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PHYTOCHEMICAL CHARACTERIZATION AND ASSESSMENT OF HYPOGLYCEMIC ACTIVITY OF HERBAL PLANT EXTRACT IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

The present study evaluated the phytochemical profile, safety, and anti-diabetic potential of *Martynia annua* seed extract. The extract was prepared using successive Soxhlet extraction with petroleum ether and methanol, with the methanol extract exhibiting a richer phytochemical composition, including alkaloids, flavonoids, glycosides, tannins, saponins, and phenolic compounds. Quantitative analysis revealed high total phenolic (86.2 mg GAE/g) and flavonoid content (73.5 mg RE/g), indicating strong antioxidant potential. Acute oral toxicity studies in Wistar rats demonstrated the extract's safety at doses up to 2000 mg/kg, with no observed mortality or adverse effects. The anti-diabetic activity was assessed in streptozotocin-induced diabetic rats over 14 days. Treatment with *Martynia annua* extract, particularly at 400 mg/kg, significantly reduced blood glucose levels, stabilized body weight, and improved lipid profiles by lowering total cholesterol, triglycerides, VLDL, and LDL-C while increasing HDL-C. These findings suggest that *Martynia annua* seed extract possesses potent anti-diabetic and cardioprotective properties, supporting its potential therapeutic application in the management of diabetes and associated metabolic complications.

KEYWORDS: *Martynia annua*, anti-diabetic, streptozotocin, phenolic compounds, flavonoids, oxidative stress.

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association, 2023). The condition is associated with long-term damage and dysfunction of multiple organs, particularly the eyes, kidneys, nerves, heart, and blood vessels. Type 1 diabetes results from autoimmune destruction of pancreatic β-cells, leading to absolute insulin deficiency, whereas type 2 diabetes, the most prevalent form, arises due to insulin resistance combined with relative insulin deficiency (Zimmet et al., 2014). Globally, the prevalence of diabetes is increasing at an alarming rate, with over 537 million adults affected in 2021, projected to rise to 783 million by 2045, imposing substantial socioeconomic and healthcare burdens (International Diabetes Federation, 2021). Chronic hyperglycemia in diabetes often leads to dyslipidemia, oxidative stress, and inflammation, which further exacerbate cardiovascular complications and metabolic disturbances (Forbes & Cooper, 2013).

Despite the availability of conventional anti-diabetic drugs such as sulfonylureas, biguanides, and insulin, their long-term use is often associated with limitations including side effects, high cost, and incomplete control of diabetic complications (Nathan, 2015). Consequently, there is growing interest in identifying safe, effective, and affordable alternative therapies, particularly from natural sources, which may offer multi-targeted approaches for glycemic control, lipid regulation, and antioxidant protection. Medicinal plants have historically played a vital role in the management of diabetes, providing bioactive compounds such as alkaloids, flavonoids, phenolics, saponins, and terpenoids that exhibit hypoglycemic, hypolipidemic, and antioxidant activities (Patel et al., 2012).

Martynia annua L., belonging to the family Martyniaceae, is an ethnomedicinal plant traditionally used for its anti-inflammatory, analgesic, and wound-healing properties (Kumar et al., 2018). Preliminary phytochemical investigations have indicated the presence of flavonoids, phenolic compounds, alkaloids, glycosides, and saponins, which are known to contribute to its pharmacological activities (Wang et al., 2009). Flavonoids and phenolics, in particular, possess potent antioxidant properties, scavenging free radicals and mitigating

oxidative stress, a key contributor to pancreatic β-cell dysfunction and insulin resistance in diabetes (Babbar et al., 2011).

Given the increasing demand for plant-based anti-diabetic therapies and the promising phytochemical profile of *Martynia annua*, this study was designed to evaluate the safety, phytochemical content, and in vivo anti-diabetic potential of its seed extract. The investigation focused on assessing its effects on blood glucose levels, body weight, and lipid profile in streptozotocin-induced diabetic rats, aiming to provide scientific evidence for its therapeutic application in diabetes management.

2. Plant collection

A total of 350 g of *Martynia annua* was collected, thoroughly cleaned, and shade-dried at room temperature for three days to preserve its phytochemicals. The material was then oven-dried at 45 °C to remove residual moisture and stored in sterile, airtight glass containers under cool, dry conditions to prevent microbial contamination. The plant species was taxonomically authenticated by a qualified botanist to ensure correct identification and purity for experimental use.

