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Evaluation of Anti-diabetic Activity of *Tecoma Stans* Stem Extract in Induced Diabetic Albino Rats

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ABSTRACT

Tecoma stans, from the family Bignoniaceae is an important medicinal plant. All parts are widely used in ayurvedic system of medicines. The present study is designed to investigate the antidiabetic activity of *Tecoma stans* stem extract in alloxan induced diabetic albino rats. The ethanolic extract of *Tecoma stans* contains Saponins, Flavonoids and Mono terpenoid alkaloids such as tecostanine and tecomine which is having hypoglycemic effect.The ethanolic extract (200mg/kg) once a day orally given to the alloxan induced diabetic albino rats for 15 days. The glibenclamide 2.5mg /kg body weight once a day orally to the alloxan induced diabetic albino rats for 15 days by using an intragastric tube which acts as standard. Blood samples were collected for collected for day 1, 5, 10, 15 and estimated for glucose levels. At the end of the study (15th day) the glibenclamide (2.5 mg/kg.) and ethanolic extract of *Tecoma stans* stem (200 mg/kg) showed statistically more significant in decrease blood glucose level.Based upon the results of the present investigation, we conclude that the ethanolic extract of *Tecoma stans* stem showed the potential antidiabetic activity.

Keywords: *Tecoma stans*, Antidiabetic activity, Glibenclamide, .

INTRODUCTION

"Diabetes" is a metabolic syndrome of multiple etiologies characterized by chronic hyperglycemia with abnormalities in carbohydrate, fat and protein metabolism due to defect in insulin secretions (Alemzadeh R and Wyatt DT *et al*., 2007; ADA, 2009). Diabetes is associated with long term damage such as malfunction of eyes, kidneys, nerves, heart and blood vessels. It is associated with health complications including renal failure with risk of foot ulcers, including sexual dysfunction, heart disease, stroke and blindness. On the basis of etiology two main categories of diabetes are recognized, Primary diabetes, Secondary diabetes.

(1) Primary diabetes (EC, 2003; Tamborlane WV *et al*., 2008)

It is divided into two types. Juvenile onset diabetes which is Type1 or Insulin dependent diabetes mellitus (IDDM) In Juvenile onset diabetes there is a profound decrease in the number of b cells in the islet of Langerhans and thus there is absolute deficiency of insulin. The main treatment for this type is insulin. Maturity onset diabetes which is also referred as Type II /Non-insulin dependent diabetes mellitus (NIDDM). The patients are usually obese and the treatment is usually dietary, though supplementary oral hypoglycaemic drugs. It is diagnosed by blood or urinary glucose measurement. Insulin resistances as well as loss of insulin secretion contribute to the onset of disease.

2) Secondary Diabetes (Skyler JS, 2007)

The symptoms result from the following pancreatic dysfunction (pancreatitis, pancreatectomy). Hormonal imbalance (eg: Acromegaly, Pheochromacytoma, Cushing's syndrome, glucagonoma). Drugs or chemical induced reactions (eg: glucocorticoids, anticancer agents, streptozotocin or diazoxide, thiazide, some psychoactive agents).

Insulin receptor abnormalities (Voltarelli JC *et al*., 2007)

Certain genetic syndromes (Hyperlipidemea and muscular dystrophy).

Malnutrition (Pirart J, 1973; Christlieb AR *et al*., 1981; Mogensen CE *et al*., 1982).

Diagnosis of early Diabetes Mellitus In moderately severe early diabetes, following features are present. Hyperglycemia, Glycosuria, Loss of weight due to

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increased breakdown of fat and tissue protein. Increased production of ketone bodies by liver and their incomplete utilization by the tissue leading to their accumulation in blood (Ketosis) and elimination in urine (Ketonuria). Lowering of PH of blood due to circulating keto acids (acidosis). Dehydration due to elimination of large amounts of water with glucose in urine. Increased levels of lipid, fatty acids and cholesterol in blood (lipemia). Increased tendency to develop cataract in the eye and atheromatous and artherosclerotic lesions of blood vessels.

AIM OF STUDY

The aim of the study is to evaluate the hypoglycaemic activity of ethanolic extract of *Tecoma stans* stem in induced diabetic albino rats.

