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## Research



### Antidiabetic activity of methanolic extracts of leaves of samanea saman on alloxan induced diabetes in rats

Kallem Krishnaveni\*, N. Rajashekar, Dr. D. Swathi

Department Of Pharmacology, Samskruti College Of Pharmacy, Ghatkesar, Telangana. 501301.

\*Author for Correspondence:: Kallem Krishnaveni

Email: krishnavenir38@gmail.com

	<b>Abstract</b>
Published on: 19 Oct 2023	<p>Samanea Saman, belongs to the family Fabaceae, have pharmacological actions like Antioxidant, Antimicrobial, anti-inflammatory , anti-ulcer.</p>
Published by: DrSriram Publications	<p><b>Objetive:</b> To investigate Antidiabetic activity of methanolic leaf extract of Samanea Saman, on alloxan induced diabetic rats.</p> <p><b>Methods:</b> Alloxan is administered as an inducer for diabetes. Thirty Wistar albino rats were randomly divided into five groups. Either sex of Wister strains of albino rats were divided in to 5 groups were Group 1 served as normal control, Group 2 served as alloxan control, Group 3 were administered with standard drug (Glibenclamide), Group 4 and Group 5 were administered to different doses of methanolic extract of Samanea Saman, (i.e. 200 and 300 mg/kg/Kg body weight).The anti-diabetic activity was determined by glucometer in both normal and alloxan-induced diabetic rats. The methanolic extract of <i>Samanea Saman</i> showed significant reduction in blood glucose levels due to the presence of phytochemicals such as alkaloids in extracts.</p>
<p>2023  All rights reserved.</p>  <p><a href="#">Creative Commons Attribution 4.0 International License.</a></p>	<p><b>Results:</b> Altered levels of the FBGL and OGTT in alloxan induced rats were brought back to normal on treatment with methanolic extract of Samanea Saman., Thus the positive results suggest that <i>Samanea Saman</i> extract should be further studied to determine the bioactive chemical compounds as well as to understand the possible mechanism of action and evaluate their toxicity looking towards pharmaceutical actions.</p> <p><b>Conclusion:</b> It was concluded from the result that the methanolic extract of Samanea Saman., showed significant antidiabetic activity in a dose dependent manner.</p> <p><b>Keywords:</b> Samanea Saman., Antidiabetic activity, Glibenclamide, FBGL and OGTT.</p>

## INTRODUCTION

### Diabetes Mellitus (DM)

Diabetes is one of the most common non-communicable diseases and a serious life-long condition appearing worldwide. The etiology of diabetes is a complex interaction of genetic and environmental factors. It is a heterogeneous group of metabolic disorders characterized physiologically by dysfunction of pancreatic beta cells and deficiency in insulin secretion or insulin activity and clinically by hyperglycemia or impaired glucose tolerance and other manifestable disorders. It is an endocrinological syndrome abnormally having high levels of

sugar in the blood. This may be either due to insulin not being produced at all, is not made at sufficient levels, or is not as effective as it should be.

Diabetes is still a serious health problem all over the world since it is associated with increased morbidity and mortality rate. When compared with the general population, mortality and morbidity increase in diabetes is mainly due to the associated chronic complications both specific (microvascular) and nonspecific (macrovascular). Since the disease prevails in both genders and in all age groups, the general public has a concern about its control and treatment<sup>1</sup>.

### Classification of DM

Diabetes is classified by underlying cause. The most common forms of diabetes are categorized as

**Type 1**, or insulin-dependent diabetes mellitus (IDDM) - an autoimmune disease in which the body's own immune system attacks the pancreatic beta cells, rendering it unable to produce insulin and

**Type 2**, or non-insulin-dependent diabetes mellitus (NIDDM) - in which there is resistance to the effects of insulin or a defect in insulin secretion.

Type 2 diabetes commonly occurs in adults associated with obesity. There are many underlying factors that contribute to the high blood glucose levels in these individuals. An important factor is the resistance to insulin in the body essentially ignoring its insulin secretions. A second factor is the decreased production of insulin by the cells of the pancreas. Therefore, an individual with Type 2 diabetes may have a combination of deficient secretion and deficient action of insulin. In contrast to Type 2 diabetes, Type 1 diabetes most commonly occurs in children and is a result of the body's immune system attacking and destroying the beta cells. The trigger for this autoimmune attack is not clear, but the result is the end of insulin production.