2.1 Extraction of plant material by Soxhalation process

In this study, extraction of *Martynia annua* was performed using the continuous hot percolation method with a Soxhlet apparatus. Finely powdered plant material was placed in the extractor thimble and first extracted with petroleum ether (non-polar solvent) at 60 °C to remove fats and waxes. Once the siphon tube showed no color, indicating complete extraction, the defatted marc was air-dried and re-extracted with methanol (polar solvent) under similar conditions. Extraction was continued until the solvent became colorless, confirming exhaustion. The collected extracts were concentrated under reduced pressure using a rotary vacuum evaporator (Buchi model) at 40 °C. The dried extracts were weighed, and the percentage yield was determined using the formula:

$$\% \text{ Yield } = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \text{x100}$$

The prepared extracts were examined for organoleptic characteristics (percentage yield, color, and odor) and then packaged in an airtight container and labeled for future use **(Abubakar and Haque 2020).**

2.2 Phytochemical investigation

The dried petroleum ether and methanolic extracts of *Martynia annua* obtained through Soxhlet extraction were subjected to preliminary phytochemical screening to detect major bioactive constituents. Standard qualitative tests were performed to identify alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, steroids, and phenolic compounds. Positive results were indicated by characteristic color changes or precipitate formation. These analyses provided insight into the plant's chemical composition and guided further pharmacological evaluation (Wang et al., 2009).

➢ **Tests for Alkaloids**

- **Dragendorff's Test:** One milliliter of *Martynia annua* extract was mixed with alcohol and a few drops of acetic acid, followed by the addition of Dragendorff's reagent. The mixture was well agitated, and the formation of an orange-red precipitate confirmed the presence of alkaloids in the extract.
- **Wagner's Test:** One milliliter of *Martynia annua* extract was dissolved in acetic acid, followed by the addition of a few drops of Wagner's reagent. The appearance of a reddish-brown precipitate indicated the presence of alkaloids in the extract.
- **Mayer's Test:** One milliliter of *Martynia annua* extract was dissolved in acetic acid and treated with a few drops of Mayer's reagent. The formation of a dull white precipitate indicated the possible presence of alkaloids in the extract.
- **Hager's Test:** One to two milliliters of Martynia annua extract was dissolved in acetic acid and treated with 3 mL of Hager's reagent. The formation of a yellow precipitate confirmed the presence of alkaloids in the extract.

➢ **Test for Carbohydrates**

- **Molisch's Test:** The 1 ml water-based solution of *Martynia annua* extract was mixed with a few drops of Molish reagent (naphthol), then conc. H2SO4 (sulphuric acid) was added dropwise along the test tube's wall. When two liquids mix, a purple-colored ring forms at the confluence. It identifies the presence of carbs.
- **Fehling's Test:** Two milliliters of aqueous Martynia annua extract were added to 1 mL each of Fehling's solutions A and B. The mixture was boiled in a water bath for 5–10 minutes. The formation of a reddish-brown precipitate of cuprous oxide confirmed the presence of reducing sugars in the extract.

- **Benedict's test:** Equal volumes of Benedict's reagent and Martynia annua extract were mixed in a test tube and heated in a water bath for 5–10 minutes. A color change to green, yellow, or red, depending on the concentration of reducing sugars, indicated their presence in the extract.
- **Barfoed's Test:** In One milliliter of Benedict's solution was added to the aqueous Martynia annua extract, and the mixture was heated to boiling. The appearance of a red color, due to the formation of cuprous oxide, indicated the presence of monosaccharides in the extract.

➢ **Test for Saponins**

- **Froth Test:** 1ml of *Martynia annua* extract was mixed with distilled water and shaken thoroughly. The presence of saponin was detected by steady foam formation.

➢ **Test for Triterpenoids and Steroids**

- **Libermann-Burchard Test:** The Martynia annua extract was dissolved in chloroform, and 1 mL each of acetic acid and acetic anhydride were added. The mixture was heated in a water bath, cooled, and then treated with a few drops of concentrated sulfuric acid along the test tube wall. The appearance of a bluish-green color indicated the presence of steroids in the extract.
- **Salkowski Test:** The Martynia annua extract was dissolved in chloroform, and an equal volume of concentrated sulfuric acid was added. The presence of steroids was indicated by a bluish-red to cherry-red color in the chloroform layer and green fluorescence in the acid layer.

➢ **Test for Tannin and Phenolic Compounds**

- **Ferric Chloride Test:** *Martynia annua* extract was dissolved in distilled water. Add a few drops of a diluted ferric chloride solution. The intense blue color showed the existence of tannins.
- **Gelatin Test:** A measured amount of Martynia annua extract was dissolved in distilled water, and 2 mL of 1% gelatin solution containing 10% sodium chloride was added. The formation of a white precipitate indicated the presence of phenolic compounds in the extract.

- **Lead Acetate Test:** A small amount of *Martynia annua* extract was mixed in a test tube with distilled water, followed by a few drops of lead acetate solution. The formation of white precipitate suggests that phenolic chemicals are present.

➢ **Test for protein and amino acids**

- **Biuret's test:** The Martynia annua extract was treated with 1 mL of 10% sodium hydroxide solution in a test tube and heated. A drop of 0.7% copper sulfate solution was then added. The appearance of a violet or pink color indicated the presence of proteins in the extract.
- **Ninhydrin test:** 3ml of extract was heated with 3drops of 5%ninhydrin solution in a water bath for 10 minutes. Formation of blue colour indicates the presence of amino acids.

➢ **Test for Flavonoids**

- **Lead Acetate test:** The extract was treated with few drops of lead acetate solution. Formation of yellow color precipitate may indicate the presence of flavonoids.
- **Alkaline reagent test:** The Martynia annua extract was treated with a few drops of sodium hydroxide in a test tube. The development of an intense yellow color, which disappeared upon the addition of dilute acid, indicated the presence of flavonoids in the extract.

