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Evaluation of anti-diabetic activity of *Triumfetta rhomboidea* in alloxan induced Wistar rats

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ABSTRACT

Triumfetta rhomboidea is used ethno medically since ancient times in the treatment of diabetes mellitus. In the present study, the antidiabetic effect of ethanolic extract of *Triumfetta rhomboidea* was investigated in alloxan induced diabetes rats. Oral administration of ethanolic extract of *Triumfetta rhomboidea* at the doses of 100, 200, and 400 mg / kg body weight was studied in alloxan induced diabetic rats. The antidiabetic effect of plant extract was increasing with increase in dose. At 400 mg/kg body weight ethanolic extract showed significant decrease in the blood glucose levels. The activity of the plant extracts was comparable with glibenclamide, a well known antidiabetic drug. The result clearly suggests that ethanolic extract of *Triumfetta rhomboidea* possesses significant antidiabetic activity.

Keywords: Antidiabetic activity, *Triumfetta rhomboidea*, Diabetes mellitus, glibenclamide

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INTRODUCTION

Diabetes is a metabolic disorder characterized by symptoms like hyperglycemia, altered metabolism of lipids, carbohydrates and proteins. India has the highest diabetic population with an estimated 32 million people suffering from it. In conventional therapy, Type 1 diabetes is treated with exogenous insulin and Type 2 with oral hypoglycemic agents (sulphonylureas, biguanides etc) [1]. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes, there is an increased demand by patients to use natural products with antidiabetic activity [2]. Since time immemorial, patients with non-insulin dependent diabetes have been treated orally by folk medicine, with a variety of plant extracts. In India, a number of plants are mentioned in ancient literature (Ayurveda) for the treatment of diabetic conditions.

Triumfetta rhomboidea, locally called as Dehkki, Chiru chitrika [3] is an erect and woody herb found at the edges of fields, fruit orchards, dry scrub forest and waste places throughout tropical and subtropical India [4]. *Triumfetta* (*Tiliaceae*) species are used in the folk medicine for the treatment of various diseases, such as diabetes, leprosy, diarrhoea, demulcent, etc. *Triumfetta rhomboidea* is locally used as antidiabetic, aphrodisiac, tonic, galactogenic, roots as diuretic, barks in diarrhoea, leaves and flowers as astringent [5]. The *Triumfetta rhomboidea*, ethno medically used for treatment of diabetes without any systematic scientific studies, thus an attempt was made to investigate the antidiabetic activity of this plant.

MATERIALS AND METHODS

Chemicals

Alloxan was purchased from Crescent Trading Company, Hyderabad. Glibenclamide was obtained as a gift sample from Arandy Laboratories Ltd, Hyderabad. All other chemical and reagents used were of analytical grade.

Plant Material

The dried whole plant powder of *Triumfetta rhomboidea* was supplied and authenticated by Dr. K. Madhav Chetty, Assistant Professor, Dept. of Botany, Sri Venkateswara University, Tirupati.

Preparation of plant extract

The dried powder of *Triumfetta rhomboidea* whole plant was extracted with Ethanol (95%) by Soxhlation process [6]. The filtrates were concentrated at reduced pressure by rotary flash vacuum evaporator and air dried. Phytochemical studies have been performed to identify the presence of various phytoconstituents. [7,8].

Experimental Animals

Healthy albino Wistar rats (200–225 g) of either sex, in-house bred at the Animal House of Shadan College of Pharmacy, Hyderabad, India were used for the study. Rats were housed in polypropylene cages lined with husk in standard environmental conditions (temperature $25\pm 2^{\circ}\text{C}$, relative humidity $55\pm 10\%$ and 12:12 light:dark cycle). The rats were fed on a standard pellet diet ad libitum and had free access to water [9]. The experiments were performed after approval of the protocol by the Institutional Animal Ethics Committee (IAEC) and were carried out in accordance with the current guidelines for the care of laboratory animals.

