Research Article

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ASSESSMENT OF ANTIDIABETIC ACTIVITY OF ETHANOL EXTRACT OF *GREWIA FLAVESCENS* JUSS LEAVES AGAINST ALLOXAN INDUCED DIABETES IN RATS

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ABSTRACT

The aim of the present study was to investigate the anti-diabetic and preliminary phytochemical properties of Grewia flavescens Juss. The anti diabetic potentials of ethanol extract of Grewia flavescens Juss (Tiliaceae) (EEGF) were investigated against alloxan induced diabetes I n rats. Decreased blood glucose levels of the test animals showed that the extract exhibit significant anti diabetic activity when compared to diabetic control group. The results also indicated the dose dependent effect. The anti-diabetic activity produced by the extract may be due to inhibition of intestinal absorption of glucose. The present study supports the use of this herbal drug as anti-diabetic.

Keywords: Antidiabetic, Grewia flavescens, Ethanol extract, Glibenclamide, Alloxan.

1. INTRODUCTION:

Diabetes mellitus is a chronic endocrine disorder characterized by metabolic derangements of carbohydrate, fat and protein, there by developing complications such as nephropathy, retinopathy, neuropathy and cardiomyopathy over a period of time. Traditional medicines derived mainly from plants play major role in the management of diabetes mellitus[1]. In recent past, many medicinal plants possessing experimental and clinical antidiabetic activity has been used in traditional systems of medicine [2]. Ayurvedic literatures like Charak Samhita and Sushruta Samhita, have reported the use of the some of the plant grains for the management of diabetes mellitus[3]. On the otherhand, many synthetic hypoglycemic agents were introduced for maintenance of type 2 diabetes. Yet, the diabetes and the related complications continued to be major medical problem all over the world. The prevalence of diabetes mellitus is estimated to be more than 300 million by 2025. The disease is a major degenerative ailment in the world today, affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders [4].The WHO has recommended the evaluation of traditional plant treatments for management of diabetes as they are effective, less toxic with minimum or no side effects and are considered to be excellent candidates for oral therapy [5].

Grewia flavescens (Tiliaceae) popularly known as "donkeys berry", is a shrub or small tree, often seen in groups along the edges of roads, river banks and dry rivers, growing in large uniform groups. The plant parts are being used in Indian folk medicine. The leaves were reported to be useful in ulcerated tongue, colic pain, wounds, cholera and dysentery. *Grewia flavescens*is a multi-stemmed shrub or small tree, up to 5 m high. Its bark is dark grey-brown belongs to Tiliaceae family. The plant is used as Anthelmentic, CNS depressant [6], anti-inflammatory, antimalarial,

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antidiabetic and analgesic in Indian traditional system of medicine. The berries of *Grewia flavescens* are soaked in water for two or three days to make a refreshing drink [7].

However, the above plant is claimed to possess antidiabetic activity, but no scientific evidence is provided. Therefore, study of antidiabetic effect of *Grewiaflavescens*was undertaken to evaluate the potential of the activity against alloxan induced diabetis in rats.

2. MATERIALS AND METHODS

2.1. Plant Material

Grewia flavescens naturally occurs in bushveld, open woodland and thicket. It is frequently associated with termite mounds, rocky koppies, riverine fringes and the margins of forests. It is widespread at medium to low altitude, in temperate climates with moderate summer rainfall. Its distribution in southern India.Flowering: July-August.The plant was authenticated by Dr. Madhava Chetty, Taxonomist, S.V. University, Tirupathi, India.

