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EFFECTS OF ETHANOLIC EXTRACT OF *TECOMA STANS* LEAF ON REPRODUCTIVE PERFORMANCE IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

The present study was formulated to Effects of Ethanolic Extract of *Tecoma stans* leaf on reproductive performance in streptozotocin-induced diabetic rats. Effects of ethanolic extract of *Tecoma stans* leaf extract were studied on physicochemical parameters, successive solvent extraction, and phytochemical screening and reproductive performance in streptozotocin-induced diabetic rats. After acute oral toxicity study, streptozotocin-induced diabetic albino rats are grouped into four groups, Normal control albino rats (Group I) received saline solution, Positive control albino rats (Group II) received alloxan mono hydrate, Standard group albino rats (Group II1) Received alloxan mono hydrate + glibenclamide2.5mg/kg) and Treated group (Group IV) received alloxan mono hydrate + *Tecoma stans* leaf 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* leaf (200 mg/kg) showed statistically more significant in decrease blood glucose level .The ethanolic extract shows (157.5±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* leaf may be due to potentiation of insulin secretion from β-cells of pancreas, i.e., pancreatotropic action.

KEYWORDS: The *Tecoma stans* were collected from the garden of College of Pharmacy.

INTRODUCTION

Diabetes mellitus is a condition in which the body either does not produce enough or does not properly respond to insulin hormone produced by pancreas. Insulin enables cells to absorb glucose in order to turn it into energy. In diabetes the body either fails to properly respond to its own insulin, does not make enough insulin or both.[1-3] This causes glucose to accumulate in the blood, often leading to various complications. The complications are less common and less severe in people who have well-controlled blood sugar levels. In fact, the better the control, the lower the risk of complications. Hence patient education, understanding and participation are vital. Healthcare professionals who treat diabetes also address other health problems that may accelerate the deleterious effects of diabetes.[4] These include smoking (abstain), elevated cholesterol levels (control with diet, exercise or medication), obesity (even modest weight loss can be beneficial), high blood pressure and lack of regular exercise can cause further complications. According to the American Diabetes Association, approximately 18.3% (8.6 million) of Americans age 60 and older have diabetes. Diabetes mellitus prevalence increases with age, and the numbers of older persons with diabetes are expected to grow as the elderly population increases in number.[5]

Yellow Trumpet bush is an attractive plant that is cultivated as an ornamental. It has sharply-toothed, lance-shaped green leafs and bears large, showy, bright golden yellow trumpet-shaped flowers. It is drought-tolerant and grows well in warm climates. The flowers attract bees, butterflies, and hummingbirds.[6] The plant produces pods containing yellow seeds with papery wings. The plant is desirable fodder when it grows in fields grazed by livestock. Yellow Trumpet bush is a ruderal species, readily colonizing disturbed, rocky, sandy, and cleared land and occasionally becoming an invasive weed. The leafs and roots of the plant contain bioactive compounds, especially monoterpenes, which may have medicinal uses; Honey bees are attracted to it, but-unlike most flowering plants-the honey produced from Yellow Trumpet bush's nectar/pollen is poisonous.[7] 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. Flowers having analgesic and antipyretic effect. Roots are used for Antispasmodic effect, Antimicrobial activity, Antifungal activity, Anti-Inflammatory. Aerial parts having antioxidant activity. Leaf is used for diabetes, Anti-Inflammatory.[8]

The plant *Tecoma Stan* was widely used in traditional for antidiabetic. The *Tecoma stans* leaf contains Monoterpinoid alkaloids having potential antidiabetic activity. So the objective of this research is to determine the hypoglycaemic activity of ethanolic extract of *Tecoma stans* leaf.[9]

MATERIAL AND METHOD

The *Tecoma stans* were collected from the garden of College of Pharmacy, SSSUTMS Shore Madhya Pradesh, and India. The plant was authenticated from PBRI, Bhopal Madhya Pradesh. The plant material was carefully washed with tap water and left to dry at room temperature.

