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Assessment of Antihyperglycemic, Antihyperlipidemic and Antioxidant Activities of *Saraca Asoca* (Roxb.) Wilde Flower in Alloxan Induced Diabetic Rats

P. S. Tresina[1], **M. Santhiya Selvam**[2], **K. Paulpriya**[3], **A. Doss**[1] and **V. R. Mohan***[1]
[1]Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Thoothukudi, Tamil Nadu.
[2]Research Scholar (19212232262039), PG & Research Department of Botany, V.O.Chidambaram College, Thoothukudi, Tamil Nadu, India, Affiliated to Manonmaniam Sundaranar University, Tirunelveli
[3]Department of Botany, Seethalakshmi Achi College for Women, Pallathur, Karaikudi, Tamil Nadu.
***Corresponding author's** E-mail: vrmohanvoc@gmail.com

ABSTRACT
The objective of the present study was to investigate the effect of ethanolic extract of Saraca asoca flower (EESAF) as an antihyperglycemic, antihyperlipidemic and antioxidant in diabetic rats induced with alloxan monohydrate (150mg/kg). The EESAF at a dose of 150, 300 and 600 mg/Kg of body weight was administrated at single dose per day to diabetes induced rats for a period of 30 days. The effect of EESAF on blood glucose, insulin, urea, creatinine, HbA$_1$C, serum protein, albumin, globulin, serum enzymes [serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP)], serum lipid profile, [total cholesterol (TC), triglycerides (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C)] and lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP$_X$) and reduced glutathione (GSH) were assessed in the diabetic rats. The EESAF elicited significant decline of blood glucose ($p<0.01$), lipid parameters except HDL-C, serum enzymes and LPO and significantly reduced insulin ($p<0.01$), HDL-C, SOD, CAT, GPx and GSH at the dose of 600mg/kg was compared with the standard drug glibenclamide. Based on the results, it is concluded that ethanol extract (600 mg/Kg) of S. asoca flower exhibited significant antihyperglycemic, antihyperlipidemic and antioxidant effect in alloxan induced diabetic rats. Thus this effect of the EESAF supported the traditional claim of the plant.
***Keywords**: Saraca asoca, Alloxan, Insulin, HbA$_1$C, HDL-C, LPO, SOD, GSH.*

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INTRODUCTION

One of the major metabolic disorders currently associated with considerable morbidity and mortality is Diabetes Mellitus (DM). This metabolic disorder leads to several long-term complications in the affected individuals. Insufficient insulin secretion or insulin resistance causes hyperglycemia. In India, there is a rapid rise in the percentage of people affected by DM. India alone represents nearly 20% of the total diabetes population worldwide with more than 40 million people affected in India. The blood glucose levels in diabetic patients were controlled by the use of oral hypoglycemic/antihyperglycemic agents and insulin[1-3]. However, the limited efficiency of all these treatments has been reported to be associated with undesirable side effects. The adverse effects associated with synthetic drugs makes natural medicine a safe, cheap and effective alternative to modern medicine [4]. Therefore, traditional antidiabetic plants found in natural medicine can be explored. For ages, many indigenous plants have been used successfully for DM throughout the world. Some of those indigenous plants have been evaluated experimentally and their active ingredients have been isolated[5, 6].

Antihyperglycemic activity of the plants is caused by the plants ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. There are over 400 plant species which have been proven to contain hypoglycemic activity according to available literature. However, the search for new antidiabetic drugs from natural plants remains lucrative since they contain substances which clearly demonstrate alternative and safe effects on DM [7, 8].

