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Original Research Paper

# Antidiabetic and In-vitro Antioxidant Activity of Successive Extracts of Cleome rutidosperma DC (Cleomaceae) leaves in Alloxan- and Streptozotocin-Induced Diabetic Wistar Rats

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## Abstract

Background: Cleome rutidosperma DC (Cleomaceae), a fringed spider-flower widely distributed across India and parts of Africa, is used in folk medicine for the management of diabetes, inflammation, pain, skin diseases and microbial infections, yet its blood-glucose-lowering and antioxidant potential in chemically induced diabetic models remains inadequately characterised. Objective: The present work was designed to evaluate the antidiabetic activity of four successive solvent extracts of C. rutidosperma leaves and to assess the in-vitro antioxidant capacity and total phenolic content of the most active extracts. Methods: Shade-dried leaves were extracted successively with petroleum ether, chloroform, methanol and water using a Soxhlet apparatus. Acute toxicity was studied in albino mice by the up-and-down method. Antidiabetic activity was tested at 200 mg/kg in normoglycemic, glucose-loaded, alloxan-induced (single and multi-dose) and streptozotocin-induced (single and multi-dose) Wistar rat models, with glibenclamide (10 mg/kg) as the reference standard. In-vitro antioxidant activity was determined by DPPH and superoxide radical scavenging assays and total phenolic content was estimated by the Folin-Ciocalteu method. Results: The methanol extract produced a significant ($p<0.01$) fall in blood glucose of 29.85% (alloxan, acute), 37.76% (alloxan, multi-dose), 31.11% (streptozotocin, acute) and 39.47% (streptozotocin, multi-dose). The methanol extract scavenged 69.59% of DPPH radical and 61.57% of superoxide radical at 100 micrograms per millilitre, with IC50 values of 37.78 and 55.26 micrograms per millilitre respectively, and contained 30.66 mg gallic acid equivalents per gram of total phenolics. Conclusion: The methanol and aqueous extracts of C. rutidosperma leaves possess demonstrable antidiabetic and antioxidant activity, plausibly linked to their flavonoid, steroid, saponin and phenolic constituents, and support the folk use of this plant in diabetes management.

**Keywords:** ***Cleome rutidosperma; Cleomaceae; antidiabetic; alloxan; streptozotocin; DPPH; superoxide; total phenolics; glibenclamide.***

## 1. Introduction

Diabetes mellitus is a chronic endocrine disorder characterised by persistent hyperglycaemia that results from defective insulin secretion, impaired insulin action, or a combination of both [1]. The World Health Organization estimates that 70 to 80 per cent of the population in developing nations still depends on plant-based preparations for primary health care, and the search for safer hypoglycaemic agents from indigenous plants has gained momentum over the last three decades [2,3]. Modern oral hypoglycaemic agents such as sulfonylureas, biguanides, alpha-glucosidase inhibitors, thiazolidinediones, dipeptidyl peptidase-IV inhibitors and incretin mimetics offer symptomatic relief but seldom reverse the disease, and each class carries a characteristic profile of adverse effects ranging from hypoglycaemia and lactic acidosis to gastrointestinal intolerance and cardiovascular concerns [4,5].

Plant-derived natural products continue to provide lead structures for drug discovery. Of the small-molecule drugs approved between 1981 and 2002, a substantial fraction trace their origin to natural products or their derivatives, and ethnopharmacological leads have repeatedly delivered clinically useful medicines such as artemisinin, vinblastine, vincristine and metformin [6,7]. In India, traditional systems such as Ayurveda, Siddha and Unani have catalogued several hundred plants with claimed antidiabetic activity, and the Central Drug Research Institute, Lucknow, alone has screened more than 2,500 plant species over a 20-year period [8]. Such a backdrop justifies the systematic pharmacological evaluation of indigenous plants that

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have a credible ethnic claim but limited modern scientific validation.

Cleome rutidosperma DC (family Cleomaceae), commonly known as the fringed spider-flower, is a small herb distributed across Africa and the warmer parts of India, where it grows as a weed of damp, shady localities, wastelands, roadsides and abandoned land [9,10]. The plant is known in vernacular systems as Pilitalvani (Gujarati), Naikkaduku (Tamil), Nayibela (Kannada) and Kukkavaminta (Telugu). The tribal and rural populations of West Bengal, Odisha, Cameroon, Mozambique and Argentina use the leaf infusion orally for diabetes, chest pain, dysentery and dysmenorrhoea, and apply it externally for skin diseases and infected wounds [11,12]. Earlier pharmacological reports on C. rutidosperma describe anti-inflammatory, analgesic, anticonvulsant, antimicrobial, diuretic, laxative, antiplasmodial, wound-healing, anticancer and CNS-depressant activities of various solvent extracts [13,14,15,16,17,18]. A bioassay-guided fractionation study has also indicated antidiabetic potential of the aqueous extract in streptozotocin-treated rats [19], yet the comparative activity of successive solvent extracts in both alloxan- and streptozotocin-induced models, together with the in-vitro antioxidant and total phenolic profile, has not been reported in detail.

With this background, the present investigation was undertaken to evaluate the antidiabetic activity of the petroleum ether, chloroform, methanol and aqueous extracts of C. rutidosperma leaves in normoglycaemic, glucose-loaded, alloxan-induced and streptozotocin-induced Wistar rats, and to characterise the more active extracts in terms of DPPH and superoxide radical scavenging capacity and total phenolic content. The selection of successive extraction was deliberate: it solubilises constituents of differing polarity in distinct fractions and allows a more rational identification of the carrier of biological activity than a single-solvent extract [20].

## 2. Materials and Methods

### 2.1 Plant material

Fresh leaves of Cleome rutidosperma DC were collected during October 2018 from the local areas of Khurda district, Odisha (India). The botanical identity was confirmed by Dr. P. C. Panda, Principal Scientist, Regional Plant Resource Centre, Bhubaneswar, and a voucher specimen (V No. UIH-22548) was deposited in the herbarium of Hi-Tech College of Pharmacy, Hi-Tech Medical College and Hospital, Bhubaneswar for future reference. The leaves were washed under running tap water, dried in a hot-air oven at 40 degrees Celsius for 48 hours and pulverised with a mechanical grinder to a coarse powder.

### 2.2 Chemicals and instruments

Alloxan monohydrate and streptozotocin (Sigma Chemical Co., USA), glibenclamide (Aventis Pharma Ltd., Goa), DPPH, Folin-Ciocalteu reagent, NBT, EDTA and ascorbic acid (Sigma/Himedia), petroleum ether (40 to 60 degrees Celsius), chloroform, methanol and other analytical-grade reagents (Merck/Qualigen/S.D. Fine-Chem) were used. Absorbance was recorded on a Shimadzu 1700 UV-Vis spectrophotometer (Japan). Blood glucose was measured with an Accu-Chek Active glucometer (Roche Diagnostics, USA) based on the glucose oxidase-peroxidase principle.

