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Research Article

EVALUATION OF PRELIMINARY PHYTOCHEMICAL PROPERTIES AND HYPOGLYCEMIC ACTIVITY OF *CLEOME GYNANDRA* L

KARIMULLA SHAIK[a*], AFSAR SHAIK[a], DEEPAK KUMAR[b], DEVI KADIRVEL[a]

[a]Department of Pharmacology, [b]Department of Pharmacognosy, Gokula Krishna College of Pharmacy, Sullurpet - 524121, Nellore Dist, Andhra Pradesh, India. Email: karim.cro@gmail.com

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ABSTRACT

The objective of the present study was to investigate the hypoglycemic effect of Cleome gynandra L Ethanolic leaf extract (EECG) on normal and non insulin dependent diabetes mellitus in rats. The study was further carried out to investigate the effect of extract on blood glucose and hepatic enzyme level. Diabetes was induced by intraperitonial injection of Alloxon (150 mg/kg, b.wt). EECG was administered at 100 and 200 mg/kg, b.wt orally for 14 days. The blood glucose was estimated on 0, 7 and 15 days of drug treatment. Glibenclamide (0.4 mg/kg, b.wt) served as positive control. Other parameters such as serum cholesterol, triglycerides, HDL, LDL, VLDL levels were also estimated. Biochemical observations were supplemented with histopathological examination. Administration of 200 mg/kg of EECG significantly ($p<0.01$) reduced the amount of blood glucose (118.24±4.67 mg dL) when compared to diabetic control (289.42±5.23 mg dL). An increase in HDL and reduction in Triglycerides, Total cholesterol, LDL and VLDL of EECG treated groups against diabetic control evidenced its hypolipoproteinemic activity. The results revealed the beneficial role of EECG as a potential hypoglycemic gent against Alloxon induced diabetes mellitus in rats.

Keywords: Hypoglycemic effect, Glibenclamide, *Cleome gynandra* L, Alloxon.

INTRODUCTION

Diabetes mellitus (DM) is the most common endocrine disorder. It affects more than 100 million people worldwide and its incidence is increasing steadily with changes in life styles [1]. It is not a single disease entity, but rather a group of metabolic disorders sharing the common underlying feature of hyperglycemia. Hyperglycemia result from an absolute deficiency of insulin caused by pancreatic β-cell destruction or by a combination of peripheral resistance to insulin action and an inadequate secretary response by the pancreatic β-cells [2]. Insulin has proved to be effective to some extent in increasing the life expectancy of diabetic patients, but is not a permanent solution science there are many drawbacks of this therapy. Also the therapy with oral hypoglycemic agents is not satisfactory. Thus, the search for a new therapeutical agent devoid of adverse effect originating from plants used in traditional medicine would be of interest.

Plants have been used in traditional medicine since ancient times for the treatment of various diseases of man and animals. However, still a large number of local herbs claimed to be useful in the treatment of many diseases including diabetes have not been screened in addition increased awareness to the unwanted effects of allopathic drugs has encouraged people to look alternative drugs [3].

One such ethno botanically important plant, *Cleome gynandra* (Cleomaceae), a plant drug of traditional systems of medicine in India i.e., Ayurveda and siddha is used for the treatment of diabetes mellitus. In the present study the authors made an effort to pharmacologically evaluate the plant for its anti-diabetic property.

MATERIALS AND METHODS

Collection and authentication of plant material

The leaf of *Cleome gynandra* L was collected from the town venkatagiri, Nellore (Dt), A.P, India. The plant material was identified and authenticated by Prof. Dr. A. Srinivasulu, Department of botany, Government Degree College, Naidupeta, SPSR Nellore Dt, AP.

Experimental animals

Inbred adult Wistar albino rats (150-280 g) of either sex were obtained from Sri Venkateswara Enterprises, Bangalore. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet fed and tap water was provided *ad libitum* throughout experimentation period. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments.

Preparation of ethanolic extract

About 2000 g of the powdered material was subjected to soxhlation and exhaustively extracted with 80% ethanol for 48 h. The solvent was distilled off at low temperature under reduced pressure using rotary flash evaporator. The semisolid mass obtained was dried in an oven at 400C, powdered, labeled as EECG (Ethanolic extract of *Cleome gynandra*) and stored in desiccator.

Preliminary Phytochemical Analysis: [4]

The EECG is subjected to different chemical tests separately for the identification of various active constituents. The results were recorded in Table 1.

