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EVALUATION OF ANTI-DIABETIC ACTIVITY OF *CAESALPINIA SAPPAN* WOOD AGAINST ALLOXAN INDUCED DIABETIC RATS

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Abstract

The *Caesalpinia sappan* L. (*Fabaceae*) was authenticated and identified. The aim of this study is to evaluate the anti-diabetic activity of ethanolic extract of *Caesalpinia sappan* wood in albino wistar rat models (in-vivo) and inhibition of α-glucosidase enzyme method (in-vitro). The ethanol extract dose of 100mg/kg and 200mg/kg was significantly reduced blood glucose levels in diabetic rats after 21 days were evaluated for anti-diabetic activity in alloxan induced diabetic rats. The results were determined by blood parameters and the histopathological study showed significant anti diabetic activity. Invitro method of anti-diabetic activity, the ethanolic extract was prepared 25 µg/ml to 1000µg/ml. The invitro study shown better inhibition of α-glucosidase enzyme was determined by % inhibition. The ethanolic extract of *Caesalpinia sappan* showed IC_{50} is 215.95±7.52 and standard drug showed 183.46±5.85. *Caesalpinia sappan* shows that alkaloids and flavanoids present in this extract may be possibly responsible for the antidiabeic activities.

Keywords: *Caesalpinia sappan* wood, Anti-diabetic activity, Alloxan induced diabetic rats, Albino wistar rats, α-glucosidase inhibition.

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by high blood glucose levels that result from defects in insulin secretion, or action, or both. Elevated levels of blood glucose (hyperglycemia) lead to spillage of glucose into the urine, hence the term sweet urine. Normally, blood glucose levels are tightly controlled by insulin, a hormone produced by the pancreas.[1] Insulin lowers the blood glucose level. When the blood glucose elevates insulin is released from the pancreas to normalize the glucose level. In patients with diabetes, the absence or insufficient production of insulin[2] causes hyperglycemia. After you eat a meal, your body breakes down the foods you eat into glucose and other nutrients, which are then absorbed into the bloodstream from the gastrointestinal tract. The glucose level in the blood rises after a meal and triggers the pancreas to make the hormone insulin and release it into the bloodstream. But in people with diabetes, the body either can't respond to insulin properly. Sappan wood is a small thorny tree, 6-9m in height and 15-25cm in trunk diameter with a few prickly branches. Coloring matter of sappan wood appears to be identical to the brazilin obtained from brazilwood.[3] The heartwood is bitter, astringent, sweet, acrid, refrigerant, vulnerary, depurative, constipating, sedative and haemostatic. It is useful

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in vitiated conditions of pitta, burning sensation, wounds, ulcers, leprosy, skin diseases, diarrhea, dysentery, epilepsy, convulsions, menorrhagia, leucorrhoea, diabetes, haemoptysis, hemorrhages, stomatopathy and odontopathy.[4]

Materials and methods

Plant materials

The species for the proposed study that is wood of *Caesalpinia sappan* collected carefully from National park of Mathikettan, Idukki district, Kerala. The plant was positively identified by Dr.Prabakaran. M.sc, M.ed; PhD, Professor and head of the department of botany, Vivekanandha college of Arts and Science for women, Elayampalayam, Tiruchengode, Namakkal. The plant was authenticated as *Caesalpinia sappan* of family *Fabaceae* from available literature.[5, 6]

Drugs and chemicals

Alloxan monohydrate (LOBA Chemie, Mumbai, India) was purchased, preserved at 25ºC and used for this study. Glibenclamide is an oral antidiabetic preparation[7] with an efficient hypoglycemic action. Daonil (Glibenclamide) manufactured by Aventis Pharma Ltd. Goa, India, was collected from market and preserved at room temperature.

Animals

Male albino-Wistar rats weighing 150-250g were used in the present study. All rats were kept at room temperature of 22-25ºc in the animal house. Prior to the experiments, rats were fed with standard food for 1 week in order to adapt to the laboratory conditions. All animal procedures were performed after approval from the institutional ethics committee and in accordance with the recommendations for the proper care and use of laboratory animals. Institutional IAEC number: 1158/PO/ac/07/CPCSEA.

