



Anti-hyperglycemic and hypolipidemic effects of *Saraca asoca* (Roxb.) Wild. flowers in alloxan-treated diabetic rats

[Efectos antihiper glucémicos e hipolipidémicos de flores de *Saraca asoca* (Roxb.) Wild. en ratas diabéticas tratadas con aloxano]

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Abstract

Context: *Saraca asoca* (Leguminosae) has been widely used in the Ayurvedic system of medicine for various ailments, and it has been used to treat diabetes as a folk medicine.

Aims: To investigate the anti-hyperglycemic and anti-hyperlipidemic effect of ethanolic extracts of *S. asoca* (EESA) flowers in alloxan-induced diabetic rats.

Methods: The anti-hyperglycemic activity of EESA was evaluated by using normal and alloxan-induced (120 mg/kg, i.p.) diabetic rats. In the sub-chronic animal model of diabetes mellitus, EESA was orally administered to normal and alloxan-induced-diabetic rats at doses of 200 and 400 mg/kg p.o. per day for 28 days.

Results: Fasting blood glucose (FBG), insulin, glycated hemoglobin (HbA1c) levels, lipid profiles, alkaline phosphatase (ALP), and body weights were monitored at the end of 28 days in the EESA treated diabetic rats. The anti-hyperglycemic effect of EESA was more pronounced at the doses of 200 and 400 mg/kg in alloxan-treated diabetic rats as compared with vehicle-treated rats. EESA also showed a significant ($p < 0.05$) increase in serum insulin levels and body weights, while there was a significant reduction in the levels of ALP, HbA1c, serum triglyceride and total cholesterol. EESA also showed a significant anti-hyperlipidemic effect, as evidenced by the increased HDL-c level of alloxan-induced diabetic rats.

Conclusions: The results of the current investigation indicate that EESA possesses a significant anti-hyperglycemic effect and anti-hyperlipidemic effect.

Keywords: alloxan; anti-hyperglycemic; diabetes mellitus; hypolipidemic; Oral glucose tolerance test; *Saraca asoca*.

Resumen

Contexto: *Saraca asoca* (Leguminosae) se ha utilizado ampliamente en el sistema de medicina ayurvédica para diversas dolencias y para tratar la diabetes como medicina popular.

Objetivos: Investigar el efecto antihiper glucémico y antihiperlipidémico de extractos etanólicos de flores de *S. asoca* (EESA) en ratas diabéticas inducidas por aloxano.

Métodos: Se evaluó la actividad antihiper glucémica de EESA utilizando ratas diabéticas normales e inducidas por aloxano (120 mg/kg, i.p.). En el modelo animal subcrónico de diabetes mellitus se administró EESA por vía oral a ratas normales y con diabetes inducida por aloxano en dosis de 200 y 400 mg/kg p.o. por día durante 28 días.

Resultados: La glucosa en sangre en ayunas (FBG), la insulina, los niveles de hemoglobina glucosilada (HbA1c), los perfiles de lípidos, la fosfatasa alcalina (ALP) y los pesos corporales se controlaron al final de los 28 días en las ratas diabéticas tratadas con EESA. El efecto antihiper glucémico de EESA fue más pronunciado a las dosis de 200 y 400 mg/kg en ratas diabéticas tratadas con aloxano en comparación con ratas tratadas con vehículo. EESA también mostró un aumento significativo ($p < 0,05$) en los niveles de insulina sérica y el peso corporal, mientras que hubo una reducción significativa en los niveles de ALP, HbA1c, triglicéridos séricos y colesterol total. EESA también mostró un efecto antihiperlipidémico significativo, como lo demuestra el aumento del nivel de HDL-c de ratas diabéticas inducidas por aloxano.

Conclusiones: Los resultados de la investigación actual indican que EESA posee un efecto antihiper glucémico significativo y un efecto antihiperlipidémico.

Palabras Clave: aloxano; antihiper glucémico; diabetes mellitus; hipolipidémico; prueba tolerancia glucosa oral; *Saraca asoca*.

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Abbreviations: EESA: ethanolic extracts of *Saraca asoca*; OGTT: oral glucose tolerance test; ALP: alkaline phosphatase; HbA1c: glycated hemoglobin; TGs: triglycerides; TC: total cholesterol; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; VLDL- very low-density lipoprotein; i.p: intraperitoneal; FBG: fasting blood glucose; DM: diabetes mellitus; GLUT2: Glucose transporter 2.