Saraca asoca (Roxb.) Wilde belongs to the family Caesalpiniaceae which is an evergreen tree most commonly referred as Ashoka. Practitioners of Ayurveda and Unani medicine hailed the Ashoka tree as a miracle tree for women as the tree's medicinal properties were helpful in alleviating many gynaecological issues [9]. The bark from *Saraca asoca* had immense antimicrobial activity to fight against a wide range of bacterial pathogenic organisms[10]. The flowers from the tree were useful in the treatment of disorders such as uterine toxicity, improper digestion, stomach pain, constipation, hemorrhagic dysentery and diabetes. The leaves and flowers from the plant were used for treating fever, colic, ulcers and pimples [11]. Ashokarishta is an ayurvedic preparation from *Saraca asoca* which cures various diseases in women. It reduces headache, leucorrhoea and excessive bleeding [12]. A large scale investigation is done on *Saraca asoca* for its antiinflammatory and analgesic properties [13, 14] antipyretic activity [13], chemopreventive activity [15] and molluscicidal activity [16]. The antihelmintic activities of ethanolic and methanolic extracts from the bark of *Saraca asoca* were also reported owing to the presence of phytochemical constituents such as glycosides, alkaloids, tannins, flavonoids and terpenoids [17] The antimutagenic and antigenotoxic properties of the *Saraca asoca* bark extract is mainly due to the presence of gallic acid which is one of the major compounds [18, 19].

There is no experimental evidence supporting the antidiabetic effect of the *Saraca asoca* flower as revealed by the literature survey. Therefore, the present study was undertaken to examine the antihyperglycemic, antihyperlipidemic and antioxidant activities effect of ethanol extract from the *Saraca asoca* flower (EESAF) injected in alloxan induced diabetic rats.

MATERIAL AND METHODS

Collection of Plant Material

Saraca asoca (Roxb.) Wilde flower was collected from Bryant Nagar, Tuticorin, Tamil Nadu. The plant samples were identified with the help of local flora and authenticated by the Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen of collected plants was deposited in the Ethnopharmacology Unit, PG & Research Department of Botany, V.O.Chidambaram College, Thoothukudi District, Tamil Nadu.

Preparation of Plant Extract for Antidiabetic Activity

The flower of *Saraca asoca* were cut into small pieces, washed and then dried at room temperature; thereafter the dried flower was powdered in a Wiley mill. Hundred grams of the powdered flower was separately packed in a Soxhlet apparatus and then extracted with ethanol. This ethanol extract was concentrated in a rotary evaporator. The concentrated ethanol extract of the flower (EESAF) was used for antidiabetic activity. The extract was given a qualitative test with the purpose of identifying various phytochemical constituents as per the standard procedures [20-22].

Animals

Normal and healthy male wistar albino rats were housed under standard environmental conditions with temperature at 25±2°C and light and dark at 12:12h. The rats were fed with the standard pellet diet and water *ad libitum.* All the studies were performed in accordance with the guidelines for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Ethical Committee, CPSEA, India (Approval No. 1012/C06/CPSEA-Corres-2008-2009).

Acute Toxicity Study

Acute oral toxicity study was performed according to the OECD – 423 guidelines. Through random sampling, albino rats of both sexes were selected and were used for acute toxicity study. The animals were kept for an overnight of fasting and were provided only with water, after which the extracts of *Saraca asoca* were administered orally at 5 mg/Kg body weight by gastric intubations. Then the rats were observed for 14 days. The dose given was assigned as toxic dose when mortality was observed in two out of three animals. When mortality was seen in one animal, then the same dose was repeated again to confirm the toxic dose. When mortality was not observed, the procedure was done again using higher doses such as 50, 100 upto 2000 mg/Kg body weight.

Induction of Experimental Diabetes

Rats were induced with diabetes by the administration of simple intra peritoneal dose of alloxan monohydrate (150 mg/kg). Two days after injecting alloxan monohydrate, the rats were screened for diabetes. The rats having glucosuria and hypoglycemia with blood glucose level of 200-260 mg/100 ml were considered for the study. Room temperature was maintained in the plastic cage, where the animals had free access to water and pellet diet.

