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SCIENTIFIC VALIDATION OF ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACTS OF TECOMA STANS (L) JUSS. LEAF

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ABSTRACT

Diabetes mellitus (DM) currently is a major health problem of the world and due to chronic metabolic syndrome resulting from a variable interaction of hereditary and environmental factors and is characterized by abnormal insulin secretion or insulin receptor or post-receptor events, affecting metabolism involving carbohydrates, proteins and fats in addition to damaging liver, kidney and beta-cell of pancreas. The *Tecoma Stans(L)* juss is not scientifically validated and which was traditionally practicing herb. The work provides scientific validation for use of leaf against diabetes mellitus. The current study is help to develop a plant based diabetic drug which will be evaluated by using *in-vivo* streptozocin induced diabetes in rats. The leaves of plant, dried under shade are carefully removed and grinded using a blender. The coarse power so obtained was used for the extraction by successive solvent extraction by Soxhlet apparatus using various solvents. The extract with maximum number of phyto-constituents and extractive value identified (ethanolic) is used in the further evaluations. Toxicity study shows the safety nature of the extract and also acute and sub-acute toxicity study does not produce any toxic symptoms upto 500 mg/kg. The extract was pre-clinically evaluated against STZ induced diabetic rats models for its antidiabetic activity. The extract showed insulin mimetic activity and control of blood sugar level which are comparable to the reference drug glibenclamide at a dose of 10mg/kg. As the *in-vivo* results indication has been concluded 50% ethanolic extract of *Techoma Stanus* (L), which may be containing structurally insulin resembled compounds. In conclusion the extract is safe and can be used to treat diabetic condition without any harmful effects.

Keywords: Diabetes mellitus, *Tecoma Stans (L),* 50% ethanolic extract, leaf, streptozocin, antidiabetic activity.

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1. INTRODUCTION

Diabetes mellitus is one of the most common and challenging disease conditions of 21st century. It is a chronic complex progressive and multi-systemic disorder with life threatening micro and macrovacular complications [1]. WHO defined Diabetes mellitus as a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [2]. It is a major cause of morbidity and mortality. Prevalence of DM is about more than 150 million diabetics across the world and more than one fifth of them are Indians. International Diabetes Federation, India has been declared India as "Diabetic Capital of the World" at the recent Conference in Paris [3]. Diabetes mellitus consists of a group of syndrome characterized by hyperglycemia, altered metabolism of lipids, carbohydrates, and proteins; resulting from defects in insulin secretion, its action, or both [4]. DM is a complex, heterogeneous and polygenic metabolic disease where there will be an absolute lack of insulin, decreased sensitivity to insulin or both and which results in abnormal glucose homeostasis and subsequent hyperglycemia. Mutual interaction between genetic and environmental factors plays an important role in the pathogenesis of

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diabetes mellitus [5]. It is found that the developing countries adopt the western life styles like decreased physical activity and over consumption of cheap, energy dense food for past 20 years and as a result the rate of obesity has tripled in developing countries. Such changes have a direct influence on the child health of the country; the prevalence of obesity among them ranges from 2 to 10% and the prevalence of overweight ranges from 10 to 25% [6]. Diabetes mellitus (DM) currently is a major health problem of the world and due to chronic metabolic syndrome resulting from a variable interaction of hereditary and environmental factors and is characterized by abnormal insulin secretion or insulin receptor or post-receptor events, affecting metabolism involving carbohydrates, proteins and fats in addition to damaging liver, kidney and beta-cell of pancreas [7] .

Ayurvedic practitioners treat diabetes with a multi-pronged approach, using diet modification, Panchkarma to cleanse the system, herbal preparations, yoga and breathing exercises. The common herbs which can be used against diabetes include turmeric, neem, *coccinea indica*, amalaki, triphala, bitter gourd, rose, apple, leaves of bilva, cinnamon, gymnema, fenugreek, bay leaf and aloe vera. The Ayurvedic preparations 'Vasanta Kusumakar Ras' and 'Chandra prabhavati' are used to treat diabetes mellitus. Proprietary Ayurvedic medications are also used to treat diabetes [8]. Nature always stands as a golden ark to exemplify the outstanding phenomenon of symbiosis. The biotic and abiotic elements of the nature are all independent. The plants were indispensible to man, for his life. A nest of other useful products are supplied to him by the plant kingdom. Nature has provides a complete range of remedies to came an ailments of mankind. The knowledge of drugs has accumulated thoughts of years of a result of meaning inquisitive nature so that today we possess many affective of causing health care. Archaeological evidence indicates that the use of medical plants data of least the paleotic, approximately 60,000 years age. In India, medicinal plants are widely used in traditional systems of medicine like Ayurvedic, Unani, Siddha and Homeopathy. India with it's valuable resources of natural flora has always been one of the richest sources of medicinal plants in the world [9-12].

Streptozotocin or streptozocin or Izostazin or zanosar (STZ) is a synthetic glucopyranose derivative isolated by the fermentations of *Streptomyces achromogenes* which possess anti-tumor antibiotic activity. It can be used to induce both type 1 and type 2 diabetes. Chemically it is (2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose).
The frequently used single i.v dose in adult rats to induce IDDM by immune system activation was found to be in between 40 and 60mg/kg. NIDDM can also be induced in rats by intravenous or intraperitoneal treatment with 100mg/kg b.w. STZ on the day of birth [13].

Mohammed Salem *et al.* (2013), studied the antioxidant and antibacterial activity of *Tecoma stans* leaves and branches against the growth of some human bacterial strains using the disc diffusion and minimum inhibitory concentration (MIC) methods and antioxidant activity using 2,2-dimethyl-1-picrylhydrazyl (DPPH) method. Very significant activities were exhibited by the samples. These findings provide scientific evidence to support traditional medicines uses of *Tecoma stans* and indicate a promising potential for the development of an antibacterial and antioxidant agent from *T. stans* [14]. Kameswaran *et al.* (2013), studied Hepato protective activity of *Tecoma stans* extract against the liver injury induced by paracetamol, carbon tetrachloride and thioacetamide. Results revealed that the extracts significantly reduce the elevated serum levels of Asparate amino transferase, alanine amino transferase, alkaline phosphate and bilirubin. The ethanolic extract at the dose of 500 mg/ kg more effective than 250 mg/kg but his excluded in paracetamol induced liver damage. In chronic liver injury induced by CCl_4, ethanolic extracts at the dose of 500 mg/kg. P.O. was found to be more effective than the extract of the dose of 250 mg/kg. Histological examination of the liver tissues supported the hepato protective activity of the extracts [15]. Kameswaran Sugavanan *et al.* (2012), studied the CVS depressant potential of different extracts of *Tecoma stans* flowers. Namely chloroform, methanol and water on Albino mice of both sexes. The study conform that the different extracts of *Tecoma stans* flowers exhibit CNS depressant activity [16]. Medicinal Uses *Tecoma stans of* aerial parts used in the treatment of stomach problems, gastritis, Diarrhea, roots are used as diuretic, vermifuge, tonic, beer making, a remedy in snake's bits, scorpion sting and in the treatment of syphilis and flowers possess narcotic and analgesic activity [14-16].

