Quantitative determination of phytochemical constituents and evaluation of acute antihyperglycemic activity of four various extracts of Cleome viscosa whole plant in STZ-induced diabetic rats

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Abstract
Cleome viscosa is traditionally used in the management of diabetes mellitus. While this claim has not been investigated technically, the aim of this study was to estimate the phytochemical constituents and investigate the antidiabetic property of the methanolic extract (MeCV), aqueous extract (AgCV), ethyl acetate (EaCV) and n-hexane (NhCV) extract of Cleome viscosa whole plant in streptozotocin (STZ)-induced diabetic rats. Diabetes was induced by using a single intra peritoneal injection of STZ (45 mg/kg b.w.). Determination of FBG levels at 0 hour (before administration of extract) and 1 h, 3 h, 5 h and 7 h (after administration of extract) by using accucheck glucometer. Quantitative determination of phytochemicals was done by using standard methods. We observed, MeCV showed a maximum effectiveness with a dosage of 400 mg/ kg b.w. at 5 h which is about -300.38±42.91% diminution in FBG level over zero time values. The FBG-decreasing effect of MeCV extract was more rapid than that of the remaining three extracts when compared to diabetic control; the diminishing rates were -50.70±2.22% after 1 h, -179.42±33.31% after 3 h, -300.38±42.91% after 5 h and -92.64±16.08% after 7 h. After 5 h (-300.38±42.91%), the effect of MeCV on FBG levels was significantly similar mode to that of reference drug glibenclamide (-324.24±33.21%) in diabetic rats. These results suggest that whole plant of MeCV extract is valuable in the management of diabetes by diminution of blood glucose levels presumably due to the presence of phytochemical constituents such as alkaloids, saponins and tannins.

Keywords: Cleome viscosa, Diabetes mellitus, fasting blood glucose level, saponins, tannins

1. Introduction
Nutritional substrates play a key role in physiological system controlling intermediary metabolism and different defects in various human diseases, including cardiovascular, hyperlipidemia and other metabolic diseases, hypertension, and diabetes [1]. Diabetes mellitus is affected by several disorders in intermediary metabolism, such as disarrangements in carbohydrates, proteins, and fat metabolism resulting from the complete or moderately deficient insulin secretion and movement [2]. Diabetes mellitus is classified into two types: types-1 and type-2. Type-1 diabetes is also called as insulin dependent diabetes mellitus. In type 1 Kuppm his state, the body does not fabricate insulin; for that reason, patients with type-1 diabetes should get every day insulin injections to remain alive. Type-1 diabetes is also accounts for 5-10 percent of diabetes cases. Type-2 is also known as non-insulin dependent diabetes mellitus; in this state, body does not produce enough amount of insulin or does not appropriately utilize this body and also it is a most common type of disease. In adults, type-2 diabetes accounts for regarding 90-95% of all diagnosed. Yet, 9 out of 10 peoples of this collection don’t recognize that they have pre-diabetes [3]. Reasons for this increase incorporate enlarge in inactive lifestyle, utilization of energy-rich diet, fatness, higher life span, etc. [4]. Type-2 diabetes is widely circulated in China and India, where diabetes mellitus rates increase with more than 200 million people suffering from the disease worldwide. The WHO (World Health Organization) estimates that the amount of patients with diabetes mellitus will go beyond 360 million by the year of 2030 [5]. Although the present prevalence rate of diabetes in world 60.3 per 1000 persons in 2004 and has shown a 10-fold enhance from the prevalence rate of 20 years ago [6]. Management methods of diabetes contain diet treatment, exercise therapy simultaneously with
Pharmacotherapy, and clinically, administration of insulin, α-glucosidase inhibitors, biguanide, sulphonylurea and troglitazone. However, lethal side effects such as hypoglycemia and lactic acidosis contain reported through use of therapeutic treatments [7]. For the reason that of disadvantage to maintain standard blood glucose levels during folk medicine and natural food, slightly than with therapeutic treatments that deserve lethal effects and tolerance, several researchers are keenly conducting studies to increase matter from natural resources and food constituents that can diminish blood glucose [8].