2.2. Extraction

The fresh leaves were collected in the month of November from the surroundings of Vontimitta, Kadapa (District), India. The leaves were washed with tap water, shade dried for two weeks and pulverized, sieved (10/44) and stored in air-tight containers. About 1000 g of powdered drug was extracted with ethanol by using soxhlet apparatus until the phytoconstituents were completely exhausted. The ethanol extract was evaporated through rotary evaporator (Buchi type, Mumbai, India) under reduced pressure at 40°C.and labeled as EEGF. (5.4%)

2.3. Phytochemical investigation [8]

Phytochemical analysis of extract was carried out to find out the presence of phytoconstituents viz flavonoids, phytosterols, phenolics, carbohydrates, tannins, triterpenoids etc

2.4 Animals

Albino Wistar Rats of 8 to 10 weeks old weighing 200–250 g supplied by National Institute of Nutrition, Hyderabad, India, were individually housed in polypropylene cages lined with husk renewed every 24 h in well-ventilated rooms at 22±3°C and %RH between 50 to 60, under artificial lighting12:12 h light and dark cycle in hygienic condition for at least five days prior to the study. The rats were fed with standard laboratory pellet diet and water ad libitum. The commercial diet and water were provided.

3. ACUTE TOXICITY STUDIES

The acute oral toxicity test of the extract was determined according to OECD (Organisation for Economic Co-operation and Development) Guidelines 425.

4. EXPERIMENTATION

4.1 Diabetes induction:

Diabetes was induced by using Alloxan monohydrate (100mg/kg) is dissolved in chilled normal saline and given intraperitonially to a overnight fasted animals. The rats were kept for the next 24hrs on 10% of glucose solution to prevent hypoglycemia and death. After 48 hrs of inducing, fasting blood glucose levels were measured. The animals which didn't show blood glucose levels of more than 250mg/dl were rejected [9,10].

4.2. Oral Glucose Tolerance Test for EEGF

Wistar rats of either sex were divided into six groups with each group containing six animals. Group I animals served as normal and received glucose (2 g/kg), animals in group II, received standard drug of glibenclamide (2 mg/kg) with glucose (2 g/kg). Animals in group III were treated with test EEGF (100 mg/kg) and glucose (2 g/kg), group IV were treated with test EEGF (200 mg/kg) and glucose (2 g/kg), group V were treated with test EEGF (400 mg/kg) and glucose (2 g/kg).The animals were fasted overnight and treated with above dosage schedule orally. The EEGF and glibenclamide was administered half an hour before administration of glucose solution.

4.3. Antidiabetic activity on alloxan induced diabetic rats

About 36 animals were selected for the experiment out of which six animals were kept separately as normal (Group I). Remaining 30 animals were made diabetic by a single intraperitoneal injection of alloxan at dose of 100 mg/kg dissolved in citrate buffer (0.01 M, pH 4.5). The rats were provided with 5% glucose solution bottles in their cages for the next 24 h to prevent hypoglycaemia. The blood glucose levels were measured before and after 72 h of alloxan injection to confirm the development of diabetes.

The rats were divided into groups each consisting of six animals.

Group-I animals were served as control and received 1%w/v sodium CMC

Group-II diabetic induced animals were treated only with vehicle 1%w/v sodium CMC

Group-III diabetic induced animals were treated with glibenclamide at dose of 5 mg/kg

Group-IV diabetic induced animals were treated with EEGF 100 mg/kg,

Group-V diabetic induced animals were treated with EEGF 200 mg/kg.

Group-VI diabetic induced animals were treated with EEGF 400 mg/kg.

The study was conducted for the period of 21 days by dosing once daily.

4.4. Biochemical analysis

Blood samples were collected from the animals prior to the treatment with above schedule and after 30 min of glibenclamide /ethanol extracts administration on 7^{th}, 14^{th} and 21^{st} day. Blood was obtained from the retro orbital venous plexus of rats under ether anesthesia using a glass capillary tube and was centrifuged (2,500 rpm/10 min) to separate serum. The serum was used for biochemical analysis of blood glucose levels, TG, HDL cholesterol, LDL Cholesterol, SGOT, SGPT and ALP.

3.5. Statistical analysis

Data was expressed as mean ± SEM, (n=6). Statistical analysis was done using one-way analysisof variance (ANOVA) followed by Tukey's multiple comparison. Values were considered statistically significant when at $p<0.05$.