The diabetes mellitus prevalence was increased day by day, due to metabolic disorder, life style changes, improper food intake and less physical activity .symptoms of high blood sugar, left untreated; diabetes mellitus can cause many complications. Synthetic drugs have many side effects and harmful to the health. Over the centuries, they are traditionally practicing medicinal plants used to the treatment for various diseases but no scientific validation. Several literatures are indicated that the herbal drugs have lesser side effects when compared to synthetic medicines. The *Tecoma Stans (L)* juss is not scientifically validated and which was traditionally practicing herb. The work provides scientific validation for use of leaf against diabetes mellitus. The current study is help to develop a plant based diabetic drug which will be evaluated by using *in-vivo* streptozocin induced diabetes in rats. *Tecoma stans* is a species of flowering perennial large shrub or small, much-branched, tree usually growing 1.5 to 5m tall, but occasionally reaching up to 10 m in height in the trumpet vine family, Bignoniaceae, that is native to the southern USA, Mexico, the Caribbean, Peru and Ecuador. Tecomastans is the official flower of the United States Virgin Islands and the floral emblem of the Bahamas. Yellow trumpet bush is an attractive plant that is cultivated as an ornamental. The plant is desirable fodder when it grows in fields grazed by livestock. Yellow trumpetbush is a ruderal species, readily colonizing disturbed, rocky, sandy, and cleared land and occasionally becoming an invasive weed [16-18]. It is used as firewood and charcoal, in the construction of buildings and the leaf infusion can be taken orally for diabetes and stomach pains. A strong leaf and root decoction is taken orally as a diuretic, to treat syphilis or for intestinal worms. It is a strong shading plant and can be planted as a live hedge.

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Fig. 1: *Tecomastans* (L.) Leaf

2. MATERIALS AND METHOD

2.1 Plant Material

The plant leaves was collected locally from herbal store and botanical garden of the garden of the botany central council for Research Ayurvedic and Sidha Govt. of India. The plant was identified and authenticated by comparison with herbarium specimens. The leaf of *Tecomastans* (L.) juss ex kunth was authenticated by comparison with herbarium specimens and authentification No. BSI/SRC5/23/2016/Tec/1993. The weighed coarse powder was used for the extraction by successive solvent extraction by Soxhlet apparatus using various solvents.

2.2 Animals

Wistar rats (150 – 250 g) used for the study were obtained from the animal house of the Department of Pharmacology, Karpagam College of Pharmacy, Coimbatore, Tamil Nadu. The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The animals were housed three per cage in a polypropylene cage and maintained in standard laboratory conditions with free access to food and water *ad libitum [18]*. All animal experiments were conducted in compliance with (Organization for Economic Cooperation and Development) OECD Guideline and approved by the Institutional Animal Ethics Committee, Karpagam College of Pharmacy.

2.3 Chemicals, Drugs and Instruments

Streptozotocin, citric acid, sodium citrates were collected from a private chemical store Coimbatore (Ponmani and co). Other important chemical used in phytochemical analysis like alcohol, hydrochloric acid, ∞-napthol, Sulphuric acid, Fehling A&B, Benedict reagent, sodium hydroxide, nitric acid, ammonia, lead acetate, ninhydrin, sudan red III reagent, glycerin, picric acid, chloroform, acetic anhydride, ferric chloride, zinc, dragendroff's reagent, Wagner's reagent, Mayer's reagent, sodium chloride and bromin water were collected from the store of Karpagam College of Pharmacy. All the chemicals used in the study are of analytical grade.

2.4 Extraction Procedure

The leaves of plant, dried under shade are carefully removed and grinded using a blender. The coarse power so obtained was used for the extraction by successive solvent extraction by Soxhlet apparatus using various solvents. The assembly of Soxhlet apparatus is as shown in the figure [19-26].

