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EXPERIMENTAL EVALUATION OF ANTI-DIABETIC ACTIVITY AND ANTI HYPERLIPIDEMIC EVALUATION OF LEAF EXTRACTS OF *SENNA ALATA* IN ALLOXAN INDUCED DIABETIC RATS

Rama Swamy Nanna*, Archana Pamulaparthi, Vamshi Ramana Prathap[1], Mahitha Banala

*Plant Biotechnology Research Group, Department of Biotechnology, Kakatiya University, Warangal-506009, TS, India.

[1]Jawaharlal Nehru Technological University, Hyderabad- 500085, TS, India.

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***Correspondence for Author**
Rama Swamy Nanna
Plant Biotechnology Research Group, Department of Biotechnology, Kakatiya University, Warangal-506009, TS, India.

ABSTRACT

Senna alata (Syn. *Cassia alata*) is an ethnomedicinal plant belonging to the family Fabaceae. A number of bioactive constituents which contribute to the medicinal properties are attributed to the species. The present study is aimed to evaluate the anti diabetic activity attributed to the species and also to evaluate the anti hyperlipidemic potential of the species using various standard experimental models available. Acute toxicity studies of aqueous leaf extracts of *S. alata* were performed upto a dose of 2500mg/kg bodyweight of rats and a dose of 200 mg/kg was selected for the present study. Aqueous leaf extracts of *S. alata* showed a significant ($P<0.01$) anti diabetic and anti hyperlipedimic potential in alloxan induced diabetic rats within 15 days of induction of diabetes and the antidiabetic potential of the species may be due to the presence of flavonoids.

KEYWORDS: *Senna alata,* Alloxan induced diabetes, Anti Diabetic, Anti Hyperlipedemic.

INTRODUCTION

Diabetes mellitus is a heterogeneous group of metabolic disorders caused by the disturbance in the metabolism of carbohydrates, fats and proteins. It is characterized by classical symptoms such as weight loss, polyuria, polydipsia and polyphagia[I]. It is one of the major metabolic disorders and the only non infectious disease affecting about 10% of the world

population[II]. According to WHO more than 180 million people are suffering from diabetes globally and the number may double by the year 2030[III] and is one of the five leading causes of deaths worldwide [IV,V].

There are several causes of diabetes; some of them include unhealthy diet, sedentary lifestyle, urbanization, aging and obesity [VI]. If left untreated, diabetes mellitus may lead to complications like cardiovascular risks, Diabetic Retinopathy, Diabetic Ketosis, Renal failure and cataract formation[VII-X].

A number of animal models have been developed in the past few decades for studying diabetes mellitus and testing antidiabetic agents which include chemical, surgical and gene manipulation procedures [XI,XII]. Two most widely used models for induction of diabetes by chemical method are Streptozotocin induced diabetes and Alloxan induced diabetes. Alloxan is a diabetogenic agent that induce Type-I diabetes. It is a urea derivative that causes selective necrosis of β- cells of islets of langerhans in pancreas.

Currently available hyperglycemic drugs like sulphonylureas or other oral hyperglycemic drugs and Insulin administration have severe adverse effects like Insulin allergy, hyperglycemia when insulin is administered at higher doses, Insulin resistance, edema, lipoatrophy, lipohypertrophy, lactic acidosis, diahorrea, etc. So, there is a need for the development of safer agents with minimal side effects and which could be used for longer duration[XIII, XIV].

In the present investigation, we have attempted to evaluate theaqueous leaf extracts of *S. alata* on antidiabetic and antihyperlipedemic activities in alloxan induced rats.

The species *Senna alata* is an ethnomedicinal plant belonging to family Fabaceae. The plant is known to possess a wide range of medicinal properties [XV-XVII]. Anti diabetic potential of methanolic extracts of *S.alata* have been carried out through α-glucosidase inhibitory activity by Varghese et al. [XVIII]. So far no reports have been published on antidiabetic activity of aqueous leaf extracts of *S.alata* and the anti hyperlipedemic profile associated with diabetes on alloxan induced diabetes in rats. Hence, the present study is undertaken to screen the antidiabetic properties of *S.alata* aqueous leaf extracts and to study the antihyperlipidemic profile of the species.