As traditional medicine, herbal plants are usually used as anti-hyperglycemic agent because of they are composed phytochemical compounds acted as antioxidants with minimum or no side effects in medical consign and also demand comparatively lower expenses than oral synthetic hypoglycemic agents [9]. According to ethno botanical information, around 800 floras might demonstrate antidiabetic properties [10]. As such, several plants enclosed suggested to treat diabetes [11]. The most important chemical compounds isolated and recognized from plants are proteins, glycans and mucilages. Other compounds, such as phenolics, flavonoids, alkaloids, steroids, and triterpenoids with antihyperglycemic activity, have been extracted from established medicinal plants with different crude solvents [12]. Cleome viscosa (C. viscosa) as one of the major species of Cleome (Cleomaceae) is medicinally used to treat hypertension, inflammation, hypoglycemic, antimicrobial, hepatoprotective, antipyretic, antihelminctic, and also treat piles, snake bite and malaria [13].

Previous studies indicated that the ethanol extract of the aerial parts of C. viscosa is effectively used to treat gastric lesions and methanol extract are more potent than ethyl acetate and n-hexane extracts [14, 15].

Cleome viscosa L. (Cleomaceae), called “kukkavamitta” in Telugu, is commonly found in road sides and wastelands. It is commonly known as Asian spider flower or tick weed is an annual herb, up to a meter high. Although the treatment of this plant, it has not been prevalence to compose the study of the effects of C. viscosa whole plant extracts by polar to non-polar solvents with antidiabetic potential. These studies were thus formulate with the aim to investigate the acute effect of MeCV, AqCV, EaCV and NhCV extracts of C. viscosa whole plant on blood glucose level of STZ-induced diabetic rats and measuring the percentage of alkaloid, saponin and tannin contents.

2. Materials and methods

2.1 Collection of plant materials

Fresh C. viscosa whole plant was collected in Dravidian University surroundings, Kuppam, India. A voucher specimen (Voucher No: CV-01) was deposited in the herbarium of the Botany Department, Sri Venkateswara University, Tirupati, India. The whole plant material was cut into pieces and washed with running tap water. Visibly damaged plants were removed and rinsed with distilled water. The fully dried plant material was grind into powder by using an electric grinder. The obtained powder was vacuum-packed and stored at 4°C until further analysis.

2.2 Preparation of plant extracts

The powdered (250 g) materials were successively extracted by using Soxhlet apparatus for 6 Hrs with methanol, aqueous, ethyl acetate and n-hexane solvents (1:5 ratio W/V) was extracted by using were concentrated to dehydration under the vacuum 70-80 °C. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in vacuum at 35 °C-40 °C and dry powder was obtained.

2.3 Chemicals

As a standard anti-diabetic drug, Glibenclamide (purchased from local market) was used as positive control. Streptozotocin (STZ; Sigma–Aldrich Chemical Co.,) was used to induce diabetes. Other chemicals, solvents and reagents were of analytical grade and were procured from approved organizations.

2.4 Quantitative Determination of Phytochemical Constituents

2.4.1 Determination of Alkaloids

Quantitative analyses of alkaloids were estimating according to the method by Harborne [16]. Exactly 200 mL of 10% acetic acid in ethanol was added to each plant extracts (2.50 g) in a 250 mL beaker and allowed to stand for 4 h. The extracts were vigorous on a water bath to one-quarter of the original volume followed by addition of 15 drops of concentrated NH2OH drop wise to the extracts until the precipitation was complete immediately after filtration. After 3 h of mixture sedimentation, discarded the supernatant and the precipitate was washed with 20 mL of 0.1M of NH2OH and then filtered by using filter paper. The residues were dried in an oven and the percentage of alkaloids is calculated by using following formula:

\[
\% \text{ Alkaloids} = \frac{\text{Weight of alkaloids}}{\text{Weight of samples \times 100}}
\]