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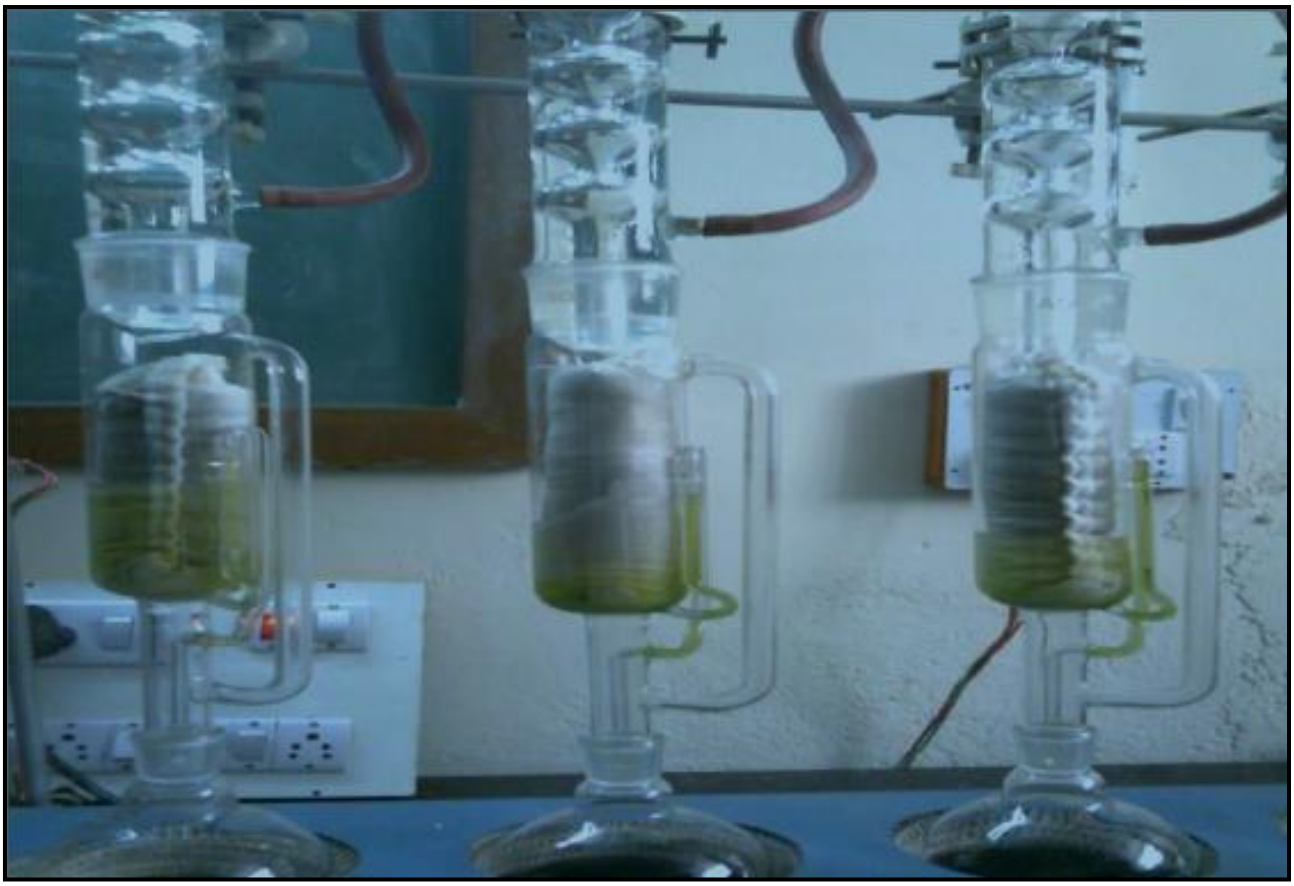

Fig. 2: Extraction using Soxhlet apparatus assembly

Alcoholic extract

Marc obtained from the above extract was dried and extracted with 2.5 litres of ethanol (90%) in soxhlet apparatus for 36 hours .Then the extract obtained were collected and concentrated by vacuum distillation .The concentrated extract were then dried by in a vacuum desiccators.

2.5 Phytochemical Analysis

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. Phyto-constituents are the contributors of pharmacological activities of a plant. The individual extracts are subjected to qualitative tests for identification of various plant constituents [14-27].

2.5.1 Test for Carbohydrates

I. Molisch Test:

To the aqueous extract, 1ml of ∞-napthol solution was added and Conc. Sulphuric acids were added along the sides of the test tube.

II. Fehling Test:

To the aqueous extract, equal quantities of Fehling A & B were added upon heating gently.

III. Benedict's test:

To 5ml of Benedict reagent, 8 drops of solution under test was added to the test solution mixed well. Then it was boiled vigorously for 2 minutes and cooled.

2.5.2 Test for Proteins

I. **Biuret Test:**

To the aqueous extract, 1ml of 40% NaOH and 2 drops of 1% copper sulphate solution was added.

II. **Xanthophoretic Test:**

To the aqueous extract, 1ml of conc. Nitric acid was added. When a white precipitate was formed, it is boiled and cooled. Then 20% of NaOH or ammonia was added.

III. Lead acetate Test: To the aqueous extract, 1ml of lead acetate solution was added.

2.5.3 Test for Amino acids Ninhydrin Test:

2 drops of freshly prepared 0.2% ninhydrin reagent was added to the aqueous extract and heated.

2.5.4 Test for Fats and Oils

Place a thick section of drug on glass slide. Add a drop of Sudan Red III reagent. After two minutes, wash with 50 % alcohol. Mount in glycerin. Observe under microscope.

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2.5.5 Test for Steroids

I. **Liebermann Burchard Test:**

The aqueous extract was dissolved in 2ml chloroform in dry test tube. 10 drops of acetic anhydride and 2 drops of conc. sulphuric acid were added.

II. **Salkowaski Test:** The aqueous extract was dissolved in chloroform and equal volume of sulphuric acid was added to it.

2.5.6 Test for Cardiac glycosides

Keller-killiani Test: Test sample was dissolved in acetic acid containing traces of ferric chloride and transferred to the surface of conc. Sulphuric acid.

2.5.7 Test for Saponins Foam Test: About 1ml of aqueous extract is diluted separately with distilled water to 20ml and shaken in a graduated cylinder for 15 minutes.

2.5.8 Test for Flavonoids

I. Sulphuric Acid Test: On addition of sulphuric acid (66% or 80%) flavons and flavonols dissolves into it and give a deep yellow solution.

II. Heat the test solution with Zinc and HCl, pink to red colour observation shows the presence of flavonoids.

2.5.9 Test for Alkaloids

I. Dragendroff's Test: To the aqueous extract, add 1ml of Dragendroff's reagent.

II. Wagner's Test: To the aqueous extract, add1 ml of Wagner's reagent.

III. Mayer's Test: To the aqueous extract, add 1ml of Mayer's reagent.

2.5.10 Test for Phenolic compounds and Tannins

Small quantities of alcoholic and aqueous extracts in water were tested for the presence of phenolic compounds and tannins with dilute ferric chloride solution (5%), 1% solution of gelatin containing 10% sodium chloride, 10% lead acetate and bromine solutions.

2.6 Acute Toxicity Study

Experimental Protocol:

Guideline: OECD-423

CPCSEA Ref. No: KU/IAEC/M.Pharm/169

Test: Limit test

Species: *Rattusnorvegicus*

Strain: Albino Wistar rats

Number of animals: 24 animals (6 for each group)

Sex: Female

Initial dose: 5mg/kg

Route of administration: Oral

Duration: 3hr close observation, followed by 14 days observation

Others: Body weight, mortality status

Parameters: CNS, ANS and behavioural changes [28-30].

Table 1: Experimental Design of Acute Toxicity Study

GROUP	Number of Animals	DOSE (mg/kg)
Group 1		5
Group 2		50
Group 3	**6 each**	300
Group 4		2000

2.7 Selection of Test animals.

Female adult Wistar rats of 8-12 weeks are selected. Nulliparous and non-pregnant animals were obtained from the centralized animal house of Karpagam College of pharmacy, Coimbatore and they are acclimatized for holding 1 week prior to dosing.