➢ **Test for Glycosides**

- **Borntragers Test:** Three milliliters of the Martynia annua test solution were mixed with dilute sulfuric acid and boiled for 5 minutes. After cooling, the filtrate was mixed with an equal volume of benzene or chloroform and shaken thoroughly. The organic layer was separated, and ammonia was added. The appearance of a pink to crimson color in the ammonical layer indicated the presence of anthraquinone glycosides in the extract.
- **Keller Killiani Test:** In a test tube, 2 mL of the Martynia annua test solution was mixed with 3 mL of glacial acetic acid and one drop of 5% ferric chloride. Then, 0.5 mL of concentrated sulfuric acid was carefully added. The appearance of a blue color in the acetic acid layer indicated the presence of cardiac glycosides in the extract.

2.3 Quantitative Phytochemical Estimation

To further assess the bioactive potential of *Martynia annua*, quantitative estimation of key phytochemicals was conducted on the methanolic and petroleum ether extracts. This evaluation was aimed at determining the concentration of major secondary metabolites such

as total phenolics, flavonoids, alkaloids, and tannins, which are often associated with therapeutic activities.

2.3.1 Total phenolic content (TPC)

The total phenolic content of *Martynia annua* extracts was determined using the Folin–Ciocalteu colorimetric method. A 0.2 mL aliquot of the extract was mixed with 2.5 mL of Folin–Ciocalteu reagent and 2 mL of 7.5% sodium carbonate, then diluted to 7 mL with distilled water. The mixture was incubated at room temperature for 2 hours, and the absorbance was measured at 760 nm using a UV-Visible spectrophotometer. Total phenolics were quantified against a gallic acid standard curve (20–100 µg/mL) and expressed as mg gallic acid equivalents per gram of dry extract (mg GAE/g). The blue-colored complex formed results from the reduction of Folin–Ciocalteu reagent by phenolic compounds, with color intensity proportional to phenolic content (Babbar et al., 2011).

2.3.2 Total flavonoid content (TFC)

The total flavonoid content of *Martynia annua* extracts was measured using the aluminium chloride colorimetric method. A 0.5 mL aliquot of extract was mixed with 2 mL distilled water and 0.15 mL of 5% sodium nitrite, allowed to stand for 6 minutes, then 0.15 mL of 10% aluminium chloride was added and left for another 6 minutes. Finally, 2 mL of 4% sodium hydroxide was added, and the mixture was thoroughly shaken. The absorbance of the resulting pink complex was measured at 510 nm using a UV-Visible spectrophotometer. Total flavonoid content was calculated from a rutin standard curve (20–100 µg/mL) and expressed as mg rutin equivalents per gram of dry extract (mg RE/g). This assay is based on flavonoid–aluminium complex formation, which exhibits maximum absorbance at 510 nm, enabling precise quantification (Ghafar et al., 2017).

2.3.3 Acute Toxicity Study

The acute oral toxicity of the test substance was assessed using the Acute Toxic Class Method in accordance with standard regulatory guidelines. In this approach, small groups of animals (typically three of the same sex per step) were administered the test compound orally at fixed dose levels of 5, 50, 300, or 2000 mg/kg body weight. Animals were closely observed for signs of toxicity, including mortality and severe adverse effects. Based on observed responses—no toxicity, partial toxicity, or death—the dose for the next group was adjusted upward or downward as per the guidelines. This stepwise method allows determination of the

approximate lethal dose while minimizing the number of animals used, providing a reliable toxicity profile for the test substance (Halim et al., 2011).

2.4 Experimental work of Antidiabetic Activity

- **Animals Protocol**

All animal experiments were performed in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The study protocol, including animal care, handling, and experimental design, was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) before initiation. Healthy adult Wistar rats of either sex, weighing 250–300 g, were obtained from a registered breeding facility and housed under standard laboratory conditions (temperature 22 ± 2°C, relative humidity 55 ± 10%, 12-hour light/dark cycle). Animals received standard pellet diet and water ad libitum. All procedures were conducted to minimize animal suffering and reduce the number of animals used, ensuring that acute toxicity and pharmacological evaluations adhered strictly to ethical norms.

- **Animal used**

Weight - 250 ± 60 gm

Strain - Wistar rat

Sex – Either sex

- **Streptozotocin induced diabetes**

Streptozotocin (STZ) [Batch No. T1829656] was obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Diabetes was induced by freshly preparing STZ in 0.1 M citrate buffer (pH 4.5) and administering it orally at 50 mg/kg body weight. The solution was injected within 15 minutes of preparation using a 1 mL tuberculin syringe with a 24-gauge needle, with each animal receiving 0.4 mL. Control animals received only citrate buffer (0.4 mL) under the same conditions. Fasting blood glucose levels were measured on the third day post-administration, and animals with glucose levels above 250 mg/dL were considered diabetic and selected for further pharmacological evaluation (Prasad et al., 2009).