Multiple risk factors for the development of Type 2 diabetes mellitus:

- Family history (parents with diabetes).
- Obesity (i.e.,  $\geq 20\%$  over ideal body weight or body mass index  $\geq 25\text{kg/m}^2$ ).
- Habitual physical inactivity.
- Impaired glucose tolerance.
- Hypertension ( $\geq 140/90\text{mm Hg}$  in adults).
- High density lipoprotein (HDL) cholesterol  $\leq 35\text{mg/dl}$  and/or triglyceride level  $\geq 250\text{mg/dl}$ .

### History

The term "Diabetes" was first used around 250 B.C. It is a Greek word meaning "to syphon", reflecting how diabetes seemed to rapidly drain fluid from the affected individual. The Greek physician Aretaeus noted that affected individuals passed increasing amounts of urine as if there was "liquefaction of flesh and bones into urine". The complete term "diabetes mellitus" was coined in 1674 by Thomas Willis. Mellitus is Latin for honey, which is how Willis described the urine of diabetics.

Historical accounts reveal that as early as 700-200 BC, diabetes mellitus was a well recognized disease in India and was even distinguished as two types, a genetically based disorder and other one resulting from dietary indiscretion. Ancient Hindu writings document how black ants and flies were attracted to the urine of diabetics. The Indian physician Sushruta in 400 B.C. described the sweet taste of urine from affected individuals, and for many centuries to come, the sweet taste of urine was a key to the diagnosis.

Physicians have observed the effects of diabetes for thousands of years. One of the effects of diabetes is the presence of glucose in the urine (glucosuria). For much of the time, little was known about this fatal disease that caused weight loss of body, extreme thirst, and frequent urination. It was in 1922 that the first patient was successfully treated with insulin. Till the mid-1800s, the treatments offered for diabetes varied tremendously. A breakthrough in the puzzle of diabetes came in 1889. German physicians Joseph von Mering and Oskar Minkowski surgically removed the pancreas from dogs. The dogs immediately developed diabetes. Now that a link was established between the pancreas and diabetes, research focused on isolating the pancreatic extract that could treat diabetes. Dr. Frederick Banting succeeded in his experiments of isolating a pancreatic extract. The diabetic dog was kept alive for eight days by regular injections until supplies of the extract, at that time called "isletin", was exhausted. Experiments on dogs showed that extracts from the pancreas caused a drop in blood sugar, caused glucose in the urine to disappear, and produced a marked improvement in clinical condition.

A young boy, Leonard Thompson, was the first patient to receive insulin treatment in the year 1922 and lived for thirteen years. Over the next 70 years, insulin was further refined and purified. A revolution came with the production of recombinant human DNA insulin in 1978. Instead of collecting insulin from animals, new human insulin could be synthesized. In 1923, Banting and Macloed were awarded the Nobel Prize for the discovery of insulin. In his Nobel Lecture, Banting concluded the following about their discovery: "Insulin is not a cure for diabetes; it is a treatment."

## MATERIALS AND METHODS

The designing of methodology involves a series of steps taken in a systematic way in order to achieve the set goal(s) under the prescribed guidelines and recommendations. It includes in it all the steps from field trip to the observation including selection and collection of the medicinal plant, selection of dose value, standardization of protocol, usage of instruments, preparation of reagents, selection of specific solvents for extraction, formation of protocols and final execution of the standardized protocol. All this requires good build of mind and a good and soft technical hand to handle the materials and procedure in a true scientific manner.

### Drugs and Chemicals

Drugs and Chemicals used in this study were of analytical grade and of highest purity procured from standard commercial sources in India.

**Table 1: Drugs and Chemicals**

S.No	Materials	Company Name
1.	Alloxan	Quali Kems Fine Chem Pvt, Ltd, Vadodara.
2.	Methanol	ChangshuYangyuan Chemicals, China.
3.	Glibenclamide	Sanofi India Ltd, Ankleshwar.

### Instruments

Following instruments were required for the study

**Table 2: List of Instruments used for study**

Name of the instrument	Source
Centrifuge	Dolphin
Digital weighing balance	Horizon
Glucometer	Horizon
Heating mantle	ASGI®
Refrigerator	Videocon
Soxhlet extractor	ASGI®
Condenser	ASGI®
Burette stand	Dolphin
Round bottom flask	ASGI®, Amar
Mixer	Videocon
Oven	ASGI®
Water bath	ASGI®
Stirrer/glass rod	ASGI®
Watch glass	ASGI®
Whatmann filter paper	Manipore microproducts, Ghaizabad.
Butter paper	ASGI®
Spatula	ASGI®
Rubber pipes	ASGI®

### Experimental animals

Healthy adult albino wistar rats weighing 200-250grams of either sex were selected for the study. Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory Animal Diet) and water ad libitum. They were fasted overnight before the day of experiment, after 72hours of fasting from the day of Alloxan introduction. Animals were housed within the departmental animal house and the room temperature was maintained at 27° C. Animal studies had approval of IAEC.

