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

A Dissertation submitted to
THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY,
CHENNAI– 600 032

In partial fulfillment of the requirements for the award of the Degree of
MASTER OF PHARMACY
IN
BRANCH –VI - PHARMACOLOGY

Submitted by
Mr. BHAVAN KUMAR
REGISTRATION No.261526158

Under the guidance of
Prof. G. NAGARAJAPERUMAL, M.Pharm., (Ph.D).,
Professor & Head
Department of Pharmacology

DEPARTMENT OF PHARMACOLOGY
KARPAGAM COLLEGE OF PHARMACY
COIMBATORE-641 032

MAY - 2017

CERTIFICATE

This is to certify that the dissertation entitled **"Scientific Validation of Antidiabetic Activity of Ethanolic Extract of Tecoma Stans (L) Juss.Leaf"** being submitted to The Tamil Nadu Dr. M.G.R Medical University, Chennai was carried out by **Mr. Bhavan Kumar.A** to The Tamil Nadu Dr. M.G.R Medical University, Chennai in partial fulfillment for the degree of **Master of Pharmacy** in **Pharmacology** is a bonafied work carried out by candidate under my guidance and supervision in the Department of Pharmacology, Karpagam College of Pharmacy Coimbatore – 32.

I have fully satisfied with his performance and work. I have forwarded this dissertation work for evaluation.

Station: **G.NAGARAJA PERUMAL M Pharm.,(Ph.D).,**

Date : Professor & Head

Department of Pharmacology

CERTIFICATE

This is to certify that the dissertation entitled **"Scientific Validation of Antidiabetic Activity of Ethanolic Extract of Tecoma Stans (L) Juss.Leaf"** being submitted to The Tamil Nadu Dr. M.G.R Medical University, Chennai was carried out by **Mr. Bhavan Kumar. A** to The Tamil Nadu Dr. M.G.R Medical University , Chennai in partial fulfillment for the degree of **Master of Pharmacy** in **Pharmacology** is a bonafied work carried out by candidate under the guidance of **Prof. G. Nagarajaperumal, M.Pharm., (Ph.D).,** in the Department of Pharmacology, Karpagam College of Pharmacy, Coimbatore – 32.

I have fully satisfied with her performance and work. I have forwarded this dissertation work for evaluation.

Station: **Dr. S. MOHAN, M.Pharm, Ph.D.,**

Date :

Principal

DECLARATION

I hereby declare that this dissertation **"Scientific Validation of Antidiabetic Activity of Ethanolic Extract of Tecoma Stans (L) Juss.Leaf"**submitted by me , in partial fulfillment of requirements for the degree of **Master of Pharmacy** in **Pharmacology** to The Tamil Nadu Dr.M.G.R Medical University, Chennai is the result of my original and independent research work carried out under the guidance of **Prof .G.Nagarajaperumal.,M.Pharm.,** Professor & Head Department of Pharmacology, Karpagam College of Pharmacy, Coimbatore -32,& Co-guide **Dr. Hashim K.M., U Win Life Science,** during the academic year 2016-2017.

Station : **BHAVAN KUMAR.A**

Date : **Reg . No: 261526158**

EVALUATION CERTIFICATE

This is to certify that disseration work entitled **"Scientific Validation of Antidiabetic Activity of Ethanolic of Tecoma Stans (L) Juss.Leaf"** submitted by **Mr.BhavanKumar.A**, bearing Reg. No : 261526158 to The Tamil Nadu Dr. M.G.R Medical University, Chennai in the partial fulfillment for the degree of **Master of Pharmacy** in **Pharmacology** is a bonafied work carried out during the academic year 2016-2017 by the candidate at Department of Pharmacology, Karpagam College of Pharmacy, Coimbatore and evaluated by us.

Examination centre:

Date:

Internal Examiner **Convenor of Examination**

External examiner

ACKNOWLEDGEMENT

First of all I would like to thank God for his blessings to do this research work successfully. With immense pleasure and pride I would like to take his opportunity in expressing my deep sense of gratitude to my beloved guide **Prof. G. Nagarajaperumal, M.Pharm., (Ph.D).,** Professor and Head, Department of Pharmacology, Karpagam College of Pharmacy under whose active guidance, innovative ideas , Constant inspiration and encouragement of the work entitled **"Scientific Validation of Antidiabetic Activity of Ethanolic Extract of *Tecoma Stans* (L) Juss. Leaf"**carried out.

I wish to express my deep sense of gratitude to **Dr.R.Vasanthakumar,** Chairman of Karpagam Group of Institutions for the facilities provided me in this institution.

My sincere thanks to our respected and beloved **,Dr.S.Mohan, M Pharm ,Ph.D**, Principal, Karpagam College of Pharmacy for his encouragement and also providing all facilities in this institutions to the fullest possible extent enabling me to complete this work successfully.

My sincere thnanks to **Mr. Muthukumar, M.Pharm.,** Assistant Professor, a Department of Pharmacology and **Ms. Mary Priya,M.Pharm.,** Assistant Professor, Department of Pharmacy Practice for theirindispensable support which enable me to complete this work successfully.

I am also conveying my thanks to **Dr. M.Karpagavalli**, **M. Pharm., Ph.D.,** Associate Professor, Department of Pharmaceutical Chemistry, for encouragement and valuable suggestion during this work.

I take this opportunity with pride and immense pleasure expressing my deep sense of gratitude to my co guide **Dr.Hashim,K.M,** Director of U WIN LIFE SCIENCES, whose innovative ideas,guidance, inspiration, tremendous encouragement, help and continuous supervision has made the dissertation a grand and glaring success to complete.

My glorious acknowledgement to **Mr.N. Shafi** and **Mujeeb** Lab Assistant of U WIN LIFE SCIENCES for encouraging us in a kind and generous manner to complete his work.

I express my sincere thanks to **Mr. K. Simon**, Lab Assistant, Department of Pharmaceutical Chemistry for his kind support.

I convey my gratitude to **Mr. S. Antony Das**, Lab Assistant, Department of Pharmaceutics for his kind support.

I am duly bound to all my **Non Teaching Staffs** of Karpagam College of Pharmacy for their valuable advices and co-operation.

Above all, I am remain indebted to my seniors and class mates **(Anoopa, Amirtha, Shanavas, Mohammed Shanavas, Habeeb, Sijad, Ubaid),** to my beloved parents who inspired and guided me and also for being that back bone for all my successful endeavors in my life.

BHAVAN KUMAR

(261526158)

ABBREVIATIONS

SL.No.	ABBREVIATIONS	EXPLANATIONS
1.	OECD	Organization For Economic Co-Operation And Development
2.	CPCSEA	Committee For The Purpose of Control And Supervision of Experiment On Animal
3.	IAE	Institutional Animal Ethics Committee
4.	CTRI	Clinical Trial Registry of India
5.	ml	Milli Litter
6.	2N	2 Normal
7.	g	Gram
8.	^{0}C	Degree Celsius
9.	mg	Milli Gram
10.	mm	Milli Meter
11.	ng	Nano Gram
12.	dl	Desi Litter
13.	cm	Centi Meter
14.	kg	Kilo Gram
15.	hr	Hour
16.	µg	Micro Gram
17.	Ppb	Parts Per Billion
18.	PHF	Poly Herbal Formulation
19.	H_2SO_4	Sulphuric Acid
20.	HNO_3	Nitric Acid
21.	CNS	Central Nervous System
22.	ANS	Autonomic Nervous System

CONTENTS

LIST OF TABLES

LIST FIGURES

1. INTRODUCTION

1.1. Diabetes Mellitus (DM)

Diabetes mellitus is one of the most common and challenging disease conditions of 21st centuary. It is a chronic complex progressive and multisystemic 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 are 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 diabetes mellitus[5]. DM Has been characterized by a variety of causes such asobesity, abdominal adiposity, genetic, ethnicity etc.

Obesity and increased BMI have a great impact on diabetogenesis. The association between increased BMI and weight gain and risk of Diabetes mellitus is significant among Asians. Waist circumference (WC) cut point for Indians for any cardio- metabolic risk factors is 87cm for men and 82cm for women whereas that of BMI is 23 kg/m^2 in both sexes[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].

DM has a strong genomic association, genome-wide association studies has catalogued a number of gene that have an influence on DM (with modest odds ratio ranges between 1.2 to 1.5) and they include TCF7L2, HHEX, CDKAL1, SLC30A8etc[5].

In India nearly 75% of the Type IIDM has first degree family history of diabetes indicating a strong familial aggregation. Prevalence of insulin resistance is found to be high in Asian Indians and they need higher amount of insulin to maintain normoglycemia. Comparison of Asian Indians, Europeans and other ethnic groups have shown that the former have higher insulin response than others, at fasting and in response to glucose. Asian Indians also have some factors which decreases the insulin sensitivity such as central obesity and high percentage of body fat in comparison to many other populations.

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].

1.2. Ayurveda treatment for DM

Ayurveda which means 'Science of life' has derived from the Sanskrit words 'Ayur' meaning life and 'Veda' meaning knowledge.Ayurveda is based on the concept that everything in the universe has composed of five basic elements such as space, air, energy, liquid and solid and they are called The PanchaMahabhuta, or "five great elements". Ayurveda believes in the theory of tridoshas, namely vata (ether and air), pitha (fire) and kapha (earth and water). Ayurveda aims to keep the structural and physiological entities in a state of equilibrium, which signifies good health. Any imbalance in tridosha may leads to disease.