- **Experimental design**

The experimental animals were randomly divided into five groups, each consisting of an equal number of rats (n = 6). Treatments were administered once daily via oral intubation using a feeding tube. The grouping and treatment protocols were as follows:

Group 1 – Normal Control

Received 1 ml of distilled water orally and served as the non-diabetic control group.

Group 2 – Diabetic Control

Diabetes was induced with streptozotocin (50 mg/kg body weight, orally). This group received no further treatment and served as the untreated diabetic control.

Group 3 – Standard Drug Treatment

Diabetic rats in the standard reference group were administered glibenclamide orally at a dose of 10 mg/kg body weight daily, dissolved in water, for 30 consecutive days each morning.

Group 4 – Test Group I

Diabetic rats were treated with *Martynia annua* extract at a dose of 200 mg/kg body weight per day. The extract was administered orally prior to feeding each morning for 14 consecutive days.

Group 5 – Test Group II

Diabetic rats were administered *Martynia annua* extract at a higher dose of 400 mg/kg body weight per day, using the same oral route before meals, for a period of 14 consecutive days.

- **Collection of Blood Samples for Glucose Analysis**

Blood glucose levels were monitored in overnight-fasted rats (12 hours) via tail vein sampling at predetermined time points following streptozotocin administration. The initial measurement, taken three days post-STZ injection (day 0), confirmed diabetic status. Subsequent readings were recorded on days 1, 3, 5, 7, and 14 during treatment. For each sample, a small incision was made at the tail tip, the first drop of blood was discarded, and the second drop was applied to a glucose test strip and measured using an Accu-Chek glucometer (Roche Diagnostics, Germany). The puncture site was cleaned with ethanol after each collection to reduce infection risk.

- **Biochemical Parameters**

To assess the effects of treatment on lipid metabolism, blood samples were collected from overnight-fasted rats on days 0 and 14. Under light diethyl ether anesthesia, blood was drawn via retro-orbital plexus puncture and allowed to clot at room temperature for 30 minutes. The clotted blood was centrifuged at 2500 rpm for 10 minutes at 25°C to obtain serum. The serum

samples were subsequently analyzed for total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C).

Additional lipid parameters were calculated using standard formulas:

- Very Low-Density Lipoprotein (VLDL-C) was estimated as:

$$\textbf{VLDL-C = TG / 5}$$

- Low-Density Lipoprotein (LDL-C) was calculated using the equation:

$$\textbf{LDL-C = TC – (HDL-C + VLDL-C)}$$

These parameters provided insight into the lipid profile alterations associated with diabetic conditions and the potential lipid-modulating effects of *Martynia annua* extracts.

3. RESULTS

3.1 Percentage Yield

Percentage yield is an important parameter in phytochemical extraction, reflecting the efficiency of extraction for a given plant, plant part, or solvent. Table 3 presents the extract yields obtained from *Martynia annua*.

Table 3: Percentage Yield of crude extracts of *Martynia annua* extract.

S. No	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1	*Martynia annua*	Pet ether	350	1.15	0.32%
2		Methanol	290.85	5.54	1.90%

3.2 Preliminary Phytochemical study

Table 4: Phytochemical testing of extract petroleum ether.

S. No.	Experiment	Presence or absence of phytochemical test
		Petroleum ether extract
1.	**Alkaloids**	
1.1	Dragendroff’s test	Absent (- ve)
1.2	Mayer’s reagent test	Absent (- ve)
1.3	Wagner’s reagent test	Absent (- ve)
1.3	Hager’s reagent test	Absent (- ve)
2.	**Glycoside**	
2.1	Borntrager test	Present (+ ve)
2.2	Legal’s test	Present (+ ve)
2.3	Killer-Killiani test	Present (+ ve)
3.	**Carbohydrates**	
3.1	Molish’s test	Absent (- ve)
3.2	Fehling’s test	Absent (- ve)

3.3	Benedict's test	Absent (- ve)
3.4	Barfoed's test	Absent (- ve)
4.	**Proteins and Amino Acids**	
4.1	Biuret test	Present (+ ve)
4.2	Ninhydrin test	Present (+ ve)
5.	**Flavonoids**	
5.1	Alkaline reagent test	Present (+ ve)
5.2	Lead Acetate test	Present (+ ve)
6.	**Tannin and Phenolic Compounds**	
6.1	Ferric Chloride test	Present (+ ve)
7.	**Saponin**	
7.1	Foam test	Absent (- ve)
8.	**Test for Triterpenoids and Steroids**	
8.1	Salkowski's test	Absent (- ve)
8.2	Libbermann-Burchard's test	Absent (- ve)