Preparation of extract

About 350 gm of air dried powdered material was taken in 1000ml soxhlet apparatus and extracted with ethanol as solvent, till colour disappeared. The temperature was maintained at 55ºC-65ºC. The final solution was evaporated to dryness.

Preliminary phytochemical screening

In order to determine the presence of phytconstituents, a preliminary phytochemical study with EECS was performed.

Acute Oral Toxicity Study

The procedure was followed by using OECD guidelines 423 (Acute toxic class method).Twelve albino-wistar rats (150-250gm) were selected for studies. Depending on the mortality or moribund status of the animals. The testing dose of ethanolic extracts of *Caesalpinia sappan* Linn. 2000mg/kg,b.w,p.o, was administered. After giving the dose the animals toxic or death was observed upto 14 days.

Induction of Diabetes

The adult albino-wistar rats (150-250gm) were overnight fasted and determine the fasting blood glucose level. The sequence blood glucose level of animals were selected and used to induce diabetes by single i.p injection of 120 mg/kg of alloxan monohydrate.[8] Hyperglycemia is to be confirmed by elevated blood glucose levels, determined at by one touch glucometer. The threshold value of fasting blood glucose level >200mg/dL was taken as diabetic animal and rats found with permanent diabetes were used for the antidiabetic study.

Experimental Design

Experimental rats were divided into 5 groups of six rats (n=6). Animals were induced diabetic except control and treated for 21days .Group 1 Normal control rats fed with vehicles only. (Normal saline with 1%CMC). Group 2 Diabetic controls rats. Group 3 and 4 Diabetic rats treated with ethanolic extract of *caesalpinia sappan*, 100 and 200mg/kg,bw,po., dissolved in 1% carboxy methyl cellulose (CMC). Group 5 Diabetic rats treated with standard drug, Glibenclamide 3mg/kg bw,po. Fasting blood glucose (FBG) of all rats was determined before the start of the experiment. Blood sample was collected at weekly intervals from tail vein puncture till the end of study. In the continuous 21 days of drug treatment, a blood glucose level of all animals was determined at the 0, 7, 14, 21 day by using one touch glucometer. On day 21, overnight fasted animals were under mild ether anaesthesia, the blood was collected by direct cardiac puncture and was collected in tubes and evaluated biochemical parameters. The pancreas tissues were excised and rinsed in ice-cold saline and kept in formalin solution for further Histopathological studies.[9]

Invitro antidiabetic activity

Inhibition of alpha glucosidase enzyme

The inhibitory activity was determined by incubating a solution of starch substrate (2% w/v maltose) 1ml with 0.2M Tris buffer pH 8.0 and various concentration of plant extract for 5 min at 37ºC. The reaction was initiated by adding 1 ml of α-glucosidase enzyme (1U/ml) to it followed by incubation for 10min at 37ºC. Then, the reaction mixture was heated for 2 min in boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidase peroxidase method.[10]

$$\%\ of\ inhibition = \frac{(\text{Absorbance of control} - \text{Absorbance of extract} \times 100}{\text{Absorbance of control}}$$

Statistical analysis

All the values were expressed as mean ± S.E.M and were analyzed for significance by ANOVA and groups were compared by Tukey-Kramer multible comparison test. Differences between groups were considered significant at P<0.05 level.[11]

Results and discussion

Analytical Parameters

The analytical parameters were investigated and reported as, total ash value (6.2%w/w), acid insoluble ash value (1.8%w/w), water soluble ash value (0.9%w/w), and loss on drying (12.9%w/w).

Preliminary phytochemical screening

The extract of *Caesalpinia sappan* revealed the presence of alkaloids, glycosides, flavonoids, carbohydrates, phenolic compounds, proteins, amino acids.[12]

Acute Oral Toxicity Study

No toxicity or death was observed for these given dose levels, in selected and treated animals. So the LD_{50} of the ethanolic extract of *Caesalpinia sappan* as per OECD guidelines-423 is greater than 2000mg/kg (LD_{50}>2000mg/kg). Hence the biological dose was fixed at two levels, 100 and 200mg/kg body weight for the extract.

Changes in animal body weight

Alloxan caused body weight reduction, which is slightly reversed by ethanolic extract of *Caesalpinia sappan* treated (100mg/kg – 200mg/kg) groups after 21 days. While, significant (p<0.01, p<0.001) increase in body weight was observed in normal rats treated with ethanolic extract of *Caesalpinia sappan*.The EECS treated diabetic rats (200mg/kg) were slightly increased the body weight level and compare to *Glibenclamide* **(tab.1, fig.1)**.

