Research Paper

# Biochemical Evaluation of the Hypoglycemic Effects of Extract and Fraction of *Cassia fistula* Linn. in Alloxan-induced Diabetic Rats

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**Various extracts of flowers of *Cassia fistula* Linn (Leguminosae) such as petroleum ether (60-80°), chloroform, acetone, ethanol, aqueous, and crude aqueous extracts and two fractions of ethanol extract were tested for antihyperglycemic activity in glucose-overloaded hyperglycemic rats. The effective antihyperglycemic extracts and fraction were tested for their hypoglycemic activity at two dose levels, 200 and 400 mg/kg, respectively. To confirm their utility in higher models, the effective extracts and fraction of *C. fistula* were subjected to antidiabetic study in an alloxan-induced diabetic model at two dose levels, 200 and 400 mg/kg, respectively. Biochemical parameters like glucose, urea, creatinine, serum cholesterol, serum triglyceride, high-density lipoprotein, low-density lipoprotein, hemoglobin, and glycosylated hemoglobin were also assessed in experimental animals. The petroleum ether and ethanol extracts of *C. fistula* and the water-soluble fraction of ethanol extract were found to exhibit significant antihyperglycemic activity. The extracts, at the given doses, did not produce hypoglycemia in fasted normal rats, and the fraction exhibited weak hypoglycemic effect after 2 h of the treatment. Treatment of diabetic rats with ethanol extract and water-soluble fraction of this plant restored the elevated biochemical parameters significantly ($P<0.05$) to the normal level. No activity was found in the petroleum ether extract of the plant. Comparatively, the water-soluble fraction of ethanol extract was found to be more effective than the ethanol extract, and the activity was comparable with that of the standard, glibenclamide (5 mg/kg).**

**Key words: *Cassia fistula* Linn, antidiabetic activity, biochemical analysis, alloxan**

Diabetes mellitus, a chronic metabolic disorder, has now become an epidemic with a worldwide incidence of 5% in the general population. More than 100 million of the world's population has already reached the diabetic mark and the number of people suffering from is expected to soared upto 366 million[1]. Decreased physical activity, increasing obesity, stress, and changes in food consumption have been implicated in this increasing prevalence in the past two decades[2]. Overt diabetes affects 2-3% of the total world population. In conventional therapy, type I diabetes is treated with exogenous insulin and type 2 with oral hypoglycemic agents[3]. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes, there is an increase demand by patients to use natural products with antidiabetic activity[4]. Since time immemorial, patients with noninsulin-requiring diabetes have been treated orally in folk medicine with a variety of plant extracts. In India, a number of plants are mentioned in ancient literature (*Ayurveda*) for the cure of diabetic conditions.

Flowers of *Cassia fistula* Linn (Indian laburnam) belonging to the family Leguminosae is commonly used by many traditional healers in most of the herbal preparations for diabetes[5]. Traditionally, it is used to treat diabetes, constipation, skin diseases, fever, abdominal pain, and leprosy[6]. The reported uses of this plant are antibacterial[6], antioxidant, and antiinflammatory activities[7]. The constituents reported in this plant are alkaloids[8], flavonoids, and anthraquinone glycosides[6].

The traditional use of the flowers of *C. fistula* for diabetes has not been proven so far. Moreover, the researchers focus mainly on ethanol and

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aqueous extracts for diabetes, but a considerable number of studies state that the petroleum ether, benzene, and chloroform extracts were also found active against diabetes[9-11]. Knowing the effective extract and isolating the active fraction from the effective extract would be a useful technique in the development of new drugs. The standard fraction of an active extract may prove better therapeutically than the extract and less toxic and inexpensive compared to pure isolated compounds. Keeping these facts in mind, the present study aimed to prove its traditional use, to identify the active antidiabetic extract of *C. fistula* in diabetes-associated complications and to identify the active antidiabetic fraction of the active extract.

