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ISSN: 0975-7538

Research Article

## Investigation of hypoglycemic and hypolipidemic activity of ethanolic extract of *Grewia serrulata* DC on high fat diet (HFD) - streptozotocin (STZ) induced diabetic rats

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### ABSTRACT

The present study was aimed to investigate the antihyperglycemic activity of ethanolic extract of aerial parts of *Grewia Serrulata* DC (EEGS) on high fat diet (HFD) - streptozotocin (STZ) induced diabetic rats. Male wistar albino rats were fed with a high fat diet for a period of two weeks prior to the administration of streptozotocin (50mg/kg) intraperitoneally. The long term effect of the extract was studied by treating diabetic rats with vehicle (0.5% CMC) and EEGS (200 and 400 mg/kg b wt) for a period of 28 days. The effects of EEGS on fasting blood glucose and serum insulin levels were studied. In addition, body weight changes, lipid profile and serum biomarkers of liver and kidney function were also studied. Results revealed that EEGS-400mg shows excellent antihyperglycemic activity when compared to lower dose of the extract 200mg and also shows better antihyperlipidemic activity. The elevated serum urea and creatinine levels also decreased by the treatment with extract. Hence the results justify that *Grewia serrulata* DC possess antidiabetic activity.

**Keywords:** *Grewia serrulata* DC; Anti-diabetic activity; Streptozotocin

### INTRODUCTION

Diabetes is a chronic metabolic disorder of carbohydrates, proteins and fats due to absolute or relative decrease in insulin resistance (Jarald E et al., 2008). It is an epidemic disease with a frequency of 5% around the world (Turben H et al., 2002). In India more than 30 million people are suffering with diabetes mellitus and the frequency is accelerating (Shankar P et al., 2001). As the incidence is alarming and due to adverse effects of synthetic medicine usage, there is a need for the development of indigenous, inexpensive botanical source of antidiabetic crude drug. (Venkatesh S et al., 2003). In ayurvedic system of medicine, plants specified for the treatment of diabetes mellitus have been tested on experimental animals. (Grover JK et al., 2002). One among such ethnomedicinal plants is *Grewia serrulata* DC.

*Grewia Serrulata* DC (Family: Tiliaceae) is a small tree with slender branches. It is a cuisine of the popular

edible fruit *phalsa* (Plant list 2011). Traditionally the root juice is taken as expectorant and wood part is applied for skin diseases. In ayurveda root juice is used for controlling bleeding and bronchitis. Latest common pharmacological findings indicate fruits are used as cardio tonic (Ripu M Kunvar et al., 2010). It is one of the medicinal plants for diabetic complications used in Pankaj Oudhia's Herbal Formulations (Pankaj oudhia 2011). Some of these ethno medical and reported biological activities may be due to the antioxidant nature of aerial parts of *Grewia serrulata* DC (Sarath Chandiran I et al., 2013). Hence in the present investigation ethanolic extract of aerial parts of *Grewia serrulata* DC (EEGS) was screened for antidiabetic activity in high fat diet (HFD) - streptozotocin (STZ) induced diabetic rats.

### MATERIALS AND METHODS

#### Plant material

The aerial parts of *Grewia Serrulata* DC were collected from Tirumala hills, Tirumala, Chittoor DT, A.P, India. The plant was identified and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, A.P, and India.

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Received on: 17-01-2014

Revised on: 24-02-2014

Accepted on: 25-02-2014

### Preparation of extract

After shade drying the aerial parts of *Grewia serrulata* DC were then blended in to fine powder with a blender and used for the preparation of ethanol extract. Ethanol extract was prepared by using Soxhlet extractor for 18-20 h. The extract obtained, was concentrated and dried under reduced pressure at controlled temperature (40-50 C) (Srinivasan R *et al.*, 2007).

### Drugs and chemicals

Streptozotocin (STZ) was purchased from Sigma Ulrich, USA and pioglitazone was a gift sample from Dr. Reddy's Laboratories. All the other chemicals were of analytical grade.

### Experimental animals

Male Wistar Albino rats (200-250 gm) were used in the study. Animals were housed individually in polypropylene cages in a ventilated room under ambient temperature of  $22 \pm 2$  C and 45-65 % relative humidity, with a 12 hour light followed by 12 hour dark. All the animals were acclimatized for at least 7days to the laboratory conditions prior to experimentation. Tap water and food pellets were provided ad libitum. Food pellets were with held overnight prior to dosing.

