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EVALUATION OF ANTIDIABETIC ACTIVITY OF LEAVES OF *SAMANEA SAMAN* ON STREPTOZOTOCIN-INDUCED DIABETES IN RATS

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Abstract:

*In India, the number of people suffering from diabetes is believed to be rising steadily and the current antidiabetic therapies are frequently reported to have adverse side effects. Ethno medicinal plant use has shown promise for the development of cheaper, cost-effective antidiabetic agents with fewer side effects. Te aim of this study was to investigate the antidiabetic activity and mechanism of action of Ethanolic extract prepared from **Samanea saman**. Since this claim has not been investigated scientifically, the aim of this study was to evaluate the antidiabetic effect and phytochemical screening of Streptozotocin -induced diabetic Rats. The leaves of **Samanea saman** have been used in traditional health systems to treat diabetes mellitus. However, the antidiabetic activity of this medicinal plant is not scientifically validated and authenticated. The present study aimed to investigate the in vitro and in vivo anti-diabetic activity of flower crude extract and solvent fractions of **Samanea saman**.*

*The in vitro α–amylase inhibition of the crude extract and solvent fractions of **Samanea saman**. Blood glucose lowering activity of 80% Ethanolic crude extract and solvent fraction was studied in animal models: Hypoglycemic rats model, oral glucose loaded rat model, dose-treated Streptozotocin -induced diabetic Rat model. The effect of the crude extract on diabetic lipid profile was studied.*

*The acute toxicity study of **Samanea saman** leaves extract did not show mortality in the animals at the limit dose during the observation period. The result of α–amylase enzyme inhibition activity was found in a dose-dependent manner, the strongest activity was shown by Crude extract fraction (89.60 % inhibition at 1000 µg/mL) compared to the standard acarbose having 97.19% inhibition at 1000 µg/mL. The crude extract of **Samanea saman** showed significant blood glucose-lowering effect on hypoglycemic rats and oral glucose loaded rats. In Streptozotocin -induced diabetic rats model, the crude extract fraction significantly decreased the fasting blood glucose level after 14 days of treatment.*

*The result demonstrated the beneficial biochemical effects of **Samanea saman** extract by inhibiting α–amylase improving serum lipid profile levels. The leaves crude extract are effective in lowering blood glucose levels in diabetic and hypoglycemic rats. The claimed traditional use as antidiabetic has scientific ground.*

Key words: *Diabetes mellitus, Herbal medicine, Samanea saman, Streptozotocin, Anti diabetic activity.*

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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 [1].

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 [2].

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 [5].

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."

Epidemiology:

Present status projects that incidence of diabetes is on rise. Present number of diabetics worldwide is 150 million and according to new estimates from researchers at the World Health Organization (WHO), there will be an increase of about 300 million or more by the year 2030 (Warner, 2004). Only in year 2001, about 441,004 deaths were registered and 49,855 of them provoked by diabetes, representing 11.2% of the total population. In United States, diabetes is the sixth leading cause of death. The prevalence of diabetes mellitus is rapidly increasing worldwide and India is estimated to have 31 million diabetics from the total population of the world. Diabetes is predicted to become one of the most common diseases in the world within a couple of decades, affecting at least half a billion people. The driving force behind the high prevalence of diabetes is the rise of obesity, sedentary lifestyle, consumption of energy rich diet, etc. The diabetes epidemic is accelerating in the developing world, with an increasing proportion of affected people in younger age groups. The prevalence of Type 2 diabetes is now at epidemic proportions. Type 2 diabetes has a significant impact on the health, quality of life, and life expectancy of patients, as well as on the health care system. Type 2 diabetes accounts for about 90-95 % of population while Type 1 diabetes accounts for about 5 -10% of the total population. In the past, Type 2 was rarely seen in the young, but recent reports describe Type 2 diabetes being diagnosed even in children and adolescent.

SIGNS AND SYMPTOMS:

In both the types of diabetes, signs and symptoms are more likely to be similar as the blood sugar is high, either due to less or no production of insulin, or insulin resistance. In any case, if there is inadequate glucose in the cells, it is identifiable through certain signs and symptoms. These are quickly relieved once the diabetes is treated and also reduce the chances of developing serious health problems.

Type 1 Diabetes: In type 1 the pancreas stops producing insulin due to autoimmune response or possibly viral attack on pancreas. In absence of insulin body cells don't get the required glucose for producing ATP (Adenosine Triphosphate) units which results into primary symptom in the form of nausea and vomiting. In later stage, which leads to ketoacidosis, the body starts breaking down the muscle tissue and fat for producing energy hence, causing fast weight loss. Dehydration is also usually observed due to electrolyte disturbance. In advance stages, coma and death is witnessed.