Diabetes mellitus and its clinical features were known to ancient Indian physicians and they reported an elaborate description of Diabetes mellitus and its management. 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, coccineaindica, amalaki, triphala, bitter gourd, rose apple, leaves of bilva, cinnamon, gymnema, fenugreek, bay leaf and aloe vera. The Ayurvedic preparations 'Vasanta Kusumakar Ras' and 'Chandraprabhavati' are used to treat diabetes mellitus. Proprietary Ayurvedic medications are also used to treat diabetes[8].

In ayurveda, diabetes mellitus is known as 'madhumeha' (madhu means "honey" and meha means "urine").

Madhumeha is characterized by Deterioration of the body with impairment of vata (vatajaprameha). Since it deteriorates maximum of dhatus (body tissues) all the vital organs will be affected. Impaired

digestion leads to accumulation of certain specific digestive impurities in pancreas and thereby abnormal insulin production. Madhumeha is a Maharoga (major disease) lead to several complications like retinopathy, neuropathy, nephropathy, joint pain, impotency, sexual and urologic problems[9].

1.3. Prevalence of DM in India[10]

India is currently experiencing an epidemic of diabetes mellitus. To study the consequences of diabetes and the importance of diabetic care in India we need a thorough study on Epidemiology of Diabetes in different regions of India. Epidemiology of diabetes in India has an extensive history. Long back a national study reported that prevalence of DM in urban areas was 2.1 % and that in rural area was 1.5%. The available studies show that there is a sharp rise in the prevalence of DM in both urban and rural areas, among these southern India having the sharpest increase.

Although in rural India the prevalence of diabetes is much lower than in the urban population, even here the prevalence of diabetes is rapidly rising. Diabetes is fast becoming the epidemic of the 21st century. Type II diabetes, which is more prevalent (more than 90% of all DM cases) and the main driver of the diabetes epidemic, now affects 5.9% of the world's adult population with almost 80% of the total in developing countries. World Health Organization (WHO) reported that 32 million Indians had diabetes in the year 2000. The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and it will rise to 69.9 million by the year 2025. The earliest documented diabetes prevalence study of India was done in Calcutta (now Kolkata) in the year 1938. They found that only 1% of subjects have DM with glycosuria. In Bombay (now Mumbai) reports on test for detection of diabetes in large number of subjects were first available in 1959. Another population based study - National Urban

Diabetes Survey (NUDS) was conducted in six large cities from different regions of India in 2001. This study was done on 11,216 subjects aged over 20 years from all socio-economic strata.

The WHO criterion was used for diagnosis diabetes after an Oral Glucose Tolerance Test using capillary blood. The study showed that the age standardized prevalence of type II diabetes was 12.1%. The prevalence was the highest in Hyderabad (16.6%), followed by Chennai (13.5%), Bengaluru (12.4%), Kolkata (11.7%), New Delhi (11.6%) and Mumbai (9.3%). The prevalence of IGT was 16.8% in Chennai, 14.9% in Bengaluru (formerly Bangalore), 29.8% in Hyderabad, 10% in Kolkata, 10.8% in Mumbai and 8.6% in New Delhi. The Prevalence of Diabetes in India Study (PODIS) was carried out in 108 centers (49 urban and 59 rural) in different parts of India to look at the urban-rural differences in type 2 diabetes and glucose intolerance.

The prevalence of DM was found to be more in urban areas of northern region. Urban area of Chandigarh was the first site to study the prevalence of DM in this region. The study conducted in rural areas reported that the prevalence of diabetes in a rural locality near Delhi was 1.5 % in the year 1991. The prevalence has been reported to vary between 1.5% in Delhi (1991) and 3.7% in Nagpur (2007) in rural areas. Regarding urban prevalence, an increasing trend is observed in the northern part of India since late 1960's, which has escalated from 2.9% in Chandigarh (1966) to 20.1% in Jaipur (2007).

The first study done in South India was a hospital based study at Vellore in 1964, which showed a prevalence of 2.5%. The study conducted in Hyderabad showed a high prevalence of 4.1%. However, the studies in rural areas were conducted since 1972. In 1984, house to house surveys were conducted in Tenali, a small town in Andhra Pradesh (urban) and rural population of Pondicherry (now Puducherry), which reported a prevalence of 4.7% and 1.8% respectively. The prevalence in south India has been reported to vary between 0.7% in Pondicherry to 19.5% in Kochi in urban areas, while the prevalence in rural areas range from 1.3% in

Trivandrum to 13.2% in Godavari. The Chennai Urban Rural Epidemiology Study (CURES) investigators had a unique opportunity to compare prevalence rates of diabetes in Chennai city with three earlier epidemiological studies carried out in the same city using similar methods. It has been shown that Indians have a younger age of onset of diabetes compared to other ethnic groups.

The Chennai Urban Population Study (CUPS) and CURES state that prevalence of coronary artery disease was 21.4 per cent among diabetic subjects compared to 9.1 per cent in subjects with normal glucose tolerance. The Impaired Glucose Tolerance subjects showed 14.9 per cent of prevalence of CAD. It was also seen that there is a rising prevalence of atherosclerosis with an increased intimal medial thickness.

The prevalence of diabetes in urban areas has increased from 2.3% in 1975 to 11.7% in the year 2000. The prevalence of diabetes in peri-urban population was found to be 4.0%. Mumbai and Ahmedabad are the main site f study. Urban prevalence has an escalating trend from 1.5% (1963) to 9.3% in Mumbai (2001). A similar trend is observed in the rural areas - an increase from 3.9% in 1991 to 9.3% in 2006.

1.4. Mechanism of diabetes mellitus induction[2]

1. β- cell destruction (Type 1 diabetes - IDDM)
   (a) Immune mediated
   (b) Idiopathy
2. Insulin resistance (Type 2 diabetes - NIDDM)
3. Genetic defects of β- cell function
   (a) Glucokinase
   (b) Hepatocyte nuclear transcription factor – 4 α
   (c) Insulin promoter factor
   (d) Mitochondrial DNA
   (e) Proinsulin or insulin conversion
4. Genetic defects in insulin processing or insulin actions defects in

(a) Proinsulin conversion.

(b) Insulin gene mutation

(c) Insulin receptor mutation

5. Exocrine pancreatic defects
6. Endocrinopathy

(a) Acromegaly

(b) Cushing syndrome

(c) Hyperthyroidism

(d) Pheochrmocytoma

(e) Glucocanonama

7. Infections

(a) Cytomegalovirus

(b) Coxhacivirus

8. Genetic syndrome associated with diabetes

- (a) Down‘s syndroo generate reactive oxygen species, which also contribute to DNA fragmentation. The formation of superoxide anions results from both STZ action on mitochondria and increased activity of xanthine oxidase.

STZ induced DNA damage activates poly ADP ribosylation leading to the depletion of cellular NAD^+ and ATP content and thereby inhibition of insulin biosynthesis and secretion. Calcium, which may also induce necrosis, does not seen to play a significant roleme

(b) Kleinfelter‘s syndrome

(c) Turner‘s syndrome

9. Drugs

(a) Glucocorticoid

(b) Thyroid hormone

(c) Thiazides

(d) Phenytoins

1.5. Types of Diabetes

There are three main types of diabetes

1.5.1 Type Ⅰ Diabetes

Insulin-dependent diabetes (IDDM; Type I diabetes) is one of the most common metabolic disorders characterized by pancreatic beta cell destruction, it may be due to autoimmune attack. Genetic and environmental factors play a part and HLA- DR3 and HLA-DR4 confer susceptibility to Type 1 Diabetes Mellitus.

1.5.2 Type Ⅱ Diabetes:

Non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes. Resulting from the combination of resistance to insulin action, inadequate insulin secretion and excessive or inappropriate glucagon secretion.

1.5.3 Gestational Diabetes:

Gestational diabetes mellitus (GDM) affects ~ 7% of all pregnancies and it may also be defined as carbohydrate intolerance during gestation. The condition can be associated with several maternal and fetal complications, such as macrosomia, birth trauma, cesarean section and hypocalcaemia, hypoglycemia and hyperbilirubinemia in newborns.

1.6. Etiology/Contributing Factors

- Insulin resistance in the hepatic and skeletal muscle, increased hepatic glucose synthesis, over production of free fatty acids and relative insulin deficiency.
- Beta cells failure.
- Contributing factors:
- Obesity
- Racial/ ethnic background.

- Age (onset of puberty is associated with increased insulin resistance).
- Sedentary lifestyle.
- Genetic predisposition.
- Conditions associated with insulin resistance, (e.g., polycystic ovary syndrome)

1.6.1 Risk Factors of Diabetes

The risk factors for diabetes may be categorized as modifiable risk factors and non-modifiable risk factors.

1.6.2 Modifiable Risk Factors

- Obesity (via BMI and WHR) : A meta-analysis demonstrated the pooled relative risks for incident diabetes of 1.87 (95% confidence interval (CI): 1.67-2.10), 1.87 (95% CI: 1.58-2.20) and 1.88 (95% CI: 1.61-2.19) per standard deviation of body mass index, waist circumference and waist/hip ratio and these three results in obesity and may leads to diabetes.

- Physical Inactivity: The protective effect of physical activity in subjects with an excessive BMI and elevated glucose levels; Diabetes can be prevented by physical activity and weight control in peoples with both normal and impaired glycemic control.