MATERIALS AND METHODS

Preparation of the extracts

Mature leaves of *S.alata* were collected from the medicinal garden, Department of Biotechnology, Kakatiya University, Warangal, TS, India. The samples were authenticated by Prof. N. Rama Swamy, Department of Biotechnology, Kakatiya University, Warangal TS, India. The leaves were shade dried, powered using a mechanical blender. The obtained coarse powder was further sieved to obtain a fine powder. 2-3 gms of the leaf powder was macerated overnight using 100ml of distilled water. The extract was then filtered and evaporated to dryness at room temperature to obtain the final yield.

Animals

Wistar rats of either sex weighing 200-250 gms, 10-11 weeks of age were used for the study. The animals were housed and maintained at 22ºC under a 12 light/12 dark cycle with free access to the standard diet and water ad libitum. Efforts were made to minimize animal suffering and all the experiments were performed based on the guidelines of ethical standards for the investigation in animals. All the experiments were carried out following the approval of Institutional Animal ethical committee (IAEC) and the ethical norms were strictly followed for all the experimental procedures (Ref no. 25 CARE/IAEC-2013).

Drugs and Instruments

Alloxan Monohydrate (Sigma Aldrich), Sodium Citrate (Sigma Aldrich) and one touch Glucometer (Accu Check) were used in the present investigation.

Acute toxicity studies

Acute toxicity studies were conducted according to OECD guidelines (no. 423). In the present investigation, acute toxicity and gross behavioural studies were carried out in rats after administration of aqueous leaf extract of *S. alata*. The animals fasted for 4 hrs before the test. The mice received the test dose of aqueous extract in the form of suspension at a dose of 10mg/kg to 2500mg/kg orally (P.O.). The behavior (awareness, grooming, irritability, motor activity) and mortality of mice was observed continuously and carefully observed for 4 hours, followed occasionally for next 24 hours.

Oral glucose tolerance test

The animals were divided into 5 groups of 6 rats each. All animals were fasted overnight but had free access to water. Group I consisted of 6 rats did not receive any treatment and served

as normal control group. Group II rats served as vehicle group and were given oral treatment of distilled water. Group III rats received only glucose treatment. Group IV and V animals were given oral treatment of aqueous extracts of *S. alata* (200mg/Kg bodyweight). The rats of only group IV were loaded with glucose (3g/Kg P.O.) 30 min after the administration of test extract. Blood samples were collected from the retro orbital sinus using a capillary tube prior to the administration and 30, 90, 150 min. after the administration of glucose by subjecting the animal to mild ether anesthesia. Serum glucose level was measured using a one touch glucometer.

Testing for anti-diabetic activity

The animals were divided into 4 groups of 6 rats each. All animals were fasted overnight but had free access to water. Group I rats served as normal control group and were given oral treatment of distilled water. Group II rats were given alloxan treatment and served as Diabetic control. Group III rats received both alloxan and standard drug Glibenclamide (5mg/kg) and served as standard group. Group IV animals were given oral treatment of alloxan and aqueous leaf extracts of *S.alata* (200mg/Kg bodyweight).

Induction of diabetes

Rats were made diabetic by a single intraperitonial injection of Alloxan monohydrate (150mg/kg) [XIX]. Alloxan was dissolved in normal saline just prior to the injection. Rats with plasma glucose level >140mg/dl were selected and included in the study after alloxan injection.

Collection of blood sample and estimation of blood glucose level

Blood was collected from the tail vein of rats by pricking with a sterile lancelet on1st, 7th and 15th day of the study. Blood glucose estimation was done using one touch horizon glucometer. On 15th day of the study, blood was collected from overnight fasted animals from the retro orbital plexus under mild ether anesthesia and fasting glucose level was estimated [XX].