Changes in blood glucose and HbA1c

A significant increase in the level of blood glucose and HbA1c was observed in diabetic control rats when compared to control rats. Administration of EECS and *Glibenclamide* to diabetic rats significantly decreased the level of blood glucose and HbA1c to near control level.[13, 14] **(tab.2, fig.2)**.

Changes in total cholesterol, triglycerides, HDL, LDL and VLDL

The level of HDL decreased in diabetic animals when compared to control animals. After EECS treatment, HDL was increased to near control. The level of cholesterol, triglyceride, LDL and VLDL increased in diabetic animals when compared to control animals. After EECS treatment, the higher level cholesterol, triglyceride, LDL and VLDL were decreased to near control. The showed that treatment with EECS significantly p<0.001 improved the lipid profile in alloxan induced diabetic rats. **(tab.3, fig.3)**

Changes in SGOT, SGPT and Alkaline Phosphatase

The level of SGOT, SGPT and ALP in plasma of diabetic animals was increased. SGOT, SGPT and ALP were restored significantly near to normal in EECS treated diabetic groups this level was decreased significantly (p<0.001) in ethanolic extract of *Caesalpinia sappan* treated groups and standard drug. **(tab.4, fig.4)**

Histopathology of pancreas of rats

Examination of Pancreatic tissue of diabetic rats treated with *Caesalpinia sppan* indicated that pancreatic section appeared more (or) less like control. **(fig.5)**

***Invitro* antidiabetic activity**

***In-vitro* α- glucosidase inhibiton**

The *Caesalpinia sappan* ethanol extract revealed a significant inhibitory action on α-glucosidase enzyme. The percentage inhibition at 25-1000µg/ml concentrations of *Caesalpinia sappan* extract showed a concentration-dependent increase in percentage inhibition. The percentage inhibition varied from 78.33±0.3215 to 18.18±0.4855 for

highest concentration to the lowest concentration of 25µg/ml. The concentration required for 50% inhibition (IC_{50}) was found to be 215.95±7.52 µg/ml whereas the α- glucosidase inhibitory activity of positive control acarbose produced percentage of 21.21±0.9052 for 25µg/ml and 90.90±0.1358 for 1000µg/ml. The IC_{50} value of standard drug acarbose against α-glucosidase was found to be 183.46±5.25 µg/ml**. (tab.5, fig.6)** The result suggest that ethanol extract of *Caesalpinia sappan* wood efficiently inhibits α- glucosidase enzyme.[15]

Table No. 01: Body weight changes in ethanolic extract of *Caesalpinia sapppan* and *Glibenclamide* on control and experimental groups of rats

Group	Treatment	Body Weight (gm)			
		Day 0	Day 7	Day 14	Day 21
I	Normal control rats (vehicles only)	186 ± 3.07	189.1 ± 2.9	190.83±3.016	193.6±2.9
II	Diabetic control rats	183.6±3.47	181.6±3.2	179.6±3.15	176.5±3.54
III	Diabetic group + EECS (100mg/kg)	187.8±3.52	191.5±3.37	195.16±3.34	197±3.77**
IV	Diabetic group + EECS (200mg/kg)	188.6±3.7	191.83±3.6	194.83±3.6	199±2.91***
V	Diabetic group + Glibenclamide 3mg/kg	188.16±2.1	191±2.08	193.8±1.92	198±1.80***

Values are statistically significant at * = p<0.05; ** = p<0.01; ***=p<0.001.