## MATERIALS AND METHODS

Flowers of *C. fistula* were collected in March 2006 from Tamil Nadu, India. The taxonomical identification of the plant was done at Government Arts and Science College, Mandsaur, India. The voucher specimen (BRNCP/C/005/2006) was deposited in the Herbarium of the Department of Pharmacognosy, B. R. Nahata College of Pharmacy, Mandsaur.

Healthy Wistar rats of either sex (150-180 g) with no prior drug treatment were used for the present studies. The animals were fed with a commercial pellet diet and water ad libitum. The animals were acclimatized to laboratory hygienic conditions for 10 days before starting the experiment. The animal study was performed in the Division of Pharmacology, B. R. Nahata College of Pharmacy, Mandsaur with due permission from the Institutional Animal Ethics Committee (registration number 918/ac/05/CPCSEA). All solvents used were purchased from Merck Ltd., Mumbai.

**Preparation of extracts:**
Five hundred grams of shade-dried and powdered petals of *C. fistula* were extracted using Soxhlet, successively with petroleum ether (60-80°), chloroform, acetone, ethanol, and water for 72 h each. Crude aqueous extract of the dried and powdered petals was prepared separately by boiling the plant material (25 g) with 200 ml of water for 15 min. The solvents were evaporated in vacuum to obtain residues of the extracts. Percent yield of the various extracts are given in Table 1.

**TABLE 1: PRELIMINARY PHYTOCHEMICAL EVALUATION, AND PERCENT YIELD OF VARIOUS EXTRACTS AND FRACTIONS OF *CASSIA FISTULA***

| Plant extracts | % yield (w/w) | Constituents |
|---|---|---|
| CF-P | 2.88 | Fats and terpenoids |
| CF-C | 4.06 | Steroids |
| CF-A | 6.08 | Tannins and flavonoids |
| CF-E | 4.99 | Carbohydrates, alkaloids, flavonoids, anthraquinones, glycosides, and steroids |
| CF-Aq | 21.56 | Carbohydrates, glycosides, anthraquinones, and saponins |
| CF-CAq | 22.80 | Carbohydrates, glycosides, tannins, anthraquinones, flavonoids, and saponins |
| E-WSF | 39.00 | Carbohydrates, alkaloids, flavonoids, anthraquinones, glycosides, and steroids |
| E-WISF | 52.20 | Alkaloids and steroids |

CF=*Cassia fistula*, P=petroleum ether (60-80°), C=chloroform, A=acetone, E=ethanol, Aq=aqueous, CAq=crude aqueous, WSF=water-soluble fraction, WISF=water-insoluble fraction

**Fractionation of ethanol extract:**
Fractionation of ethanol extract was done using its solubility profile[12-14]; 15 g of dried ethanol extract was taken in a stoppered flask containing 200 ml of water and shaken mechanically for 1-2 h in a flask shaker. The ethanol extract was not completely soluble in water. The water-insoluble portion of ethanol extract was separated using filtration and both the fractions (water-soluble and water-insoluble) were dried and their percent yield with respect to ethanol extract was determined (Table 1). The extracts and fractions that were not soluble in water were suspended in 1% Tween 80 just before administration to the rats.

**Preliminary phytochemical screening:**
To determine the presence of alkaloids, glycosides, flavones, tannins, terpenes, sterols, saponins, fats, and sugars, a preliminary phytochemical study (color reactions) with various plant extracts and fractions was performed[15,16].

**Acute toxicity studies:**
The acute toxicity test of the extracts and fractions was determined according to the Organization for Economic Co-operation and Development (OECD) guideline number 420. Female Wistar rats (150-180 g) were used for this study. After the sighting study, a starting dose of 2000 mg/kg (P.O.) of the test samples was given to the various groups consisting of five animals in each group. The treated animals were monitored for 14 days for mortality and various responses like behavioral, neurological, and auto-nomic responses. No death was observed till the end of the study. The test samples were found to be safe up to the dose of 2000 mg/kg, and

from the results, 400 mg/kg dose was chosen for further experimentation as the maximum dose.