Table 5: **Phytochemical testing extract of methanol.**

S. No.	**Experiment**	**Presence or absence of phytochemical test**
		Methanol extract
1.	**Alkaloids**	
1.1	Dragendroff's test	Present (+ ve)
1.2	Mayer's reagent test	Present (+ ve)
1.3	Wagner's reagent test	Present (+ ve)
1.3	Hager's reagent test	Present (+ ve)
2.	**Glycoside**	
2.1	Borntrager test	Present (+ ve)
2.2	Legal's test	Present (+ ve)
2.3	Killer-Killiani test	Present (+ ve)
3.	**Carbohydrates**	
3.1	Molish's test	Present (+ ve)
3.2	Fehling's test	Present (+ ve)
3.3	Benedict's test	Present (+ ve)
3.4	Barfoed's test	Present (+ ve)
4.	**Proteins and Amino Acids**	
4.1	Biuret test	Absent (- ve)
4.2	Ninhydrin test	Absent (- ve)
5.	**Flavonoids**	
5.1	Alkaline reagent test	Present (+ ve)
5.2	Lead Acetate test	Present (+ ve)
6.	**Tannin and Phenolic Compounds**	
6.1	Ferric Chloride test	Present (+ ve)
7.	**Saponin**	
7.1	Foam test	Present (+ ve)
8.	**Test for Triterpenoids and Steroids**	
8.1	Salkowski's test	Absent (- ve)
8.2	Libbermann-Burchard's test	Absent (- ve)

Preliminary phytochemical screening showed that the methanol extract of *Martynia annua* contained a wider range of bioactive compounds—including alkaloids, glycosides, carbohydrates, flavonoids, tannins, and saponins—due to its polar nature. In contrast, the petroleum ether extract contained only glycosides, proteins, amino acids, flavonoids, and phenolic compounds, with alkaloids, carbohydrates, saponins, and steroids absent, reflecting the limited extraction capacity of the non-polar solvent. Triterpenoids and steroids were absent in both extracts. Overall, the methanol extract exhibited a richer phytochemical profile, making it more suitable for further pharmacological studies.

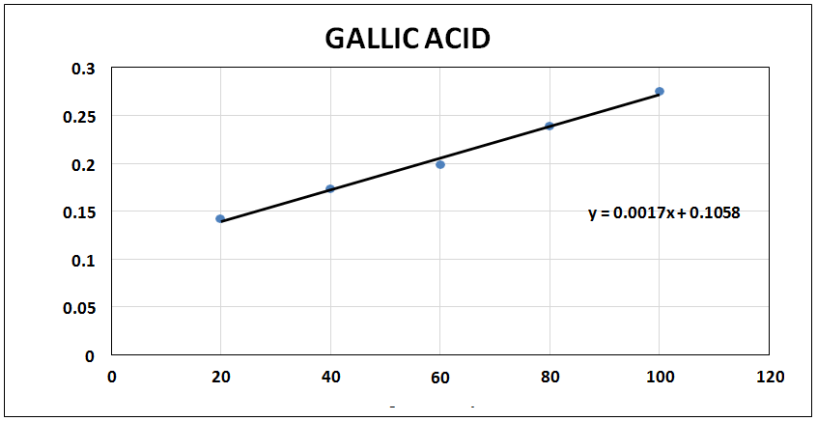
3.3 Quantitative Analysis

Initial analysis of the crude *Martynia annua* extract indicated substantial amounts of biologically active secondary metabolites, particularly flavonoids and phenolic compounds. Targeted spectrophotometric methods were employed to quantify total flavonoid and phenolic content, providing an estimate of the extract's potential antioxidant and health-promoting properties.

3.3.1 Total Phenolic content (TPC) estimation Table 6: Standard table for Gallic acid

Table 6: Standard table for Gallic acid.

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.142
2.	40	0.173
3.	60	0.198
4.	80	0.239
5.	100	0.275



2.3.1.1 Total Phenolic Content in extract

Table 7: Total Phenolic Content.

S. No	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1	0.151	86.2 mg/gm
2	0.195	
3	0.230	

Table 8: Total Phenolic Content of extract *Martynia annua.*

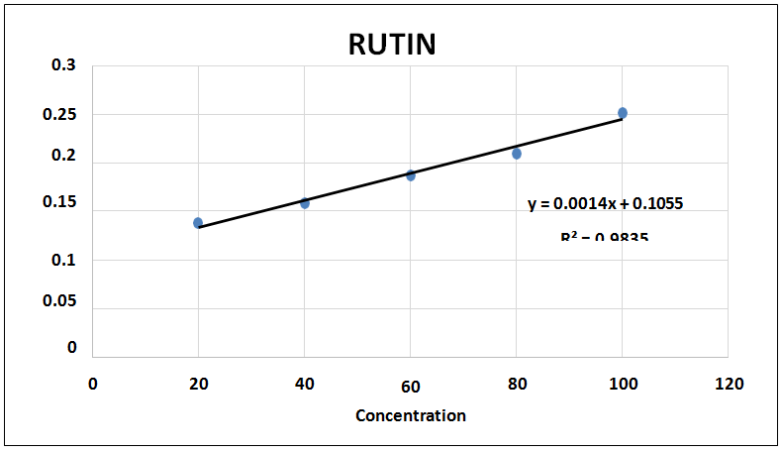
Extracts	Total Phenolic content (mg/gm equivalent of Gallic acid)
Methanol	86.2

The *Martynia annua* extract exhibited a total phenolic content of 86.2 mg/g gallic acid equivalent, reflecting a high concentration of phenolic compounds. Quantification using the Folin–Ciocalteu method and a gallic acid standard curve confirmed significant antioxidant potential, supporting the extract's prospective therapeutic applications.