2.4.2 Determination of Saponin

Quantitative determination of saponin was carried out by using the method reported by Obadoni and Ochuko and Ejikeme et al. [17]. Exactly 100 mL of 20% aqueous ethanol was added to 5 g of each extract in a 250 mL conical flask. The mixture was heated over a water bath for 4 hours with constant centrifugation at 55°C. The filtrate of the mixture was reextracted with another 100 mL of 20% aqueous ethanol, after filtration then heated for 4 hours at 55°C with constant centrifugation. The collective extracts was evaporated to 40 mL over water bath at 90°C. Diethyl ether (20 mL) was added in 250 mL separator funnel and vigorously agitated from which the aqueous layer was recovered while the ether layer was discarded. This purification process was repeated twice. 60 mL of n-butanol was added and extracted twice with 10 mL of 5% NaCl. After discarding the NaCl layer the remaining solution was heated in a water bath for 30 minutes, after which the solution was transferred into a crucible and was dried in an oven to a constant weight. The saponin content was deliberate as a percentage.

\[
\% \text{ Saponin} = \frac{\text{Weight of saponin}}{\text{Weight of sample \times 100}}
\]

2.4.3 Determination of Tannin

Analytical method for determination of tannin quantitative analyses by according to Ejikeme et al. [17] and Amadi et al. [17]. By dissolving 50 g of sodium tungstate in 37 mL of distilled water, Folin-Denis reagent was prepared. To the above prepared reagent, 10 g of phosphomolybdic acid and 25 mL of orthophosphoric acid were added. Two-hour reflux of the mixture was carried out, cooled, and diluted to 500 mL with distilled water. 1 g of each extract was added to 100 mL of distilled water. This was heated gently for 1 hour on a hot
plate and filtered by using Whatman filter paper. Addition of 5 mL Folin-Denis reagent and 10 mL of saturated Na₂CO₃ solution into 50 mL of distilled water and 10 mL of diluted extracts (aliquot volume) was carried out after being pipetted for colour development. The solution was allowed to stand for 30 minutes in a water bath at 25°C after thorough agitation. Suspension of 200 mg of tannic acid in distilled water and dilution to 200 mL mark (1 mg/mL) was used to get tannic standard curve. Varying concentrations (200–1000 µg/mL) of the standard tannic acid solution were pipetted into five different test tubes to which 5 mL of Folin-Denis reagent and 10 mL of saturated Na₂CO₃ solution was added and prepared to the 100 mL mark with distilled water. The solution was kept for 30 minutes in a water bath at 25°C. Optimal density was measured at 700 nm with the help of spectrophotometer. Optimal density versus tannic acid concentration was plotted. The following formula was used in the calculation:

Tannic acid (mg /100 g) = C × extract volume × 100 Aliquot volume × weight of sample,

Where C is concentration of tannic acid read off the graph.

2.5 Acute antihyperglycemic activity
2.5.1 Animals
Healthy Wistar strain male albino rats weighing 180-200 g were used in this study. The rats were kept in polypropylene cages under standard conditions: 22 ± 3°C and a 12 h/12 h light/dark cycle. The rats were fed with a commercial diet and allowed free access to water ad libitum. All experimental procedures involving animals were conducted in accordance with the guidelines for the care and use of laboratory animals, as approved by the Animal Ethical Committee, Sri Krishnadevaraya University, Andhra Pradesh, India. (1889/GO/Re/S/16/CPCSEA SKU/ZOO/02/2018) to perform this animal work.

2.5.2 Induction of diabetes
Diabetes was induced in healthy Wistar Albino male rats, with body weights ranging from 180 to 200 g, by a single intra peritoneal injection of freshly prepared STZ (45 mg/kg/b.w) dissolved in ice cold 0.1M citrate buffer (pH 4.5) after allowing the rats for overnight fasting for 12–15 h as per the method followed by Rakieten et al. (1963) [18]. 8 h after STZ administration the rats were kept for next 24 h on given 15% glucose solution to prevent hypoglycemia, as STZ is capable of producing fatal hypoglycemia due to destruction of β-cells which in turn results into massive pancreatic insulin release. After 48 hours of STZ administration, glucose level was measured in the tail vein punctured by using an Accu-check glucometer (Roche Diagnostics Co., USA). In this study, the rats with fasting blood glucose levels (FBGL) > 270 mg/dL were considered diabetic and taken in the study. After a week, when the condition of diabetes was stabilized, rats with marked hyperglycemia (fasting blood glucose level ≥250 mg/dL) were selected. The percentage change in blood glucose was calculated thus:

Percentage of glycemic change = (Gx − Gi)/Gx × 100

Where, Gx is the glycemia at time x and Gi is the glycemia at initial time (i).