INTRODUCTION

Diabetes mellitus (DM) affects nearly 4% of the population worldwide and is expected to increase by 5.4% in 2025 (Whiting et al., 2001). It is recognized as a clinical disorder that is diagnosed by hyperglycemia due to a deficiency of insulin secretion and function. Hyperglycemia has been associated with increased levels of triglycerides, free fatty acids, urea, creatinine, and total cholesterol (Ravi et al., 2004). Lipid alteration is another unique clinical feature of DM, manifested mainly by high serum triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL) and low high-density lipoprotein cholesterol (HDL) levels (Yang et al., 2006). In DM, an increased level of lipid parameters is generally stated that raises the cardiovascular risks and other associated complications (Grover et al., 2002). Currently, DM is managed and treated using a new class of therapeutic drugs. However, all the currently available drugs are not lacking side effects, and it triggers to a search of new antidiabetic drugs from natural products (Koehn and Carter, 2005). An estimate of about 80% of the diabetic people in the world's population presently depends upon herbal medicine for their successive treatments (Saxena and Vikram, 2004). Recently, several plants have been testified, which may possess antidiabetic potentials (Shukia et al., 2000). The use of modern oral hypoglycemic drugs has various side effects (Nathan et al., 2006). Traditionally medical plans and polyherbal formulations have been used effectively for the management of diabetes that is now being accepted worldwide (Thakur et al., 2010).

Saraca asoca (Roxb.) Wild. (family *Leguminosae*) has been widely used in the Ayurvedic (Traditional Indian) system of medicine, especially due to its wound healing property. *S. asoca* (Ashoka) is a highly valued endangered medicinal tree species from Western Ghats of India. There are extensive uses of this plant in Ayurvedic, Unani, and Siddha systems of alternative medicine. The ethnobotani-

cal information reports about 800 plants that may possess antidiabetic potential (Manisha et al., 2007). *S. asoca* is an important plant of the Indian system of medicine for its chemical constituents and its well-known pharmacological activities. *S. asoca* is an important medicinal tree that has been well known for its effectiveness in menorrhagia and dysmenorrhea (Swar et al., 2017). Besides treating cardiac and circulatory problems, *S. asoca* provides immense relief in gynecological disorders.

The medicinal property of *Saraca* is attributed to ketosteroids, flavonoids, and phenolic and aldehydes compounds present in leaves, bark and stem. Phytochemical studies of *S. asoca* have shown the presence of major compounds such as (+)-catechin, (-)-epicatechin, procyanidin B-2, 11'-deoxyprocyanidin B4 and leucocyanidin. It is also known to contain tannin, essential oil, catechol, hematoxylin, phenolic glycosides, saponins, and a fair amount of gallic acid and (-)-epicatechin. These constituents are believed to impart the plant its characteristic medicinal property. It has also been reported to possess antibacterial, anticancer, antioxidant, antidepressant, analgesic and antipyretic (Ghosh et al., 1999; Pandey et al., 2011; Ahmad et al., 2016). Ayurvedic literature has reported the use of the *S. asoca* for the management of diabetes mellitus. Considering the variety of uses and therapeutic applications and limited information on the anti-hyperglycemic effect of *S. asoca*, we have undertaken this study to investigate the antidiabetic activities of the ethanolic extract of *S. asoca* (EESA) flowers.

MATERIAL AND METHODS

Chemicals

Alloxan and glibenclamide were purchased from Sigma-Aldrich (St Louis, MO, USA). Electronic glucose meter and Accu-Chek active glucose strips were purchased from Roche Diagnostics (GmbH, Germany). The serum biochemical esti-

mation kits were purchased from Span Diagnostic Ltd (Span Divergent Ltd, India). The rest of the analytical grade reagents and solvents were purchased from SD Fine-Chem Ltd (SDFCL, Mumbai, India).

Plant materials

The *S. asoca* flowers were collected in the month of March 2018 from the village of Kaaripatti, Salem district, Tamilnadu (11.6645° N, 78.2828° E). The flowers of *S. asoca* was identified and authenticated as *S. asoca* (Roxb.) Wild. (BSI/SRC/5/23/2018-12/Tech-1979). The plant was identified and authenticated by the Department of Botany Research Office (Botanist), Botanical Survey of India (BSI), Agricultural University, Tamilnadu, Coimbatore. An authentic voucher specimen (DP-PCOG/2018/MARCH-324SI-F) was deposited in the Herbarium of Division of Pharmacognosy, Department of Pharmacognosy, JKK Munirajah Medical Research Foundation, College of Pharmacy, Tamil Nadu, India.

Extract preparation

The freshly collected flowers of *S. asoca* (200 g) were air dried under shade at room temperature (25°C), powdered crude flower was extracted with 70% ethanol (500 mL) in a Soxhlet extractor. The extraction was continued until the color of the final drop of the extract became colorless. Then, ethanol was removed from the extract using a rotary vacuum evaporator (Superfit, India) at 55 - 60°C under reduced pressure. The resulting dry alcoholic extract has a percentage yield of 9.26% (*w/w*). The extract was kept in a refrigerator until used for the experiment. The residue was lyophilized and stored at -70°C. The presence of preliminary phytochemicals in the ethanolic extract of *Saraca* was performed using the standard methods (Debiyi and Sofowora, 1978).

