



RESEARCH PAPER

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Antidiabetic activity of *in-vivo* and *in-vitro* plants of *Cleome gynandra* L. in streptozotocin-induced-diabetic rats

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Abstract

The aim of the present study is to evaluate the therapeutical potential of *in-vivo* and *in-vitro* plants of *Cleome gynandra* L. in alleviating diabetes by assessment of body weight and blood glucose level of STZ-induced diabetic rats. Ethanolic shoot extracts of *C. gynandra* used in the traditional management of diabetes. Nicotinamide 120mg/kg was used as a positive control. A glucometer was used for blood glucose level measurement and body weight was measured by weighing balance at intervals. Total cholesterol and triglycerides of the lipid profile were analyzed. Treatment of STZ-induced Wistar diabetic with the ethanolic plant extracts of *C. gynandra* (500mg/kg) compared to the glibenclamide standard drug, the *in-vitro* plant extracts of *C. gynandra* were best reacted against body weight reduction and when compared to *in-vivo* shoots caused significant ($P < 0.001$) reductions in the Blood Glucose Level (BGL). Administration of *in-vitro* shoot extracts showed a higher reduction in TC and TG levels than *in-vivo* shoot extracts TC and TG and control rats TC and TG. The drug consumption of diabetic-induced rats' body weight and blood glucose level shows successful treatment. The ethanolic shoot extracts of *in-vivo* and *in-vitro* plants *C. gynandra* demonstrate anti-hyperglycemic activity, thereby confirming anti-diabetic potential and validating traditional medicine.

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Introduction

Diabetes mellitus was characterized by hyperglycemia and glucose intolerance, either due to the relative deficiency in insulin secretion or impaired the effectiveness of insulin's action to enhance glucose uptake. Diabetes mellitus is a chronic medical condition which though can be controlled lasts a lifetime (Day and Bailey, 1998; Gray and Flatt, 1997; Swanson-Flatt *et al.*, 1991). The diabetes of chronic hyperglycemia is associated with long-term damages of dysfunction and failure of various organs, especially the kidneys, eyes, heart, blood vessels and nerves. Two forms of diabetes (Types 1 and 2) differ in their pathological process, but both have hyperglycemia as a common hallmark. In type 2 diabetes, hyperglycemia caused due to impairment in insulin secretion combined with or without impairment of insulin action (Rajkumar and Govindarajulu, 1991; Lin and Sun, 2010). The World Health Organization reported that the worldwide global population is in the midst of a diabetes epidemic.

The people in Southeast Asia and the western Pacific are being under greater risk, and the majority of patients have type 2 diabetes. Insulin resistance usually precedes the onset of type 2 diabetes and is generally accompanied by other cardiovascular risk factors like dyslipidemia, hypertension, and prothrombotic factors (Tomoda *et al.*, 1985; Reher *et al.*, 1991 and Yallow *et al.*, 1960). The majority of folklore medicines in anti-diabetic plants await proper scientific and medical evaluation for their ability to improve blood glucose management. In Indian folklore medicine systems, the number of medicinal plants has been used since ancient time to effectively treat diabetes (Dongdong *et al.*, 2021; Mukherjee *et al.*, 2006).

According to World Health Organization (WHO), up to 80% of the world's population in developing countries relies on traditional medicine practices for their primary health care needs (WHO, 2015; Huang *et al.*, 1992). Plants have always been medicine for treat many diseases throughout the world. They contain a great diversity of bioactive compounds

which makes them a possible source for different types of drugs (Musila *et al.*, 2002). The chemical composition of herbal products and potency depends on the plant extract derivative, the age of the plant part used, season when harvested and the methods of processing (Mahmood and Qureshi, 2012). Herbal medicines are cheap, more readily available to people and are assumed to be less toxic due to their long term clinical experience. However, a lot needs to be done to establish their efficacy and safety profiles, which in common practice is presumed from historical traditional uses (Berhan and Awgichew, 2021).

Spider plant (*C. gynandra* L.) is one of the most important traditional medicines in African countries and also Asian regions. *Cleome* is a genus under the family Cleomaceae (formerly Capparaceae) is a large group of angiosperms, consisting many species present in tropical and sub-tropical areas of the world (Orech *et al.*, 2005). It is also used for vegetables as worldwide use. Leafy vegetables like *Cleome gynandra* are an excellent source of protein, vitamins and minerals, and dietary fibre (Partha Pradip Adhikari and Satya Bhusan Paul, 2018). It has a high nutritive value and contains phenolic compounds that are essential in reducing or preventing the occurrence of chronic and infectious diseases. The pharmacology property of this plant is also described in Ayurvedic pharmacopoeia of India and also in other traditional medical texts. Traditionally, ayurvedic medicine is a chief constituent in Narayana Churna (Mishra *et al.*, 2011). The anti-hyperglycemic activities of different extracts of most plants of this genus have been validated by several studies (Ali *et al.*, 2016; Chanda, 2014). This study summarizes the anti-diabetic activity of *in vivo* and *in vitro* regeneration shoots of *Cleome gynandra* in STZ induced diabetic rats (Mohtasheem *et al.*, 2011).