### 2.3 Successive solvent extraction

Five hundred grams of the coarse powder was packed in a Soxhlet apparatus and extracted successively with petroleum ether, chloroform and methanol for 24 hours each [21]. After each solvent was exhausted, the marc was air-dried and re-extracted with the next solvent. The final marc, obtained after methanol extraction, was heated with distilled water at 80 degrees Celsius for 20 minutes, cooled to room temperature and filtered. All extracts were concentrated under reduced pressure in a rotary evaporator and stored in a desiccator. The yields were calculated as percentage weight per weight of dried starting material, and the colour and consistency of each extract were recorded.

### 2.4 Preliminary phytochemical screening

All four extracts were screened for the presence of alkaloids, glycosides, saponins, flavonoids, steroids and triterpenoids, tannins and polyphenolics, carbohydrates and reducing sugars, and amino acids and proteins using standard qualitative tests described by Kokate and Mukherjee [22,23]. Mayer's, Wagner's, Dragendorff's and Hager's reagents were used for alkaloids; Molisch's, Fehling's, Benedict's and Barfoed's tests for carbohydrates and reducing sugars; ferric chloride, lead acetate and gelatin tests for tannins; foam and haemolysis tests for saponins; Shinoda's test with magnesium turnings and concentrated hydrochloric acid for flavonoids; and Liebermann-Burchard and Salkowski tests for steroids and triterpenoids.

### 2.5 Acute toxicity study

Acute toxicity was evaluated in albino mice (20 to 25 g) of either sex by the up-and-down method of Bruce and Dixon, using the OECD guideline for acute oral toxicity testing [24,25,26]. The animals were fasted overnight with free access to water, and the extracts were administered orally as aqueous suspensions with 5 per cent Tween 80 at a limit dose of 2000 mg per kg body weight. The animals were observed continuously for the first 4 hours and then every 24 hours for 14 days for mortality, behavioural changes, salivation, convulsions, lacrimation, sedation and tremor. As no mortality was observed at the limit dose, one-tenth of the safe dose, i.e. 200 mg per kg, was selected as the therapeutic dose for pharmacological studies.

### 2.6 Experimental animals and grouping

Wistar albino rats (150 to 200 g) of either sex were procured from the Department of Veterinary Science, Odisha, through M/s Chakraborty Enterprise, Kolkata. They were housed in polypropylene cages at room temperature (25 plus or minus 2 degrees Celsius) with a 12-hour light and dark cycle, and provided with standard rat pellets (Hindustan Lever, Kolkata) and water ad libitum. The protocol was reviewed and approved by the Institutional Animal

Ethics Committee and the experiments were performed in accordance with the CPCSEA guidelines. For each antidiabetic model, six groups of six rats each were used: group I received the vehicle (2 ml per kg, aqueous Tween 80), group II received glibenclamide (10 mg per kg), and groups III to VI received the petroleum ether, chloroform, methanol and aqueous extracts respectively at 200 mg per kg orally.

**2.7 Antidiabetic activity**

**2.7.1 Effect on normoglycaemic rats**

Overnight-fasted normal rats were treated as per the grouping scheme. Blood samples were collected from the tail tip at 0, 1, 2, 4 and 8 hours, and glucose was measured with the glucometer [27].

**2.7.2 Oral glucose tolerance test**

Overnight-fasted rats were treated with vehicle, glibenclamide or the test extracts. Thirty minutes after treatment, a glucose load of 2 g per kg was administered orally to all animals. Blood glucose was measured at 0, 30, 60, 90 and 120 minutes [28].

**2.7.3 Alloxan-induced diabetes (acute and multi-dose)**

Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate at 150 mg per kg in overnight-fasted rats [29,30]. Animals with fasting blood glucose above 200 mg per dL on the third day were considered diabetic and included in the study. For the acute study, a single dose of vehicle, glibenclamide or test extract was administered orally, and blood glucose was measured at 0, 1, 2, 4, 8 and 24 hours. For the multi-dose study, the treatments were repeated daily for 14 consecutive days and blood glucose was measured on days 0, 1, 2, 4, 7 and 14.

**2.7.4 Streptozotocin-induced diabetes (acute and multi-dose)**

Streptozotocin (60 mg per kg, freshly dissolved in 0.1 M citrate buffer pH 4.5) was injected intraperitoneally into overnight-fasted rats [31,32]. Stable hyperglycaemia (fasting blood glucose above 200 mg per dL) was confirmed on the third day. The single-dose and multi-dose treatment protocols and sampling schedules were identical to those used for the alloxan model.

**2.8 In-vitro antioxidant activity**

**2.8.1 DPPH radical scavenging**

The hydrogen-donating capacity of the methanol and aqueous extracts was measured by the DPPH assay [33,34]. A 0.004 per cent w/v DPPH solution was prepared in 95 per cent methanol. Stock solutions (100 micrograms per millilitre) of the test extracts and ascorbic acid were serially diluted to give 12.5, 25, 50, 75 and 100 micrograms per millilitre. Equal volumes (1.5 mL) of DPPH solution and the test solution were mixed, incubated in the dark for 30 minutes at room temperature, and the absorbance was read at 517 nm against a methanol blank. A control with DPPH and methanol was run in parallel. The percentage inhibition was calculated, and the IC50 was derived from the regression equation.

**2.8.2 Superoxide radical scavenging**

Superoxide scavenging was assessed by the alkaline DMSO method [35]. Potassium superoxide was allowed to react with dry DMSO for 24 hours, and the filtrate (200 microlitres) was added to 2.8 mL of an aqueous solution containing NBT (56 micromolar), EDTA (10 micromolar) and potassium phosphate buffer (10 millimolar, pH 8.0). Methanol and aqueous extract solutions at 12.5 to 100 micrograms per millilitre were added separately, incubated for 150 minutes, and the absorbance was read at 560 nm. Ascorbic acid served as the reference standard, and the percentage inhibition and IC50 were calculated as above.

**2.9 Total phenolic content**

Total phenolic content was estimated by the Folin-Ciocalteu method [36]. One and a half millilitres of each extract (1 mg per mL in methanol) was mixed with 10 mL of distilled water and 1.5 mL of Folin-Ciocalteu reagent in a 25 mL volumetric flask. After 5 minutes, 4 mL of 20 per cent sodium carbonate was added and the volume was made up with double-distilled water. The absorbance was measured at 760 nm after 30 minutes, and the phenolic content was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE per g) using a calibration curve constructed with gallic acid.

**2.10 Statistical analysis**

All values are reported as mean plus or minus standard deviation (n equals 6). Statistical differences between treatments and the solvent control were tested by one-way analysis of variance (ANOVA) followed by Dunnett's t-test. Probability values less than 0.05 were considered statistically significant and those less than 0.01 were considered highly significant. The F values from ANOVA are reported alongside the data.

**3. Results**

**3.1 Extractive values, colour and consistency**

Successive Soxhlet extraction of 500 g of dried C. rutidosperma leaf powder yielded four extracts of distinct polarity. The methanol extract gave the highest yield of 7.4 per cent w/w, followed by the aqueous extract at 5.8 per cent, the petroleum ether extract at 2.6 per cent and the chloroform extract at 1.8 per cent (Table 1). The colours and consistencies were also distinct, the petroleum ether and chloroform extracts being yellow to pale yellow and the methanol and aqueous extracts being brown to light brown; these organoleptic features served as a useful check on extract identity during storage.