- Plasma Lipids and Lipoproteins Level: There are reports like the blood level of LDL, VLDL, TGL are high and that of HDL is low in Diabetic people. According to American Diabetes Association LDL Cholesterol should be <100 mg/dl; HDL Cholesterol: >60 mg/dL; and Triglycerides: <150 mg/dl.
- Hypertension: It has been reported that the incident of diabetes for hypertensive patient is 2.21 greater.

- Dietary Habits: It is suggested that whole grains are rich resources of dietary fiber, fat, vitamin, antioxidant nutrients, minerals, lignans,

starch, and phenolic compounds that have been linked to the reduced risk of insulin resistance, dyslipidemia, obesity, T2DM, heart diseases and dietary fructose specifically increases de novo lipogenesis, promotes dyslipidemia, decreases insulin sensitivity, and increases visceral adiposity in overweight/obese adults.

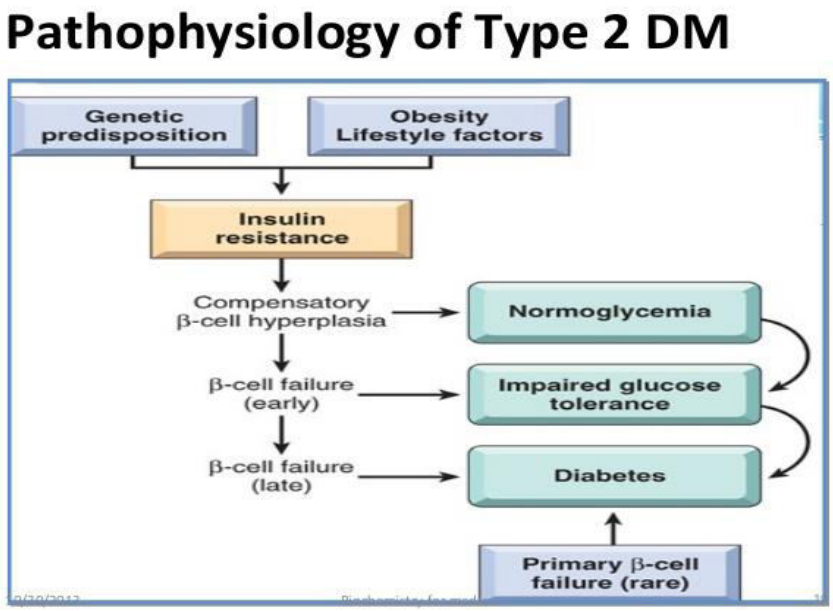
1.6.2 Non-modifiable Risk Factors

- Family History: First degree family history of diabetes was found in an approximate of 75% of the T2DM patients.
- Genetic factors: Genome-wide association studies show a strong association between genetic factor and Diabetes.
- Low/High Birth Weight: Both Low and High Birth Weight are risk factors of Diabetes.

1.7 Pathophysiology of non-insulin dependent DM (Type-II)

Type-2 diabetes mainly occurs due to insulin resistance and/or insulin deficiency. Insulin resistance result in the impaired insulin mediated glucose uptake in the peripheral tissues like muscle and fat, fail to suppress hepatic glucose production and TGL reuptake by fat cells. To overcome the insulin resistance, beta islet cells try to increase the amount of insulin secreted. Beta cell destruction reduces insulin production. Therefore DM-2 has to occur, two defects are necessary: insulin resistance and insulin deficiency relative to the resistance.

Fig. No: 01: Pathophysiology of Type-II Diabetes mellitus



1.7.1 Clinical Manifestations of Type 2 Diabetes Mellitus:[11]

- Polydipsia – increased thirst.
- Polyphagia – increased hunger.
- Polyuria – increased urine.
- Slow healing infections.
- Blurred vision.
- Impotence in men

1.7.2. Criteria

Overweight (BMI ≥ 85th percentile for age and gender, weight for height ≥ 85th percentile or weight ≥ 120% of ideal for height).

Plus any two of the following risk factors:

- Family history of type 2 diabetes in first- or second degree relative
- Race/ethnicity (American Indian, African American, Hispanic, Asian/Pacific Islander)
- Signs of insulin resistance or conditions associated with insulin resistance (acanthosisnigricans, hypertension, dyslipidemia, polycystic ovary syndrome)

1.7.3 Age of initiation:

Age 10 years or at onset of puberty if puberty occurs at a younger age.

1.7.4. Frequency:

Every 2 years.

1.7.5. Test:

Fasting plasma glucose is the preferred method for screening.

* Clinical judgment should be used to test for diabetes in high-risk patients who do not meet these criteria.

1.8. Type 2 dm management in India

The patient should receive appropriate medical care along with self-management to keep the diabetes under control. The main treatment goals are

1. To achieve optimal glycemic level.
2. To reduce other cardiovascular risk factors, including hypertension, hyperlipidemia, and overweight and obesity; and
3. To diminish micro and macro vascular complications.

Diabetics are treated with oral hypoglycemic agents with appropriate diet and exercise. Some patients may require insulin therapy with or without oral hypoglycemic agents.

1.9 Treatment[12]

Fig. No : 02. Image showing the effect of different anti diabetic drugs

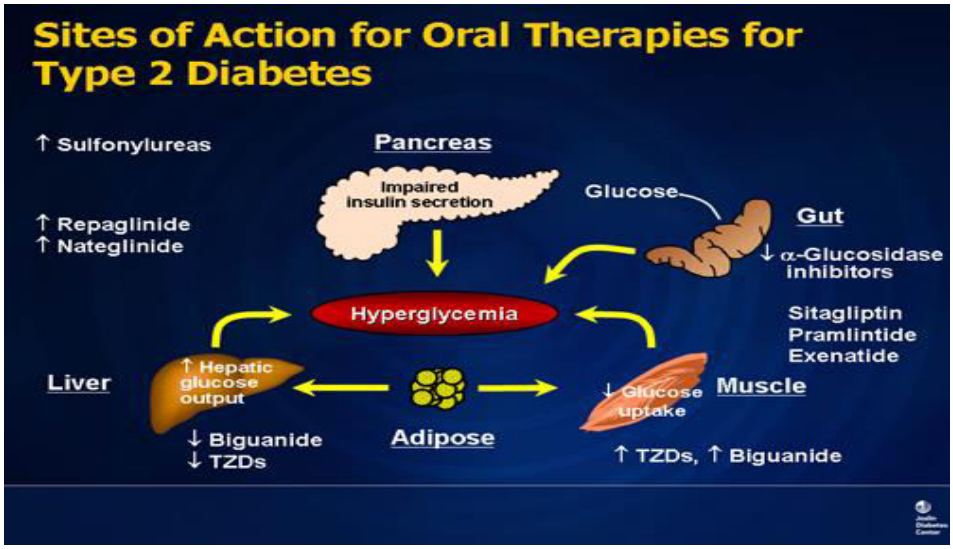


Table 01: Classification of Oral Hypoglycaemic Drugs

ORAL HYPOGLYCAEMIC DRUGS		
Si No	**CLASS**	**DRUGS**
1	**Sulfonylureas**	**First generation;** Tolbutamide, Chlorpropamide. **Second generation;** Glibenclamide, Glipizide
2	**Biguanide**	Metformin, Gliclazide, Glimepiride
3	**Meglitinide/Phenylalanine analogues**	Repaglinide, Nateglinide
4	**Thiazolidinediones**	Rosiglitazone, Pioglitazone
5	**Glucosidase inhibitors**	Acarbose, Miglitol
6	**Dipeptidyl peptidase-4 (DPP-4) inhibitor**	SitagliptinVildagliptin

1.9.1 Sulfonylureas

Drugs: Tolbutamide, chlorpropamide these are the drugs in the first generation. In second generation drugs like glibenclimide, glipizide, gliclazide and glimepiride.

1.9.2 Side Effects

- Low blood sugar,
- An upset stomach,
- Skin rash or itching,
- Weight gain.

1.9.3 Biguanides

Drugs: Metformin

1.9.4 Side Effects

- Abdominal pain
- Nausea
- Metallic taste
- Mild diarrhea
- Anorexia

1.9.5 Alpha-glycosidase inhibitors

Drugs: Acarbose and miglitol

1.9.6 SideEffects

- May cause stomach problems such as gas, bloating and diarrhea

1.9.7 Thiazolidinediones Drugs

Drugs:Pioglitazone and troglitazone

1.9.8 Side Effects

- Plasma volume expansion

- Edema
- Weight gain Headache
- Myalgia
- Mild anemia

1.9.9 Meglitinides

Drugs: Repaglinide and nateglinide

1.9.10 Side Effect

- Weight gain
- Low blood sugar

1.10 Medical nutrition therapy [11]

A meal plan with regular meals and snacks and carbohydrate goals that are moderately less than their usual intake will often help lower the elevated blood.Since most of the teens diagnosed with type II diabetes are obese, they have to identify carbohydrate-containing foods and monitor carbohydrate intake thereby cessation of weight gainmay occur.

1.10.1 General Guidelines for Food Intake

- Eat 3 meals and 1 snack on a regular schedule.
- Try not to skip meals.
- Follow carbohydrate goals for meal planning from the dietitian.
- Try to eat about the same amount of carbohydrate at the same time each day.
- Decrease saturated fat intake.
- Work towards a healthy weight.
- Eat smaller portions at meals.

1.10.2 Ways to Limit Carbohydrate Intake

- Drink calorie-free beverages (e.g., water, tea, diet soda).

