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RESEARCH ARTICLE

Anti-Hyperglycemic Activity of *Tridax Procumbens* Root Extract in Streptozotocin Induced Hyperglycemia in Rats

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ABSTRACT

Tridax procumbens L (asteraceae) have been used, traditionally, for the treatment of various disorders of cancer, wound healing, diabetes, protective activity. The present study was to investigate the possible anti-hyperglycemic activity of *Tridax procumbens* root [TPR] extracts in streptozotocin induced hyperglycemia in rats. Methanolic extract were administered to the streptozotocin (STZ) [55mg/kg, body weight, intra peritoneal (i.p)] induced hyperglycemia rats for 21 days to study anti-hyperglycemic activity. Blood was collected from retro orbital puncture using capillary tubes. Serum obtained by immediate centrifugation of blood samples using remi ultra cooling centrifuge at 3000 rpm for 15 minutes at room temperature and was directly used for estimating serum glucose. The acute toxicity values of methanol extract after oral administration in mice were found to be 5000 mg/kg. The results concluded that *Tridax procumbens* methanolic extract (500 mg/kg) have greater anti-hyperglycemic activity than aqueous extract in streptozotocin induced hyperglycemia model and when compared with Glibenclamide treated group. Hence to conclude that methanolic extract of *Tridax procumbens* root has potent anti- hyperglycemic activity in streptozotocin induced diabetic rats.

KEYWORDS

Anti-Hyperglycemia, Tridax procumbens root, streptozotocin, methanolic extract

INTRODUCTION

Diabetes mellitus is a serious disorder with a number of vascular complications. An ancient periodic literature indicates that diabetes was well known in India[1]. Diabetes mellitus is a disease in which a patient passes sweet urine and exhibits sweetness all over the body. Diabetes mellitus is the most significant disease and gaining importance throughout the world.

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Diabetes mellitus type 2 (formerly noninsulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes) is a metabolic disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency. This is in contrast to diabetes mellitus type 1, in which there is an absolute insulin deficiency due to destruction of islet cells in the pancreas. The classic symptoms are excess thirst, frequent urination, and constant hunger. Type 2 diabetes makes up about 90% of cases of diabetes with the other 10% due primarily to diabetes mellitus type 1 and gestational diabetes. Obesity is thought to be the

primary cause of type 2 diabetes in people who are genetically predisposed to the disease.

An increase in ageing population, the daily consumption of food materials rich in more fat and more calories and obesity are the common factors for the development of diabetes[2]. In addition to restriction of energy intake and exercise promotion, the usefulness of functional foods and herbal medicines during the daily life has been shown. For this purpose, several studies on functional foods and medicinal plants as well as their active components have been carried out to ascertain their usefulness in controlling the diabetes and their complications[3,4].

For the controlling of the diabetes mellitus a few herbs are natural remedies with higher safety and efficacy. In India a number of varieties of medicinal herbs because of varieties of climatic conditions and favorable for growth of many plant varieties.

Among the large number of herbal drugs in India, *Tridax Procumbens* is a highly valuable weed and it is present with a more number of pharmacological activities like hepato protective activity, anti-inflammatory, wound healing, antidiabetic activity, dysentry, and antimicrobial activity against both gram positive and gram negative bacteria. The leaf extract juice shows anti-septic, insecticidal, parasiticidal propertis.

Tridax procumbens leaves are used traditionally for diabetes activity[5,6,7]. There is no scientific study reported about the root system of *Tridax procumbens*. Therefore present study was under taken to establish the acute toxicity study and scientifically evaluate the anti-hyperglycemic activity of the methanol extracts of *Tridax procumbens* root (TPR) in streptozotocin induced hyperglycemia model in Albino rats.

Streptozotocin (STZ) is a naturally occurring chemical particularly toxic to the insulin producing beta cells of pancreas in mammals. It is used in medicine for treating certain cancers of the islets of langerhans and used medical research to produce an animal model for type-1 diabetes in large dose as well as type 2 diabetes with multiple doses. Streptozotocin is similar enough to glucose to be transported into the cell by the glucose transport protein GLUT2; this explains its relative toxicity to beta cells.