**Table 1. Extractive values, colour and consistency of successive extracts of Cleome rutidosperma DC leaves.**

| Sl. No. | Extract | Yield (% w/w) | Colour | Consistency |
|---|---|---|---|---|
| 1 | Petroleum ether (PE) | 2.6 | Yellow | Non-sticky, soft |
| 2 | Chloroform (CL) | 1.8 | Pale yellow | Non-greasy, semi-solid |
| 3 | Methanol | 7.4 | Brown | Non-viscous, |

| | | | | |
|---|---|---|---|---|
| | (ME) | | | solid |
| 4 | Aqueous (AQ) | 5.8 | Light brown | Non-viscous, solid |

### 3.2 Preliminary phytochemical screening

Qualitative phytochemical analysis (Table 2) showed that the methanol extract contained alkaloids, glycosides, saponins, flavonoids, steroids and triterpenoids, tannins and polyphenolics, and amino acids and proteins; only carbohydrates were absent. The aqueous extract shared most of these constituents except steroids and triterpenoids, while also testing positive for carbohydrates and reducing sugars. The petroleum ether extract contained only steroids and triterpenoids, and the chloroform extract contained only alkaloids. The polar methanol and aqueous extracts therefore carried the broadest phytochemical spectrum, which formed the basis for their selection for antioxidant evaluation.

**Table 2. Qualitative phytochemical screening of successive extracts of Cleome rutidosperma DC leaves.**

| Chemical group | PE extract | CL extract | ME extract | AQ extract |
|---|---|---|---|---|
| Alkaloids | - | + | + | + |
| Glycosides | - | - | + | + |
| Saponins | - | - | + | + |
| Flavonoids | - | - | + | + |
| Steroids / Triterpenoids | + | - | + | - |
| Tannins / Polyphenolics | - | - | + | + |
| Carbohydrate / Reducing sugars | - | - | - | + |
| Amino acids / Proteins | - | - | + | + |

*+ denotes present; - denotes absent. PE = petroleum ether, CL = chloroform, ME = methanol, AQ = aqueous.*

### 3.3 Acute toxicity

All four extracts were well tolerated at the limit dose of 2000 mg per kg in albino mice. No mortality, no change in skin and fur texture, no alteration of mucous membranes and no abnormal behavioural or autonomic responses were recorded during the 14-day observation period. Based on these observations, 200 mg per kg (one-tenth of the safe dose) was selected as the screening dose for the antidiabetic studies.

### 3.4 Effect on normoglycaemic rats

In normoglycaemic rats, glibenclamide (10 mg per kg) produced a steady fall in blood glucose from 88.65 mg per dL at 0 h to 56.25 mg per dL at 8 h, a fall of 36.54 per cent (Table 3). None of the four test extracts lowered the blood glucose significantly in normal rats; the methanol and aqueous extracts showed a marginal, non-significant trend, while the petroleum ether and chloroform extracts were indistinguishable from the solvent control. The absence of hypoglycaemia in normoglycaemic animals is itself a meaningful observation and is discussed later.

**Table 3. Effect of Cleome rutidosperma DC leaf extracts on blood glucose levels (mg/dL) in normoglycaemic Wistar rats.**

| Group | Treatment | 0 h | 1 h | 2 h | 4 h | 8 h | F value |
|---|---|---|---|---|---|---|---|
| I | Control (2 mL/kg) | 89.16 +/- 5.01 | 91.37 +/- 14.16 | 93.25 +/- 4.62 | 92.66 +/- 6.11 | 91.52 +/- 4.50 | 0.21 |
| II | Glibenclamide (10 mg/kg) | 88.65 +/- 8.56 | 75.83 +/- 8.56* | 69.66 +/- 10.52* | 63.24 +/- 8.14* | 56.25 +/- 5.56* (36.54) | 38.45 |
| III | PE (200 mg/kg) | 90.50 +/- 6.15 | 93.64 +/- 6.47 | 91.16 +/- 4.33 | 94.50 +/- 4.78 | 92.33 +/- 5.92 | 1.24 |
| IV | CL (200 mg/kg) | 96.50 +/- 5.29 | 98.33 +/- 6.11 | 94.23 +/- 6.89 | 95.66 +/- 4.52 | 97.82 +/- 5.16 | 1.21 |
| V | ME (200 mg/kg) | 90.16 +/- 6.52 | 88.24 +/- 6.86 | 86.32 +/- 6.70 | 82.68 +/- 6.18 | 90.03 +/- 3.31 | 1.79 |
| VI | AQ (200 mg/kg) | 88.07 +/- 5.11 | 85.20 +/- 5.69 | 87.16 +/- 5.52 | 89.83 +/- 5.11 | 94.50 +/- 4.92 | 1.30 |

*Values are mean +/- SD (n = 6); analysed by one-way ANOVA followed by Dunnett's t-test. *p < 0.05; **p < 0.01. Figure in parenthesis indicates % fall in blood glucose compared with 0 h.*

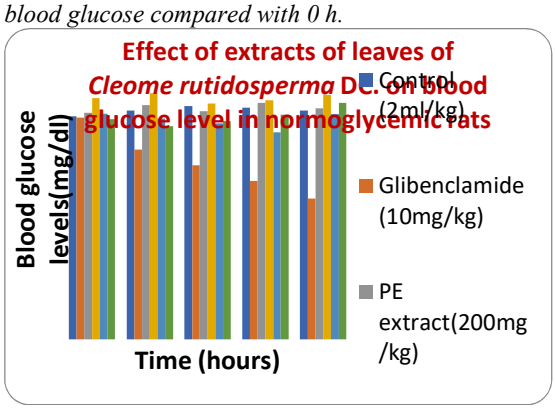


*Figure 1. Effect of Cleome rutidosperma DC leaf extracts on blood glucose levels in normoglycaemic rats.*

### 3.5 Oral glucose tolerance test

In the oral glucose tolerance test, the methanol and aqueous extracts produced a significant and progressive fall in the post-prandial blood glucose elevation when compared with the solvent control (Table 4). The methanol extract reduced blood glucose by 11.90, 14.78, 21.35 and 26.68 per cent at 30, 60, 90 and 120 minutes respectively, while the

aqueous extract produced reductions of 7.63, 10.73, 17.30 and 21.64 per cent. Glibenclamide produced a maximum fall of 32.10 per cent at 120 minutes. The petroleum ether and chloroform extracts were inactive. The statistical significance for both polar extracts was p less than 0.05 at 30 minutes and p less than 0.01 from 60 minutes onwards.