2.5.3 Effect of acute oral administration of extracts and Glibenclamide on STZ-induced diabetic rats
33 rats were taken for this experiment. The rats were divided into 11 groups, each group containing 3 animals. Group I-Normal control, Group II-Diabetic Control, Group III-Diabetic+Glibenclamide-20 mg/kg b.w treated, Group IV-Diabetic+MeCV-200 mg/kg b.w treated, Group V-Diabetic+MeCV- 400 mg/kg b.w treated, Group VI-Diabetic+AgCV-200 mg/kg b.w treated, Group VII-Diabetic+AgCV-400 mg/kg b.w treated, Group VIII-Diabetic+EaCV- 200 mg/kg b.w treated, Group IX-Diabetic+EaCV- 400 mg/kg b.w treated, Group X-Diabetic+NhCV-200 mg/kg b.w treated and Group XI-Diabetic+NhCV-400 mg/kg b.w treated. Blood samples were collected from the tail vein to measure the blood glucose level at 0 h (before treatment) and at 1, 3, 5, and 7 h after the plant extracts were administered; fasting blood glucose levels were determined in all groups.

2.6 Statistical analysis
Values were represented as mean ± SEM. Data were statistically analyzed with One-way ANOVA followed by DMRT test by using SPSS (version 16). P value < 0.01 was considered as significant.

3. Results
3.1 Quantitative Determination of Phytochemical Constituents
![Figure 1: Percentage of alkaloids content on the four various extracts of Cleome viscosa whole plant.](Image)

The amount of phytochemicals which are found in the four various extracts of C. viscosa whole plant was quantitatively determined by using standard procedures. All the extracts of C. viscosa demonstrated different quantity of phytochemicals. Among the three components saponin and alkaloid content was uppermost in the chosen MeCV, AgCV, EaCV and NhCV followed by tannin compounds (Figure 1, 2 and 3). The quantity of tannin was low in the whole plant of MeCV, AgCV, EaCV and NhCV extracts.

![Figure 2: Percentage of saponins content on the four various extracts of Cleome viscosa whole plant.](Image)
3.2 Acute antihyperglycemic activity
3.2.1 Effect of the acute/ oral administration of extracts and Glibenclamide on STZ-induced diabetic rats

The oral single administration, the MeCV, AqCV, EaCV and NhCV extracts of C. viscosa whole plant decreased the fasting blood glucose levels at the two doses of 200 mg and 400 mg/kg body weight (Table 1). The oral administration of 200 mg and 400 mg/kg b.w of MeCV, AqCV, EaCV and NhCV extracts of C. viscosa whole plant lowered blood glucose levels from the 1st h to 7th h in a dose-independent manner. A gradual decrease was then observed from the 5th h at the doses of 200 mg and 400 mg/kg b.w when compared to diabetic control.

Data were given as mean ± SE for six animals in each group. Values not sharing a common letter differ significantly at $P<0.01$ by DMRT in column.

The 5th h, MeCV, AqCV, EaCV and NhCV extracts have lowered the fasting blood glucose levels to -300.38±4.91%, -182.38±9.22%, -53.07±6.84%, and -51.36±4.40% respectively over zero time values for the dose of 400 mg/kg b.w, compared to -324.24±33.21% for the commercial oral drug, glibenclamide (Table 2). Among this four extracts at the two doses, MeCV extract had decreased the maximum percentage of fasting blood glucose levels and NhCV extract had showed the minimum percentage of fasting blood glucose levels in compared to remaining extracts at all time intervals. This is a dose-independent response. The reduction in fasting blood glucose levels when compared to the diabetic control fasting blood glucose levels was statistically significant ($P<0.01$) in all groups.