### Plant Material Collection

The leaves of *Samanea Saman* was collected

## Preparation of plant extracts

### Preparation of methanolic Extract

Fresh leaves of *Samanea Saman* were collected and washed under tap water. The leaf extract used was prepared by taking 20gms of finely cut leaves into 250ml beaker containing 200ml of methanol. The contents were mixed well and then the mixture was boiled upto 50-60°C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.

### Selection of dose for animal study

The dose considered for the experiment on rats was obtained from conversion of human dose of *Samanea Saman* (3-5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats and 0.002 for mice (Ghosh 1984). Hence the calculated dose for the rats (considering human dose 3 and 5 g/kg) is 200 and 300 mg/kg. Acute toxicity was done at dose of 2000mg/kg body weight.

## Pharmacological evaluation

### Preparation of extracts

The aqueous and alcoholic extracts of *Samanea Saman* suspended in water in presence of 3%v/v Tween-80 solution.

All the drugs were administered orally for experimental purpose. Each time preparations of the extracts were prepared when required. The drugs were administered at a constant volume of 10ml/kg for each animal.

### Acute oral toxicity

The acute oral toxicity of methanolic extracts of *Samanea Saman* was determined by using Albino wistar rats (200-250g) which were maintained under standard conditions. The animals were fasted 12 hour prior to the experiment, up and down procedure OECD guideline no. 425 were adopted for toxicity studies. Animals were administered with single dose of individual extract upto 2000mg/kg and observed for its mortality during 2days and 7days study period (short term) toxicity and observed upto 7days for their mortality, behavioral and neurological profiles.

### Assessment of Anti-diabetic Activity in Normal and Alloxan induced Rats

**Table 3: Assessment of hypoglycemic activity on normal rats. Group Classification**

Group	Treatment	Dose(mg/kg)
Group 1	Normal control received distilled water	10ml/kg
Group 2	Standard group received Glibenclamide	10ml/kg
Group 3	Methanolic extract of <i>Samanea Saman</i>	200
Group 4	Methanolic extract of <i>Samanea Saman</i>	300

### Procedure

Animals were grouped and divided randomly into four groups of four and each was fasted to overnight. The blood samples were withdrawn by tail vein at 0hour i.e. before I.P administration of extracts/standard/vehicle. Then blood was collected at an interval of 1, 2, 4, and 8 hour after the administration, and according to procedure blood glucose levels were measured by glucometer (ONE TOUCH glucometer). The administration of drug was continued upto fifteen days.

### Oral glucose tolerance test(OGTT) in normal rats

On the next day (16<sup>th</sup> day) after the assessment of hypoglycemic activity OGTT was carried out in same normal animals.

### Procedure

All the animals in each group were administered 2g/kg of glucose one hour after extract/ glibenclamide/ vehicle administration. The blood samples were collected by tail vein at 0 hour, 0.5 hour, 1 hour, 1.5 hour and 2 hour after the administration of glucose load. Blood glucose levels were measured by glucometer.

## Assessment Of Anti-Diabetic Activity In Alloxan Induced Diabetic Rats

### Induction of Diabetes

Albino wistar rats of either sex weighing 200-250 g were selected for the study. All the animals were allowed free access to water and pellet diet and maintained at room temperature in rat cages.

Alloxan was dissolved in normal saline immediately before use. Diabetes was induced in 16 hour fasted rats by single intraperitoneal injection of 120 mg/kg body weight of freshly prepared alloxan in normal saline.

The rats after alloxanization were given 5% w/v glucose solution in feeding bottles for next 24 hours in their cages to prevent hypoglycemia. After 72 hours rats with fasting blood glucose levels greater than 200 mg/dl were selected and used for further studies

All the animals were observed for seven days for consistent hyperglycemia (fasting blood glucose level greater than 200 mg/dl and lesser than 400 mg/dl) and such animals were selected and divided into six groups of four each and used for the study of the following experimental models.