Preparation of extraction

The *Tecoma stans* leaf is dried in room temperature; leaf was dried under shade until complete removal of moisture content. Such dried leaf was powdered by using pulverized and passed through sieve no80, dried powder extract is obtained with ethanol solvent at 70-80 Degree temperature, after the ethanol extraction the marc was dried and the percentage yield is calculated.[10]

Extraction of crude drugs

The successive extraction of powdered leaf of *Tecoma stans* was done by using soxhlet apparatus.[11]

By using soxhlet apparatus

For extraction, 200gm of powdered leaf was packed in thimble containing Whitman filter paper and extracted with ethanol 90% (70˚C-80˚C) in sox let apparatus for the period till all the crude substances and were extracted. The extract thus obtained was concentrated with the help of rotatory vacuum evaporator.[12-13]

Phytochemical Screening

Identification of the chemical constituents was carried out on the powdered *Tecoma stans* and on the ethanol extracts using chemical methods.[14]

Experimental Design

Animals

Adult male and female rats of albino waster strain weighing between 200-300g were obtained from the Laboratory Animal House, PBRI Bhopal (M.P.). 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°C–24°C with 12 hour day and night cycle.[15] 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.[16] *Rates norvegicus* adapts readily to breeding and living in laboratory conditions. Almost 3 to 5 million rats were used annually in laboratories all over the world. This accounts for almost 10-15 percent of the total number of laboratory animals of different species used.[17]

Acute toxicity study and dose selection

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 behavior and changes in body weight and mortality[18] It was found that the extract doesn't produce any significant toxicity up to the dose of 200 mg/kg. Thus the extract was highly tolerable unto 200 mg/kg. The mortality and signs of toxicity of treated groups were monitored for 14 days.

Induction of diabetes in albino wister strain

Diabetes was induced by intraperitoneal injection of streptozotocin (5% w/v) in physiological saline at a dose of 150 mg/ kg body weight. The diabetic state was confirmed 48hours after alloxan injection by glucosuria and hyperglycemia. Rats with a fasting blood glucose level higher than 200 mg/dl were selected for the study.[19]

Administration 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 alloxan induced diabetic albino rats.[20]

Procedure

The albino rats were weighed and housed in metabolic cages and left for 2 weeks 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 over night but allowed to access to water. The fasting blood sugar levels were measured in all rats to confirm that the valves are with in normal range. At the commencement of the experiment the rats were again fasted over night. Later to the divided groups accept to group1 (normal) to all groups the alloxan is administrated through intra peritoneal 150mg/kg body weight.

Experimental animals are divided in four groups each groups having six animals (albino rat-Wister 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 alloxan 150mg/kg body weight, Administered through intra-peritoneal.

Group II1. 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* leaf 200 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 eppamtroff tubes. The blood samples were collected for day 1,5,10,15 and estimated for parameters.[21-25]

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.[26]

Determination of blood glucose

Glucose was estimated by glucose estimation kit by GOD-POD method.

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.[28-30]

RESULTS AND DISCUSSION

Phytochemical Tests for Ethanolic Extract of *Tecoma Stans*

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

Table 1: Preliminary phytochemical study of Tecoma stans.

Phytochemical constituents	Ethanolic extract of *Tecoma stans* leaf
Alkaloids	+
Glycoside	+
Tannins and phenolic compounds	+
Steroids	-
Saponins	+
Flavonoids	+
Proteins and amino acids	+
Carbohydrates	+
Gums and mucilage	_

(+) presence (-) absence

Table 2: Effect of ethanolic extract of *Tecoma Stan* leaf 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**	157.5±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 alloxan mono hydrate, Standard group albino rats (Group II1) Received alloxan mono hydrate + glibenclamide2.5mg/kg) and Treated group (Group IV) Received alloxan mono hydrate +*Tecoma stans* extract (200 mg/kg). Diabetic control was compared with the vehicle control and extract treated, glibenclamide treated were compared with the alloxan diabetic control. *P < 0.05 - Statistically significant;** P< 0.01 –more significant.

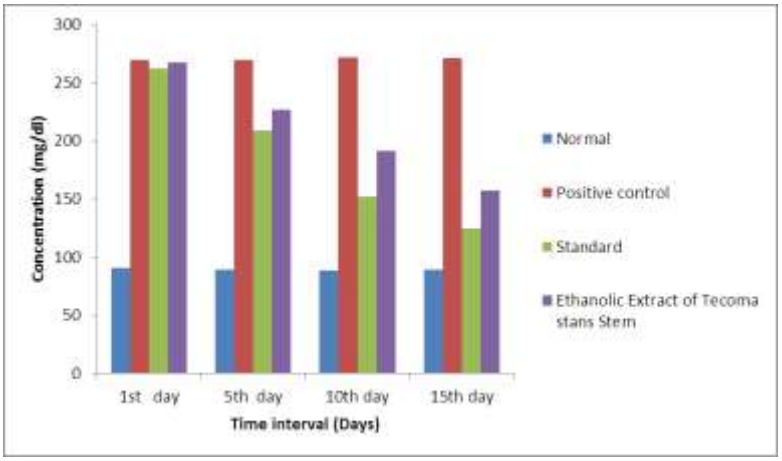

Figure 1: Shows Effect of *Tecoma Stan* leaf extract on glucose level.