Experimental Design

The rats were divided into six groups and each group consisted of six rats:

Group I: Untreated normal rats received a standard pellet and sterilized water for 30 days

Group II: Untreated Diabetic Rats received a standard pellet and sterilized water for 30 days

Group III: Diabetic rats treated with 150 mg/Kg b.w. of EESAF orally for 30 days
Group IV: Diabetic rats treated with 300 mg/Kg b.w. of EESAF orally for 30 days
Group VI: Diabetic rats treated with 600 mg/Kg b.w. of EESAF orally for 30 days
Group VII: Diabetic rats treated with 600 μg/Kg b.w. of standard drug glibenclamide orally for 30 days

Biochemical Analysis

The animals were decapitated after the experimental period of 30 days. Blood was collected and sera separated by centrifugation. Serum glucose was measured by the O-toluidine method. [23] Insulin level was assayed by Enzyme Linked Immunosorbant Assay (ELISA) kit [24] Urea estimation was carried out by the method of [25]; serum creatinine was estimated by the method of [26]. Glycosylated haemoglobin (HBA_1C) estimation was carried out by a modified colorimetric method of [27]. Serum total cholesterol (TC) [28], total triglycerides (TG) [29], low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) [30], high density lipoprotein cholesterol (HDL-C) [31] and phospholipids [32] were analyzed. Serum protein [33] and serum albumins was determined by quantitative colorimetrically method by using bromocresol green. The total protein minus the albumin gives the globulin, serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by utilizing the method of [34]. Serum alkaline phosphatase (ALP) was measured by the method of [35]. Lipid peroxidation (LPO) [36], Glutathione peroxidase (GPX) [37], reduced glutathione (GSH) [38], superoxide dismutase (SOD) [39] and catalase (CAT) [40] were analyzed in the serum, of normal, diabetic induced and drug treated rats.

Methodology for Tissue Antioxidant Preparation of Tissue Homogenate

After the experimental regimen, the animals were sacrificed under mild chloroform anesthesia. Liver and kidney were excised and immediately washed with cold saline. The tissue was weighed and 10% tissue homogenate was prepared with 0.025 M Tris-Hcl buffer (pH 7.5). After centrifugation at 10,000×g for 10 minutes, the resulting supernatant was used for the estimation of non enzymatic and enzymatic antioxidants. Lipid peroxidation (LPO) [41], superoxide dismutase (SOD) [42], catalase (CAT) [43], glutatione peroxidase (GPx) [44] and reduced glutathione (GSH) [45] were also analyzed in the liver and kidney.

Statistical Analysis

Using the student's t-test statistical methods, the data was analyzed. For the statistical tests *p* values of less than 0.001, 0.01 and 0.05 were taken as significant.

RESULTS

Phytochemical Screening

The presence of alkaloid, coumarin, catechin, flavonoid, saponin, steroid, glycoside, terpenoid, phenol, tannin, sugar and xanthoprotein were found after conducting phytochemical analysis of EESAF.

Acute Toxicity Studies

There was no mortality up to the dose of 2000mg/Kg body weight as per this study. For long term administration, the extracts have been found to be safe.

Blood Glucose Level

Compared to control rats, alloxan induced diabetic rats showed significant elevation in the blood glucose level according to the present study. From the first week to the fourth week, blood glucose levels were significantly reduced after administering EESAF and glibenclamide. But from the second week onwards, there was significant reduction of blood glucose levels than diabetic control rats when EESAF was administered in the dosage of 300 mg/Kg and 600 mg/Kg. (Table 1).

Insulin, Glucose, Urea, Creatinine and HbA1C Levels

Table 2 showed the levels of blood glucose, plasma insulin, urea, creatinine and glycosylated haemoglobin of control, diabetic induced and plant extracts treated rats. When compared to normal control, the alloxan induced diabetic rats showed a significant elevation in levels of blood glucose (p<0.001), urea (p<0.01), creatinine (p<0.05) and glycosylated haemoglobin (p<0.001), but there was a decrease in the level of plasma insulin (p< 0.01). Administration with the EESAF at 300mg/Kg and 600mg/Kg body weight doses and glibenclamide tends to bring the above said parameters towards normal significantly (p<0.001; p<0.01).