2.8 Housing and feeding conditions for Experimental Animals.

Temperature - As per OECD guideline-420 the temperature of animal house were maintained at 23°C±5°C.

Humidity - The relative humidity of animal room maintained at 50-60% preferably not exceeds 70% (OECD guidelines-423, 2001). Otherwise there may be chances of developing lesions such as ring tail and food consumption may be increased.

Light – The sequence of light used was 12 hrs light and 12 hrs dark.

Caging – Polypropylene cages with solid bottom and walls. The lids are made up of stainless steel grill which is capable to hold both feed and water.

Feeding condition and feed – Sterile laboratory feed (*ad libitum*) and water daily. The feed used were brown colored chow diet.

2.9 Drug administration

Animals are fasted prior to dosing (food but not water should be withheld for overnight).After that animals are weighed and the test substance administered. The healthy rats has been taken and divided into 4 different groups. The test substance was administered in a single dose by oral gavages, using a curved and ball tipped stainless steel feeding needle.

2.10 Experimental Design

In this study, 4 groups of 6 rats each were given with 5, 50 and 300 and 2000 mg/kg of the extract (p.o.). After drug administration the food is withheld for 3 hours. The animals are observed continuously for the first 2 hours, then occasionally up to 6 hours and then daily up to 14 days, post treatment to observe for any symptoms of toxicity and mortality. Daily observations on the changes in skin and fur, eyes and mucus membrane (nasal), autonomic effects (salivation, lacrimation, gauntness and piloerection) and central nervous system (gait, tremors and convulsion) were carried out and changes were noted (OECD, 2001).

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2.11 Clinical observation

All animals were monitored continuously with special attention for 4 hrs after dosing for signs of toxicity. Additional observations are also done for the next 14 days for any other behavioral or clinical signs of toxicity. Weight changes are calculated. At the end of the test animals are weighed. LD50 values are established using the formula.

2.12 Dose Calculation Equation LD50= higher dose − Σ (a x b)/n

Where, **a =** dose difference

b = animal died **n =** No. of animals in each group

2.13 Pharmacological Studies

2.13.1 Selection of Test animals. Male wistar rats weighing 150-200g were used for the present work.

The animals used for the experiment were maintained under standard laboratory conditions in an animal house of Karpagam College of Pharmacy approved by the committee for the purpose of control and supervision on experiments on animals (IAECNO.KU/IAEC/M.Pharm/169) under 12 h dark/light cycle and controlled temperature 24±2°C. They had free access to food and water *ad libitum.* The animals were acclimatized to the laboratory for a period of 7 days, before the commencement of experiment [31].

2.13.2 Induction of Diabetes in Experimental Animals

Experimental diabetes was induced by single intra-peritonial injection of 25 mg/kg of streptozotocin (STZ), freshly dissolved in cold citrate buffer (pH 4.5) after 15 min of intra-peritonial 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.

2.14 Assessment of diabetes

Diabetes was confirmed after 48 hr of streptozotocin injection, the blood samples were collected through tail vein and plasma glucose levels were estimated by glucose oxidase method (accu check active glucometer). The rats having fasting plasma glucose levels more than 200mg/dL were selected and used for the present study.

2.15 Glucose Tolerance Test

The Oral Glucose Tolerance test (OGTT) measures the body's ability to use glucose, which is the body's main source of energy. Oral glucose tolerance test was performed in overnight fasted (18 hours) normal rats.

2.16 Experimental Design

Normal rats were divided into four groups, each consisting of six rats.

Group I was normal control (distilled water).

Group II and III animals received different concentrations of extract viz., 200 mg/kg and 300 mg/kg respectively.

Group IV animals are standard receiving Glibenclamide (GL) 10 mg/kg body weight.

Groups II and III animals were treated orally with a single dose of extract at a dose of 200mg/kg and 300 mg/kg p.o. respectively. Glucose (2 g/kg) was fed orally through oro-gastric tubes 30 min after the administration of the drug.

Control animals were administered with equal volume of water. Blood was withdrawn from the tail vein at 0, 1, 2, 3 and 4 hr of glucose administration. The percentage induced glycaemia (%IG) following oral glucose load at different time intervals was calculated for the control and treated groups as follows.

%IG = (Gx-Go)/Go × 10

Where Go is the initial glycemia (mg/dL) and Gx the glycemia (mg/dL) at different time intervals after the oral glucose load.

2.17 Hypoglycaemic Activity

On the basis of the OGTT studies in normal and diabetic rats, dose was selected for STZ-induced diabetic rat model studies.

2.18 Experimental Design:

All hyperglycaemic rats were randomly divided into four groups of six rats in each group, 24rats (18 diabetic rats and 6 normal rats).

Group I – Normal control (Distilled Water)

Group II – Diabetic control (Distilled Water)

Group III – Streptozotocin + Glibenclamide (10 mg/kg p.o)

Group IV – Streptozotocin + Ethanolic extract (300 mg/kg p.o.)

The test drug was administered orally using an oral feeding needle once daily for 28 days. The body weight, food and water intake behaviour of the animals were measured at the onset of the study and at the regular intervals of every week up to 28 days.

Group I animals (normal rats) were administered orally with distilled water whereas group II animals (diabetic) received distilled water, group III animals (diabetic) received glibenclamide (10 mg/kg p.o) and group IV animals (diabetic) received extract 300 mg/kg body weight for 28 consecutive days. The blood samples collected from the tail vein of rats on 0, 7, 14, 21 and 28 days after administration of formulation. The blood glucose levels were determined by the glucose oxidase method using glucometer (Accucheck active) [32-38].

2.19 Statistical Analysis

All values are expressed as mean ± SEM. Statistical analysis was performed by One-way Anova, analysis of variance (ANOVA) followed by Dunnet's t-test. A 'p' value less than 0.05 was considered significant.

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3. RESULTS AND DISCUSSION

3.1. EXTRACTION

The dried powdered course blend of leaf form *Tecoma Stans* are undergone successive solvent extraction using alcohol and water as solvents. A comparatively greater extractive value was obtained in alcoholic extract of the leaf.

3.2. PHYTOCHEMICAL EVALUATION

Phytochemicals are bioactive substances of plants that have been associated in the protection of human health against chronic degenerative diseases [39]. Phytochemical analysis of ethanol extract shows alkaloids, carbohydrates, saponins, proteins, amino acids, flavonoids and tannins. The combination of above mentioned phytochemicals may be the reason behind the ant diabetic properties of the plant.