- Limit fruit juice to 1 cup/day.
- Limit carbohydrate servings to 3-4/meal. If necessary decrease to 1-2 at breakfast.
- Check blood glucose level 2 hours after eating. (If >180 mg/dl, you ate more carbohydrate than your body could handle).

1.10.3 Benefits of Exercise

- Helps you feel better and increases your energy
- Reduces HbA1c
- Improves insulin sensitivity
- Helps in reaching a healthy weight
- Increases strength and flexibility
- Decreases risk factors for heart disease
- Educes body fat and increases muscle mass[1]

The aim of treatment is to maintain glycemic level with in normal range. Additional considerations are:

- Maintain healthy weight.
- Minimize hyperglycemia and hypoglycemia.
- Achieve normal lipid levels.
- Prevent and delay complications.
- Promote optimal health and well-being.

Treatment regimen depends on the type of diabetes, medical nutrition therapy, frequent blood glucose monitoring to identify and evaluate blood glucose patterns, and comprehensive education in diabetes, self-management and decision-making skills at diagnosis and follow-up visits. Target blood glucose goals for teens are listed in Table 2.

Table 02 : Blood Glucose Goals for Adolescents

Biochemical Index	Normal	**Goal Action**	**Indicated**
Average premeal BG (mg/dl)	<110	80-120	<80 or >140
Average 2 hour postmeal BG for rapid-acting insulin only	<120	150-180	>180
Average bedtime BG (mg/dl)	<120	100-140	<100 or >160
Average 3:00 am BG (mg/dl)	<110	80-100	<80 or >120
HbA1c (%)	<6	<7	>8

Adapted from: Orr, DP. Contemporary management of adolescents with diabetes mellitus. Part 1: Type 1 diabetes. Adolescent Health Update 2000;12(2), Table1, p 2.

1.11 Medicinal Plants

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.

1.11.1 Importance of herbal drugs

Antidiabetic allopathic drugs have their own side effect & adverse events like hypoglycaemia, nausea, vomiting, hyponatremia, flatulence, diarrhoea or constipation, alcohol flush, headache, weight gain, lactic acidosis, pernicious anaemia, dyspepsia, dizziness, joint pain. So instead of allopathic drugs, herbal drugs are a great choice which is having more or less no side effect & adverse effects. Around 800 Indian herbs possess ant diabetic activity. Though complementary & alternative medicine (CAM) treatments are popular, scientific evidence support their application to diabetes care is scare. Instead of focusing on single modalities CAM practitioners prescribe complex, multi dietary intervention. Ayurvedic interventions may benefits patients with higher base line HbA1c value, warranting further research.[14]

Natural origin and fewer side effectspromote the use of herbal drugs in bothdeveloping and developed countries. In the last few years there has been an exponential growth in the use of herbal drugs. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter.According to World Health Organization (WHO) there are about 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are found in India. Indiais called as botanical garden of the world because of the rich herbal medicine resources. Very recently, two exhaustive reviews have been published based on global literature survey on 150 plants and 343 plants from different parts of the world. some plants like Allium cepa (Onion, piyaj), Allium sativum (garlic, lasun), Syzygiumcumini (Syn. Eugenia jambolana; (black plum; jamun), Momordicacharantia (bitter gourd; karela) Gymemasylvestre (Gurmar), Pterocarpusmarsupium (Vijay-) sar) etc. are well noticed by scientists as well as laymen, in recent years [15].

Biological actions of the plant products used against diabetes are related to their phytochenistry. Herbal products or plant products are rich in phenolic compounds, flavonoids, terpenoids, coumarins, and other constituents which reduces the blood glucose levels[16].

Our Vedic literatures like CharakSamhita already report the use of herbs and herbal derivatives for treatment of diabetes mellitus. According to CharakSamhitsa more than 400 plants are used in 700 recipes which are used to treat diabetes mellitus in almost two thirds of the world population. A large number of in vivo studies have been conducted on animals to test the claimed activity have demonstrated the hypoglycemic property of many plants, already reported in various literatures. The plant families, including the species most studied for their confirmed hypoglycemic effects include, Leguminoseae, Lamiaceae, Liliaceae, Cucurbitaceae, Asteraceae, Moraceae, Rosaceae, Euphorbiaceae and Araliaceae[17].

1.12 Animal Models [13]

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.

- STZ decrease insulin biosynthesis and secretion by impairing glucose oxidation. STZ at first abolished the B cell response to glucose. Temporary return of responsiveness than appears which is followed by its permanent loss and cells are damaged. STZ is taken up by pancreatic B cells via glucose transporter mainly GLUT-2. Intracellular action of STZ

causes in changes of DNA I pancreatic B cells compromising its fragmentation. Alkylation of DNA is the main reason for the STZ induced B cells death.

- STZ inhibits the Krebs cycle and decreases oxygen consumption by mitochondria and strongly limit mitochondrial ATP production and cause depletion of this nucleotide in B cells. Augmented ATP dephosphorylation increases the supply of substrate for xanthine and enhances for uric the final product of ATP degradation.

- STZ is a Nitric oxide (NO) donor and NO was found to bring about the destruction of pancreatic islet cells. Hence it produces DNA damage. STZ is however not a spontaneous nitric oxide donor. STZ was found to generate reactive oxygen species, which also contribute to DNA fragmentation. The formation of superoxide anions results from both STZ action on mitochondria and increased activity of xanthine oxidase.

- STZ induced DNA damage activates poly ADP ribosylation leading to the depletion of cellular NAD^+ and ATP content and thereby inhibition of insulin biosynthesis and secretion. Calcium, which may also induce necrosis, does not seen to play a significant role.

2. LITERATURE REVIEW

Mohammed Salem et al (2013).,[14] 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*

Kameswaran et al (2013).,[15] 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.

Kameswaran Sugavanan et al (2012).,[16] 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.

Amad Ali-Azzawi et al (2012).,[17] studied the genotoxic potential of *Tecoma stans* by *in vivo* and *in vitro* system. This study examined the genotoxic activity of aqueous and ethanolic extracts on bone marrow cells from BAL B/C mice through evalution of milotic index and chromosome aberration and cytotoxic effect of two extracts on mouse embryo fibroplast (MFF) cell line. No alteration in the total no. of chromosomal aberration were observed and percentage of miotic index at the concentrations tested remained unchanged. The higher concentrations of the plant extracts had a cytotoxic effect on MFF cell line. Both extracts had no significant elastogenic effect *in vivo,*but showed cytotoxic effects on mouse embryo.

Thirumal et al (2012).,[18] studied the crude leaf extract of *Tecoma stans*. These were examined for their anticancer activity. To determine *in vitro* Anticancer activity, different concentration of crude extract were tested in MCD - of cancer cell line by 3- (4,5- dimethylthiazole-2-yl)-2,5-dimethyl tetrazolium bromide (μTT) assay. *Tecoma stans* leaf extract showed significant anti-proliferative activity and a dose dependant effect was observed. Minimum inhibition of 14.6% was shown by extract at concentration 7.5 μg/ ml and maximum inhibition (95.9%) was observed at 1000 μg/ml.

Govindappa et al (2012).,[19] studied the antibacterial and antioxidant activity of ethanol, methanol and aques extract of *Tecoma stans*. These three solvent fractions possessed strong radical scavenging activity when analyzed using FRAP and DPPH. It was ranged from 1443.79 to 3841.17 g/ml. The results indicate that the plant is a potential candidate to be used as an antimicrobial and antioxidant.

Kameswaran et al (2012).,[20] evaluated the anticancer activity of methanolic flower extract of *Tecoma standin vitro* and *in vivo* methods. Extract was subjected to preliminary qualitative phytochemical

investigation by using standard procedures and *In vitro* antitumor activities were evaluated by the MTT Assay method using vero and HFP - 2 cell lines. The extract was subjected to *in vivo* anticancer activity using enrich as cities carcinoma (EAC) tumor model. The activity was assessed increase in life span, average increase body weight, changes in food intake, Tumor volume, tumor weight, variable cell count, non-variable cell count, PCV, total cell count and hematological studies. The potency of extract was compared with standard 5-flurouracil (20 mg/kg). *In vitro* anticancer activity was exhibited and significant cytotoxic activity against both cell lines, at different concentration. Oral administration of the extracts at the doses of 200 and 400 mg/kg, significantly ($P<0.001$) increase the survival time. Non-viable cell count, decreased the average body weight and food intake, viable cell count of the tumor bearing mice. After 14 days of inoculation, METS was able to release the changes in the haemotological parameters, protein and P<V consequent to tumor inoculation.

Raju et al (2011).,[21] received on the ethno-pharmacological, phytochemical and therapeutic potential of *Tecoma stans*. An exhaustive survey of literature revealed that alkaloids, flavanoids, saponins, phenols, steroids, anthraquinone tannis, terpenes and glycosides constitute the major classes of phytoconstituentspresent in the plant. Pharmacological report revealed that is having antidiabetic, anticancer, antioxidant, antispasmodic, antimicrobial, antifungal properties and extensively used in the treatment of diabetes.

Krishna et al (2009).,[22] studied the phytochemical screening analysis of n-hexane, ethyl acetate, ethanol and aqueous extracts of Tecoma stans, which indicates, the presence of alkaloids, saponins and tannins, In the n-hexane extract, the constituents were absent.

Lin et al (2008).,[23] found that, the flavanoids from the plant extractspossesssubstantial antimicrobial and antioxidant activities.

