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Evaluation of hypoglycemic effect of ethanolic leaf extracts of *Grewia Umbellifera*

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ABSTRACT

Grewia umbellifera is an Indian traditional medicinal plant of the Tiliaceae family .The present study was investigated for anti-diabetic of Grewia umbellifera in streptozotocin induced diabetes in rats. In the present study thirty rats were randomly classified into five groups. The first group was served as a control. The second group was served as Diabetic control. The third group was served as test 1 administered with 250 mg/kg and forth group was served as test 2 administered with 500 mg/kg of Ethanolic leaf extracts of Grewia umbellifera in a daily oral dose for 28 days. The fifth group was served as Standard (Glibenclamide 0.6 mg/kg) Type II diabetes was induced in overnight fasted rats by a single intra peritoneal injection of 35mg/kg streptozotocin. Hyperglycemia was confirmed by the elevated glucose levels >250 mg dL-1 in plasma. Treatment of animals with ethanolic leaf extracts of Grewia umbellifera in a daily dose of 250 and 500 mg/kg for 28 consecutive days significantly mitigated the induced changes in the glucose and body weight parameters. Conclusively, ethanolic leaf extract of Grewia umbellifera treatment exhibited marked beneficial effects against streptozotocin induced diabetes.

KEYWORDS : leaf extract of Grewia umbellifera, Diabetes, hypoglycemic effect

Introduction

The chronic disease diabetic mellitus takes an ever-increasing proportion of national and international health care budgets as the number of people with diabetes is escalating worldwide. Inherited and/or acquired deficiency in the production of insulin by pancreas or the ineffectiveness of the insulin produced, causes diabetes mellitus [1]

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels [2.].

The International Diabetes Federation predicts that the number of people living with diabetes will rise from 366 million in 2011 to 552 million by 2030 [3].

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 formulations for oral therapy [5].

A number of herbs are traditionally used in different countries during drug or toxin induced in hepatic, renal and cardiac disorders (6). *Grewiaumbelliferea* (Tiliaceae) (GU) is herbaceous medicinal plant that has been distributed in Kanniyakumari district, Tamilnadu, India (7)

Extensive phytochemical investigations shows that the presence of many chemical constituents including palmitic and linoleic acid such as n-Hexadecanoic acid, 9,12-Octadecatrienoic acid (Z,Z,Z) -, and oleic acid, which are considered significant for Hypocholesterolemic property (8) (9) (10). It is used as CNS depressant (11), hypotension and antidiuretic agent (12).

Material and Methods

Plant Material *Grewia umbellifera's* aerial part plant collected and authenticated by Dr.V.Chelladurai (Research Officer) Botany (C.C.R.A.S) Government of India. Voucher specimen (SIVET C-453/2012-2013) has been retained in the Dept of Biochemistry, S.I.V.E.T College of Arts & Science, Chennai. Materials were cleaned with water and dried in the shade until a constant weight was obtained.

Animals

Studies were carried out using Wistar albino male rats (150–200 g), maintained at animal house SBST VIT, Vellore, Tamilnadu, India. The animals were housed in polyacrylic cages (38 cm_23 cm_10 cm) and maintained under standard laboratory conditions (temperature 25_20_C) with dark/light cycle (12/12 h). The animals were fed with standard pellet diet and fresh water ad *liibitum*. All the animals were acclimatized to lab conditions for a week before commencement of the experiment. All the procedures described were reviewed and approved by the Animal's Ethical Committee.

Extraction

The whole plant was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieves and extracted with ethanol (95% v/v) in Soxhlet's apparatus at 60_C.The solvent was completely removed and obtained dried crude extract which was used for investigation. (13)

Qualitative Phytochemical Analysis:

Ethanolic extracts of *Grewia Umbellifera* Leaf were analyzed for the tannins, sterols, lipids, glycosides, terpenoids, phenols, carbohydrates, anthraquinones, resins, reducing sugar, saponins, flavanoids and alkaloids [14].