5. RESULTS and DISCUSSION

5.1. Phytochemical studies

As per the results of phytochemical study, EEGF have exhibited the presence of flavonoids, glycosides, phenolics, proteins, tannins, carbohydrates, saponins and phytosterols in appreciable amounts. The results are given in Table 1.

5.2. Effect on oral glucose tolerance

The blood glucose levels in the control group (Group I) were found to increase maximum levels within 30 min after glucose load and normal glucose levels were observed over a period of 150 min. In Group-III (glibenclamide treated group), GroupV(EEGF 400 mg/kg treated group) and the blood sugar levels returned to normal within 30 min. Group III and IV showed significant decrease in blood glucose levels at 90 min. Results in table 2& Fig 1, suggested that the EEGF have not decreased the blood glucose levels below normal levels.

5.3. Biochemical Analysis

Groups G-IV to G-VI (EEGF 100, 200 and 400 mg/kg) have shown a dose dependent decrease in the serum glucose levels on7th, 14th and 21st day. The EEGF at the dose of 400 mg/kg showed an efficient anti-diabetic activity and its efficacy was found to be on par with glibenclamide. The plausible mechanism behind the anti-diabetic potential of EEGF could be due to the presence of flavanoids, phytosterols and tannins which would have increased the activity of enzymes responsible for utilization of glucose by insulin-dependent pathway. Lowering of blood glucose level in alloxanised rats after administration of the extracts indicated that the extracts possessed extra-pancreatic effects or regeneration of β-cells in pancreatic islets[11-16]. Lipid profile experimental result of EEGF at 400 mg/kg have shown a significant decrease in TC, TG, SGOT, SGPT,ALP and the progressive decrease in lipid levels were observed over 21 days period of treatment which was in dose-

dependent manner and progressively increase in HDLC level was also noted during the study period. The devoid levels of lipids in serum could be due to the erratic affects of lipolytic compounds on adipose matter, majorly due to insulin. In general conditions, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. Hyper triglyceridemia and hypercholesterolemia caused due to inactivation of lipoprotein lipase in insulin deficiency [16, 17]. The altered serum lipid profile was returned to normal after treatment with ethanol extracts. Liver and muscle glycogen contents were increased significantly over 21 days of treatment of ethanol extracts in a dose-dependent manner. Results in table 3,4& Fig 2,3. From the results obtained EEGF has shown effective anti-diabetic activity against alloxan induced diabetic Wistar rats at the dose of 400 mg/kg. It could be due the presence of relatively more amounts of flavonoids, tannins and phytosterols.

In conclusion, EEGF has demonstrated a significant anti-diabetic potentials and which could be *via* restoration of the pancreatic functions, activation of the beta cells or decreased absorption of glucose. However, further studies are required to assess the antioxidant properties, isolation and characterization of the bioactive compounds/enzymes responsible for anti-diabetic activity and the establishment of the exact mechanism(s) of action.

Table 1: Phytochemical analysis of EEGF

Phytoconstituents	EEGF
Alkaloids	-ve
Flavanoids	+ ve
Phytosterols	+ ve
Phenolics	+ ve
Carbohydrates	+ ve
Tannins	+ ve
Triterpenoids	+ ve
Proteins	+ ve

+ve: positive; -ve: negative

Table 2: Effect of EEGF blood glucose levels

Group	Blood glucose levels mg/dl			
	Initial	30 min	90 min	150 min
I	93.5±4.4	94.22±3.5	94.5±2.9	91.5±1.8
II	299.8±8.2	255.5±5.6	170.6±6.6	119.8±4.5
III	298.9±6.3	271.5±8.4*	220.8±4.3**	195.1±6.6**
IV	299.4±5.6	265.8±6.4**	210.6±5.4*	180.2±5.7*
V	297.6±7.9	267.9±7.9*	198.7±6.6*	135.9±6.6*

All the values are shown as Mean±SEM,n=6, ***indicates p<0.001,**indicates p<0.01,*indicates p<0.1when compared to normal.