**Antihyperglycemic activity in glucose-loaded animals:**

Antihyperglycemic activity was studied in glucose overloaded hyperglycemic rats[17]. The animals were divided into various treatment groups (n=5) as mentioned in Tables 2 and 3. Glibenclamide (5 mg/kg) was used as the reference standard and the negative control group animals received only vehicle. The remaining groups were treated with 400 mg/kg of various extracts and fractions of plant suspended in 1% Tween 80. The $0^{th}$ hour blood sugar level was determined from overnight-fasted animals. After 30 min of the drug treatment, the animals were fed with glucose (4 g/kg) and blood glucose was determined after half, one, two, and three hours of the glucose load. Blood glucose concentration was estimated by the glucose oxidase enzymatic method using a commercial glucometer and test strips (Accu-Chek® Active test meter).

**Hypoglycemic activity:**

The animals were classified into eight groups (n=5). Group 1 was kept as control and received a single dose of 0.5 ml/100 g of the vehicle, group 2 was treated with glibenclamide (5 mg/kg) as the hypoglycemic reference drug. Groups 3 to 8 were treated with petroleum ether and ethanol extracts and water-soluble

**TABLE 2: EFFECT OF VARIOUS EXTRACTS OF *CASSIA FISTULA* IN GLUCOSE-LOADED HYPERGLYCEMIC RATS**

| Treatments | Dose mg/kg | Blood glucose concentration (mg/dl) | | | | |
|---|---|---|---|---|---|---|
| | | 0 h | 1/2 h | 1 h | 2 h | 3 h |
| G control | - | 89.80±3.02 | 144.40±4.85 | 150.60±4.01 | 124.40±3.32 | 105.20±4.77 |
| Glibenclamide | 5 | 94.20±3.59 | 105.20±3.49* | 92.20±4.60* | 78.40±4.20* | 66.20±3.68* |
| CF-P | 400 | 83.60±4.88 | 105.20±5.30* | 105.80±6.20* | 98.40±3.22* | 85.60±2.80* |
| CF-C | 400 | 86.00±4.58 | 132.40±4.95 | 129.20±4.44 | 107.80±6.20 | 96.40±4.19 |
| CF-A | 400 | 84.60±2.40 | 128.40±8.20 | 128.20±5.34 | 109.00±5.36 | 95.80±4.44 |
| CF-E | 400 | 88.60±2.88 | 110.80±4.12* | 104.40±5.10* | 89.60±4.60* | 86.40±4.18* |
| CF-Aq | 400 | 83.60±3.22 | 130.00±4.40 | 125.40±6.10 | 108.20±4.20 | 90.80±5.48 |
| CF-CAq | 400 | 82.20±2.18 | 126.80±3.86 | 121.80±2.38 | 95.80±6.80 | 90.20±6.28 |

Each value represents the mean±standard error of mean of five observations, *$P$<0.05 versus control (at respective hours), G=glucose, CF=*Cassia fistula*, P=petroleum ether (60-80°), C=chloroform, A=acetone, E=ethanol, Aq=aqueous, CAq=crude aqueous

**TABLE 3: EFFECT OF FRACTIONS OF ETHANOL EXTRACT OF *CASSIA FISTULA* IN GLUCOSE-LOADED HYPERGLYCEMIC RATS**

| Treatments | Dose mg/kg | Blood glucose concentration (mg/dl) | | | | |
|---|---|---|---|---|---|---|
| | | 0 h | 1/2 h | 1 h | 2 h | 3 h |
| G control | - | 85.80±3.20 | 131.00±4.46 | 110.00±4.22 | 85.40±2.20 | 83.80±4.20 |
| Glibenclamide | 5 | 82.40±2.15 | 85.40±1.93* | 71.80±2.49* | 63.80±1.85* | 58.80±1.85* |
| CF-E | 400 | 82.20±2.35 | 90.20±5.42* | 89.20±3.40* | 80.00±2.09* | 81.20±3.60 |
| CF-WSF | 400 | 82.40±2.22 | 89.00±5.12* | 85.40±3.24* | 80.00±4.26 | 74.20±2.42* |
| CF-WISF | 400 | 80.60±2.10 | 128.80±5.40 | 111.20±4.80 | 91.40±5.24 | 83.40±2.34 |

