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INVESTIGATION FOR POSSIBLE ANTIDIABETIC EFFECTS OF TRIDAX PROCUMBENS IN RATS

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Abstract:
Tridax procumbens have different medicinal properties due to its active phytochemical constituents and May able to treat diabetes & diabetics complications. Ethanolic extracts of Tridax procumbens was prepared from plant parts are subjected to acute oral toxicity studies and found that the ethanolic extract of Tridax procumbens is safe to use up to the dose of 2000mg/kg. The ethanolic extract of Tridax procumbens was found to be in dose dependent way against alloxan induced diabetes in rats. The reduction of the elevated blood glucose levels in diabetic rats on treatment with the extract at two different concentrations confirmed that ethanolic extract of Tridax procumbens posses Antidiabetic activity & has shown significant effect when compared to Alloxan administration. It needs comprehensive investigations for developing a safe and effective herbal drug. Further research is required to isolate the biomolecules responsible for the antidiabetic and antidiabetic complications.

Keywords: *Tridax procumbens, ethanolic extracts, anti diabetic etc.*

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INTRODUCTION:

Diabetes mellitus is one of the most common endocrine diseases in all populations and all age groups. It is a syndrome of disturbed intermediary metabolism caused by inadequate insulin secretion or impaired insulin action, or both. Diabetes mellitus comprises of heterogeneous group of disorders characterized by hyperglycemia, altered metabolism of carbohydrates, lipids and proteins. Diabetes mellitus is associated with complications such as nephropathy, retinopathy, neuropathy and cardiovascular disease. [1]

Diabetes is mainly classified into three types as: Type-I (Insulin-Dependent Diabetes Mellitus, IDDM) and Type-II (Non- Insulin-Dependent Diabetes Mellitus, NIDDM), Type-III(Gestational diabetes. Both these types are associated with excessive morbidity and mortality. Type I diabetes accounts for 5% to 10% of diabetes, usually occurs in children or young adults. This disease is caused by autoimmune destruction of the pancreatic β-cells that secrete insulin. The process involves a smoldering destructive process that can persist for several years and ultimately leading to failure of insulin secretion. Patients with type I diabetes require insulin therapy for survival and most patients ultimately develop devastating complications of this disease. [1]

Type II diabetes accounts for 90% to 95% of all patients with diabetes and is increasing in prevalence. Some of the known environmental factors that contribute to development of type–II diabetes are obesity, a sedentary lifestyle, and aging. Insulin resistance is a characteristic metabolic defect in the great majority of patients with type II diabetes. As a consequence of insulin resistance, the β-cell produces increased amounts of insulin, and, if sufficient, the compensatory hyperinsulinemia maintains glucose levels within the normal range.

Tridax procumbens, commonly known as coat buttons or Tridax daisy, is a species of flowering plant in the daisy family. It is best known as a widespread weed and pest plant. It is native to the tropical Americas, but it has been introduced to tropical, subtropical, and mild temperate regions worldwide. It is listed as a noxious weed in the United States and has pest status in nine states. The plant is rich in minerals such as iron, copper, manganese, sodium and zinc and other trace minerals such as magnesium, phosphorous, potassium, selenium and calcium[2]. The aqueous extract contains phytochemicals such as alkaloids, steroids, carotenoids, flavonoids (catechins and flavones), saponins and tannins. While organic solvent extraction with ethyl acetate has flavonoids (centaureidin and centaurein) and bergenin. Some of the 2° metabolites present are fatty acid derivatives, sterols, lipid constituents, luteolin, glucoluteolin, quercetin, isoquercetin and fumaric acid[3].

MATERIALS:

Sodium citrate, Diethyl ether, Methanol, Normal saline, Formaldehyde, Chloroform, Alloxan monohydrate, Metformin all are provided by Vyas labs-Medchal, Hyderabad.

METHODOLOGY:

Collection and Authentification of Plant Material:
The Leaves powder of *Tridax procumbens* is purchased from amazon (Shrisha Herbal Jayanti Veda sold by Shrisha Organics.)

Animal Ethics permission:
The housing of the animals were carried out in the animal house of the Teja College of pharmacy-kodad, the treatment and sample collection, analysis of samples carried out in VYAS LABS, Medchal, Malkajgiri with approved CPCSEA registration number-2085/PO/RCBIBT/S/19/CPCSEA

Extraction of Plant Material:
The plant is grinded in to a coarse powder with the help of suitable grinder.

Cold Extraction (Ethanol Extraction)[8]:
In this work the cold extraction process was done with the help of ethanol. About 200gms of powdered material was taken in a clean, flat bottomed glass container and soaked in 750 ml of ethanol. The container with its contents were sealed and kept for period of 7 days accompanied by continuous shaking with the shaker. The whole mixture then went under a coarse filtration by a piece of a clean, white cotton wool.