2.3.2 Total Flavonoids content (TFC) estimation

Table 8: Standard table for Rutin.

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.138
2.	40	0.159
3.	60	0.187
4.	80	0.210
5.	100	0.252



2.3.2.1 Total Flavonoid Content in extract

Table 10: Total Flavonoid Content.

S. No	Absorbance	TFC in mg/gm equivalent of Rutin
1	0.139	73.5 mg/gm
2	0.192	
3	0.206	

Table 9: Total Flavonoid Content of extract *Martynia annua.*

Extracts	Total Flavonoid content (mg/gm equivalent of rutin)
Methanol	**73.5**

The methanol extract of *Martynia annua* exhibited a total flavonoid content of 73.5 mg/g rutin equivalent, determined using the aluminum chloride colorimetric method with rutin as the standard. This high flavonoid content highlights the extract's potential antioxidant and anti-inflammatory properties, suggesting promising therapeutic applications.

2.4 *In vivo* acute oral toxicity (OECD 423)

Table 12: General appearance and behavioral observations of acute oral toxicity study for control and treated groups.

Observations	Control	5 mg/kg	50 mg/kg	300 mg/kg	2000 mg/kg
Food intake	Normal	Normal	Normal	Normal	Normal
Body weight	Normal	Normal	No change	No change	No change
Temperature	Normal	Normal	Normal	Normal	Normal
Changes in skin and fur	Nill	Nill	Nill	Nill	Nill
Urination	Normal	No effect	No effect	No effect	No effect
Diarrhoea	Absent	Absent	Absent	Absent	Absent
Death	Alive	Alive	Alive	Alive	Alive

The acute oral toxicity study showed that *Martynia annua* extract was safe at doses up to 2000 mg/kg, with no mortality or signs of toxicity observed. Animals exhibited normal behavior, food intake, body temperature, and appearance, with no diarrhea, urinary issues, or skin changes. Body weight remained stable, indicating a high safety margin and supporting the extract's suitability for further biological evaluations.

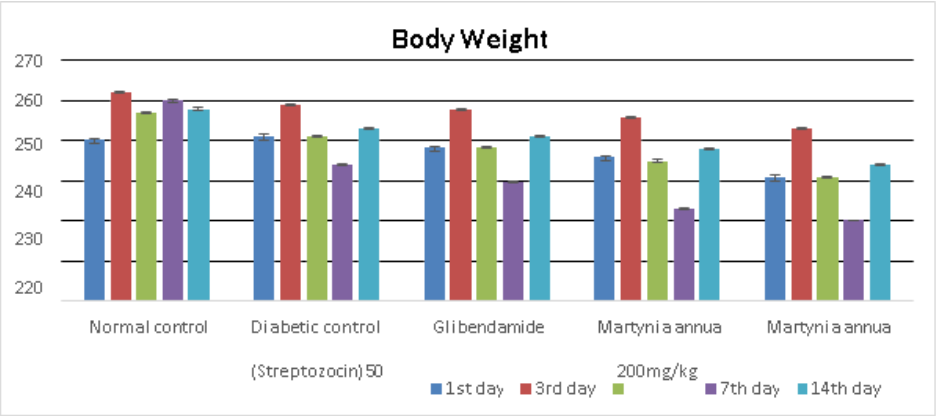
2.5 Streptozotocin induced diabetes Model

Figure 18: Oral Dosing of Streptozotocin induced diabetes Model in rats.

2.6 *In vivo* anti -diabetic study

Table 13: Effect of *Martynia annua* extract on Body weight of the rats.

Body weight(gms)						
Groups	**Treatments**	**1 day**	**3 days**	**5 days**	**7 days**	**14 days**
Group 1	**Normal control**	250.12±0.262	251.23±0.234	248.19±0.221	245.99±0.214	240.98±0.253
Group II	**Diabetic control (Streptozocin) 50 mg/kg**	261.89±0.210	258.76±0.145	257.65±0.185	255.87±0.251	253±0.262
Group III	**Glibenclami de (10 mg/kg)**	256.93±0.249	251.10±0.271	248.203±0.187	244.87±0.204	240.89±0.272
Group IV	**Martynia annua 200mg/kg**	259.90±0.223	243.99±0.263	239.65±0.394	233.02±0.157	230.05±0.241
Group V	**Martynia annua 400mg/kg**	257.91±0.255	253.01±0.207	250.99±0.138	247.87±0.121	243.98±0.228