Toxicological evaluation: [5]

Determination of LD50 value by acute oral toxicity study in rats

In the present study, LD_{50} value of the EECG was determined by the 'Acute oral Toxicity Test'**.** The procedure was followed by using OECD-423 (Acute Toxic Class Method) (OECD Guide lines 423, 1996). The acute toxic class method is a step wise procedure with three animals of a single sex per step. Depending on the mortality or moribund status of the animals and the average two to three steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use number of animals while allowing for acceptable data based scientific conclusion. The method used to defined doses (5, 50, 500, 2000 mg/kg body weight) the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemicals which cause acute toxicity.

Pharmacological studies

Induction of diabetes mellitus in experimental animals [6]

Adult inbred male Wistar albino rats (32 numbers) of either sex were overnight fasted and received a freshly prepared solution of alloxan, [S.d.fine chemicals Ltd], (150 mg/kg) in distilled water injected intraperitonially in a volume of 1 ml/kg .After injection the animals had free access to food and water and were given 5% glucose in their drinking water for the first 24 hours to counter any initial hypoglycemia. Normal rats (6 numbers) received 1ml 1% Sodium carboxymethyl cellose (SCMC) as vehicle. The development of diabetes was confirmed after 48 hours of the alloxan injection. The animals with fasting blood glucose level more than 200 mg/dl were selected for the experimentation. Out of 32 animals subjected

for diabetes induction, 6 animals died before grouping and two animals were omitted from the study, because of sub diabetic condition (118mg/dl) and (122mg/dl). Of the remaining 24 animals, 4 groups of 6 animals were formed and used for the experimentation. In the present study, glibenclamide (0.4 mg/kg body weight) was used as the standard drug.

Determination of the blood glucose levels

Blood was collected from tip of the tail vein and fasting blood glucose level was measured using single touch glucometer (One Touch Ltd) based on glucose oxidase method.

Effect of EECG on normoglycemic and glucose fed-hyperglycemic rats [NG-OGTT] [7]

A combined methodology is preferred for the activity assessment of extract in order to avoid wasting animals; there are some modifications incorporated in the time pattern for blood glucose level determination. After overnight fasting (16 h) the blood glucose level of rats were determined and then were given the test samples and standard.

The animals were divided in to four groups of 6 rats in each.

Group I - Animals received 1% Sodium Carboxy methyl cellulose (SCMC)

Group II - Animals received glibenclamide 0.4mg/kg b.w/p.o.

Group III - Animals received EECG 100mg/kg b.w/ p.o.

Group IV - Animals received EECG 200mg/kg b.w/ p.o.

Test samples and standard were given immediately after the collection of initial blood samples. The blood glucose levels were determined in the following pattern: 30 and 60 min to access the effect of test samples on normoglycemic animals. The rats were then loaded orally with 2g/kg glucose and the glucose concentrations were determined at 60, 90 and 210 min after glucose load.

Biochemical estimations

At the end of the study, all the animals were sacrificed under light ether anesthesia. The rats were sacrificed by decapitation and blood was collected by bleeding of carotid artery and serum was separated to study the biochemical parameters like serum protein, total cholesterol, triglycerides, HDL, LDL, VLDL and creatinine. All analyses were carried out using Flexor junior Automated Clinical Chemistry Analyser.

Histopathological changes

The relevant organ like pancreas was removed dissected out and washed with ice-cold saline. The pancreatic tissues were preserved in 10% Formalin solution for histopathological studies.

Statistical analysis

Data was analysed statistically using graph pad prism 0.5 Version as Mean ± standard error using one way analysis of variance (ANNOVA) followed by Dunnett test.

RESULTS

Preliminary phytochemical analysis of ethanolic extract of leaf of *Cleome gynandra* L.

The result of preliminary phytochemical analysis of leaf extract of *Cleome gynandra* L is shown in Table 1.

Table 1: Phytochemical Screening of Ethanolic extract of leaf of *Cleome gynandra* L

S. No.	Constituents	Ethanolic Extract
1.	Alkaloids	Absent
2.	Carbohydrates	Present
3.	Protein	Absent
4.	Steroids	Present
5.	Phenols	Absent
6.	Tannins	Present
7.	Flavanoids	Present
8.	Gums and Mucilage	Absent
9.	Glycosides	Present
10.	Sterols	Present
11.	Saponins	Present
12.	Terpenes	Present

Acute oral toxicity study

The acute oral toxicity study was done according to OECD 423 guide lines (Acute toxicity class method). A single administration of a starting dose of 2000 mg/kg bw/p.o, of EECG was administered to 3 female rats and observed for 14 days. There was no considerable change in body weight before and after treatment of the experiment and no signs of toxicity were observed. The results are shown in Table 2.

