EVALUATION OF ANTIDIABETIC ACTIVITY OF TRIUMFETTA PILOSA ROTH IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

* D Ramakrishna [1], G Vidyasagar [2], K Pavan Kumar [1], I Madhusudhana Reddy [3], VSS S Gupta Atyam [4]

[1] Department of Pharmaceutical Sciences, JJT University, Jhunjhunu, Chudela, Rajasthan 333 001

[2] Department of Pharmaceutical Sciences, Veerayatan institute of pharmacy, Bhuj, Jakhania Kutch, Gujarat 370 460

[3] Department of pharmaceutical chemistry, Malla reddy college of pharmacy, Dhulapally village, Hyderabad 500014

[4] Department of pharmacology, Joginpally B.R College of pharmacy, Yenkapally, Hyderabad 500072, India

Abstract:

The present study aims to evaluate medicinal use of Triumfetta pilosa for the treatment of diabetes. Antidiabetic activity of ethanolic extract of Triumfetta Pilosa Roth was evaluated for in-vivo hypoglycemic activity using Streptozotocin induced diabetic rats. Different biochemical parameters were used to determine the blood glucose levels using streptozotocin induced diabetes and analyzed it effect in kidneys after 21 days treatment. Ethanol extract had shown significant protection and lowered the blood glucose levels when compared to normal in glucose tolerance test. In kidney the changes caused after induction of diabetes showed degeneration's in proximal tubular epithelial cells in the cortex of kidneys, hemorrhage in the interstitial area and periglomerular lympolytic infiltration and hyalinization of the arterioles which was reduced after feeding with Triumfetta Pilosa .Ethanolic extract of Triumfetta Pilosa prevented alteration in kidney pathology.

Key Words: Triumfetta Pilosa, Antidiabetic activity, blood glucose, Kidney, Streptozotocin- Induced diabetes.

INTRODUCTION

Diabetes mellitus, is a chronic metabolic disorder characterized by a high blood glucose concentration-hyperglycemia (fasting plasma glucose > 7.0 mmol/l or plasma glucose > 11.1 mmol/l 2 hours after a meal) – caused by insulin deficiency, often combined with insulin resistance. The body has to maintain the blood glucose levels at very narrow range, which is done with insulin and glucagons. Insulin is a hormone produced by special cells (called beta cells) in pancreas. The pancreas is located deep in the upper part of the abdomen, behind the stomach attached to the duodenum. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of active research. Furthermore, after the recommendations made by WHO on diabetes mellitus, investigations on hypoglycemic agents from medicinal plants has become more important [1-4].

The plants and herbs are being used as decoctions or in other extracted forms for their blood sugar lowering properties. Many useful herbs introduced in pharmacological and clinical trials have confirmed their blood sugar lowering effect, repair of ß-cells of islets of Langerhans. There are some useful reviews on Indian medicinal plants having blood sugar lowering potentials. India is well known for its herbal wealth. Medicinal plants like *Andrographis paniculata , Azadirachta indica, Ocimum sanctum, Trigonella foenum graecum,Swertia chirayita, Pterocarpus marsupium, Aegle marmelos, Helitropium zeylanicum, puntia ficus, Caralluma attenuata, Salacia reticulate, Raphanus sativus, Amarathus spinosus*,have been studied for the treatment of diabetes.However,detailed study on the efficacy, mechanism of action and safety of plant extracts are needed [5-7].

Many herbal medicines have been recommended for the treatment of diabetes. Traditionally plant medicines are used throughout the world for a range of diabetic conditions.The whole plant *Triumfetta pilosa* Roth, family *tiliaceae* is traditionally used for Antidiabetic. The present study is an attempt to investigate Antidiabetic activity on ethanolic extract of plant *Triumfetta pilosa* Roth. as to provide a scientific proof for the activity.

MATERIAL AND METHODS

Collection of plant

The dried plant of *Triumfetta pilosa* Roth. was collected from Herbal garden Tirupathi, Andhra Pradesh, India in the month of January 2009 and authenticated by Dr. K. Madhav Chetty, Assistant Professor, Dept. of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh. The whole plant was cleaned, air dried and grounded into powdered separately. The dried powdered plant material was passed through sieve 60 and stored in air tight containers.

