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ANTI DIABETIC ACTIVITY OF METHANOLIC EXTRACT OF *Caesalpinia sappan* Linn. ON ALLOXAN INDUCED DIABETES MELLITUS IN RATS

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ABSTRACT

Management of diabetes mellitus is a global concern and successful treatment is very essential for preventing or at least delaying the onset of long-term complications of the disorder. It is believed that the traditional medicines used for the treatment of diabetes mellitus to attenuate the progression of complications of the disease. The search for the effective herbal drugs for the treatment of diabetes based on ethno medical clues still continues and in the long run has yielded us invaluable herbal remedies. To prove the ethno medical use of such folkloric traditional medicines, we have selected such ethno botanically important *Caesalpinia sappan* Linn, a plant used in the traditional systems of medicine in India for various uses. Methanolic extract of *Caesalpinia sappan* Linn. (MECS) was used at two dose levels 200mg/kg and 400mg/kg body weight and administered orally for 21 days to Alloxan induced diabetic rats. They significantly ($p<0.001$) reduced the blood glucose, total cholesterol and triglyceride levels and regulation in serum total proteins levels when compared with the standard Glibenclamide 10 mg/kg body weight.

Keywords: Diabetes Mellitus, Alloxan, *Caesalpinia sappan* Linn., Glibenclamide.

INTRODUCTION

The term 'Diabetes' (Greek word for siphon) was coined by Greek physician Artaeus around 2 AD. Artaeus noticed that patients with diabetes had a disease that caused the siphoning of the structural components of the body into the urine, after it was named as 'Diabetes Mellitus' by physician named Willis in 1674 [1]. Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Without enough insulin, body tissues in particular the liver, muscle and adipose tissues fail to take and utilize glucose from the blood circulation results in elevated blood glucose levels, a condition known as hyperglycemia [2]. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people above 65 years of age. In the modernized world, the changes in dietary habits and life style of the people leading to the cause of non-communicable diseases such as diabetes mellitus, heart diseases, strokes, cancer and lung diseases [3]. It is projected that India would become the world capital of diabetes mellitus by the year 2030. It is estimated that the number of people with diabetes between 45 to 64 years of age will be more than 82 million in developing countries and more than 48 million in developed countries [4].

Caesalpinia sappan Linn. (family: Caesal piniaceae) is a small to medium sized tree grows up to 10 meters in height. Leaves compound with 8-10 pairs of oblong, small leaflets the flowers are yellow in color and axillary panicles. Fruits small woody pods, with 2-3 seeds. Stem covered with woody thons. The woody part (heart wood) was used for various types of ailments like wounds,

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ulcers, leprosy, diarrhea, dysentery, convulsion, skin diseases and also used as bitter agent. The review of literature shows that the plant extract has immunosuppressive activity and used to prevent focal cerebral ischemia [5], antimicrobial activity [6], vasorelaxing effect and antioxidant activities [7]. The present study has been under taken to evaluate antidiabetic activity of methanol extract of *Caesalpinia sappan* Linn. in alloxan induced diabetic rats.

MATERIALS AND METHODS

Chemical Source

Alloxan and Glibenclamide were procured from Hetero laboratories Hyderabad, India. Glucose, Total cholesterol, Triglyceride and protein kits were procured from SS Pharma, Warangal.

Procurement of Plant Materials

The whole plant of *Caesalpinia sappan* Linn. was collected from different regions of Chittor district, after proper identification by an expert taxonomist Prof. Madhava Shetti, Department of Botany, Sri. Venkateshwara University, Chittor. The sample specimen (SAM/06/2011) was deposited at St.John College of Pharmacy for future reference.

Preparation of plant extracts

Commercial coarse *Caesalpinia sappan* Linn. powder 10 g was macerated in 400 ml methanol in 1000ml round bottom flask. They were placed at room temperature and shaken twice for a day and continued for 7 days. Then the extract was filtered, evaporated under vacuum to dryness. The percentage yield of extract from solvent extraction was calculated [8]. The methanolic extract of *Caesalpinia sappan* Linn. was subjected to preliminary phytochemical screening for the identification of phytocostituents.

