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Antidiabetic and Protective Effects of Bioactive Fractions of *Abelmoschus ficulneus* in Streptozotocin-induced Diabetic Rats

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Abstract

Background: Diabetes mellitus is a chronic metabolic disorder causing hyperglycaemia and organ damage. Limitations of current therapies highlight the need for safer, plant-based alternatives. The antidiabetic and organ-protective potential of *Abelmoschus ficulneus* remains largely unexplored. **Aim:** To evaluate the antidiabetic, hepatoprotective, and nephroprotective effects of *A. ficulneus* leaf extracts and fractions in streptozotocin-induced diabetic rats. **Methods:** Acute oral toxicity of *A. ficulneus* leaf extracts was assessed up to 2000 mg/kg. Diabetes was induced in Wistar rats using streptozotocin (60 mg/kg). Animals were allocated to control, diabetic control, glimepiride-treated, extract-treated, and fraction-treated groups. Blood glucose and body weight were monitored on days 0, 7, 14, and 21. At study completion, blood and urine samples were analysed for renal (serum creatinine, serum total protein, urine total protein) and hepatic (SGPT, SGOT, serum bilirubin) biomarkers using commercial kits. Liver and kidney tissues were examined histopathologically. **Results:** No mortality or toxicity was observed up to 2000 mg/kg. Streptozotocin caused marked hyperglycaemia (375.17 ± 1.60 vs. 64.16 ± 2.71 mg/dL in controls) and body weight loss (−19.41 ± 0.72 g). Treatment with *A. ficulneus* significantly reduced blood glucose, with the aqueous extract (200 mg/kg) lowering levels to 99.5 ± 3.69 mg/dL, comparable to glimepiride (97.83 ± 1.04 mg/dL). Weight loss was attenuated, with methanol fraction AF008-F5 (200 mg/kg) showing the greatest improvement (−0.46 ± 0.02 g). Elevated hepatic markers were significantly reduced by AF008-F5 (SGPT: 52.36 ± 0.52 IU/L, SGOT: 53.51 ± 0.21 IU/L, bilirubin: 1.01 ± 0.01 mg/dL). Histopathological findings confirmed protection against diabetes-induced liver and kidney damage. **Conclusion:** *A. ficulneus* leaf extracts, particularly the bioactive methanol fraction AF008-F5, demonstrated significant antihyperglycaemic, hepatoprotective and nephroprotective effects in diabetic rats without observable toxicity. These findings support the potential of *A. ficulneus* as a promising natural therapeutic candidate for diabetes and its associated complications.

Major Finding: *Abelmoschus ficulneus* fractions significantly ameliorated streptozotocin-induced hyperglycemia and associated organ dysfunction in rats. Among the tested fractions, the methanolic fraction (AF011-F5, 200 mg/kg) exhibited the greatest efficacy by lowering blood glucose levels, improving body weight and restoring renal and hepatic biomarkers.

Keywords: *Abelmoschus ficulneus*, Antidiabetic Activity, Hepatoprotective and Nephroprotective Effects, Methanol Fraction, Streptozotocin-induced Diabetes

Abbreviations: D – Diabetic; AF- *Abelmoschus ficulnesus*, F – fraction; Hex – hexane; EA - ethyl acetate; ME – Methanol; AF02 - 50% Ethanol; AF008 - 100% Water; SGPT - Serum Glutamate Pyruvate Transaminase/ALT; SGOT - Serum Glutamic Oxaloacetic Transaminase/AST.

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1. Introduction

Diabetes mellitus is a long-term metabolic disease marked by elevated blood glucose levels due to impaired insulin production, reduced insulin sensitivity, or a combination of both conditions. This metabolic imbalance affects the metabolism of carbohydrates, fats, and proteins, which may eventually result in serious complications involving various organs and body systems. Over the past few decades, the prevalence of diabetes has risen significantly, establishing it as a critical global health challenge. Current reports suggest that nearly 589 million adults are affected by diabetes globally, with the number expected to rise beyond 850 million by the year 2050[1,2]. The prevalence has nearly doubled over the past three decades, particularly in low- and middle-income countries[3].

Diabetes is associated with a wide range of complications, including cardiovascular diseases, nephropathy, neuropathy, and retinopathy, which significantly contribute to morbidity and mortality. Chronic hyperglycaemia induces oxidative stress and inflammatory pathways that play a central role in the progression of these complications[4–6]. Despite the availability of various synthetic antidiabetic agents such as sulfonylureas and biguanides, their long-term use is often limited by adverse effects, reduced efficacy, and secondary treatment failure[7,8]. Therefore, the search for safer and more effective therapeutic alternatives remains an important area of research.

In recent years, medicinal plants have attracted considerable attention as potential sources of antidiabetic agents. These plants are rich in bioactive compounds such as flavonoids, phenolics, and alkaloids, which exhibit antihyperglycaemic, antioxidant and cytoprotective properties[9–12]. Several studies have demonstrated that plant extracts can significantly improve glycaemic control and reduce oxidative stress in experimental diabetic models[13–15]. However, only a limited number of medicinal plants have undergone rigorous scientific validation for their efficacy and safety.

Abelmoschus ficulneus (AF), belonging to the family *Malvaceae,* is a traditionally used medicinal plant known for its therapeutic potential. Preliminary phytochemical investigations indicate the presence of bioactive constituents that may contribute to its pharmacological properties, including possible antidiabetic and protective effects. However, its efficacy in managing diabetes and associated complications has not been extensively explored.

Streptozotocin-induced diabetes is a well-established experimental model widely used for evaluating antidiabetic agents, as it selectively destroys pancreatic β-cells and induces hyperglycaemia similar to human diabetes[16–18]. Glimepiride is considered to have a lower risk of causing severe hypoglycaemia, as a significant part of its glucose-lowering action is attributed to its extra-pancreatic mechanisms in addition to its pancreatic effects[19].

