ANTI DIABETIC ATIVITY OF PETROLIUM ETHER EXTRACT OF 
TRIUMFETTA RHOMBOIDEA ON STREPTOZOTOCIN INDUCED 
DIABETIC RAT MODEL.

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ABSTRACT

Objective: The present study investigates the ant diabetic effect of petroleum ether extract of *Triumfetta Rhomboidea* (tiliaceae) in adult albino rats. Methods: The plant was collected locally and washed, dried, powdered and extracted in standard laboratory conditions. Diabetes induction was done by single intra peritoneal administration of streptozotocin in cold citrate buffer. After a week time all the animals who had blood glucose levels more than 200 mg/dl were taken for the study. All the diabetic animals were randomly divided into five groups and given with vehicle, glibenclamide (10 mg/kg), the prepared extract (100, 200, 300 mg/kg) respectively for 28 days. Blood glucose levels were checked in regular intervals using glucometer. Results: At a dose of 200 and 300 mg/kg p.o., *Triumfetta Rhomboidea* extract showed a hypoglycemic effect at a varying degree of significance (P<0.05-0.001) in diabetic rats in comparison with respective control group. Conclusion: The results shows that petroleum ether extract of *Triumfetta Rhomboidea* has significant hypoglycaemic activity in diabetic rats.

KEYWORDS: *Triumfetta Rhomboidea*, Glucometer, hypoglycaemic, streptozotocin.

1. INTRODUCTION

Diabetes mellitus (DM) is commonly referred to as diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. As the disease progresses tissue or vascular damage ensues leading to severe diabetic complications.
such as retinopathy, neuropathy, nephropathy, cardiovascular complications and ulceration. Thus, diabetes covers a wide range of heterogeneous diseases.

Drugs are used primarily to save life and alleviate symptoms. Secondary aims are to prevent long-term diabetic complications and by eliminating various risk factors, to increase longevity. Oral hypoglycaemics and insulin therapy are the majorly used in treating diabetes. World Health Organization (2003) has predicted that by 2030 the number of adults with diabetes would have almost doubled worldwide, from 177 million in 2000 to 370 million. Decreased physical activity, increasing obesity, stress and changes in food consumption have been implicated in its increasing prevalence over the past two decades. Although different types of oral hypoglycemic agents are available along with insulin for the management of diabetes mellitus, there is a growing interest in herbal remedies due to the side effects associated with these therapeutic agents.

Triumfetta Rhomboidea (tiliaceae) is an erect woody herb 75-150 cm in height. Stems glabrous longitudinally grooved. Leaves simple, alternate; blade ovate to rhomboid in shape with 3-5 lobes, flowers small yellow clustered on the leaf’s axis. Fruits are subglobose bur with the body diameter, covered with hooked spines. The plant is famous for its usage as demulcent, astringent, antidiarrheal, treating dysentery, gonorrhoea, womens use this in the later stages of pregnancy to facilitate childbirth. Scientific studies substantiating the use of Triumfetta Rhomboidea or their extract in treatment of diabetes are lacking. Therefore this study we report the antidiabetic activity of petroleum extract of Triumfetta Rhomboidea using recommended laboratory animal models.

2. MATERIALS AND METHODS
2.1 Plant Material
The plant material was collected from the herbal store of Ayurveda College Coimbatore and it was authenticated by Chelladurai. V, research officer, Botany Central Council for Research in Ayurveda and Siddha, Govt of India. The freshly collected sample were thoroughly cleaned and soaked in fresh water repeatedly and the whole plant is allowed to dry on shade. The dried plant is made into small pieces of about 2-3 cm in size, then it is powdered by mechanical grinding. The dried powdered plant material (500 g) is extracted with 2.5 litres of petroleum ether (60-80ºc) by continues hot percolation using soxhlet apparatus. This is continued upto 24 hours. Now the solvent petroleum ether was extracted out and followed by
extraction, the liquid extracts were separately concentrated under vacuum. This extract is used to proceed the following study.

2.2 Animals
Healthy albino rats of either sex of 2-2½-months-old of body weight 125-150 g were housed in polypropylene cages at 25±2°C with light dark cycle of 12 hours in the Animal House of RVS College of Pharmaceutical Sciences. It was acclimatized for seven days. All animals were given standard rat feed and water ad libitum.[5] The experiments were performed after approval of the protocol by the Institutional Animal Ethics Committee (IAEC) and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (Registration No. 1012/c/06/CPCSEA).