Type 2 Diabetes:

Increased fatigue: due to inefficiency of the cell to metabolize glucose, reserve fat of body is metabolized to gain energy. When fat is broken down in the body, it uses more energy as compared to glucose; hence body goes in negative calorie effect, which results in fatigue.

Polydypsia: As the concentration of glucose increases in the blood, brain receives signal for diluting it and, in its counteraction we feel thirsty.

Polyuria: Increase in urine production is due to excess glucose present in body. Body gets rid of the extra sugar in the blood by excreting it through urine. This leads to dehydration because along with the sugar, a large amount of water is excreted out of the body.

Polyphagia: The hormone insulin is also responsible for stimulating hunger. In order to cope up with high sugar levels in blood, body produces insulin which leads to increased hunger.

Weight fluctuation: Factors like loss of water (polyuria), glucosuria, metabolism of body fat and protein may lead to weight loss. Few cases may show weight gain due to increased appetite.

Blurry vision: Hyperosmolar, hyperglycaemia, nonketotic syndrome is the condition when body fluid is pulled out of tissues including lenses of the eye; this affects it's to focus, resulting blurry vision.

Irritability: It is a sign of high blood sugar of the inefficient glucose supply to the brain and other body organs, which make us, feel tired and uneasy.

Infections: The body gives few signals whenever there is fluctuation in blood sugar(due to suppression of immune system) by frequent skin infections like fungal or bacterial or UTI(urinary tract infection).

MATERIALS AND METHODS:

List of Chemicals used for study:

Name of the Chemical	Source
Samanea saman	Tirumala hills, Tirupati, Andhra Pradesh. India
Glibenclamide	Sanofi
Streptozotocin	Loba Chemie
Ethanol	Honeywell

a) Plant collection:
The aerial part of *Samanea saman* was collected from Tirumala hills, Tirupati, Andhra Pradesh. India. It was identified and authenticated by Prof. Madhava Chetty, K., Taxonomist, S.V. University, Tirupati, Andhra Pradesh, India. A voucher specimen has been kept in our laboratory for future reference.

The leaves of *Samanea saman* were collected and authenticated by Department of Botany. After shade-dried (Temp<40°C.), plant material was grounded into a moderately coarse powder. The extract was made by maceration and the ethanolic extract was made by using soxhlet apparatus. The extract was allowed to dry. Both the extracts were preserved in the refrigerator till further use.

***Invitro* antidiabetic activity of *Samanea saman* leaves extracts:**
Alpha-amylase inhibition assay:
The a-amylase inhibition assay was performed using the 3,5-dinitrosalicylic acid (DNSA) method.50 The crude and solvent fractions of *Samanea saman* were dissolved in buffer ((Na2HPO4/ NaH2PO4 (0.02 M), NaCl (0.006 M) at pH 6.9) to give concentrations ranging from 50 to 1000 mg/mL. A volume of 200 mL of a-amylase solution (Molychem) (2 units/mL) was mixed with 200 mL of the extract and was incubated for 10 minutes at 30 C. Thereafter, 200 mL of the starch solution (1% in water w/v) was added to each tube and incubated for 3 minutes. The reaction was terminated by the addition of 200 mL DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM 3,5-DNSA solution) and was boiled for 10 minutes in a water bath at 85°C. The mixture was cooled to ambient temperature and was diluted with 5 mL of distilled water, and the absorbance was measured at 540 nm using a UV-visible spectrophotometer (Agilent Technologies). The blank with 100% enzyme activity was prepared by replacing the plant extract with 200 mL of the buffer. A blank reaction was similarly prepared using the plant extract at each concentration in the absence of the enzyme solution. A positive control sample was prepared using acarbose (Bayer) and the reaction was performed similarly to the reaction with plant extract as mentioned above. The inhibition of a-amylase was expressed as percentage of inhibition and was calculated by the following equation: Inhibition (%) ¼ [(Ac -Acb) (As Asb) / (Ac -Acb)] × 100, where Ac is the absorbance of control; Acb is the absorbance of control blank; As is the absorbance of sample; and Asb is the absorbance of sample blank. The % a-amylase inhibition was plotted against the extract concentration and the IC50 values were obtained from the graph.
Preliminary phytochemical screening of Ethanolic leaves extract of *Samanea saman*[52]
The Ethanolic leaves extract of *Samanea saman* was used for testing preliminary phytochemical screening in order to detect major chemical groups.
Test for carbohydrates:
Molisch's test: Dissolved small quantity of 300mg alcoholic and dried leaves extract powder of Pimenta dioica separately in 4ml distilled water and filtered. The filtrate was subjected to Molisch's test.
Fehling's test: Dissolve a small portion of extract in water and treat with Fehling's solution.
Phenols test: The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapours.
Test for flavanoids
Shinoda test: To 2 to 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added.
Lead acetate test: To 5ml of extract 1ml of lead acetate solution was added.
Test for tannins
Braemer's test: To a 2 to 3ml of extract, 10% alcoholic ferric chloride solution was added.
Test for steroid/terpenoid
Liebermann-Burchardt test: To 1ml of extract, 1ml of chloroform, 2 to3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added.
Test for alkaloids
Draggendorf's test: A drop of extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Draggendorf's reagent.
Hager's test: The extract was treated with few ml of Hager's reagen.
Wagner's test: The extract was treated with few ml of Wagner's reagent.
Tests for Glycosides
Legal's test: Dissolved the extract [0.1g] in pyridine [2ml], added sodium nitroprusside solution [2ml] and made alkaline with Sodium hydroxide solution.
Test for Saponins
Foam test: 1ml of extract was dilute with 20ml of distilled water and shaken with a graduated cylinder for 15 minutes.
Test for Anthraquinones
Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml of concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia.
Test for Amino acids