Each value represents the mean±standard error of mean of five observations, *$P$<0.05 versus control (at respective hours), G=glucose, CF=*Cassia fistula*, E=ethanol, WSF=water-soluble fraction, WISF=water-insoluble fraction

**TABLE 4: EFFECT OF ACTIVE ANTIHYPERGLYCEMIC EXTRACTS AND WATER SOLUBLE FRACTION OF ETHANOL EXTRACT OF *CASSIA FISTULA* IN NORMAL RATS**

| Treatments | Dose mg/kg | Blood glucose concentration (mg/dl) | | | | |
|---|---|---|---|---|---|---|
| | | 0 h | 1/2 h | 1 h | 2 h | 3 h |
| Normal control | - | 84.40±2.32 | 83.00±1.80 | 81.80±2.42 | 82.20±1.70 | 80.20±2.38 |
| Glibenclamide | 5 | 82.00±2.15 | 45.20±3.86* | 36.60±1.43* | 33.20±1.39* | 35.00±3.16* |
| CF-P | 200 | 83.40±2.10 | 85.20±3.10 | 80.40±3.01 | 81.20±1.90 | 80.00±3.93 |
| | 400 | 81.00±1.80 | 82.40±2.72 | 80.40±2.44 | 79.80±3.31 | 80.20±2.10 |
| CF-E | 200 | 80.20±2.24 | 81.00±3.12 | 82.00±3.11 | 78.40±2.80 | 79.40±2.15 |
| | 400 | 82.40±2.80 | 83.40±2.92 | 84.20±2.88 | 82.00±2.66 | 80.00±3.12 |
| WSF | 200 | 84.40±2.10 | 82.20±2.80 | 72.40±2.80* | 71.20±2.28* | 65.80±2.32* |
| | 400 | 83.20±1.89 | 71.20±2.80* | 69.40±3.10* | 60.80±4.02* | 55.00±3.32* |

Each value represents the mean±standard error of mean of five observations, *$P$<0.05 versus control (Dunnett's test), CF=*Cassia fistula*, P=petroleum ether (60-80), E=ethanol, WSF=water-soluble fraction of ethanol extract

fraction of ethanol extract at two dose levels (200 and 400 mg/kg) as mentioned in Table 4. Blood samples were collected from the tail tip at zero (before oral administration), half, one, two, and three hours after vehicle, sample, and drug administration[18]. The blood sugar level was measured using Accu-Chek® Active test strips in Accu-Chek® Active test meter.

**Antidiabetic activity in alloxan-induced diabetes model:**

Alloxan-induced diabetic models were selected to confirm the utility of the active antihyperglycemic extracts and fractions in diabetic conditions. Diabetes was induced in rats by injecting 120 mg/kg of alloxan monohydrate intraperitoneally in 0.9% w/v sodium chloride (NaCl) into overnight fasted rats. The rats were then kept for the next 24 h on 10% glucose solution bottles, in their cages to prevent hypoglycemia. After 72 h of the injection, fasting blood glucose level was measured. Animals which did not develop more than 300 mg/dl glucose levels, were rejected[19,20]. The selected diabetic animals were divided into 8 groups (n=5) and one group of normal non-alloxanized animals was also included in the study. Group 1 was kept as normal control (non-alloxanized rats) and received a single dose of 0.5 ml/100 g of the vehicle, group 2 was kept as negative control, alloxan-induced, and received a single dose of 0.5 ml/100 g of the vehicle, group 3, diabetic-induced was treated with glibenclamide (5 mg/kg) as reference drug. Groups 4 to 9, diabetic-induced, were treated with petroleum ether and ethanol extracts and water-soluble fraction of ethanol extract that exhibited antihyperglycemic activity at two dose levels (200 and 400 mg/kg) as mentioned in Table 5. Treatment was continued for seven consecutive days (P.O.). At the end of seventh day, the rats were fasted for 16 h and blood parameters were determined.