Table 3: Effect of ethanolic extract of *Tecoma Stan* leaf 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**	164.27± 2.51**

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 alloxan mono hydrate, Standard group albino rats (Group II1) Received alloxan mono hydrate + glibenclamide2.5mg/kg) and Treated group (Group IV) Received alloxan mono hydrate +*Tecoma stans* extract (200 mg/kg). Diabetic control was compared with the vehicle control and extract treated, glibenclamide treated were compared with the alloxan diabetic control. *P < 0.05 - Statistically significant; ** P< 0.01 –more significant.

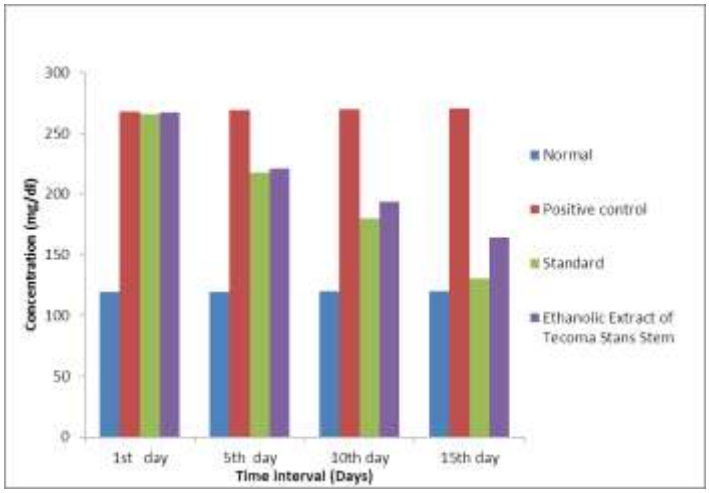

Figure 2: Shows Effect of *Tecoma Stans* leaf extract on total cholesterol level.

Table 4: Effect of ethanolic extract of *Tecoma Stan* leaf 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.

Normal control albino rats (Group I) received saline solution, Positive control albino rats (Group II) Received alloxan mono hydrate, Standard group albino rats (Group II1) Received alloxan mono hydrate + glibenclamide2.5mg/kg) and Treated group (Group IV) Received alloxan mono hydrate +*Tecoma stans* extract (200 mg/kg). Diabetic control was compared with the vehicle control and extract treated, glibenclamide treated were compared with the alloxan diabetic control. *P < 0.05 - Statistically significant; ** P< 0.01 –more significant.

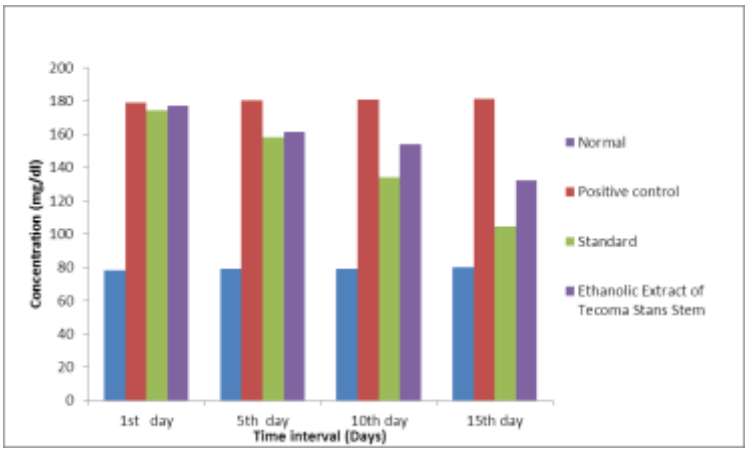

Figure 3: Shows Effect of *Tecoma Stans* leaf extract on triglycerides.

Table 5: Effect of ethanolic extract of *Tecoma Stan* leaf 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.