MATERIALS AND METHODS
Animals

Adult male and female rats of albino wistar strain weighing between 200-300g were obtained from the Laboratory Animal House, Padmavathi College of Pharmacy and Research Institute. They were kept in polypropylene cages and allowed to get acclimatized to a standard laboratory diet. The animals were adapted to laboratory condition for prior to the experiments and constant room temperature at 22–24°C with 12 hour day and night cycle. Feed and drinking water were provided *ad libitum*. The studies were performed with the approval of Institutional Animal ethics committee (IAEC) following the guide lines of CPCSEA.

Albino Rats

Albino rat is one of the most widely used species of laboratory animals. Its popularity is next only to that of albino mice. Like the mouse, rat is found all over the world, especially in association with human habitation. *Rattus norvegicus* adapts readily to breeding and living in laboratory conditions. Almost 3 to 5 million rats were used annually in laboratories all over world.

Acute toxicity study and dose selection (Belhekar SN *et al*., 2009; Das SN *et al*., 1995)

The extract was investigated for its acute toxicity studies according to the OECD guidelines (425). The extract was given at different doses to the group of six animals at 100 mg/kg, 200mg/kg, 400mg/kg and 600mg/kg orally. The animals were observed for regular three hours after the dose administration and after 24 hours and 48 hours for the changes in behaviour and changes in body weight and mortality. It was found that the extract doesn't produce any significant toxicity upto the dose of 200 mg/kg. Thus the extract was highly tolerable upto 200 mg/kg. The mortality and signs of toxicity of treated groups were monitored for 14 days.

Induction of diabetes in albino wistar strain (Belhekar SN *et al*., 2009)

Diabetes was induced by intraperitoneal injection of monohydrate (5% w/v) in physiological saline at a dose of 150 mg/kg body weight. The diabetic state was confirmed 48 hours after injection by glucosuria and hyperglycemia. Rats with a fasting blood glucose level higher than 200 mg/dl were selected for the study.

Adminstration of Extract

Suspension of ethanolic extract was prepared in 2% carboxyl methyl cellulose. The ethanolic extract was administered in a dose of 200 mg/kg to induced diabetic albino rats.

Procedure (Costantino L *et al*., 2003; Tanko Y *et al*., 2007)

The albino rats were weighed and housed in metabolic cages and left for 2weeks to acclimatize under room temperature. They were allowed to free access of standard food and water. Later they are randomized into four groups 6 animals in each group. Before starting the experiment they were fasted overnight but allowed to access to water. The fasting blood sugar levels were measured in all rats to confirm that the values are within normal range. At the commencement of the experiment the rats were again fasted overnight. Group1 (normal) and to all other groups administrated through intra peritoneal 150mg/kg body weight.

Experimental animals are divided in four groups each groups having six animals (albino rat-wistar strain)

Group I. Normal group Received 1 ml of saline and served as control. Once a day orally for 15 days by using an intragastric tube.
Group II. Positive control group received 150mg/kg body weight, Administered through intra-peritoneal.
Group III. Standard group received glibenclamide 2.5mg /kg body weight once a day orally for 15 days by using an intragastric tube.
Group IV. Received Ethanol extraction of *Tecomastans* stem200 mg/kg body weight once a day orally for 15 days by using an intra gastric tube.

Blood collection

Blood samples were collected from experimental animals by retro orbital puncture method by using capillary tube and stored in eppandroff tubes. The blood samples were collected for day 1, 5, 10, 15 and estimated for parameters.

Blood Biochemical Analysis

The serum levels of glucose, total-cholesterol, high density lipoprotein (HDL), low density lipoproteins (LDL), and triglycerides (TGs) were estimated using the biochemical kits (Beacon Diagnostics) by auto analyzer.

Statistical analysis

All the experimental results were expressed as Mean ± Standard Error Mean (S.E.M.). The values were analyzed for statistically significance using one-way analysis of variance (ANOVA), comparison was done by using Dunnett's test. P* values < 0.05 were considered as

significant and P** values < 0.01 were considered as more significant.

RESULTS

Phytochemical Tests for Ethanolic Extract of *Tecoma Stans*

The ethanolic extract of *Tecoma stans* was subjected for phytochemical screening.

