PRECLINICAL EVALUATION OF ANTI-DIABETIC AND ANTIHYPERLIPIDEMIC ACTIVITY OF METHANOLIC EXTRACT OF *Sterculia foetida* LEAVES BY USING WISTAR ALBINO RATS

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ABSTARCT

The Anti-diabetic and Antihyperlipidemic activity of the Methanolic extract of the leaves of *Sterculia foetida* (jangli badam) (family: Sterculiaceae) was investigated on alloxan induced diabetic albino rats. A comparison was made between both plant extract and a known Antidiabetic drug Glibenclamide (10mg/kg). The dried leaves of Sterculia foetida was subjected to extraction by continuous hot extraction method using methanol as a solvent. Phytochemical estimation was done for the presence of phytoconstituents. Dose selection was made on the basis of acute oral toxicity study (100mg/kg, 200mg/kg, and 400mg/kg bodyweight) as per OECD 423 guidelines. Oral administration of extract of *Sterculia foetida* for 21 days resulted in significant reduction in blood glucose level. The biochemical parameters were analysed. All rats in the diabetic groups had FBG levels well within the diabetic range (>200 mg dL-1) at the initial stage of the experiment but after three weeks of treatment with extracts or Glibenclamide the FBG significantly dropped in dose-dependent manner, and also corrected the lipid profile and liver enzymes. The results suggest that the Methanolic extracts of the leaves of *Sterculia foetida* (200mg/kg and 400mg/kg) possess significant anti-diabetic and antihyperglycemic activities and restored the metabolic changes in alloxan-induced diabetic rats, but the percentage reduction was higher in Glibenclamide group than in test groups.

KEY WORDS: *Sterculia foetida*, Glibenclamide, Alloxan, anti-diabetic activity, antihyperlipidemic activity.

1. INTRODUCTION

Diabetes mellitus is a group of metabolic disorders of heterogeneous etiology characterized by persistent elevated blood glucose levels (hyperglycemia) with disturbances of carbohydrate, fat and protein metabolism as a result of defects in insulin secretion, impaired effectiveness of insulin action, or both. Globally, as of 2010, an estimated 285 million people had diabetes representing 6.6% of the world's adult population, today there are 382 million people living with diabetes. A further 316 million with impaired glucose tolerance are at high risk from the disease–an alarming number that is set to reach 471 million by 2035.

Study of *Sterculia foetida* leaf extracts yielded 46 compounds, including 36 flavonoids, 4 coumarins, 6 organic acids, and 3 steroids compounds, that include derivatives of methoxyflavone and Quercetin, which were proved to have antihyperglycemic activity. Since the plant materials used for the evaluation is of natural origin and also edible by nature, so their usage is not harmful to the humans and other animals.

In the light of the above information the present investigation was undertaken to evaluate the anti-diabetic potential of *Sterculia foetida* Linn. leaves extracts on fasting blood sugar and serum biochemical analysis.

2. MATERIALS AND METHODS

Chemicals: Alloxan monohydrate, glibenclamide, glucose, all other chemicals and reagents used were analytical grade.

Plant material: The leaves of *Sterculia foetida L* (jangli badam) belonging to Sterculiaceae were collected from Tirumala Hills, Chithoor district (A.P). The plant material was identified and authenticated by Dr.K.Madhava Chetty, Professor, Dept. of Botany, Sri Venkateswara University, Tirupati, India.

Preparation of plant extraction: The collected leaves were shade dried and powdered in mixer grinder to get coarse powder. The powdered plant material (120gms) was extracted with methanol (95%v/v) by using soxhlet apparatus. The extract was air dried to evaporate solvent.

Phytochemical screening: The preliminary phytochemical screening of methanolic extract of *Sterculia foetida* was carried by using standard procedures.

Animals Used: Wistar albino rats (200-250 g) of both sexes were procured from the Mahaveer Enterprises, Bagh Amberpet, Hyderabad. Before and during the experiment rats were fed with standard diet. After randomization to various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard

environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 hours *ad libitum*.

