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Research Article

HYPOLIPIDEMIC, HYPOGLYCEMIC AND ANTIOXIDANT POTENTIAL OF *SARACA ASOCA* ETHANOLIC LEAVES EXTRACT IN STREPTOZOTOCIN INDUCED- EXPERIMENTAL DIABETES

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ABSTRACT

Objective: To investigate the hypolipidemic, hypoglycemic and antioxidant potential of *Saraca asoca* ethanolic leaves extract in streptozotocin induced- experimental diabetes.

Method: Experimental hyperglycemia was produced in rats by single dose of Streptozotocin (55 mg/kg). Assessment of blood glucose, TBARS and GSH was used as marker of hyperglycemia and oxidative stress respectively. Serum lipid profile (total cholesterol, HDL and triglyceride) were used as markers of dyslipidaemia.

Result: Treatment with *Saraca asoca* ethanolic extract normalizes the altered lipid profile and reduced the elevated glucose level in dose dependent manner. Further it attenuates the diabetes-induced renal oxidative stress.

Conclusion: Concurrent administrations of *Saraca asoca* ethanolic extract reducing the lipid alteration, decreasing the renal oxidative stress and certainly provide hypolipidemic, hypoglycemic and antioxidant effect.

Keywords: *Saraca asoca*, Hyperglycemia, Diabetes, Antioxidant, Hypolipidemia

INTRODUCTION

Diabetes mellitus (DM) is a metabolic syndrome characterized by hyperglycemia, polyuria, polydipsia and weight loss in spite of polyphagia, glycosuria, ketosis and acidosis [1] classified as insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM). Persistent hyperglycemia results in microvascular and macrovascular complications including retinopathy, neuropathy, cardiomyopathy, coronary artery disease, peripheral vascular disease, cerebrovascular disease and nephropathy [2]. The International Diabetes Federation recently reported that the number of people with diabetes will escalate from 246 million at present to 380 million by 2025[3].

Saraca asoca (Roxb.) De Wilde, sacred tree belonging to family caesalpinaceae, found almost throughout India, except North-Western India, upto 750m also found in the Andaman Islands used for various ailments like diabetes, emollient, stomachic, blood disease, ulcers, menorrhagia, dyspepsia, inflammation, dysentery, hemorrhoids etc. Leaves extracts has been reported for antidepressant, analgesic, antipyretic, anti-inflammatory, antibacterial, antihelminthic, antimicrobial and antidiabetic activity. In present scenario many researchers explored and identified various antidiabetic traditional medicinal plants but a large number of medicinal plants are yet to be investigated. *Saraca asoca* is enriched with carbohydrates, proteins, amino acids, flavanoids, tannin, saponin, steroids. So, the present study was designed to investigate hypolipidemic, hypoglycemic and antioxidant potential of *Saraca asoca* ethanolic leaves extract in streptozotocin induced-experimental diabetes [4].

MATERIALS & METHOD

Drugs and chemicals

Streptozotocin was purchased from Sigma-Aldrich, St. Louis, USA. Glibenclamide (standard drug) was gift samples from Wokhardt Pvt. Ltd. All other chemicals used in the present study were of analytical grade.

Selection and aclimization of animals

Age matched healthy Albino Wistar rats of either sex, weighing about 190-230g were used for the study. The experimental protocol MMCP/IEC/10/13 was approved by the Institutional Animal Ethical Committee which is certified by the Committee for the purpose of control and supervision of experiments on animals, India (CPCSEA); Reg. No-1355/AC/10/ CPCSEA. The animals were kept in clean and dry polycarbonate cages and were maintained under standard laboratory conditions (22 ± 3°C), with pellated food and tap water ad libitum during 8 weeks of experimental period. They were acclimatized in institutional animal house and were subjected to normal cycles of day and night.

Plant Material

The leaves of *Saraca asoca* was collected from Kurukshetra University, Haryana, India and authenticated by Dr. H.B. Singh; Director, Department of Raw Material Herbarium & Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India (Ref. NISCAIR/ RHMD/Consult/-2010-11/1652/250). The leaves were dried in shade at room temperature and the dried leaves were powdered by using grinder to coarse powder and stored at room temperature in an air tight container for further use.

Preparation of the Extract

Coarsely powdered leaves were packed into Soxhlet column and extracted with 90% ethanol till the plant material produced no coloration. The excess of solvent was removed by using rotatory evaporator. The obtained crude extract was stored in airtight container in below 10^0C for further studies. The extracts were dissolved in 0.5% Carboxy methyl cellulose (CMC) and used for study. The yield of extract was found to be 9.2%.

Phytochemical Screening

The extract was subjected to various chemicals tests to determine the active phytoconstituents like carbohydrates, proteins, amino acids, flavonoids, tannin, saponin and steroids may present in the ethanolic leaves extracts of *Saraca asoca* (Roxb.) De Wilde [5].