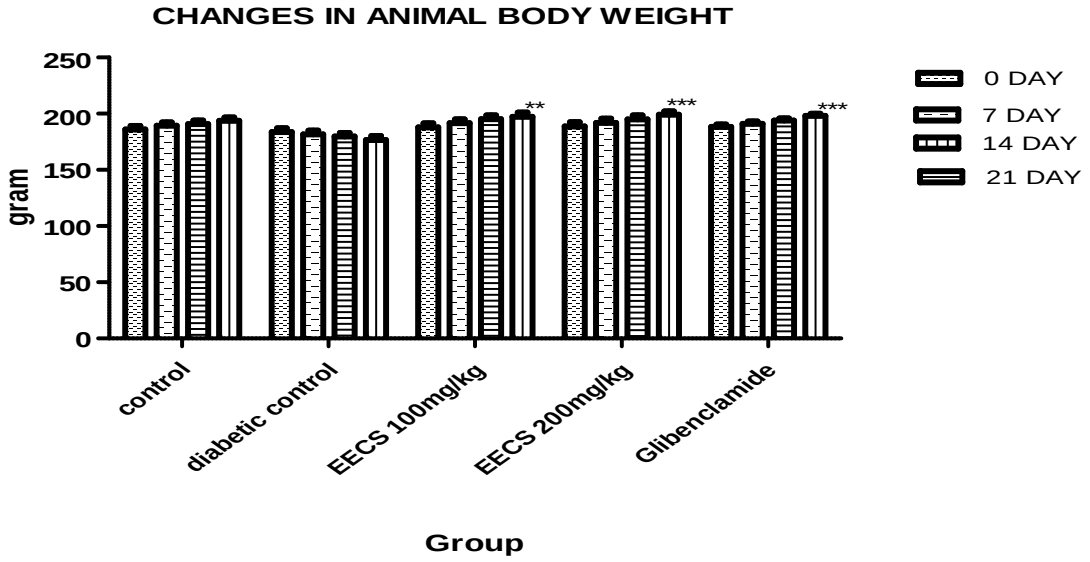

Fig. No. 01: Body weight changes in ethanolic wood extract of *Caesalpinia sappan* L. and *Glibenclamide* on control and experimental groups of rats

Table No. 02: Effect of ethanolic extract of *Caesalpinia sappan* and *Glibenclamide* on blood

Group	Treatment	Blood Glucose Level (mg/dL)				HbA1c (%)
		Day 0	Day 7	Day 14	Day 21	
I	Normal control rats (vehicles only)	91.66±3.40	96±3.83	88.16±2.91	91±2.97	5.52±0.22
II	Diabetic control rats	429.33±3.12***	436.5±2.11***	444.33±2.02***	453±2.38***	7.86±0.28***
III	Diabetic group + EECS (100mg/kg)	396.16±4.26***	340±4.47***	291.5±3.35***	220.16±4.4***	6.72±0.14**
IV	Diabetic group + EECS (200mg/kg)	405±4.36**	316.6±4.56***	236.6±4.4***	171.5±4.7***	5.77±0.17***
V	Diabetic group + Glibenclamide 3mg/kg	406.6±4.89**	314.8±4.2***	230.83±3.29***	149.5±1.38***	5.6±0.22***

Values are statistically significant at * = p<0.05; ** = p<0.01; *** =p<0.001.

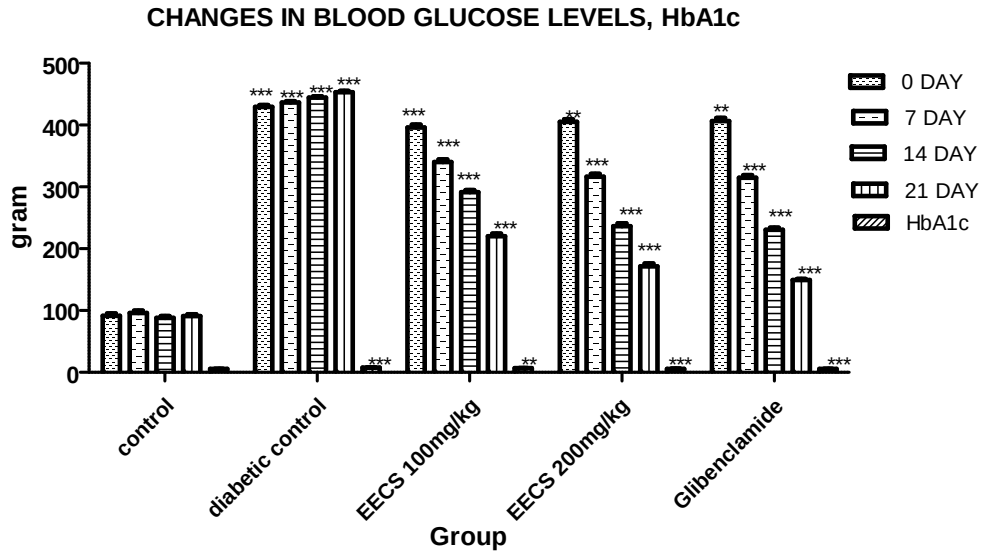

Fig. No. 02: Effect of ethanolic wood extract of *Caesalpinia sappan* L. and *Glibenclamide* on blood glucose and HbA1c level

Table No. 03: Effect of ethanolic extract of *Caesalpinia sappan* and *Glibenclamide* in Total cholesterol, Triglycerides, HDL, LDL, VLDL of control and experimental groups of rats.