Alkaloids	+
Glycoside	+
Tannins and Phenolic compounds	+
Steroids	-
Saponins	+
Flavonoids	+
Proteins and Amino acids	+
Carbohydrates	+
Gums and mucilage	-

(+) presence (-) absence

Table.1 Effect of ethanolic extract of *Tecoma Stan* stem on glucose level

Time interval	Normal	Positive control	Standard	Ethanolic extract
1st day	90.5±3.9	269.7±8.2*	262.3 ± 6.4	267.3±5.8
5th day	89.42±3.7	270.1±9.8*	209.3±5.4*	226.6±4.0*
10th day	88.72±4.2	271.9±8.3*	152.5±5.7**	191.7±4.6*
15th day	89.12±3.7	271.5±8.7*	124.6±3.9**	147.5±4.4**

n = 6/group. Values are expressed Mean ± S.E.M. Normal control albino rats(Group I) received saline solution, Positive control albino rats (Group II) Received mono hydrate, Standard group albino rats (Group III) Received mono hydrate + glibenclamide2.5mg/kg) and Treated group (Group IV) Received mono hydrate + *Tecoma stans* extract (200 mg/kg). Diabetic control was compared with the vehicle control and extract treated, glibenclamide treated was compared with the diabetic control. *P < 0.05 - Statistically significant;** P< 0.01 –more significant.

Table.2 Effect of ethanolic extract of *Tecoma stans* stem on total cholesterol level

Time interval	Normal	Positive control	Standard	Ethanolic extract
1st day	119.11 ± 094	268.01±1.80*	265.96± 1.16	267.14± 1.72
5th day	119.34±0.86	268..94±1.38*	217.62± 1.75*	221.09± 1.80*
10th day	119.94±0.61	269.72±1.40*	179.93± 1.71*	193.81± 1.88*
15th day	120.27±1.19	270.34±1.46*	130.63± 1.48**	154.27± 2.51**

n= 6/group. Values are expressed Mean ± S.E.M. *P < 0.05 - Statistically significant; ** P< 0.01 –more significant

Table.3 Effect of ethanolic extract of *Tecoma stans* stem on triglycerides

| Time interval	Normal	Positive control	Standard	Ethanolic extract
1st day	78.26± 1.11	179.17±1.18*	174.46± 1.61	177.15±2.15
5th day	78.91± 1.51	180.43± 0.56*	158.21±2.62*	161.31±2.37
10th day	79.12± 1.20	180.94± 1.12*	134.23±1.26*	153.85±2.64*
15th day	79.94± 1.61	181.43± 2.05*	104.53±2.26**	132.41±1.21**

n= 6/group. Values are expressed Mean ± S.E.M.). *P < 0.05 - Statistically significant; ** P< 0.01 –more significant.

**Table.4 Effect of ethanolic extract of *Tecoma stans* stem on high density lipoproteins**

Time interval	Normal	Positive control	Standard	Ethanolic extract
1st day	54.32±0.45	28.25±1.09*	29.95 ± 0.34	28.78±0.47
5th day	54.95±0.60	27.41±0-71*	34.72±0.53*	29.75±0.45
10th day	55.28±0.91	27.92±0.49*	46.10±0.36*	37.63±0.44*
15th day	55.65±0.75	26.23±0.84*	57.19±0.67**	42.78±0.53**

n= 6/group. Values are expressed Mean ± S.E.M. *P < 0.05 - Statistically significant; ** P< 0.01 –more significant.

Table.5 Effect of ethanolic extract of *Tecoma stans* stem on low density lipoproteins

Time interval	Normal	Positive control	Standard	Ethanolic extract
1st day	46.81±0.76	207.12± 1.60*	204.30± 1.59	206.57± 1.10
5th day	46.38± 1.28	207.68± 1.77*	158.32± 1.90*	174.35± 1.95*
10th day	47.21± 1.21	208.12± 1.68*	109.30± 1.60**	127.83± 2.81*
15th day	47.94± 1.17	208.42± 2.02*	58.42± 1.94**	83.16± 2.47**