**Table 4. Effect of Cleome rutidosperma DC leaf extracts on blood glucose levels (mg/dL) in glucose-loaded rats (oral glucose tolerance test).**

| Group | Treatment | 0 min | 30 min | 60 min | 90 min | 120 min |
|---|---|---|---|---|---|---|
| I | Control (2 mL/kg) | 90.50 +/- 3.22 | 132.25 +/- 3.95 | 127.33 +/- 3.60 | 123.65 +/- 3.28 | 120.50 +/- 5.21 |
| II | Glibenclamide (10 mg/kg) | 87.45 +/- 6.55 | 114.76 +/- 4.53 ** (13.22) | 103.15 +/- 3.65 ** (18.99) | 89.60 +/- 7.24 ** (27.53) | 80.46 +/- 4.58 ** (32.10) |
| III | PE (200 mg/kg) | 94.25 +/- 4.33 | 135.30 +/- 6.50 | 140.50 +/- 4.35 | 131.66 +/- 4.72 | 125.25 +/- 7.15 |
| IV | CL (200 mg/kg) | 95.06 +/- 5.22 | 133.50 +/- 4.55 | 128.40 +/- 3.62 | 126.25 +/- 6.73 | 122.45 +/- 5.88 |
| V | ME (200 mg/kg) | 90.50 +/- 4.08 | 116.50 +/- 5.75 * (11.90) | 108.50 +/- 3.85 ** (14.78) | 97.25 +/- 5.34 ** (21.35) | 88.35 +/- 7.51 * (26.68) |
| VI | AQ (200 mg/kg) | 88.67 +/- 3.07 | 122.15 +/- 4.90 * (7.63) | 113.66 +/- 6.02 ** (10.73) | 102.25 +/- 5.82 ** (17.30) | 97.64 +/- 3.25 * (21.64) |

*Values are mean +/- SD (n = 6); analysed by one-way ANOVA followed by Dunnett's t-test. Glucose (2 g/kg p.o.) was administered 30 min after treatment. *p < 0.05; **p < 0.01. Figure in parenthesis indicates % fall in BGL compared with solvent control.*

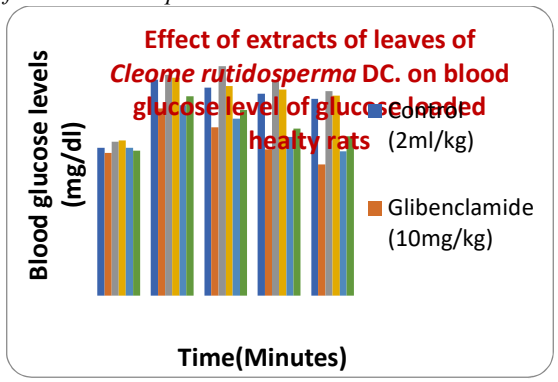


*Figure 2. Effect of Cleome rutidosperma DC leaf extracts on blood glucose levels in glucose-loaded rats.*

**3.6 Alloxan-induced diabetes (acute, single-dose)**

In alloxan-diabetic rats treated with a single dose of the test extracts, the methanol and aqueous extracts produced a clear, time-dependent fall in blood glucose (Table 5). At 8 hours the methanol extract had lowered blood glucose from 242.75 to 170.35 mg per dL, a fall of 29.85 per cent, while the aqueous extract had lowered it from 247.83 to 182.65 mg per dL, a fall of 26.30 per cent. Glibenclamide produced a 43.40 per cent fall over the same period. The petroleum ether and chloroform extracts did not differ from the solvent control. The methanol extract was significant at p less than 0.01 from 2 hours onwards, and the aqueous extract from 4 hours onwards.

**Table 5. Effect of Cleome rutidosperma DC leaf extracts on blood glucose levels (mg/dL) in single-dose treated alloxan-induced diabetic rats.**

| Group | Treatment | 0 h | 1 h | 2 h | 4 h | 8 h | 24 h | F value |
|---|---|---|---|---|---|---|---|---|
| I | Control (2 mL/kg) | 235.50 +/- 13.45 | 242.25 +/- 12.56 | 246.50 +/- 10.05 | 249.16 +/- 6.47 | 251.85 +/- 7.08 | 258.45 +/- 10.68 | 1.21 |
| II | Glibenclamide (10 mg/kg) | 240.00 +/- 9.20 | 218.00 +/- 8.59* | 194.50 +/- 17.94** | 153.16 +/- 21.47** | 135.83 +/- 22.09** (43.40) | 246.33 +/- 9.30 | 49.38 |
| III | PE (200 mg/kg) | 243.76 +/- 11.57 | 245.60 +/- 11.34 | 247.50 +/- 10.82 | 250.00 +/- 8.92 | 255.33 +/- 6.65 | 258.00 +/- 10.33 | 1.26 |
| IV | CL (200 mg/kg) | 236.35 +/- 13.12 | 244.72 +/- 12.89 | 241.37 +/- 11.97 | 245.16 +/- 10.53 | 247.64 +/- 9.10 | 251.00 +/- 6.75 | 1.28 |
| V | ME (200 mg/kg) | 242.75 +/- 12.78 | 237.33 +/- 13.39 | 226.50 +/- 11.62* | 187.50 +/- 9.37** | 170.35 +/- 7.22** (29.85) | 236.65 +/- 10.78 | 47.67 |
| VI | AQ (200 mg/kg) | 247.83 +/- 7.46 | 240.50 +/- 7.47 | 233.15 +/- 5.45* | 204.86 +/- 4.87* | 182.65 +/- 5.95** (26.30) | 248.00 +/- 6.57 | 40.28 |

*Values are mean +/- SD (n = 6); analysed by one-way ANOVA followed by Dunnett's t-test. *p < 0.05; **p*

*< 0.01. Figure in parenthesis indicates % fall in BGL compared with 0 h.*

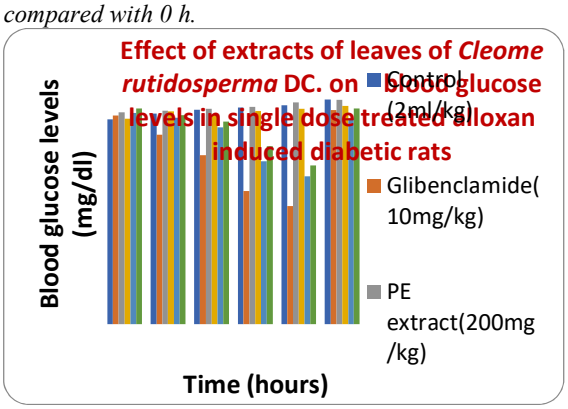


*Figure 3. Effect of Cleome rutidosperma DC leaf extracts on blood glucose levels in single-dose treated alloxan-induced diabetic rats.*

**3.7 Alloxan-induced diabetes (multi-dose, 14-day)**

When the treatment was continued for 14 days, the methanol extract lowered blood glucose from 235 to 150.5 mg per dL (37.76 per cent fall) and the aqueous extract from 234.5 to 164.16 mg per dL (32.53 per cent fall), while glibenclamide produced a 63.51 per cent fall (Table 6). Both polar extracts were significant at p less than 0.05 from day 2 and p less than 0.01 from day 4 onwards. The petroleum ether and chloroform extracts showed a gradual rise in blood glucose similar to the solvent control, indicating absence of activity in long-term hyperglycaemia.