Table 2: Sign of toxicity, mortality and mean body weight results of acute oral toxicity observations of EECS in rats

Treated group	Dose	Sign of toxicity (ST/NB)[a]	Mortality (D/S)[a]	Mean body weight (g)[a]		
				Day 0	Day 7	Day 14
Group-I: vehicle	10ml/kg b.w	0/3	0/3	218 ± 2.6	230 ±4.3	276 ± 2.6
Group-II: EECG	2000 mg/kg b.w	0/3	0/3	220 ± 2.2	232 ± 3.6	278 ± 3.2

[a] Values are expressed as animal numbers; **ST**, Sign of toxicity; **NB,** Normal behavior; **D,** Died; **S,** Survived

Effect of EECG on blood glucose levels in normoglycaemic and glucose induced hyperglycemic rats. [NG-OGTT]

The EECG at a dose level 100mg/kg b.w/p.o did not exhibit significant hypoglycemic effect in fasted normal rats after 30 minutes of adminstration and a high dose of 200mg/kg b.w/p.o reduced blood glucose in normal rats significantly after 60 min of drug administration ($p<0.01$). In the same group of rats which are loaded with glucose (2gm/kg b.w/p.o) after 60 min of drug administration a low dose of 100mg/kg bw reduced blood glucose level with less significance ($p<0.05$) but a high dose of 200mg/kg/b.w reduced blood glucose significantly ($p<0.01$) .The standard drug glibenclamide (0.4 mg/kg b.w/p.o) treatment showed significant reduction in blood glucose levels in both normal and glucose induced hyperglycemic rats ($p<0.01$). Results are shown in Table 3.

Table 3: Effect of EECG extract of whole plant on blood glucose in alloxan induced diabetic rats [NG-OGTT]

Groups	Test Sample (mg/kg)	Blood glucose levels (mg/dl)					
		0 min	30 min	60min (glucose load)	120min	150min	270min
I	Control (1% SCMC)	75.38±1.8	80.2±2.3	75.9±0.8	127.18±0.51	100.23±0.55	78.23±0.24
II	Std-0.4	73.11±2.7	50.9±1.7**	41.88±0.6**	90.8±0.3**	71.5±0.52**	55.8±0.52**
III	EECG-100	65.5±1.2	70.2±2.3ns	61.85±0.71*	122.28±0.5ns	83.01±0.45*	62.46±0.65*
IV	EECG-200	74.7±1.9	72.01±0.8ns	59.03±0.27**	110.15±0.5*	75.95±0.5**	60.6±0.52**

The values are expressed as mean ± SEM. Statistical significance test was done by ANOVA followed by Dunnet's test. The blood glucose values of group II, III and IV are compared with control animal values. *-p< 0.05, **-p< 0.01, ns-non significant.

Effect of sub-acute treatment of EECG on body weight changes in Alloxan induced diabetic rats

The EECG at oral dose level of 100mg/kg do not show significant improvement in the body weight of Alloxan induced diabetic rats on 10th day of the treatment and shows a slight significance in the body weight improvement on 15th day ($p<0.05$). An oral dose of 200mg/kg b.w shows significant improvement in the body weight of Alloxan induced diabetic rats on 10th day and 15th day of treatment ($p<0.01$). The standard drug glibenclamide (0.4 mg/kg b.w /p.o) also produced significant improvement in body weight of Alloxan induced diabetic rats.($p<0.01$) .Results are shown in Table 4.

Effect of sub-acute treatment of EECG on blood glucose level in Alloxan induced diabetic rats

In the sub-acute study, Alloxan induced diabetic rats were treated with EECG 100mg and 200 mg/kg b w.t /p.o for a duration of 14 days. Treatment with EECG 100mg significantly ($p<0.01$) decreased the blood glucose level after 14th day onwards. Treatment with EECG 500mg produced a significant ($p<0.01$) drop in blood glucose level after 10th day onwards. Treatment with glibenclamide 0.4 mg/kg produced a significant ($p<0.01$) decrease in blood glucose level after 10th onwards and thereafter. Results are shown in Table 5.