Biochemical Parameters Level

In diabetic control rats, the decreased protein, albumin and globulin levels were noticed when compared to control rats (Table 3). Administration of EESAF 150, 300 and 600 mg/kg and glibenclamide increased protein, albumin and globulin levels compared to diabetic control rats. Alloxan induced diabetic rats showed significantly elevated SGPT, SGOT and ALP levels than control rats. Administration of EESAF

(600mg/Kg) and glibenclamide treatment significantly ($p<0.01$) reduced above parameters compared to diabetic control rats (Table 3).

Lipid Profile Level

The normal lipid profile levels were significantly altered after the induction of diabetes in the present study. Administration of EESAF (300mg/Kg and 600mg/Kg body weight) and glibenclamide significantly ($p<0.01$, $p<0.05$) decreased TC, TA, LDL, VLDL and PL levels and also significantly ($p<0.05$) increased the HDL level compared to diabetic control rats. After orally administering EESAF in the dosage of 600 mg/Kg, elevated lipid profile levels were greatly reduced in diabetic rats than when EESAF was given in the dosage of 300 mg/Kg (Table 4).

Antioxidants Level

The serum, liver and kidney of diabetic control rats showed significant elevation of LPO and reducing GPx, GSH, SOD and CAT levels was also observed. Administration of EESAF (300mg/kg and 600mg/kg) and glibenclamide significantly ($p<0.01$, $p<0.05$) decreased the LPO and increased the GP_X, GSH, SOD and CAT levels compared to diabetic control rats in serum, liver and kidney (Table 5, 6 & 7).

Table 1 Effect of EESAF extract on the Insulin, blood glucose, urea, creatinine and HbA_1C level of normal, diabetic induced and drugs treated rats

Parameter	Insulin (MIu/ml)	Glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	HbA_1C (%)
Group I	14.21±0.43	67.31±0.91	11.24±0.18	0.63±0.27	2.84±0.11
Group II	8.16±0.24**	206.15±3.46***	28.46±0.11**	1.84±0.17*	8.16±0.12***
Group III	9.34±0.12*	174.22±3.16**	21.54±0.24*	0.93±0.27ns	6.39±0.34*
Group IV	13.46±0.37a	119.36±3.92ns	19.36±0.48ns	0.84±0.22ns	5.11±0.18a
Group V	15.81±0.35aa	98.21±0.34aaa	16.15±0.92a	0.69±0.29a	4.04±0.29aa
Group VI	16.22±0.46aa	81.33±0.18aaa	14.54±0.77a	0.73±0.15a	2.98±0.11aa

Each Value is SEM of 6 animals * $p < 0.05$; ** $p < 0.01$ ***; $p < 0.001$ Significance between normal control vs diabetic induced control, drug treated group; a $p < 0.05$; aa $p < 0.01$ Significance between diabetic induced control vs drug treated group ns: not significant

Table 2 Effect of EESAF extract on blood glucose level of normal, diabetic induced, and drug treated rats at different time intervals

Groups	Blood glucose level in mgs/dl				
	0 day	1 week	2 week	3week	4 week
Group I	70.96±1.17	73.66±1.15	79.31±0.98	74.18±1.05	67.31±0.91
Group II	224.00±5.18***	231.63±6.95***	227.31±3.61***	216.35±4.16***	206.15±3.46***
Group III	208.15±1.68***	201.56±6.24***	193.16±2.84***	182.15±3.16**	174.22±3.16**
Group IV	213.16±3.84***	173.91±4.18**	162.96±2.16*a	131.65±2.67ns a	119.36±3.92ns a
Group V	205.27±1.67***	159.31±4.16**	132.68±2.46ns aa	117.56±2.98aa	98.21±0.34aaa
Group VI	211.67±3.1***	138.31±2.16*	121.36±1.27ns aa	103.65±2.34aa	81.33±0.18aaa