Estimation of blood glucose level

Serum was separated by centrifugation for the estimation of total cholesterol[XXI], triglycerides[XXII]and HDL and LDL cholesterol[XXIII].

Administration of standard drug

Glibenclamide is administered orally at a dose of 10mg/kg body weight of rats for 15 consecutive days.

Administration of test drug

Aqueous leaf extracts of *S.alata* were administered orally at a dose of 200 mg/Kg bodyweight of rats with the help of oral feeding tube for 15 consecutive days.

Data Analysis

The study was carried out for a period of 15 days. The blood glucose concentration was monitored on 0, 5, 10, 15 days. The statistical analysis was done following the method of Sinha & Pillai[XXIV].

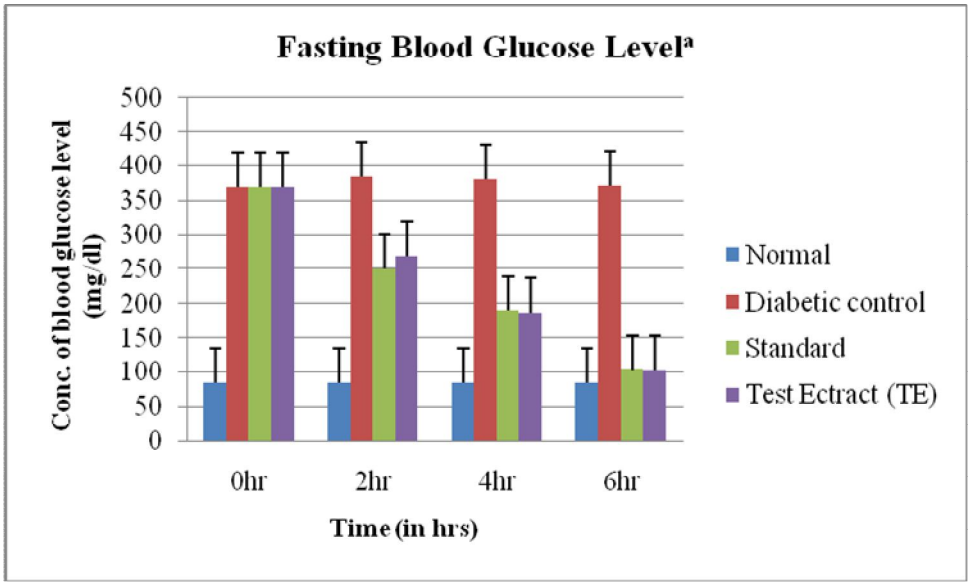
RESULTS

Acute toxicity studies

The aqueous leaf extracts of *S. alata* was found to be safer for the dose tested and there was no mortality upto a dose of 2000mg/kg body weight. Doses upto 2000mg/kg did not show any significant changes such as mortality, behavioral changes, salivation, tremors etc., thus it was conformed that there is no potential toxic activity.