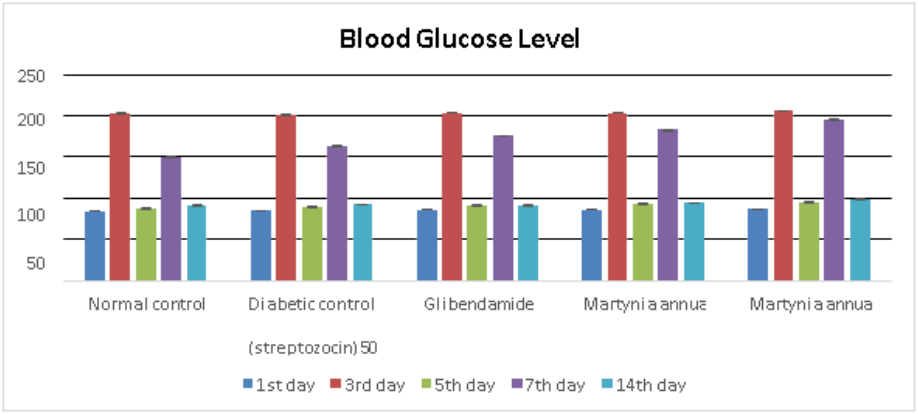


Graph 1: Graphical representation of effect of *Martynia annua* extract on Body weight of the rats.

2.7 Blood Glucose Level

Table 14: Effect of test samples of extract on Blood Glucose Level in experimental rats.

Blood Glucose Level (gms)						
Groups	**Treatment**	**1 day**	**3 days**	**5 days**	**7 days**	**14 days**
Group 1	**Normal control**	84.07±0.192	84.45±0.208	85.98±0.334	85.99±0.194	86.76±0.269
Group II	**Diabetic control (streptozocin) 50 mg/kg**	202.89±0.179	200.87±0.260	203.76±0.238	204.65±0.306	205.89±0.228
Group III	**Glibenclami de (10 mg/kg)**	88.05±0.226	89.76±0.104	90.88±0.496	92.89±0.287	95.78±0.093
Group IV	**Martynia annua Dose-200mg/kg**	150.67±0.095	162.89±0.601	175.98±0.201	182.89±0.235	195.45±0.208
Group V	**Martynia annua 400mg/kg**	90.99±0.351	92.76±0.264	91.43±0.121	94.21±0.256	98.90±0.34

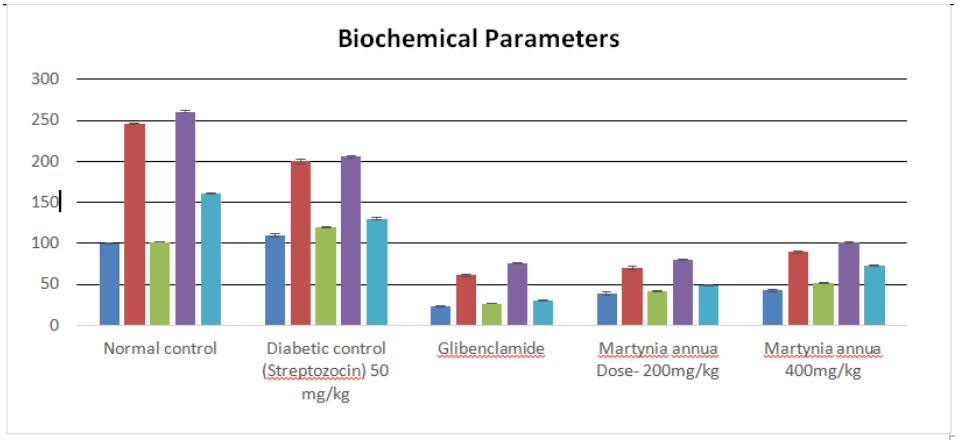


Graph 2: Graphical representation of effect of *Martynia annua* extract on Blood Glucose Level of the rats.

2.8 Biochemical Parameters

Table 15: Effect of test samples of extract on Biochemical Parameters in experimental rats.

Treatment groups	Biochemical parameters				
	TC(g/l)	TG(g/l)	VLDL(g/l)	HDL-C(g/l)	LDL-C(g/l)
Normal control	100±1.22	110±0.91	23.52±0.24	38.67±0.81	43±0.45
Diabetic control (Streptozocin) 50 mg/kg	245.65±2.02	200.14±2.78	60.82±0.55	69.90±1.64	89.73±0.98
Glibenclamide (10 mg/kg)	101.54±1.11	119.54±0.99	26.98±0.26	41.78±0.89	51.67±0.66
Martynia annua Dose- 200mg/kg	260.76±1.99	205.65±2.01	75.65±0.50	79.56±1.25	100.87±1.15
Martynia annua 400mg/kg	160.54±1.50	130.24±1.50	29.87±0.34	48.89±0.99	72.87±1.16

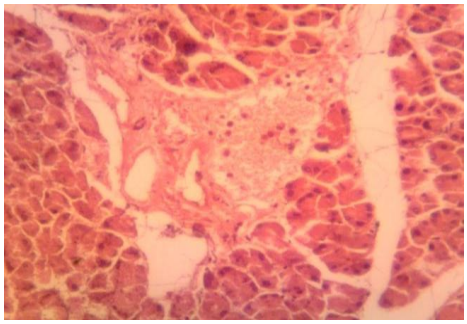


Graph 3: Graphical representation of effect of *Martynia annua* extract on Biochemical Parameters of the rats.