Animals

Male and female Wistar albino rats weighing about 180 - 220 g b.wt were used in the current investigation. Animals were collected from the breeding colony and acclimatized to the laboratory condition for two weeks. They were housed in

macrolon cages under standard laboratory conditions (12 h light and 12 h dark cycle, 21 ± 2°C, and relative humidity 55 - 70%). The animals were fed with a commercial diet from Hindustan Lever Ltd. (Bangalore, India) and free access to water (*ad libitum*) during the experiments. The animal experiments were performed in accordance with the "CPCSEA guidelines for Laboratory Animal Facility" (Committee for the Purpose of Control and Supervision on Experiments on Animals). This animal study was further approved by the Institutional Animal Ethical Committee (IAEC) of the JKK Munirajah Medical Research Foundation, College of Pharmacy, India (JKKMMRFCP/1158/PO/ac/07CPCSEA).

Acute oral toxicity study by toxic class method (OECD-423)

The oral acute toxicity investigation was approved to be conducted in accordance with OECD guideline 423 and IAEC, which instruct the use of only three male and female rats (OECD, 2000; Jonsson et al., 2013). Three of the test rats were fasted overnight (~12 h) and weighed. Test doses of EESA were administered 2000 mg/kg orally in relation to the body weight of every fasted rat. The animals were continuously and separately monitored for general behavioral changes and other toxicity signs after dosing for the first 24 h, with particular attention being given during the first 4 h. Thereafter, monitoring was sustained daily for 14 days of the toxicity experiment (Nana et al., 2011).

Oral glucose tolerance test (OGTT)

OGTT for rats was performed according to the standard method (Bonner-Wier, 1988). Group I served as normal control, and the oral 2 h glucose tolerance test was carried out via estimating glucose of blood sample from the tail vein by using a glucometer (Accu-chek active, Roche Diagnostics, Mannheim, Germany) at 0, 30, 60, 90 and 120 min. An oral glucose load of 2 g/kg b.wt was administered for the test. Group II rats were glucose load control, and similar OGTT was conducted on these rats. Group III and IV rats were treated by EESA at the doses of 200 and 400 mg/kg body weight

(b.wt), followed by OGTT. Group IV rats were administered by glibenclamide orally at a dose of 10 mg/kg b.wt, followed by OGTT.

Induction of diabetes

Diabetes was induced in rats that had been fasted for 12 h by the intraperitoneal (i.p) administration of alloxan (120 mg/kg body weight), freshly dissolved in sterile normal saline, after anesthesia with ethyl ether (King, 2012). After alloxan administration, animals were fed up with a 5% glucose solution in order to prevent hypoglycemic shock for 18 h. The diabetic state was assessed by measuring FBG concentration 72 h after alloxan treatment. The rats with serum glucose above 300 mg/dL, as well as with polydipsia, polyuria, and polyphagia, were selected for the experiment. The oral treatments (by gavage) of all groups were carried out at the same time (in the morning) and under the same conditions. Blood samples were drawn at weekly intervals until the end of the study (i.e., 4 weeks). FBG weights were measured on days 1, 7, 14, 21 and 28 days of the study.

Experimental design

Male Wistar albino rats were divided into five groups, each group containing six animals and treated for 28 days as Group I: Normal control rats fed with vehicles only; Group II: Diabetic control rats treated with alloxan monohydrate 120 mg/kg (i.p) body weight, dissolved in normal saline; Group III: Diabetic rats treated with EESA 200 mg/kg (p.o), dissolved in CMC; Group IV: Diabetic rats treated with EESA 400 mg/kg (p.o), dissolved in CMC; Group V: Diabetic rats treated with the standard drug, glibenclamide 3 mg/kg (p.o), dissolved in CMC.

The doses used in this experiment were chosen according to previous studies for extracts of this species in which the maximum dose of 500 mg/kg b.wt was used (Sasmal et al., 2012; Gupta et al., 2014).

Estimation of biochemical parameters

FBG levels were estimated by glucose oxidase-peroxidase reactive strips (Accu-chek active,

Roche Diagnostics, Germany). On day 28, blood was collected by cardiac puncture under mild ether anesthesia from overnight fasted rats. The serum was further used for the estimation of biochemical parameters such as ALP, HbA1c, TC, TGs, and HDL cholesterol using Span Diagnostic Kit (Vital Diagnostics Pvt Ltd, India). LDL cholesterol and very low-density lipoprotein (VLDL) cholesterol were estimated by standard formula (Friedewald et al., 1972).