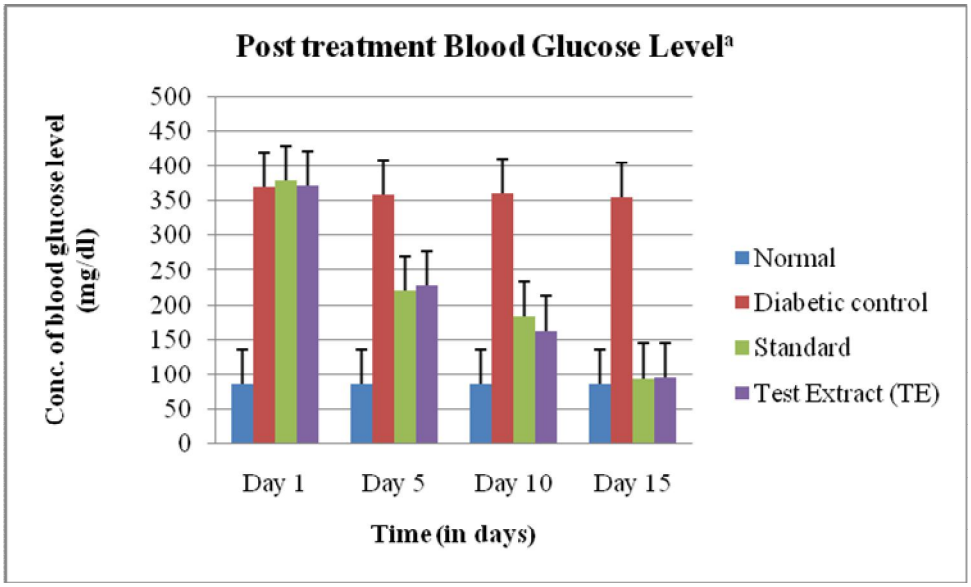
Anti diabetic activity

The effect of *S.alata* aqueous leaf extracts on the antidiabetic activity in alloxan induced diabetic rats is given in Figs 1-2. Effect of the extracts on both the fasting glucose levels and post treatment blood glucose levels was determined. The results showed a change in the level of fasting blood glucose levels in diabetic and experimental groups (standard and test groups) after the oral administration of glucose (3g/kg) (Fig 2). In the test group, where the animals were treated with test extract (200mg/kg) the blood glucose levels decreased considerably ($P<0.001$) after regular intervals of 2, 4 and 6 hrs (Fig. 1) which was comparable to that of the standard drug glibenclamide (10mg/kg).



[a]Mean±Standard Error

Fig. 1: Effect of *S. alata* leaf extract on fasting blood glucose levels in Alloxan induced diabetic rats



[a]Mean±Standard Error

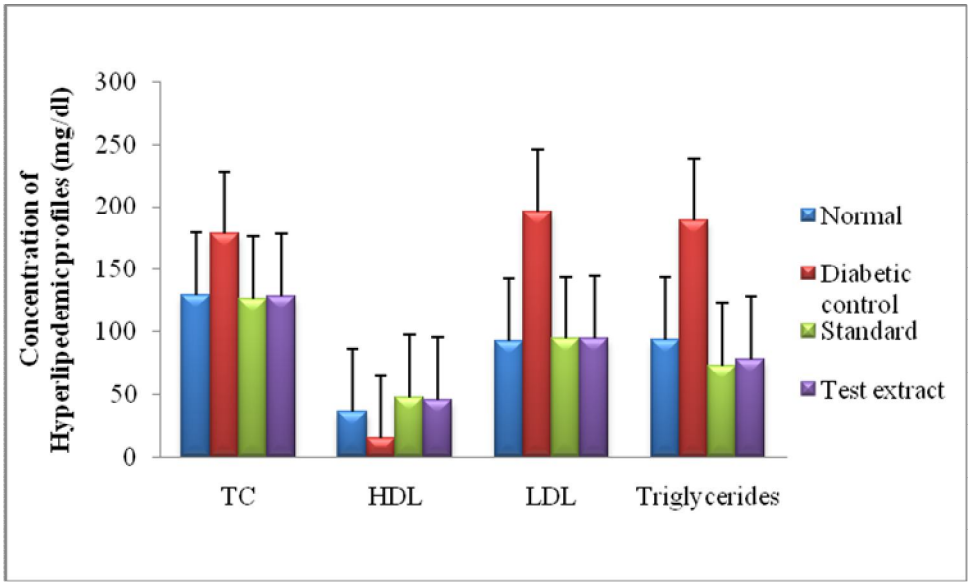
Fig. 2: Effect of *S.alata* leaf extract on post treatment blood glucose levels in Alloxan induced diabetic rats

Hyper lipedemic profile

The antihyperglycemic effect of leaf extracts of *S. alata* on post treated blood glucose levels in the diabetic rats was assessed on day 1st, 7th and 15th day. The results of the study are shown in Fig 2. The decrease in the blood glucose levels of the extract (200mg/kg) was comparable with that of the standard drug glibenclamide. The leaf extract showed a

considerable decrease in the blood glucose level (95.2±0.15mg/dl) which is very nearer to that of the standard drug glibenclamide (94.3±0.38) in comparison to the diabetic control group(355.6±0.11).