Materials and methods

The efficacy of *in vivo* and *in vitro* shoot extracts of *C. gynandra* was evaluated for antidiabetic activities through STZ induced wistar albino rats through pharmacological studies. *Cleome gynandra* L. was collected from Thanthonimalai villages of Karur District, Tamilnadu, India used as an *in-vivo* shoot sample.

From *in vitro* plants were produced through micropropagation in our laboratory (Sudan and Karthikeyan, 2019).

Extraction of samples for pharmacological studies

The *in vivo* and *in vitro* regenerated shoots were washed, shade dried and powdered in a mechanical grinder. Twenty grams of dry powder of each plant was soaked in 200 ml ethanol. The flask was shaken, this process periodically repeated for 3 days (Bassey *et al.*, 2010). Then it was filtered and evaporated at room temperature. Now the prepared sample is subjected to analysis.

Experimental Design

Adult male albino Wistar rats (6 weeks), weighing 150 to 200g were used for the present antidiabetic study. The animal divided into five groups of five animals each. The six weeks animals are kept overnight fasting. Streptozotocin was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal physiological saline. The animals with blood glucose concentration more than 250mg/dl will be used for the study (Masiello *et al.*, 1998; Murugan and Pari 2006). The total experimental period was 28 days and the animal was grouped into 6 groups and the treatment for each group as follows:

Study Design

The total experimental period was 28 days and the animal was grouped into 6 groups and the treatment for each group as follows:

Group 1: Control only normal saline.

Group 2: Only Streptozotocine 60mg/kg/b.w. (IP) and Nicotinamide 120mg/kg(po) only

Group 3: Streptozotocin (60mg/kg) and Nicotinamide 120mg/kg (po) rats treated with Glibenclamide 20mg/kg (po)

Group 4: Streptozotocin (60mg/kg) and Nicotinamide 120mg/kg (po) rats treated with *in-vivo* plant extracts 500mg/kg

Group 5: Streptozotocin (60mg/kg) and Nicotinamide 120mg/kg (po) rats treated with *in-vitro* plant extracts 500mg/kg

Diabetic rats given, glibenclamide and plant extracts are daily using an intragastric tube for 28 days. The fasting animal body weight, blood glucose level was estimated on 1st, 7th, 14th, 21st and 28th day and estimation of lipid parameters of TC and TG were recorded.

Measurement of body weight

Body weight of the rats was measured in grams on a mechanical balance on 1st weeks, 2nd weeks, 3rd weeks and 4th weeks.

Estimation of blood glucose

Blood sample were collected from tip of rat tail vein and glucose levels were estimated using a glucose oxidase-peroxidase reactive strips and a glucometer (Accu-chek, Roche Diagnostics, USA).

Total cholesterol (TP)

CHOP-PAP: Enzymatic photometric test is following (Artiss and Zak, 1997)

Triglycerides (TG)

Colorimetric enzymatic test using glucerol-3-phosphate-oxidase (GPO) method is following (Trinder, 1969).

Statically analysis

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA), and the group's means were compared by Turkey multiple comparison test. Values were considered statistically significant if $p < 0.001$.

Results and discussion

Body weight in STZ induced diabetic

Body weight of streptozotocin (STZ) induced diabetic rats found to be significantly ($P < 0.05$) decreased when compared to control rats. Administration of *in vivo* and *in vitro* plant extracts of *C. gynandra* (500mg kg⁻¹) produced a significantly increase in body weight of Streptozotocin induced diabetic rats (Table 1). The initial body weight in the first week were recorded 151.5g in control, 162.7g in STZ administration rats, 162.7g in STZ with standard, 163.12g in STZ with *in*

in vivo shoot extracts and 165.8g in STZ with *in vitro* shoot extracts. After 4 weeks, the animal body weights were recorded 178g in control, 99.67g in

STZ administration rats, 165.7g in STZ with standard drug, 139.75g in STZ with *in vivo* shoot extracts and 124g in *in vitro* shoot extracts.