Table 2: Mean percentage change in blood glucose levels of four various extracts of Cleome viscosa whole plant administered in STZ- induced diabetic rats.

Data were given as mean ± SE for six animals in each group. Values not sharing a common letter differ significantly at $P<0.01$ by DMRT in column. The percentage of decreasing or increasing levels of the blood glucose regarding to baseline of Fasting Blood Glucose.

Discussion

This study investigated the acute antihyperglycemic effect of C. viscosa whole plant extracts in STZ-induced diabetes; in the majority of experiments, the effect of the plant extracts were compared to those of glibenclamide, which was used as a positive control. The Wistar strain male albino rats were used as an experimental model. In this study, we have used one of the commonly used animal models of human disease, where diabetes is induced by discriminating damage of the pancreas β-cells with a single intraperitoneal injection of STZ [19]. Using this diabetic model, we screened serial polar to non-polar C. viscosa extracts to establish their antihyperglycemic effects. The STZ-induced diabetic rats show 0 h to 7 h increase of fasting blood glucose levels (355.33±3.71

Fig 3: Percentage of tannins content on the four various extracts of Cleome viscosa whole plant.

Table 1: Acute antihyperglycemic activity of four various solvent extracts of Cleome viscosa whole plant on fasting blood glucose levels in streptozotocin-induced diabetic rats.

<table>
<thead>
<tr>
<th>Treated groups</th>
<th>FBG level (mg/dL)</th>
<th>Time (h) after a single dose of plant extracts administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>1 h</td>
</tr>
<tr>
<td>Normal Control</td>
<td>81±5.85</td>
<td>89.33±1.45</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>355.33±3.71</td>
<td>361.33±4.25</td>
</tr>
<tr>
<td>Diabetic+Glibenclamide-20mg/k.b.w</td>
<td>352.66±6.60</td>
<td>177.66±11.11</td>
</tr>
<tr>
<td>Diabetic+MeCV-200mg/k.b.w</td>
<td>354.33±6.56</td>
<td>239.33±2.72</td>
</tr>
<tr>
<td>Diabetic+MeCV-400mg/k.b.w</td>
<td>357.66±4.48</td>
<td>237.33±2.02</td>
</tr>
<tr>
<td>Diabetic+AquEV-200mg/k.b.w</td>
<td>359.33±5.56</td>
<td>319.33±12.24</td>
</tr>
<tr>
<td>Diabetic+AquEV-400mg/k.b.w</td>
<td>347.33±5.04</td>
<td>244.33±2.29</td>
</tr>
<tr>
<td>Diabetic+EaCV-200mg/k.b.w</td>
<td>360.33±5.78</td>
<td>353.66±5.60</td>
</tr>
<tr>
<td>Diabetic+EaCV-400mg/k.b.w</td>
<td>349±5.50</td>
<td>340±5.03</td>
</tr>
<tr>
<td>Diabetic+NhCV-200mg/k.b.w</td>
<td>364.66±4.05</td>
<td>358±4.16</td>
</tr>
<tr>
<td>Diabetic+NhCV-400mg/k.b.w</td>
<td>370.33±10.68</td>
<td>351±6.08</td>
</tr>
</tbody>
</table>

Data were given as mean ± SE for six animals in each group. Values not sharing a common letter differ significantly at $P<0.01$ by DMRT in column.
mg/dL to 379.33±5.54 mg/dL) when compared to the normal control rats. All of the extracts except the NhCV extract significantly (P<0.01) reduced the blood glucose levels of the diabetic rats after the treatment. In this study, C. viscosa extracts did not significantly affect FBG at an acute dose of 200 and 400 mg/kg b.w until 1 h; after 3 h, these extracts significantly decreased FBG (P<0.01).