### Induction of diabetes mellitus

Wistar albino rats of male sex were fed with high fat diet (HFD) that consists of 20% fat, 46% carbohydrate and protein (w/w). Two weeks later HFD fed rats were administered streptozotocin at a dose of 50mg/kg b wt intraperitoneally and allowed to free access to food and water (Reed MJ *et al.*, 1999). Fasting blood glucose levels were measured 3days after STZ administration. The rats with fasting glucose  $\geq 200$ mg/dl were considered diabetic and selected for the study.

### Experimental design

Normal and HFD fed –STZ-diabetic rats were divided in to five groups of six in each. Group I: Normal rats treated with 0.05 %CMC (p.o). Group II: Diabetic rats treated with 0.05 % CMC (p.o). Group III: Diabetic rats administered with pioglitazone (2mg/kg b wt p.o). Group IV: Diabetic rats treated with EEGS 200mg/kg b wt p.o. Group V: Diabetic rats treated with EEGS 400mg/kg b wt p.o. The above dosage schedule should be given for 28 days. Blood glucose levels and body weights were monitored on day 1, 7, 14, 21 and 28.

### Estimation of biochemical parameters

At the end of the experimental period, rats were fasted overnight and blood was collected by cardiac puncture. The serum samples were analyzed for various biochemical parameters lipid profile and serum biomarkers of liver and kidney. The serum insulin was measured by ELISA kit. The rats were sacrificed by cervical dislocation and samples of pancreas, liver and kidney were collected immediately, stored in 10% formalin and send for histological assessment.

### Statistical analysis

The statistical analysis were carried out by one way ANOVA followed by Turkey's multiple comparison test for all groups using Graph Pad prism 5.0. The results were expressed as the mean  $\pm$  S.E.M. to show variations in a group. Differences are considered significant when  $p$  value  $< 0.05$ .

## RESULTS

### Effect of EEGS on body weights of rats

All the rats did not show any significant changes in their body weights and the results were not statistically significant at  $p < 0.05$  as illustrated in figure 1. Treatment with pioglitazone and extract did not improve the body weight of diabetic rats.

### Effect of EEGS on fasting blood glucose

The fasting blood glucose levels are in normal range in non diabetic rats until the end of experimental period and is significantly ( $p < 0.05$ ) high in untreated diabetic rats when compared to all other groups. Diabetic rats treated with EEGS 200 & 400 mg/kg b wt for 28 days period exhibited a significant decrease in fasting blood glucose on day 28 as compared to that of untreated diabetic rats. The results are depicted in table 1.

### Effect of EEGS on serum insulin

In the present study the insulin levels in diabetic rats are almost similar to that of normal rats. Diabetic rats treated with pioglitazone, EEGS 200 & 400 mg/kg showed decrease in serum insulin levels, while EEGS 400 mg treated rats exhibited significantly ( $p < 0.05$ ) reduced serum insulin levels similar to that of standard group. The details are shown in figure 2.

### Effect of EEGS on serum lipid parameters

Untreated diabetic rats showed significant hypercholesterolemia, hypertriglyceridemia, elevated LDL-C, VLDL-C and decrease in HDL –C as compared with normal control. Standard dose and test dose of EEGC 200 & 400mg showed significant results ( $p < 0.001$ ) for all lipid parameters when compared with diabetic group. EEGS at dose 200mg/kg dose not shown significant decrease in VLDL-C levels compared to that of diseased group as depicted in table 2.

### Effect of EEGS on serum biomarkers of liver and kidney

Serum biomarkers like SGOT and SGPT significantly increased in diseased rats compared to that of normal rats. There was no significant difference among the diabetic groups for SGPT and total proteins after the treatment with standard and test drugs. Extract of both doses 200 & 400 mg shows a very good significant decrease in creatinine ( $p < 0.001$ ) and urea ( $p < 0.05$ ) levels in diseased group compared to that of standard as shown in table 3.