**Collection of blood and estimation of biochemical parameters:**

The blood sugar level was measured using Accu-Chek® Active test strips in Accu-Chek® Active test meter by collecting the blood from rat tail vein. For other plasma profiles, blood was collected from retro-orbital plexus of the rats under light ether anesthesia using capillary tubes into Eppendorf Tubes® containing heparin. The plasma was separated by centrifugation (5 min at 5000 rpm) and was analyzed for lipid profiles (cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol), creatinine, and urea. Whole blood was used to determine hemoglobin and glycosylated hemoglobin. The plasma profiles were measured by standard enzymatic methods with an automatic analyzer[11] and glycosylated hemoglobin by colorimetric method[21].

**Statistical analysis:**

The values are expressed as mean±standard error of mean (SEM). The results were analyzed for statistical significance using one-way analysis of variance (ANOVA) followed by Dunnet's test; $P<0.05$ was considered significant.

## RESULTS

The phytochemicals present and percent yield of various extracts and fractions of *C. fistula* are given in Table 1.

**Effect of extracts and fractions in glucose-loaded hyperglycemic animals:**

Tables 2 and 3 shows the antihyperglycemic effect in glucose-loaded hyperglycemic rats after administration of plant extracts and fractions at a dose of 400 mg/kg. After half an hour of the glucose load, there

**TABLE 5: BIOCHEMICAL PARAMETERS OF NORMAL AND EXPERIMENTAL ANIMALS ON DAY 7, POST TREATMENT**

| Groups | Parameters | | | | |
|---|---|---|---|---|---|
| | Blood glucose | Hemoglobin | Gly hemoglobin | Triglycerides | Cholesterol |
| Normal control | 81.40±3.22* | 1.92±0.17* | 11.20±0.33* | 33.40±3.45* | 34.00±1.74* |
| Diabetic control | 512.00±15.29 | 5.70±0.37 | 6.90±0.41 | 123.00±6.63 | 84.00±4.94 |
| CFP: 200 mg/kg | 462.00±15.30 | 5.20±0.44 | 7.00±1.01 | 128.00±7.53 | 78.80±5.94 |
| CFP: 400 mg/kg | 500.80±17.30 | 6.00±0.57 | 6.20±0.81 | 116.20±4.44 | 80.20±4.86 |
| CFE: 200 mg/kg | 266.60±12.22* | 3.80±0.24* | 9.20±0.57* | 100.00±3.80* | 53.20±3.50* |
| CFE: 400 mg/kg | 171.40±9.80* | 3.60±0.25* | 9.60±0.43* | 79.00±3.84* | 49.20±1.93* |
| WSF: 200 mg/kg | 188.80±14.24* | 3.80±0.45* | 10.20±1.84* | 40.20±2.62* | 54.20±1.13* |
| WSF: 200 mg/kg | 131.40±8.80* | 2.00±0.35* | 11.00±0.64* | 32.20±2.80* | 36.00±2.64* |
| Glibenclamide (5 mg/kg) | 124.40±7.84* | 2.00±0.15* | 10.99±0.47* | 38.20±1.90* | 32.20±2.49* |

Each value represents the mean±standard error of mean of five observations, *$P<0.05$ versus diabetic control [analysis of variance (ANOVA) followed by Dunnett's test], Gly=glycosylated, CFP=*Cassia fistula* petroleum ether extract, CFE=*Cassia fistula* ethanol extract, WSF=water-soluble fraction of ethanol extract

was a significant rise in the blood glucose levels of control animals and at the end of the second hour, the glucose level declined. The antihyperglycemic activity of any extract would be determined by its ability to lower the increasing blood glucose after a glucose load. The plant studied for the activity was found to exhibit significant antihyperglycemic activity ($P<0.05$) at one, two, and three hours after the glucose load compared to control. Petroleum ether and ethanol extracts of the plant exhibited significant antihyperglycemic activity. Among the fractions of ethanol extract, only the water-soluble fraction was found to produce significant activity ($P<0.05$) and it was also found to produce hypoglycemia at the end of the third hour. Comparatively, the water-soluble fraction of ethanol extract of *C. fistula* was found to be more active than ethanol extract (Table 3).