Experimental design

Antidiabetic activity of *Triumfetta rhomboidea* ethanolic extract was assessed in normal, and alloxan induced diabetic rats [10]. In all studies, the animals were fasted overnight for 16hrs with free access to water.

Acute Toxicity Studies

Acute toxicity of *Triumfetta rhomboidea* ethanolic extract was performed on albino rats, according to OECD Guidelines 425 [11]. The first group was treated after fasting overnight with a oral dose of 1000 mg/ kg body weight with the ethanolic extract of the plant "*T. rhomboidea*", suspended in 0.6 % sodium carboxy methyl cellulose and extracts were given in two different groups. Animals were monitored continuously for 2-3 hrs for general behavioral, neurological, autonomic, toxic effects and finally for death after 24 hrs. There was no mortality and no signs of toxicity and the extracts were found to be safe at this dose level, and then treated with higher dose of 2000 mg/ kg body weight. For the assessment of all the biological activities, three dose levels were chosen in such a way that, middle dose was approximately one tenth of the maximum dose during acute toxicity studies, and a low dose, which was 50 % of the one tenth of maximum dose, and high dose was 200% of one tenth of maximum dose. (100 mg/ kg, 200 mg/ kg, 400 mg/ kg).

Induction of Diabetes

Diabetes was induced by injecting 120 mg/kg of Alloxan monohydrate intraperitoneally in 0.6% w/v CMC to overnight-fasted rats. After 72 h of injection, fasting blood glucose level was measured. The animals that did not develop more than 250 mg/dl glucose levels were rejected [12]. In all studies, the animals were fasted overnight for 16hrs with free access to water throughout the duration of experiment.

Evaluation of extract on Alloxan-induced diabetic rats

The selected diabetic animals were divided into five groups ($n = 6$) (Ghosh M.N, 2005) and one more group of normal non-alloxanised animals was also added to the study. Group 1

was kept as normal control (non-alloxanised rats) received only distilled water; Group 2 was kept as negative control, alloxan induced and received only distilled water; Group 3, 4, and 5 are diabetic induced and treated with 100mg/kg, 200mg/kg, 400mg/kg b.w. of ethanolic extract respectively; Group 6 was diabetic induced and treated with glibenclamide and considered as standard. The treatment was continued for 7 consecutive days (p.o) at the end of 7th day, the rats were fasted for 16h and blood glucose level was determined. The determination of blood glucose levels is done by tail tipping method using Accu chek-sensor glucometer [13]. The results were compared with Group 6 which was treated with 5mg/kg b.w. of glibenclamide.

Statistical analysis

All values are expressed as mean \pm S.E.M. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. The results were considered statistically significant if $P < 0.05$.

RESULTS

Preliminary phytochemical screening: The percentage yield ethanolic extract were found to be 10.53% w/w respectively. The ethanol extract gave positive tests for steroids, alkaloids, flavonoids, glycosides and tannins. Effect of *T. rhomboidea* ethanolic extract in alloxan induced diabetic rats. The effect of different doses of ethanolic extract of plant on fasting glucose level of both normal and diabetic rats are given in Table.1. The fasting blood glucose levels of diabetic untreated rats (Group 2) were significantly higher than those of normal untreated rats (Group1). The ethanolic extract of *T.rhomboidea* at a dosage of 400 mg/kg b.w. produced the maximum fall of 43.73% in the blood glucose levels of diabetic rats after 7th day of treatment. Other doses of ethanolic extract of *T.rhomboidea* also shows fall in blood glucose level, and the fall in blood glucose level are proportional to increase in the doses of ethanolic extract of *T.rhomboidea*. Treatment with glibenclamide at a dosage of 5 mg / kg b.w. resulted in 52.47 % fall in the blood glucose levels of diabetic rats after 7 days of treatment. The graphical representation is given in fig.1.