Acute Toxicity Study: Toxicity studies were performed according to OECD-423guidelines.

Experimental model: Alloxan monohydrate was weighed individually for each animal according to their body weight and solubilised with saline just prior to injection. Diabetes was induced by injecting it at a dose of 150 mg/kg body weight intraperitoneally. The rats were then kept for the next 24 h on 10% glucose solution bottles, in their cases to prevent hypoglycemia. After 72 h of injection, rats with marked hyperglycemia (fasting blood glucose > 200 mg/dl) were separated and divided into six different groups for experimental studies, with each group containing six animals.

Experimental design: The rats were divided into six groups each consist of six rats. Significant hyperglycaemia was achieved within 72 hrs after alloxan (150 mg/kg body weight i.p) injection.

Group I: normal control rats, received Tween 80.

Group II: diabetic control received Alloxan in single dose (150 mg/kg.i.p.).

Group III: rats received Glibenclamide (10mg/kg/day, p.o.) treatment for 21 days.

Group IV: rats received MESF (Methanolic Extract of *Sterculia foetida*) (100mg/kg/day, p.o) treatment for 21 days.

Group V: rats received MESF (200mg/kg/day, p.o) treatment for 21 days.

Group VI: rats received MESF (400 mg/kg/day, p.o) treatment for 21 days.

Assessment of Extracts on Alloxan-Induced Diabetic Animals: Treatment with plant extracts was started 72 hours after alloxan injection. Blood sample were drawn at weekly intervals till end of study (i.e. 3 weeks). Fasting blood glucose estimation and body weight measurement were done on day of 0, 1, 7, 14 and 21 of the study. On day 21, blood was collected by cardiac puncture under mild ether anesthesia from overnight fasted rats and fasting blood sugar was estimated. Serum was separated and analyzed for SGOT, SGPT, serum cholesterol, serum triglycerides by enzymatic colorimetric method, serum HDL, serum LDL, serum creatinine, total protein and serum alkaline phosphatase.

Statistical Analysis: All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean ± standard error of mean (SEM). The result of the study were subjected to one way analysis of variance (ANOVA) fallowed by Dunnet's test for multiple comparisons.

3. RESULTS

Phytochemical Screening: Phytochemical screening of methanolic extract of *Sterculia foetida* showed the presence of various chemical constituents mainly alkaloids, Proteins, glycosides, phytosterols and saponins. The results obtained were comparable and satisfied the standard literature.

Acute oral toxicity studies: In the present study the MESF was subjected for toxicity studies. For the LD_{50} dose determination MESF was administered the dose level of 2000 mg/kg body weight and the dose did not produce any mortality. Hence $1/20^{th}$, $1/10^{th}$ and $1/5^{th}$ of the dose tested i.e. 100mg/kg, 200mg/kg and 400mg/kg body weight was selected for the study. It was decided to evaluate experimental design of Antidiabetic activity and Anti-hyperlipidemic activity by Alloxan induced model.

Anti-diabetic activity:

Alloxan induced diabetic model: The Anti-diabetic effect of MESF in alloxan induced diabetic animals is presented. The results showed that after the administration of the extract in individual group of alloxan induced diabetic rats, there was a significant reduction in serum glucose levels throughout the entire period of study (21 days) as compared to diabetic control group. Vehicle control animals were found to be stable in their body weight while diabetic rats showed significant reduction in body weight during 21 days (Table 1). Alloxan caused weight reduction, which was reversed by methanolic extract of *Sterculia foetida* after 21 days of treatment. Other Biochemical parameter levels were decreased significantly by Glibencalmide ($p<0.01$), methanolic extract ($p<0.01$) of *Sterculia foetida*, after 21 days of treatment compared with diabetic control

Glibenclamide at an oral dose 10 mg/kg reduced serum glucose level at '0'day ($P<0.01$), 1st day ($P<0.01$), 7th day ($P<0.01$), 14th day ($P<0.01$) and 21st day ($P<0.01$) significantly when compared with control respectively.