Evaporation of Solvent:
The filtrates (ethanol extract) obtained were evaporated using Rotary evaporator in a porcelain dish. They rendered a gummy concentrate of greenish black. The extract was kept in vacuum desiccator for 7 days.

Preliminary Phytochemical Screening:
Preliminary phytochemical screening of the *Tridax procumbens* extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, Flavonoids, as per the standard methods [4, 10, 11].

Animals:
Healthy Adult Male wistar rats of 8-10 weeks old with Average weight in the range of 150-180gms were selected. Animals are housed 4 per cage in temperature controlled (27 ^{0}C ±3 ^{0}c) room with light/dark cycle in a ratio of 12:12 hrs is to be maintained. The Animals are allowed to acclimatize to the environment for seven days and are supplied with a standard diet and water *ad libitum*. The prior permission was sought from the Institutional Animal Ethics Committee (IAEC) for conducting the study.

Acute toxicity studies:
The Acute oral toxicity test of the extracts was determined prior to the experimentation on animals according to the OECD (Organization for Economic Co-operation and Development) guidelines no 423. Female Albino wistar rats were taken for the study and dosed once with 2000 mg/kg of the extract. The treated animals were monitored for 14 days to observe general clinical signs and symptoms as well as mortality. No mortality was observed till the end of the study revealing the 2000 mg/kg dose to be safe. Thus, 1/10 and 1/20 doses of 2000 mg/kg i.e. 100 mg/kg and 200 mg/kg were chosen for subsequent experimentation.

Induction of diabetes:
Diabetes mellitus or hyperglycemia was induced in rats by administration of alloxan monohydrate (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-primidine tetr-one) at dose of 120mg/kg intraperitoneally in normal saline. After one hour of alloxan administration the animals were given feed ad libitum. The animals were kept fasting overnight and blood glucose levels were estimated before and after 72hrs of alloxan treatment. Animals showing blood glucose levels of >200mg/dl is considered as diabetic and were used for study [12].

Experimental Study Design:
Diabetic rats were divided in to five groups with four animals each.
Group-I: rats served as normal control group
Group-II: served as diabetic/disease control.
Group-III: Diabetic rats treated with *Tridax procumbens* plant extract at a dose 120mg/kg(low dose).
Group-IV: Diabetic rats treated with *Tridax procumbens* plant extract at a dose of 200mg/kg (high dose).
Group V: Diabetic rats treated with Metformin (standard drug) at 450mg/kg.
The treatment was given for 14days and blood samples were collected at different intervals.

EVALUTION PARAMETER:
Glucometer Method: the newly popular method which is used to check blood glucose levels by using digital glucometer. In this method the tail of the animal is first sanitized and cleaned then by tail pricking method one drop of blood is collected by the use of a micropipette and the blood sample is placed on the strip of the digital glucometer and the readings are noted.

Waste disposal:
Wastage removed regularly and frequently in a safe and sanitary manner and will be incinerated, animal tissues, carcasses also incinerated if they have to be stored they will be packed in a leak proof plastic bag and stored in required temperature avoiding decomposition and contamination, if hazardous chemicals used first they are neutralized and disposed.

STATISTICAL ANALYSIS:
All the values will be expressed as mean ±standard deviation (S.D). Statistical comparisons between different groups will be done by using one way analysis of variance .P value <0.05 will be considered as statistically significant.

RESULTS & DISCUSSION:
% Yield of ethanolic Extract from Aerial Parts of *Tridax procumbens* was found to be **26.77%**

Preliminary Phytochemical Screening:
Investigation revealed the presence of Alkaloid, Tannin, Saponin, Phenol in Ethanolic Extract of *Tridax procumbens* while only Phenol were present in Phenolic Extract of *Tridax procumbens*

Table no.1. Preliminary Phytochemical Screening

Phytochemical	Results
Steroid	-
Alkaloid	+
Tannin	+
Carbohydrate	-
Phenol	+
Flavonoid	+
Saponin	+