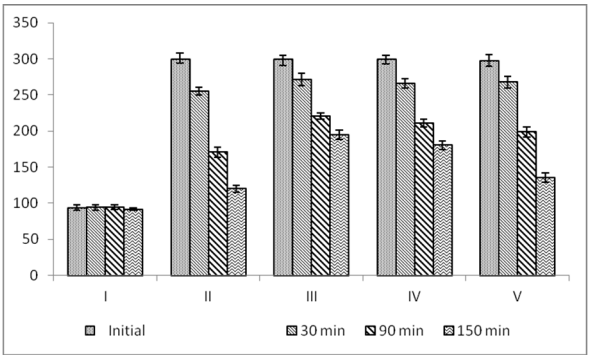

Fig 1: Effect of EEGF blood glucose levels

Table 3: Effect of EEGF on plasma lipid profile

Groups	TC	TG	HDL cholesterol	LDL cholesterol
I	58.97±2.82	64.24±2.14	60.01±2.95	92.26±3.36
II	104.5±1.84	124.25±2.48	88.6±3.21	199.61±3.25
III	53.62±2.34***	50.24±2.48***	48.65±2.27	72.86±2.12***
IV	57.24±2.13	58.68±2.37**	54.02±2.27	85.02±2.35*
V	55.91±2.73	53.2±1.81**	52.27±2.87	79.27±1.92*
VI	53.96±3.62***	48.23±2.88***	47.25±3.16***	73.89±2.25***

All the values are shown as Mean ± SEM, n=6, ***indicates p<0.001, **indicates p<0.01,*indicates p<0.1when compared to normal.

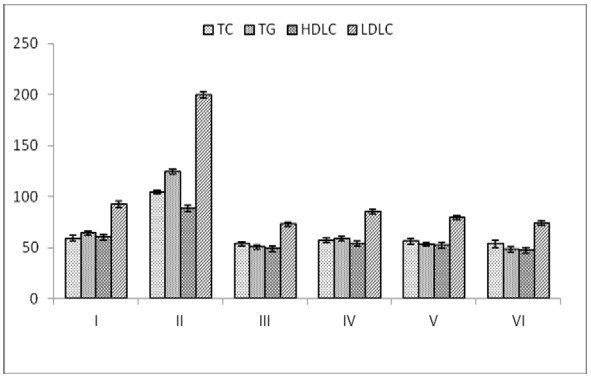

Fig 2: Effect of EEGF on plasma lipid profile

Table 4: Effect of EEGF on Biochemical parameters

Groups	SGOT	SGPT	ALP
I	151.26±6.25	52.17±2.71	143.23±5.31
II	192.7±4.58	76.7±3.36	310.25±6.25
III	145.25±7.9***	47.26±4.26***	133.36±4.97***
IV	152.91±6.23**	51.26±3.45	141.3±5.36*
V	150.27±7.47	50.35±2.21**	138.25±6.34**
VI	146.41±6.85**	46.25±3.24**	134.23±5.41***

All the values are shown as Mean±SEM,n=6, ***indicates p<0.001,**indicates p<0.01,*indicates p<0.1when compared to normal.

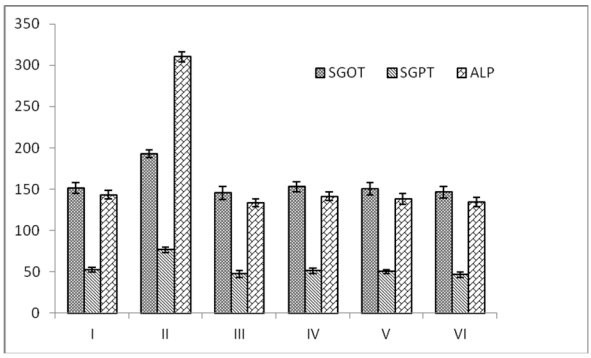

Fig 3: Effect of EEGF on Biochemical parameters

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