Normal control albino rats (Group I) received saline solution, Positive control albino rats (Group II) Received alloxan mono hydrate, Standard group albino rats (Group II1) Received alloxan mono hydrate + glibenclamide2.5mg/kg) and Treated group (Group IV) Received alloxan mono hydrate +*Tecoma stans* extract (200 mg/kg). Diabetic control was compared

with the vehicle control and extract treated, glibenclamide treated were compared with the alloxan diabetic control. *P < 0.05 - Statistically significant; ** P< 0.01 –more significant.

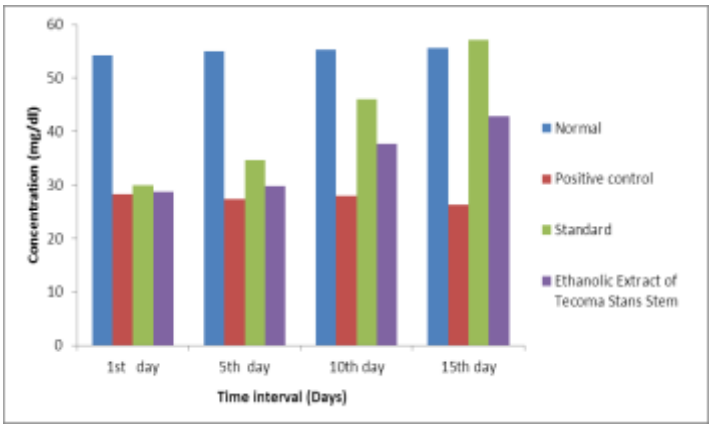

Figure 4: Shows Effect of *Tecoma Stans* leaf extract on high density lipoproteins.

Table 6: Effect of ethanolic extract of *Tecoma Stan* leaf 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.

Normal control albino rats (Group I) received saline solution, Positive control albino rats (Group II) Received alloxan mono hydrate, Standard group albino rats (Group II1) Received alloxan mono hydrate + glibenclamide2.5mg/kg) and Treated group (Group IV) Received alloxan mono hydrate + *Tecoma stans* extract (200 mg/kg). Diabetic control was compared with the vehicle control and extract treated, glibenclamide treated were compared with the alloxan diabetic control. *P < 0.05 - Statistically significant;** P< 0.01 –more significant.

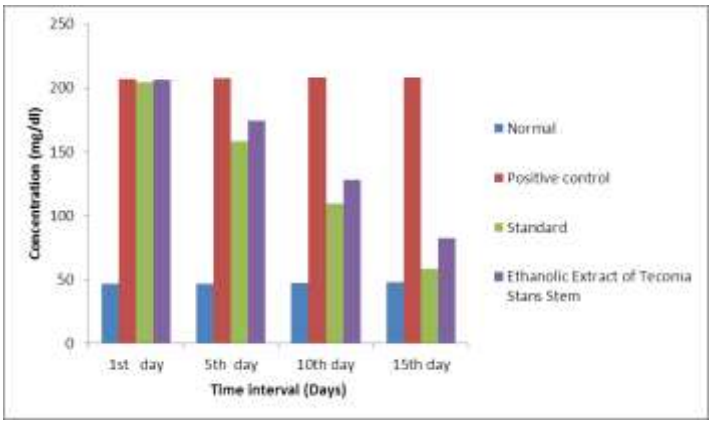

Figure 5: Shows Effect of *Tecoma Stans* leaf extract on low density lipoproteins.

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 streptozotoc in induced diabetic animals. Alloxan 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 alloxan mono hydrate, Standard group albino rats (Group II1) Received alloxan mono hydrate + glibenclamide2.5mg/kg)and Treated group (Group IV) received alloxan mono hydrate +*Tecoma stans* leaf ethanolic extract (200 mg/kg). The ethanolic extract group was compared with standard group and positive control group. At the end of the study (15^{th} day) the glibenclamide (2.5 mg/kg.) and ethanolic extract of *Tecoma stans* leaf (200 mg/kg) showed statistically more significant in decrease blood glucose level .The ethanolic extract shows (157.5±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* leaf may be due to potentiation of insulin secretion from β-cells of pancreas, i.e., pancreatotropic action.

In streptozotocin 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).Where as ethanolic extract shows (164.27± 2.51) significant value is (** p< 0.01.)For triglycerides the standard group shows (104.53±2.26) and significant value (**p<0.01).Where as 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). Where as 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).Where as ethanolic extract shows (83.16± 2.47) and significant value (**p< 0.01). The hypolipidemic activity of ethanolic extract of *Tecoma stans* leaf 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 flavonoids, saponins and alkaloids present in ethanolic extract of *Tecoma stans* leaf.

CONCLUSION

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

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