**Table 6. Effect of Cleome rutidosperma DC leaf extracts on blood glucose levels (mg/dL) in multi-dose treated alloxan-induced diabetic rats.**

| Group | Treatment | Day 0 | Day 1 | Day 2 | Day 4 | Day 7 | Day 14 | F value |
|---|---|---|---|---|---|---|---|---|
| I | Control (2 mL/kg) | 235.50 +/- 13.45 | 241.55 +/- 12.50 | 243.60 +/- 11.20 | 247.16 +/- 8.40 | 248.85 +/- 7.80 | 253.45 +/- 10.50 | 1.21 |
| II | Glibenclamide (10 mg/kg) | 237.00 +/- 9.50 | 215.00 +/- 8.80** | 190.50 +/- 9.40** | 150.50 +/- 8.20** | 92.50 +/- 7.10** | 90.36 +/- 6.50** (63.51) | 198.45 |
| III | PE (200 mg/kg) | 238.00 +/- 11.00 | 240.00 +/- 10.50 | 243.00 +/- 10.20 | 246.50 +/- 9.10 | 250.00 +/- 8.40 | 254.00 +/- 9.50 | 1.18 |
| IV | CL (200 mg/kg) | 234.50 +/- 12.50 | 238.00 +/- 11.40 | 242.00 +/- 10.80 | 245.50 +/- 9.80 | 248.00 +/- 8.60 | 252.00 +/- 8.20 | 1.16 |
| V | ME (200 mg/kg) | 235.0 +/- 10.50 | 225.0 +/- 9.80* | 200.5 +/- 9.20** | 175.0 +/- 8.50** | 160.0 +/- 7.40** | 150.50 +/- 6.80** (37.76) | 89.32 |
| VI | AQ (200 mg/kg) | 234.50 +/- 11.50 | 228.00 +/- 10.80* | 210.50 +/- 9.80** | 190.00 +/- 8.80** | 175.50 +/- 7.60** | 164.16 +/- 6.50** (32.53) | 65.45 |

*Values are mean +/- SD (n = 6); analysed by one-way ANOVA followed by Dunnett's t-test. *p < 0.05; **p < 0.01. Figure in parenthesis indicates % fall in BGL compared with day 0.*

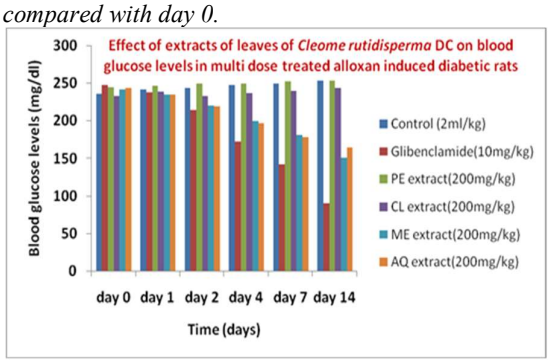


*Figure 4. Effect of Cleome rutidosperma DC leaf extracts on blood glucose levels in multi-dose treated alloxan-induced diabetic rats.*

**3.8 Streptozotocin-induced diabetes (acute, single-dose)**

In the streptozotocin model, the methanol extract lowered blood glucose from 237.38 to 168.25 mg per dL (31.11 per cent) and the aqueous extract from 239.16 to 173.86 mg per dL (26.89 per cent) at 8 hours, while glibenclamide produced a 45.04 per cent fall (Table 7). The methanol extract was significant at p less than 0.01 from 2 hours, and the aqueous extract reached the same level of significance from 4 hours. The non-polar extracts were again inactive.

**Table 7. Effect of Cleome rutidosperma DC leaf extracts on blood glucose levels (mg/dL) in single-dose treated streptozotocin-induced diabetic rats.**

| Group | Treatment | 0 h | 1 h | 2 h | 4 h | 8 h | 24 h | F value |
|---|---|---|---|---|---|---|---|---|
| I | Control (2 mL/kg) | 238.50 +/- 12.50 | 243.20 +/- 11.80 | 247.30 +/- 10.50 | 251.40 +/- 9.80 | 254.20 +/- 8.90 | 258.50 +/- 10.20 | 1.15 |
| II | Glibenclamide (10 mg/kg) | 231.82 +/- 9.50 | 210.50 +/- 8.80* | 185.40 +/- 9.20** | 155.20 +/- 8.50** | 139.83 +/- 7.80** (45.04) | 245.50 +/- 9.10 | 55.32 |
| III | PE | 24 | 24 | 24 | 25 | 255 | 25 | 1. |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | (200 mg/kg) | 0.20 +/- 11.50 | 3.50 +/- 10.80 | 7.80 +/- 10.20 | 1.20 +/- 9.50 | .40 +/- 8.60 | 9.00 +/- 9.80 | 18 |
| IV | CL (200 mg/kg) | 236.50 +/- 12.00 | 240.20 +/- 11.50 | 245.00 +/- 10.50 | 249.50 +/- 9.80 | 253.50 +/- 8.90 | 257.50 +/- 10.20 | 1.16 |
| V | ME (200 mg/kg) | 237.38 +/- 10.80 | 228.50 +/- 9.80 | 210.50 +/- 9.20** | 185.50 +/- 8.50** | 168.25 +/- 7.60** (31.11) | 238.00 +/- 9.50 | 48.65 |
| VI | AQ (200 mg/kg) | 239.16 +/- 11.20 | 232.50 +/- 10.50 | 220.50 +/- 9.80* | 198.50 +/- 8.80** | 173.86 +/- 7.80** (26.89) | 240.50 +/- 9.80 | 38.25 |

*Values are mean +/- SD (n = 6); analysed by one-way ANOVA followed by Dunnett's t-test. *p < 0.05; **p < 0.01. Figure in parenthesis indicates % fall in BGL compared with 0 h.*

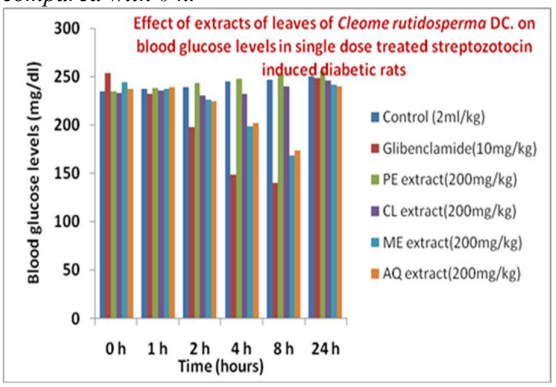


*Figure 5. Effect of Cleome rutidosperma DC leaf extracts on blood glucose levels in single-dose treated streptozotocin-induced diabetic rats.*

### 3.9 Streptozotocin-induced diabetes (multi-dose, 14-day)

Repeated dosing over 14 days produced the most pronounced antidiabetic effect observed in the study. The methanol extract lowered blood glucose from 234.88 to 146.55 mg per dL (39.47 per cent) and the aqueous extract from 235.5 to 165.24 mg per dL (33.63 per cent), compared with a 64.65 per cent fall for glibenclamide (Table 8). The difference between the methanol extract and the solvent control was significant at p less than 0.01 from day 2 onwards. The sustained activity over two weeks indicated that the active constituents of the polar extracts maintained their effect on the residual beta-cells or extrapancreatic glucose handling throughout the dosing interval.