Statistical analysis

All the values of body weight, fasting blood glucose level, and estimation of biochemical parameters were expressed as mean \pm standard error of the mean (S.E.M) and was analyzed for significance by one way ANOVA, and groups were compared by Dunnett's multiple comparison tests using GraphPad Prism (Version 7.0) and the p values are considered significant when $p < 0.05$ or $p < 0.01$.

RESULTS

Effect on acute oral toxicity

A phytochemical test was performed according to the standard methods with EESA flowers, and the result manifested the presence of flavonoids, polyphenolic compounds, phytosterols, saponins, carbohydrates, tannins, amino acids and proteins. There was no lethality or any toxic reactions found at any of the doses selected until the end of the study period. No sign of toxicity was observed in the wellness parameters during the 14-day observation period. A similar observation was made in the second set of female rats treated with 2000 mg/kg of the extract. Acute toxicity studies revealed the non-toxic nature of the EESA. According to OECD guidelines for acute oral toxicity, an LD₅₀ dose of 2000 mg/kg and above is characterized as unclassified, and hence the drug is found to be safe in experimental rats.

Effect on oral glucose tolerance test (OGTT)

Fig. 1 illustrates the anti-hyperglycemic effects of single oral administration of the extracts at 200

and 400 mg/kg on OGTT of normal rats. The EESA at the dose of 400 mg/kg produced a maximum fall of fasting blood glucose at 60 min after a glucose load. The standard drug prevented the drastic elevation of blood glucose 1 h after the glucose administration and decreased the blood glucose level even below the normal values after the glucose loading. The fasting blood glucose levels of the normoglycemic rats reached a peak at 1 h after the oral administration of glucose load and progressively reduced to the normal level. Effect of four-week daily oral dose treatment of EESA and glibenclamide on serum fasting blood glucose results in alloxan-induced diabetic male rats are graphically illustrated in Fig. 2.

Effect on fasting blood glucose (FBG)

The EESA treatment of alloxan-treated diabetic rats showed a dose-dependent decrease in fasting blood glucose levels. The serum fasting glucose level of the EESA administered groups were significantly on 28th days ($p < 0.01$) when compared to the diabetic control group. The sharp decrease in fasting blood glucose levels after EESA treatment showed in a time-dependent manner across the study. The EESA was administered orally to alloxan-induced diabetic rats for 28 days, and it reversed the fasting blood glucose levels to near normal. Administration of EESA to alloxan-treated diabetic rats showed a significant decrease in the fasting blood glucose and an increase in the serum insulin levels.

Effect on glycosylated hemoglobin (HbA1c)

Alloxan-treated diabetic rats exhibited a significant decrease in body weight during 28 days of experiment when compared to the normal control rats. EESA administered at 200 and 400 mg/kg to alloxan-induced diabetic rats produced a significant rise in body weights when compared to alloxan-diabetic control rats at the end of 28 days (Fig. 3). Effect of four-week daily oral dose treatment of EESA and glibenclamide on blood HbA1c level of alloxan-induced diabetic male rats are graphically illustrated in Fig. 4. Alloxan significantly increased the level of HbA1c as compared to the corresponding normal healthy rats. Moreover, EESA and

glibenclamide significantly reduced the level of HbA1c as compared to diabetic rats. Continuous administration of glibenclamide and EESA considerably elevated serum insulin levels as compared to the alloxan-treated diabetic rats (Fig. 5).

Effect on liver enzymes and lipid levels

The liver enzyme (ALP) in blood serum was assessed for an increasing level in the liver because this enzyme level is elevated due to hepatic cell dysfunction and leakage in liver cells membrane. The elevated liver enzymes levels were significantly reduced in the treatment of diabetic rats with EESA extract to a near normal state comparable to that of normal and alloxan-treated diabetic rats (Fig. 6). The elevated levels of serum triglycerides (TG), low-density lipoprotein (LDLc), total cholesterol (TC), and very low-density lipoprotein (VLDLc) with a simultaneous reduction in high-density lipoprotein (HDLc) in alloxan-induced diabetic rats was significantly reversed by the EESA in a dose-dependent manner (Fig. 7). These lipid levels were compared significantly with the vehicle control and glibenclamide treated groups. Treatment with EESA at both the doses produced a significant reversal of all these biochemical changes towards the normal. However, the standard drug glibenclamide showed better activity than EESA in all the above biochemical parameters.