n= 6/group. Values are expressed Mean ± S.E.M. *P < 0.05 - Statistically significant;** P< 0.01 –more significant.
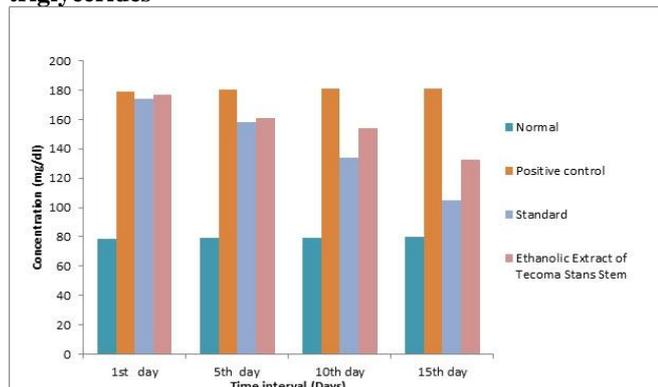
Graph.1 Shows Effect of *Tecoma Stan* stem extract on glucose level
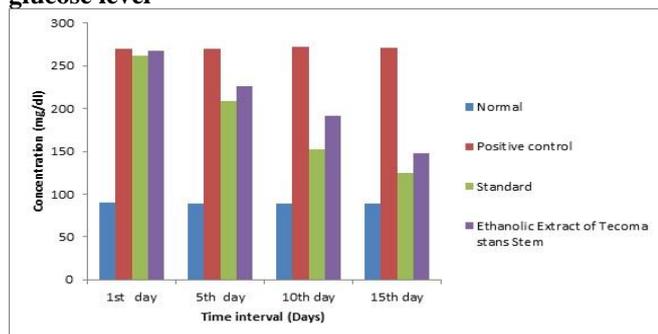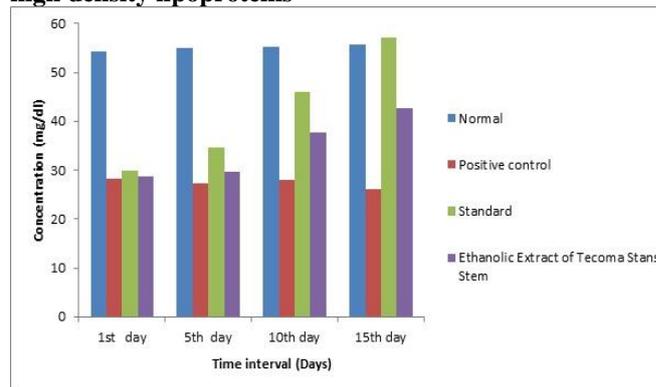

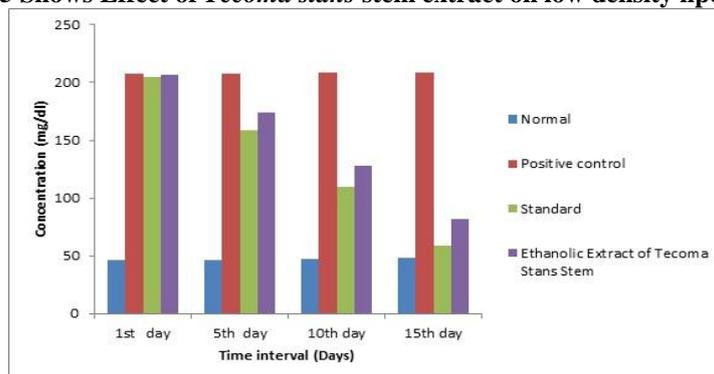**Graph.2 Shows Effect of *Tecoma stans* stem extract on total cholesterol level**
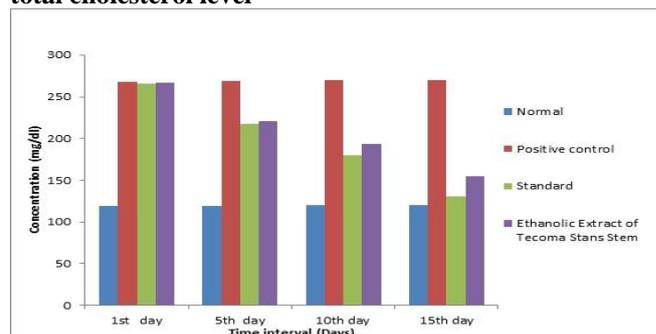
Graph.3 Shows Effect of *Tecoma stans* stem extract on triglycerides

Graph.4 Shows Effect of *Tecoma stans* stem extract on high density lipoproteins

Graph.5 Shows Effect of *Tecoma stans* stem extract on low density lipoproteins

DISCUSSION

The fundamental mechanism underlying hyperglycemia involves over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues. The results of the present study indicate that *Tecoma stans* ethanolic extract (200 mg/kg) was found to reduce the glucose level in monohydrate induced diabetic animals. mono hydrate has been shown to induce free radical production and cause tissue injury.