3.2.1. Test for Carbohydrates

I. Molisch Test:

Purple or reddish violet colour at the junction between the two liquids indicates the presence of carbohydrates.

II. Fehling Test: A brick red precipitate indicates the presence of carbohydrates.

III. Benedict's test: Red precipitate indicates the presence of carbohydrates.

3.2.2. Test for Proteins

I. Biuret Test: A violet colour indicates the presence of proteins.

II. Xanthophoretic Test: Orange colour indicates the presence of aromatic acids.

III. Lead acetate Test: A white precipitate indicates the presence of proteins.

3.2.3 Test for Amino acids

I. Ninhydrin Test: A blue colour indicates the presence of proteins, peptides or amino acids.

3.2. 4. Test for Fats and Oils

I. Red globules in the section when viewed under the microscope show the presence of fats or oils.

3.2.5. Test for Steroids

I. Layer assumes marked green fluorescence indicates the presence of steroids.

3.2.6. Test for Cardiac glycosides

II. Keller-killiani Test:

At the junction, reddish brown colour was formed, which gradually becomes blue indicates the presence of cardiac glycosides.

3.2.7. Test for Saponins

I. Foam Test: A1cm layer of foam indicates the presence of saponins.

3.2.8. Test for Alkaloids

I. Dragendroff's Test: An orange red coloured precipitate indicates the presence of alkaloids.

II. Wagner's Test: Reddish brown coloured precipitate indicates the presence of alkaloids.

III. Mayer's Test: A dull white coloured precipitate indicates the presence of alkaloids.

3.2.9. Test for Phenolic compounds and Tannins

I. The respective observations may be deep blue black colour, white precipitate, white precipitate, decolouration of bromine water showing the presence of tannins and phenolic compounds.

Table 2: preliminary phytochemical evaluation of *Tecomastans* (l.) juss. Ex Kunth leaf extracts

S. No	Phytoconstituents	*Results*
1.	Alkaloids	+
2.	Carbohydrates & Glycosides	+
3.	Phytosterols	_
4.	Fixed oils	-
5.	Saponins	+
6.	Tannins and Phenols	+
7.	Proteins and Amino acids	+
8.	Gums and Mucilage's	-
9.	Flavonoids	+
10.	Tannins's	+

(+) – Presence, (-) – Absence

3.3. ACUTE TOXICITY STUDY

There was no mortality or signs of toxicity up to the limit dose of 2000 mg/kg in treated rats. All 24 rats were normal throughout the study and survived until the end of the 14-day experiment period. Animal wellness parameters were observed continuously for the first 2 hours, then occasionally up to 6 hours and then daily up to 14 days as per paragraph 24 and 25 of OECD Guideline 423. Experimental observations are recorded systematically for each group. The parameters considered are changes in skin and fur, eyes and mucous membrane and also respiratory and circulatory, autonomic and central nervous system, somatomotor activity and behavioral pattern. Special attention is given for the observations of tremor, convulsion, salivation, diarrhoea, lethargy, sleep and coma.

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3.4. PHARMAACOLOGICAL STUDIES

3.4.1. Effect of ethanolic extract on Glucose-Loaded Rat (OGTT Model)

Table 3: Effect of ethanolic extract on Glucose-Loaded Rat (OGTT Model)

· Effect of ethanolic extract on serum glucose levels in OGTT model in normal rats

S.No	Drug/Control	Body weight	Blood glucose level (mg/dL)				
			0 hour	1 hour	2 hour	3 hour	4 hour
1	Group-1 control(distilled water)	180.0 ±2.0	92.0± 2.5	132.0 ±3.5	117.0± 0	119.0± 1.0	100.5± 1.5
2	Group-2 extract (200mg/kg)	164.1 ±2.7	102.0 ±1.0 **	123±0 **	107.0± 2.0 **	101.0± 3.0 *	98.0± 2.0 *
3	Group-3 exotract (400mg/kg)	152.6 ±3.4	99.0± 1.5 **	120.0 ±1.5 **	100.0± 2.5 **	96.0± 3.0 *	88.5± 1.5 *
4	Group-4 GL (10 mg/kg body wt)	151.3 ±2.3	111.0 ±4.5 **	121.0 ±3.1 **	117.0± 3.6 **	114.0± 2.6 *	112.5± 1.2 *

Values are represented as mean ± SEM (n=6 rats).
Values are statistically significant at *P < 0.05,** P < 0.01.
GL = Glibenclamide.

