Research Paper

Antihyperglycemic Effects of *Tragia plukenetii* Ethanolic Extract

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ABSTRACT

Plants represent a major potential source of drugs for treating diabetes. The study of plants having antidiabetic activity may give a new approach in the treatment of diabetes mellitus. *Tragia plukenetii* is traditionally claimed to be useful in the treatment of diabetes. The present study was intended to evaluate the antihyperglycemic activity of aqueous ethanolic extract on normal fasted, glucose loaded and alloxan induced diabetic rats, at an oral dose of 75, 150 and 300 mg/kg in male Wistar rats. The alcoholic extract has not produced any hypoglycemia in normal fasted rats. The ethanolic extract has displayed a significant dose dependent antihyperglycemic activity in oral glucose tolerance test and in alloxan induced diabetic rats at an oral dose of 150 and 300 mg/kg. The ethanolic extract has effectively scavenged the stable free DPPH radical *in-vitro*. It is concluded that *Tragia plukenetii* aerial parts alcoholic extract is effective in controlling blood glucose levels in diabetic rats.

KEYWORDS: *Tragia plukenetii*, Hypoglycemia, Antidiabetic activity, Alloxan, Glucose, Ethanolic extract, DPPH.

Introduction

Plants have been the basis of many traditional systems of medicines throughout the world for thousands of years and continue to provide mankind with new remedies. Plants represent a major potential source of drugs for treating diabetes (Alarcon-Aguilar et al., 1998). Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, which affects the metabolism of carbohydrates, fats and proteins. This metabolic disorder is associated with absolute or relative deficiency in insulin action (Cunha et al., 2008). The recommendation of WHO committee on diabetes mellitus is encouraging research on hypoglycemic agents of plant origin used in traditional medicine has greatly motivated the researchers (Malalavidhane et al., 2000). In several studies treatment with traditional medicine in the form of plant extract has been reported to give remarkably good results. Available ethanobotanical information reports about 800 plants which may possess antidiabetic potential. However, most orally active hypoglycemic remedies extracted from plant materials are not scientifically evaluated and incompletely characterized.

*Tragia plukenetii* R. Smith. (Euphorbiaceae) is an herb or undershrub and commonly known as Chinnadulagondi. The ethanolic extract of *Tragia plukenetii* is reported to possess antioxidant and antitumor properties when tested in *Ehrlich ascites* carciomacells (Muthuraman et al., 2008). The aqueous juice of *Tragia plukenetii* aerial parts is claimed to be useful in treatment of diabetes at Tirupati region of Andhra Pradesh, India. *Tragia cannabina* is claimed to be a cure for diabetes in traditional medicines (Sivajothi et al., 2008). The alcoholic extract of *Tragia cannabina* was reported to possess a significant antihyperglycemic effect in streptozotocin induced diabetic rats at a dose of 250 mg/kg (Sivajothi et al., 2007). In view of the reported antihyperglycemic activity of other *Tragia* species and traditional claim, *Tragia plukenetii* is screened for antihyperglycemic activity with the aim of developing a natural antidiabetic drug.

Materials and Methods

Plant material

The aerial parts of *Tragia plukenetii* were collected from Vallur village of Kadapa district, Andhra Pradesh. The botanical identification of plant is performed by Prof. Rama Krishna, Head, Department of Botany, P.G College of Science, Hyderabad. The voucher specimen (TRP-303-09) is being maintained in Department of Phytochemistry and Pharmacognosy.

Preparation of extract

The dried aerial parts powder (470 gm) was extracted with 80% aqueous ethyl alcohol by maceration for seven days. The contents were filtered and concentrated under reduced pressure in rotary flash evaporator, yielding 9.26% of extract. The extract was subjected to

ABBREVIATIONS: DPPH- 2,2-diphenyl-1-picrylhydrazyl; CMC- Carboxy methyl cellulose
preliminary phytochemical analysis by test tube and TLC reactions (Khandelwal, 2004; Trease and Evans, 1983).