Each Value is SEM of 6 animals * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ Significance between normal control vs diabetic induced control , drug treated group; a $p < 0.05$; aa $p < 0.01$ Significance between diabetic induced control vs drug treated group ns: not significant

Table 3 Effect of EESAF extract on the protein, albumin, globulin, SGOT, SGPT and ALP level of normal, diabetic induced, and drug treated rats

Groups	Parameters					
	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	SGPT (u/l)	SGOT (u/l)	ALP (u/l)
Group I	8.31±0.67	4.67±0.15	3.64±0.23	19.56±1.84	20.33±0.46	179.33±2.65
Group II	7.08±0.13*	4.19±0.31ns	2.89±0.10*	148.36±4.61***	151.26±1.67**	246.37±6.37***
Group III	7.56±0.18ns	4.26±0.58	3.30±0.23	119.24±2.81***	102.68±3.91**	216.31±6.94**
Group IV	7.96±0.32ns	4.31±0.73	3.65±0.41	62.16±3.40*a	50.22±2.18ns a	178.31±3.88aa
Group V	8.16±0.18a	4.42±0.91	3.74±0.54a	25.89±1.54aa	26.27±1.67aa	168.16±2.67aa
Group VI	8.24±0.15a	4.54±0.34	3.70±0.29a	26.18±1.17aa	21.65±0.98aa	143.98±3.16aa

Each Value is SEM of 6 animals *$p < 0.05$; ** $p < 0.01$ ***; $p < 0.001$ Significance between normal control vs diabetic induced control, drug treated group; a $p < 0.05$; aa $p < 0.01$ Significance between diabetic induced control vs drug treated group ns: not significant

Table 4 Effect of EESAF extract on the TC, TG, LDL-C and PL in the plasma of normal, diabetic induced, and drug treated rats

Groups	Parameter					
	TC (mg/dl)	TG(mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	PL (mg/dl)
Group I	143.96±1.24	112.63±2.16	51.31±1.26	70.12±2.13	22.53±1.16	196.12±3.29
Group II	228.31±4.16***	163.29±2.88**	30.22±0.96**	165.43±3.56***	32.66±1.35*	271.20±3.13**
Group III	206.67±3.16**	136.36±1.28ns	38.16±2.61ns	141.24±4.11**	27.27±0.98ns	251.94±2.86**
Group IV	173.93±2.86ns	123.31±4.31aa	41.54±0.98ns a	107.75±2.56ns	24.64±1.02ns	222.80±3.11a
Group V	159.65±1.98aa	118.31±5.48aa	43.66±1.27ns a	92.33±1.98aa	23.66±0.87ns	210.09±2.84aa
Group VI	137.65±1.68aa	109.56±1.24aa	44.93±0.98ns a	70.81±1.36aa	21.91±1.28a	190.51±2.56aa

Each Value is SEM of 6 animals * $p < 0.05$; ** $p < 0.01$ *** $p < 0.001$ Significance between normal control vs diabetic induced control, drug treated group; a $p < 0.05$; aa $p < 0.01$ Significance between diabetic induced control vs drug treated group ns: not significant

Table –5 Effect of EESAF extract on serum LPO, GPX, GSH, SOD and CAT in the normal, diabetic and drug treated rats

Groups	Parameters				
	LPO (nanomol/mg protein)	GPX (u/mg protein)	GSH (u/mg protein)	SOD (u/mg protein)	CAT (u/mg protein)
I	1.73±0.026	698.37±11.24	51.31±0.96	481.22±6.36	92.68±2.06
II	7.36±0.66***	436.16±11.13***	20.22±0.67***	293.15±3.84***	39.27±1.24**
III	7.04±0.046**	493.68±5.67** ns	25.18±0.54*	384.16±5.27**	56.37±1.98*
IV	3.04±0.056*	616.36±7.36ns aa	30.26±1.67ns	402.76±3.18ns a	73.16±2.26a
V	2.13±0.054ns a	711.67±4.16ns aa	46.22±1.29aa	448.66±4.31aa	89.22±3.18aa
VI	1.92±0.017ns a	726.31±6.92ns aa	49.36±1.36aa	492.36±5.81aa	98.36±2.86aa