DISCUSSION

Diabetes mellitus, a pervasive and multifactorial metabolic syndrome, is characterized by defects in insulin secretion and insulin receptor (Marin-Penalver et al., 2016). Management of diabetes without any major side effects remains a challenge. Medicinal herbs are significant sources of therapeutic drugs as evidenced by the discovery of metformin from natural sources. Despite the great interest in the development of new drugs to prevent the burden of diabetes mellitus and its complications and the raised interest in the research community to evaluate natural products in experimental animal models. Over several years, researchers were fascinated to find out the effect of natural compounds, mainly polyphenolic com-

pounds for their excellent biological activity as an antioxidant agent against diabetes mellitus (Qader et al., 2011). Phenolic compounds are an important class of phytoconstituents with considerable antioxidant properties, which play a potential role in

managing diabetes mellitus. These compounds were shown to significantly regulate various insulin and diabetes-associated pathologies in diabetics (Ahangarpour et al., 2019).

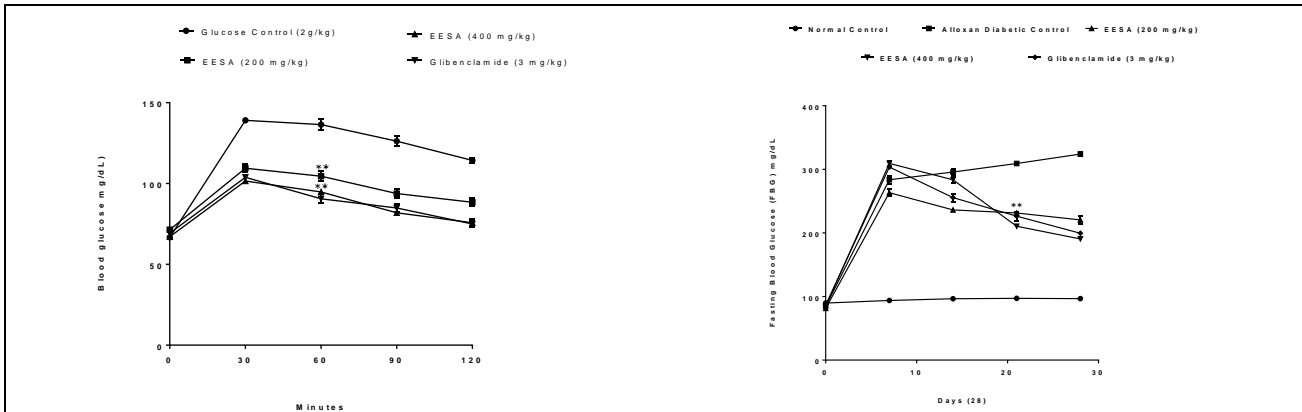


Figure 1. Effect of ethanolic extracts of *S. asoca* (EESA) on oral glucose tolerance test of normal healthy rats.

Values are expressed as mean ± S.E.M for five groups of six animals each. EESA (200 and 400 mg/kg b.wt) treated diabetic groups (III and IV) shown statistically significant differences ($p < 0.01$) when these were compared with the diabetic control group.

Figure 2. Effect of ethanolic extracts of *S. asoca* (EESA) on FBG levels of alloxan-treated experimental rats.

Values are expressed as mean ± S.E.M for five groups of six animals each. EESA (200 and 400 mg/kg b.wt) treated diabetic groups (III and IV) shown statistically significant differences ($p < 0.01$) when these were compared with the diabetic control group.

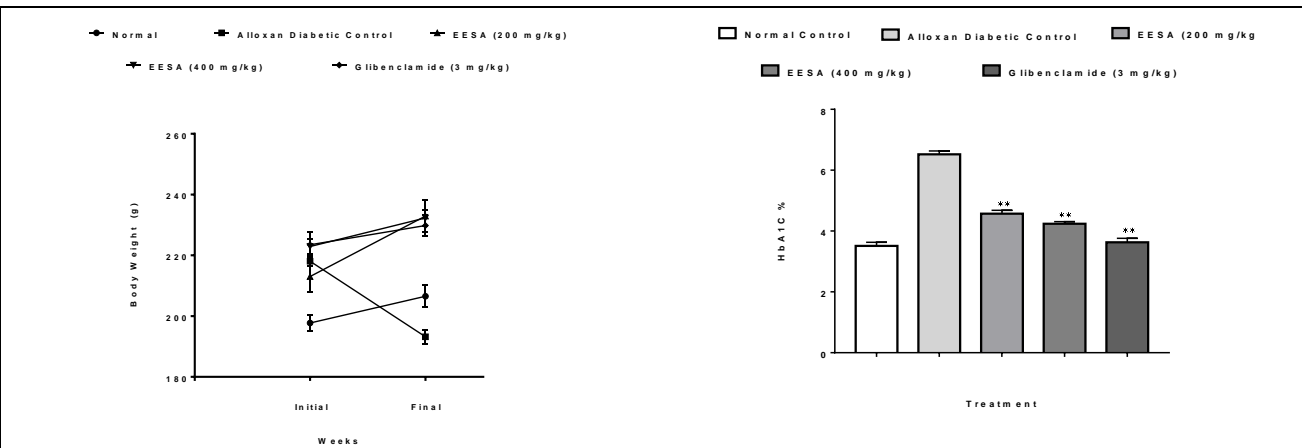


Figure 3. Effect of ethanolic extracts of *S. asoca* (EESA) on body weights of alloxan-treated experimental rats.