Experimental animals

The animal experimental protocol was approved by the Institutional Animal Ethics Committee of G.Pulla Reddy College of Pharmacy, Hyderabad, India. Male Wistar rats (180-200 gm) were used in the experiment and were maintained under standard environmental conditions of temperature, relative humidity and dark/light cycle with free access to standard diet and water. Animals described as fasted have been deprived of food for 16 hr but have been allowed free access to water. The alcoholic extract of *Tragia plukenetii* was administered orally as a fine aqueous suspension of 0.5% w/v of CMC to experimental animals.

Acute toxicity

To determine the acute toxicity, a single oral administration of the alcoholic extract of *Tragia plukenetii* at different doses (0.5, 1, 2, 3.0 g/kg body weight) was administered to different groups of mice. Each group consists of six animals. Control group received the vehicle. The animals were observed continuously for the initial period of 2 hr, intermittently for the next 6 hr and 24 hr and 48 hr following oral administration of different doses of drug for death and abnormality in behavioral changes (Ghosh MN, 1984).

Effect of *Tragia plukenetii* on normal fasted rats

Fasted rats were divided in to four groups of six in each, group I served as normal and received vehicle CMC. The animals of group II-IV, received the alcoholic extract of *Tragia plukenetii* at an oral dose of 75, 150 and 300 mg/kg, respectively. The blood samples were collected by retro orbital puncture under light ether anesthesia just prior to and at 1, 2 and 3rd hr after extract administration (Venkatesh et al., 2003). Plasma was separated and blood glucose levels were estimated by glucose oxidase method (Trinder, 1969), using commercially available diagnostic kit (Span Diagnostic, India) at 505 nm.

Effect of *Tragia plukenetii* on glucose tolerance test

The fasted Wistar rats were divided in to four groups of six animals each, group I served as diabetic control, received vehicle, groups II-IV animals received the alcoholic extract of *Tragia plukenetii* at an oral dose of 75, 150 and 300 mg/kg, respectively. After 30 min of extract administration, the rats of all groups were orally loaded with glucose at 2 g/kg dose. Blood samples were collected just prior to glucose administration and at 30, 60 and 120 min after glucose loading. Plasma were separated and glucose levels were measured immediately (Sachdewa et al., 2001).

Effect of *Tragia plukenetii* on alloxan induced diabetic rats

Male Wistar rats were made diabetic by single intra peritoneal injection of alloxan monohydrate, 120 mg/kg (S.D Fine chemical, India) in normal saline (Deniz et al., 2008). Five days after alloxan injection, rats with marked hyperglycemia (fasted blood glucose > 250 mg/dl) were separated and divided into three groups of six animals each. Group I served as diabetic control and received CMC. Groups II and III received alcoholic extract of *Tragia plukenetii* orally at a dose of 150 and 300 mg/kg, respectively. Blood samples were collected for the measurement of plasma blood glucose levels just prior to and at 1, 2 and 5th hr after extract administration.

Statistical analysis

All values were expressed as mean ± SEM. The results were analyzed statistically by using analysis of variance (ANOVA) followed by Dunnett’s test. Values of p < 0.05 were considered significant.

Determination of DPPH radical scavenging activity

A commercially available and stable free radical DPPH is soluble in methanol was used (Aquino et al., 2001). To 1 ml of different concentrations of alcoholic extract in methanol (10-100 µg/ml) was added to 2 ml freshly prepared methanolic solution of 90 µM DPPH and volume was made up to 4 ml with methanol. The resultant solution was incubated at room temperature for 30 min and the absorbance was measured at 570 nm. The percentage inhibition of DPPH in the reaction medium was calculated by comparing with control. All the tests were performed in triplicate and the graph was plotted with mean value. Ascorbic acid served as standard.

Results

The qualitative phytochemical analysis of *Tragia plukenetii* showed the presence of steroids and/or triterpenoids and their glycosides, tannins, carbohydrates. In oral acute toxicity test, no mortality and abnormal behavioral changes were observed in mice up to 3 g/kg body weight. Further the antihyperglycemicstudies were carried out at an oral dose of 75, 150 and 300 mg/kg.