2.3 Drugs And Chemicals
Streptozotocin (Sigma Chemical Co., St. Louis, MO, USA), Glibenclamide, petroleum ether used in this study were collected from ponmani and co coimbatore. All other solvents and drugs used in the present study is of analytical grade.

2.4 Induction of Diabetes in Experimental Animals
Experimental diabetes was induced by single i.p. injection of 60 mg/kg of streptozotocin (STZ), freshly dissolved in cold citrate buffer (pH 4.5) after 15 min of i.p. injection of nicotinamide (110 mg/kg) prepared in normal saline. Rats with marked glycosuria (fasting blood glucose level greater than 200 mg/dL) after one week of administration of STZ were used for the study[6].

2.5 Acute toxicity test[7]
Acute toxicity study was done on healthy Wistar albino rats of both sexes weighing between 120-150 g maintained under standard laboratory conditions, according to the Organization for Economic Cooperation and Development (OECD) guidelines 423 (OECD guideline, 2002). A total of twelve animals of equal numbers of male and female rats were used and each received a single oral-dose of 1000 mg/kg body weight of extract. Animals were kept overnight fasting prior to drug administration by oral gavage. After administration of drug sample, food was withheld for further 3-4 h. Animals were observed individually at least once during first 30 min after dosing, periodically during first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days.
2.6 Evaluation Of Extract On Streptozotocin Induced Diabetic Rats

For this study we first randomly divide the diabetes induced wistar rats of both sexes weighing between 120-150 g into six groups of six rats. Also a group of six normal healthy wistar rats, six in number are used as control group.

Group I – Normal control (0.5% w/v CMC sol.)
Group II – Diabetic control (0.5% w/v CMC sol.)
Group III – Streptozotocin + Glibenclamide (10 mg/kg p.o)
Group IV – Streptozotocin+Extract (100 mg/kg)
Group V – Streptozotocin+ Extract (200 mg/kg)
Group VI – Streptozotocin+ Extract (400 mg/kg)

Group I was kept as normal control (normal animals) who receivs only the vehicle ie, 0.5% w/v CMC solution. Group II is the negative control, streptozotocin induced diabetic rats receiving vehicle, 0.5 % w/v CMC solution. Group III being the standard control is treated with glibenclamide 10 mg/kg body weight. Group IV, V, VI are induced diabetic rats treated with 100 mg/Kg, 200 mg/Kg, 400 mg/Kg of plant extract. This regimen is continued for 28 days. The body weights of the animals were checked before the study and regularly thereafter on every weeks during the study^8^.

2.7 Statistical Analysis^2^

Statistical analysis was done using one way ANOVA followed by the post hoc Dunnett’s test (SPSS version 17), where the data were compared with the control. All data points are expressed as the Mean±SD. Value of p<0.05 was considered statistically significant.

3. RESULT

3.1 Acute toxicity study

There were no Triumfetta Rhomboidea extract-treatment related mortalities recorded in animals treated with a single dose of 1000mg/kg body weight. Therefore, the approximate lethal dose (LD_{50}) of Triumfetta Rhomboidea extract in the experimental rats was higher than 1000 mg/kg. There were no clinical signs in the skin and fur, eyes and mucus membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure), autonomic effects (salivation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) among rats administered with 1000 mg/kg body weight of the extract.
Effect of extract on serum glucose level of streptozotocin induced diabetic rats

<table>
<thead>
<tr>
<th>SI NO</th>
<th>TEATMENT</th>
<th>DAY 1 (INITIAL)</th>
<th>DAY 7</th>
<th>DAY 14</th>
<th>DAY 21</th>
<th>DAY 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control (0.5% w/v CMC sol.)</td>
<td>93.0±1.7</td>
<td>82.3±1.8</td>
<td>93.0±3.0</td>
<td>93.3±2.8</td>
<td>87.0±1.5</td>
</tr>
<tr>
<td>2</td>
<td>Diabetic control (0.5% w/v CMC sol.)</td>
<td>223.0±2.5</td>
<td>242.0±2.6</td>
<td>295.0±3.4</td>
<td>344.3±5.2</td>
<td>375.0±3.2</td>
</tr>
<tr>
<td>3</td>
<td>Streptozotocin+Glibenclamide (10 mg/kg p.o)</td>
<td>231.6±1.7</td>
<td>210.6±2.9*</td>
<td>176.3±1.8***</td>
<td>140.6±2.8***</td>
<td>128.0±4.3***</td>
</tr>
<tr>
<td>4</td>
<td>Streptozotocin+Extract (100 mg/kg)</td>
<td>221.3±3.7</td>
<td>252.6±4.4</td>
<td>268.6±3.1**</td>
<td>282.0±3.0***</td>
<td>295.3±2.9***</td>
</tr>
<tr>
<td>5</td>
<td>Streptozotocin+Extract (200 mg/kg)</td>
<td>234.6±2.9</td>
<td>266.3±4.0</td>
<td>220.6±3.1***</td>
<td>230.6±3.1***</td>
<td>225.3±1.3***</td>
</tr>
<tr>
<td>6</td>
<td>Streptozotocin+Extract (300 mg/kg)</td>
<td>236.0±3.0</td>
<td>233.6±4.0</td>
<td>186.6±3.3***</td>
<td>167.0±1.5***</td>
<td>140.6±1.7***</td>
</tr>
</tbody>
</table>