**Table 4: Group Classification**

Group	Treatment	Dose(mg/kg)
Group 1	Normal control received distilled water	10ml/kg
Group 2	Diabetic control received distilled water	10ml/kg
Group 3	Standard group received Glibenclamide	10ml/kg
Group 4	Methanolic extract of <i>Samanea Saman</i>	200
Group 5	Methanolic extract of <i>Samanea Saman</i>	300

### Effect of Methanolic extracts of *Samanea Saman* on blood glucose levels in alloxan induced diabetic rats

All the animals of above groups were administered as per treatment protocol mentioned above. The blood samples were collected by retro orbital puncture at 0,1,2,4 and 8 hour after the administration. The treatment was continued for next 22 days. Again blood samples were also collected on 4<sup>th</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day after 1 hour administration for sub acute study. Blood glucose level was estimated at various time intervals by subjecting the collected blood to cold centrifugation for serum separation. Serum obtained was used for estimating glucose level using GOD/POD (span) kit.

### Oral glucose tolerance test (OGTT) in alloxan induced diabetic rats

On the 8<sup>th</sup>, 15<sup>th</sup> and 22<sup>nd</sup> day OGTT was carried out on the same alloxan induced diabetic animals used for assessment of anti-diabetic activity studies.

#### Procedure

All the animals in each group were administered 2g/kg of glucose one hour after extract/ Glibenclamide/ vehicle administration. The blood samples were collected by retro orbital puncture at 0 hour, 0.5 hour, 1 hour, 1.5 hour and 2 hour after the administration of the glucose load. Serum was treated with solutions of GOD/POD kit and according to procedure blood glucose levels were measured under by Biochemical analyzer.

### Statistical analysis

The values were expressed as mean  $\pm$  SEM data was analyzed using one-way ANOVA followed by T-test. Two sets of comparison had made. i.e.

1. Normal control Vs All treated groups.
2. Diabetic Control Vs All treated groups.

Differences between groups were considered significant at  $P < 0.001$  and  $P < 0.05$  levels.

## RESULTS

### Acute toxicity testing

Acute toxicity studies revealed that the alcoholic extracts of *Samanea Saman* were safe up to 2000 mg/kg of body weight and approximate LD 50 is more than 2000 mg/kg. No lethality or any toxic reactions was observed up to the end of the study period.

### Anti-diabetic activity in alloxan induced diabetic rats

Fasting blood glucose levels (FBGL) in normal rats were in range of 90-100 mg/dl. Treatment with alloxan (120 mg/kg, I.P.) had increased the FBGL to range of 252-266 mg/dl after 72 hours. These values on subsequent days got stabilized by day seven on an average between 255 mg/dl.

Changes in the fasting blood glucose levels in different groups are tabulated in Table No: -----. This data shown that blood glucose level of normal control animals has maintained throughout the study period.

The group 1 which is the diabetic control group has shown significant increase in fasting blood glucose levels during this 21<sup>st</sup> day study period.

The group 2 Glibenclamide (10mg/kg) treated group has shown ( $p < 0.005$ ) significant decrease in fasting blood glucose level during 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day of study period.

#### Effect of MESS on antidiabetic activity in alloxan induced diabetic rats

The animals treated with 200 and 300 mg/kg of MESS shown significant decrease ( $P < 0.005$ ) in FBGL on 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day of treatment when compared to other groups of animals. These extract have reduced more (%) in FBGL when compared to control group. The detailed results are summarized in Table 5.

**Table 5: Effect of MESS on fasting blood glucose level (FBGL) in Alloxan induced diabetic rats.**

Treatment	Dose(mg/kg)	Blood glucose level(mg/dl)			
		0 day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day
Normal control	-	100±3	98±2	95±1	91±1
Diabetic control	10	240±1	267±2	274±3	285±2
Glibenclamide	10	99±2	95±3	78±1	71±1
MESS	200	88±1	78±2	70±1	62±2
MESS	300	95±1	83±1	78±2	60±3

Values are expressed as mean ± S.E.M. n=3. Significant values were compared with  $P < 0.005$ . Normal control Vs all groups. Paranthesis indicates % reduction in BGL.

#### Oral glucose tolerance test (OGTT) on 16<sup>th</sup> day

MESS (200 and 300 mg/kg) significantly ( $P < 0.005$ ) suppress the rise in FBGL after glucose load (2g/kg) in rats, at first half-an-hour and upto 2hr time period as compare with other groups extract glibenclamide on 15<sup>th</sup> day. While MESS produced significant reduction in FBGL. Glibenclamide (10mg/kg) showed ( $P < 0.005$ ) significant suppression in FBGL rise at 1<sup>st</sup>, 8<sup>th</sup>, 15<sup>th</sup> & 22<sup>nd</sup> day normalized FBGL within 2hr. The detailed results are summarized in Table 6.