Group	Treatment	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL Cholesterol (mg/dL)	LDL Cholesterol (mg/dL)	VLDL Cholesterol (mg/dL)
I	Normal control group (vehicles only)	86.30±0.68	86.75±0.59	45.84±0.97	75.30±1.35	18.70±0.611
II	Diabetic control rats	181.98±2.79***	111.59±1.20***	36.60±1.23***	112.01±2.40***	35.8±1.09***
III	Diabetic group + EECS (100mg/kg)	121.8±2.86***	98.68±0.70***	44.3±1.40**	91.02±1.01***	28.17±0.76***
IV	Diabetic group + EECS (200mg/kg)	117.55±1.5***	94.69±0.53***	47.61±1.16***	86.30±0.98***	26.75±0.16***
V	Diabetic group + glibemclamide (3mg/kg)	94.19±1.5***	93.49±0.34***	45.02±1.25***	74.24±0.76***	23.20±0.84***

Values are statistically significant at * = p<0.05; ** = p<0.01; *** =p<0.001.

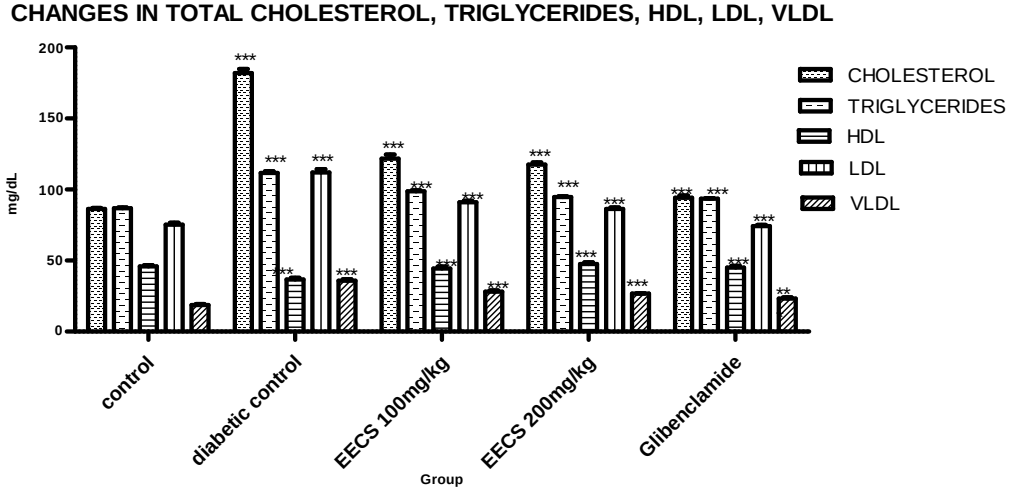

Fig. No. 03: Effects of ethanolic extract of *Caesalpinias sappan* L. *glibenclamide* on total cholesterol, triglycerides, HDL, LDL, VLDL levels of control and experimental groups of rats.

Table No. 04: Effect of ethanolic extract of *Caesalpinia sappan* and *Glibenclamide*in SGOT, SGPT, ALP of control and experimental groups of rats

Group	Treatment	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
I	Normal control group (vehicles only)	36.70±0.88	55.79±0.88	114.98±0.76
II	Diabetic control rats	84.12±0.93***	85.93±0.74***	183.69±0.83***
III	Diabetic group + EECS (100mg/kg)	46.96±0.82***	56.44±0.67***	132.91±0.70***
IV	Diabetic group + EECS (200mg/kg)	44.57±0.46***	54.64±0.75***	125.14±0.80***
V	Diabetic group + glibemclamide (3mg/kg)	42.14±0.57***	54.55±0.95***	122.64±0.75***

Values are statistically significant at * = p<0.05; ** = p<0.01; *** = p<0.001.