Acute Toxicity Study:

The ethanolic extract (400, 800, 2000 and 5000 mg/kg body weight) was administered to twenty albino rats divided into four groups of five rats each by oral gavage using a incubation cannula, and were observed for signs of toxicity and death once in first 30 min., after 24 hour and thereafter for a total of 14 days including changes are; body weights of rats before and after drug administration, onset of toxicity and sign of toxicity like change in

skin, fur, eyes, mucous membrane and also respiratory, circulatory, autonomic, central nervous system and somatomotor activity, behavior pattern, sign of tremors, convulsions, salvation, lethargy, sleep and coma [6].

Induction of experimental diabetes

Diabetes is induced by single dose of streptozotocin (55 mg/kg *i. p.*) dissolved in cold citrate buffer (pH 4.5) administration to overnight fasted animals. After 1 week of STZ administration animals having random serum glucose more than 240 mg/ dL were considered as diabetic.

Experimental Design

The animals were randomly divided into 6 groups of 6 animals each after the induction of diabetes.

Group A: Normal control rats given 0.5% CMC for 7 weeks.

Group B: Diabetic control rats given 0.5% CMC for 7 weeks.

Group C: Diabetic rats given Ethanolic leaf extract of *Saraca asoca*, 100 mg/kg body weight, orally, daily for 7 weeks.

Group D: Diabetic rats given Ethanolic leaf extract of *Saraca asoca*, 200 mg/kg body weight, orally, daily for 7 weeks.

Group E: Diabetic rats given Ethanolic leaf extract of *Saraca asoca,* 400 mg/kg body weight, orally, daily for 7 weeks.

Group F: Diabetic rats given glibenclamide 10 mg/kg body weight, orally, daily for 7 weeks.

Assessment of streptozotocin-induced diabetes and dyslipidaemia

The blood samples were collected and serum was separated. The serum samples were frozen until analyzing the biochemical parameters. The serum glucose concentration was estimated by glucose oxidase-peroxidase (GOD-POD) method [7] using commercially available kit (Erba diagnostic kit). The serum total cholesterol was estimated by cholesterol oxidase peroxidase (CHOD/PAP) method [8] using commercially available kit (Erba diagnostic kit). The serum triglyceride was estimated by Glycerol Phosphate Oxidase-Trinder method [9] using commercially available kit (Erba diagnostic kit). The serum high density lipoprotein (HDL) was estimated by phosphotungstic acid method [10] using commercially available kit (Erba diagnostic kit).

Assessment of intrarenal oxidative stress

At the end of 8th week, the animals were sacrificed by cervical dislocation and kidneys were dissected and washed with ice cold isotonic saline and weighed. The kidney tissue was then minced and homogenate (10% w/v) was prepared in chilled 1.15% potassium chloride for estimation of TBARS and GSH. TBARS, an index of lipid peroxidation was estimated according to the method of Ohkawa et al, 1979 [11], GSH in kidney was estimated using the method of Ellman, 1959 [12].

Statistical Analysis

All values were expressed as mean ± S.E.M. All data were statistically analyzed using one way ANOVA followed by Tukey's multiple comparison tests. The p value <0.05 was considered to be statistically significant.

RESULTS

Phytochemical screening revealed the presence of alkaloids, carbohydrates, phytosterols, phenols, tannin and flavonoids. Animal does not shown any signs of toxicity and mortality for a total of 14 days with ethanolic extract (400, 800, 2000 and 5000 mg/kg body weight) administration of plant extract so, the extract can be used for long term administration.

The significant increase in serum concentration of glucose, total cholesterol and triglycerides, and decrease in HDL level was noted in diabetic rats as compared to normal rats. Treatment with ethanolic extract of *Saraca asoca (*100, 200, 400 mg/kg *p.o.*, 7 weeks) and Glibenclaimide (10 mg/kg p.o., 7 weeks) improves glucose, cholesterol and triglycerides and HDL level significantly (Table 1). The marked increase in tissue TBARS concentration and decrease in reduced form of glutathione (GSH) were noted in kidney of diabetic rats as compared to normal rats. Treatment with ethanolic extract of *Saraca asoca* (100, 200, 400 mg/kg *p.o.*, 7 weeks) significantly prevented diabetes-induced increase in renal TBARS level and decrease in GSH level. Moreover, treatment with *Saraca asoca* at the dose of 200 mg/kg/day and 400 mg/kg/day markedly reduced diabetes-induced increase in renal TBARS concentration as compared to Glibenclaimide (10 mg/kg p.o., 7 weeks) (Table 2).

