RESEARCH ARTICLE

Anti-diabetic activity of leaf extract of *Tridax procumbens*

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A study of ancient literature indicates that diabetes was fairly well known and well conceived as an entity in India. Plant-based drugs have been used against various diseases since a long time. The nature has provided abundant plant wealth for all the living creatures, which possess medicinal virtues. The essential values of some plants have long been published, but a large number of them have remained unexplored to date. Therefore, there is a necessity to explore their uses and to conduct pharmacognostic and pharmacological studies to ascertain their therapeutic properties. In fact, nowadays, diabetes is a global problem. Hence, the present study aims to open new avenues for the improvement of medicinal uses of *Tridax procumbens* (Compositae) for the selected area for diabetes. Another important objective of such a study is to bring the anti-diabetic medicinal plants sector on a firm scientific footing, raise awareness and add value to the resource. Dried aqueous, alcoholic, and petroleum ether (60-80°C) extracts of leaves of *Tridax procumbens* were subjected for hypoglycaemic activity in Wistar rats (150-200 g). Blood sugar level was determined using digital glucometer. The oral administration of leaf extracts at doses of 200 mg kg^{-1} lead to a significant blood glucose reduction. This laid the foundation to study the active compounds of such anti-diabetic plants that are responsible for the hypoglycemic activities. It also proves the traditional claim of Mandesh region with regard to *Tridax procumbens* for its anti-diabetic activity.

Key words: Alloxan-induced diabetes, hypoglycaemic activity, *Tridax procumbens*

INTRODUCTION

A study of ancient literature indicates that diabetes (Madhumeha/Prameha) was fairly well known and well conceived as an entity in India. The knowledge of the system of diabetes mellitus, as the history reveals, existed with the Indians since prehistoric age. 'Madhumeha' is a disease in which a patient passes sweet urine and exhibits sweetness all over the body, i.e., in sweat, mucus, breathe, blood, etc. The practical usage of juices of various plants achieved the lowering of blood glucose by 10-20%.[1] Diabetes mellitus occurs throughout the world; however, it is more common in the more developed countries. Diabetes is in the top 10, perhaps in the top 5, of the most significant diseases in the developed world and is still gaining significance.[2]

Therefore, it is advised to allow such remedial measures as supplements to other modes of therapy. Plant-based drugs have been used against various diseases since long time. The primitive man used herbs as therapeutic agents and medicament, which they were able to procure easily. The nature has provided abundant plant wealth for all living creatures, which possess medicinal virtues. The essential values of some plants have long been published; however, a large number of them remain unexplored as yet. Therefore, there is a necessity to explore their uses and to conduct pharmacognostic and pharmacological studies to ascertain their therapeutic properties. In fact, nowadays, diabetes is a global problem. The present study aims to open new avenues for the improvement of medicinal uses of *Tridax procumbens* (Compositae) for diabetes. Further, information regarding the traditional phytotherapy (Mandesh region) is obtained, which provides the base for clinical research to study the active compounds of such anti-diabetic plants. Another important objective is to bring the anti-diabetic medicinal plants sector on a firm scientific footing, raise awareness, add value to the resource and contribute to the socio-economic well being of our country particularly on the national and international levels.

A weed named *Tridax procumbens* Linn. (compositae) present throughout India and is employed as indigenous medicine for a variety of ailments, including jaundice.[3] It is commonly known as 'Ghamra' and in English popularly called 'coat buttons' because of the appearance of its flowers. It has been extensively used in Indian traditional medicine as anticoagulant, antifungal and insect repellent; in bronchial catarrh, diarrhoea and dysentery.[4] Moreover, it possesses wound healing activity and promotes hair growth.[5] *Tridax procumbens* is also dispensed as 'Bhringraj', which is well known Ayurvedic medicine for liver disorders.[6] Antioxidant properties[16] have also been demonstrated. The

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Table 1: Acute toxicity studies of extract for 48 h

Group	Dose (mg)	Extract toxicity (48 h)					
		Motor reflex			Behaviour pattern		
		Aqueous	Alcoholic	Petroleum ether	Aqueous	Alcoholic	Petroleum ether
Test	200	Normal	Normal	Normal	Normal	Normal	Normal
Control	-	Normal	Normal				

Table 2: Acute toxicity studies of extract for 72 h

Group	Dose (mg)	Extract toxicity (72 h)					
		Motor reflex			Behaviour pattern		
		Aqueous	Alcoholic	Petroleum ether	Aqueous	Alcoholic	Petroleum ether
Test	200	Normal	Normal	Normal	Normal	Normal	Normal
Control	-	Normal	Normal				

present study aims at studying the hypoglycaemic activity of leaf extract of *Tridax procumbens* in the model of alloxan-induced diabetes in rats.

MATERIALS AND METHODS

Plant Material

Tridax procumbens leaves were collected from Mandesh region (Atpadi, Dist: Sangli, M.S., India). Botanical identification was performed by Dr. Madhukar Bachulkar, Department of Botany, Vivekanand College, Kolhapur.