The effect of *Tragia plukenetii* alcoholic extract on plasma glucose levels in normal fasted rats were presented in figure 1. The alcoholic extract has not produced any hypoglycemia in normal fasted rats, at all test dose levels. A rise in glucose concentration was observed in control rats after glucose load. The ethanolic extract of *Tragia plukenetii* produced a significant antihyperglycemic activity in glucose loaded rats at all three tested dose levels. The maximum activity was observed at 60 min after glucose administration. The percent decrease in glucose levels in comparison to control rats was found to be 34.16, 40.94 and 47.71 of 75, 150 and 300 mg/kg, respectively. The mean serum glucose levels were decreased to initial (pre glucose) levels in animals treated with alcoholic extract with a dose of 300 mg/kg. However, there is no decrease in blood glucose levels were observed with test doses of 75 and 150 mg/kg. Similarly there is no activity is observed after 30 min of drug administration. Effect of ethanolic extract.
of *Tragia plukenetii* aerial parts on glucose tolerance test in rats were presented in Table 1.

![Blood Glucose Levels](image)

**Fig. 1.** The effect of *Tragia plukenetii* alcoholic extract on plasma glucose levels in normal fasted rats.

The plasma glucose levels markedly raise over three folds in diabetic control when compared to normal rats in alloxan induced diabetic rats. The fasting blood glucose levels in diabetic rats were 365-375 mg/dl. The results were presented in Table 2. The ethanolic extract of *Tragia plukenetii* has produced a significant dose dependent antiglycemic activity. The significant antiglycemic activity was observed 1 hr after drug administration. The maximum percent decrease in glucose concentration is observed at 3rd hr after drug administration. The percent reduction in glucose levels at 3rd hr is 16.85 and 27.22 of 150 and 300 mg/kg of *Tragia plukenetii*, respectively. In the untreated diabetic animals the blood glucose levels did not change significantly.

DPPH is commercially available free radical and widely used to determine the scavenging capability of drugs. The IC₅₀ value of standard ascorbic acid is higher than the *Tragia plukenetii* extract, the concentration of alcoholic extract in 50% inhibition of free radical is 7 µg/ml, the standard ascorbic acid showed 4.4 µg/ml (Figure 2).

### TABLE 1

Effect of ethanolic extract of *Tragia plukenetii* aerial parts on glucose tolerance test in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood Glucose Concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial 30 min 60 min 120 min</td>
</tr>
<tr>
<td>I</td>
<td>Control - Untreated</td>
<td>79.36 ± 1.26 108.45 ± 1.92 192.23 ± 1.8 108.52 ± 1.82</td>
</tr>
<tr>
<td>II</td>
<td>ETOH Extract (75 mg/kg)</td>
<td>92.81 ± 1.86 172.36 ± 1.7*** 126.56 ± 1.45*** (34.16) 116.41 ± 1.59***</td>
</tr>
<tr>
<td>III</td>
<td>ETOH Extract (150 mg/kg)</td>
<td>82.75 ± 1.61 131.22 ± 1.45*** 113.53 ± 1.81*** (40.94) 129.38 ± 1.05***</td>
</tr>
<tr>
<td>IV</td>
<td>ETOH Extract (300 mg/kg)</td>
<td>79.51 ± 1.57 99.65 ± 1.38** (8.11) 100.50 ± 1.82*** (47.71) 86.72 ± 1.05*** (20.08)</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M.; n = 6, *p < 0.05, **p < 0.01, ***p < 0.001 vs. control. Figures in parenthesis indicate % decrease in blood glucose level.