**Table 8. Effect of Cleome rutidosperma DC leaf extracts on blood glucose levels (mg/dL) in multi-dose treated streptozotocin-induced diabetic rats.**

| Group | Treatment | Day 0 | Day 1 | Day 2 | Day 4 | Day 7 | Day 14 | F value |
|---|---|---|---|---|---|---|---|---|
| I | Control (2 mL/kg) | 236.50 +/- 12.00 | 240.20 +/- 11.50 | 244.50 +/- 10.80 | 248.50 +/- 9.80 | 252.00 +/- 8.90 | 256.50 +/- 10.20 | 1.18 |
| II | Glibenclamide (10 mg/kg) | 226.35 +/- 9.50 | 200.50 +/- 8.80** | 170.50 +/- 8.20** | 135.20 +/- 7.50** | 98.50 +/- 6.80** | 87.66 +/- 6.20** (64.65) | 215.40 |
| III | PE (200 mg/kg) | 238.20 +/- 11.50 | 241.50 +/- 10.80 | 245.20 +/- 10.20 | 249.50 +/- 9.50 | 253.50 +/- 8.60 | 258.00 +/- 9.80 | 1.16 |
| IV | CL (200 mg/kg) | 235.50 +/- 12.00 | 239.20 +/- 11.50 | 243.50 +/- 10.80 | 247.50 +/- 9.80 | 251.50 +/- 8.90 | 256.00 +/- 10.20 | 1.15 |
| V | ME (200 mg/kg) | 234.88 +/- 10.50 | 218.50 +/- 9.80** | 190.50 +/- 9.20** | 170.50 +/- 8.50** | 155.20 +/- 7.60** | 146.55 +/- 6.80** (39.47) | 102.50 |
| VI | AQ (200 mg/kg) | 235.50 +/- 11.20 | 225.50 +/- 10.50** | 205.50 +/- 9.80** | 188.50 +/- 8.80** | 175.20 +/- 7.80** | 165.24 +/- 6.50** (33.63) | 78.60 |

*Values are mean +/- SD (n = 6); analysed by one-way ANOVA followed by Dunnett's t-test. *p < 0.05; **p < 0.01. Figure in parenthesis indicates % fall in BGL compared with day 0.*

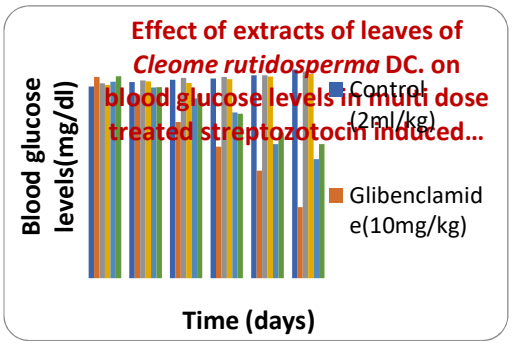


*Figure 6. Effect of Cleome rutidosperma DC leaf extracts on blood glucose levels in multi-dose treated streptozotocin-induced diabetic rats.*

**3.10 In-vitro antioxidant activity**

Because the methanol and aqueous extracts displayed the strongest antidiabetic activity, they were tested for in-vitro radical scavenging capacity. Both extracts showed a concentration-dependent inhibition of the DPPH radical (Table 9) and of the superoxide anion (Table 10). The methanol extract scavenged 69.59 per cent of DPPH and 61.57 per cent of superoxide at 100 micrograms per millilitre, with IC50 values of 37.78 and 55.26 micrograms per millilitre respectively. The aqueous extract scavenged 66.01 per cent of DPPH and 57.45 per cent of superoxide, with IC50 values of 58.96 and 79.17 micrograms per millilitre. Ascorbic acid, the reference standard, gave 83.93 and 77.93 per cent inhibition at the same concentration with IC50 values of 8.08 and 11.37 micrograms per millilitre.

**Table 9. DPPH radical scavenging activity of ascorbic acid and the methanol and aqueous extracts of Cleome rutidosperma DC leaves.**

| Sample | Conc. (ug/mL) | Absorbance | % Inhibition | IC50 (ug/mL) |
|---|---|---|---|---|
| Ascorbic acid | 12.5 | 0.416 +/- 0.003 | 51.90 +/- 0.32 | 8.08 |
| Ascorbic acid | 25 | 0.375 +/- 0.011 | 56.64 +/- 0.86 | 8.08 |
| Ascorbic acid | 50 | 0.307 +/- 0.006 | 64.50 +/- 0.25 | 8.08 |
| Ascorbic acid | 75 | 0.206 +/- 0.012 | 76.18 +/- 0.98 | 8.08 |
| Ascorbic acid | 100 | 0.139 +/- 0.014 | 83.93 +/- 0.59 | 8.08 |
| ME extract | 12.5 | 0.501 +/- 0.019 | 42.08 +/- 0.81 | 37.78 |
| ME extract | 25 | 0.475 +/- 0.008 | 45.08 +/- 1.05 | 37.78 |
| ME extract | 50 | 0.392 +/- 0.016 | 54.68 +/- 1.19 | 37.78 |
| ME extract | 75 | 0.327 +/- 0.024 | 62.19 +/- 0.98 | 37.78 |
| ME extract | 100 | 0.263 +/- 0.027 | 69.59 +/- 1.56 | 37.78 |
| AQ extract | 12.5 | 0.607 +/- 0.025 | 29.82 +/- 0.85 | 58.96 |
| AQ extract | 25 | 0.572 +/- 0.011 | 33.87 +/- 1.32 | 58.96 |
| AQ extract | 50 | 0.461 +/- 0.023 | 46.70 +/- 1.67 | 58.96 |
| AQ extract | 75 | 0.349 +/- 0.008 | 59.63 +/- 0.96 | 58.96 |
| AQ extract | 100 | 0.294 +/- 0.016 | 66.01 +/- 1.22 | 58.96 |

*Values are mean +/- SD of triplicate experiments. IC50 was derived by regression analysis at 95% confidence level. ME = methanol extract; AQ = aqueous extract.*

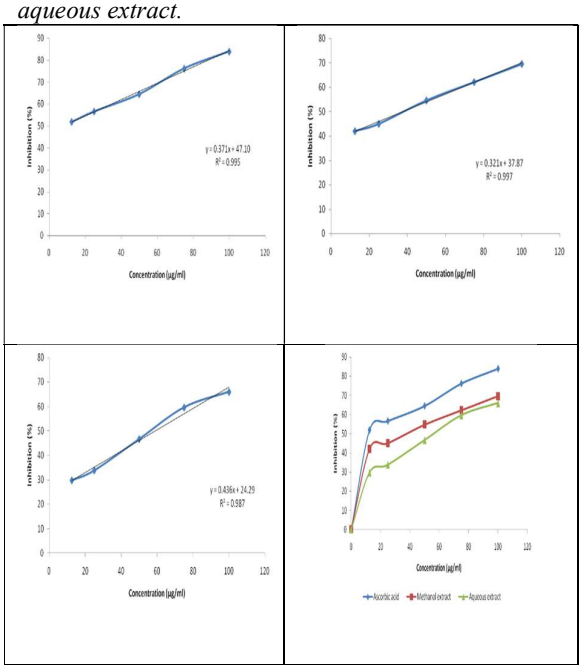

*Figure 7. DPPH radical scavenging activity of ascorbic acid, methanol and aqueous extracts of Cleome rutidosperma DC.*