Acute Toxicity Studies: Acute oral toxicity study was performed as per Organization for Economic Cooperation and Development (OECD) guidelines 423 [15]. Administration of stepwise dose of GULE (50 mg/kg - 2000 mg/kg b.w), animals were observed individually at least once during the first 30 minutes and periodically during the first 24 hours, with special attention given during the first 4 hours and dai-

ly thereafter, for 28 days. The dose 2000 mg/kg was found to be safe and no toxicity was observed. One-fifth and one-tenth of upper limit dose were selected as the label for examination of antidiabetic activity.

Oral Glucose Tolerance Test (OGTT): The oral glucose tolerance test was performed in overnight fasted normal rats. Twenty four rats were divided into four groups (n = 6),

Group-I was administered with citrate buffer (0.1M) at a dose of 2ml/kg,

Groups II was administered with ethanolic extract of *Grewia Umbellifera* (GULE) at a dose of 250 mg/kg.

Group III was administered with ethanolic extract of *Grewia Umbellifera* (GULE) at a dose of 500 mg/kg.

Group-IV was administered with glibenclamide at a dose of 0.6 mg/kg

Glucose (2g/kg) was fed 30 min after the administration of extracts. Blood sample were taken at time periods of 0, 30, 60 and 120 min after glucose administration. Plasma was separated from the collected blood samples after centrifugation at 4000 rpm for 15 min. Blood glucose level in plasma was measured using glucose oxidase and peroxidase method [16].

Hypoglycemic Activity: Tests were performed in overnight fasted normal rats. 30 rats were divided into five groups (n = 6).

Group-I was administered with citrate buffer (0.1M) at a dose of 2ml/kg,

Groups II was administered with ethanolic extract of *Grewia Umbellifera* (GULE) at a dose of 250 mg/kg.

Group III was administered with ethanolic extract of *Grewia Umbellifera* (GULE) at a dose of 500 mg/kg.

Group-IV was administered with glibenclamide at a dose of 0.6 mg/kg

Blood (0.3 ml) was withdrawn at a time periods of 0, 2, 4 and 6 hours after drug administration [17,18].

Experimental induction of diabetes

Diabetes was induced in the animals fasted overnight by a single intraperitoneal (ip) injection of freshly prepared solution of STZ (Sigma, USA) 35 mg kg^{-1} body weight in 0.1M cold citrate buffer pH4.5 (19,20,21) The animals were allowed to drink 5% glucose solution to overcome the drug-induced hyperglycemia. (22,24) Control rats were injected with citrate buffer (0.1M) alone. Animals were considered diabetic if the blood glucose values were >250 mg dL^{-1} on the third day after STZ injection. After a fortnight, rats with moderate diabetes having glycosuria (indicated by Benedict's test for urine) and hyperglycemia with blood glucose range of 200 – 300 mg dL^{-1} were used for the experiment.

Experimental design

Rats were divided into five groups each have 6 rats as follows after the induction of STZ-induced diabetes. Diabetes was induced in rats two weeks before starting the treatment.

Group I: animals were considered as control rats.
Group II: animals were treated as diabetic STZ-induced rats.
Group III: diabetic-induced animals were fed with 250 mg kg_1 of ethanolic extract of GU for 28 days.
Group IV: diabetic-induced animals were fed with 500 mg kg_1 of ethanolic extract of GU for 28 days.
Group V: diabetic rats were given glibenclamide orally (0.6 mg kg_1) in distilled water daily for 28 days.

Assessment of Antidiabetic Activity in Streptozotocin induced Diabetic Rats:

The fasting blood glucose levels were determined on 1, 7, 14, 21 and 28 days using GOD-POD method [23, 24].

Histopathological Studies: After the end of the study all the rats were sacrificed by cervical dislocation under mild ether anesthesia and pancreas, liver, and kidney were isolated, washed with cold saline and preserved in 10% formalin solution in buffered form. Blocks from tissues were routinely processed and embedded in paraffin. Thin sections were cut by using rotary microtone and stained with hematoxylin and eosin for histomorphology evaluation [25].