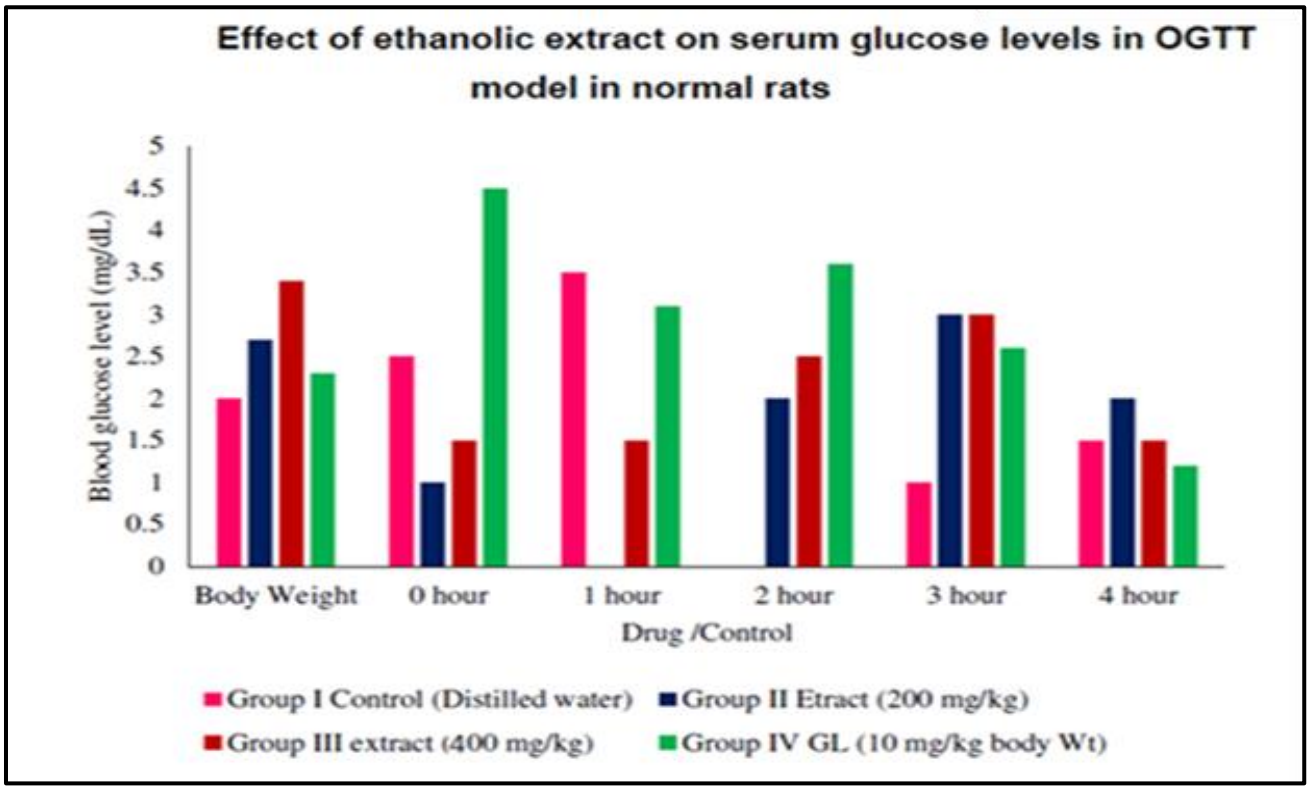

Fig.3: Effect of ethanolic extract on Glucose-Loaded Rat (OGTT Model)

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Vehicle treated group and GL (10 mg/kg body wt) treated group showed significantly rise in serum glucose level (SGL) after one hour of glucose administration, whereas groups II and III showed significantly increase in SGL respectively. From the study, it is found that both 200 mg/kg and 400 mg/kg of ethanolic extract possess significant hypoglycemic activity in normal rats. It is found that 200 mg/kg of ethanolic extract showed a significant reduction in blood glucose at second hour and 400 mg/kg of ethanolic extract shows more significant reduction at the same time interval compared to control group and GL group respectively. Hence, ethanolic extract 400 mg/kg dose was selected for further study in STZ-induced diabetic rat model. However, all groups of animals almost normalized the SGLs within three hours indicating that the pancreas of animals was healthy to clear out the glucose load from the body.

3.4.2. Effect of ethanolic extract on serum glucose level of diabetic rats

Table 4: Effect of ethanolic extract on serum glucose level of diabetic rats

Effect of 27 days treatment of ethanolic extract on serum glucose levels of STZ-induced diabetic rats

S.No	Treatment	Initial	7th day	14th day	21st day	28th day
1	Normal control	89.3±3.8	91.0± 1.5	95.0±1.0	92.8± 2.1	89.0± 1.7
2	Diabetic control	221.5±3.2	267.3± 3.5	310.3± 2.2	383.0± 2.8	405.3± 3.2
3	Diabetic+Glibenclamide (10mg/kg)	281.0±1.9 ***	261.0± 3.6 **	153±3.8 ***	140.1± 3.1 ***	129.5± 2.7 ***
4	Diabetic+ extract (400 mg/kg)	240.1±2.2 ***	210.6± 3.3 ***	160.3± 3.7 ***	121.3± 1.4 ***	96.8± 1.7 ***

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

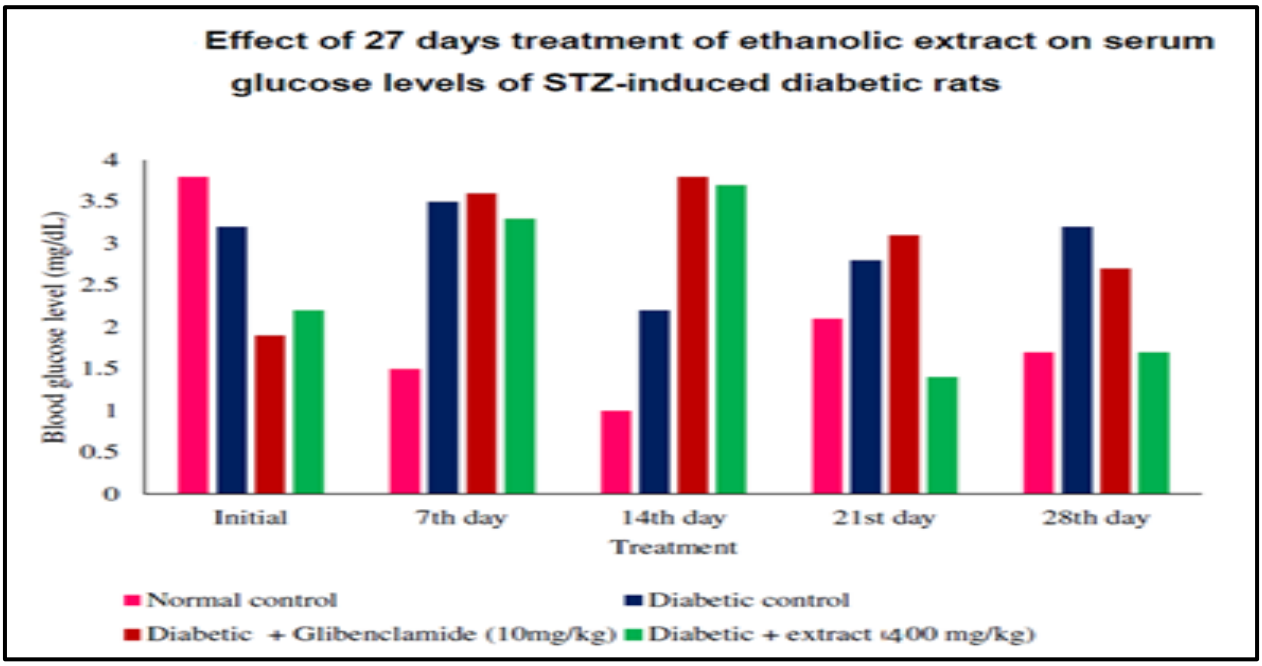

Fig. 4: Effect of ethanolic extract on serum glucose level of diabetic rats

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Diabetic control rats showed consistent and gradual rise in SGL during the study. GL (10 mg/kg body wt) and ethanolic extract 400 mg/kg treated rats showed a significant reduction 7th, 14th, 21st, and 28th day of the study and the results were found to be statistically significant (P<001) as compared to diabetic control. The effect was found to be time dependent up to 28th day of the study. Decrease in SGL was more significant (P<0.001) on 28th day when compared with standard drug.