DISCUSSIONS

In the present study, ethanolic extract of the whole plant of *T.rhomboidea* at a dose level of 400mg/kg b.w. showed a significant fall in blood glucose level by about 46% in diabetic rats, after 7 days of treatment. The ethanolic extract showed increased anti diabetic activity with increase in concentration, this clearly states that the ethanolic extract contains active principles with antidiabetic activity. The phytochemical study reveals the presence of flavonoids, alkaloids, steroids, glycosides and tannins.

Flavonoids, alkaloids, and other phenolic compounds are found to be bioactive antidiabetic principles [14-17]. Flavonoids have found to regenerate the damaged beta cells in alloxan induced diabetic rats [18]. Thus, the significant antidiabetic effect of *Triumfetta rhomboidea*

ethanolic extract could be due to the presence of various phytoconstituents detected in the phytochemical screening which alone or in synergism can impart therapeutic effect.

The present study also reveals the antihyperglycemic activity of glibenclamide in alloxan induced rats is an indication of the presence of some beta cells, as glibenclamide is known to stimulate insulin secretion from beta cells. Thus the ethanolic extract could have regenerated the beta cells or would have increased the secretion of insulin from the remnant beta cells for the possible antihyperglycaemic effect.

Table 1: Antidiabetic Activity of ethanolic extract of *Triumfetta rhomboidea*

Days	1 st Day	3 rd Day	7 th Day
Normal Control	106.16 ± 1.47	109.83 ± 2.48	112.83 ± 2.16
Diabetic Control	259.08 ± 3.51	256.002 ± 4.21	248.67 ± 1.065
Ethanolic Extract 100 mg/Kg	251.83 ± 3.15	231.50 ± 4.023*	212.17 ± 4.70 **
Ethanolic Extract 200 mg/Kg	250.02 ± 2.68 *	212.17 ± 3.416 **	182.50 ± 1.035 **
Ethanolic Extract 400 mg/Kg	248.50 ± 1.256**	198.004 ± 2.64***	139.64 ± 2.49 ***
Standard (Glibenclamide 5 mg/kg)	248 ± 3.114**	151.17 ± 4.325***	117.864 ± 3.169***

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

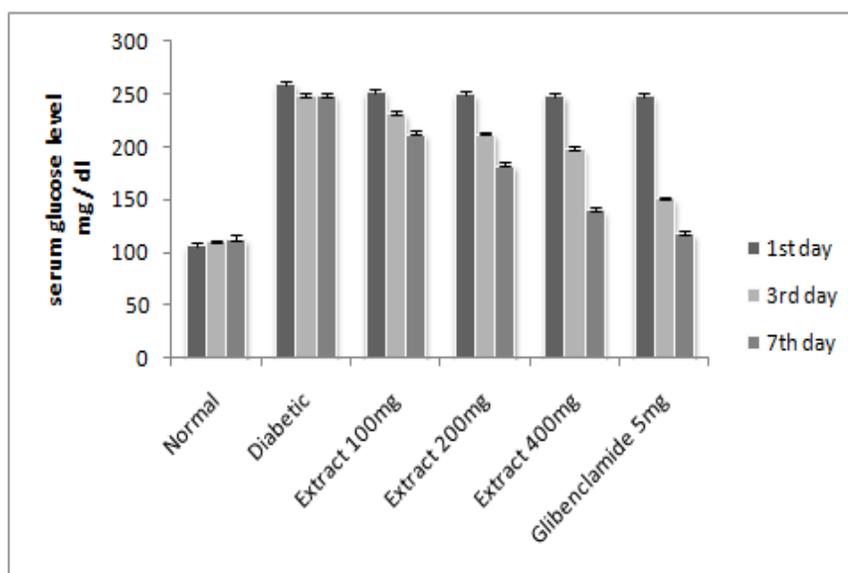


Fig.1. Effect of *Thomboidea* extract on blood glucose levels of alloxan induced diabetic rats. Each value is expressed as mean ± S.E.M. (n = 6).



CONCLUSION

The present investigation clearly reveals the importance of *Triumfetta rhomboidea* as an antidiabetic agent. Further studies will be focused on the determination of the mechanism(s) of action, as well as on the isolation of bioactive principles.

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