Each Value is SEM of 6 animals * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ Significance between normal control vs diabetic induced control , drug treated group; a $p < 0.05$; aa $p < 0.01$ Significance between diabetic induced control vs drug treated group ns: not significant

Table 6 Effect of EESAF extracts on Liver LPO,GPX,GSH,SOD and CAT in the normal, diabetic and drug treated rats

Groups	Parameters				
	LPO (nanomol/mg protein)	GPX (u/mg protein)	GSH (u/mg protein)	SOD (u/mg protein)	CAT (u/mg protein)
Group I	0.076±0.021	67.31±2.93	42.39±1.13	19.36±1.16	13.68±1.08
Group-II	0.639±0.053***	29.22±1.67**	16.81±0.73*	9.31±0.84**	4.36±0.36***
Group-III	0.316±0.056***a	51.36±1.54 ns a	21.63±1.03*	14.22±0.94ns	5.35±0.18*
Group-IV	0.204±0.037***a	56.22±1.36aa	23.16±1.26 a	16.92±0.68 a	8.56±0.39 ns
Group-V	0.093±0.013ns aa	60.26±3.16aa	26.18±2.04aa	22.67±1.08aa	10.26±0.18a

Each Value is SEM of 6 animals * $p < 0.05$; ** $p < 0.01$ *** $p < 0.001$ Significance between normal control vs diabetic induced control, drug treated group; a $p < 0.05$; aa $p < 0.01$ Significance between diabetic induced control vs drug treated group ns: not significant

Table –7 Effect of EESAF extracts on Kidney LPO, GPX, GSH, SOD and CAT in the normal, diabetic and drug treated rats.

Groups	Parameters				
	LPO (nanomol/mg protein)	GPX (u/mg protein)	GSH (u/mg protein)	SOD (u/mg protein)	CAT (u/mg protein)
Group I	0.091±0.036	5.98±0.22	38.31±0.24	30.26±0.86	28.16±0.18
Group-II	0.291±0.016***	3.54±0.16*	16.84±0.18**	8.46±0.54***	10.96±0.36**
Group-III	0.126±0.011**	4.91±0.26ns	22.16±0.26*	11.67±0.84*	18.31±0.92*
Group-IV	0.112±0.036ns a	5.36±0.11a	26.84±0.16ns a	14.16±0.34ns	20.31±0.88ns
Group-V	0.101±0.024ns aa	5.88±0.36a	32.67±1.06aa	14.86±0.16ns	21.67±0.26a
Group-VI	0.084±0.006 ns aa	6.11±0.24a	36.11±0.18aa	26.84±0.24ns aa	33.16±0.36aa

Each Value is SEM of 6 animals * $p < 0.05$; ** $p < 0.01$ *** $p < 0.001$ Significance between normal control vs diabetic induced control , drug treated group; a $p < 0.05$; aa $p < 0.01$ Significance between diabetic induced control vs drug treated group ns: not significant

DISCUSSION

Diabetes mellitus of long duration is associated with several complications such as atherosclerosis, myocardial infarction, neptropathy, etc., [46]. These complications are usually related to chronically elevated blood glucose level. Alloxan caused by the destruction of β – cells of the islets of langerhans, thereby inducing hyperglycemia [9]. Daily administration of the EESAF for 30 days resulted in decrease in the blood glucose levels of alloxan induced diabetic rats. The possible hypoglycemic mechanism of EESAF may be through potentiation of pancreatic secretion of insulin from β – cell of islets or due to enhanced transport of blood glucose to the peripheral tissues. This may be due to the presence of secondary metabolites and the hypoglycemic action of EESAF and glibenclamide, thereby stimulating the insulin release and inhibition of glucagon secretion [47]. In a similar study, [48] reported the dried extract of *Coccinia indica* to exert beneficial hypoglycemic effect in experimental animals and human diabetic subject possibly through an insulin secreting effect or through influence of enzymes involved in glucose metabolism.