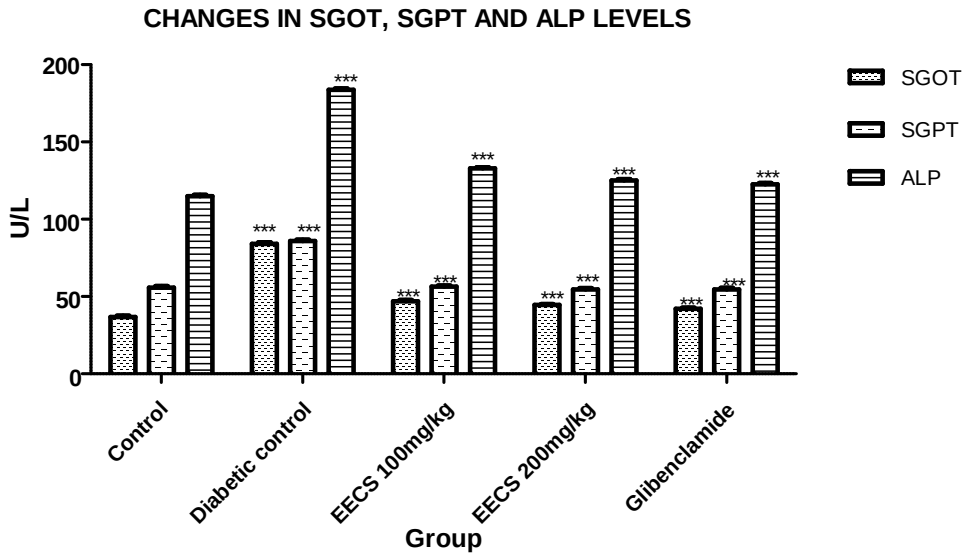

Fig. No. 04: Effect of ethanol extract of *Caesalpinia sappan* L. and *Glibenclamide* on SGOT, SGPT and ALP levels of control and experimental groups of rats.

Table No. 05: percentage of α- glucosidase inhibition of ethanolic extract of *Caesalpinia sappan* compared with standard acarbose

S.No	Concentration (µg/ml)	% of inhibition of ethanolic extract	% of inhibition of std	LC_{50} of ethanolic Extract (µg/ml)	LC_{50} of std(µg/ml)
1	25	18.18±0.4855	21.21±0.9052	215.95±7.52	183.46±5.85
2	50	22.42±0.9151	26.61±0.1352		
3	100	41.81±0.5126	33.33±0.4851		
4	250	58.18±0.1362	68.48±0.1532		
5	500	64.24±0.2564	72.72±0.2154		
6	1000	78.33±0.3215	90.90±0.1358		

All determinations were carried out in triplicate manner and values are expressed as the mean±SEM. The IC_{50} value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions.