Table 4: Effect of sub-acute treatment of EECG on body weight changes in alloxan induced diabetic rats

Group	Treatment	Dose (Kg^{-1} b.w)	Body weight (gm)		
			0th Day	10th Day	15th Day
I	Control(1% Sodium Carboxymethyl cellose (SCMC))	2 ml	192.45±1.24	196.92 ± 1.2	201.5 ±1.5
II	Disease control(Alloxan)	150mg	210.24±0.99	167.89±1.4**	153.5±0.7**
III	Standard(Glibenclamide+Alloxan)	0.4mg	183.13±2.64	185.47±3.2*	187.9±1.5**
IV	Test I (EECG+ Alloxan)	100mg	197.62 ± 4.3	166.6±5.4ns	173.2± 4.2*
V	Test II (EECG+Alloxan)	200mg	189.92 ± 1.7	175.6 ±1.3*	187.6±4.1**

The values are expressed as mean ± SEM. Statistical significance test was done by ANOVA followed by Dunnet's test.The body weights of group II, III and IV are compared with control.*-p< 0.05, **-p<0.001, ns-non significant.

Table 5: Effect of sub-acute treatment of EECG on blood glucose in Alloxan induced diabetic rats

Group	Treatment	Dose (Kg - 1 Body Weight)	Blood Glucose (mg/dl)		
			0th Day	10th Day	15th Day
I	Control (1% SCMC)	2 ml	76.27±1.27	79.27±1.93	82.35±9.7
II	Disease control (Alloxan)	150 mg	246.51±5.3	266.07±5.3**	289.42±5.23**
III	Standard (Glibenclamide+Alloxan)	0.4mg	231.12±4.8	168.65±4.2**	108.74±2.51**
IV	Test I (EECG+Alloxan)	100 mg	233±3.4	179.69±3.06*	132.67±4.1**
V	Test II (EECG+Alloxan)	200mg	229.97±3.2	169.89±4.11**	118.24±4.67**

The values are expressed as mean ± SEM. Statistical significance test for comparision was done by ANOVA, followed by Dunnet's test.a-Group II is compared with Group I .b-groups III, IV, V are compared with group. **P<0.01, *P<0.05

Effect of sub-acute treatment of EECG on Biochemical parameters in Alloxan induced diabetic rats

In biochemical parametric evaluation the standards, and EECG (100mg and 200mg/kg b.wt) treated diabetic rats showed significant decrease in Total cholesterol, Triglycerides, LDl-cholesterol, VLDL-cholesterol and creatinine levels and significant increase in total proteins and HDL-cholesterol levels in contrast to that of those levels in diabetic control rats the results are shown in Table 6.

Histopathological changes

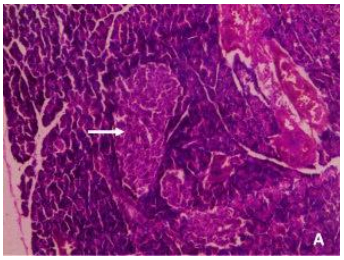
The effect of EECG on histopathological is as shown in the Table 7 and Figure A to E.

Table 6: Effect of sub-acute treatment of EECG on Biochemical parameters

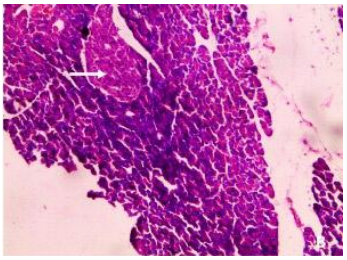
Groups	Treatment	Dose (kg^{-1} b.w	Serum total protein (mg/dL)	Total Cholesterol (mg/dL)	HDL-Cholesterol (mg/dL)	Triglycerides (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	Creatinine (mg/dL)
I	Control (1% SCMC)	2 ml	6.5593± 0.1556	116.505± 3.8146	41.06± 2.87	142.33± 3.45	48.00± 4.58	28.47± 0.63	0.72± 0.061
II	Diabetic control(Alloxan)	150 mg	3.6388± 0.0916**	167.43± 3.0334**	27.61± 1.69**	188.36± 3.46**	102.18± 4.99**	37.51± 0.97**	1.72± 0.05**
III	Standard (Glibenclamide + Alloxan)	0.4mg	6.038± 0.2600**	121.422± 3.119**	37.00± 2.45*	151.85± 3.02**	55.56± 4.56**	32.39± 0.90*	1.23± 0.03**
IV	Test I (EECG +Alloxan)	100mg	5.1215± 0.5387*	127.147± 3.81**	34.3± 3.14*	168.1± 3.44*	83.75± 3.15*	33.87± 0.70*	1.36± 0.02*
V	Test II (EECG + Alloxan)	200mg	5.2698± 0.7055**	141.77± 4.397**	39.60± 2.47**	150.8± 3.21**	55.46± 3.08**	32.56± 0.65*	1.28± 0.04*

Table 7: Histopathological analysis of pancreas (200x)