RESULTS

Phytochemical Screening: Phytochemical investigation of GULE revealed the presence of steroids, sqaulene ,vitamin E, fatty ester, alkaloids, carbohydrates, flavonoids, tannins, glycosides, polyphenols. [14]

Acute Oral Toxicity Study: From the acute studies no toxicity was found to dose of 2000mg/kg hence, 250 mg/kg b.w and 500 mg/kg b.w of this dose was selected for further study.

Hypoglycemic Effect of Ethanolic Extract of *Grewia Umbllifera:* The results from the study clearly indicated that the ethanolic extract 250 and 500 mg/kg exhibited significant hypoglycemic activity at 4^{th} and 6^{th} hours in fasted normal rats. The hypoglycemic activity of GULE was found to be dose dependent. Standard drug glibenclamide indicated a significant decrease of blood glucose levels (Table1).

Effect on Oral Glucose Tolerance Test: The effects of ethanolic extract of GULE (250 mg/kg and 500 mg/kg) on glucose tolerance are shown in (Table 2). By administration of glucose (3 g/kg) produced significant change in blood glucose level of normal rats. The treatment groups with GULE 250 mg/kg, 500 mg/kg and glibenclamide 0.6 mg/kg showed significant reduction in plasma glucose level at 60, 120 minutes when compared to normal control group.

Effect of GULE on Streptozotocin Induced Diabetic Rats: There was a significant increase in blood glucose level in diabetic rats when compared with normal controls due to injection of STZ. In the study, daily administration of the extract for 6 weeks led to fall in blood glucose levels. At the end of experiment (28^{th}) blood glucose level was (130.30±5.06) and (118.8±3.99) mg/dl at the doses of 250 and 500 mg/kg of GULE respectively. The antidiabetic effect of GULE on the blood glucose levels in diabetic rats is also shown in (Table 3).

Effect of GULE on Body Weight: The body weight of the diabetic controls (group II) significantly decreased compared with the normal controls (group I). During the observation of the ethanoic leaf extract of GU treated diabetic rats at doses of 250 mg/kg and 500 mg/kg, there were significant (p <0.05) weight gains on day 28 relative to day 0 as shown in (Table 4).

Histopathological Studies of Pancreas: Histopathological examination of pancreas showed the destruction of β-cells in the diabetic control group and by treating with GULE (250 and 500 mg/kg) and glibenclamide (0.6 mg/kg) showed recovery of damaged tissues when section of treated groups compared with diabetic control.

Histopathology: The histopathological changes in control, Diabetic control and Ethanolic extract of *Grewia umbellifera* treated groups is shown in Fig. 1.1 to 1.5.

DISCUSSION

Disturbances in Glucose metabolism, altered lipid levels and Oxidative stress are important risk factors for diabetes, cardiovascular, oncologic and many other diseases [26].

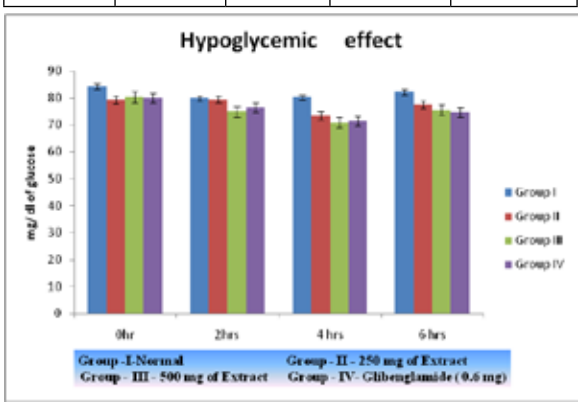
In diabetic condition, elevated blood glucose, reduced body weight, polyuria, polydipsia and polyphagia are commonly observed. In present study, induction of diabetes by STZ produced increase in blood glucose levels. This may be due to insulin deficiency or resistance state in diabetic control rats [27]. GULE treatment significantly reduced blood glucose level in diabetic rats which represents reversal of insulin resistance or increasing insulin secretion possibly by regeneration of damaged pancreatic β -cells in STZ induced diabetic rats. These effects may be attributed to either inhibition of increase in insulin output, inhibition of the intestinal absorption of glucose and increase in glucose metabolism because GULE contains flavonoids, Triterpene, steroids, phenols and esters. Which have been proved to be antidiabetic activity by different mechanisms of action [28].