Values are expressed as mean ± S.E.M for five groups of six animals each. EESA (200 and 400 mg/kg b.wt) treated diabetic groups (III and IV) shown statistically significant differences ($p < 0.01$) when these were compared with the diabetic control group.

Figure 4. Effect of ethanolic extracts of *S. asoca* (EESA) on serum HbA1c levels of alloxan-treated experimental rats.

Values are expressed as mean ± S.E.M for five groups (n=6). EESA (200 and 400 mg/kg b.wt) treated diabetic groups (III and IV) shown statistically significant differences ($p < 0.01$) when these were compared with the diabetic control group.

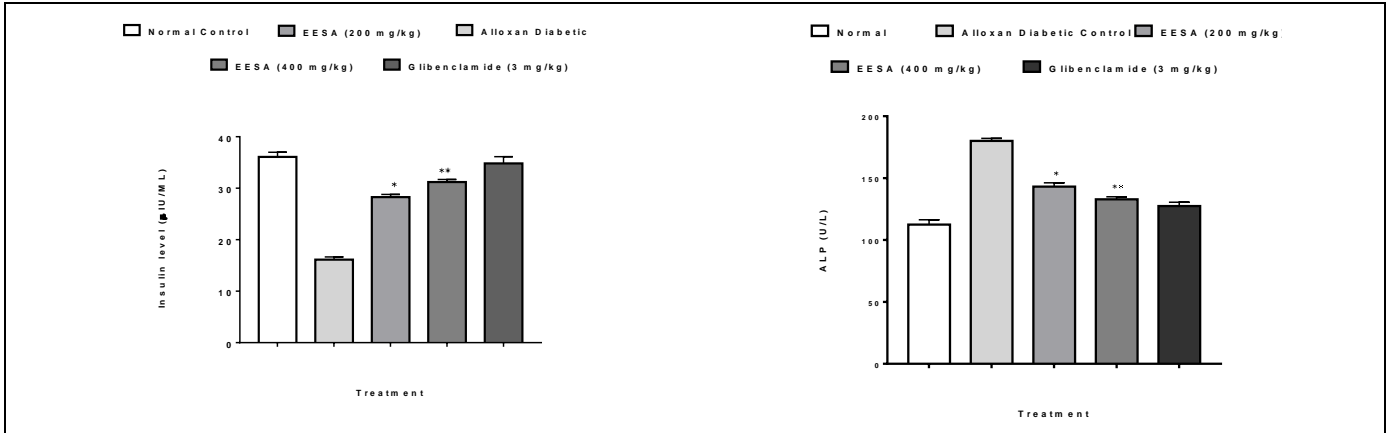


Figure 5. Effect of ethanolic extracts of *S. asoca* (EESA) on plasma insulin levels of alloxan-treated experimental rats. Values are expressed as mean ± S.E.M for five groups of six animals each.

Values are expressed as mean ± S.E.M for five groups (n=6). EESA (200 and 400 mg/kg b.wt) treated diabetic groups (III and IV) shown statistically significant differences (**p<0.01 and *p<0.05) when these were compared with the diabetic control group.

Figure 6. Effect of ethanolic extracts of *S. asoca* (EESA) on serum alkaline phosphatase levels of alloxan-treated experimental rats.

Values are expressed as mean ± S.E.M for five groups (n=6). EESA (200 and 400 mg/kg b.wt) treated diabetic groups (III and IV) shown statistically significant differences (**p<0.01 and *p<0.05) when these were compared with the diabetic control group.