(+) Present. **(-)** Absent

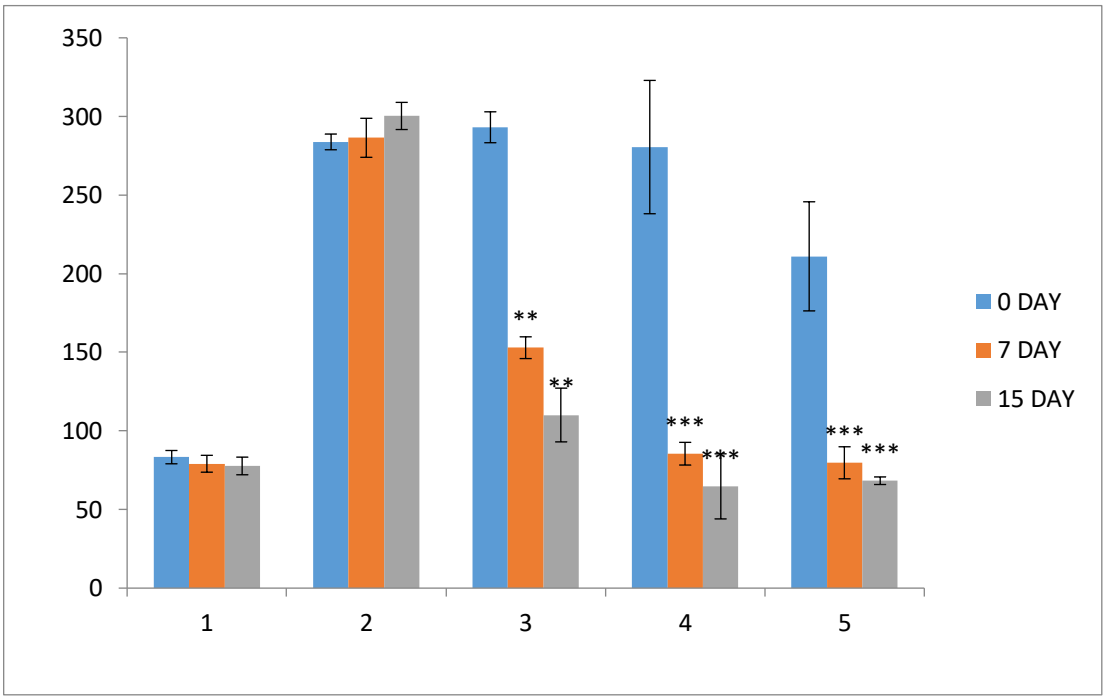
Acute toxicity studies:

As per (OECD) draft guidelines 423 adopted, male wistar rats were administered with *Tridax procumbens* and doses was be selected in the sequence (1.75- 5000) using the default dose progression factor, for the purpose of toxicity study. Animals are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours and daily thereafter, for a total of 14 days,. In all the cases, no death was observed within 14 days. Additional observations like behavioral changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems and somato motor activity and behavior pattern were also found to be normal. Attention was also given to observation of tremors and convulsions, salivation, diarrhoea, lethargy, sleep and coma.

GLUCOSE:

Table 2: Effect of *Tridax procumbens (EETP)* on serum glucose levels (mg/dl) in diabetic rats

Groups/Interval	0^{th} Day	7^{th} Day	15^{th} Day
Normal	83.3±4.23	79.1±5.36	77.7±5.62
Diabetic control	283.8±5.01	286.4±12.4	300.3±8.64
EETP (120mg/kg)	293.1±9.83	192.1±12.3**	100.3±12.5**
EETP (200mg/kg)	280.5±42.4	185.2±11.2***	94.2±7.2***
Metformin (450mg/kg)	271.0±13.5	80.2±6.4***	70.1±6.3**



*All the values of mean±SD; n=6; ** indicates p<0.01, *** indicates ᵃp<0.001 vs diabetic control.*

Figure 1: Effect of EETP on serum glucose levels (mg/dl) in diabetic rats

*All the values of mean±SD; n=6; ** indicates p<0.01, *** indicates ᵃp<0.001 vs. diabetic control.*

The present study was aimed to evaluate the anti diabetic, of *Tridax procumbens*. The activity was measured by estimating various biomarkers like blood glucose levels, in experimental rats. In the previous studies it was shown that alloxan induced to diabetes mellitus. When given in a dose of 120mg/kg to rats intraperitoneally as evidenced in study. [23] In the present study alloxan was administered in a single dose to induce diabetes mellitus in rats at the dose of 120mg/kg. The *Tridax procumbens* has reported anti-microbial properties but the effect of the plant extract on antidiabetic, were not reported yet and so the plant

was chosen for the study. Alloxan forms an increased glucose levels that generates diabetes. Pretreatment with *Tridax procumbens* produced significant decrease in glucose levels indicating the protective effect of tissue. On alloxan treatment a dose dependent decrease in glucose levels were observed. Pretreatment with *Tridax procumbens* and metformin produced significant alteration in levels.

CONCLUSION:

Tridax procumbens have different medicinal properties and May able to treat diabetes & diabetics complications. Subjected to acute oral toxicity studies and found that the *Tridax procumbens* is safe to use up to the dose of 1000mg/kg. The *Tridax procumbens* was found to be in dose dependent way against alloxan induced diabetes in rats. The reduction of the elevated blood glucose levels in diabetic rats on treatment with the extract at two different concentrations confirmed that ethanolic extract of *Tridax procumbens* possesses Antidiabetic activity & has shown significant effect when compared to alloxan administration. It needs comprehensive investigations for developing a safe and effective drug. Further research is required to confirm the antidiabetic and antidiabetic complications.

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Conflicts of interest:
The authors express no conflicts of interest regarding the publication, all the authors worked and provided support equally and credited equally.

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