MATERIALS AND METHOD

Plant Material

The fresh roots of Tridax procumbens L were collected from Chithur district (A.P) in the months of Dec-Jan. The trailing herb was authenticated by Dr. K. MadhavaChetty, Dept. of Botany, Sri Venkateswara University, Tirupati, and A.P. Voucher specimen number 558-5 (dated 09-03-2013). The roots are crushed and air dried under shadow and then grounded into coarsely powdered in grinder and powder material was passed through 120 meshes to remove fine powders and coarse powder was used for extraction.

Protocol for Successive Extraction

The coarse powder of *Tridax procumbens* root (100 gm) was extracted by using successive soxhlet extraction using methanol for 72 hrs. After completion of extraction, solvent was distilled off and concentrated extract was air-dried[8]. Methanol extract was mixed with 0.5% Sodium carboxy methyl cellulose (CMC) and which was used for the anti-hyperglycemic activity.

Phytochemical Screening

The crude extract obtained by using various solvents was analyzed for alkaloids, carotenoids, saponins, flavonoids, and phenolic compound using standard procedure of analysis[9].

Chemicals

Streptozotocin was purchased from Albino labs, Hyderabad, India. Glucose kit (GOD-POD Method), were purchased from Classic Enterprises, Rajahmundry-533296, India. All solvents used for extraction purchased from Classic Enterprises, Rajahmundry-533296, India.

Animal

Wistar albino male rats weighing 200-220g and

albino mice (Either sex) weighing 20-25g were selected and housed in polypropylene cages in a room where the congenial temperature was 27°C ±1°C and 12 hrs light and dark cycles were maintained. The animals were allowed to acclimatize to the environment for 7 days and supplied with a standard pellet diet (VRK nutritional solution, Pune) and water *ad libitum*.

Acute Toxicity Study

Albino mice (Either sex) of 10 animals per group and weighing 20-25 g were administered graded dose (100-5000 mg/kg body weight, p.o.) of the methanolic extracts of TPR. After administration of the extract the mice were observed for toxic effects for 48 hr. The toxicological effects were observed in terms of mortality expressed as LD_{50}. The number of animals dying during a period was noted. The LD_{50} of the extract was determined by Litchfield and Wilcoxon (Litchfield and Wilcoxon, 1949) method[10].

Anti-hyperglycemic Activity

Hyperglycemia was induced in Wistar albino male rats by single intraperitoneal injection of streptozotocin (55 mg/kg, body weight, i.p) in citrate buffer solution pH 4.8 after overnight fasting for 18 hours[11,12]. The rats were divided into five groups of six rats in each group and were treated with single dose/day, *per os* (p.o.) of standard drug or extracts of TPR. The first group was given Standard pellet diet, 0.5% Sod.CMC, and water (Served as normal control). The second group was given a single dose of streptozotocin (55 mg/kg), i.p. After 72 hours of streptozotocin injection, this group received a daily dose of 0.5 % Sod. CMC (p.o.). Third group was administered with Standard Glibenclamide 10 mg/kg p.o. for 21 days (served as standard). The fourth and five group was administered a daily dose of TPR Methanol extract at a dose 300 mg/kg and 500 mg/kg suspended in 0.5% Sod. CMC, body weight, p.o. (served as treatment groups) for 21 days, after inducing hyperglycemia.

Collection of Blood Samples

On 1st, 7th, 14th, 21st day blood was collected by retro orbital sinus puncture, under mild ether anesthesia in plane capillary tubes. Serum obtained by immediate centrifugation of blood samples using remi ultra cooling centrifuge at 3000 rpm for 15 minutes at room temperature and was directly used for estimating serum glucose. All samples were stored at 4°C until analysis.

Biochemical Analysis

Serum glucose levels were carried out using respective diagnostic commercial kits (Transasia Bio-medicals Ltd, Solan, India) in semi auto analyzer.