There was a decrease in the total cholesterol, HDL, LDL and triglyceride levels in the test group when compared with the diabetic control group (Fig. 3) on the 15th day in alloxan induced diabetic rats. The cholesterol content was totally reduced in aqueous extract (128±1.09) compared to diabetic control (178 ± 0.94). Triglyceride levels were found significantly higher (189±0.38) in diabetic control in comparison to the test (78±1.72) and standard (73±1.62) groups. Similar reduction was also observed in the serum LDL levels in both test (94.6±1.63) and standard groups (94.1±1.53) than the diabetic control group (196±0.77). It was also observed that the HDL levels were also balanced at the end of the 15th day in the test (46±1.87) and standard groups (48±1.13) when compared to the diabetic control group (25±0.83).



[a]Mean±Standard Error

Fig. 3: Effect of aqueous leaf extracts of *S. alata* on total cholesterol (TC), serum HDL, serum LDL and triglyceride levels in alloxan induced diabetic rats

DISCUSSION

Anti diabetic activity of aqueous leaf extracts of *S.alata* is determined by alloxan induced diabetes in rats. Treatment with *S.alata* aqueous leaf extracts (200mg/kg) continuously for 15 days showed a considerable decrease in the fasting blood glucose level, post treatment glucose levels. The extract may be effective either by increasing the glucose uptake or by

increasing the pancreatic secretions. Presence of bioconstituents like flavonoids attribute to the diabetic potential of the species[XXV]. Alloxan also induced Hypercholesterolemia and Hypertriglyceridemia in the animals. A significant decrease in cholesterol levels, HDL, LDL and triglyceride levels (Fig. 3). Therefore it is evident from our study that aqueous leaf extracts of *S. alata* (200mg/kg) may have stimulated glycogenesis or inhibited glycogenolysis in rats and reduced the complications associated with lipid profile as reported by Sharma *et al.* [XXVI] in ethanolic seed extracts of *Eugenia jambolana* in alloxan induced diabetic rats**.**

Hypercholesterolemia and Hypertriglyceridemia are other major complications reported in alloxan treated diabetic rats [XXVII, XXVIII]. Hypercholesterolemia is a metabolic complication in clinical and experimental diabetes[XXIX]25. It is a condition which arises due to a decrease in the concentration of Insulin which leads to increase in lipolysis. Increased lipolysis leads to a release of more free fatty acids into the blood leading to an increase in the concentration of Acetyl Co A and cholesterol in the blood stream[XXX]. The increase in the blood cholesterol leads to an decrease in the fluidity of the cell membrane, as the phospholipids, which are an integral part of the cell membrane are sensitive to the O^{2-} and OH^{*} free radicals generated by alloxan treatment[XXXI].

Hypertriglyceridemia occurs in individuals with type-I diabetes. It is also observed in people with Insulin resistance. Patients with diabetes or insulin resistance develop elevated triglycerides due to overproduction of VLDL and reduced triglyceride lipolysis. Increased production of fatty acids by adipose tissues results in the packing of them into triglycerides into VLDL, which are transported out of the liver. In patients with insulin resistance, lipoprotein lipase which is essential for breaking down triglycerides in the blood circulation is less effective or non functional[XXXII].

CONCLUSION

From the results presented above it can be concluded that aqueous leaf extracts of *S. alata* showed a gradual decrease in the diabetes from 5th day of administration of the test extract. The blood glucose levels returned to normal by the 15th day indicating that the aqueous leaf extracts possess a potent diabetic activity. The extracts also showed a reduction in the hyperlipidemic profile associated with diabetes. Thus, it can be suggested that the aqueous leaf extracts with optimal dose may be administered for human beings to cure type-I diabetes.

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