Therefore, the present study aims to investigate the antidiabetic and protective effects of bioactive fractions of *A. ficulneus* leaf extract in streptozotocin-induced diabetic rats, thereby providing scientific validation for its traditional use and exploring its potential as a natural therapeutic agent.

2. Materials and Methods

2.1 Plant Material

The leaves of *A. ficulneus* were collected from the forest regions surrounding Nanded district, Maharashtra, and subsequently dried under shade conditions. The botanical authentication of the plant material was carried out by Dr. V. R. Marathe, Department of Botany, N. E. S. Science College, SRTM University, Nanded.

2.2 Reagents

Streptozotocin was procured from Sigma-Aldrich (St. Louis, Missouri, USA) and was freshly prepared by dissolving it in ice-cold citrate buffer (p^H 4.5) immediately before administration. Standard drug Glimepiride obtained from the Supra Chemicals Limited, Mumbai. Organic solvents used for the extraction of the plant material were all purchased from Rankem. All other chemicals used were of analytical grade.

2.3 Extraction and Fractionation of Plant Material

Extraction of *A. ficulneus* was performed based on solvent polarity using aqueous and ethanol solvents to obtain a broad spectrum of phytoconstituents. The 50% ethanol extract showed higher yield, whereas the

100% aqueous extract exhibited superior α-amylase inhibitory activity. Based on these findings, extracts AF002 (ethanol) and AF008 (aqueous) were selected for further purification.

Fractionation of AF002 was carried out using silica gel column chromatography (60–120 mesh; 40 mm × 60 mm), packed by the slurry method and pre-equilibrated with hexane. The extract (5 g), previously adsorbed onto silica gel, was subjected to elution at a flow rate of 5 mL/min using solvent systems with gradually increasing polarity. The solvents employed included 100% hexane, hexane:ethyl acetate (50:50), ethyl acetate:methanol (80:20), ethyl acetate:methanol (50:50), and finally 100% methanol.

Purification of the aqueous extract (AF011) was performed using a similar procedure (batch AF002) up to column packing. Elution was carried out using ethyl acetate:methanol mixtures of increasing polarity: 85:15 (AF011-F1), 65:35 (AF011-F2), 50:50 (AF011-F3), 20:80 (AF011-F4), and methanol (100%) (AF011-F5). All fractions were collected, monitored by Thin-Layer Chromatography (TLC), pooled based on similarity and concentrated using a rotary evaporator.

2.4 Experimental Animals

Adult Wistar rats (150–200 g) were used for the study, with six animals in each group. The animals were procured from a certified breeding facility, Systemic Life Sciences and Research Private Limited, Aushapur, Telangana, India. The animals were maintained under standard laboratory conditions with a controlled temperature of 22 ± 2 °C, relative humidity of 55 ± 5%, and a 12-hour light and dark cycle. Before the initiation of the experiment, the animals were acclimatised to the laboratory environment for a period of seven days. Standard pellet feed and water were supplied freely throughout the study. All experimental protocols were performed in compliance with the guidelines prescribed by the Committee for the Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was reviewed and approved, and the study plan was approved with 19/IAEC-II/SLSRPL/2024 approval.

2.5 Diabetes Induction in Wistar Rats

Before diabetes induction, the rats were fasted overnight. Experimental diabetes was induced by intraperitoneal administration of streptozotocin (STZ) at a dose of 60 mg/kg, freshly prepared in 0.1 M sodium citrate buffer[20]. Following STZ administration, the animals were provided with standard rat feed and water ad libitum and were observed routinely throughout the experimental period.

Hyperglycaemia was confirmed 48 hours after STZ induction by determining fasting blood glucose levels using a glucometer (Accu-Chek). Blood samples were obtained from the tail veins of rats after 16 hours of fasting[21]. Animals exhibiting fasting blood glucose levels above 250 mg/dL along with classic diabetic symptoms such as polyphagia, polydipsia, polyuria and loss of body weight were considered diabetic and included in the study.

2.6 Collection of Blood and Determination of Blood Glucose Levels

The rats were anesthetised with ketamine (60 mg/kg) and xylazine (10 mg/kg), following which approximately 5 mL of blood was collected from each animal. Blood samples were obtained from the tail tip of the rats both before administration and 90 minutes after treatment with the extracts, fractions, or standard drugs. A glucometer (Accu-Chek) was used to measure blood glucose levels. Urine samples were collected over 24 h using metabolic cages.

2.7 Weight Assessment

Rats' body weight was regularly measured and tracked using a compression spring balance, which served as a gauge of the animals' physical health during the trial.

2.8 Estimation of Renal and Hepatic Biochemical Parameters

Serum creatinine was estimated by the Jaffe's alkaline picrate method at 520 nm and expressed in mg/dL; serum and urine total protein were determined by the Biuret method at 540 nm and expressed in g/dL. Hepatic function markers, including SGPT (ALT) and SGOT (AST), were estimated by the Reitman and Frankel method at 505 nm and expressed in IU/L, while serum bilirubin was determined by the Diazo method at 540 nm and expressed in mg/dL.

2.9 Evaluation of Hypoglycemic Effect

In the experiment, a total of seventy-two (150-200 g) diabetic rats were used. All experiments were carried

out using six animals per group. Thirteen groups (n = 6) were created from the rats. The rats were labelled and classified as follows.