Statistical Analysis

The results were expressed as mean ± SEM. The Streptozotocin control was compared with normal and the experimental results were compared with Streptozotocin control. Data were evaluated with GRAPH PAD PRISM 6 software hypothesis testing methods include t-test and one-way ANOVA test. Differences below $P<0.05$ implied statistically significance.

RESULTS

Phytochemical Screening

Table 1: Phytochemical screening of *Tridax procumbens root* extract

Chemical constituents	Methanolic extract
Carbohydrates	++
Tannins	++
Alkaloids	-
Saponins	++
Flavonoids	++
Glycosides	+
Steroids	++
Oleonic acid	++
Calcium	+

NOTE: +: Presence of Chemical constituents, -: Absence of Chemical constituents, ++: Maximum Presence of Chemical constituents.

The results of phytochemical screening in table 1. Indicated the presence of maximum amount of Carbohydrate, Flavonoids, Tannins, Saponins, steroids is present in Methanolic

extract contains Carbohydrate, Flavonoids, Saponin and Oleonic acid.

Acute Toxicity Study

The acute toxicity studies of chloroform, methanolic extract of the TPR were found to be non-toxic up to the dose 5000 mg/kg and did not show any mortality.

Anti-hyperglycemic Activity Methodology

The results of present study are given in Table 2. The rats treated with streptozotocin showed significant increase in serum glucose level from 70 mg/dl to 120 mg/dl in rats. Treatment with TPR methanolic extract at the doses of 300 mg/kg and 500 mg/kg reduced the serum glucose levels when compared to the control and standard group rats.

Induction of diabetes with STZ is associated with a characteristic loss of body weight, which is due to increased muscle wasting[15] and loss of tissue proteins[16]. Diabetic rats treated with the *Tridax procumbens root* methanolic extract showed an increase in body weight as compared to the diabetic control, which may be due to its effect in controlling muscle wasting, i.e., by reversal of antagonizing[17].

Tridax procumbens methanolic extract (500 mg/kg, body weight, p.o) has shown more anti-hyperglycemic activity than extraction of (300mg/kg, body weight, p.o.) compared to control and standard (Glibenclamide) groups in streptozotocin (55 mg/kg) induced diabetic rats.

Table 2: Showing Decrease in Blood Glucose Level in All Extracts

Sr.no	Groups	Conc.	blood glucose levels (mg/dl)			
			0day	7day	14day	21 Day
1	Control	0.5% CMC	115.3±1.03	118.6±0.30	117.9±0.48	124.0±2.18
2	Diabetic+ control	0.5% CMC	367.0±1.24	369.4±1.69*	364.9±2.06*	368.9±0.40*
3	Diabetic+standard	10mg/kg	373.2±2.70	210.5±2.40*	173.6±1.70*	105.3±1.28*
4	Diabetic +TPME	300mg	373.3±1.90	194.5±1.50*	178.2±0.40*	133.9±0.40*
5	Diabetic +TPME	500mg	370.0±0.40	173.3±0.30*	143.6±0.12*	110.5±0.23*

TPME: *Tridax procumbens* Root Methanolic Extract;
Values are expressed as Mean ± SEM of 6 rats in each group.
*P < 0.05 is compared to control group.

DISCUSSION

The present work has detected the antidiabetic effect of the methanolic extract of tridax procumbens root in streptozotocin induced type-II diabetic rats. Streptozotocin injection caused diabetes mellitus, probably due to destruction of the β-cells of the islets of Langerhans of the pancreas[13]. Over-production of glucose and decreased utilization by the tissues form the fundamental basis of hyperglycemia in diabetes mellitus[14].

CONCLUSION

The methanolic extract of the roots of tridax procumbens has antidiabetic activity as it lowers serum glucose levels in diabetic rats. Hence to conclude that methanolic extract of *Tridax procumbens* roots have potent anti-hyperglycemic activity.

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