Ninhydrin test: Dissolved a small quantity of the extract in few ml of water and added 1ml of ninhydrin reagent.

Test for fixed oils and fats

Press small quantity of the petroleum ether extract between two filter paper.

Note: the results for the above experiments can be noted as follows.

- If the response to the test is high it can be noted as +++ which indicates that the particular group is present as the major class.
- If the response is average then note it as ++ indicates the presence in moderate quantity.
- If the response is very small then note it as + indicating the presence of only in traces.
- If no response is then negative.

g) Acute oral toxicity study

In a research study when a drug is administered to a biological system there will be some interactions may happen .In most case these are desired and useful, but many effects are not advantageous. Acute, sub acute and chronic toxicity studies are performed by the manufacturers in the investigation of a new drug. Acute toxicity is involved in estimation of LD50 (It is the lethal dose (causing death) to 50% of tested group animals).

LD50 (median lethal oral dose)

LD 50 (median lethal oral dose) is a statistically derived oral dose of a substance that can be expected to cause death in percent of animals when administered by the oral route. The LD50 value is expressed in terms of weight of test substance per unit weight of animal (mg/kg).

In this study acute oral toxicity study was carried out in Rats. The procedure was followed by using OECD 423(Acute toxic class method).The Rats are fasted overnight, prior to dosing. The three dose levels are administered by orally the help of oral feeding needle over the prior of 24 hours. After the drug has been administered, food may be with held for a further 3-4 hours in Rats. The purpose of sighting study is to allow selection of the appropriate starting dose for main study.

The test substance is administered to a single animal in a sequential manner following from the fixed dose levels of 5, 50, 300 and 2000mg/kg. The interval between dosing of each level is determined by the mortality/onset, duration and severity of toxic signs over the period of 24 hours, special attention given during the first 4 hours. Four hours after the drug administration, provide the food and water for 14 days and daily observed some parameters such as food intake, water intake, mortality, onset, Duration and severity of toxic signs. The animal weight is recorded on weekly once. On the day fourteen all the animals are sacrificed, to isolate the organs and observe the histopathological changes. Based on the mortality result of sighting is decided and carried out with five animals per dose level (5 or 50 or 300 or 2000mg/kg).Based on the mortality result on 14th day of observation, the doses for *in vivo* study are selected.

***Invivo* antidiabetic activity of *Samanea saman* leaves extract in Streptozotocin induced diabetic Rats.**

Prior to the experiment the rats were housed in a clean polypropylene cages (6 Rats / cages) for a period of 7 days under standard temperature (25 - 30ºC), relative humidity (45 – 55%), dark / light cycle (12 /12 hrs). The studies were performed with the approval of Organizational Animal Ethics Committee (OAEC) (DAEC/TNA/965/345/16). The animals were put in overnight fasting were deprived of food for 16 hrs but allowed free access of water.

Hypoglycemic Test Groupings were done as follows:

Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g Rats)

Group II served as Positive control – Glibenclamide (2mg /kg)

Group III served as ethanolic extract of *Samanea saman* – (200mg/kg)

Group IV served as ethanolic extract of *Samanea saman* – (400mg/kg).

Blood samples were collected by the tail nipping method and glucose level checked by glucometer. After drug Administration blood samples have been collected different time intervals at 30, 60 and 120.

Oral Glucose Tolerance Test Groupings were done as follows:

Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g Rats)

Group II served as Positive control – Glibenclamide (2 mg /kg)

Group III served as Ethanolic extract of *Samanea saman* – (200mg/kg)

Group IV served as Ethanolic extract of *Samanea saman* – (400mg/kg).