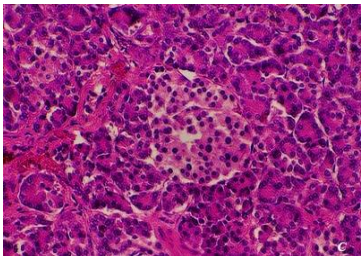
Figure No.	Report
A Normal control	H & E stained section shows pancreas with normal islets and acinar cells.
B Diabetic control	H & E stained section shows damaged and atrophic islet with acni.
C Glibenclamide treated	H & E stained section shows preserved pancreatic islet cells.
D EECG 100 mg/kg body weight	H & E stained section shows small islet cells
E EECG 200 mg/kg body weight	H & E stained section shows hyperplastic islet with acni



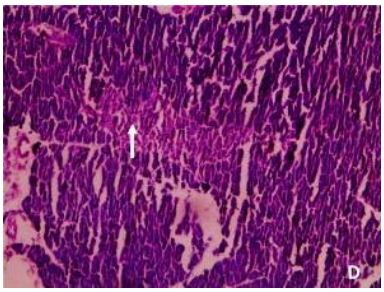
A: Normal Control (200x)



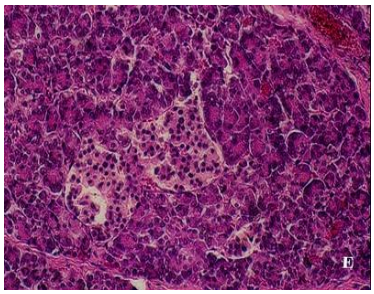
B: Diabetic Control (200x)



C: Glibenclamide treated (200x)



D: EECG 100 mg treated (200x)



E: EECG 200mg treated (200x)

DISCUSSION

Plants have been used as source of drugs for the treatment of diabetes mellitus in developing countries where the cost of conventional medicines represents a burden to the population. Many species have been reported to present antidiabetic activity [8].Working on the same line, we have undertaken a study on *Cleome gynandra* L for its antidiabetic property. Preliminary phytochemical analysis of the EECG showed that the plant has a rich possession of phytochemicals like flavonoids and steroids. Acute oral toxicity studies reveal that EECG did not produce any mortality or signs of toxicity at the dose of 2000 mg/kg b.w. p.o, in experimental rats. The EECG at doses 100 and 200 mg/kg bw.po.did not significantly suppress blood glucose levels in overnight fasted normoglycaemic animals but showed significant improvement in glucose tolerance in glucose fed hyperglycemic normal rats. Such an effect may be accounted for, in part, by a decrease in rate of intestinal glucose absorption, achieved by an extra pancreatic action including stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic process [9].

Alloxan is the most commonly employed agent for the induction of experimental diabetic animal models of human insulin dependent diabetes mellitus [10].There is an increasing evidence that alloxan causes diabetes by rapid depletion of β-cells, by DNA alkylation and accumulation of cytotoxic free radicals that is suggested to result from initial islet inflammation, followed by infiltration of activated macrophages and lymphocyte in the inflammatory focus [11] In the sub-acute study, glibenclamide treatment brought down the sugar levels from the first day of the treatment. EECG 100 mg and 200mg treatment produces significant reduction in blood glucose levels from 10th of treatment and a steady decrease was observed thereafter. Histopathological studies that showed prominent islets cell hyperplasia and regeneration of islet cell show a proof for the possible antidiabetic property of the leaf extract of *Cleome gynandra* L.

Lipids play an important role in the pathogenesis of diabetes mellitus. The level of serum lipids is usually raised in diabetic condition and such an elevation poses to be a risk factor for cardiovascular diseases like coronary heart disease and two to four fold risk for.

Atherosclerosis which constitutes the main cause of morbidity and mortality in diabetes mellitus [12]. In the present study an elevated serum total cholesterol and reduced HDL-cholesterol, triglycerides, LDL-cholesterol, VLDL-choleseterol, creatinine was observed in Alloxan-induced diabetic rats. The glibenclamide treatment, EECG 100mg and EECG 200 mg treatment in diabetic animals produced beneficial improvement in the lipid profile.

CONCLUSION

Based on the obtained results and observations, we can infer that the leaf of *Cleome gynandra* L could be used for the supportive treatment of diabetes mellitus, as the plant also offers effective protection against free radicals that form the basis for the development of diabetic complications Further studies are required to establish the anti-hyperglycemic activity of *Cleome gynandra* L in terms of molecular mechanism(s) involved in the activity.

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