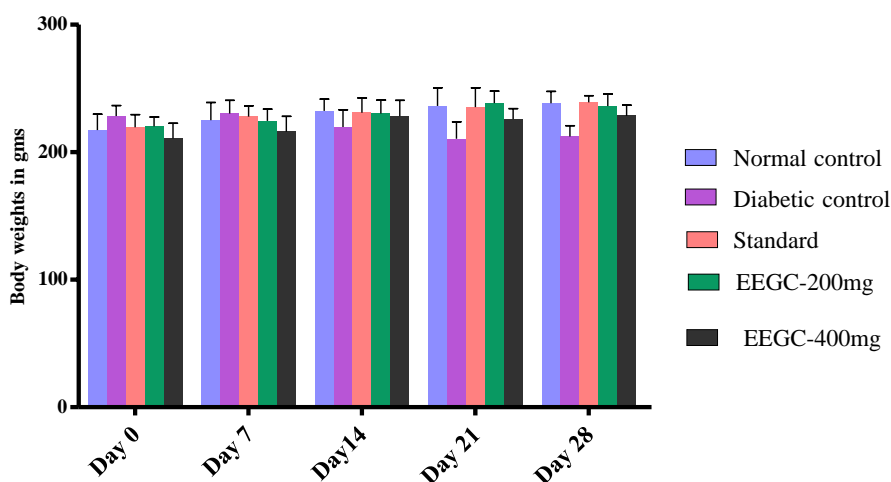


Figure 1: Effect of EEGS on body weight

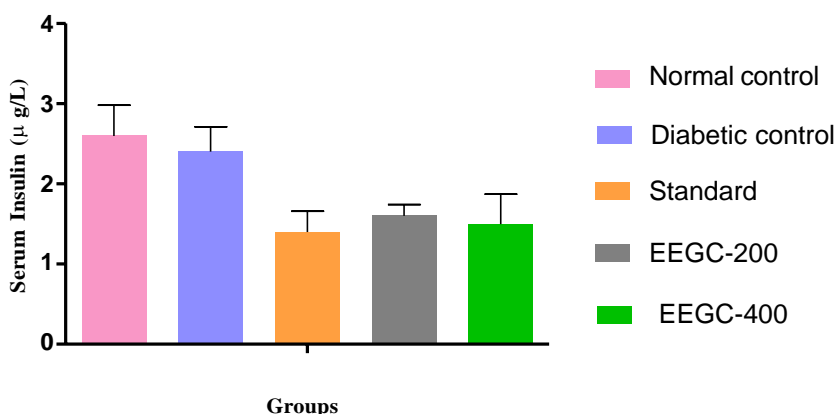


Figure 2: Effect of extract on serum insulin on day 28

Table 1: Effect of EEGS on fasting blood glucose

Group	Treatment (mg/kg)	Blood glucose (mg/dl)				
		Day 0	Day 7	Day 14	Day 21	Day 28
I	Normal	72.4±1.60	73.72±1.3	74.26±2.43	76.82±3.69	78.12±1.9
II	Diabetic control	262.25±2.83†	256.49±3.51†	252.34±6.29†	255.74±8.29†	260.20±2.6†
III	Pioglitazone-2	260.85±4.67	99.48±4.29***	89.72±3.67***	78.35±5.82***	70.11±3.21***
IV	EEGS-200	251.36±1.92	220.95±8.24**	102.62±4.13**8	96.4±2.54***	92.43±6.03***
V	EEGS-400	259.41±1.71	208.24±9.23***	98.23±2.82***	82.53±5.32***	76.22±4.52***

All values are expressed as mean ±SEM; †= p<0.001 compared to normal. \*= p<0.05 when compared to diabetic control.

**Effect of EEGS on organ histology**

All the organ tissues are magnified with 40X in figure 3.

**Pancreas**

In Normal group a normal cyto-architecture of pancreatic tissue was found under higher magnification (40X). In diseased group Pancreas tissue shows structural damages like vacuolization and necrosis where as in standard group regenerative changes takes place in pancreatic tissue and shows similar to normal cyto-

architecture. Similar regenerative changes were also found in pancreas of extract treated groups.

**Liver**

The normal architecture of the Liver tissue was found in normal rats. In Disease group degenerative changes in Liver tissue degage of Central Vein with congestion and necrotic changes were found. Regeneration of tissue in rats belonging to test groups of both doses of extract show similar to normal with central vein. In standard group liver tissue was regenerated and shows

**Table 2: Effect of EEGS on lipid profile**

Group	Treatment(mg/kg)	TGs (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
I	Normal	76.12±3.49	79.16±3.31	49.53±2.11	14.40±2.29	15.22±1.06
II	Diabetic control	105.43±4.32 <sup>†</sup>	106.24±4.62 <sup>†</sup>	32.18±1.96 <sup>†</sup>	52.97±1.96 <sup>†</sup>	21.08±1.02 <sup>†</sup>
III	Pioglitazone-2	83.62±3.16 <sup>**</sup>	83.51±3.10 <sup>**</sup>	46.75±2.83 <sup>**</sup>	20.03±3.06 <sup>***</sup>	16.72±1.04 <sup>*</sup>
IV	EEGS-200	89.43±4.01 <sup>*</sup>	87.84±4.03 <sup>*</sup>	42.45±2.74 <sup>*</sup>	27.50±2.84 <sup>***</sup>	17.89±0.13
V	EEGS-400	86.34±2.19 <sup>**</sup>	85.63±3.17 <sup>**</sup>	45.70±1.92 <sup>**</sup>	22.66±1.90 <sup>***</sup>	17.27±0.90 <sup>*</sup>

All values are expressed as mean ±SEM; <sup>†</sup>= p<0.001 compared to normal. <sup>\*</sup>= p<0.05 when compared to diabetic control.