All the groups of animals were fasted for 24h and blood samples were collected before drug or solvent treatment. The drug, extract and solvent, have been administered to different groups and 30mins later all the groups of Rats were treated with glucose orally at dose 10gm/kg body weight by using oral feeding needle. Blood samples were collected by the tail nipping method and glucose level checked by glucometer. After drug Administration blood samples have been collected different time intervals at 30, 60 and 120.

Induction of diabetes to animals

A single dose (100 mg/kg b.w., i.p.) of Streptozotocin dissolved in sodium citrate buffer was used for the induction of diabetes in Rats after overnight fasting. After 1 hr of Streptozotocin administration, the animals were given feed and libitum and 5% dextrose solution was also given in feeding bottle for a day to overcome early hypoglycaemic phase. The animals were stabilized for a week and animals showing blood glucose level more than 200 mg/dl were selected for the study.

Experimental design

Five groups of Rats six in each groups received the following treatment schedule for 14 days.

GROUP I - Normal control (normal saline 10 ml /kg, P.O)

GROUP II - Streptozotocin treated control (100 mg/kg, I.P)

GROUP III - Streptozotocin (100 mg/kg, I.P) + Standard drug Glibenclamide (2 mg/kg, P.O).

GROUP IV - Streptozotocin (100 mg/kg, i.p.) + EESS (200 mg/kg, P.O)

GROUP V - Streptozotocin (100 mg/kg, i.p.) + EESS (400 mg/kg, P.O)

Plant leaves extract, standard drug and normal saline were administered with the help of oral feeding needle. Group I serve as normal control which received normal saline for 14 days. Group II to Group V were diabetic control Rats. Group IV and Group V (which previously received Streptozotocin 100mg/kg) were given fixed doses of ethanol leaves extract (200 mg/kg, P.O, 400 mg/kg, P.O) of *Samanea saman* and group III received standard drug Glibenclamide (2 mg/kg,P.O) for 14 consecutive days. (EESS- Ethanolic extract of *Samanea saman* Leaves).

Collection of blood samples

Fasting blood samples were drawn from retro orbital puncture of Rats at weekly intervals till the end of the study 1, 7, and 14 days.

Estimation of biochemical parameters Serum blood glucose

On 1, 7, and 14 days fasting blood samples were collected and analyzed the blood glucose.

Blood glucose level

The blood glucose level test measures the amount of glucose in the blood sample obtained from the animals. The test is usually performed to check for elevated blood glucose levels which can be an indication of diabetes or insulin inhibition.

Statistical analysis

Statistical analysis was done by using GRAPHPAD PRISM 5.0.All the values of Biochemical parameters and body weight were expressed as Mean ± Standard Error Mean (SEM). The values were analyzed for statistical significance using one- way analysis of variance (ANOVA), comparison was done by using Dunnett's t test. P values < 0.05 were considered as significant, P values < 0.01 were considered as very significant, P values < 0.001 were considered as highly significant and ns were considered as not significant.

RESULTS:

a) Appearance and percentage yield of EESS (Ethanolic Extract of *Samanea saman* Leaves)

Table: a-Amylase Inhibitory Activities of the Crude Extract and Solvent Fractions.

	Percentage inhibition				
Concentration (mg/mL)	**Chloroform fraction**	**Ethyl acetate fraction**	**Aqueous fraction**	**Crude extract**	**Acarbose**
50	4.52 + 0.2	17.25 + 0.95	26.03 + 1.03	31.20 + 0.36	57.65 + 0.79
100	13.51 + 0.10	23.65 + 0.51	32.11 + 0.11	43.01 + 1.42	68.10 + 0.46
200	26.26 + 0.03	28.99 + 2.58	40.65 + 0.56	60.12 + 0.98	76.93 + 1.53
400	31.31 + 0.12	44.98 + 0.20	57.92 + 0.19	73.84 + 0.76	88.51 + 0.17
600	40.09 + 0.35	52.15 + 1.47	70.82 + 0.56	80.19 + 0.24	93.06 + 0.26
800	47.26 + 0.64	66.09 + 0.12	77.60 + 0.74	83.55 + 0.19	96.27 + 0.17
1000	55.39 + 0.33	72.69 + 0.41	84.21 + 0.22	88.98 + 0.74	97.19 + 0.92
IC50	33.25 + 0.19	23.56 + 0.82	15.36 + 0.69	9.69 + 0.91	3.34 + 0.14

Abbreviation: IC50, half maximal inhibitory concentration.

Each value of percentage inhibition of a-amylase is presented as means + standard error of the mean (SEM), n ¼ 3.