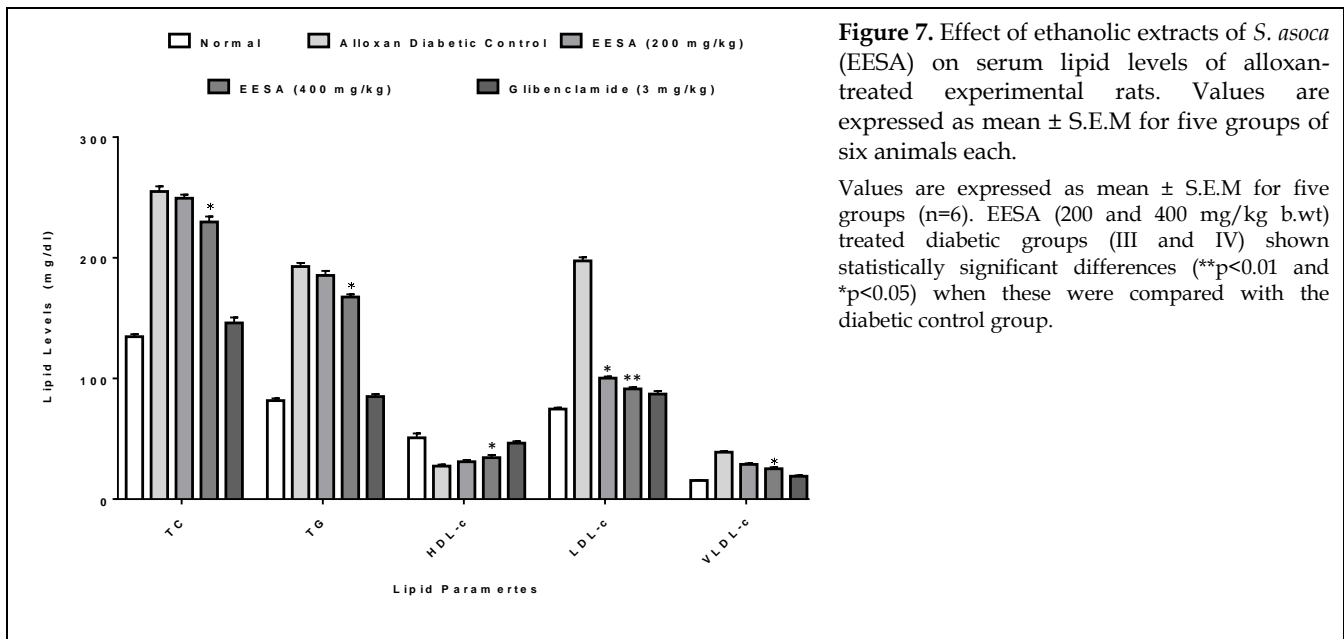


Figure 7. Effect of ethanolic extracts of *S. asoca* (EESA) on serum lipid levels of alloxan-treated experimental rats. Values are expressed as mean ± S.E.M for five groups of six animals each.

Values are expressed as mean ± S.E.M for five groups (n=6). EESA (200 and 400 mg/kg b.wt) treated diabetic groups (III and IV) shown statistically significant differences (**p<0.01 and *p<0.05) when these were compared with the diabetic control group.

Alloxan is most commonly used for the induction of diabetes mellitus apart from other cytotoxic chemicals, and it has a pancreatic beta-cell destructive action. It may cause a remarkable decrease in insulin secretion by the destruction of β-cells, thereby inducing hyperglycemia (Lenzen, 2008). The targeting of mitochondrial DNA, thereby impairing the signaling function of β-cell mitochondria

also explains how alloxan is able to inhibit glucose-induced insulin secretion. The induction of hyperglycemia by alloxan not only alters the glucose metabolism and insulin function, but diabetic animals also increased the various lipids, which causes metabolic abnormalities (Ceriello, 2000).

Oral glucose tolerance test designated that EESA considerably enhanced the glucose tolerance in alloxan-treated diabetic treated rats. The results of elevated fasting blood glucose from hyperglycemic rats induced by alloxan are established in this study. The mechanism behind this elevation is explained to be through the inhibition of insulin secretion and damage to β -cells. Administration of EESA or metformin significantly reduced elevated blood glucose levels, possibly through increased release of insulin from existing survival and/or regenerated pancreatic β -cells (Im Walde et al., 2002). The oral treatment of EESA recovers normal body weight as paralleled to alloxan-induced diabetic rats, which shows the protective action of EESA on structural protein degradation. Briefly, β -cells expressing the GLUT2 transporter are susceptible to alloxan-induced cytotoxicity and death through reactive oxygen species (ROS) due to the uptake of alloxan by this specific glucose transporter isoform. Further, hyperglycemia manifested to appear with the elevation of free radicals in the body by alloxan (Szkudelski, 2001).

The elevated fasting blood glucose level after alloxan treatment is due to insulin deficiency by beta cell destruction. Further, to understand the mechanism of action of the EESA, we have estimated the insulin levels. The decreased plasma insulin levels of alloxan-treated diabetic rats were significantly elevated after the treatment with EESA signifying potential β -cell regenerative and/or stimulatory (secretagogue) activity of the EESA. Treatment with EESA significantly decreased fasting blood glucose level in alloxan administered diabetic rats, which signify a reversal of beta-cell damage, as evidenced by improved insulin secretion after EESA treatment (Malviya et al., 2010). Eventually, after administering diabetic rats with EESA, the lowering in the fasting serum glucose levels have been established. Following the administration of EESA to diabetic rats, the decrease in weight loss was arrested.