**Table 10. Superoxide radical scavenging activity of ascorbic acid and the methanol and aqueous extracts of Cleome rutidosperma DC leaves.**

| Sample | Conc. (ug/mL) | Absorbance | % Inhibition | IC50 (ug/mL) |
|---|---|---|---|---|
| Ascorbic acid | 12.5 | 0.415 +/- 0.013 | 49.69 +/- 1.12 | 11.37 |
| Ascorbic acid | 25 | 0.376 +/- 0.017 | 54.42 +/- 0.68 | 11.37 |
| Ascorbic acid | 50 | 0.301 +/- 0.008 | 63.51 +/- 0.89 | 11.37 |
| Ascorbic acid | 75 | 0.242 +/- 0.023 | 70.66 +/- 0.78 | 11.37 |
| Ascorbic acid | 100 | 0.182 +/- 0.006 | 77.93 +/- 1.15 | 11.37 |
| ME extract | 12.5 | 0.572 +/- 0.078 | 30.66 +/- 1.09 | 55.26 |
| ME extract | 25 | 0.424 +/- 0.004 | 48.60 +/- 1.78 | 55.26 |
| ME extract | 50 | 0.406 +/- 0.055 | 50.78 +/- 0.86 | 55.26 |
| ME extract | 75 | 0.375 +/- 0.098 | 54.54 +/- 2.05 | 55.26 |
| ME extract | 100 | 0.317 +/- 0.027 | 61.57 +/- 1.17 | 55.26 |
| AQ extract | 12.5 | 0.608 +/- 0.031 | 26.30 +/- 0.13 | 79.17 |
| AQ extract | 25 | 0.546 +/- 0.005 | 33.81 +/- 0.16 | 79.17 |
| AQ | 50 | 0.509 +/- | 38.30 +/- | 79.17 |

| | | | | |
|---|---|---|---|---|
| extract | | 0.061 | 0.92 | |
| AQ extract | 75 | 0.422 +/- 0.092 | 48.84 +/- 0.19 | 79.17 |
| AQ extract | 100 | 0.351 +/- 0.011 | 57.45 +/- 0.17 | 79.17 |

*Values are mean +/- SD of triplicate experiments. IC50 was derived by regression analysis at 95% confidence level. ME = methanol extract; AQ = aqueous extract.*

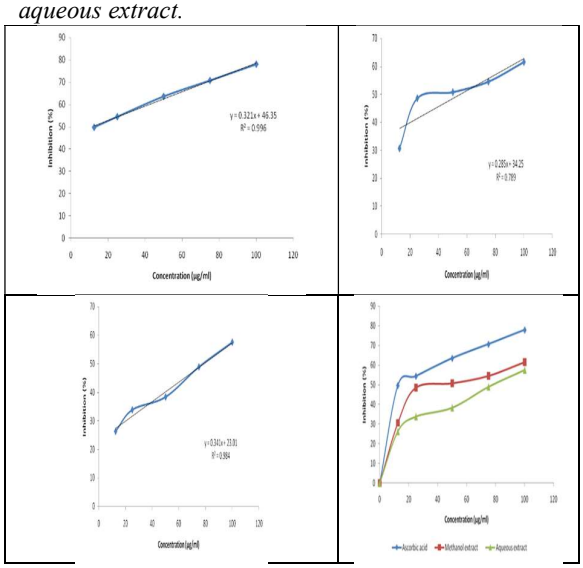

*Figure 8. Superoxide radical scavenging activity of ascorbic acid, methanol and aqueous extracts of Cleome rutidosperma DC.*

### 3.11 Total phenolic content

Total phenolic content, expressed as gallic acid equivalents, was 30.66 mg GAE per g for the methanol extract and 9.33 mg GAE per g for the aqueous extract (Table 11). The methanol extract therefore carried roughly three times the phenolic load of the aqueous extract, and this difference paralleled the differences in both antidiabetic and antioxidant activity between the two extracts.

**Table 11. Total phenolic content of the methanol and aqueous extracts of Cleome rutidosperma DC leaves.**

| Extract | Concentration (ug/mL) | Absorbance | Total phenolics (mg GAE/g) |
|---|---|---|---|
| Methanol extract | 100 | 0.0296 +/- 0.014 | 30.66 +/- 1.09 |
| Aqueous extract | 100 | 0.0264 +/- 0.027 | 9.33 +/- 1.16 |

*GAE = gallic acid equivalent. Values are mean +/- SD of triplicate experiments.*

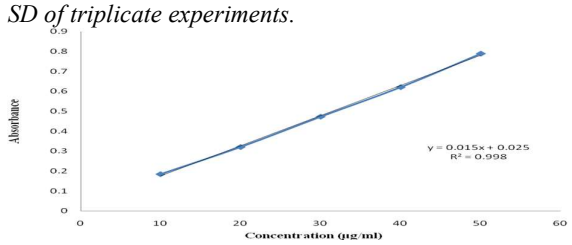


*Figure 9. Standard calibration curve of gallic acid used for total phenolic estimation. [Insert original Figure 4.15 from thesis here]*

## 4. Discussion

The present study was designed to provide a comparative pharmacological profile of the four successive solvent extracts of Cleome rutidosperma DC leaves, since the published literature on this species has so far concentrated on its anti-inflammatory, analgesic, antimicrobial and wound-healing properties with limited systematic work on chemically induced diabetes. The choice of successive Soxhlet extraction with solvents of increasing polarity, namely petroleum ether, chloroform, methanol and water, allowed a polarity-based fractionation of the leaf constituents and provided four extracts with markedly different phytochemical fingerprints. The methanol extract, with the highest yield of 7.4 per cent and the widest phytochemical spectrum, contained alkaloids, glycosides, saponins, flavonoids, steroids and triterpenoids, tannins and polyphenolics, and amino acids and proteins, while the non-polar petroleum ether and chloroform extracts were essentially mono-component. The broad chemical composition of the polar extracts is consistent with the profile reported for other Cleome species [9,10,11] and provides a chemical rationale for the differential biological activity observed in this study.

The acute toxicity study gave a clear safety signal. None of the extracts produced mortality or observable behavioural changes at the limit dose of 2000 mg per kg in albino mice, which permitted the use of one-tenth of this dose, i.e. 200 mg per kg, as the screening dose in all the antidiabetic models. This dose also corresponds to the conventional one-tenth selection rule widely used in rodent pharmacology [26].

In normoglycaemic rats, neither the methanol nor the aqueous extract lowered blood glucose beyond the normal range, in contrast to glibenclamide which produced a 36.54 per cent fall by 8 hours. Sulfonylureas such as glibenclamide stimulate insulin release from pancreatic beta-cells and predictably cause hypoglycaemia, even in non-diabetic animals, when given in pharmacological doses [37]. Biguanides, in contrast, do not cause hypoglycaemia in the normal state because their action depends on the presence of insulin resistance and on suppression of hepatic gluconeogenesis [38]. Plant extracts that behave like sulfonylureas reduce blood glucose in normoglycaemic animals, whereas those that behave like biguanides do not [39,40,41,42]. The present results, in which the test extracts left the normoglycaemic blood glucose essentially unchanged, therefore suggest a biguanide-like rather than a sulfonylurea-like mode of action.