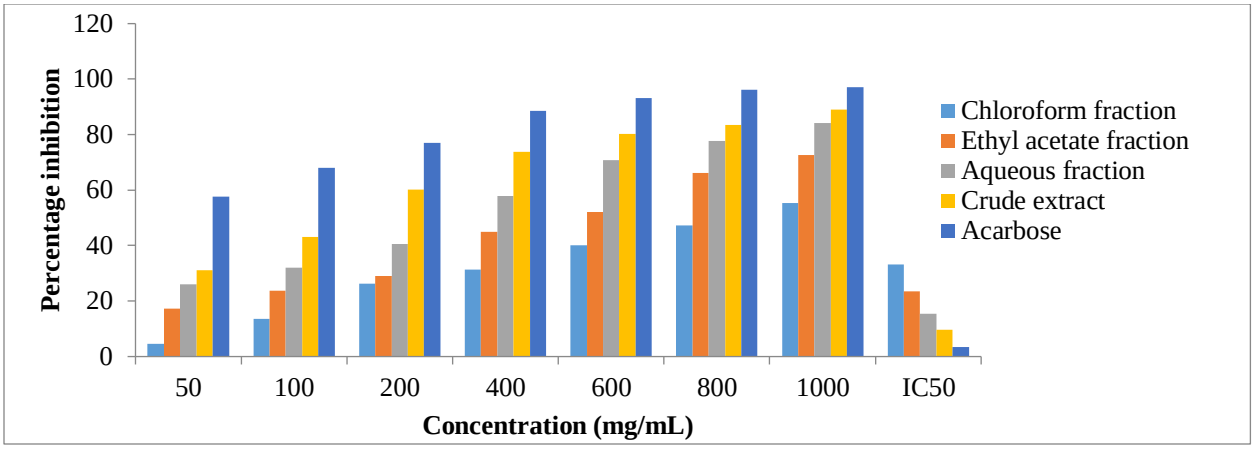

Figure : a-Amylase inhibitory activity of the ripe crude extract and solvent fractions of *Samanea saman*

TABLE: Hypoglycemic Test

TREATMENT	DOSE mg/kg	BLOOD GLUCOSE LEVEL (mg/dl)		
		0 min	30min	1hr
CONTROL Carboxyme Thyl Cellulose (CMC)	0.5%	70.25±1.361	69.15±4.320	72.21±1.261
Positive Control Glibenclamide	2	69.36±3.209	51.62±1.492**	31.96±2.415***
Ethanolic Extract of *Samanea saman*	200	68.10±1.251	58.20±3.482*	56.14±1.111*
Ethanolic Extract of *Samanea saman*	400	67.08±3.420	51.35±3.281**	35.2+±2.810***

Table: Oral Glucose Tolerance Test

Treatment	DOSE mg/kg	Blood Glucose Level (mg/dl)						
		0 min	0.5hr	1hr	1.5hr	2hr	2.5hr	3hr
Control (CMC)	0.5%	68.05±1.141	143.2±1.325	184.3±1.120	173.2±10.42	153.2±4.121	150.0±3.142	128.3±9.36
Positive Control Glibenclamide	2	70.24±0.21	104.1±3.154***	112.1±3.24***	93.10±1.121***	80.14±3.011**	74.03±1.201***	70.50±3.512***
AEESS	200	65.10±1.109	123.5±1.001	142.3±1.121*	131.5±0.162*	121.3±0.101*	110.12±0.20**	103.0±2.106**
AEESS	400	67.01±2.141	111.2±0.156**	120±4.116**	100.0±2.211***a	90.12±3.251*** a	83.01±1.02***a	79.03±201***a