3.4.3. Effect of ethanolic extract treatment on body weight

Table 5: Effect of ethanolic extract treatment on body weight

Effect of ethanolic extract treatment on body weight in STZ-induced diabetic rats on 21st day and 28th day

S.No	Drug/Control	Body weight(g)		
		Baseline	21st day	28th day
1	Normal control	180.0±2.0	180.9±3.2	182.2±3.1
2	Diabetic control	164.1±7.1	155.0±7.0	123±10.2 **
3	Diabetic+ Glibenclamide (10mg/kg)	152.6±8.4	153.8±9.5 **	155.1±6.7 ***
4	Diabetic + extract (400mg/kg)	151.3 ±7.3	152.0±5.1 **	156.0±7.3 ***

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

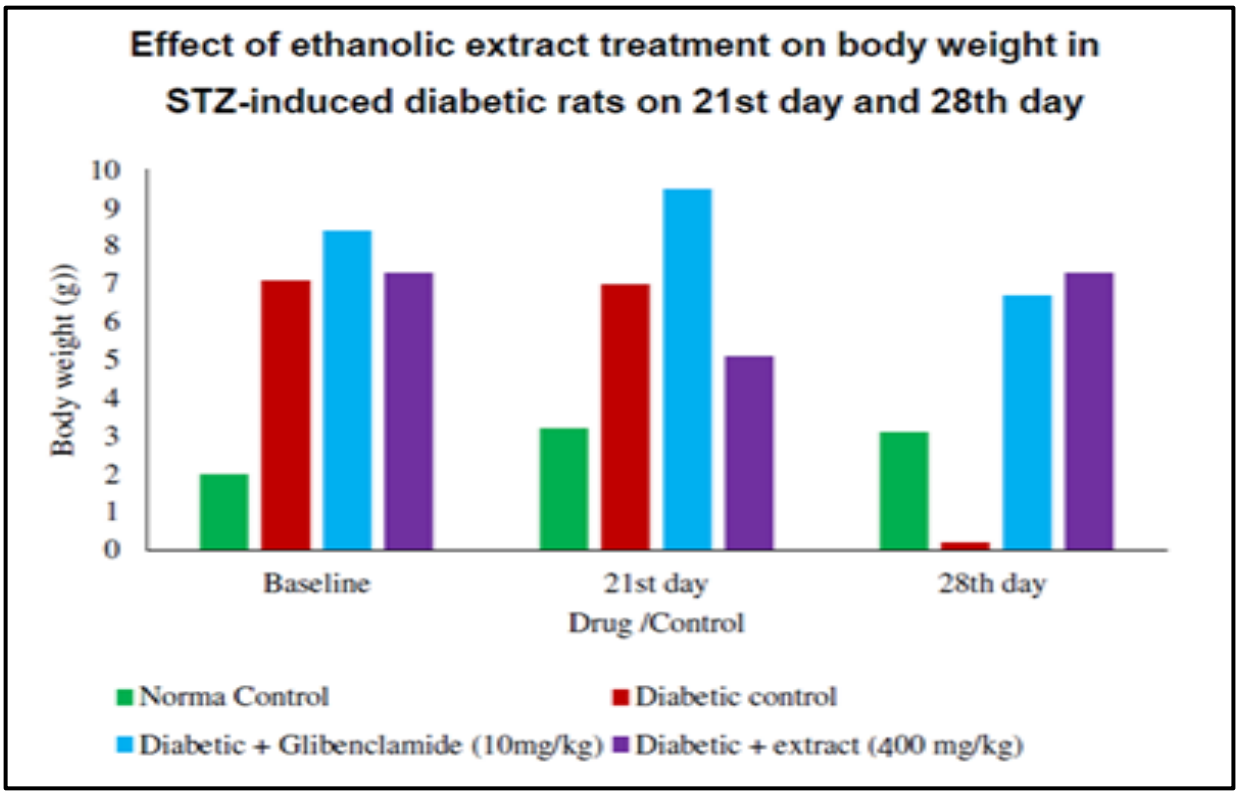

Fig. 5: Effect of ethanolic extract treatment on body weight

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There was also a significant reduction in body weight in diabetic animals, however, the animals treated with 400 mg of ethanolic extract and GL showed significant (P<0.001) check on the loss of body weight on days 21 and 28 in comparison to the day of onset of the study. This effect may be attributed to increased insulin secretion and food consumption.

These results implied that the developed ethanolic extract can reduce the complications of body weight and associated cardiovascular risk factors during diabetes.

The body's ability to maintain the glycemic level may be measured by OGTT in normal rats. The method is usually used to test DM, insulin resistance, beta cell function [40] and sometime reactive hypoglycaemia, acromegaly or rarer disorders of carbohydrate metabolism. Glucose tolerance was first described in 1923 by *Jerome et al.* [41]. In the present study the blood samples were collected at a time interval of 0, 1, 2, 3 & 4 hours. The glycemic level of extract treated groups at different doses is compared with control groups. Vehicle treated group and Glibenclamide (10 mg/kg body weight) treated group showed 43.4% and 9.0% rise in serum glucose level (SGL) after one hour of glucose administration whereas groups II and III showed 20.5% increase and 21% increase in SGL respectively. From the study, it was found out that both 200 mg/kg and 400 mg/kg of extract possess significant hypoglycemic activity in normal rats. It is found that 200 mg/kg of extract showed a 13% reduction in blood glucose at second hour and 400 mg/kg of ethanolic extract shows 16.5 % reduction at the same time interval compared to 11.3% decrease and 3.4% decrease in control group and GL group respectively. Hence, ethanolic extract of 400 mg/kg dose was selected for further study in STZ-induced diabetic rat model. However, all groups of animals almost normalized the SGLs within three hours indicating that the pancreas of animals was healthy to clear out the glucose load from the body. After OGTT the anti-hyperglycemic effect of ethanolic extract was checked in streptozotocin induced diabetic Wistar rats after an 18 hours fasting. Glibenclamide 10 mg/kg is used as a standard. The diabetic rats were subjected for 28 days study ad libitum. Diabetic control rats showed consistent and gradual rise in SGL during the study. GL (10 mg/kg body weight) and extract 400 mg/kg treated rats showed a reduction in SGL. Diabetic control rats showed consistent and gradual rise in SGL during the study. GL (10 mg/kg body wt) and extract 400 mg/kg treated rats showed a reduction in SGL by 7.1%, 45.5%, 50.1, 53.9%; and 12.3%, 33.3%, 49.5%, 59.7% on 7th, 14th, 21st, and 28th day of the study and the results were found to be statistically significant (P<001) as compared to diabetic control. The effect was found to be time dependent up to 28th day of the study. Decrease in SGL was more significant (P<0.001) on 28th day when compared with standard drug.