**Effect of extract and fraction in fasted normal rats:**
Based on the antihyperglycemic activity, the active petroleum ether extract, ethanol extract, and water-soluble fraction were subjected to hypoglycemic studies at two dose levels (200 and 400 mg/kg), and the results are given in Table 4. Both the petroleum ether and ethanol extracts did not produce any hypoglycemic effect. Only the water-soluble fraction of ethanol extract showed hypoglycemic activity after two hours of administration ($P<0.05$).

**Effect of extracts and fraction in alloxan-induced diabetic rats:**
The basal blood glucose levels of all the groups were statistically not different from each other. Three days after alloxan administration, blood glucose values were fivefold higher in all the groups and were not statistically different from each other. After seven days, values of blood glucose were decreased in the groups treated with ethanol extract and its water-soluble fraction, and the diabetic rats showed a slight increase in blood glucose level. Petroleum ether extract treatment did not produce any difference in the diabetic animals. The administration of ethanol extract, fraction, and glibenclamide to the diabetic rats restored the level of blood glucose significantly ($P<0.05$) (Table 5).

The levels of total hemoglobin, glycosylated hemoglobin, serum urea, serum creatinine, and lipid profiles of different experimental groups are also represented in Table 5. The diabetic rats showed a significant decrease in the level of total hemoglobin and significant increase in the level of glycosylated hemoglobin. The administration of ethanol extract, fraction, and glibenclamide to the diabetic rats restored the changes in the level of total hemoglobin and glycosylated hemoglobin to near-normal levels ($P<0.05$). Petroleum ether extract was found inactive.

Alloxan-induced diabetic rats showed significant hypercholesterolemia as compared to control. Treatment with ethanol extract and fraction showed a significant decrease in cholesterol levels ($P<0.05$), with an increase in HDL cholesterol at the same time. Hypercholesterolemia was associated with hypertriglyceridemia as compared with control animals. Hypertriglyceridemia also was significantly prevented by the treatment with plant extract and fraction ($P<0.05$). The diabetic control rats showed a significant increase in creatinine and urea levels as compared with control animals. Treatment with ethanol extract and water-soluble fraction of ethanol extract of *C. fistula* reduced these values significantly ($P<0.05$). Both extract and fraction were found effective in alleviating diabetes and diabetes-related complications. Petroleum ether extract did not produce any effect over these levels in the diabetic rats. The activity of the water-soluble fraction was better than that exhibited by 400 mg/kg of ethanol extract, and the activity was comparable with that of the standard drug, glibenclamide (Tables 5 and 6).

## DISCUSSION

This study was performed to validate traditional claims and to determine the active antihyperglycemic fraction isolated from the active extract of this plant. Also, the extracts obtained by successive solvent extraction method were compared with crude aqueous extract of the same plant prepared in a traditional manner. To establish the mechanism behind their activity, the active antihyperglycemic extract and fraction were subjected to hypoglycemic studies, and to check the effect of the extract and fraction in diabetes-associated complications, biochemical parameters were also assessed.

Diabetes is a major health problem affecting major populations worldwide. Epidemiological studies and clinical trials strongly support the notion that hyperglycemia is the principal cause of complications.