Preparation of extract

The dried powder of *Triumfetta pilosa* plant (200gms) was successively extracted with Ethanol (80%) at room temperature by Soxhlet extraction process**.** Each time before extracting with the solvent, dry the powdered material in oven below 50^0c. Concentrate the extract at reduced pressure by Rotary Flash Vacuum Evaporator. Weigh the extract obtained with the solvent and calculate its percentage in terms of the air-dried weight of the plant material. Further the concentrated extract was dried in desiccator and stored in vacuum sealed air tight containers. The extract was suspended in 0.9% normal saline as a vehicle solution and is used [8].

Test animals

Male wistar albino rats (160 – 200 g) were used in the experiment. Animals maintained under standard environmental conditions, were fed with a standard diet (Hindustan Lever, India) and water ad libitum. The animals were fasted for 16h before experimentation but allowed free access to water. All the chemicals were for study were analytical grade.

THE FIVE GROUPS ARE AS FOLLOWS:

Group I: Normal control rats received normal saline as a vehicle p.o.

Group II: Diabetic control rats received normal saline p.o.

Group III: Diabetic + Effective dose 100mg/kg b.w of whole plant extract in normal saline p.o.

Group IV: Diabetic + Effective dose 200mg/kg b.w of whole plant extract in normal saline p.o.

Group V: Diabetic + glibenclamide (2.5 mg/kg b.w) in normal saline p.o.

BIOCHEMICAL TESTS

After 21-days treatment, blood from all the groups were collected by retro-orbital puncture under mild anesthesia for estimating blood glucose levels , total cholesterol levels, total triglyceride levels , serum urea, serum creatinine, serum insulin levels , HDL, LDL & VLDL levels. Then the animals of all groups' kidneys were decapitated, isolated and fixed in 10% neutral formalin for histology.

Acute Toxicity Studies:

In acute toxicity study, there were no behavioral changes seen up to 4hrs and no mortality was observed up to the end of 48hrs even at the maximum tested dose level of 2000mg/kg per oral, it is considered as LD50. Thus, $1/10^{th}$ of LD50 is taken as the effective dose. As a result, two doses of 100mg/kg and 200 mg/kg b.w was taken as an effective dose for the study 9-10.Different biochemical data were represented in table [9-10]

Table 1: Glucose Tolerance Test

GROUPS	0 min	30 min	90 min	150 min
Control (normal saline)	**101.43±5.7**	**191.8±2.0**	**312.8±33.7**	**304.2±35**
Extract treated (T.p) (100 mg/kg)	**97.4±5.7**	**113.8±2.3****	**78.2±9.17****	**89.4±10.3****
Extract treated (T.p) (200 mg/kg)	**93.3±3.3**	**106.5±33.4****	**72.4±11.5*****	**79.6±11.3*****

Extract Treated = Triumfetta pilosa (T.p) Plant

Values are given as mean ± SEM for groups of six animals in each group.

Extract treated rats were compared with normal control rats.

***P<0.05, **P<0.01, ***P<0.001 was considered significant comparing to Diabetic control group.**

Table 2: BLOOD GLUCOSE LEVELS (mg/dl)
Effect of Ethanolic extract of *Triumfetta pilosa* plant
on 1st, 7th, 14th & 21st day in rats

GROUPS	1st Day	7th Day	14th Day	21st Day
Normal Control	**71.66±1.30**	**77.83±1.72**	**83.16±1.75**	**82±1.06**
Diabetic Control	**290..83±6.34**	**307.16±6.76**	**319.33±5.99**	**335.5±4.49**
Extract Treated (T.p) 100 mg/kg	**263.33±4.5**	**218.16±3.12***	**185.33±2.22****	**150.83±1.8****
Extract Treated (T.p) 200 mg/kg	**265±4.69**	**219.16±3.57***	**182.83±2.73****	**142.33±1.45****
Standard Drug Treated	**270±5.32**	**233.33±6.39***	**176.5±3.2****	**135.33±1.2*****

Extract Treated = Triumfetta pilosa (T.p) Plant

Values are given as mean ± SEM for groups of six animals in each group.

Diabetic control rats were compared with normal control rats. Diabetic + *Triumfetta pilosa* and Diabetic + glibenclamide treated rats were compared with Diabetic control rats.

ANOVA followed by Dunnet's *t*-test.