However, among this four extracts, the MeCV extract (400 mg/kg b.w) induced the highest decrease on fasting blood glucose level at 5 h (-300.38±42.91%) and NhCV extract induced the smallest amount decrease on fasting blood glucose level at all-time intervals when compared diabetic control rats. The polarity of methanol is 5.1 compared with that of aqueous, ethyl acetate and n-hexane solvents differentiate by moderate polarity. Hence, with reference to this result it is expected that the extracts with a balanced concentration of active compounds obtain antihyperglycemic properties compared with high and low polarity concentrations of active compounds that extracted by other solvents. The present study also supports the majority of earlier reports that polar extracts can decrease the blood glucose levels [20, 21]. This result suggested that the MeCV extract grouping might be used to isolate the active antidiabetic compound in C. viscosawhole plant. For that reason, the MeCV extract can be considered as a good contender to develop novel antidiabetic natural products or consistent herbal formulation.

The blood glucose lowering property of this plant extracts might be endorsed to the occurrence of alkaloids, saponins, tannins, phenols, flavonoids, and terpenoids that contain be connected with antihyperglycemic activity [22]. The phytochemical determination of the whole plant extracts (using MeCV, AqCV, EaCV and NhCV) of C. viscosa showing the occurrence of alkaloids, saponins and tannin compounds. Results indicated that MeCV extract showed the most significant antihyperglycemic property which was possibly due to the attendance of some secondary metabolites and their synergistic properties. Reported that the most prospect of antihyperglycemic property of the ethanol root extract of Nuclea latifolia was the presence of tannins, flavonoids, and alkaloids [23]. Generally alkaloids have been supposed to inhibit α-glucosidase and diminish glucose transport during the intestinal epithelium [29]. Based on our results, major content of alkaloids present in the whole plant of MeCV extract might be responsible for the blood glucose levels lowering effect in STZ-induced diabetic rat management. Alkaloids have also been severally reported to have antihyperglycemic effect. Alkaloids such as bebecrine, berberine, bebeerine, buxine, cissammpareine, cissamine, hyatin, pareirubrine A and B, pareitropone, tetrandine and trandrine have been reported in C. pareira various biological activities [25, 30]. Alkaloids tetrandine and berberine have been account to exhibit antioxidant property and this property might be dependable for the different biological activities linked with this plant together with antidiabetic activity.

Saponin contains Kalopanax pictus has been revealed to show antihyperglycemic activity in STZ-induced diabetic rats [27]. In our results demonstrated that the whole plant of MeCV extract had more content of saponins are present when compared remaining three extracts. In other studies, saponins have been publicized to decrease blood glucose levels in diabetic rats [28]. Similar studies were done on saponins obtained from the roots of Garcinia kola (bitter kola) on alloxan-induced diabetic rats [29] and significant quantities of saponins are established in Anogeissus leiocarpus, Kaempferia galanga, Dichrostachys cinerea, and Allanblackia floribunda having anti-diabetic properties [30]. Tannins is one of the main energetic components establish in plant based medicines [31]. Tannin has been reported to selectively inhibit antiviral, antibacterial, and antitumor activities and HIV replication [32]. The aqueous leaf extracts of the Cissampelos pareira plant enclosed tannins that are recognized to have antihyperglycemic activity [33]. The tannin epigallocatechin-3-gallate exhibit hypoglycemic activity as demonstrated by Broadhurst et al. [34]. The hypoglycemic activity (α-amylase and α-glucosidase inhibition activities) of tannin extracted from several vegetables, cereals, legumes, and oil seeds have been studied [35] and also results have publicized supporting property. Therefore, the above listed whole plant of MeCV extract has potentially effect on diabetic rats.

5. Conclusion
This study established that C. viscosa whole plant posses’s antihyperglycemic property in STZ-induced diabetic rats, thus systematically validating its sustained utilize in the administration of diabetes mellitus. The antihyperglycemic property was due to collective consequence of phytochemicals present in the plant extracts including alkaloids, saponins and tannins. However, supplementary research should be made focusing on isolating the bio-active compounds in responsible for the antihyperglycemic property of this plant during bioassay guided fractionation.

6. Acknowledgement
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