Group 1: Normal group
Group 2: Diabetic rats Group
Group 3: Diabetic rats + Glibenclamide (10 mg/kg)
Group 4: Diabetic rats + AF02 -50% ethanol (100 mg/kg)
Group 5: Diabetic rats + AF02 -50% ethanol (200 mg/kg)
Group 6: Diabetic rats + AF08 -100% water (100 mg/kg)
Group 7: Diabetic rats + AF08 -100% water (200 mg/kg)
Group 8: Diabetic rats + AF02-F2 (hex:Ea) (100 mg/kg)
Group 9: Diabetic rats + AF02-F2 (hex:Ea) (200 mg/kg)
Group 10: Diabetic rats + AF011-F4 (EA:ME) (100 mg/kg)
Group 11: Diabetic rats + AF011-F4 (EA:ME) (200mg/kg)
Group 12: Diabetic rats + AF011-F5 ME (100 mg/kg)
Group 13: Diabetic rats + AF011-F5 ME (200mg/kg)

2.10 Statistical Analysis

The blood glucose levels (mg/dL) and body weights (g) of the experimental rats recorded before and after diabetes induction were statistically evaluated using analysis of variance (ANOVA), and the obtained data were tabulated accordingly. All experimental findings were expressed as mean ± standard error of the mean (SEM). Comparisons between groups were performed using ANOVA, and differences among multiple groups were assessed through analysis of variance methods. A probability value of $p < 0.05$ was considered to indicate statistical significance.

3. Results

3.1 Acute Toxicity Study

Oral administration of AF02 (50% ethanolic extract) and AF008 (aqueous extract) of *A. ficulneus* leaves at a dose of 2000 mg/kg did not produce any sign of toxicity or mortality in rats during the observation period. All animals remained normal, indicating the safety of the extracts at the tested dose. Based on these findings, experimental doses of 100 and 200 mg/kg were selected for subsequent pharmacological studies.

3.2 Effect on Blood Glucose Levels

As shown in Table 1, streptozotocin (STZ)-induced diabetic rats exhibited a marked increase in blood glucose levels (341.50 ± 0.88 to 375.17 ± 1.60 mg/dL) compared to the normal control (59.83–64.16 mg/dL). Treatment with glimepiride significantly reduced glucose levels to 97.83 ± 1.04 mg/dL by day 21. Among the extracts, AF008 (200 mg/kg) showed better antihyperglycemic activity (99.5 ± 3.69 mg/dL) than AF02. Among the fractions, **AF011-F5 (200 mg/kg)** produced the maximum reduction (98.66 ± 0.98 mg/

Table 1. Effect of *Abelmoschus ficulneus* extracts and fractions on blood glucose levels in streptozotocin-induced diabetic rats

Sr. No.	Groups	0th Day	7th Day	14th Day	21st Day
1	Control (saline water) negative control	59.83±1.195	60.66±0.42	61.16±1.49	64.16±2.71
2	Positive control (Streptozotocin dose)	341.50±0.885	346.16±0.47	354.01±0.57	375.17±1.60
3	Standard (D + Glimepiride)	326.66±1.453**	275.83±1.376**	172.83±0.70**	97.83±1.04**
4	D + AF02-50% Ethanol 100 mg/kg	340.33± 0.42**	294.33± 0.55**	197.33 ± 1.73**	111.83 ±1.88**
5	D + AF02-50% Ethanol 200 mg/kg	339.05 ± 0.42**	293.34 ± 2.15**	191.66± 1.25**	105.33 ± 1.60 **
6	D + AF008-100% Water 100 mg/kg	334.5 ± 0.42**	283.5± 0.49**	184.01 ±1.5**	105.16 ± 3.42**
7	D + AF008-100% Water 200 mg/kg	330.01±0.57**	278.16±1.81**	173.66±2.26**	99.5±3.69**#
8	D + AF02-F2 (hex:EA) 100 mg/kg	340.66 **±0.84****	292.16 ±1.42**	192.17**±1.41**	106.66**±1.43**
9	D + AF02-F2 (hex:EA) 200 mg/kg	336.5± 1.14**	289.33 ±0.66**	189.66±0.71	103.66±1.02
10	D + AF011-F4 (EA:ME)100 mg/kg	334.33±1.68**	283.83±1.35**	184.66±1.60**	104.83±1.30**
11	D + AF011-F4 (EA:ME) 200 mg/kg	330.5±0.99**	280.83±0.79**	181.15±0.61**	100.01±1.18**
12	D + AF011-F5 (ME) 100 mg/kg	333.66±1.33**	278.83±0.60**	176.33± 1.83**	100.83±1.19**
13	D + AF011-F5 (ME) 200 mg/kg	329.16 ±1.16**	276.16±1.01**	173.93±2.08**	98.66±0.98**#

Data are presented as Mean ± SEM (n = 6). Statistical evaluation was performed using ANOVA followed by Tukey's post hoc test. A value of *$p < 0.05$ was considered statistically significant, while **$p < 0.001$ indicated a highly significant difference in comparison with the diabetic control group. Values of #$p > 0.05$ were regarded as statistically non-significant when compared with the standard treatment group.

dL), comparable to the standard, followed by AF011-F4, indicating AF011-F5 as the most active fraction.

3.3 Effect on Body Weight

As shown in Table 2, streptozotocin-induced diabetic rats exhibited a significant decrease in body weight (−12.18±1.04 to −19.41±0.72 g) compared to the normal control group, which showed a gradual increase (3.17±0.57 to 5.49±0.13 g). Treatment with glimepiride significantly improved body weight (2.83±0.23 g on day 21).

Among the extracts, AF008 (200 mg/kg) showed better improvement (−1.41±0.31 g) compared to AF02. Among the fractions, AF011-F5 (200 mg/kg) exhibited the maximum protection against weight loss (−0.46±0.02 g), followed by AF011-F4, indicating its superior efficacy in ameliorating diabetes-induced weight reduction.