***P<0.05, **P<0.01, ***P<0.001 was considered significant compared to Diabetic control group.**

Table 3: Effect of Ethanolic extract of *Triumfetta pilosa* plant on Serum Biochemical Parameters after 3 weeks treatment

GROUPS	SERUM INSULIN (IU/ML)	SERUM UREA (mg/dl)	SERUM CREATININE(mg/dl)
NORMAL GROUP	**14.34±1.86**	**30.83±1.04**	**0.53±0.07**
DIABETIC GROUP	**4.25±0.54**	**71±2.59**	**1.33±0.11**
EXTRACT GROUP(T.p)100 mg/kg	**10.96±0.36***	**39.33±2.33**	**0.71±0.03***
EXTRACT GROUP(T.p) 200 mg/kg	**11.78±0.179***	**33±1.78***	**0.52±0.02****
STANDARD GROUP	**14.48±1.63****	**26.16±2.71****	**0.49±0.02*****

Extract Treated = Triumfetta pilosa (T.p) Plant

Values are given as mean ± SEM for groups of six animals in each group.

Diabetic control rats were compared with normal control rats. Diabetic + *Triumfetta pilosa* and Diabetic + glibenclamide treated rats were compared with Diabetic control rats.

ANOVA followed by Dunnet's *t*-test.

***P<0.05, **P<0.01, ***P<0.001 was considered significant compared to Diabetic control group.**

Table 4: Effect of Ethanolic extract of *Triumfetta pilosa* plant on

Serum Biochemical Parameters after 3 weeks treatment

GROUPS	Serum Total Cholesterol (TC)	Serum Total Triglycerides (TG)
NORMAL GROUP	117±2.29	82.33±2.75
DIABETIC GROUP	221±2.76	138.5±3.24
EXTRACT GROUP (T.p) 100mg/kg	141.16±3.070*	100.16±2.70*
EXTRACT GROUP (T.p) 200mg/kg	120.66±2.445*	95.16±1.51*
STANDARD GROUP	101.83±2.53**	88.83±2.49**

Extract Treated = Triumfetta pilosa (T.p) Plant

Values are given as mean ± SEM for groups of six animals in each group.

Diabetic control rats were compared with normal control rats. Diabetic + *Triumfetta pilosa* and Diabetic + glibenclamide treated rats were compared with Diabetic control rats.

ANOVA followed by Dunnet's *t*-test.

***P<0.05, **P<0.01, ***P<0.001 was considered significant compared to Diabetic control group.**

Table 5: Effect of Ethanolic extract of *Triumfetta pilosa* whole plant on Serum Biochemical Parameters after 3 weeks treatment

GROUPS	SERUM HDL LEVELS	SERUM LDL LEVELS	SERUM VLDL LEVELS
NORMAL GROUP	65.83±4.35	78.16±1.47	16.46±0.55
DIABETIC GROUP	19.33±2.37	176.16±2.77	27.61±0.65
EXTRACT GROUP 100mg/kg	37.66±6.439*	112.66±3.63*	20.03±0.54*
EXTRACT GROUP 200mg/kg	47.83±5.45*	98.66±2.37*	19.03±0.30*
STANDARD GROUP	67.66±1.89**	93.33±1.89**	17.76±0.49**

Extract Treated = Triumfetta pilosa (T.p) Plant

Values are given as mean ± SEM for groups of six animals in each group.

Diabetic control rats were compared with normal control rats.

Diabetic + *Triumfetta pilosa* and Diabetic + glibenclamide treated rats were compared with Diabetic control rats.

ANOVA followed by Dunnet's *t*-test.

***P<0.05, **P<0.01, ***P<0.001 was considered significant compared to Diabetic control group.**

HISTOPATHOLOGY OF KIDNEYS

Group I: Normal Control showed normal structure of glomeruli and proximal and distal convoluted tubules in kidneys.

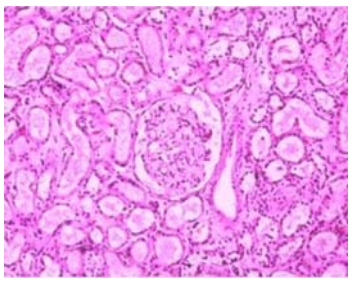
Group II: Diabetic Control kidneys showed an increase in the mesangial cell and matrix of glomeruli and hyalinization of arterioles.

Group III: *Triumfetta pilosa* treated group showed kidney with less increase in the mesangial cell matrix of glomeruli and few hyalinized arterioles.

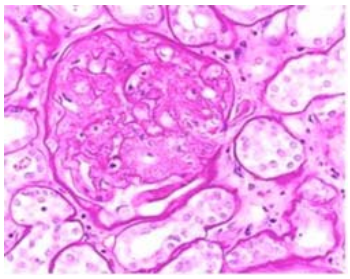
Group IV: *Triumfetta pilosa* treated group showed kidney with increase in the mesangial cell matrix of glomeruli and hyalinized arterioles.