Socolwsji et al (2008).,[24] studied the effects of temperature and light on the control of seed germination in *Tecoma stands*. The influence of constant temperature from 10-40°C, with 5°C intervals under the white light and darkness were tested. The optimum temperature for the seed germination were found to be between 25 and 30° C for both the light and dark treatment. The maximal germination was reached in the range of 19-38°C under the light and 20-40°C during the darkness. The seeds showed highest synchronization of the germination near the optimal temperatures. The germination in the field was tested under the two light conditions. The highest percent of germination occurred under the Canopy (69%). However under the Canopy, the seedling presented 1.9% of the recruitment, while under the direct sunlight, 96.9% results showed that *T. stans* seeds germinated well in open areas with the occurrence of high seedling recruitment indicating the invasion potential of the speciesunder such light conditions.

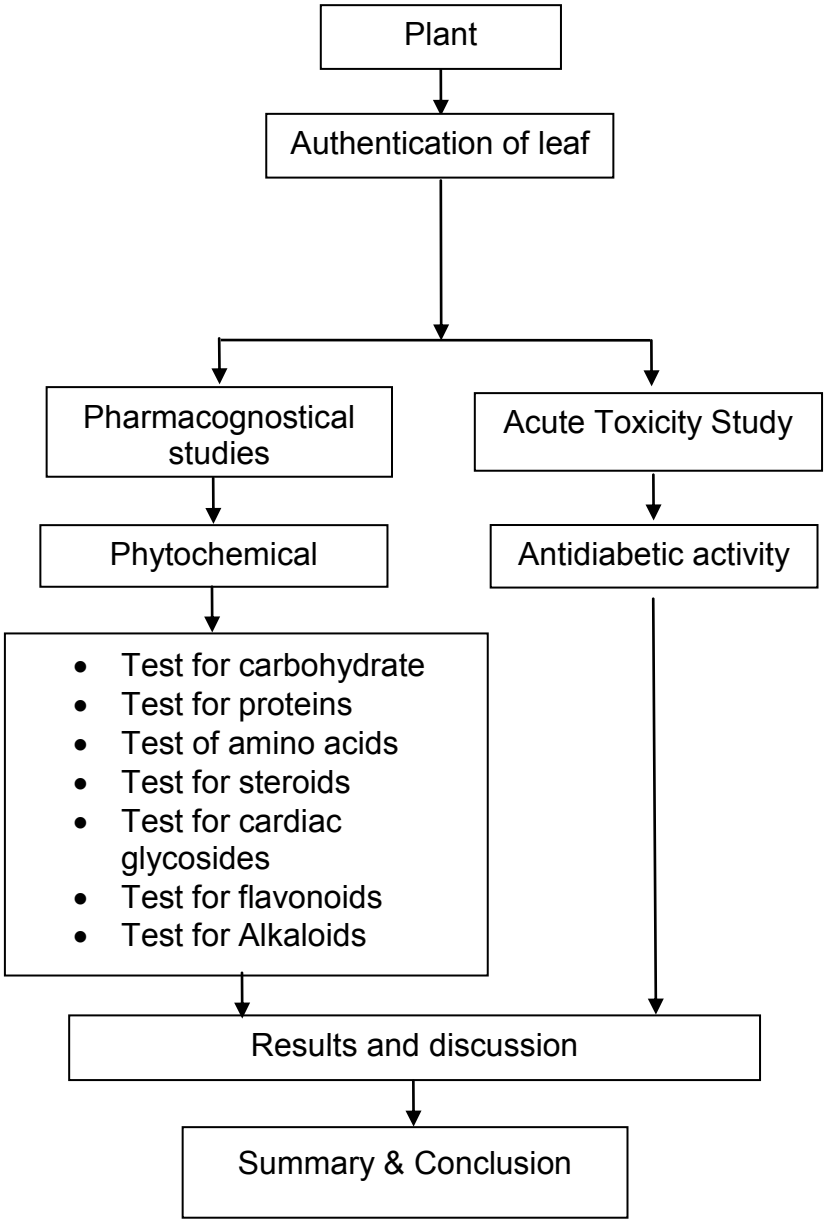
Gharib Naseri . et al (2007).,[25] have reported antispasmodic effect of *Tecoma stans* hydro alcoholic leaf extract on rat ileum.

3. AIM AND OBJECTIVE

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 *TecomaStans(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 *invivo* streptozocin induced diabetes in rats

4. PLAN OF WORK



5. PLANT PROFILE

5.1 Tecomastans (l.)Juss.exkunth

Fig No:03. Palnt of *TecomaStans*(L.)

Kingdom : *Plantae*

Sub kingdom : *Tracheobionta*

Super division: *Spermatophyta*

Division : *Magnoliophyta*

Class : *Magnoliopsida*

Subclass : *Asteridae*

Order : *Scrophulariales*

Family : *Bignoniaceae*

Genus : *Tecoma* Juss.

Species : *Tecoma stans* (L.) Juss.exKunth

5.2 Vernacular names

Tamil	:	Manjarali, Naagasmbagam, Soonnapati,Sornapati
English	:	*Trumpet Flower, Yellow Elder, Yellow Trumpet Bush.*
Spanish	:	*Lluvia De Oro, Trompeta, Tronafrente, Tronadora*
French	:	*Tecomajaune*
Portuguese	:	*Amarelinho, Ipê-Mírím*
Germany	:	*Aufrechtetrompetenwinde*
Italy	:	tecomagiallo
Pacific Islands		:*piti*

Tecomastans is a species of flowering perennial large shrub or small, much-branched, tree usually growing 1.5 to 5 m 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. 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.

5.3 General description

5.3.1 Stem

Full and cylindrical. The younger stems are smooth, glabrous and greenish in colour. They are slightly quadrangular and turn pale brown or reddish-brown in colour as they age. The bark on the main stem is light brown to pale grey in colour, furrowed, and relatively rough in texture, covered in light greyish to brown barks.

5.3.2 Leaf

Fig No:04: *Tecomastans*(L.) Leaf

Borne on petioles 3-5 cm long. Compound, opposite, 10-25 cm long, with 3-13 leaflets, but usually 3-7 leaflets. Leaflets lanceolate to elliptic, 2-10 cm long and 1-4 cm wide, apexlong-acuminate, base cuneate. Margins irregularly and finely toothed. Both sides of the leaf blade are smooth and mostly glabrous, though a few hairs may be present on the undersides near the midrib.

Inflorescence: Erect or inclined several-flowered clusters (5-15 cm long), produced terminally (at the ends of the branches), and then later, in the leaf axils near the tips of the branches.

5.3.3 Flowers

Showy bright yellow, tubular (trumpet-shaped), borne on short pedicels somewhat curved or twisted. Corolla tube 3-5 cm long with five rounded lobes, 8-30 mm long. Presence of several faint reddish lines in the throat of the flower, which is slightly ridged and hairy.

Fig. 5 : Flower of *Tecomastans*(L.)

5.3.4. Fruits

The fruits are large, linear capsules, somewhat flattened, 10-20 cm long and 0.5-2 cm wide, brown at maturity, they split open to release numerous seeds, 3-5 mm each.

Fig No: 03 Fruit of *Tecomastans*(L.)

5.3.5. Seed

Numerous. The seeds are very flat, oblong in shape (7-8 mm long and about 4 mm wide), and have a transparent wing at each end (the size of entire seed including the wings is about 20 x 6 mm)

Fig No: 03 Seed of *Tecomastans*(L.)

5.3.5 Trunk

Tecomastans has a tendency to grow with several trunks. As an ornamental it can be trained to grow with a single trunks. The bark on the main trunk is light brown, hard, lose grained and become corky with age.

5.4 Chemical Constituents

The plant contains triterpenes, hydrocarbons, resins and volatile oil.

1. Leaves contain flavanoids, chryseriol, Luteolin, Hyperoside, Indoleoxygenase. Alakloid like Tecomanine, Tecostamine, 4-noroctidine, N- hormethyl-skytarthine and S- skytanthine.
2. Flowers contain p- carotene and Zeaxanthin. Methanolic extract of the flowers showed the presence of flavonone , 7, 8 dihydroxy-5,6-dimethoxy flavones and Kaemperferol .
3. Seeds contain fatty oil and the compositionis plamitic acid, stearic acid octadecenoic acid, octadienoic acid, octadecatrienoic acid and octadeccatetranoic acid.
4. Ethanolic extract of fruit contains monoterpenic alkaloids, 7-hydroxyskytenthine, 5-hydroxytecomanine, 5-hydroxyskytenthine .

5.5 Medicinal Uses

- Aerial parts used in the treatment of stomach problems, gastritis, diarrhoea
- Roots are used as diuretic, vermifuge, tonic, beer making, a remedy in snakes bits, scorpion sting and in the treatment of syphilis .
- Flowers possess narcotic and analgesic activity.

6. MATERIALS AND METHOD

6.1 Plant Material

The plant leaves was collected locally from herbal store and botanical garden of the garden of the botany central council for Research Ayurvedicand Sidha Govt. of India .The plant was identified and authenticated by comparison with herbarium specimens. The leaf of *Tecomastans* (L.)juss ex kunth were authenticated by comparison with herbarium specimens and authentification No. BSI/SRC 5/23/2016/Tec/1993.

The weighed coarse powder was used for the extraction by successive solvent extraction by Soxhlet apparatus using various solvents.

6.2 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 acclimatisation 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

6.3 Chemicals, Drugs and Instruments

Streptozotocin, citric acid, sodium citrate 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.