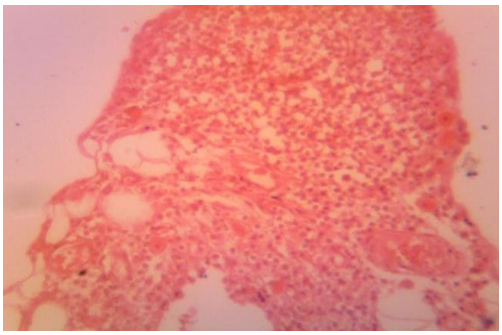
The in vivo study of *Martynia annua* extract demonstrated significant anti-diabetic effects in streptozotocin-induced diabetic rats. Diabetic control rats exhibited progressive weight loss and elevated blood glucose, confirming diabetes induction. Administration of the extract at 400 mg/kg stabilized body weight and markedly reduced blood glucose levels by day 14, showing effects comparable to glibenclamide, while the 200 mg/kg dose produced moderate improvements. Biochemical analysis revealed that the 400 mg/kg dose improved lipid profiles by decreasing total cholesterol, triglycerides, VLDL, and LDL-C, and increasing HDL-C, indicating both anti-diabetic and cardioprotective potential. These results highlight the extract's dose-dependent efficacy in controlling hyperglycemia and improving lipid metabolism.

2.9 Histopathological examination

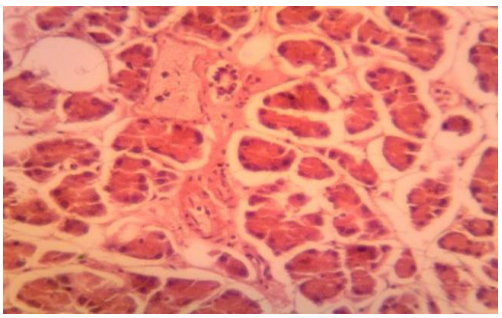
GROUP 1



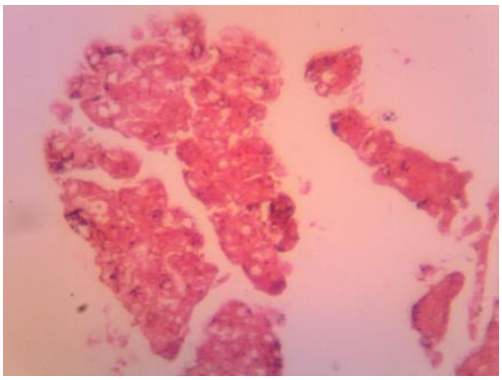
GROUP 2



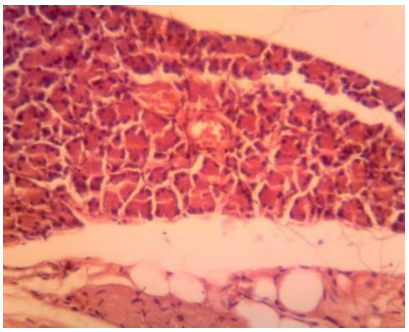
GROUP 3



GROUP 4



GROUP 5



The in vivo anti-diabetic study in rats demonstrated that *Martynia annua* seed extract positively influenced body weight, blood glucose, and lipid profile. Diabetic control rats experienced continuous weight loss, while extract-treated rats showed dose-dependent effects, with the 400 mg/kg group exhibiting the greatest weight reduction. Blood glucose levels remained elevated in untreated diabetic rats but declined significantly in extract-treated groups, especially at 400 mg/kg by day 14. Diabetic rats displayed dyslipidemia, including increased total cholesterol, triglycerides, VLDL, and LDL-C, alongside decreased HDL-C. Treatment with *Martynia annua* extract moderately improved these lipid parameters, with the higher dose yielding more pronounced benefits. Overall, the findings suggest that *Martynia annua* seed extract, particularly at 400 mg/kg, possesses notable anti-diabetic activity by enhancing glycemic control, improving lipid metabolism, and mitigating diabetes-induced weight loss.

CONCLUSION

The present study demonstrates that *Martynia annua* seed extract possesses significant anti-diabetic potential with additional benefits on lipid metabolism and overall metabolic health. Phytochemical analysis revealed a rich presence of bioactive compounds, particularly flavonoids and phenolics, which contribute to its antioxidant and therapeutic properties. Acute oral toxicity studies confirmed the extract's safety at doses up to 2000 mg/kg, indicating a wide safety margin for biological applications. In streptozotocin-induced diabetic rats, the extract, especially at 400 mg/kg, effectively reduced blood glucose levels, mitigated diabetes-associated weight loss, and improved lipid profiles by lowering total cholesterol, triglycerides, VLDL, and LDL-C while increasing HDL-C. These findings collectively suggest that *Martynia annua* seed extract is a safe, potent, and promising candidate for managing diabetes and its associated metabolic complications, warranting further investigation for potential clinical application.

Conflict of interest

The authors declare no conflict of interest related to the formulation, analysis, or reporting of the data presented in this study.

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