Experimental animals

Adult Wistar rats of either sex weighing 180-220gms were used in present study. The in bred animals were procured from the animal house in Teena Biolabs Pvt Ltd. (Reg, No. 177/99 CPCSEA) Hyderabad. Animals were housed at CPCSEA approved in animal house of St. John College of Pharmacy (1278/AC/09/CPCSEA), Warangal. The animals were maintained in a well-ventilated room at 12:12 hr light:dark cycle in polypropylene cages and maintained at 22±1°C temperature with humidity at 55±5%. The animals were fed with standard balanced rat pellet diet and mineral water *ad libitum* throughout the experimental period. The experimental protocol was approved by the Institutional Animal Ethics Committee of St.John college of Pharmacy (IAEC No. 003/IAEC/StJCOP/2011).

Acute oral toxicity study

The procedure was followed according to the OECD 423 guidelines (acute toxic class method). The acute toxic class method is a step wise procedure with three animals of single sex per group. Depending on the mortality and moribid states of the animals on an average 2-4 step may be necessary to allow judgment on the acute toxicity of testing substance. It was observed that the test extract was not mortal even at 2000mg/kg dose.

Induction of experimental diabetes mellitus

Diabetes was induced in rats by injecting 150 mg/kg of Alloxan monohydrate intraperitoneally in 0.9% w/v NaCl to over-night fasted rats. The rats were then allowed for 10% glucose solution for the next 24h to prevent hypoglycemia. After 72 h of injection, rats with marked hyperglycemia (fasting blood glucose > 250 mg/dl) were selected and used for the study [9]. The selected diabetic animals were divided into four groups (n = 6) and one more group of normal non-alloxanized animals was also added in the study as control group.

Grouping of animals

Group I : served as normal control
Group II: served as diabetic control and received alloxan monohydrate 10ml/kg/p.o.
Group III: Diabetic rats treated with alloxan monohydrate and Glibenclamide 10mg/kg/p.o. Served as Standard.
Group IV: Diabetic rats treated with alloxan monohydrate and methanol extract of *Caesalpinia sappan* (MECS) 200 mg/kg/p.o.
Group V: Diabetic rats treated with alloxan monohydrate and methanol extract of *Caesalpinia sappan* (MECS) 400 mg/kg/p.o.

Fasting blood glucose estimation was done at 0, 2, 4 and 6 hr after the treatment. Drug treatment was continued for 21 consecutive days. The fasting blood glucose levels were estimated on days 0, 1, 7, 14, and 21 [8-11].

Collection of Blood Samples

Blood samples were collected from the retro orbital plexus of rats, by inserting a fine capillary gently in the inner angle of the eye. The capillary is slided under the eye ball at 45^0angle and over the bone socket to rupture the fragile venous capillaries of the ophthalmic venous plexus. After collecting the desired volume, capillary is removed with simultaneous release of pressure by fore finger and thumb. Any residual blood droplet around the eyeball is wiped off by dry cotton wool [12].

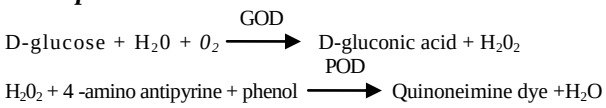
Estimation of Biochemical Parameters

On day 21, blood was collected from retro-orbital plexus of the overnight fasted rats under light ether anesthesia and kept aside for 1/2 h for clotting. Serum was separated by centrifuging the sample at 6000 rpm for 20 min. The serum was analyzed for total protein (Biuret method), cholesterol (CHOD-PAP % method), and triglyceride (GPO method) [9].