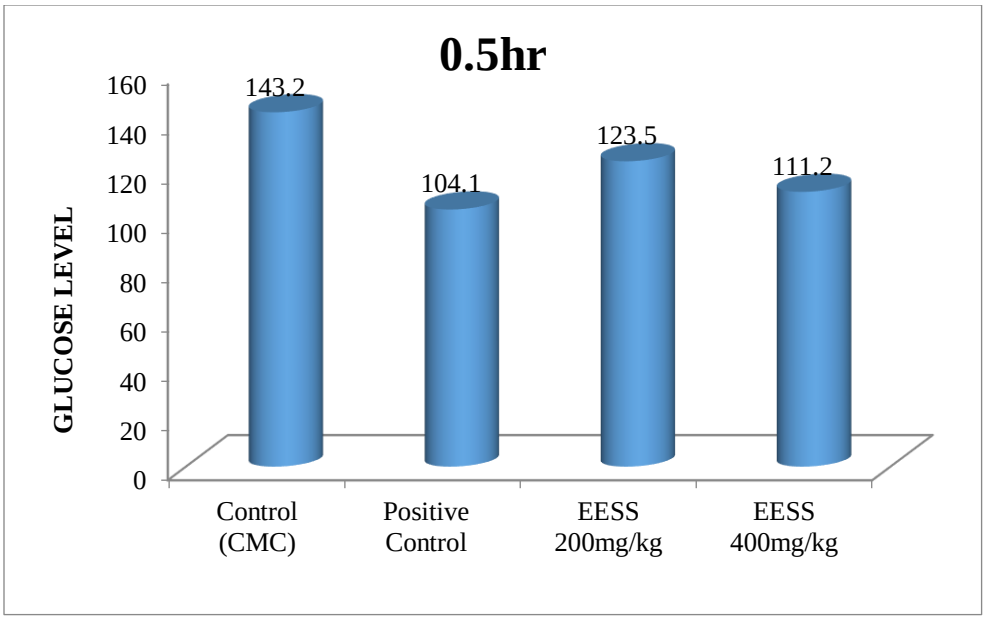

Fig: Blood Glucose Level (mg/dl) 0.5hr

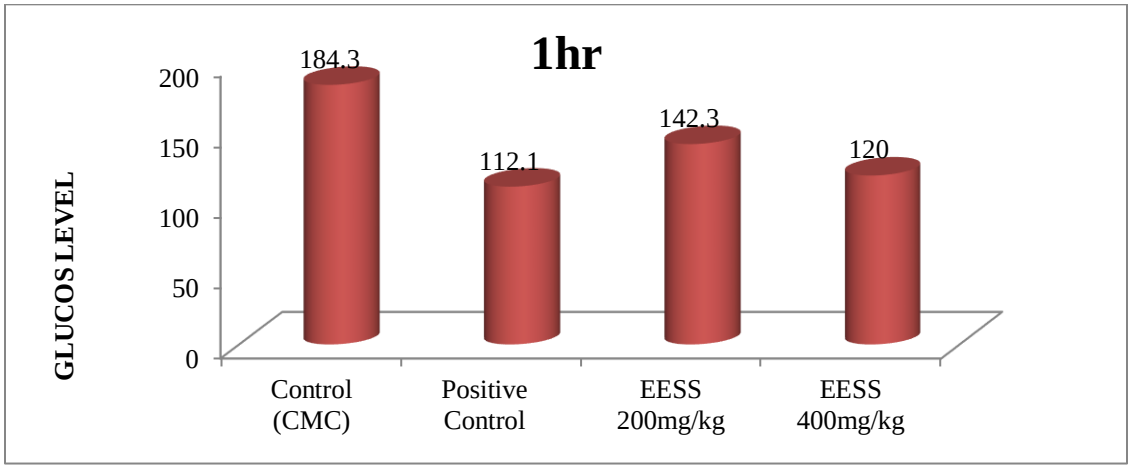

Fig: Blood Glucose Level (mg/dl) 1hr

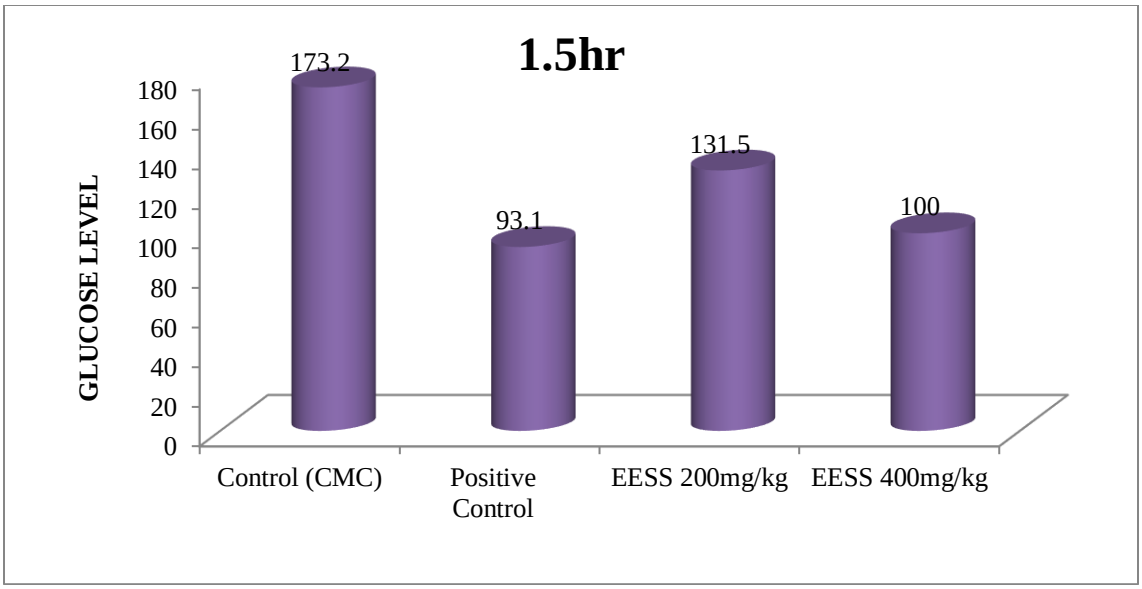

Fig: Blood Glucose Level (mg/dl) 1.5hr

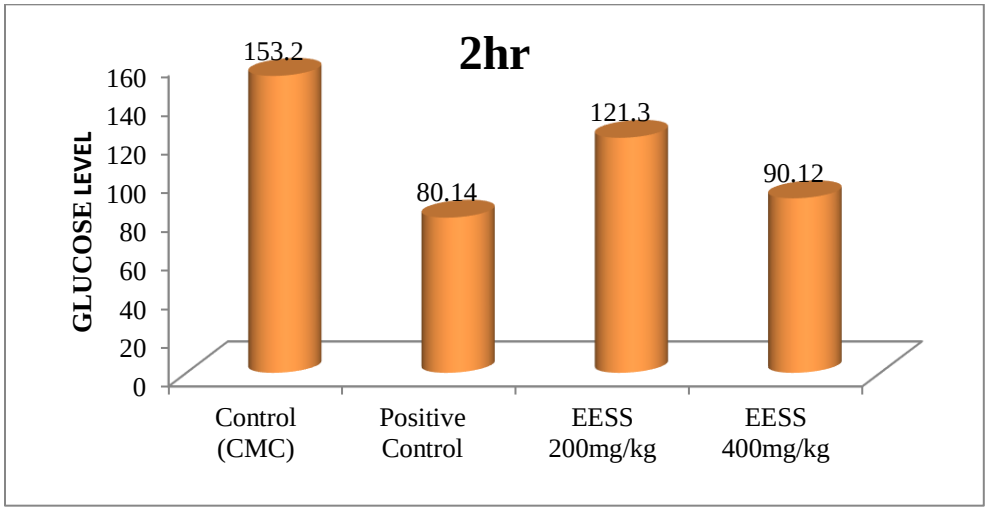

Fig: Blood Glucose Level (mg/dl) 2hr

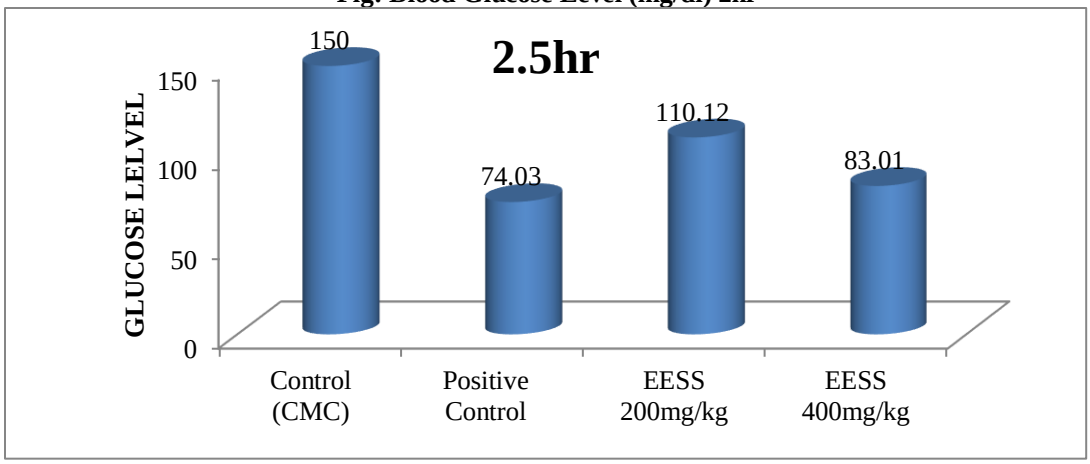

Fig: Blood Glucose Level (mg/dl) 2.5hr

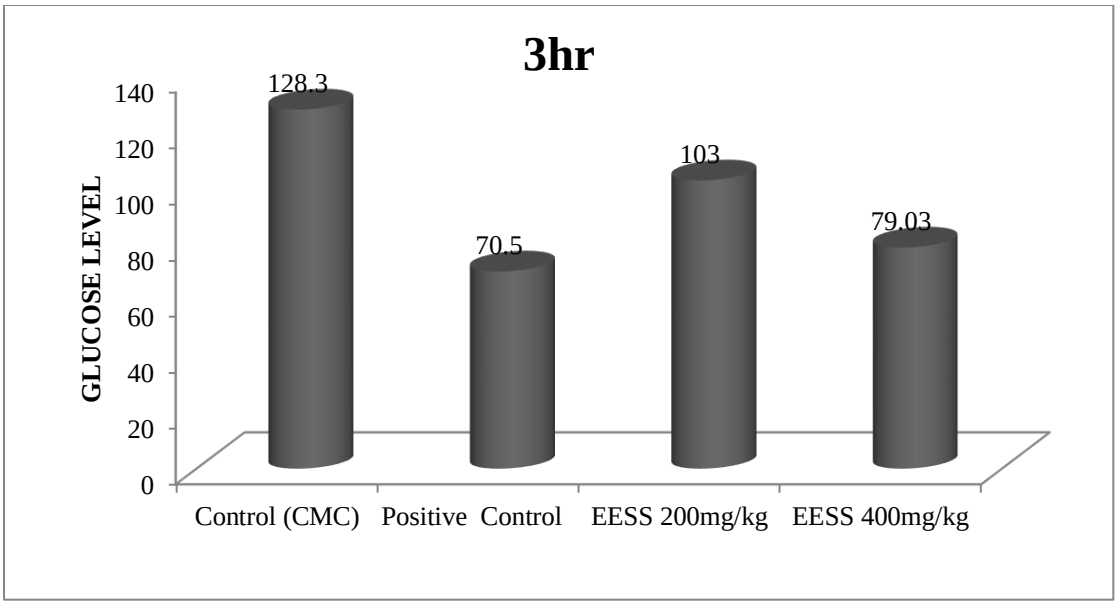

Fig: Blood Glucose Level (mg/dl) 3hr

Oral Glucose Tolerance Test (OGTT) results have been expressed on Table. Half hour after the glucose treatment, all the groups of animal blood glucose levels were significantly increased. The blood glucose levels were significantly decreased for, Ethanolic extract of *Samanea saman* 200 & 400 mg/kg when compared to control and positive control at 1hour and each and every ½ hour blood glucose levels (200 mg/kg were changes in the dose dependent manner extract treated group of animals compared to control and positive control but 400mg/kg produce the equipotent activity.

DISCUSSION:

Invitro study is on the principle of Inhibition of α-amylase, enzyme that plays a role in digestion of starch and glycogen are considered a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes. Pancreatic αamylase is a key enzyme in the digestive system and catalyses the initial step in hydrolysis of starch to a mixture of smaller. Sequential extraction was done according to increasing polarity order. Each extracts were tested for α-amylase inhibition to get the extraction with minimum IC50 value. As per the above mechanism all the extract have concentration dependent affinity towards the inhibition of α-amylase. Finally acarbose extract was observed as more active extract.