The current anti-diabetic drug research is facing complex challenges. As times go on it demands an integrated approach towards the health care system. There has been a growing interest in natural medicinal plant related research. They are many differences in their philosophical and epistemological foundation concerted frame work and practical outlook. In case of diabetes both the system of medicine have different type of treatment approaches based on the severity of the diseases. By using medicines reduces the signs and symptoms of the disease. Once diabetes mellitus is diagnosed, the patient should take medication lifelong. In modern medical system long duration treatment of diabetes is risky, because the side effects of the drugs are severe. But in the case of ayurvedic medical system the side effects of drugs are less compared to modern medical system, because they are natural in origin. Phytochemicals are bioactive substances of plants that have been associated in the protection of human health against chronic degenerative diseases. Phytochemical analysis of ethanol extract shows alkaloids, carbohydrates, saponins, proteins, amino acids, flavonoids and tannins.

The combination of above mentioned phytochemicals may be structural similarity of compound of the pant. In the toxicity studies ethanolic extract did not show any signs or symptoms of toxicity in rats at doses up to 2000 mg/kg p.o., indicating that it has no toxicity at the maximal doses tested in this work. Although herbal medicinal products are widely considered to be of lower risk compared with synthetic drugs, they are not completely free from the possibility of toxicity or other adverse effects [40]. Thus, toxicological evaluation of plants derived products, including extracts forms an essential part of scientific validation of medicinal plants. Although, poisonous plants are ubiquitous [41], herbal medicine is used by up to 80% of the population in the developing countries. The safety of herbal medicine use has recently been questioned due to reports of illness and fatalities like nephrotoxicity and hepatotoxicity [42-44].

The acute toxicity study indicated that ethanolic extract at a dose 2000 mg/kg caused neither visible signs of toxicity nor mortality. The LD50 and ED50 of the drug were estimated at 2000 mg/kg and 200 mg/kg respectively. If LD50 is 2000 mg/kg, it could be generally regarded as safe (GRAS). This finding is in agreement with Clarke and Clarke [45], who reported that any compound or drug with oral LD50 estimates greater than 1000 mg/kg body weight could be considered to be of low toxicity and safe. However, it is suggested that variables such as animal species, strain, age, gender, diet, bedding, ambient temperature, caging conditions, and time of the day can all affect the LD50 values obtained and as such are considerable uncertainties in extrapolating the LD50 obtained for species to other species. This finding is suggestive that LD50 may not be considered as a biological constant [46].

Oral administration of ethanolic extract at doses of 200, 500, or 1000 mg/kg body weight daily for 28 day did not produce any signs of toxicity or mortality. The animals did not show any changes in general behavior or other physiological activities and were found normal throughout the study. 28 day study provides information on the effects of repeated oral exposure and can indicate the need for further longer term studies. It can also provide information on the selection of concentrations for longer term studies. All animals are observed for morbidity and mortality twice daily. Little or no change was observed in body weight, food consumption, and water intake in ethanolic extract (200, 500 and 1000 mg/kg)-treated groups compared with control group after 28 days of study period in rats. All animals are weighed before starting the experiment and once in a week. Measurements of food and water

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consumption are also made once weekly. Ethanolic extract caused a statistically significant (P <0.01) rise in body weight among group III animals. It is necessary to measure the water consumption at least weekly. No signs and symptoms of toxicity, changes in behavior or other physical and physiological abnormalities were observed during the experimental period. Streptozotocin is probably the most widely used agents producing insulin-dependent diabetes mellitus and non-insulin dependent diabetes mellitus in experimental animals. It is a glucosamine nitrosourea compound causes beta cells of islets of Langerhans of rats to clearly degenerate. In three days, Streptozotocin makes pancreas swell and at last causes degeneration in beta cells of islets of Langerhans and induces experimental diabetes. It also changes normal metabolism in diabetic rats in comparison with normal rats. Prolonged administration of STZ might have reduced the beta calls of islets of Langerhans to produce insulin. The observed blood glucose lowering effect of the decoction in STZ induced diabetic rats could also possibly due to increase peripheral glucose utilization. A number of other plant have also been shown to exert hypoglycemic activity through stimulation of insulin release [46-49] Consumption of water and food, volume of urine, serum glucose increases in diabetic animals in comparison with normal rats, but the levels of serum insulin, C-peptide and body weight decreases.55 The characteristic loss of bodyweight is due to increased muscle wasting in diabetes. When diabetic rats were treated with extract; the weight loss was put on check and reversed [50].

CONCLUSION

The different extracts (alcoholic and aqueous) of *Tecoma Stans* were subjected to physicochemical analysis. Tests for carbohydrates, phenols, tannins, alkaloids, flavonoids, fats, glycosides, steroids, amino acids, proteins carbohydrates, proteins, amino acids, flavonoids, saponins, phenol and tannins which may probably responsible for their expected pharmacologic action. The extract with maximum number of phyto-constituents and extractive value identified (ethanolic) is used in the further evaluations. Toxicity study shows the safety nature of the extract and also acute and sub-acute toxicity study does not produce any toxic symptoms up to 500 mg/kg. The extract was pre-clinically evaluated against STZ induced diabetic rats models for its antidiabetic activity. The extract showed insulin mimetic activity and control of blood sugar level which are comparable to the reference drug glibenclamide at a dose of 10mg/kg. As the *in-vivo* results indication has been concluded 50% ethanolic extract of *Techoma Stanus* (L), which may be containing structurally insulin resembled compounds. In conclusion the extract is safe and can be used to treat diabetic condition without any harmful effects. Further studies are required to confirm the exact mechanism behind the antidiabetic activity of the extract.

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