Streptozotocin induced diabetes is associated with the characteristic loss of body weight, which is due to increased muscle wasting and due to loss of tissue proteins. Diabetic rats treated with the GULE showed an increase in body weight as compared to the diabetic control, which may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis and may also be due to the improvement in insulin secretion and glycemic control. The results of the present investigation of GULE showed significant antidiabetic activity against STZ induced diabetic rats. Hence, GULE may be regarded as a promising natural and safe remedy for prevention of diabetic complications.

CONCLUSION

The results revealed that ethanolic leaf extract of *Grewia Umbellifera* possess significant antidiabetic activity in streptozotocin induced diabetic rats. Further studies are necessary to elucidate in detail the mechanism of action of medicinal plant at the cellular and molecular mechanism.

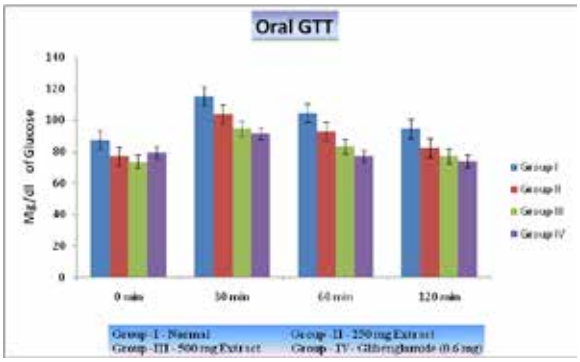
Hypoglycemic effect of Ethanolic extract of GU- Blood glucose (mg/dl) (mean ± SD)				
Groups	0hr	2hrs	4 hrs	6 hrs
Control	84.33±5.65	79.93±5.22	80.26±3.20	82.23±3.84
GUL Extract (250mg/kg)	79.40±5.66	79.49±8.77	73.49±5.69	77.53±2.66*
GUL Extract (500mg/kg)	80.25±6.78	74.86±9.28	70.80±6.04*	75.56±4.88**
Standard (0.6mg/kg)	79.96±4.84	76.26±6.97	71.36±4.36**	74.70±3.63**



Data represents mean ± S.D (n=6). *P<0.05 ** P<0.01, significant compared to control analyzed by one – way ANOVA.

Effect on Oral Glucose Tolerance Test Blood glucose (mg/dl) (mean ± SD)				
Groups	0 min	30 min	60 min	120 min
Control	87.45±7.87	115.23±7.31	104.63±7.89	94.43±7.49
GUL Extract (250mg/kg)	77.06±5.44	103.93±9.79	92.93±09.00	82.36±5.64*
GUL Extract (500mg/kg)	73.60±12.70	94.56±11.87**	83.23±11.54**	77.26±6.31**
Glibenglamide (0.6 mg/kg.bw)	79.63±6.54	91.82±9.85**	77.15±6.21***	74.07±7.31***

Data represents mean ± S.D (n=6). *P<0.05 ** P<0.01, ***P<0.001 significant compared to control analyzed by one – way ANOVA.



Effect of Streptozotocin induced Diabetic Rats Blood glucose (mg/dl)(Mean ± SD)					
Groups	1st day	7th day	14th day	21st day	28th day
Control	78.98±4.24	75.66±3.55	77.16±3.43	78.23±5.64	77.83±2.39
STZ induced Diabetic control	249.45±9.26	255.05±12.04	260.10±11.25	270.78±7.84	280.80±2.29
Dia + GULE (250mg/kg)	245.16±8.90	221.90±10.40	190.9±09.77*	165.56±10.24	130.30±5.06*
Dia + GULE (500mg/kg)	240.52±11.90	200.56±10.72	150.54±13.61**	125.62±12.64**	118.8±3.99**
Diabetic + Glibengla-mide (0.6 mg/kg.bw)	213.16±6.11	189.66±7.91**	164.83±6.17**	144.58±9.94*	138.00±2.43*

Data represents mean ± S.D (n=6). *P<0.05 ** P<0.01, ***P<0.001 significant compared to control analyzed by one – way ANOVA.