Values are represented as Mean ± SEM (n=6 rats).
Values are statistically significant at *P < 0.05, ** P < 0.01, *** P < 0.001. Diabetic + extract compared with diabetic + glibenclamide and Diabetic control rats.

Effect of *Triumfetta Rhomboidea* extract on body weight of animals.

<table>
<thead>
<tr>
<th>SI NO</th>
<th>TEATMENT</th>
<th>Body Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BASELINE</td>
</tr>
<tr>
<td>1</td>
<td>Normal control (0.5% w/v CMC sol.)</td>
<td>236.3±3.2</td>
</tr>
<tr>
<td>2</td>
<td>Diabetic control (0.5% w/v CMC sol.)</td>
<td>243.3±2.4</td>
</tr>
<tr>
<td>3</td>
<td>Streptozotocin+Glibenclamide (10 mg/kg p.o)</td>
<td>245.6±2.3</td>
</tr>
<tr>
<td>4</td>
<td>Streptozotocin+Extract (100 mg/kg)</td>
<td>244.0±2.6</td>
</tr>
<tr>
<td>5</td>
<td>Streptozotocin+Extract (200 mg/kg)</td>
<td>249.0±2.0</td>
</tr>
<tr>
<td>6</td>
<td>Streptozotocin+Extract (300 mg/kg)</td>
<td>249.6±1.2</td>
</tr>
</tbody>
</table>

Values are represented as Mean ± SEM (n=6 rats).
Values are statistically significant at *P < 0.05, ** P < 0.01, *** P < 0.001. Diabetic + extract compared with diabetic and normal control rats.

The blood glucose levels on day 1 indicates the fasting blood glucose levels of animals before the study. The study results show there are significant dose-dependent reduction in animals treated with *Triumfetta Rhomboidea* extract. 300 mg/kg dose administered animals shows more reduction compared to 100 and 200 mg/kg groups. The percentage reduction observed
in 300 mg/kg administered group were 1.2%, 20%, 10.2%, 16% respectively whereas for standard glibenclamide was 9%, 16%, 20%, 8%. A similar effect to standard marketed drug (glibenclamide) was observed in 300 mg/kg extract treated group.

4. DISCUSSION
Diabetes is a health problem affecting major population worldwide. Epidemiological studies and clinical trials strongly supports the notion that hyperglycaemia is the principle cause of complications. Hence first goal towards treatment of diabetes is to reduce complications like cardiovascular risks, retinopathy, neuropathy, nephropathy and other micro vascular complications, through sustained reduction in hyperglycaemia\textsuperscript{[9]}. This study shows the efficiency of petroleum ether extract of \textit{Triumfetta Rhomboidea} in streptozotocin induced diabetic rats. Decrease in body weight in streptozotocin induced diabetic rats are a common phenomenon and it is mostly due to loss of tissue protein\textsuperscript{[10]} and muscle wasting significant increase in weight in extract treated group of rats in comparison to vehicle treated diabetic rats, indicating the petroleum ether extract of \textit{Triumfetta Rhomboidea} had beneficial effects in preventing loss of body weight of rats.

Possible hypoglycaemic effect of \textit{Triumfetta Rhomboidea} extract may be through potentiating actions of beta cells islets or stimulation of blood glucose uptake by pheriferal tissue or inhibition of endogenous glucose production or activation of gluconeogenesis in liver and muscle. More clear idea regarding mechanism of drug action needs molecular level studies.

5. CONCLUSION
We conclude that the extract and fraction of the plant tested for antidiabetic activity have shown appreciable results in decreasing the serum glucose level and other complications associated with diabetes. This research supports the inclusion of this plant in traditional antidiabetic preparations.

6. ACKNOWLEDGEMENT
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7. REFERENCES