HbA1c is the most useful parameter in diabetes mellitus (Rohlfing et al., 2000). It is formed through the non-enzymatic binding of circulating glucose to hemoglobin. Higher levels of glucose in the blood contribute to more binding and conse-

quently increased levels of glycosylated hemoglobin (Florkowski, 2013). Increased levels of HbA1c indicate that erythrocytes are more prone to oxidative stress in diabetes patients. High levels of HbA1c are found in diabetes patients, and this increase in HbA1c is directly proportional to fasting blood glucose levels (Karnchanasorn et al., 2016). Treatment of EESA to alloxan-induced diabetic rats for 28 days normalized the HbA1c levels. It may be due to improved glycemic control and enhanced insulin secretion after the treatment with EESA. Diabetes mellitus is frequently related with increased activities of the liver-marker enzymes, such as ALP, in serum, which might be due to the leak of these enzymes from the liver into the bloodstream (Eliza et al., 2009). Decreases in ALT and AST activity in treated diabetic rats suggested that EESA exhibits therapeutic effects in hepatic disorders, in addition to its insulin secretagogue and anti-hyperglycemic activities.

Hyperlipidemia was reported as common in adults with diabetes, and it is characterized most often by increased triglyceride and reduced HDL-c levels (Nesto, 2005). The increased lipid levels have a major role in causing complications associated with diabetes mellitus, in leading micro and macrovascular disorders (Ranganathan et al., 2001). In our experiment, the LDL-c level was increased, and HDL-c level was reduced in alloxan-induced diabetic rats, which produced hyperlipidemia and diabetic associated cardiovascular complications. This is generally observed in both type 1 and type 2 diabetes, representing the defect of insulin action in each, either due to inadequate secretion or resistance. Our results establish that triglyceride, cholesterol, LDL-c, and VLDL-c had a significant reduction in alloxan-induced diabetic rats treated with EESA. The increase in HDL-c level is related to a decrease in coronary problems. EESA produced significant beneficial effects in the lipid profile in diabetic rats by restoring towards normalcy of altered lipid levels. Thus, it may improve lipid metabolism in diabetes mellitus and could prevent diabetic vascular complications.

The fruit of *Saraca* contains chemicals called anthocyanin that boost insulin, which helps control blood sugar levels (Sadhu et al., 2007; Dias et al.,

2017). Several medicinal plants have gained importance for the treatment of diabetes mellitus, many remain to be scientifically investigated (Cibin et al., 2010; Preethi et al. 2010). Preliminary phytochemical analysis of EESA revealed the presence of polyphenolic and flavonoids as a major compound in *S. asoca*. These findings showed that EESA acts mainly as anti-hyperglycemic by stimulating the secretion of insulin, consequent with increased insulin secretion and reduction of fasting blood glucose and HbA1c values. Further, the results of the current investigation reveal that oral treatment of EESA significantly lowers elevated lipid levels in alloxan-induced diabetic rats. This may be facilitated by the presence of polyphenolic and flavonoids in EESA. Further investigations are ongoing to establish the active phytoconstituents of anti-hyperglycemic effect and characterize the molecular pathways of how these phytochemicals elicit their pharmacological action in the treatment of diabetes mellitus.

CONCLUSIONS

Oral treatment with EESA at a dose of 2000 mg/kg did not produce any mortality or significant changes in behaviors for 14 days, indicating that the EESA is not toxic under the observable condition in the experimental rats. The results of the current investigation show that the ethanolic flower extract of *S. asoca* established significant anti-hyperglycemic and anti-hyperlipidemic actions, as shown in its ability to reduce blood glucose level and reversal of elevated lipid levels of alloxan-treated diabetic rats. It was confirmed from this study that the ethanolic flower extract of *S. asoca* could decrease the blood glucose and elevated lipid levels on alloxan-treated diabetic rats in dose and time-dependent manners. It may be concluded that the ethnic-traditional claim of *S. asoca* as an anti-hypoglycemic agent is justified in the experimental diabetic rats. However, additional molecular and chemical studies are necessary to establish the exact phytoconstituents and the mechanism responsible for the anti-hyperglycemic action.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTION:

Contribution	Thilagam E	Chidambaram K	Raviteja C	Vahana T	Vasudevan P
Concepts or ideas	x	x			
Design	x	x			
Definition of intellectual content	x	x			
Literature search	x		x	x	x
Experimental studies	x	x	x	x	x
Data acquisition	x	x	x	x	x
Data analysis	x	x			
Statistical analysis	x	x			
Manuscript preparation			x	x	x
Manuscript editing	x	x			
Manuscript review	x	x	x	x	x

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