochemical analysis of Annona squamosa seed extracts. International Research Journal of Pharmaceutical and Applied Sciences, 3(4), 29–31.
45. Kumar, D., Jamwal, A., Madaan, R., & Kumar, S. (2014). Estimation of total phenols and flavonoids in selected Indian traditional plants. Journal of Pharmaceutical Technology, Research and Management, 2(1), 77–86. https://doi.org/10.15415/jptrm.2014.21006.

46. Kumar, R., Srivastava, T., & Saxena, D. C. (2021). Proximate analysis of medicinally important plant *Annona squamosa* seeds. International Journal of Botany Studies, 6(4), 283–289.
47. Sachan, A., Shanker, D., Jaiswal, A. K., & Sudan, V. (2015). In vitro ovicidal assessment of methanol, ethyl acetate and chloroform extracts of *Annona squamosa* and *Chenopodium album* against caprine gastrointestinal nematodiosis. Journal of Parasitic Diseases, 39(1), 62–66. https://doi.org/10.1007/s12639-013-0288-3.
48. Brownlee, M., Vlassara, H., Kooney, A., Ulrich, P., & Cerami, A. (1986). Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. Science, 232(4758), 1629–1632. https://doi.org/10.1126/science.3487117.
49. Perez Gutierrez, R. M. (2012). Inhibition of advanced glycation end-product formation by Origanum majorana L. in vitro and in streptozotocin-induced diabetic rats. Evidence-Based Complementary and Alternative Medicine, 2012, 598638. https://doi.org/10.1155/2012/598638.
50. Wu, J. T., Tu, M. C., & Zhung, P. (1996). Advanced glycation end product (AGE): Characterization of the products from the reaction between D-glucose and serum albumin. Journal of Clinical Laboratory Analysis, 10(1), 21–34. https://doi.org/10.1002/(SICI)1098-2825(1996)10:1.
51. Kariyil, B. J., & Usha, P. T. A. (2021). Acute oral toxicity studies of seeds of *Annona muricata.* Pharmacognosy Magazine, 16(72), 757–763. https://doi.org/10.4103/pm.pm_118_20.
52. Organisation for Economic Co-operation and Development. (2002). Test No. 420: Acute oral toxicity—Fixed dose procedure. OECD Publishing. https://doi.org/10.1787/9789264070943-en
53. Sangala, R., Burra, S., Gopu, J., Kodati, D. R., & Dubasi, A. (2011). Evaluation of antidiabetic activity of Annona squamosa Linn. seed in alloxan-induced diabetic rats. International Journal of Preclinical Research, 2(1), 100–106.
54. Gupta, R. K., Kesari, A. N., Diwakar, S., Tyagi, A., Tandon, V., Chandra, R., & Watal, G. (2008). In vivo evaluation of anti-oxidant and anti-lipidimic potential of Annona squamosa aqueous extract in type 2 diabetic models. Journal of Ethnopharmacology, 118(1), 21–25. https://doi.org/10.1016/j.jep.2008.03.008.
55. Pandey, N., & Barve, D. (2011). Phytochemical and pharmacological review on Annona squamosa Linn. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2(4), 1404–1412.
56. Alali, F. Q., Liu, X.-X., & McLaughlin, J. L. (1999). Annonaceous acetogenins: Recent progress. Journal of Natural Products, 62(3), 504–540. https://doi.org/10.1021/np980406d.
57. Gajalakshmi, S., Divya, R., Deepika, V. D., & Sathiavelu, S. A. (2011). Pharmacological activities of *Annona squamosa:* A review. International Journal of Pharmaceutical Sciences Review and Research, 10, 24–29.
58. Organisation for Economic Co-operation and Development. (2002). Test No. 420: Acute oral toxicity—Fixed dose procedure. OECD Publishing. https://doi.org/10.1787/9789264070943-en.

59. World Health Organization. (2011). Quality control methods for herbal materials. World Health Organization.
60. Li, C. C., Wu, T. Y., Cheng, F. R., & Wu, Y. C. (2010). Annonaceous acetogenins: Recent progress. Journal of Natural Products, 73(3), 506–516. https://doi.org/10.1021/np900742t.
61. Wu, C. H., & Yen, G. C. (2005). Inhibitory effect of naturally occurring flavonoids on the formation of advanced glycation endproducts. Journal of Agricultural and Food Chemistry, 53(8), 3167–3173. https://doi.org/10.1021/jf048550u.
62. Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiological Research, 50(6), 537–546. https://doi.org/10.33549/physiolres.930111.