![DPPH Radical Scavenging Activity](image)

**Fig. 2.** DPPH radical scavenging activity.

DPPH radical scavenging activity of (a) *Tragia plukenetii*, (b) ascorbic acid.
TABLE 2
Effect of ethanolic extract of Tragia plukenetii aerial parts on alloxan induced diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood Glucose Concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>1st hr</td>
</tr>
<tr>
<td>I</td>
<td>Diabetic Control</td>
<td>275.38 ± 3.4</td>
</tr>
<tr>
<td>II</td>
<td>ETOH extract (150mg/kg)</td>
<td>270.41 ± 2.9</td>
</tr>
<tr>
<td>III</td>
<td>ETOH extract (300mg/kg)</td>
<td>265.28 ± 3.0</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M.; n = 6. *p < 0.05, ** p < 0.01, ***p < 0.001 vs. control. Figures in parenthesis indicate % decrease in blood glucose level.

Discussion
The aqueous juice of Tragia plukenetii aerial parts is claimed to be useful in treatment of diabetes. Results of hypoglycemic activity of Tragia plukenetii alcoholic extract presented here may help to establish a scientific basis for the utility of this plant in the treatment of diabetes. The present study indicates that alcoholic extract at an oral dose of 150 and 300 mg/kg has produced a dose dependent activity in glucose tolerance test and alloxan induced diabetic rats.

In the present investigation three different test models were used to assess the sensitivity of the evaluation procedure. These models allow us to examine the effects of test dose against different background of sugar levels. The oral route of administration was preferred as it is simple and physiological. Male Wistar rats were chosen for the experiment, because blood sugar levels of rats remains stable during experiment.

Administration of Tragia plukenetii alcoholic extract did not show any significant hypoglycemic effect in normal fasted rats at all three test dose levels. Usually synthetic drugs and insulin produces hypoglycemia on normal glucose levels. The results of present study indicate the advantage of Tragia plukenetii over side effects of synthetic drugs in regards to hypoglycemic property on normal glucose levels. The ethanolic extract of Tragia plukenetii significantly protected the glucose challenged animals, with maximum protection at 90 min after drug administration. The antihyperglycemic property was sustained even at 120 min, in animals received 300 mg/kg of test extract. The observed antihyperglycemic property of ethanolic extract may be due to potentiating the pancreatic secretion or increasing the glucose uptake.

Alloxan monohydrate is one of the most widely used chemical diabetogen, and alloxan induced diabetes in laboratory animals has become a valuable tool in diabetic research. The effect of ethanolic extract of Tragia plukenetii on alloxan induced diabetic rats was tested at 150 and 300 mg/kg dose levels. The maximum activity was observed with test dose of 300 mg/kg and 27.22 percent reduction of blood glucose levels was observed at 3rd hr. The diabetogenic property of alloxan is due to free radical production and tissue injury. Antioxidants have direct effect in scavenging the free radicals. In the present study the ethanolic extract of Tragia plukenetii effectively scavenges the free radicals of DPPH with increased concentrations. It is generally accepted that alloxan treatment causes destruction of beta cells (Halliwaee et al., 1985; Pari et al., 1999). It is therefore, conceivable that hypoglycemic principles in the Tragia plukenetii alcoholic extract exert their effect could be due to an enhancement of peripheral metabolism of glucose or even if an increase insulin release cannot be excluded.

The phytochemical analysis of Tragia plukenetii extract revealed the presence of steroids and/or triterpenoids and their glycosides, tannins and carbohydrates. Tannins or phenolic compounds are widely known for their antioxidant properties in scavenging the free radicals. The observed antihyperglycemic activity of Tragia plukenetii may be attributed to tannins, because earlier reports reveals that a tannin, epicatechin (Chakravarthy et al., 1982) and tannins of Pterocarpus marsupium (Chakravarthy et al., 1980) have pronounced antihyperglycemic activity by stimulation and generation of beta cells to secrete insulin. However, the role of steroids and/or triterpenoids and their glycosides as antihyperglycemic principles cannot be ruled out. In conclusion, this study has been able to demonstrate the antihyperglycemic potential of Tragia plukenetii alcoholic extract in diabetic rats.

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References


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