Estimation of Blood Glucose Levels

The glucose concentration in the serum samples was analyzed immediately by the glucose oxidase (GOD-POD) method using Glucose Kit (M/s Excel Diagnostics Pvt. Ltd., Hyderabad, India) and Elico UV-VIS spectrophotometer SL 164 (Elico Pvt. Ltd., Hyderabad, India)

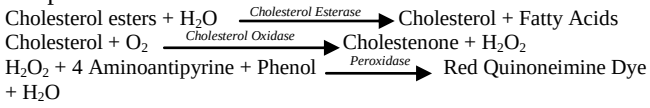
Principle:



Estimation of Total Cholesterol By CHOD/PAP Method

Principle:

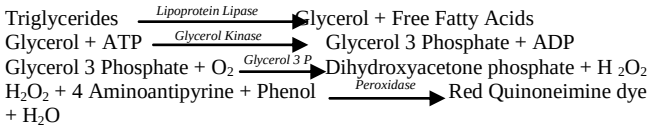
Cholesterol esterase hydrolyses esterified cholesterols to free cholesterol. The free cholesterol is oxidized to form hydrogen peroxide which further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of cholesterol present in the sample.



Eatimation of Triglycerides By GPO/PAP Method

Principle:

Lipoprotein lipase hydrolyses triglycerides to glycerol and free fatty acids. The glycerol formed with ATP in the presence of glycerol kinase forms glycerol 3 phosphate, which is oxidised by the enzyme glycerol phosphate oxidase to form hydrogen peroxide. The hydrogen peroxide further reacts with phenolic compound and 4 aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of triglycerides present in the sample.



Estimation of Total Protein

***Principle*:**

Proteins Bind with copper ions in the alkaline medium of Biuret reagent and produce a purple colored complex, whose absorbance is proportional to the Protein concentration.

Statistical Analysis

Graph Pad Prism software, version 5.0 was used in the statistical analysis of experimental data. All the values of fasting blood sugar and biochemical estimations were expressed as mean ± standard error of mean (S.E.M.). The results are analyzed for statistical significance using one-way ANOVA followed by Dunnett's t test and $p < 0.05$, $p < 0.01$, $p < 0.001$ was considered significant.

RESULTS

Effect of MECS treatment on blood glucose levels in alloxan induced diabetic rats from 0 hr to 24 hrs

Effect of MECS was evaluated at dose of 200mg/kg and 400mg/kg orally. At 0hr, 2hr, 4hr, and 24hrs extract exhibited slight significant ($p<0.05$) antidiabetic activity in case of 6th hr extract exhibited dose dependent significant ($p<0.01$, $p<0.001$) antidiabetic activity. The standard glibenclamide 10 mg/kg shown significant ($p<0.01$) antidiabetic activity in alloxan induced diabetic rats (Table 1).

Effect of MECS treatment on blood glucose levels in alloxan induced diabetic rats on day 7.

The MECS was treated with alloxan induced diabetic rats at dose of 200mg/kg and 400mg/kg b.w p.o. for the duration of 21 days .The extracts were exhibited significant ($p<0.05$, $p<0.01$,$p<0.001$) decrease in the blood glucose levels on 7th day study in diabetic rats . The standard glibenclamide 10mg/kg treatment showed significant ($P<0.01$) antidiabetic activity on 7th day in diabetic rats (Table 2).

Effect of MECS treatment on blood glucose levels in alloxan induced diabetic rats on day 14.

The extract MECS was exhibited significant ($p<0.05$, $p<0.01$, $p<0.001$) decrease in the blood glucose levels on 14th day. The standard glibenclamide 10mg/kg treatment shown significant ($p<0.01$) antidiabetic activity on 14th day in diabetic rats (Table 2).

Effect of MECS treatment on blood glucose levels in alloxan induced diabetic rats on day 21.

The extracts MECS was exhibited significant ($p<0.05$, $p<0.01$, $p<0.001$) decrease in the blood glucose levels on 21st day study in diabetic rats. The standard glibenclamide 10mg/kg treatment shown significant ($p<0.01$) antidiabetic activity on 21th day in diabetic rats (Table 2).