Administration of MESF 100, 200 and 400 mg/kg orally reduced significantly serum glucose level at '0'day ($P<0.01$), at 1st day ($P<0.01$), at 7th day ($P<0.01$), 14th day ($P<0.01$), and 21st day ($P<0.01$), when compared to (Gr. II) control respectively. Whereas the Liver Enzymes like SGOT (Serum glutamic oxaloacetic transaminase),

SGPT (Serum glutamic pyruvic transaminase), ALP (Alkaline phosphatase), Serum Creatinine, Total Protein and Serum Bilurubin were found to be $p<0.01$ on the 21st day when compared to (Gr. II) control group.

The results obtained from the present investigation demonstrated that the leaf extract of *Sterculia foetida* (Sf) constantly maintained significant reduction of the glucose level and Lipid Levels in Alloxan mediated diabetic rats throughout the experimental period suggesting the Anti-diabetic and Antihyperlipidemic property of the title plant. It was also observed that the extract reversed the weight loss of the diabetic rats.

Table 1: Effect of *Sf* leaf extract on body weight in Alloxan induced Diabetic rats

S. No	Treated Groups	Body Weight (gm)				
		0 Day	1st Day	7th Day	14th Day	21st Day
I	Normal	236.67±8.05	233.33±6.94	241.67±6.42	243.33±5.09	241.67±6.42
II	Diabetic control (alloxan 150mg/Kg)	236.33±4.36	238.67±4.36	225±3.91	215±3.12	206.67±3.85
III	Standard (Glibenclamide 10mg/Kg)	236.67±4.51**	238±3.33**	239±3.33**	240.67±1.92**	241.67±2.81**
IV	*Sf* leaf extract (100mg/Kg)	237.67±3.43ns	233.67±2.85ns	231.67±4.61ns	228.47±2.92ns	220±2.04ns
V	*Sf* leaf extract (200mg/Kg)	238.33±4.36*	237.67±3.85*	235.67±2.81*	234.67±1.92*	232±2.04*
VI	*Sf* leaf extract (400mg/Kg)	236.33±3.66**	238±2.04**	239.33±2.81**	239.67±2.81**	240±3.33**

All values are expressed as a mean ± SEM, n=6, ns = not significant; one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests, *p<0.05, **p<0.01, ***p<0.001 as compared to control.

Figure.1.Effect of *Sf* leaf extract on body weight of Alloxan induced Diabetic rats

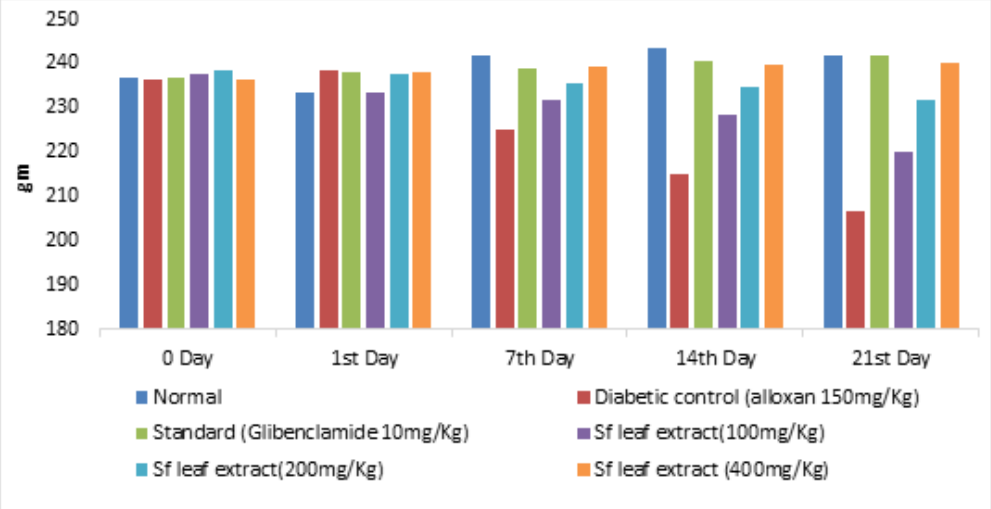


Table 2: Effect of *Sf* leaf extract on Serum Glucose levels on Alloxan induced Diabetic rats