Urea and creatinine are the prognostic indicators of renal dysfunction [49]. Urea the end product of protein metabolism is synthesized in the liver, transported by the blood to the kidney and is excreted [50]. Creatinine is endogenously produced and released into body fluids and its clearance is measured as an indicator of glomerular filteration rate [51]. In the present study, significant increase in serum urea ($p<0.01$) and creatinine ($p<0.05$) levels were observed in diabetic rats compared to normal control rats which indicate impaired renal function in diabetic rats. The treatment with EESAF lowered above parameters significantly compared to diabetic control rats and it showed protective effect of EESAF on the kidneys. An increase in the levels of serum urea and creatinine in diabetic rats were also reported by [52]. The presence of polyphenols and flavonoids in the plant extract might be responsible for the antioxidant activity; thereby a reduction in serum urea and creatinine levels [53]. In a similar study, [54] had reported that the administration of *Sarcostemma secamone* extract significantly decreased serum urea and creatinine levels in diabetic rats.

Glycosylated haemoglobin is produced by glycosylation of haemoglobin. Glycosylated haemoglobin is formed progressively and irreversibly over a period of time and is stable over the life span of the red blood cells. It is unaffected by diet, insulin or exercise, even on the day of test. Therefore, glycosylated haemoglobin can be used as an excellent marker of overall glycaemic control. Since it is formed slowly and does not dissociate easily, it reflects the real blood glucose level [55]. In this study, alloxan induced diabetic rats showed a significant increase in the level of glycosylated haemoglobin when compared with the normal rats. Following EESAF therapy indicates that the overall blood glucose level was controlled, probably due to improvement in insulin secretion. It note worthy that the serum insulin level in diabetic rats treated with EESAF also increased when compared to the diabetic control rats. Thus, it seems that EESAF stimulated increased insulin secretion in alloxan induced diabetic rats. In this respect, the mode of action of these extract is similar to those reported for extracts of *Gymnema sylvestre* [12]: *Clitoria ternatea* [13] *Pterocarpus marsupium* [56] and *Eugenia singampatiana* [57].

In diabetic condition, occurrence of reduction in protein and albumin may be due to proteinuria, albuminuria or increased protein catabolism, which is clinical markers in diabetic nephropathy [14]. The protein and albumin level was reduced after the induction of diabetes and treatment of EESAF increased both levels considerably in diabetic rats towards normal level. This action possibly is through increase in the insulin mediated amino acid uptake, enhancement of protein synthesis and or inhibition of protein degradation [15]. Also, increased serum SGOT, SGPT, and ALP levels were reported in diabetes and it may be due to liver dysfunction [16]. In this study, increased level of SGOT, SGPT, and ALP was observed in