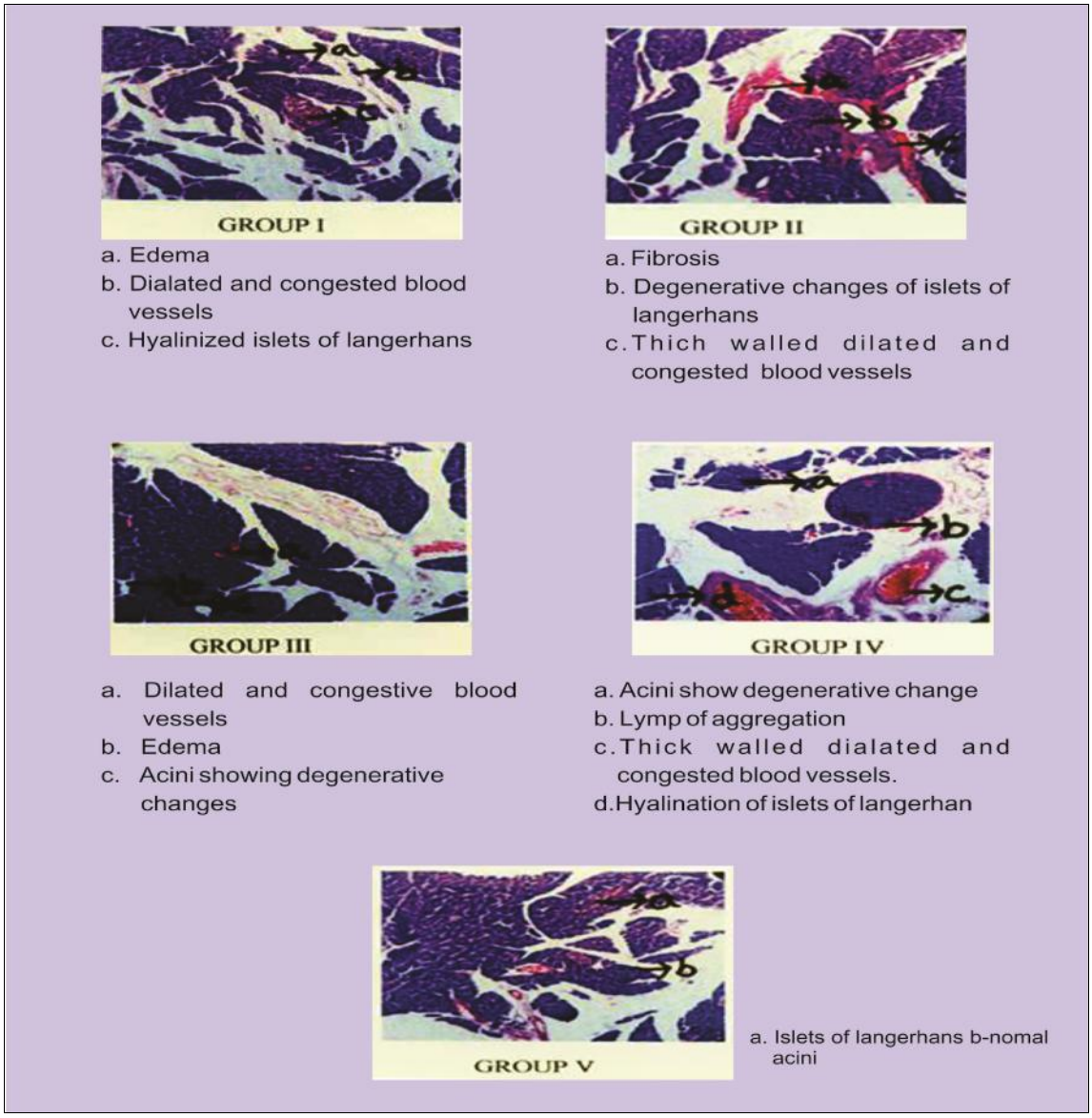

Fig. No. 05: Histopathology of pancreas

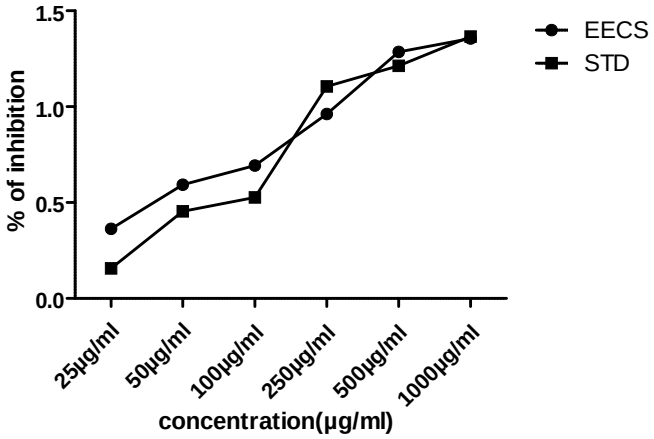

Fig. No. 06: α- glucosidase inhibition of ethanolic extract of *Caesalpinia sappan* compared with standard acarbose

Conclusion

In conclusion, in the present study on the ethanolic extract of *Caesalpinia sappan* wood having antidiabetic activity more over nearest activity of *Glibenclamide*. *Caesalpinia sappan* shows that alkaloids and flavanoids present in this extract may be possibly responsible for the antidiabetic activities. Invitro study of ethanolic extract of *Caesalpinia sappan* dose was selected 25 µg/ml to 1000 µg/ml and this doses possessed significant antidiabetic activity by inhibit the α-glucosidase enzyme.

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