The oral glucose tolerance test provided the first direct evidence of activity. The methanol extract lowered the post-prandial glucose spike by 26.68 per cent at 120 minutes and the aqueous extract by 21.64 per cent, both at p less than 0.01. Two complementary

mechanisms have been proposed for such an effect in glucose-loaded animals. The first is inhibition of endogenous glucose production, which would reduce the contribution of hepatic gluconeogenesis to the post-prandial glucose load [43]. The second is inhibition of intestinal glucose absorption, possibly mediated by dietary fibres and polyphenolics that slow carbohydrate digestion and uptake [44,45]. The presence of tannins, polyphenolics and saponins in the methanol and aqueous extracts is consistent with either mechanism. A third possibility, potentiation of insulin release from residual beta-cells, is less likely given the absence of hypoglycaemic activity in normoglycaemic rats, but cannot be excluded in the glucose-loaded state.

Alloxan and streptozotocin are the two chemical diabetogens most commonly used to screen plant extracts. Alloxan exerts its beta-cell toxicity through the generation of reactive oxygen species during redox cycling in the presence of glutathione and cysteine, with the superoxide and hydroxyl radicals produced causing direct DNA damage and beta-cell necrosis [46]. Streptozotocin, in contrast, enters the beta-cell through the GLUT2 glucose transporter and damages DNA by alkylation, with the subsequent activation of poly(ADP-ribose) polymerase depleting NAD+ and leading to cell death [47,48]. The two toxins therefore kill beta-cells by different mechanisms, and an extract that lowers blood glucose in both models is likely to act through a pathway that is independent of the specific mode of beta-cell injury. In the present work, the methanol and aqueous extracts of C. rutidosperma produced significant blood-glucose lowering in both models. The methanol extract was consistently more potent than the aqueous extract, with the magnitude of the effect ranging from 29.85 to 39.47 per cent in the alloxan model and from 31.11 to 39.47 per cent in the streptozotocin model. These values, while below those produced by glibenclamide (43 to 65 per cent), are pharmacologically meaningful for a crude extract and are in the same range as those reported for several other traditional antidiabetic plants at comparable doses [49,50,51,52].

The multi-dose studies gave additional mechanistic information. Continued treatment for 14 days produced a steady decline in blood glucose in the methanol and aqueous extract groups, whereas the solvent control and the non-polar extracts showed a gradual rise. The persistence of the effect over two weeks suggests that the active constituents act on residual beta-cells or on extrapancreatic sites of glucose handling in a sustained manner, and are not merely producing a transient osmotic or irritant effect on glucose absorption. Sulfonylureas, which act by closing ATP-sensitive potassium channels on beta-cells and stimulating insulin release, are known to be effective in alloxan-diabetic animals in which a residual beta-cell mass survives [53]. The comparable effect of the test extracts in both alloxan and streptozotocin models points to a similar residual-beta-cell-dependent or extrapancreatic mechanism.

Several classes of phytoconstituents have been linked to the antidiabetic activity of plant extracts. Flavonoids such as quercetin, luteolin and catechin have been shown to regenerate beta-cells and to inhibit alpha-glucosidase [54,55]. Steroids and triterpenoids lower blood glucose in experimental animals, and saponins have insulin-secretagogue and insulin-mimetic properties [56,57]. Polyphenolics and tannins inhibit intestinal alpha-glucosidase and alpha-amylase, slowing carbohydrate absorption, and also act as direct antioxidants that quench the reactive oxygen species generated during hyperglycaemia [58,59]. Since the methanol and aqueous extracts of C. rutidosperma leaves contain flavonoids, saponins, tannins, polyphenolics, steroids and alkaloids, the observed antidiabetic activity is plausibly the net result of several of these constituents acting on different points of the glucose handling pathway.

The in-vitro antioxidant results provide an independent line of evidence supporting the antidiabetic activity. Hyperglycaemia is associated with oxidative stress through glucose autoxidation, protein glycation and the polyol pathway, and the resulting reactive oxygen species contribute to beta-cell damage and to the long-term complications of diabetes [60,61,62]. The methanol extract of C. rutidosperma scavenged 69.59 per cent of DPPH and 61.57 per cent of superoxide radical at 100 micrograms per millilitre, with IC50 values of 37.78 and 55.26 micrograms per millilitre respectively. These values, while less potent than ascorbic acid (IC50 of 8.08 and 11.37 micrograms per millilitre), are well within the active range for crude plant extracts. The aqueous extract was less active, with IC50 values of 58.96 and 79.17 micrograms per millilitre. The phenolic content showed the same rank order, with the methanol extract containing 30.66 mg GAE per g versus 9.33 mg GAE per g for the aqueous extract, indicating a quantitative relationship between the phenolic load and the antioxidant capacity. Phenolic compounds act as primary antioxidants by donating a hydrogen atom to lipid peroxyl radicals and by chelating transition metal ions, and the position and degree of hydroxylation on the aromatic ring is the main determinant of their activity [63,64]. The strong positive correlation between total phenolics and antioxidant activity in this study is consistent with the role of phenolic compounds as the major carrier of the radical-scavenging capacity of the extracts.

Taken together, the antidiabetic and antioxidant results point to a coherent mechanistic picture. The polar extracts of C. rutidosperma leaves contain a mixture of flavonoids, polyphenolics, saponins and steroidal constituents that lower post-prandial blood glucose, suppress hyperglycaemia in chemically diabetic rats in a sustained manner over 14 days, and scavenge DPPH and superoxide radicals in vitro. The activity is plausibly the sum of an insulin-sensitising or extrapancreatic action (suggested by the absence of hypoglycaemia in normoglycaemic rats) and an antioxidant-mediated protection of residual beta-cells

from further oxidative damage. The non-polar extracts, which carry essentially only steroids and alkaloids respectively, did not produce a measurable effect in any of the antidiabetic models, indicating that the active constituents reside in the polar fraction.

These findings are consistent with the folk use of C. rutidosperma leaves in diabetes. They also extend the earlier report of Okoro and co-workers, who demonstrated antidiabetic activity of an aqueous extract in streptozotocin-diabetic rats [19], by providing a comparative analysis of four successive solvent extracts in both alloxan and streptozotocin models and by linking the activity to the phenolic and antioxidant profile of the active extracts. The study nonetheless has clear limitations. The active constituents remain unidentified, the mechanism of action has not been probed by insulin assay or by glucose uptake studies in isolated tissues, and the doses used are limited to a single screening dose. Further work, involving bioassay-guided fractionation of the methanol extract, isolation of the individual active compounds, and confirmation of the mechanism through biochemical and histopathological studies, is warranted before the extract can be considered for pharmaceutical development.

## 5. Conclusion

The present work demonstrates that the methanol and aqueous extracts of Cleome rutidosperma DC leaves possess significant antidiabetic activity in alloxan- and streptozotocin-induced diabetic Wistar rats and significant in-vitro antioxidant activity against DPPH and superoxide radicals. The methanol extract, with the broadest phytochemical profile, the highest phenolic content, and the lowest IC50 values in both antioxidant assays, was the most active fraction. The non-polar extracts were inactive. The activity is consistent with the folk use of the plant in diabetes and is plausibly mediated by the flavonoid, saponin, polyphenolic and steroidal constituents of the polar extracts, operating through a combination of an extrapancreatic (biguanide-like) mechanism and antioxidant-mediated protection of residual beta-cells. Bioassay-guided fractionation of the methanol extract, isolation of the active principles and elucidation of their mechanism of action are the logical next steps.

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