S. No	Treated Groups	Serum Glucose levels (mg/dl)				
		0 Day	1st Day	7th Day	14th Day	21st Day
I	Normal	79.5±0.87	77.5±0.81	75±0.78	71.8±0.55	75.3±0.61
II	Diabetic control (alloxan 150mg/Kg)	263.8±1.64	294.6±1.13	320.3±1.25	362.4±0.87	416.8±1.09
III	Standard (Glibenclamide 10mg/Kg)	270.2±0.89**	206.1±1.05**	163.3±1.10**	109.7±0.72**	97.5±1.31**
IV	*Sf* leaf extract (100mg/Kg)	272.3±0.79*	259.7±1.67*	224.3±2.56*	182.5±4.1*	162.3±2.38*
V	*Sf* leaf extract (200mg/Kg)	268.7±0.69**	236.8±0.45**	194.4±0.53**	158.7±0.78**	121.4±3.77**
VI	*Sf* leaf extract (400mg/Kg)	269.5±0.47**	230.3±0.72**	178.4±0.45**	128.7±0.87**	110.6±0.77**

All values are expressed as a mean ± SEM, n=6, ns = not significant; one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests, *p<0.05, **p<0.01, ***p<0.001 as compared to control.

Figure.2.Effect of *Sf* leaf extract on Serum Glucose levels on Alloxan induced Diabetic rats

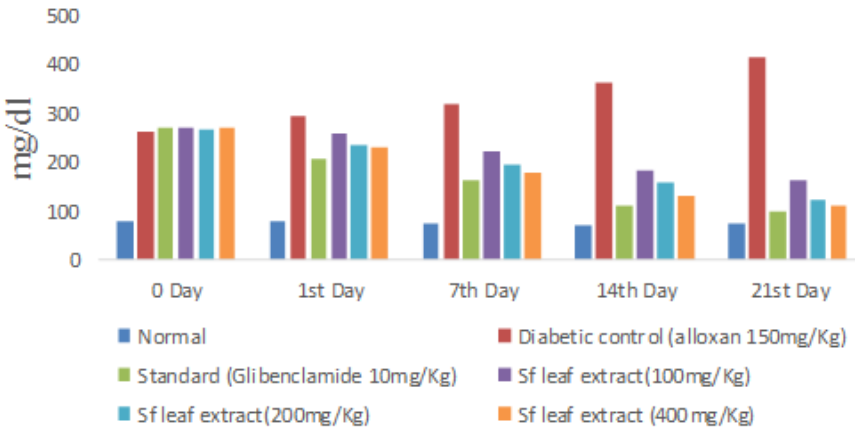


Table 3: Effect of *Sf* leaf extract on Liver Enzyme levels in Alloxan induced Diabetic rats

S. No	Treated Groups	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
I	Normal	22.4±0.805	51.42±0.659	92.66±0.707
II	Diabetic control (alloxan 150mg/Kg)	66.1±0.65	98.11±0.43	188.65±0.616
III	Standard (Glibenclamide 10mg/Kg)	25.1±0.55**	56.21±0.57**	140.87±0.635**
IV	*Sf* leaf extract (100mg/Kg)	62.59±0.42*	94.63±0.54*	179.22±0.67*
V	*Sf* leaf extract (200mg/Kg)	34.49±0.76**	74.08±0.66**	166.39±0.579**
VI	*Sf* leaf extract (400mg/Kg)	28.45±0.56**	62.16±0.67**	154.28±0.87**

All values are expressed as a mean ± SEM, n=6, ns = not significant; one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests, *p<0.05, **p<0.01, ***p<0.001 as compared to control.