Group V: Glibenclamide treated group showed normal kidney structure which appeared more or less as control.

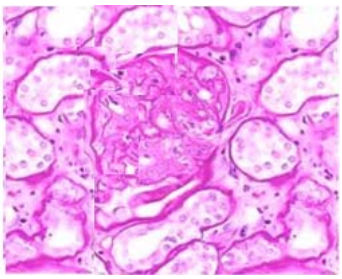
HISTOPATHOLOGICAL PICTURES OF KIDNEYS



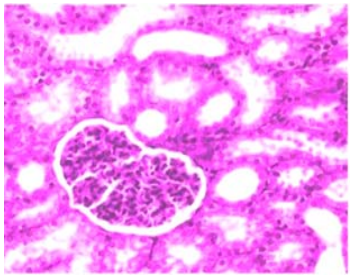
Normal Control Kidney
(Figure -1)



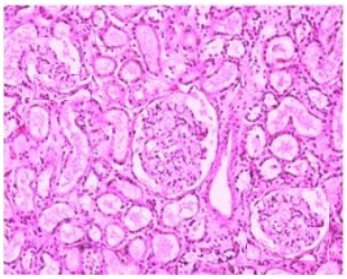
Diabetic control kidney
(Figure-2)



Triumfetta pilosa (100 mg)
Treated kidney (Figure-3)



Triumfetta pilosa (200 mg)
Treated kidney (Figure -4)



Glibenclamide treated kidney
(Figure-5)

Results & Discussion

In acute toxicity study, there were no behavioral changes seen up to 4hrs and no mortality was observed up to the end of 48hrs even at the maximum tested dose level of 2000mg/kg per oral, it is considered as LD50. Thus, $1/10^{th}$ of LD50 is taken as the effective dose. As a result, two doses of 100mg/kg and 200 mg/kg b.w was taken as an effective dose for the study. After 21-days treatment, blood from all the groups were collected by retro-orbital puncture under mild anesthesia for estimating blood glucose levels , total cholesterol levels, total triglyceride levels , serum urea, serum creatinine, serum insulin levels , HDL, LDL & VLDL levels. In Glucose Tolerance Test, the Glucose levels were estimated before drug treatment and at different intervals thereafter. In the control group the blood glucose was found to increase linearly from basal value of 101.4 mg/dl to 191.8 mg/dl in the first 30 minutes. After 60 minutes of glucose loading, the blood glucose was increased

further. The maximum value of 312 mg/dl was seen at the 90th minute. Whereas in the extract treated animals, only a little elevation in the blood glucose were seen and maximum glucose tolerance was observed at 90th minute. The extract was evaluated for in-vivo hypoglycemic activity using Streptozotocin at a dose of 60mg/kg body weight by i.p. route. 7 days after STZ injection, it was observed that there was a short phase of hypoglycemia followed by marked elevation in the blood glucose level, the diabetic rats with fasting blood glucose levels > 250 mg/dl, were selected for further studies. This is in accordance with the reports published by various authors where the increase in glucose levels has been attributed to the destruction of β cells by Streptozotocin. Damage to the Beta cells is associated with the liberation of stored insulin after which the insulin synthesis is stopped leading to a persistent diabetic state. Since insulin is no longer available, glucose absorption is impaired leading to hyperglycemia. In addition, after 21 days of treatment, the Serum insulin levels of the treated diabetic rats were significantly enhanced compared to the untreated diabetic rats. It was observed that there was an increase in Serum Urea and Serum Creatinine levels in streptozotocin induced diabetic rats. However after 21 days administration of ethanolic extract of *Triumfetta pilosa* has led to a significant fall in Serum Urea and Serum Creatinine levels when compared with the standard group. It was observed that there was an increase in Serum Total Cholesterol(TC), Serum Total Triglycerides(TG), LDL and VLDL levels and decrease in HDL levels in diabetic rats. After continuous administration of ethanolic extract for 21 days has led to significant decrease in Serum Total Cholesterol, Serum Total Triglycerides, LDL and VLDL levels, while it increased HDL levels in diabetic rats. The beneficial effect of *Triumfetta pilosa* and glibenclamide given immediately after diagnosis of diabetes which decreases serum urea, serum creatinine, serum total cholesterol, serum total triglycerides, LDL and VLDL levels and increases Serum Insulin and HDL levels was observed after 21 days treatment. Hence, the results indicate that treatment of diabetic rats with Triumfetta *pilosa* Roth**.** Prevented the alteration in kidney pathology with the return to their normal texture as shown in figures 1-5.

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