Table 1. Effect of plant extracts on body weight in STZ induced diabetic.

Treatment	Body Weight (g)			
	1 st Weeks	2 nd Weeks	3 rd Weeks	4 th Weeks
Control	151.5±1.78	158.3±2.09	173.3±3.63	178±2.68
Streptozotocin (60mg/kg)	162.7±1.33	96±3.05	107.3±3.40	99.67±3.17
Streptozotocin (60mg/kg) + Glibenclamide 20mg/kg	162.7±1.97	148.3±3.6	159±3.60	165.7±2.09
Streptozotocin (60mg/kg) + <i>in vivo</i> shoot extracts 500mg/kg	163.12±1.67	105.5±1.55	118±5.42	139.75±3.43
Streptozotocin (60mg/kg) + <i>in vitro</i> shoot extracts 500mg/kg	165.8±1.64	109.7±3.51	125±3.35	144±2.49

Values are expressed as the mean ± S.D. Statistical significance (p) calculated by one way ANOVA followed by dunnett's. ns- not significant **P < 0.05 calculated by comparing treated group with control group.

Control and each treatment were significantly changed due to the growth of rats. The STZ induced diabetic rats body weight reduced for the diagnostic effect. Further administration of drugs and plant extracts were affect the body weight positively due to the diabetic condition.

Compared to the glibenclamide standard drug, the *in vitro* plant extracts of *C. gynandra* were best reacted against body weight reduction. The drug consumption of diabetic induced rats' body weight shows successful treatment. The treatment depends upon the concentration of plant extracts and duration. After 4th week of treatment with *in vitro* shoots of *C. gynandra*, body weight had significantly increased (144g) when compared to *in vivo* shoots (139.75g).

This study was carried out to investigate the *in vivo* antidiabetic effect of the ethanolic shoot extracts of *in vivo* and *in vitro* shoot extracts of *C. gynandra* in STZ induced diabetic rats. Streptozotocin destroys and reduces the β -cells via information or reactive oxygen species like nitric oxide. The STZ induced diabetic rats had 3 to 4 times increase in blood glucose levels compared to normal control group. Body weight is indirectly related with disease recovery and health status (Gautam *et al.*, 2020). Both oral and intraperitoneal administration of the ethanolic extract of the studied plant showed hypoglycemic activity in a dose independent manner (Arika *et al.*, 2015).

Blood glucose in STZ induced diabetic

Anti-diabetic activity of ethanolic extracts of *in vivo* and *in vitro* shoots of *C. gynandra* was studied Streptozotocin-induced diabetic wistar albino rats. Strepto zotocin (60mg/kg) was used as reference standard. The blood sugar level was observed on 3rd, 7th, 14th, 21st and 28th days. In Streptozotocin induced diabetic wistar albino rats was significant increase in the mean blood sugar level on 3rd day.

Blood glucose level of diabetic wistar albino rates started decreasing from the first week of drug treatment that was continued to maintain till the end of study, which was comparable to standard drug (Glibenclamide 20mg/kg). The ethanolic extracts of *in vivo* and *in vitro* shoots, *in vitro* shoots showed better reduction in blood glucose level (93.33mg/dl) than *in vivo* (107.5mg/dl) shoots on 28th day (Table 2). All the treated animals showed blood glucose level near to the normal control animals (89.17mg/dl).

The reduction levels of Blood Glucose in the diabetic rats by glibenclamide were observed. In this study portrays an in severe state of diabetes. The continuous treatment with plant extracts for a period of 2 weeks caused significant decrease in BGL of treated rats compared to untreated diabetic rats. Diabetes is characterized by a severe loss in body weight due to loss or degradation of structural proteins (Seedevi *et al.*, 2020). This condition was alleviated by the treatment of the diabetic rats with shoot extracts of *in vivo* and *in vitro* *C. gynandra* as the treated rats were healthy and agile at the end of the study (Alema *et al.*, 2020).

Table 2. Effect of plant extracts on blood glucose level in STZ induced diabetic rats.