Figure.3.Effect of *Sf* leaf extract on Liver Enzyme levels in Alloxan induced Diabetic rats

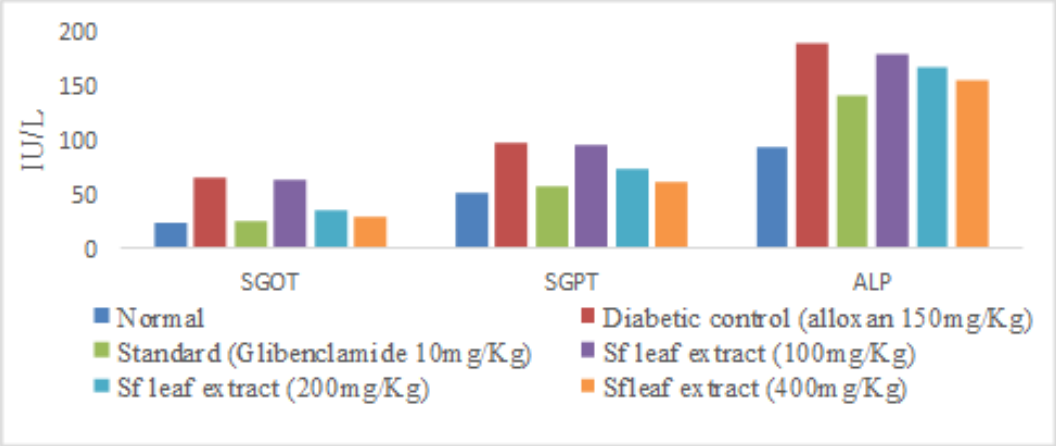


Table 4: Effect of *Sf* leaf extract on Total Protein, Serum Albumin and Serum Creatinine Levels (g/dl) in Alloxan induced Diabetic rats

S. No	Treated Groups	Total Protein	Serum Albumin	Creatinine
I	Normal	7.6±0.26	4.8±0.97	0.52±0.26
II	Diabetic control (alloxan 150mg/Kg)	2.8±0.15	2.2±0.72	1.35±0.05
III	Standard (Glibenclamide 10mg/Kg)	7.8±0.30**	5.0±0.18**	0.56±0.36**
IV	*Sf* leaf extract (100mg/Kg)	3.8±0.19*	2.7±0.76^{ns}	1.29±0.18*
V	*Sf* leaf extract (200mg/Kg)	5.1±0.32**	3.2±0.42*	0.69±0.23**
VI	*Sf* leaf extract (400mg/Kg)	6.3±0.28**	3.6±1.08**	0.61±0.09**

All values are expressed as a mean ± SEM, n=6, ns = not significant; one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests, *p<0.05, **p<0.01, ***p<0.001 as compared to control.

Figure.4. Effect of *Sf* leaf extract on Total Protein, Serum Albumin and Serum Creatinine Levels in Alloxan induced Diabetic rats