**TABLE 6: BIOCHEMICAL PARAMETERS OF NORMAL AND EXPERIMENTAL ANIMALS ON DAY 7, POST TREATMENT**

| Groups | Parameters | | | |
|---|---|---|---|---|
| | HDL | LDL | Creatinine | Urea |
| Normal control | 24.60±1.47* | 22.00±2.10* | 0.45±0.03* | 30.20±1.77* |
| Diabetic control | 10.20±1.12 | 58.80±3.22 | 1.85±0.36 | 279.00±14.01 |
| CFP: 200 mg/kg | 11.20±1.80 | 51.80±2.20 | 2.20±0.38 | 229.00±12.01 |
| CFP: 400 mg/kg | 12.20±2.14 | 60.00±3.34 | 1.95±0.46 | 301.00±13.10 |
| CFE: 200 mg/kg | 15.20±0.80* | 43.00±2.33* | 0.80±0.03* | 128.40±4.26* |
| CFE: 400 mg/kg | 18.00±1.08* | 38.40±2.80* | 0.80±0.24* | 76.40±3.60* |
| WSF: 200 mg/kg | 18.40±1.12* | 32.00±1.24* | 0.62±0.14* | 55.40±3.16* |
| WSF: 200 mg/kg | 20.60±2.22* | 24.80±2.84* | 0.46±0.08* | 46.20±2.40* |
| Glibenclamide (5mg/kg) | 25.80±0.98* | 23.60±1.90* | 0.44±0.03* | 32.80±1.39* |

Each value represents the mean±standard error of mean of five observations, *$P$<0.05 versus diabetic control [analysis of variance (ANOVA) followed by Dunnett's test], HDL=high-density lipoprotein, LDL=low-density lipoprotein, CFP=*Cassia fistula* petroleum ether extract, CFE=*Cassia fistula* ethanol extract, WSF=water-soluble fraction of ethanol extract

Effective blood glucose control is the key for preventing or reversing diabetic complications and improving the quality of life in patients with diabetes. Thus, a sustained reduction in hyperglycemia will decrease the risk of developing microvascular complications and most likely reduce the risk of macrovascular complications[22]. On the basis of this statement, we selected the glucose-induced hyperglycemic model to screen the antihyperglycemic activity of the plants extracts. Any drug that is effective in diabetes will have the ability to control the rise in glucose level by different mechanisms, and the ability of the extracts to prevent hyperglycemia could be determined by the glucose-loaded hyperglycemic model.

In the glucose-loaded hyperglycemic model, the plant tested for antihyperglycemic activity exhibited significant antihyperglycemic activity at a dose level of 400 mg/kg. Excessive amount of glucose in the blood induces insulin secretion. This secreted insulin stimulates peripheral glucose consumption and controls the production of glucose through different mechanisms[23]. However, from the study (glucose control), it was clear that the secreted insulin requires two to three hours to restore the glucose level to normal. In the case of petroleum ether extract, ethanol extract, water-soluble fraction of ethanol extract, and drug-treated groups, the glucose levels did not exceed those of the negative control group, giving an indication regarding the supportive action of the extracts, fractions, and drugs in glucose utilization. The effect of glibenclamide, the standard drug used in this study, on glucose tolerance has been attributed to the enhanced activity of beta-cells of the pancreas, resulting in the secretion of larger amounts of insulin. So, the mechanism behind this antihyperglycemic activity of plant extracts and fractions involves an insulin-like effect, probably, through peripheral glucose consumption or enhancing the sensitivity of beta-cells to glucose, resulting in increased insulin release[22]. In these contexts, a number of other plants have also been reported to have hypoglycemic effects[24].

When the active antihyperglycemic extracts and water-soluble fraction were tested for hypoglycemic activity, only the water-soluble fraction exhibited the tested hypoglycemic activity. The hypoglycemic effect produced by the fraction may be due to the increased insulin release resembling the mechanism of actions of sulfonylureas[25,26]. Alloxan produces hyperglycemia by a selective cytotoxic effect on pancreatic beta-cells. One of the intracellular phenomenon for its cytotoxicity is through the generation of free radicals demonstrated both *in vivo* and *in vitro*[27]. Our investigations indicate that the efficiency of the plant in the maintenance of blood glucose levels in alloxan-induced diabetic rats may be possibly by the above-mentioned mechanisms or the ability of the plant to prevent free radicals.