Group /Treatment	Control	Only STZ	STZ +STD	STZ + <i>in vivo</i> shoot extracts 500mg/kg	STZ + <i>in vitro</i> shoot extracts 500mg/kg
Initial blood glucose level	89.17± 4.54	88.33± 3.80	89.67± 3.43	90.83± 3.00	89.17± 3.00
3 rd day B.G.L.	85.83± 2.00	496.7± 4.24***	420± 5.75***	433.3± 4.89***	450± 4.52***
7 th day B.G.L.	85.83± 2.00	420± 9.04**	393.3± 5.81*	341.7± 5.93*	406.7± 4.55**
14 th day B.G.L.	88.33± 3.07	316.7± 1.04	375± 2.94*	353.3± 8.68*	306.7± 6.54
21 st day B.G.L.	88.33± 3.07	258.3± 9.02	241.7± 1.95	236.7± 7.78	211.7± 5.07
28 th day B.G.L.	89.17± 3.27	256.7± 8.26*	95 ± 1.97	107.5± 3.46	93.33± 1.99

Values are expressed as the mean ± S.D. Statistical significance (p) calculated by one way ANOVA followed by dunnett's. ns- not significant **P< 0.05 calculated by comparing treated group with control group.

Lipid profile in STZ induced diabetic rats

The level of serum total cholesterol and triglycerides were increased in all the diabetic groups on 0 day. A significantly (P < 0.05) increased level of TC and TG were observed after STZ-NIC induced diabetic rats than normal control rats (Table 3). Treatment with plant extracts of *in vivo* and *in vitro* shoots of *C. gynandra* and glibenclamide significantly decreased the elevated level of total cholesterol and triglycerides. Administration of *in vitro* shoot extracts showed higher reduction in TC (64.9mg/dl) and TG (90.83mg/dl) levels than *in vivo* shoot extracts TC (73.4mg/dl) and TG (102.1mg/dl) and control rats TC (80.27mg/dl) and TG (117.2mg/dl).

Table 3. Effect of plant extracts on Lipid profile in STZ induced diabetic rats.

Group / Test	Total Cholesterol (mg dl ⁻¹)	Triglycerides (mg dl ⁻¹)
Control	80.27±4.82	117.2±1.15
Only STZ	86.57±3.38	133.1±6.83
STZ +Glibenclamide	66.73±2.63	79.63±2.93*
STZ + <i>in vivo</i> shoot extracts 500mg / kg	73.4±6.09	102.1±4.19
STZ + <i>in vitro</i> shoot extracts 500mg / kg	64.9±4.32	90.83±5.57

Values are expressed as the mean ± S.D. Statistical significance (p) calculated by one way ANOVA followed by dunnett's. ns- not significant **P< 0.05 calculated by comparing treated group with control group.

The result showed that these compounds cause decrease in the solubility of cholesterol and its absorption across the intestinal barrier (Calpe-Berdiel *et al.*, 2005). Several *in vivo* studies indicate the anti-diabetic activity of cardiac glycosides present in plants (Ardalani *et al.*, 2021). The prevention of diabetic complications as well as the improvement of lipid metabolism could be induced by the

improvement of the lipid profile in diabetic animals treated with extracts (Cho *et al.*, 2002). This lipid lowering action may be due to proper stabilization of glucose level and increase in insulin level after the administration of all the treatments which may normalize the disturbed lipid metabolism in diabetic rats (Ramachandran *et al.*, 2012).

Conclusion

This study showed that these plants have hypoglycaemic effects and can be used to treat various types of secondary complications of diabetes mellitus. Various secondary metabolites and phytochemicals isolated from diverse plant species have been found to have potent antihyperglycemic and glucose suppressive effects (Rahman *et al.*, 2021). Most of the medicinal plants have been a good source of medicine for the treatment of various type of disease, still many plants and active compounds obtained from plants have not been well characterized. Evaluation of antidiabetic activity of *in vivo* and *in vitro* shoots of *C. gynandra* ethanolic extract was carried out in STZ induced diabetic rats. The plant extract which showed moderate toxicity was observed to demonstrate significant antidiabetic activity in STZ diabetic rats. The *in vitro* shoots of *C. gynandra* exhibited significant reduction of fasting glucose level as compared to the other groups. Hence the hypoglycemic activity of *in vitro* shoots of *C. gynandra* may be due to the enhancement of secondary metabolites. In conclusion, the demonstrated antioxidant and anti-lipid peroxidation effects of the extract of *C. gynandra* shoots may be the rationale behind some of its folkloric uses and also may be responsible for some of its pharmacological effects. This study justifies its use in ethnomedical medicine for the treatment of diabetes.

All the plants used in this study are of Indian origin and are well known by herbal pharmacologists for their medicinal properties. This study provides scientific evidence of their anti-diabetic effect. These plants are very affordable to the common man and can be easily incorporated in their daily diets. They can be further analyzed to develop anti-diabetic drugs free from harmful side effects. Moreover, further studies on the use of organic extracts will be necessary as it would also reveal classes of organic secondary metabolites in this plant that may be hypoglycemic.

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