Effect of MECS treatment on serum Cholesterol levels in alloxan induced diabetic rats

The serum Cholesterol levels are significant ($p<0.05$) increased in alloxan induced diabetic rats when compared to control rats. Serum Cholesterol levels of diabetic rats treated with MECS at dose of 200mg/kg and 400mg/kg were showed significant ($p<0.01$, $p<0.001$) decrease in cholesterol levels when compared to alloxan induced diabetic rats. However, standard glibenclamide 10mg/kg treatment shown significant ($p<0.01$) decrease when compared to alloxan induced diabetic rats (Table 3).

Effect of MECS treatment on Triglyceride levels in Alloxan induced diabetic rats

The serum Triglyceride levels significantly ($p<0.05$) increased in alloxan induced diabetic rats when compared to control rats. Serum Triglyceride levels of diabetic rats treated with MECS at dose of 200mg/kg and 400mg/kg showed significant ($p<0.05$, $p<0.01$, $p<0.001$) decrease in Triglyceride levels in diabetic rats when compared to alloxan induced diabetic rats. The standard drug glibenclamide 10mg/kg treatment was also shown significant ($p<0.01$) decrease when compared to alloxan induced diabetic rats (Table 3).

Effect of MECS double dose treatment of Total Protein levels in Alloxan induced diabetic rats

There is the significant ($p<0.05$) decrease in serum total Protein levels in alloxan induced diabetic rats when compared to control rats. Serum total Protein levels of diabetic rats treated with MECS at dose of 200mg/kg and 400mg/kg shown significant ($p<0.05$, $p<0.01$, $p<0.001$) increased total Protein level when compared to alloxan induced diabetic rats. The standard drug glibenclamide 10mg/kg treatment shown significant ($P<0.05$) increase when compared to alloxan induced diabetic rats (Table 3).

Table 1. Effect of MECS treatment on blood glucose levels in Alloxan induced diabetic rats from 0hr to 24 hrs (Day 1)

S.no	Groups	Treatment mg/kg	Blood glucose levels (in mg/dl)				
			0hr	2hr	4hr	6hr	24hr (Day 1)
1	I	Normal control	89.12±2.58	89.58±8.44	90.12±3.54	90.64±5.26	90.96±2.10
2	II	Diabetic control	242.30±0.71 ^{ns}	252.8±0.89*	269.30±1.05**	281.31±1.29*	289.55±1.22**
3	III	Standard drug	270.98±2.27**	256.94±3.83**	230.71±4.32**	212.58±5.12**	208.54±0.22**
4	IV	MECS 200 mg/kg p.o	226.53±4.29 ^{ns}	219.78±3.23 ^{ns}	214.18±4.55*	20 2.88±5.17***	198.22±1.54*
5	V	MECS 400 mg/kg p.o	255.98±3.98 ^{ns}	246.16±3.11 *	240.66±3.63 *	230.10±4.312 ***	224.96±2.15*

Comparisons were made between : Group I vs Group II, Group II vs Group III, IV, V.
* Values are Mean ± SEM of 6 animals Statistical Significance test for comparison was done by ANOVA followed by Dunnett's `t ` test .*p<0.05,**p<0.01,***p<0.001, ns – Non significant.

Table 2: Effect of MECS treatment on blood glucose levels in Alloxan induced diabetic rats on day 7, 14 and 21

S.No	Groups	Day 7	Day 14	Day 21
1	I	91.66±1.32	92.10±1.98	92.52±1.30
2	II	298.70±2.24 ^{ns}	310.70±1.26 ^{ns}	318.70±0.66*
3	III	175.97±1.22**	117.97±1.20**	105.97±1.24**
4	IV	182.62±0.10**	180.36±1.20**	172.36±1.38**
5	V	203.68±2.40***	176.68±2.20***	152.68±2.33**

Comparisons were made between : Group I Vs Group II and Group II vs Group III,IV,V.
* Values are Mean ± SEM of 6 animals Statistical Significance test for comparison was done by ANOVA followed by Dunnett's `t ` test .*p<0.05,**p<0.01,***p<0.001, ns – Non significant.