6.4 Extraction Procedure[26]

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.

Image No. 01: Extraction using soxhlet apparatus assembly

A. Alcoholic extract

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

6.5 Phytochemical Analysis[27]

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.

6.5.1 Test for Carbohydrates

I. **Molisch Test:** To the aqueous extract, 1ml of ∝- napthol solution was added and Conc. Sulphuric acid 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.

6.5.2 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.

6.5.3 Test for Amino acids

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

6.5.4 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.

6.5.5 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.

6.5.5 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.

6.5.7 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.

6.5.8 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.

6.5.9 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.

6.5.10 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.

6.6 *TOXICITY STUDIES* [28,29,30]

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
Blood collection	:	Not needed
Sacrifice	:	Not needed

Table No. 02 : Experimental Design of Acute Toxicity Study

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

6.7 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.

6.8 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 coloured chow diet.

6.9 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.

6.10 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 andpiloerection) and central nervous system (gait, tremors and convulsion) were carried out and changes were noted (OECD, 2001).

6.11 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 behavioural or clinical signs of toxicity. Weight changes are calculated. At the end of the test animals are weighed. LD_{50} values are established using the formula.

6.12 Dose Calculation Equation

$$LD_{50} = \text{higher dose} - \Sigma (a \times b)/n$$

Where,

a = dose difference
b = animal died

n = No. of animals in each group

$$ED_{50} = \frac{LD_{50}}{10}$$

6.13 Pharmacological Studies[31]

6.13.1 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.

6.13.2 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-peritonialinjection 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 STZwere used for the study.

6.14 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.

6.15 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.

6.16 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.

$$\%IG = (Gx-Go)/Go \times 10$$

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

6.17 Hypoglycaemic Activity

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

6.18 Experimental Design: All hyperglycaemic rats were randomly divided into four groups of six rats in each groups, 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).

6.19 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.

7. RESULTS AND DISCUSSION

7.1. EXTRACTION

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

Table No. 01 : SOXHLET EXTRACTION OF *TECOMASTANS* (L.) juss. EX KUNTH

Plant	Part used	Method of Extraction	Solvents	Average value of extractive(%W/V)
***TecomaStans*(L.) juss.exkunth**	Dried Leafs	Continuous Hot percolation by Soxhlet apparatus	Ethanol (50%)	33.2%

7.2. 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.

7.2.1. 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.

7.2.2. 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.

7.2.3 Test for Amino acids

I. Ninhydrin Test:

A blue colour indicates the presence of proteins, peptides or amino acids.

7.2. 4. Test for Fats and Oils

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

7.2.5. Test for Steroids

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

7.2.6. 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.

7.2.7. Test for Saponins

I. Foam Test:

A1cm layer of foam indicates the presence of saponins..

7.2.8. 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.

7.2.9. 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 No.02: preliminary phytochemical evaluation of *Tecomastans* (l.) juss. exKunthleaf extracts

S.No	Phytoconstituents	Ethanol
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

7.3. ACUTE TOXICITY STUDY

There were 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.

Table No: 03-Changes in wellness parameters observed for ethanolic extracttreated wistar rats.

Sl no	Response	Group1(5mg/kg)		Group 2 (50mg/kg)		Group 3 (300mg/kg)		Group 4 (2000mg/kg)	
		Before	After	Before	After	Before	After	Before	After
1	Alertness	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	Grooming	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
3	Anxiety	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
4	Roaming	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
5	Tremor	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
6	Convulsion	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
7	Depression	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	Gripping strength	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	Scratching	Present	Present	Present	Present	Present	Present	Present	Present
10	Defecation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Sl no	Response	Group1(5mg/kg)		Group 2 (50mg/kg)		Group 3 (300mg/kg)		Group 4 (2000mg/kg)	
		Before	After	Before	After	Before	After	Before	After
11	Writhing	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
12	Pupils	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
13	Urination	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
14	Salivation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
15	Skin and fur	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
16	Lacrimation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
17	Pilo erection	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
18	Nail status	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
19	Gauntness	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
20	Gait	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
21	Diarrhoea	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
22	Sleep	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
23	Coma	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
24	Lethargy	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
25	Mucous membrane	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

(+) – Presence, (-) – Absence

7.4. PHARMAACOLOGICAL STUDIES

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

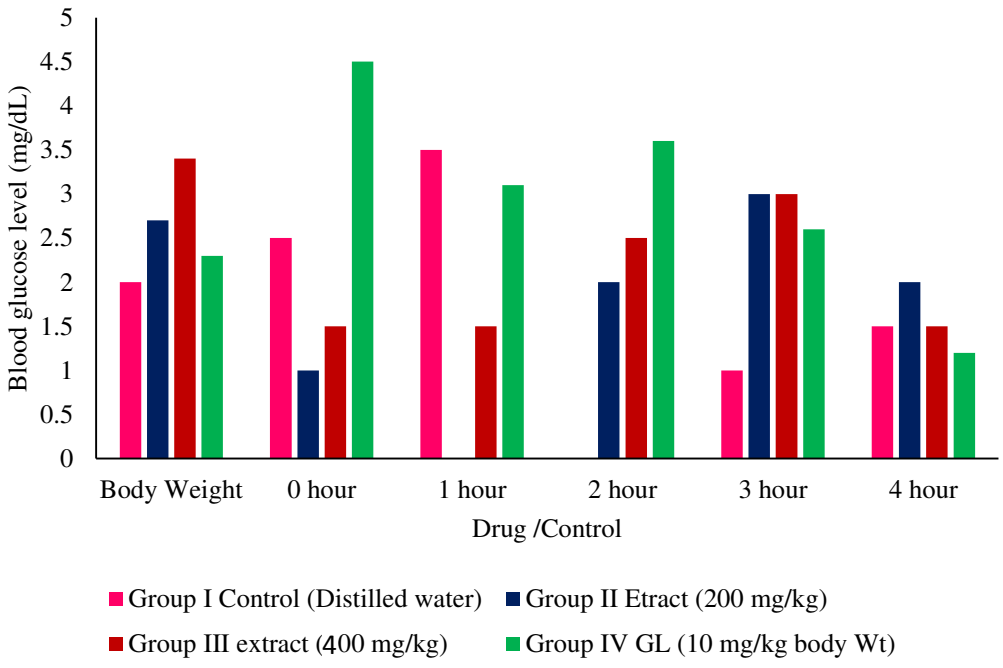
Vehicle treated group and GL (10 mg/kg body wt) treated group showed significantly rise in serum glucose level (SGL) after one hour of glucose administration, whereas groups II and III showed significantly increase in SGL respectively. From the study, it is found that both 200 mg/kg and 400 mg/kg of 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, shown in Table No 4. 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.

Table No.4: - 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.

Graph 1 - Effect of ethanolic extract on serum glucose levels in OGTT model in normal rats



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

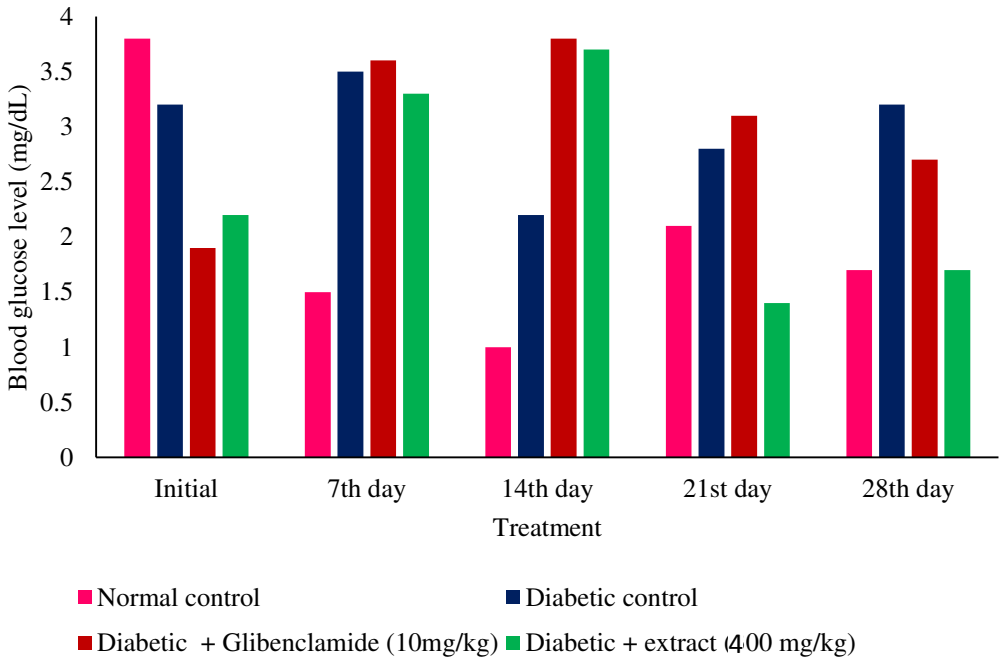
Diabetic control rats showed consistent and gradual rise in SGL during the study. GL (10 mg/kg body wt) and ethanolic extract 400 mg/kg treated rats showed a significant reduction 7th, 14th, 21st, and 28th day of the study and the results were found to be statistically significant (P<001) as compared to diabetic control which is shown in Table 5. 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.

Table No. 5: - 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	21stday	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.