alloxan induced diabetic rats which may have occurred by leakage of enzymes from the liver cytosol into the blood stream; it represents the toxicity of alloxan on liver. Diabetic rats treated with EESAF significantly reduced the above said enzyme levels which represents the protective action of EESAF on liver in diabetic condition. In the plant extract treated diabetic rats, reduction in SGOT, SGPT and ALP activities may be due to the presence of flavonoids and tannins in the plant extract which repairs the tissue damage induced by the diabetic complications [58]. In a similar study [59] had reported that *Acacia catechu* leaf extract significantly decreased the activities of SGOT, SGPT and ALP in diabetic rats.
The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [19]. The abnormal high concentrations of serum lipids in diabetic animals are mainly due to an increased mobilization of free fatty acids from peripheral fat depots [60]. In the present study, significantly increased levels of serum TC, TG, VLDL, LDL and PL as well as marked reduction of serum HDL levels in diabetic rats were noticed. LDL is the bad cholesterol since it gets deposited on the walls of blood vessels. LDL is the important predictor of atherosclerosis and coronary heart disease. HDL is an antiatherogenic lipoprotein. It is good cholesterol because of its role in the prevention of atherosclerosis by transporting the cholesterol because of its role in the prevention of atherosclerosis by transporting the cholesterol from peripheral tissues to the liver [61]. Administration of EESAF decreased levels of TC, TG, LDL, VLDL and PL levels as well as increased the level of HDL in diabetic rats. In order to prevent diabetic complications like coronary heart disease and atherosclerosis; the afore mentioned action could be beneficial. The presence of flavonoids and saponins in the plant extract contributes to the reduction in serum total cholesterol, triglyceride, LDL and VLDL levels. [62] The level of serum HDL was found to be significantly increased in the plant extract treated rats. This might be due to increase in the activity of lecithin cholesterol acyltransferase which may contribute to the regulation of blood lipids [63]. Flavonoids present in the plant extract are responsible for increasing the level of HDL [64-70].
Hyperglycemia is associated with formation of reactive oxygen species (ROS) which caused damage particularly of liver and kidney. The results of the present study showed increased lipid peroxidation (LPO) on serum liver and kidney of alloxan induced diabetic rats. A relatively high concentration of early peroxidizable fatty acids contained by the tissue may be the reason for increased lipid peroxidation. In the present study, an increase in the level of LPO was found and this level was significantly reduced after the supplementation with EESAF. This indicates that EESAF inhibit oxidative damage due to antiperoxidative effect of ingredients present in them. This could be correlated with the previous studies of [65] on *Cassia auriculata* flower, [66] on *Hamelia patens* leaves, [67] on *Mimosa pudica* leaves and [68] on *Syzygium paniculatum* indicating antiperoxidative and antihyperlipidaemic effects in diabetic rats.
Superoxide dismutase (SOD) a metalloprotein, is primarily involved in the antioxidant defence by scavenging the superoxide radicals. In hyperglycemia, glucose undergoes auto – oxidation and produces superoxide radicals which lead to lipid peroxidation [18]. Catalase, (CAT) a haemeprotein, localized in the peroxisomes or microperoxisomes catalyzed the decomposition of H_2O_2 protects the cell from oxidative damage produced by H_2O_2 [17]. An imbalance between ROS production and antioxidant scavenging systems develops as a result of decreased level of the antioxidant enzymes in diabetic rats. In the present study, SOD and CAT levels were significantly decreased in serum liver and kidney of diabetic rats. But, after the administration of EESAF, significantly increased levels of SOD and CAT were noticed in diabetic rats compared to diabetic control rats and this action supports the protective effect of EESAF on above organs against free radical mediated damage.
Reduced glutathione is an important biomolecule responsible for the elimination of reactive intermediates by reduction of hydroperoxide in the presence of glutathione peroxidase. GSH also functions as a free radical scavenger and helps to repair free radical mediated biological damage [69]. Glutathione peroxidase, catalyzed the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide and the reduction products of the hydroperoxide [70]. The decreased levels of GSH and GPx in the serum, liver and kidney of diabetic rats clearly demonstrate the defective function of GSH and GPx in diabetic rats than normal control rats. But, increased level of GSH and GPX were noticed in diabetic rats of above tissues after the administration of EESAF compared to diabetic control rats which shows the free radical scavenging ability of EESAF in diabetic condition.

CONCLUSION

Thus the long term use of ethanol extract of *Saraca asoca* flower at a dose of 600 mg/Kg body weight effectively controls blood glucose and restoration of diabetes associated changes as supported by our findings in this study. This effect may be due to the presence of tannin, saponin, flavonoids and other constituents presence in the flower, which could act synergistically or independently in enhancing the antidiabetic activity.

CONFLICTS OF INTEREST

The authors do not have any potential conflicts of interest.

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