Extraction Procedure: The leaves of *Tridax procumbens* were dried under shade and grinded in electrical grinder to coarse powder. Powdered leaves (2,5 kg) were extracted for 24 h each with aqueous (chloroform water), alcohol, and petroleum ether (60-80°C) and dried under reduced pressure and tested on alloxanised hyperglycaemic animals.[7]

Drugs

Alloxan monohydrate was purchased from Sigma chemicals (St Louis, USA). All other chemicals used for this study were of analytical grade

Animals

Wistar rats (200-225 g) of either sex were employed in this study. The rats were maintained under standard laboratory conditions at 25 ± 2°C, relative humidity 50 ± 15% and normal photo period [12 h dark/12 h light] were used for the experiment. Commercial pellet diet [Chakan oil mill, Sangli, India] and water were provided *ad libitum*. The experimental protocol has been approved by the Institutional Animal Ethics committee and by the Regulatory body of the government

Toxicity Studies

Acute and chronic toxicity studies were carried out.[8] Animals of either sex were fasted for 18 h and used. In acute toxicity studies, a dose of 250 mg/kg of dried extract were orally administered to 12 mice; additionally, 3 mice were kept as control. Then, they were observed for motor reflexes for 48 h. Since no mortality was observed and the behavioural pattern was unaffected, further studies were carried out [Tables 1 and 2]. In chronic toxicity studies, 22 animals were used. They were divided into two groups: 6 as control and 16 as test animals. In the test group, a dose of dried extract of *Tridax procumbens* of 250 mg/kg was administered daily for a period of 15 days. The body weights were recorded for both the groups at an interval of 5 days. Finally, the haematological parameters were studied in both the groups [Tables 3 and 4].

Table 3: Chronic toxicity studies

Time in days	Average body weight (g)	
	Test	Control
05	24.58	24.48
10	25.02	25.10
15	25.28	25.38

Table 4: Haematological parameters

Parameters	Test	Control
Bleeding time (min)	4.15 ± 0.09	4.16 ± 0.07
Clotting time (s)	38.90 ± 0.02	39.0 ± 0.07
Total WBC/mm^3	6010 ± 0.11	6067 ± 0.13
Total RBC/mm^3	8.52 × 10^6 ± 0.75	8.82 × 10^6 ± 0.02
Haemoglobin (mg/dl)	15.0 ± 0.06	15.1 ± 0.00

Experimental Design

Diabetes was induced using alloxan monohydrate (100 mg/kg). Only alloxanised hyperglycaemic animals were used for further studies. Animals were fasted for 18 h before the experiment and divided into 5 groups (6 animals in each group). The first group (control) received normal saline and the second group received alloxan monohydrate alone. The three test groups received 200 mg/kg of different extracts before the dose of alloxan. All the animals were regularly observed for their general behaviour.

Effects on Blood Glucose Levels

Dried aqueous, alcoholic, and petroleum ether (60-80°C) extracts of leaves of *Tridax procumbens* (200 mg/kg) were suspended in 1% bentonite and subjected for hypoglycemic activity in Wistar rats (200-225 g). Diabetes was induced by the intravenous administration of alloxan (100 mg/kg) [9,10] after anaesthesia with ethyl ether. Forty-eight hours later, the blood (1 mL) was collected from the orbital sinus into tubes and immediately used for the determination of glucose. Only animals that presented with glycaemic levels equal to or above 200 mg/dL were submitted to treatments, which consisted of a daily administration of extracts of leaves of *Tridax procumbens* for 7 days. The oral treatments (by gavage) of all groups were carried out at the same time (in the morning) and under the same conditions. One hour after the last administration, the blood was collected again for blood glucose measurements using a glucometer.

Statistical Analysis

All the values were expressed as mean ± Standard error mean (SEM). The differences were compared using one-way analysis of variance (ANOVA) followed by Dunnet's t test. *P* values < 0.01 were considered as significant.

RESULT

The effect of oral administration of aqueous, alcoholic and petroleum ether extracts of *Tridax procumbens* leaves are shown in Table 5. Experimental studies reveals that the aqueous and alcoholic extracts from *Tridax procumbens* leaves (200 mg/kg) orally administered for 7 days produced a significant decrease in the blood glucose level in the model of alloxan-induced diabetes in rats. Petroleum extract exhibits a very weak anti-diabetic activity.

DISCUSSION

Alloxan causes diabetes through its ability to destroy the insulin-producing beta cells of the pancreas.[11,12] *In vitro* studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to the induction of cell necrosis.[13,14] The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of beta cells.[15] Experimental studies revels that the aqueous and alcoholic extracts from *Tridax procumbens* leaves (200 mg/kg) orally administered for 7 days produced a significant decrease in the blood glucose level in the model of alloxan-induced diabetes in rats. Petroleum extract exhibits very weak anti-diabetic activity. It also proves the traditional claim with regard to *Tridax procumbens* for its anti-diabetic activity.

Table 5: Effect of aqueous, alcoholic and petroleum ether extract of *Tridax procumbens* leaves

Group	Blood glucose level (mg %)	
	Before treatment	After treatment
Control	93.27 ± 0.014	93.26 ± 0.013
Alloxan control	92.92 ± 0.016*	373.53 ± 0.027*
Aqueous extract	93.39 ± 0.012*	99.81 ± 0.018*
Alcoholic extract	92.68 ± 0.017*	93.72 ± 0.024*
Petroleum ether extracts	94.23 ± 0.031*	273.74 ± 0.225*

n = 5, all values are expressed as mean ± SEM, *P* < 0.01 was considered statistically significant as compared to 0 h of their respective groups

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