Graph: 2 - Effect of 27 days treatment of ethanolic extract on serum glucose levels of STZ-induced diabetic rats



7.4.3. Effect of ethanolic extract treatment on body weight

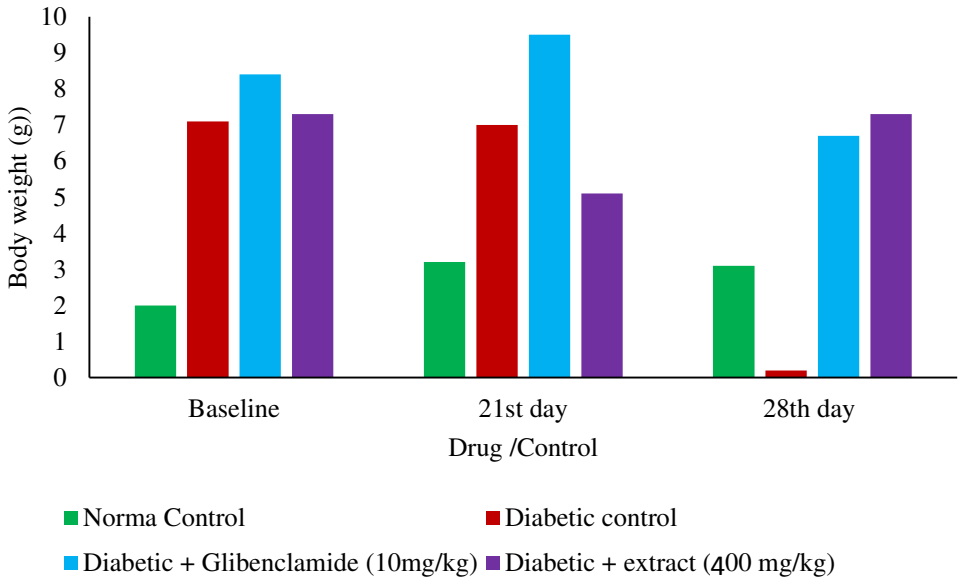
There was also a significant reduction in body weight in diabetic animals, however, the animals treated with 400 mg of ethanolic extract and GL showed significant ($P<0.001$) check on the loss of body weight on days 21 and 28 in comparison to the day of onset of the study. This effect may be attributed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

Table No.6: - 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.

Graph 3 :- Effect of ethanolic extract treatment on body weight in STZ-induced diabetic rats on 21st day and 28th day



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 etal[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 are 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 7^{th}, 14^{th}, 21^{st}, and 28^{th} 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 28^{th} day of the study. Decrease in SGL was more significant (P<0.001) on 28^{th} day when compared with standard drug.

8. SUMMARY AND CONCLUSION

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 plantrelated research[37]. They are many difference 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[27]. 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[42-43.]

The acute toxicity study indicated that ethanolic extract at a dose 2000 mg/kg caused neither visible signs of toxicity nor mortality. The LD_{50} and ED_{50} of the drug were estimated as 2000 mg/kg and 200 mg/kg respectively. If LD_{50} 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 LD_{50} 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 LD_{50} values obtained and as such are considerable uncertainties in extrapolating the LD_{50} 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 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[54] 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.[55,56]

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. [56]When diabetic rats were treated with extract, the weight loss was put on check and reversed.

The different extracts (alcoholic and aqueous) of *TecomaStans*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 do 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 *invivo* 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.

भारत सरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA
दक्षिणी क्षेत्रीय केन्द्र / Southern Regional Centre
टी,एन.ए.यू कैम्पस / T.N.A.U. Campus
लाउली रोड / Lawley Road
कोयंबत्तूर/ Coimbatore - 641 003
टेलीफोन / Phone: 0422-2432788, 2432123, 2432487
टेलीफक्स/ Telefax: 0422- 2432835
ई-मैल /E-mail id: sc@bsi.gov.in
bsisc@rediffmail.com

सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2016/Tech. / 1993 दिनांक/Date: 9th December 2016

सेवा में / To

Mr. Bhavan Kumar
II Year M. Pharmacy
Karpagam College of Pharmacy
Coimbatoe 641 032

महोदया/Madam/महोदय/Sir,

The plant specimen brought by you for identification is identified as *Techoma stans* (L.) Jus.ex Kunth (Basionym: *Bignonia stans* L.) - BIGNONIACEAE . The identified specimen is returned herewith for preservation in their college/ Department/ Institution Herbarium.

धन्यवाद/Thanking you,

भवदीय/Yours faithfully,

GVS Murthy 9/12/16.

(डॉ. जी.वी.एस. मूर्ति /Dr. G.V.S. Murthy)
वैज्ञानिक 'जी' एवं कार्यालय अध्यक्ष /
Scientist 'G' & Head of Office

वैज्ञानिक 'जी' एवंकार्यालयअध्यक्ष
SCIENTIST 'G' & Head of Office
भारतीय वनस्पति सर्वेक्षण
Botanical Survey of India
दक्षिणी क्षेत्रीय केन्द्र
Southern Regional Centre
कोयम्बत्तूर / Coimbatore - 641 003.

CERTIFICATE OF PARTICIPATION

This is to certify that Mr. / Ms. / Dr. BHAVAN KUMAR has participated as a Delegate in the educational activity titled "BEST PRACTICES IN PHARMACOVIGILANCE FOR HEALTHCARE PROFESSIONALS IN CONTEMPORARY INDIAN SCENARIO" organized by The Indian Society for Clinical Research, Indian Pharmacopoeia Commission and The Department of Pharmacology, Coimbatore Medical College, Coimbatore on 12.08.2016. This CME is awarded with 10 credit points in the Category-II by Tamil Nadu Dr. M.G.R. Medical University.

DEAN
Coimbatore Medical College
Coimbatore

ORGANISING CHAIRMAN
Coimbatore Medical College
Coimbatore

Estd 1983
RVS GROUP
Building Intellectual Capital

RVS COLLEGE OF PHARMACEUTICAL SCIENCES

Affiliated to The Tamil Nadu Dr.M.G.R Medical University, Chennai
242-B, Trichy Road, Sulur, Coimbatore - 641402. TamilNadu, India
web: www.pharmacy.rvshs.ac.in

Certificate of Participation

This is to certify that

Dr./Mr./Ms. BHAVAN KUMAR *has participated in one day*

NATIONAL SEMINAR ON "Recent Advances in Neuropharmacology and Developmental Neurobiology"

as a Speaker / Panel Member / Delegate / Presented a Poster

Held on 9th June 2016 at

RVS COLLEGE OF PHARMACEUTICAL SCIENCES SULUR, COIMBATORE

Dr.D. Benito Johnson
Convenor

Dr.R. Manavalan
Co-Chairman

Dr.R. Venkatanarayanan
Chairman

BIBLIOGRAPHY

1. HewageeganaHGSP, ArawalaLADM.2013, Phytochemical AndAnti Oxidant Activity Of Traditional Decoction Used For Type 2 Diabetes Mellitus. Universal Journal Of Pharmacy.2(2):134-137.
2. Maninder Kaur, VandanaValecha: 2014 Diabetes and Antidiabetic Herbal Formulations:2 An Alternative to Allopathy. European Journal of Medicine;6(4):226-240.
3. M.Murugan, C.UmaMaheshwaraReddy : 2009, Hypoglycemic and hypolipidemic activity of leaves of *Mucunapruriens* in alloxan induced diabetic rats. Journal of Pharmaceutical Science and Technology.1(2):69-73.
4. KeshriUmashankerPd, Satish Chandra, Janardan Sharma: 2014 Research Article Antidiabetic Efficacy Of Ethanolic Extract Of *HolarrhenaAntidysenterica* Seeds In Streptozotocin – Induced Diabetic Rats And Its Influence On Certain Biochemical Parameters. Journal Of Drug Delivery & Therapeutics.2(4):159-162.
5. Vipin Gupta: Type 2 Diabetes Mellitus In India. South Asia Network For Chronic Disease, New Delhi.1-28.
6. Lt Gen SR Mehta VSM, Col AS Kashyap, 2009 Lt Col S Das: Diabetes Mellitus in India: The Modern Scourge-review article. MJAFI ; 65 : 50-54.
7. E.D. Eze, A. Mohammed, K.Y. Musa ,Y. Tanko : 2012 Evaluation of Effect of Ethanolic Leaf Extract of *Mucunapruriens* on Blood Glucose Levels in Alloxan-Induced Diabetic Wistar Rats. Asian Journal of Medical Science.; 4(1): 23-28.
8. Kalpana Sridharan, Roshni Mohan, Sridharan, Ramaratnam, Deepak Panneerselvam: 2011 Ayurvedic Treatments For Diabetes Mellitus.Cochrane Database Syst Rev. ; 7 (12).