3.4 Effect on Renal Parameters

As summarised in Table 3, streptozotocin-induced diabetic rats showed marked renal dysfunction, evidenced by elevated serum creatinine (2.29 ± 0.03 mg/dL) and urine total protein (28.63 ± 0.44 g/dL), along with reduced serum total protein levels (3.18 ± 0.01 g/dL) compared to the normal control group (0.80 ± 0.01 mg/dL, 8.43 ± 0.13 g/dL, and 6.90 ± 0.02 g/dL, respectively). Treatment with glimepiride significantly ($p < 0.05$) restored these altered parameters toward normal values. Among the tested extracts, the aqueous extract AF008 (200 mg/kg) showed greater improvement in renal markers than the ethanolic extract. Among the fractions, AF011-F5 (200 mg/kg) exhibited the most pronounced renoprotective effect, with serum creatinine, serum total protein, and urine total protein values approaching those of the standard-treated group, followed by AF02-F2. Histopathological examination of kidney tissue further supported the biochemical findings. Diabetic control rats showed marked glomerular and tubular degeneration, whereas treatment with extracts and fractions improved renal architecture. The AF011-F5-treated group demonstrated near-normal renal histology with reduced structural damage, indicating significant nephroprotective activity. The detailed histopathological findings are presented in Figures 1(A-E) and 2(A-E), respectively.

3.5 Effect on Hepatic Parameters

As presented in Table 4, streptozotocin-induced diabetic rats showed a significant elevation in hepatic

Table 2. Effect of *Abelmoschus ficulneus* extracts and fractions on body weight in streptozotocin-induced diabetic rats

Sr. No.	Groups	Change in Body Weight (g)		
		7th Day	14th Day	21st Day
1	Control (saline water) negative control	03.17±0.57	04.13±0.32	05.49±0.13
2	Positive control (Streptozotocin dose)	-12.18±1.04	-14.20±0.08	-19.41±0.72
3	Standard (D + Glimepiride)	-2.94±0.05**	1.42±0.07**	2.83±0.23**
4	D + AF02-50% Ethanol 100 mg/kg	-11.10±0.11**	-9.93±0.04**	-6.98±0.62**
5	D + AF02-50% Ethanol 200 mg/kg	-9.88±0.07**	-8.07±0.06**	-5.78±0.47**
6	D + AF008-100% Water 100 mg/kg	-8.11±0.07**	-5.68±0.07**	-2.41±0.17**
7	D + AF008-100% Water 200 mg/kg	-6.03±0.02**	-2.83±0.03**	-1.41±0.31**
8	D + AF02-F2 (hex:EA) 100 mg/kg	-9.11±0.02**	-7.68±0.04**	-5.41±0.01**
9	D + AF02-F2 (hex:EA) 200 mg/kg	-8.42±0.02**	-6.17±0.03**	-3.64±0.02**
10	D + AF011-F4 (EA:ME)100 mg/kg	-6.83±0.01**	-4.27±0.03**	-2.41±0.02**
11	D + AF011-F4 (EA:ME) 200 mg/kg	-4.51±0.02**	-2.10±0.01**	-0.97±0.03**
12	D + AF011-F5 (ME) 100 mg/kg	-6.13±0.02**	-2.83±0.02**	-1.17±0.03**
13	D + AF011-F5 (ME) 200 mg/kg	-5.27±0.01**	-1.72±0.03**	-0.46±0.02**

Data are presented as Mean ± SEM (n = 6). Statistical evaluation was performed using ANOVA followed by Tukey's post hoc test. A value of *$p < 0.05$ was considered statistically significant, while **$p < 0.001$ indicated a highly significant difference in comparison with the diabetic control group. Values of #$p > 0.05$ were regarded as statistically non-significant when compared with the standard treatment group.

Table 3. Effect of *Abelmoschus ficulneus* extracts and fractions on renal parameters in streptozotocin-induced diabetic rats

Sr. No.	Groups	Serum Creatinine (mg/dl)	Serum Total Protein (g/dl)	Urine Total Protein (g/dl)
1	Control (saline water) negative control	0.80 ± 0.01	6.90 ± 0.02	8.43 ± 0.13
2	Positive control (Streptozotocin dose)	2.29 ± 0.03	3.18 ± 0.01	28.63 ±0.44
3	Standard (D + Glimepiride)	1.19 ± 0.01**	5.62 ± 0.01**	11.96 ± 0.27**
4	D + AF02-50% Ethanol 100 **mg/kg**	1.64 ± 0.01**	3.94 ± 0.01**	17.63± 0.15**
5	D + AF02-50% Ethanol 200 mg/kg	1.58 ± 0.01**	4.25 ± 0.01**	15.83± 0.17**
6	D + AF008-100% Water 100 mg/kg	1.70 ± 0.01**	4.27 ± 0.01**	16.23 ± 0.07**
7	D + AF008-100% Water 200 mg/kg	1.43 ± 0.01**#	5.08 ± 0.01**#	14.43**±**0.20**#
8	D + AF02-F2 (hex:EA) 100 mg/kg	1.47±0.01**	4.11±0.04**	16.15±0.22**
9	D + AF02-F2 (hex:EA) 200 mg/kg	1.38±0.01**	4.40 ± 0.08**	15.48±0.24**
10	D + AF011-F4 (EA:ME)100 mg/kg	1.61±0.02**	4.17±0.02**	15.85 ±0.16**
11	D + AF011-F4 (EA:ME) 200 mg/kg	1.54±0.01**	4.65±0.02**	14.83 ±0.17**
12	D + AF011-F5 (ME) 100 mg/kg	1.41±0.01**	4.51 ± 0.02**	14.05±0.25**
13	D + AF011-F5 (ME) 200 mg/kg	1.24±0.01**#	5.22±0.02**#	12.95**±0.31****#

Data are presented as Mean ± SEM (n = 6). Statistical evaluation was performed using ANOVA followed by Tukey's post hoc test. A value of *p < 0.05 was considered statistically significant, while **p < 0.001 indicated a highly significant difference in comparison with the diabetic control group. Values of #p > 0.05 were regarded as statistically non-significant when compared with the standard treatment group.