Table 1: Effect of *Saraca asoca* extract on Serum Glucose and lipid profile

Treatment	Blood Glucose Level (mg/dl)	Triglyceride Level (mg/dl)	HDL-cholesterol Level (mg/dl)	Total cholesterol Level (mg/dl)
Normal Control	109.81 ± 3.35	76.71 ± 2.67	41.55 ± 1.21	66.29 ± 3.28
Diabetic Control (Vehicle)	353.01 ± 3.52*	156.72 ± 2.73*	22.40 ± 0.99*	135.27 ± 2.56*
Streptozotocin + Ethanolic extract (100 mg/kg)	227.30 ± 3.96**	120.93 ± 3.55**	27.01 ± 0.79**	108.54 ± 3.07
Streptozotocin + Ethanolic extract (200 mg/kg)	157.10 ± 4.08**	99.10 ± 3.07**	32.18 ± 1.49**	96.37 ± 2.64
Streptozotocin + Ethanolic extract (400 mg/kg)	106.31 ± 4.11**	86.25 ± 3.56**	37.05 ± 1.40**	83.64 ± 3.23
Streptozotocin + glibenclamide (10 mg/kg)	136.07 ± 3.17**	83.9 ± 2.43**	36.26 ± 1.25**	93.01 ± 2.57

n=6 for all groups, Values are expressed as Mean ± S.E.M. Data was analyzed by one-way ANOVA followed by Tukey's Post-hoc test.

F (6, 35) = 720.30; p< 0.0001 (for blood glucose levels)

F (6, 35) = 68.705; p< 0.0001 (for cholesterol levels)

F (6, 35) = 65.664; p< 0.0001 (for triglyceride levels)

F (6, 35) = 43.408; p< 0.0001 (for HDL levels)

*(p<0.001) significant difference *vs* normal untreated rats.

**(p<0.001) significant difference *vs* diabetic control rats.

Table 2: Effect of *Saraca asoca* extract on renal TBARS

Treatment	TBARS Level (mg/dl)
Normal Control	2.1 ± 0.08
Diabetic Control (Vehicle)	4.13 ± 0.12*
Streptozotocin + Ethanolic extract (100 mg/kg)	3.84 ± 0.07
Streptozotocin + Ethanolic extract (200 mg/kg)	3.1 ± 0.07**
Streptozotocin + Ethanolic extract (400 mg/kg)	3.49 ± 0.09**
Streptozotocin + glibenclamide (10 mg/kg)	3.29 ± 0.1**

n=6 for all groups, Values are expressed as Mean ± S.E.M. Data was analyzed by one-way ANOVA followed by Tukey's Post-hoc test.

F (6, 35) = 49.701; p< 0.0001

*(p<0.001) significant difference *vs* normal untreated rats.

** (p<0.001) significant difference *vs* diabetic control rats.

Table 3: Effect of *Saraca asoca* extract on renal GSH

Treatment	GSH Level (mg/dl)
Normal Control	5.67 ± 0.12
Diabetic Control (Vehicle)	1.02 ± 0.08*
Streptozotocin + Ethanolic extract (100 mg/kg)	1.45 ± 0.05**
Streptozotocin + Ethanolic extract (200 mg/kg)	2.41 ± 0.08***
Streptozotocin + Ethanolic extract (400 mg/kg)	3.18 ± 0.06***
Streptozotocin + glibenclamide (10 mg/kg)	2.89 ± 0.09***

n=6 for all groups, Values are expressed as Mean ± S.E.M. Data was analyzed by one-way ANOVA followed by Tukey's Post-hoc test.

F (6, 35) = 340.86; p< 0.0001

* (p<0.001) significant difference *vs* normal untreated rats.

** (p<0.05) significant difference *vs* diabetic control rats.

***(p<0.001) significant difference *vs* diabetic control rats.

DISCUSSION

Diabetes mellitus is induced in rats by single dose of streptozocin (55 mg/kg *i.p.*) administration, which damages DNA of pancreatic-β cells by activating protein kinase-C, poly (ADP-ribose) polymerase (PARP) and NAD(P)H oxidase to generate ROS [13]. Estimation of blood glucose has been used as marker of hyperglycemia. Hyperglycemia induced-oxidative stress caused by free radical generation and decrease antioxidant defense system [14, 15] which, has been assessed to estimate the degree of oxidative stress. Dyslipidaemia is associated with elevated total cholesterol, triglycerides and low level of high density lipoprotein (HDL) [16]. Therefore, estimation of total cholesterol, triglycerides, and HDL has been used as the marker of dyslipidaemia. Treatment with *Saraca asoca* ethanolic extract normalizes the altered lipid profile and reduced the elevated glucose level in dose dependent manner. Further it attenuates the diabetes-induced renal oxidative stress, therefore, present study concludes that the concurrent administrations of *Saraca asoca* ethanolic extract reducing the lipid alteration, decreasing the renal oxidative stress and certainly provide hypolipidemic, hypoglycemic and antioxidant effect. The ameliorative effect of *Saraca asoca* ethanolic extract on hyperglycemia and lipid profile may be due its potent antioxidant activity which may be due to the presence of phytosterols, phenols, tannin and flavonoids which is consonant with previous study [17-18].

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