The albino rats are grouped into four groups, Normal control albino rats(Group I) received saline solution, Positive control albino rats(Group II) received mono hydrate, Standard group albino rats (Group III) Received mono hydrate + glibenclamide2.5mg/kg)and Treated group (Group IV) received mono hydrate +*Tecoma stans* stem ethanolic extract (200 mg/kg). The ethanolic extract group was compared with standard group and positive control group. At the end of the study

(15th day) the glibenclamide (2.5 mg/kg.) and ethanolic extract of *Tecoma stans* stem (200 mg/kg) showed statistically more significant in decrease blood glucose level .The ethanolic extract shows (147.5±4.4) more significant value (**p<0.01) when compared to positive control group and near to standard group. The standard group shows (124.6±3.9) and shows significant value (**p<0.01).The antidiabetic activity of ethanolic extract of *Tecoma stans* stem may be due to potentiation of insulin secretion from β-cells of pancreas, i.e., pancreatotropic action.

In monohydrate induced albino rats, there was an increase in the value of total cholesterol (TCH), triglycerides (TG), LDL, except HDL. While the extract treated group showed an increased value of HDL and reduced TC and TG in a significant manner by enhancement of the transcription of lipoprotein lipase similar to that of insulin, since in the normal group and positive control the level of triglycerides and cholesterol

increases due to unavailability of protein lipase which hydrolyses the triglycerides to very low density lipoproteins because of insulin deficiency. The statistically significant shows more decrease in total cholesterol, triglycerides, low density lipoproteins when compared ethanolic extract to positive control group. For total cholesterol the standard group shows (130.63± 1.48) and significant value (**p<0.01).Whereas ethanolic extract shows (154.27 ± 2.51) significant value is (**p<0.01.).

For triglycerides the standard group shows (104.53 ± 2.26) and significant value (**p<0.01). Whereas ethanolic extract shows (132.41 ± 1.21) significant value is (** p< 0.01). For High density lipoproteins the standard group shows (57.19 ± 0.67) and significant value is (**p<0.01). Whereas ethanolic extract shows (42.78 ± 0.53) and significant value is (** p<0.01). For low density lipoproteins the standard group shows (58.42 ± 1.94) significant value is (**p<0.01). Whereas ethanolic extract shows (83.16 ± 2.47) and significant value (**p< 0.01). The hypolipidemic activity of ethanolic extract of *Tecoma stans* stem may be due to the increased secretion of insulin from surviving β-cells of pancreas and consequent decrease in blood glucose level may lead to inhibition of lipid peroxidation and control of lipolytic hormones. The potential antidiabetic and hypolipidemic activity may be due to the actions of phytochemicals such as flavanoids, saponins and alkaloids present in ethanolic extract of *Tecoma stans* stem.

CONCLUSION

Based upon the results of the present investigation that the ethanolic extract of *Tecoma stans* stem showed antidiabetic activity similar to that of standard drug glibenclamide. So ethanolic extract of *Tecoma stans* stem useful in the treatment of diabetes as an antidiabetic agent. In addition ethanolic extract of *Tecoma stans* stem extract on administration reduces triglycerides, cholesterol and low density lipoproteins without modifying fasting glucose. The *Tecoma stans* stem extract contains the Saponins, Flavonoids and Mono terpenoid alkaloids such as tecostanine and tecomine which is having hypoglycemic effect. The present study reveals that the ethanolic extract of *Tecoma stans* stem showed antidiabetic effect in induced albino rats by decreased the glucose level in diabetic albino rats. In further researches based on the hypolipidemic activity of ethanolic extract of *Tecoma stans* stem the studies can be carry out for antiobesity.

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