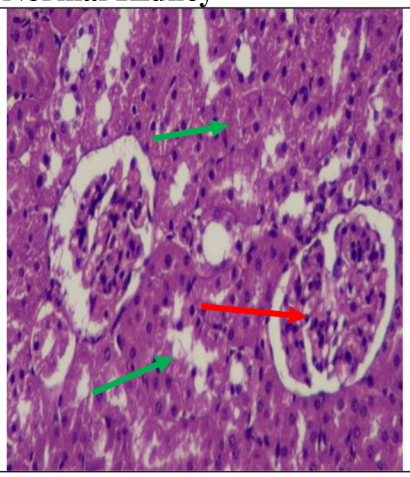
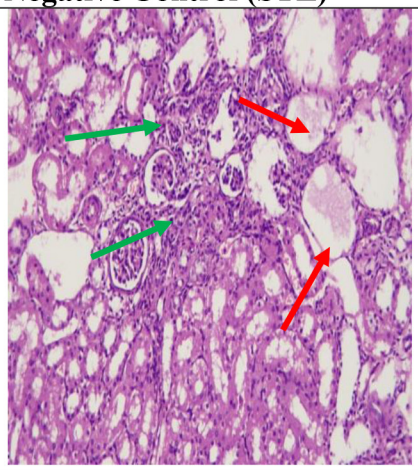
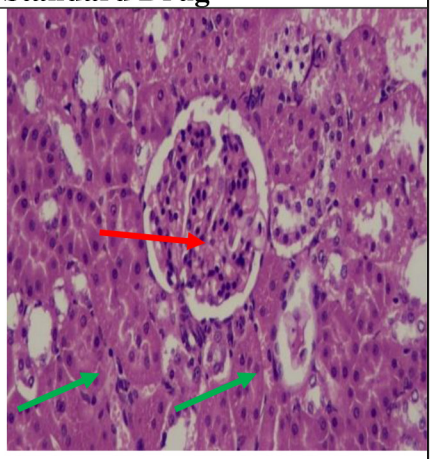
Table 4. Effect of *Abelmoschus ficulneus* extracts and fractions on hepatic parameters in streptozotocin-induced diabetic rats

Sr. No.	Groups	SGPT (IU/lit)	SGOT (IU/lit)	Serum Bilirubin (mg/dl)
1	Control (saline water) negative control	38.08±0.77	38.08±0.54	0.67 ±0.01
2	Positive control (Streptozotocin dose)	98.75±.0.47	99.83±0.51	2.41 ±0.05
3	Standard (D + Glimepiride)	51.81±0.12**	51.78±0.10**	0.91 ±0.01**
4	D + AF02-50% Ethanol 100 mg/kg	70.66±0.94**	75.43±0.73**	1.56 ± 0.05**
5	D + AF02-50% Ethanol 200 mg/kg	62.38±1.11**	70.81±0.57**	1.39± 0.03**
6	D + AF008-100% Water 100 mg/kg	65.20±0.64**	68.11±0.47**	1.44 ± 0.02**
7	D + AF008-100% Water 200 mg/kg	54.85±0.70**#	61.41±0.25**	1.08 ± 0.01**#
8	D + AF02-F2 (hex:EA) 100 mg/kg	61.81±1.02**	68.36±0.48**	1.39 ±0.02**
9	D + AF02-F2 (hex:EA) 200 mg/kg	59.28±0.66**	67.01±0.36**	1.32±0.02**
10	D + AF011-F4 (EA:ME)100 mg/kg	55.06±0.46**	60.78±0.10**	1.24 ±0.01**
11	D + AF011-F4 (EA:ME) 200 mg/kg	54.60 ±0.20**	57.56±0.19**	1.13 ±0.01**
12	D + AF011-F5 (ME) 100 mg/kg	54.25±0.44**	57.90±0.13**	1.11 ±0.02**
13	D + AF011-F5 (ME) 200 mg/kg	52.36±0.52**#	53.51±0.21**#	1.01 ±0.01**#

Data are presented as Mean ± SEM (n = 6). Statistical evaluation was performed using ANOVA followed by Tukey's post hoc test. A value of *p < 0.05 was considered statistically significant, while **p < 0.001 indicated a highly significant difference in comparison with the diabetic control group. Values of #p > 0.05 were regarded as statistically non-significant when compared with the standard treatment group.

biomarkers, including SGPT (98.75±0.47 IU/L), SGOT (99.83±0.51 IU/L) and serum bilirubin (2.41±0.05 mg/dL), compared to the normal control group (38.08±0.77 IU/L, 38.08±0.54 IU/L and 0.67±0.01 mg/dL, respectively), indicating hepatic injury.

Treatment with glimepiride significantly ($p < 0.05$) reduced these elevated levels to near-normal values (SGPT: 51.81±0.12 IU/L; SGOT: 51.78±0.10 IU/L; bilirubin: 0.91±0.01 mg/dL). Among the test extracts, AF008 (200 mg/kg) demonstrated comparatively greater

Normal Kidney	Negative Control (STZ)	Standard Drug
		
Figure 1A. Normal architecture of the glomerulus (red arrow) and renal tubules in the cortical region (green arrows) was observed. No pathological alterations or abnormalities were detected in the kidney tissue.	**Figure 1B.** Foci of cystic dilatation and degeneration of tubules with accumulation of fluids – red arrow in the cortex region. Foci of tubular/interstitial inflammation [green arrow] in the cortex region.	**Figure 1C.** Normal histological structure of the glomerulus (red arrow) and renal tubules in the cortical region (green arrows) was observed, with no detectable abnormalities in the kidney tissue.