In uncontrolled or poorly controlled diabetes, there is an increased glycosylation of a number of proteins including hemoglobin. It has been reported that in approximately 16% of diabetic patients the glycosylated hemoglobin levels were increased and was fount to be directly proportional to the fasting blood glucose levels. During diabetes, the excess glucose present in blood reacts with hemoglobin. Therefore, the total hemoglobin level is decreased in alloxan-diabetic rats[28]. The administration of ethanol extract and fraction for seven days prevented a significant elevation in glycosylated

hemoglobin, thereby increasing the level of total hemoglobin ($P<0.05$) in diabetic rats. This could be due to the result of improved glycemic control produced by the plant extract and fraction.

The levels of serum lipids are usually elevated in diabetes mellitus, and such an elevation represents a risk factor for coronary heart disease. This abnormally high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots mainly due to the action of insulin. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in the diabetic state, lipoprotein lipase is not activated due to insulin deficiency, resulting in hypertriglyceridemia[29]. Also, insulin deficiency is associated with hypercholesterolemia. Insulin deficiency may be responsible for dyslipidemia, because insulin has an inhibitory action on HMG-CoA reductase, a key rate-limiting enzyme responsible for the metabolism of cholesterol-rich LDL particles. The mechanisms responsible for the development of hypertriglyceridemia and hypercholesterolemia in uncontrolled diabetes in humans are due to a number of metabolic abnormalities that occur sequentially[30]. In our study also, the diabetic rats showed hypercholesterolemia and hypertriglyceridemia and the treatment with plant extract and fraction significantly ($P<0.05$) decreased both the cholesterol and triglyceride levels. This implies that the ethanol extract of *C. fistula* flower and water-soluble fraction of ethanol extract can prevent or be helpful in reducing the complications of lipid profile seen in some diabetics in whom hyperglycemia and hypercholesterolemia coexist quite often[31]. These findings also support the hypothesis that the activity of the plant extract and fraction may be directly attributed to improvements in insulin levels upon treatment[31].

The diabetic hyperglycemia induced by alloxan produces elevation of plasma levels of urea and creatinine, which are considered as significant markers of renal dysfunction[32]. The results in Table 6 show a significant increase in the level of plasma urea and creatinine in the diabetic groups compared to control level. These results indicate that diabetes might lead to renal dysfunction, although after treatment of alloxan-diabetic rats with ethanol extract and water-soluble fraction, the levels of urea and creatinine were significantly ($P<0.05$) decreased compared to the mean value of the diabetic group. This further confirms the utility of these plants in diabetes-associated complications[33].

Mostly it was believed that the formation of artefacts during the preparation of crude aqueous extracts would be responsible for biological activities[34]. In our study, the crude aqueous extract of *C. fistula* was found inactive. Though the main classes of active constituents are present in the aqueous and crude aqueous extract of this plant, activity was not found as in ethanol extract and its fraction, and this may be due to absence of terpenoids and alkaloids that are present in petroleum ether and ethanol extracts, respectively. Hence, the antidiabetic activity of the extracts was caused by substances that naturally exist in the plant parts, and not due to transformations induced by heating. In this study, the maximum activity was found in ethanol extract and water-soluble fraction of ethanol extract, which contains alkaloids that were not found in any other extracts (Table 1). Though the water-insoluble fraction of ethanol extract contains alkaloids, it did not produce any effect, and this may be because of the nature of the alkaloids present in it. Terpenes present in the petroleum ether extract may be responsible for its antihyperglycemic activity and these terpenes were found inactive in restoring the biochemical values induced by alloxan. This suggests that alkaloids in ethanol extract and water-soluble fraction may exhibit antidiabetic activity along with other constituents as many alkaloids are reported to have antidiabetic activity[35,36].

We conclude that the extract and fraction of the plant tested for antidiabetic activity showed appreciable results in decreasing the serum glucose level and other complications associated with diabetes. This research supports the inclusion of this plant in traditional antidiabetic preparations, and the formulations made using the identified effective extract and fraction of this plant could serve the purpose better than the existing formulations with crude aqueous extract.

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