**Table 3: Effect of EEGS on serum biomarkers of liver and kidney**

Group	Treatment(mg/kg)	SGOT (IU/L)	SGPT (IU/L)	Urea (mg/Dl)	Creatinine(mg/Dl)	Total protein (gm/Dl)
I	Normal	264.13±9.75	70.36±4.29	63.81±1.46	0.78±0.03	7.72±0.11
II	Diabetic control	286.17±14.23	78.23±8.42	76.38±2.21 <sup>†</sup>	0.97±0.04 <sup>†</sup>	7.02±0.03 <sup>†</sup>
III	Pioglitazone-2	292.38±16.52	80.36±7.03	67.20±3.18	0.76±0.02 <sup>**</sup>	6.68±0.12
IV	EEGS-200	186.14±12.56 <sup>***</sup>	60.30±4.68	68.20±1.20	0.63±0.04 <sup>***</sup>	6.79±0.19
V	EEGS-400	210.12±8.62 <sup>**</sup>	64.17±4.05	64.93±2.76 <sup>*</sup>	0.58±0.05 <sup>***</sup>	6.70±0.10

All values are expressed as mean ±SEM; <sup>†</sup>= p<0.001 compared to normal. <sup>\*</sup>= p<0.05 when compared to diabetic control.

normal cyto-architecture of central vein and other structures of tissue.

#### Kidney

In normal rats there is a normal architecture of the Kidney tissue and in disease control rats the degenerative changes in Kidney tissues shows congestion and structural damage. Standard and extract treated groups shows regeneration of tissue similar to normal cyto-architecture.

#### DISCUSSION

An immense reservoir of biologically active compounds with various chemical structures and disease preventive properties is the plant kingdom (Builders MI et al., 2012). Hence herbal drugs have received greater attention as an alternative to clinical therapy and the demand for these herbal remedies has greatly increased recently. Their utilization is often based in long term clinical experience. In the present study was aimed to investigate the antihyperglycemic activity of ethanolic extract of aerial parts of *Grewia Serrulata* DC (EEGS) on high fat diet (HFD)- streptozotocin (STZ) induced diabetic rats.

Body weights of all the animals were observed on day 1, 7, 14, 21 and 28 of the study period. All the rats including diabetic rats did not show any significant changes in the body weight. There was no mortality or signs of toxic reactions in animals and maintained their health status during the study period.