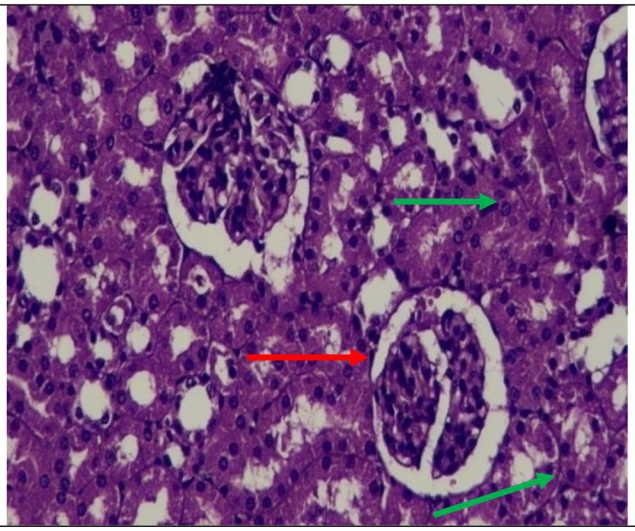
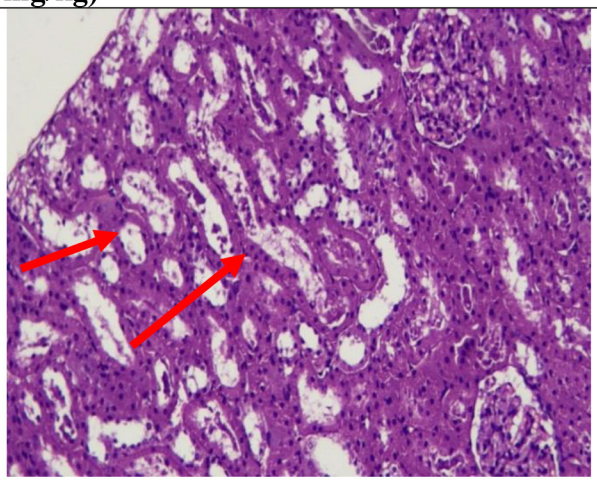
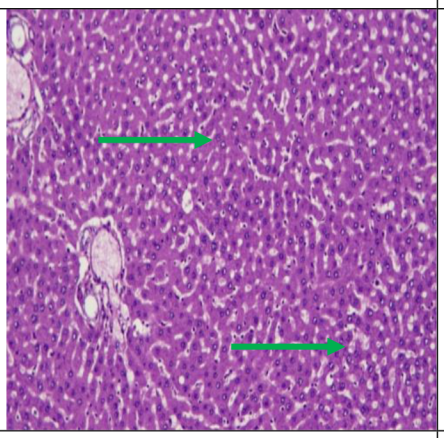
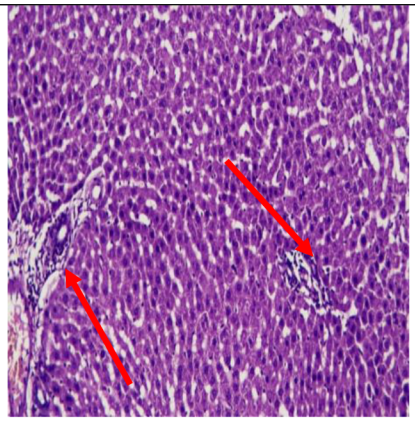
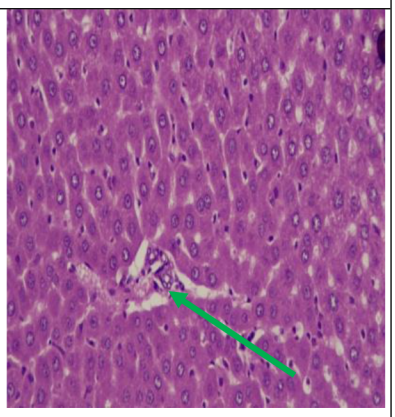
D + AF008-100% Water Extract (200 mg/kg)	D + AF011-F5 Methanol Fraction (200 mg/kg)
	
Figure 1D. Normal morphology of the glomerulus (red arrow) and renal tubules in the cortical region (green arrows) was observed.	**Figure 1E.** Moderate tubular degeneration and dilatation were observed in the cortex region of the kidney – arrow.

Figure 1. Histopathology of the kidney.

Normal Group	Negative Group(STZ)	Standard Drug
		
Figure 2A. Normal morphology of hepatocytes was observed in the portal, periportal and centrilobular region of the liver – arrow.	**Figure 2B.** Mild infiltration of inflammatory cells in peri portal region of the liver – arrow.	**Figure 2C.** Normal morphology of the portal region of the liver with the bile duct and the portal vein of the liver.

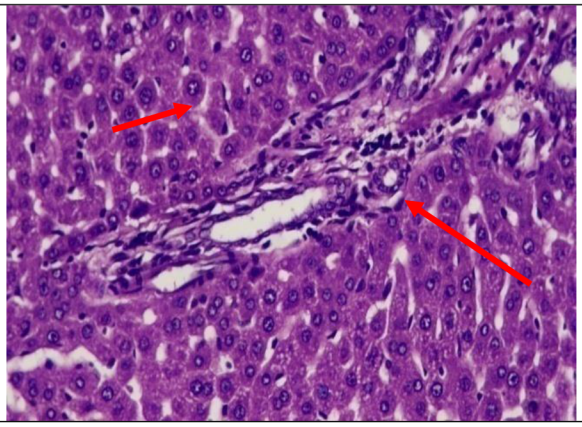
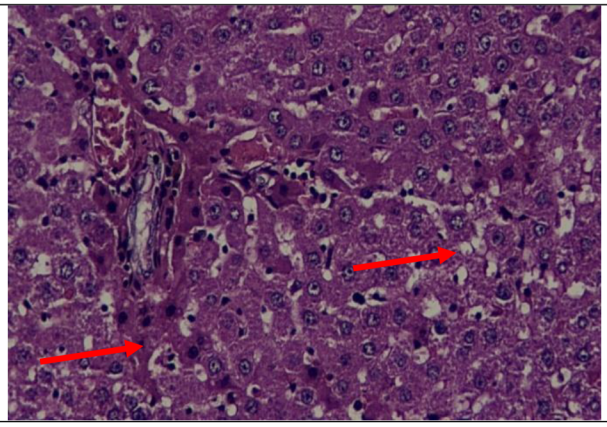
AF008 100 % Water Extract (200 mg/kg)	AF011-F5 Methanol Fraction (200 mg/kg)
	