**Table 6: Effect of extracts of *Samanea Saman* on 22<sup>nd</sup> day in normal rats.**

Traetment	Dose (mg/kg)	Blood glucose level(mg/dl)			
		1 <sup>st</sup> day	8 <sup>th</sup> day	15 <sup>th</sup> day	22 <sup>nd</sup> day
Normal control	-	105±1	150±3	121±1	133±1
Diabetic control	10	231±2	375±4	351±2	221±1
Std(Glibenclamide)	10	100±1	120±3	116±1	110±2
MESS	200	274±2	251±5	187±1	139±3
MESS	300	187±2	170±3	147±2	117±1

Values are expressed as mean ± S.E.M. n=3. Significant values were compared with  $P < 0.005$ . Normal control Vs all groups. Paranthesis indicates % reduction in BGL.

## DISCUSSION

The present study was aimed at discovering the antidiabetic activity of methanolic extracts of *Samanea Saman* at a dose of 200 and 300 mg/kg showed significant effect on glucose tolerance and the extracts also showed reduction in fasting blood glucose levels in normal and alloxan induced diabetic rats. These findings indicate that the extracts might be producing hypoglycaemic effect by a mechanism independent from the insulin secretion e.g. by the inhibition of endogenous glucose production or by the inhibition of intestinal glucose absorption. Alloxan monohydrate is one of the chemical agents used to induce diabetes mellitus in animals. It induces diabetes by dose dependent destruction of  $\beta$  -cells of islets of langerhans. It is a generator of free radicals of oxygen which cause extensive DNA damage. It was observed that single intravenous dose of alloxan exhibited significant hyperglycemia. Excessive hepatic glycogenolysis and gluconeogenesis associated with decreased utilization of glucose by tissues is the fundamental mechanism underlying hyperglycemia in the diabetic state. As the hyperglycemia induced by alloxan falls under category of mild diabetes and may reverse after a few weeks, the hypoglycemic effect of the plant in hyperglycemic rats was studied during 22 days treatment. The difference observed between the initial and final fasting blood glucose levels of extract treated hyperglycemic rats revealed

antihyperglycemic effect of *Samanea Saman* throughout the period of study. The effect of methanolic extracts of *Samanea Saman* is compared with that of reference standard, Glibenclamide and it has shown a significant results.

## CONCLUSION

In current scenario, herbs are the potent sources of medicines used in the treatment of various disease and disorders. Since, plants are used as medicine there is prompt need of evaluation of plant species, therefore, the present work was conceived to evaluate the phytochemical and pharmacological screening of few Indian medicinal plants.

The Pharmacognostical evaluation of Indian medicinal plants viz., *Samanea Saman* studied which include the morphological and physicochemical studies. The morphological studies of species plant part were studied which will be beneficial for the validation and assessment of quality control parameters of these plants to find out the presence of adulterant if any in order to establish the quality, safety and efficacy.

The data of the blood glucose level of rats treated with Alloxan (150mg/kg body weight) produced diabetes within 72 hours. After 72 hours of Alloxan administered the blood glucose levels of rats were observed. It was observed that significant lowering of sugar in alcoholic extract. The administration of MESP at a dose of 200 and 300 mg/kg showed significant anti-hyperglycaemic effect at 21st day which was evident from the 1st day on wards as compared to standard. The alcoholic extract of three extracts combination has showed better efficacy than the individual extract in all the treated extract. The anti-hyperglycaemic effect of the extract on the fasting blood sugar levels on diabetic rats is shown in table. The decreasing blood glucose levels are comparable with that of 10 mg/kg of Glibenclamide. The Glibenclamide (10 mg/kg body weight) shows significant effect on compare to the initial and more significant effect on the 7th Day compare to the initial. The methanolic extracts (200mg/kg body weight) shows significant ( $P < 0.01$ ), effect.

Results of anti-diabetic activity of extracts established the scientific basis for the utility of these plants in the treatment of diabetes. The methanolic extracts have shown significant reduction in blood glucose levels in alloxan induced diabetic rats and produced maximum anti-diabetic activity and are higher than the hypoglycaemic activity of Glibenclamide in the diabetic rats. Therefore it is obvious that the fractionation with alcohol has enriched the active principles. In glucose loaded animals, the drug has reduced the blood glucose to the normal levels. It is possible that the drug may be acting by potentiating the pancreatic secretion or increasing the glucose uptake. Alcoholic extracts in combination has reduced the glucose levels, in prolonged treatment study. In conclusion, these extract showed significant anti-diabetic effect in diabetic rats after oral administration. Thus the claim made by the traditional Indian systems of medicine regarding the use of these plants in the treatment of diabetes stands confirms.

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