In this present study acute toxicity study was carried out in Rats. The procedure was followed by using OECD 423 (Acute Toxic Class Method). The acute toxic class method is a step wise procedure with three animals of a single sex per step. Average two to three steps may be necessary. The method used to defined doses (2000, 1000, 500, 50, 5 mg/kg body weight, Up-and-Down Procedure). Observe for signs for toxicity and were noted for 14 days. The onset of toxicity and signs of toxicity also noted. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study. The principle involved in the Streptozotocin induced diabetes mellitus in Rats, Streptozotocin, a cytotoxic, diabetes induced chemical but wide variety of animal species by damaging the insulin secreting cells of the pancreas.

Literature sources indicate that the Streptozotocin induced Rats are hyperglycaemic. The treatment of lower doses of Streptozotocin (100mg/kg b.w.) produced partial destruction of pancreatic β-cells even though the animals become permanently diabetic.

Thus these animals have surviving β cells and regeneration is possible. It is well known that the sulfonylurea's act by directly stimulating the β-cells of the islets of Langerhans to release more insulin and these compounds are active in mild Streptozotocin induced diabetes. *Invivo* anti diabetic screening was performed for the confirmation of above mechanism of action was undergone the Ethanolic extract of *Samanea saman* biological system (Which was already resulted for α-amylase inhibitory activity.

At the end of the Ethanolic extract of *Samanea saman* (200 mg/kg p.o, 400 mg/kg p.o.) showed statistically significant decrease in blood glucose levels. So the Ethanolic extract of *Samanea saman* showed antidiabetic activity. This work will be useful for

further diabetes mellitus and it's related diseases research worker to develop new entity for the treatment of diabetes mellitus.

CONCLUSION:

This study revealed that the crude extract and solvent fractions of *Samanea saman* have showed significant lowering of blood glucose level on diabetic, Hypoglycemic and oral glucose loaded Rats and not permitted bodyweight loss of diabetic. The results also verified that inhibition of intestinal α-amylase by the extracts may contribute to the antihyperglycemic activity. The results give scientific support for the use of the plant in folk medicine for the management of diabetes and its associated complications. *Samanea saman* would be promising for further clinical studies in the management of DM. Further studies to find out the mechanism of this plant for its antidiabetogenic effect and there is a need for bioactivity guided investigation to isolate the lead compound responsible for the antidiabetic activity.

The present study suggested that the isolation of active constituents from Ethanolic extract of *Samanea saman* leaf and characterize the compounds by using preliminary phytochemical studies.

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