Figure 2D. Normal morphology of hepatocytes was observed in the portal, periportal and centrilobular regions of the liver – arrow; NO abnormalities were observed in the liver.	**Figure 2E.** Normal morphology of hepatocytes was observed in the portal, periportal and centrilobular region of the liver – arrow.

Figure 2. Histology of the liver.

hepatoprotective activity (SGPT: 54.85±0.70 IU/L; SGOT: 61.41±0.25 IU/L; bilirubin: 1.08±0.01 mg/dL) than AF02. Among the fractions, AF011-F5 (200 mg/kg) exhibited the most pronounced effect, significantly reducing SGPT (52.36±0.52 IU/L), SGOT (53.51±0.21 IU/L) and bilirubin (1.01±0.01 mg/dL), comparable to the standard drug, followed by AF011-F4.

Histopathological evaluation of liver tissue further supported the biochemical findings. The normal control group showed normal morphology of hepatocytes in the portal, periportal, and centrilobular regions of the liver. In contrast, diabetic control rats exhibited marked hepatic degeneration, cellular disorganisation, and inflammatory changes. Treatment with extracts and

fractions improved hepatic architecture. The AF011-F5-treated group showed normal morphology of hepatocytes in the portal, periportal and centrilobular regions of the liver (arrow), with markedly reduced tissue damage, indicating significant hepatoprotective activity.

4. Discussion

The present investigation demonstrates that *A. ficulneus* exhibits significant antihyperglycemic and organ-protective activity in streptozotocin-induced diabetic rats. Streptozotocin induces diabetes primarily through oxidative stress-mediated β-cell damage, leading to persistent hyperglycemia and metabolic disturbances[22–24]. The sustained elevation of blood glucose observed in diabetic control animals confirms successful disease induction.

Both aqueous and ethanolic extracts produced a dose-dependent reduction in blood glucose levels, with the aqueous extract showing relatively greater efficacy. Among the fractions, AF011-F5 demonstrated the strongest antihyperglycemic activity, showing effects comparable to those of the standard drug. The observed activity may be associated with the presence of bioactive phytochemicals, particularly flavonoids and polyphenolic compounds, which contribute to glucose regulation by stimulating insulin secretion, suppressing carbohydrate-hydrolysing enzymes, and reducing oxidative stress[25–27]. Loss of body weight in diabetic animals is associated with increased protein catabolism and impaired glucose utilisation. Improvement in body weight observed in treated groups, particularly with AF011-F5, suggests improved metabolic balance and glycemic control.

Renal impairment in diabetes is characterised by elevated creatinine and proteinuria along with reduced serum protein levels, reflecting compromised kidney function. The observed normalisation of these parameters indicates protection against diabetic nephropathy. These findings are consistent with previous reports showing that natural compounds mitigate renal damage through antioxidant and anti-inflammatory mechanisms[28,29].

Similarly, hepatic dysfunction in diabetic conditions, evidenced by elevated transaminases and bilirubin, was significantly attenuated following treatment. This hepatoprotective effect may be attributed to reduced oxidative stress and improved cellular integrity[23,30]. The absence of toxicity up to 2000 mg/kg supports the safety of the extracts. Overall, the findings indicate that the methanolic fraction (AF011-F5) possesses the highest biological activity, likely due to enrichment of polar bioactive constituents. The observed effects may be mediated through combined antihyperglycemic, antioxidant, and organ-protective mechanisms.

5. Conclusion

The findings of the present investigation indicate that *A. ficulneus* exhibits notable antihyperglycemic, renoprotective and hepatoprotective effects in streptozotocin-induced diabetic rats. Both aqueous and 50% ethanolic extracts exhibited dose-dependent efficacy, with the aqueous extract showing comparatively better activity. Among the fractions, the methanolic fraction (AF011-F5) displayed the most pronounced effects, significantly reducing blood glucose levels, improving body weight, and restoring altered renal and hepatic biomarkers toward normal values. The observed pharmacological effects may be attributed to the presence of bioactive phytoconstituents, likely acting through combined mechanisms involving glycemic control and attenuation of oxidative stress. The absence of toxicity at higher doses further supports the safety profile of the extracts. Overall, these findings suggest that *A. ficulneus*, particularly its methanolic fraction, holds promise as a potential therapeutic agent for the management of diabetes and its associated complications. Further studies are warranted to isolate and characterise the active constituents and to elucidate the underlying molecular mechanisms.