Table 3: **Effect of MECS treatment on serum Cholesterol levels in Alloxan induced diabetic rats**

S.No	Groups	Cholesterol mg/dl	Triglyceride mg/dl	Total protein mg/dl
1	I	89.01±0.85	88.25±1.38	6.80±0.34
2	II	156.88±0.14*	190.92±2.50 *	5.40±0.33*
3	III	91.20±1.21**	106.18±1.13**	7.13±0.17*
4	IV	139.10±1.16**	139.05±1.42**	5.94±0.04**
5	V	128.80±1.22***	125.10±2.00***	5.97±0.02**

Comparisons were made between : Group I vs Group II and Group II vs Group III,IV,V.
*Values are Mean ± SEM of 6 animals, Statistical Significance test for comparison was done by ANOVA followed by Dunnett's `t` test ,*p<0.05,**p<0.01,***p<0.001, ns – Non significant.

DISCUSSION

The currently available drug regimens for management of diabetes mellitus have certain drawbacks and therefore there is a need to find safer and more effective anti-diabetic drugs. Plants have been use to source of drugs for the treatment of anti-hyperglycemic activity. The medicinal plants or plant derived products needs extensive research as number of diabetic patients in developing countries [13]. The present work discussed about the antidiabetic effect of methanolic extract of heart wood of "*Caesalpinia sappan* Linn" in alloxan-induced diabetic rat in dose dependent fashion.

Preliminary Phytochemical analysis of the MECS of heart wood showed that the plant has a rich possession of the phytochemicals like, tannins, phenols, flavonoids, saponins, carbohydrates, proteins, glycosides and study about alkaloids, steroids, sterols, gum and mucilage, terpenes are absent in extract. Acute oral toxicity studies

reveal that non toxic nature methanol extract of *Caesalpinia sappan* Linn heart wood. There was no lethality observed or profound toxic reaction found even at dose of 2000 mg/kg b.w. and which indirectly pronounced the safety profile of the plant extracts.

Fasting blood glucose levels of untreated diabetic rats were significantly higher than those in normal rats. Excessive production of glucose due to excessive hepatic glycogenolysis and gluconeogenesis is one of the fundamental bases of hyperglycemia in diabetes mellitus[14]. Oral administration of the Methanol extract and glibenclamide for 21 days significantly ($p<0.05$ to $p<0.001$) lowered the hyperglycemia of the experimental groups. The fasting blood glucose levels in MECS 200mg/kg and 400mg/kg treated diabetic rats shown lower glucose levels from 226.53mg/dl to 172.36mg/dl and 225. 98 mg / dl to 152.68mg/dl. The standard group animals treated with glibenclamide reduced the blood glucose levels from 270.98mg/dl to 105.97mg/dl. Among the two doses of MECS 200mg/kg and 400mg/kg dose showed significant ($p<0.05$ to $p<0.001$) anti-hyperglycemic effect is comparable to that of standard drug glibenclamide (10 mg/kg). In this study Alloxan induced diabetic rats treated with MECS (200 mg/kg & 400 mg/kg b.w.) was shown that significant reduction in the serum Cholesterol, Triglyceride levels when compared to untreated diabetic rat. In case of serum total protein showed that significant increase when compared to untreated diabetic rats [14].

CONCLUSION

Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis caused by pancreatic β-cell destruction or because of insulin resistance. If blood glucose levels remain high over a long period of time, this can result in long-term damage of organs such as the kidneys, eyes, nerves, heart and blood vessels. Complications in some of these organs can lead to death. In this present research work the evaluation was carried out with the Methanol extract of *Caesalpinia sappan* Linn. in Alloxan induced Diabetic rats. From the above results we find that the MECS at two dose levels 200mg/kg and 400mg/kg shown significant reduction in serum glucose, total cholesterol and triglyceride levels and regulation in serum total proteins levels, when compared with the standard glibenclamide. From the observations it is confirmed that *Caesalpinia sappan* Linn. Extract has exerted significant anti diabetic activity. The heart wood extract of plant *Caesalpinia sappan* Linn. may be used as supportive therapy for Diabetes mellitus.

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