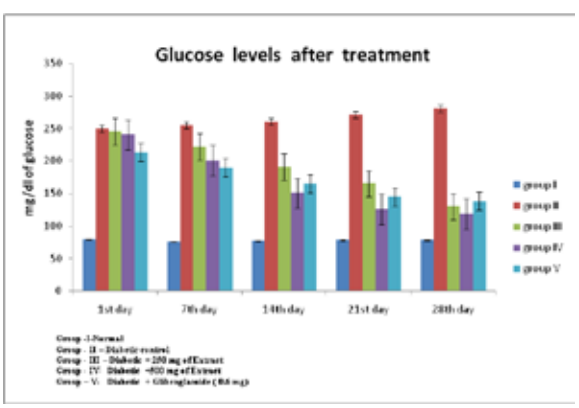
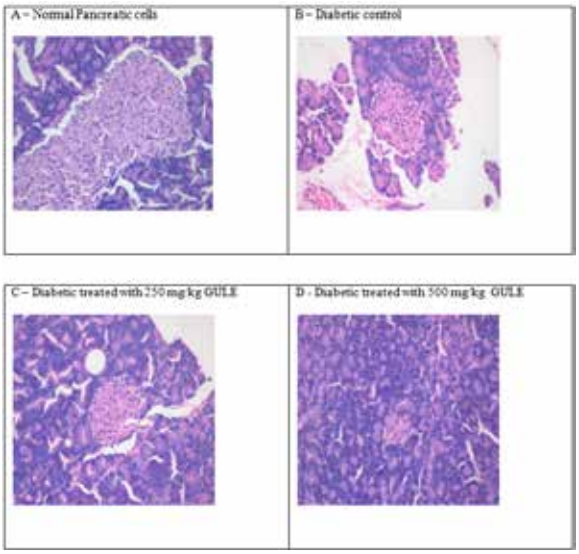


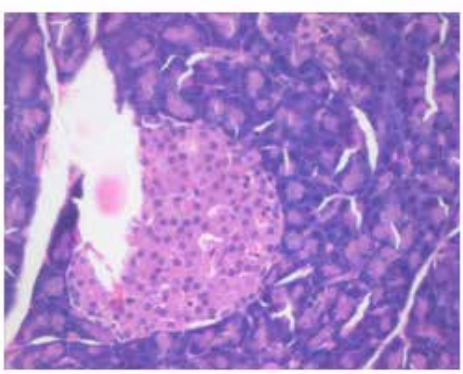
Table 4: Effect of Ethanolic leaf extract of GU on body weight

Groups	Initial weight	Final weight
Control	178.66±10.26	200.83±12.13
Diabetic control	176.33±21.57	165.50±09.00
Diabetics + GULE(250mg/kg)	178.00±09.13	182.66±08.18*
Diabetics + GULE(500mg/kg)	182.83±13.13	197.83±9.62***
Diabetics + Glibenglamide(0.6mg/kg)	194.16±8.88	226.50±10.22***

Data represents mean ± S.D. (n=6).* *P* <0.05, ** *P* <0.01, *** *P* < 0.001, Significant compared to control analyzed by one-way ANOVA



E - Diabetic + Glibenglamide (0.6 mg/kg.bw)



Histopathology of rat Pancreas.

A - Represents the Pancreatic cells of control animals showing normal histology,

B - Served as diabetic control which showed mild fatty changes and necrosis,

C - Represent the Pancreas of streptozotocin rats treated with 250 mg of Ethanolic Leaf Extract of GU showing normal histology as that of control,

D - Depicts the Pancreas of streptozotocin rats treated with 500 mg of Ethanolic Leaf Extract of GU showing normal histological structure as that of control,

E – Represent the Pancreas of streptozotocin rats treated with standard drug glibenclamide showing normal histology

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