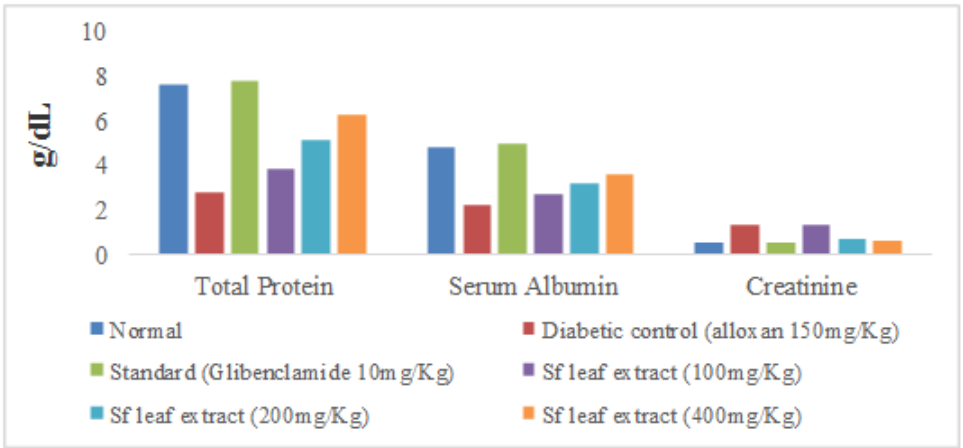
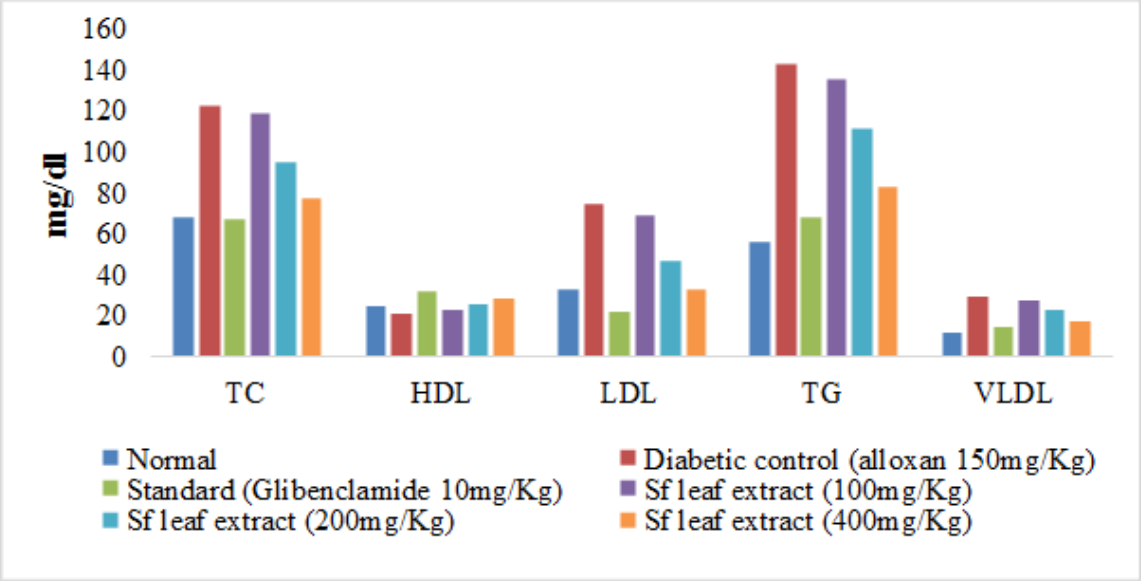


Table.5.Effect of *Sf* leaf extract on Lipid levels in Alloxan induced Diabetic rats on 21st Day of Treatment

S. No	Treated Groups	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	TG (mg/dl)	VLDL (mg/dl)
I	Normal	68.04±1.87	24.52±0.46	32.3±1.15	56.08±0.66	11.22±0.13
II	Diabetic control (alloxan 150mg/Kg)	122.9±0.73	20.18±0.26	74.12±1.12	142.85±1.62	28.6±0.32
III	Standard (Glibenclamide 10mg/Kg)	66.67±0.47**	31.81±0.45**	21.3±0.87**	67.89±1.26**	13.58±0.44**
IV	*Sf* leaf extract (100mg/Kg)	118.43±1.23*	22.55±0.36^{ns}	68.81±1.24**	135.43±0.74*	27.08±0.28^{ns}
V	*Sf* leaf extract (200mg/Kg)	94.36±2.37**	25.33±0.61*	46.79±0.93**	111.22±1.02**	22.24±0.19**
VI	*Sf* leaf extract (400mg/Kg)	76.92±0.38**	27.56±0.52**	32.85±1.02**	82.56±0.48**	16.51±0.24**

All values are expressed as a mean ± SEM, n=6, ns = not significant; one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests, *p<0.05, **p<0.01, ***p<0.001 as compared to control.

Figure.5.Effect of *Sf* leaf extract on Lipid levels in Alloxan induced Diabetic rats on 21st Day of Treatment



DISCUSSION

Diabetes mellitus is a debilitating and often life threatening disorder with increasing incidence throughout the world. Alloxan, a cyclic urea derivative, which selectively destroys insulin-producing pancreatic cells by free radical mediated damage and when administered to rodents cause an insulin-dependent diabetes mellitus. Hence it was reported as a potent diabetogenic agent and has been widely used for the induction of experimental diabetes in animals.