6. Acknowledgement

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7. References

1. International Diabetes Federation. IDF Diabetes Atlas. 11th ed. Brussels: International Diabetes Federation; 2025.

2. Genitsaridi I, *et al.* Global diabetes prevalence estimates for 2024 and projections for 2050. Lancet Diabetes Endocrinol. 2026; 14(2):149-156. https://doi.org/10.1016/S2213-8587(25)00299-2 PMid: 41412135.
3. NCD Risk Factor Collaboration. Worldwide trends in diabetes prevalence. *Lancet.* 2024; 403(10421):203-215.
4. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001; 414:813-820. https://doi.org/10.1038/414813a PMid: 11742414 PMCid: PMC13134405.
5. Forbes JM, Cooper ME. Mechanisms of diabetic complications. Physiol Rev. 2013; 93:137-188. https://doi.org/10.1152/physrev.00045.2011 PMid: 23303908 PMCid: PMC11042907.
6. Singh A, *et al.* Oxidative stress in diabetes. Biomed Pharmacother. 2021; 137:111256.
7. Campbell RK, White JR, Saulie BA. Metformin therapy. Clin Ther. 1996; 18:360-371. https://doi.org/10.1016/S0149-2918(96)80017-8 PMid: 8829014.
8. Nash D, *et al.* Diabetes therapy management. Dis Manag. 2001; 4:5-23. https://doi.org/10.1089/10935070152639036
9. Eddouks M, *et al.* Medicinal plants in diabetes treatment. J Ethnopharmacol. 2005; 97:145-150. https://doi.org/10.1201/9781482278026-01
10. Prasad SK, *et al.* Antidiabetic activity of herbal plants. Pak J Nutr. 2009; 8:551-557. https://doi.org/10.3923/pjn.2009.551.557
11. Jia Q, *et al.* Hypoglycaemic activity of plant extracts. Phytomedicine. 2009; 16:744-750. https://doi.org/10.1016/j.phymed.2008.12.012 PMid: 19464860.
12. Kumar P, *et al.* Herbal medicines for diabetes management. J Ethnopharmacol. 2022; 282:114614.
13. Noor A, *et al.* Antidiabetic activity of Aloe vera. Curr Sci. 2008; 94:1070-1076.
14. Daisy P, Eliza J. Polyherbal antidiabetic formulation. Biochem Cell Arch. 2007; 7:135-140.
15. Gupta R, *et al.* Plant-based antidiabetic therapy. Front Pharmacol. 2023; 14:1187654.
16. Szkudelski T. Mechanism of streptozotocin action. Physiol Res. 2001; 50:537-546. https://doi.org/10.33549/physiolres.930111 PMid:11829314.
17. Lenzen S. Alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008; 51:216-226. https://doi.org/10.1007/s00125-007-0886-7 PMid: 18087688.
18. Furman BL. Streptozotocin-induced diabetic models. Diabetes Res Clin Pract. 2021; 171:108615. https://doi.org/10.1002/cpz1.78 PMid: 33905609.
19. Dills DG, Schneider J. Clinical evaluation of glimepiride versus glyburide in NIDDM in a double-blind comparative study. Horm Metab Res. 1996; 28:426-429. https://doi.org/10.1055/s-2007-979831 PMid: 8911977.
20. Lal VK, Gupta PP, Pandey A. Hypoglycemic effect of *Kyllinga triceps* in STZ-induced diabetic rats. J Diabetes Metab. 2012; 3(6):1-3.
21. Chen F, Xiong H, Wang J, Ding X, Shu G, Mei Z. Antidiabetic effect of total flavonoids from *Sanguis draxonis* in type 2 diabetic rats. J Ethnopharmacol. 2013; 149(3):729-736. https://doi.org/10.1016/j.jep.2013.07.035 PMid:23933499.
22. Black HS. Oxidative stress and diabetic complications. Int J Mol Sci. 2022; 23:11903. https://doi.org/10.3390/ijms232011903
23. Singh A, *et al.* Mechanistic insight into oxidative stress-triggered pathways in diabetes. Molecules. 2022; 27:950. https://doi.org/10.3390/molecules27030950 PMid: 35164215 PMCid: PMC8840622.
24. Li X, Zou J, Lin A, *et al.* Oxidative stress and endothelial dysfunction in diabetes mellitus. Antioxid Redox Signal. 2024; 40:968-989. https://doi.org/10.1089/ars.2023.0524 PMid: 38497734 PMCid: PMC11535463.
25. Anshika, Singh VK, *et al.* Plant bioactive compounds in diabetes management. Future J Pharm Sci. 2022; 8:43. https://doi.org/10.1186/s43094-022-00443-3
26. Shamsudin NF, *et al.* Flavonoids as antidiabetic agents: mechanisms and therapeutic potential. Int J Mol Sci. 2022; 23:12605. https://doi.org/10.3390/ijms232012605 PMid: 36293459 PMCid: PMC9604264.
27. Singh H, Singh R, Kaur S, *et al.* Role of oxidative stress in diabetes-induced complications and antioxidant therapy. Arch Physiol Biochem. 2024; 130:616-641. https://doi.org/10.1080/13813455.2023.2243651 PMid: 37571852.
28. Chen XM, *et al.* Natural compounds in diabetic nephropathy management. Food Funct. 2022; 13:5630-5645. https://doi.org/10.1039/D2FO00245K PMid: 35583219.
29. Roy B. Pathophysiological mechanisms of diabetes-induced complications: role of oxidative stress. Med Sci. 2025; 13:87. https://doi.org/10.3390/medsci13030087 PMid: 40700116 PMCid: PMC12286099.
30. Rudrapal M, *et al.* Polyphenols and oxidative stress-mediated diseases. Front Pharmacol. 2022; 13:806470. https://doi.org/10.3389/fphar.2022.806470 PMid: 35237163 PMCid: PMC8882865.

Author Contribution

Akshay V. Ingle conceptualised the study, carried out the experimental work, including extraction, *in vitro* studies and data analysis, and prepared the original draft of the manuscript. Dr. Sidheshwar S. Patil supervised the research work, contributed to the study design, provided critical revisions to the manuscript and approved the final version for publication. Both authors have read and approved the final manuscript.