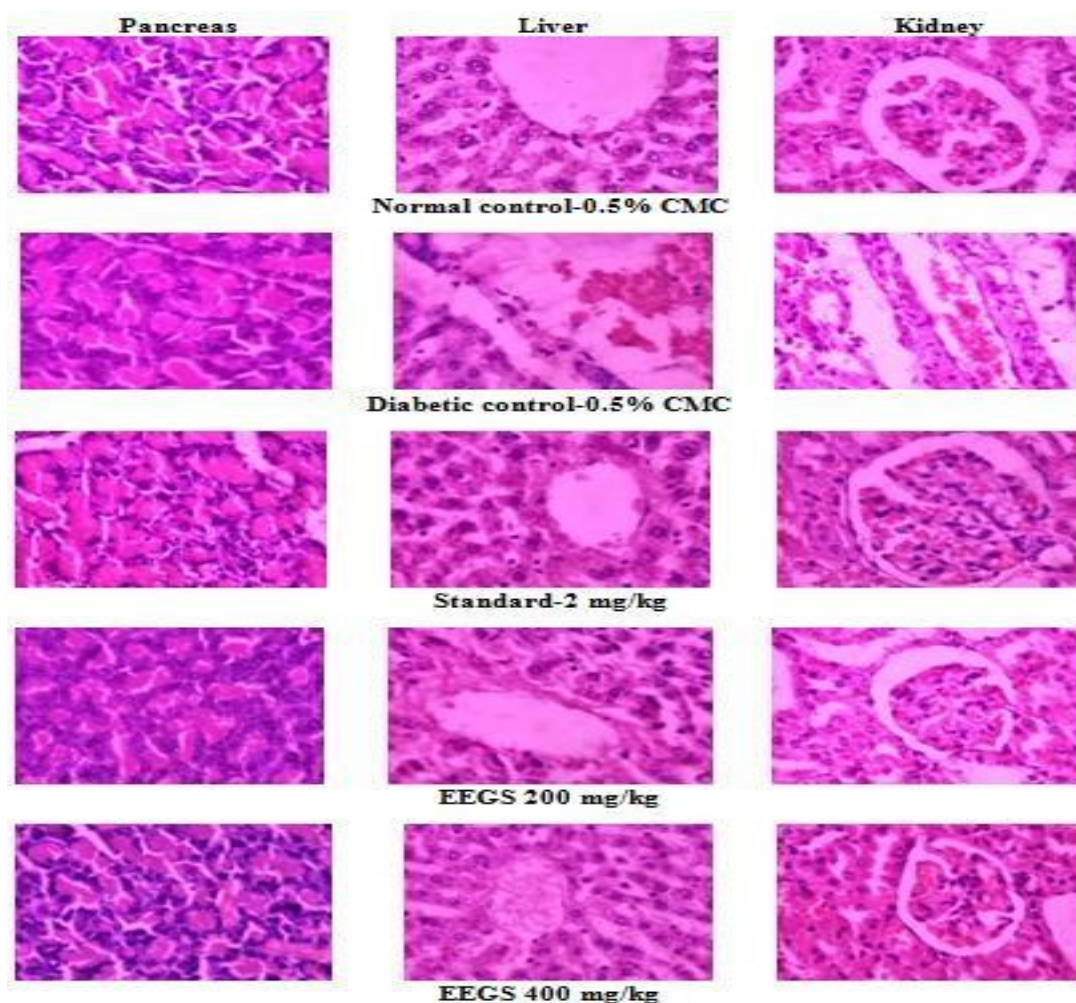
Number of plant have been reported for their hypoglycemic activity and the possible mechanism underlines could be an insulin secretion from  $\beta$ -cell of islets of langerhans or release of bounded insulin or their insu-

lin like actions (Twaij HA et al., 1998). Hypoglycemic effect of EEGS may be due one of the above said reasons. In the present study the resultant decrease in insulin levels could probably be due to the insulin sensitizing activity of the extract.

An increase in the mobilization of free fatty acids from the peripheral storage area leads to an unusually high concentration of hepatic and plasma lipids in diabetes because hormone sensitive lipase is hindered by the insulin. The distinct hyperlipidemia that distinguishes the diabetic state is considered as a significant uninhibited measure of lipolytic hormones (glucagon and catecholamine) on the fat storage area (Ravi K et al., 2005). It is stated that a deficiency in lipoprotein lipase activity in diabetics may grant to an important increase of triglycerides in blood with insulin administration; lipoprotein lipase activity is enhanced and leads to reduction of plasma triglyceride concentrations (Lopes-Virella et al., 1983). EEGS administration almost reversed these effects as it reduced triglyceride and total cholesterol concentrations, LDL concentration, and enhanced HDL, notably in combination. In this context, EEGS was found to be as effective as pioglitazone in lowering the plasma lipid profiles in the diabetic rats.

Serum urea and creatinine are elevated in diabetic hyperglycemia and are considered as significant markers related to renal dysfunction (Alamdal & Vilstrup et al., 1998). Moreover, the protein glycation in diabetes may lead to muscle wasting and increased release of purine, the main source of uric acid as well as the activity of xanthine oxidase (Anwar & Meki et al., 2003).

Hepatic serum biomarkers like SGOT and SGPT were estimated on day 28 and used for the evaluation of hepatic damage. Standard drug pioglitazone showed



**Figure 3: Photographs of histological examination of rats treated with EEGS**

elevated levels of SGOT and SGPT which are known to cause hepatic damage. There is no significant difference in total protein content.

In histopathological analysis of the pancreas of the diabetic rats treated with standard pioglitazone, EEGS 200 & 400mg/kg were comparable to normal rats in terms of the overcoming moderate degenerative changes caused by diabetes. In diabetic rats treated with extracts, the liver and kidney architecture were appeared more or less like normal control.

### CONCLUSION

The present study concealed that EEGS is an antihyperglycemic and antihyperlipidemic agent. Phytochemical constituents present in the plant are responsible for the observed activities. Preliminary phytochemical studies reveal the presence of flavonoids, terpenes, sterols and saponins in the extract which might be a responsible constituent for these activities. Our previous studies also evidenced for its antioxidant property. Further identification and isolation of active constituents are under progress.

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