In the current study, set of experiments were designed to explore the in vivo Antidiabetic potential of methanolic extract of *Sterculia foetida* leaves against alloxan induced diabetic rat model. The study was designed to evaluate the chronic effects of extract in animals induced with diabetes and to compare the results with anti-diabetic potentials of reference drug Glibencalmide.

In the present study, for the chronic study, we have made the animals diabetic by administering Alloxan and treated the animals with varying doses of extract for 21 days. The blood glucose level and body weights in animals were estimated at 0, 1st, 7th, 14th and 21st day of the study to evaluate the potency of the extract in clearing blood glucose in animals induced with diabetes. We have also studied the potentials of the extract to lower the diabetic complications by assessing the lipid profile, and liver function tests. For the evaluation of lipid profile, concentrations of total serum cholesterol and triglycerides were estimated. The liver enzymes alanine transferase and aspartate transferase were estimated to study the hepatic function.

In chronic study, diabetes was induced in all animals except normal group by administering alloxan before three days of study. Hence there was significant decrease in insulin secretion in diabetic animals due to the destruction pancreatic cells which resulted in decreased utilization of glucose and hence the blood glucose level was elevated. But in therapeutic groups treated with standard drug Glibenclamide, MESF (100mg/kg, 200mg/kg and 400mg/kg), significant increase in insulin release and subsequent decrease in blood glucose concentration was found.

The association between liver disease and diabetes mellitus is well known, the overall prevalence being significantly higher than that expected by a chance association of two very common diseases. The liver damage leads to its malfunctioning and it is characterized by the increased concentrations of liver enzymes ALT and AST in the blood. In the present study, animals treated with extract at higher dose have shown the normal blood concentrations of those enzymes.

The diabetes mellitus is a chronic metabolic disorder and it is also associated with several secondary complications such as hyperlipidemia, atherosclerosis, hypertension, diabetic nephropathy, diabetic neuropathy and diabetic keto acidosis. Hyperlipidemia is one of such common complication of diabetes which is characterized by increase in serum total cholesterol (TC), triglycerides (TG), LDL and VLDL. The azotemia is condition which is due to the accumulation of nitrogenous waste products like urea and creatinine in blood and usually found during diabetic nephropathy.

In our present study administration of alloxan in control animals caused elevation of serum cholesterol, triglycerides, and urea as a consequence of secondary complications of diabetes. In animals of therapeutic groups treated with MESF (200mg/kg and 400 mg/kg) have shown significant reduction in above serum parameters.

4. CONCLUSION

The study was performed to find out the beneficial effects of methanolic leaf extracts of *Sterculia foetida* in alloxan induced diabetic rats and the results reveal that the plant has beneficial effects on blood glucose levels of alloxan induced diabetic rats and the results alsoreveal that the plant extract significantly protect from other metabolic aberrations found in diabetes, physiological as well as biochemical aberrations.

From the toxicity studies it was concluded that the extracts are safe and don't show any sort of toxic reactions up to the oral doses of 2000 mg/kg of body weight. The results indicate that the methanolic extract is potent in lowering the fasting blood glucose level of the diabetic rats; the effect is dose dependent. Moreover, the extracts show improvement in parameters like body weight. The

extracts also lowered serum SGPT, SGOT, and ALP levels which show the effect of the extracts in reversing the organ damage due to diabetes which is clearly observed by high levels of SGOT and SGPT in diabetic control. The extracts also lower the levels of serum lipids like triglycerides and cholesterolResult from the phytochemical analysis of *Sterculia foetida* revealed the presence of alkaloids, tannins, saponins, terpenoids, flavonoids, phenolics and glycosides as the possible biologically active principles, which have also been isolated from the other plants and found to possess antidiabetic activity.

Thus the leaves of *Sterculia foetida* possesses significant Antidiabetic activity which is the first claim in this respect. And it also has a potent Antihyperlipidemic activity.

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