9. Dr. Amritha Rajeev Bams : Life Style Disorder Diabetes - Management Through Ayurveda And Yoga Pg Diploma In One Health. Assignment For The Course Oh 001: Concepts Of One Health :1-19.
10. Dr V. Mohan, Dr R. Pradeepa: 2009 Epidemiology Of Diabetes In Different Regions Of India. Health Administrator ; Vol: Xxii Number 1& 2: 1- 18.
11. Emily Loghmani: 2005 Diabetes Mellitis: Type 1 And Type 2, Stang J, Story (eds) Guidelines for Adolescent Nutrition Services.:167-182.
12. Murugesh Shivashankar,Dhandayuthapani Mani: 2011 A Brief Overview Of Diabetes .International Journal Of Pharmacy And Pharmaceutical Sciences; 3(4) :22-27.
13. Szkudelski .T. 2001 the mechanism of alloxan and streptozotocin action in B cells of the rat pancreas**.** *Physiological research*.;50: p.536-546.
14. Mohammed Z.M.salem .Yousry.M., 2013 African University of microbiology research 7(1):39
15. Kameswaran.S,kothariar, Jotimanivannan. M and Senthilkumar.R, Pharmacologia 2013,4(5):236-246
16. Kameswaran Sugavanam, Suresh Velayutam, Arunachalam Ganeshan 2012,Asian journal of Traditionl medicine 7(1):39
17. Al-Azzawi,Amad,2012.journal of Biological;15(2):92
18. Thirumal M,kishore G,prathika R ,Sampadass and Nithya 2012 chemical and biological sviences 2(4):488-493
19. Govindappa M,Sadanada TS, Channabasavar and Vinay B.Ragevendra ,2012 International Journal of Pharma and Bio Sciences .275-285
20. Kameswaran S,Suresh v ,Mohan raj M,2012 Pharmatutor-art,antiacancer potential of *Tecomastanus* extract.1(2)1275

21. Raju S, Kavimani S,Uma Maheswra Rao,Sreeramlu reDdy k,2011 Journal of Pharamaceutical and bio medical Sciences,8(7):1-5
22. Krishna A V L N, vineela P A S and ravikmar, 2009 phytochemical screening analysis of *Tecoma Stanus* extracts .int j chem. Si(2):1439-1438
23. Linn j ,Gross D,Paul M,2008 antimicrobial and antioxidant studies of plant extracts, International Journal Of Medical Sciences 146-149
24. Socolwsji , Mongaddam M A,dhadorams. 2008 anti spasmodic effect of *Tecomastanus* (L)jugs leaf extract on rat ileum123-12
25. Gharib Naseri , Mognaddam M A, Dhadroms 2007. anti spasmodic effect of *Tecoma Stans*.Leaf extract 123-8
26. Rakhi Mishra, MohdShuaib, Shravan, PremShanker Mishra: 2011 A review on herbal antidiabetic drugs. Journal of Applied Pharmaceutical Science ;01 (06): 235-237.
27. Manish Gunjan, Ravindran M, Goutam K. Jana: 2011 A Review on Some Potential Traditional Phytomedicine with Antidiabetic Properties. International Journal of Phytomedicine; 3: 448-458 .
28. M.Upendra Rao, M.Sreenivasulu, B.Chengaiah, K.Jaganmohan Reddy, C.Madhusudhana Chett:2010 Herbal Medicines for Diabetes Mellitus: A .Review. International Journal of Pharm Tech Research CODEN (USA).2010;2(3): 1883-1892.
29. Chauhan, P. K. Sharma, P. Srivastava, N. Kumar, R. Dudhe: Plants Having Potential Antidiabetic Activity: A Review . Der Pharmacia Lettre,;2(3): 369-387.
30. KeshriUmashankerPD, SatishChandra, Janardan Sharma. 2012 Antidiabetic efficacy of ethanolic extract of *Holarrhena antidysenterica* seeds in streptozotocin–induced diabetic rats and its influence on certain biochemical parameters. J. Drug Delivery Ther;2:159-62.
31. Kokate CK., Purohit AP., Gokhale SB.,Pharmacognosy, NiraliPrakashan, Pune, 2006, 35, p 106-107.

32. Madhulika Singhand EktaSharma. 2013 Preliminary Phytochemical Investigation Of *BerberisAristata*, *Acacia Catechu* And *FicusBenghalensis*- Important Medicinal Plants For Photoprotection. International Journal of Biological & Pharmaceutical Research; 4(9): 614-617

33. Organization for Economic Cooperation and Development (OECD). 2001. Guideline For Testing Of Chemicals 423: Acute Oral Toxicity – Acute Toxic Class Method.

34. Organization for Economic Cooperation and Development (OECD). 2002. Guidelines for the Testing of Chemicals /Section 4, Health Effects Test No. 423, Acute Oral toxicity - Acute Toxic Class Method

35. Organization for Economic Cooperation and Development (OECD). 2008. Guidelines For The Testing Of Chemicals 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents.

36. Shawn Tomy, Sandra Celine, UjwalaTk, Sam Johnson UdayaChander J, CS Kandasamy, P Arulraj, R Venkatanarayanan. 2015 Anti-Diabetic Effect of Polyherbal Formulation in Ogtt and Streptozotocin-Induced Diabetic Rat Model. IJPPS; 7(10)

37. Aaron R.Hansen,Donna M.Graham,GregoryR.PondandLillian L.SiuI. 2014 Phase 1Trial Design:Is 3 + 3 the Best?. Cancer Control; 21(3): 200-208.

38. MSViswanathan,PMUnnikrishnan,KalsukoKomalsu,HiroloshiFushimi,Pur usotam Basnet: 2003 A Brief Introduction Of Ayurvedic System Of Medicine & Some Of Its Problems.International Journel Of Traditional Knowledge;2(2):159-169.

39. UshaKuamari J., Navas M, Mathew Dan, Rajasekharan S: 2009 Pharmacognostic studies on *Acrotremaarnottianum* Wight – A promising ethnomedicinal plant. Indian Journal of Traditional Knowledge;8(3): 334-337

40. Sauvaire Y ,et al.,: 1998 4-hydroxyisoleucine:a novel amino acid potentiator of insulin secretion. Diabetes; 47(2): 206-210.
41. Jordan S.A, Cunningham D.G, MarlesR.J, 2010: Assessment of herbal medicinal products: challenges, and opportunities to increase the knowledge base for safety assessment. Toxicology and Applied Pharmacology ; 243, 198–216.
42. John M Kingsbury ,prentice-hall, Englewood cliffs 1964: Poisonous Plants of the United States and Canada.NJ, USA;145(3639):1425-1426.
43. Stewart M J, Moar J J, Steenkamp P, Kokot M, 1999: Findings in fatal cases of poisoning at- tributed to traditional remedies in South Af- rica. Forensic Sci Int;101:177–83.
44. Cosyns J P, 2003: Aristolochic acid and 'Chinese herbs nephropathy,A review of the evi- dence to date. Drug Saf.;26: 33–48.
45. Saad B, Azaizeh H, Abu-Hijleh G, Said O, 2006: Safety of traditional Arab herbal. Evid Based Complement Alternat Med;3: 433–9.
46. Clarke M L, Clarke E G C, 1967: Garner's Veterinary toxi-cology. London: Bailliere Tindall:1-154.
47. Zbinden G, Roversi F, 1981 : Significance of the LD50 test for the toxicological evaluation of chemical substances. Arch Toxicol; 47: 77–99.
48. Ashafa et al., 2009 : Effects of the extract from the leaves of *Chrysocomaciliata L*. on some biochemical parameters of Wistar rats. African Journal of Biotechnology; 8: 1425-1430.
49. Ode et al., 2011: The Biochemical Changes in Rats Following Chronic Toxicity with *Cassia Singueana* Leaf Extract. Journal of Pharmaceutical and Biomedical Sciences.;8:1-4.

50. Yakubu et al., 2002: Comparative effect of administration of halofantrine hydrochloride and dihydroartemisinin on some enzymes of selected rat tissues. NISEB Journal;2:175-180.

51. VasudevanD.M, Sreekumari S ,2007: Texbook of Biochemistry for Medical students, fifth ed. JaypeeBrothers Medical Publishers Ltd, New Delhi.:1-552.

52. Stanley P, Prince M, Menon VP, 2000: Hypoglycemic and other related action of *Tinosporacardiifilo* in alloxan induced diabetic rats. J. Ethnopharmacol.;70:9-15.

53. DeFronzo RA, Abdul-Ghani M, 2011 : Assessment and treatment of cardiovascular risk in prediabetes: impaired glucose tolerance and impaired fasting glucose. American Journal of Cardiology;108

54. Teschke R, Wolff A, Frenzel C ,Schulze J, 2014 : Review article: herbal hepato- toxicity-an update on traditional Chinese medicine preparations. Alimentary Pharmacology & Therapeutics;40: 32–50.

55. Kamala Kannan N, Prince PSM, 2006 :Atihyperglycemic and antioxidant effect of rutin, a polyherbal flavonoid in streptozotocin induced diabetic wistar rats. Basic ClinPharmacolToxicol;98:97-103.

56. Pari L, Uma Maheswari J, 200: Antihyperglycemic activity of *Amausasapientum* flowers: Effect on lipid peroxidation on alloxan diabetic rats. PhytotherapyRes;14(2):136-138.

57. TeerapornKatisart, 2011 :Transient Receptor Potential Function In Bladder From Control and StreptozotocinTreated Rats.Ph.DThesis, Faculty of Health and Human Sciences, University of Hertfordshire,UK:1-233.

58. A Akbarzadeh,et al., 2007 : Induction Of Diabetes By Streptozotocin In Rats. Indian Journal of Clinical Biochemistry; 22 (2):60-64.

59. Ogbe et al. 2012: Aqueous Ethanolic Extract of *Mangiferaindica* Stem Bark Effect on the Biochemical and Haematological Parameters of Albino Rats. Archives of Applied Science Research;4:1618-1622.

60. Teschke R, Frenzel C, Glass X ,Schulze J, Eickhoff A, 2013: Herbal hepatotoxi- city: a critical review. British Journal of Clinical Pharmacology ;75: 630–636.