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Anti Diabetic Activity of Methanolic and Chloroform Extracts of *Solanum seaforthianum* In Alloxan Induced Diabetes Rats

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Abstract: Diabetes mellitus is a syndrome characterized by elevated glucose levels, polyurea, polydipsia and weakness due to insufficient entry of available glucose into the cells of the body. There are many allopathic drugs available to manage diabetes such as Sulfonylureas, biguanides, gliptins etc., but associated with hypoglycemia, sweating, dizziness, confusion, hunger, weight gain on long term usage and tolerance will be developed which are further treated using multiple drugs. According to our ancient system of medicine there are many medicinal plants available to treat diabetes with the same potency of allopathic drugs. Natural products are considered to be an effective alternative for the treatment and management of diabetes, because of higher efficacy which initiated the present study of anti diabetic activity of *Solanum seaforthianum* in an alloxan induced diabetes male albino rat. Diabetes was successfully induced with the help of alloxan at a dose of 120 mg/kg body weight and the animals which are possessing the glucose levels more than 250 mg/dL included in the study. Diabetic induced rats were treated with methanolic and chloroform extracts (200 and 400 mg/kg/kg body weight) of *Solanum seaforthianum* for 10 days. The collected blood samples on 1st, 4th, 7th, and 10th day were used to determine serum biochemical parameters such as cholesterol, triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL) cholesterol, urea, creatinine and total protein. Results: The treatment with extracts of *Solanum seaforthianum* significantly reduced the elevated blood glucose levels and reversed other altered parameters of Cholesterol, Triglycerides, HDL, LDL, Urea, Creatinine and Total Protein. Among the two extracts of *Solanum seaforthianum* chloroform extract has shown comparatively significant ($p<0.001$) anti diabetic activity in alloxan induced diabetes in albino rats.

Keywords: *Solanum seaforthianum*, Diabetes, Alloxan, Albino rat, chloroform extract, Methanolic extract

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1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic syndrome characterized by hyperglycemia, hypertriglyceridemia and hypercholesterolemia.[1] It is caused by inherited and/or acquired deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced.[2] Diabetes is crudely grouped into two types: insulin dependent diabetes mellitus (IDDM) and Non – insulin dependent diabetes mellitus (NIDDM), both these types are associated with excessive morbidity and mortality.[3] Reports from World Health Organization indicate that diabetes mellitus is one of the major killer of our time, with people in South East Asia and Western Pacific being most at risk.[4] India has today become the diabetic capital of the world with over 20 million diabetics and this number is set to increase to 57 million by 2025.[5] Diabetes mellitus is a multifactorial disease characterized by hyperglycemia[6], lipoprotein abnormalities[7], raised basal metabolic rate[8] defect in reactive oxygen species scavenging enzymes[9] and high oxidative stress induced damage to pancreatic beta cells.[10] Diabetes mellitus is ranked seventh among the leading causes of death and ranked third when it's fatal complications are taken into account.[11] There are many allopathic/ synthetic drugs are available to manage diabetes but all those were associated with side effects and on the other hand persistence of hyperglycemic conditions for a longer period of time resulting in serious complications and damages to the heart, blood vessels eyes, kidney and nerves, moreover, increases the risk of heart diseases and stroke[12] and long term usage of the same allopathic anti diabetic drugs develop tolerance and need to change the combinations periodically to avoid the development of tolerance. Plants are the major source of drugs and are available in the market as extracts directly or indirectly from the plant sources.[13] The plants were used as medicine for the purpose of preventive and curative reasons in various parts of the world. Medicinal herbs were used to treat diabetes in large proportion all over the globe because of the easy availability and affordability.[14] *Solanum seaforthianum* is commonly known as Brazilian nightshade belonging to the family Solanaceae belongs to a single genus, Solanum. There is however, no report on the antidiabetic activity of *Solanum seaforthianum* in the literature. Yet, this plant is known for its possession of various medicinal alkaloids and flavonoids[15]. This *Solanum seaforthianum* has been reported to possess antibacterial activity.[16] Hence, this study was aimed at investigating the anti-diabetic activity of leaves of *Solanum seaforthianum* by albino rats.

2. MATERIALS & METHODS

2.1 Plant material

The fresh leaves of *Solanum seaforthianum* belonging to family Solanaceae were obtained from the forests of Tamilnadu in the Kancheepuram region and the sample specimen was authenticated by Dr. Madhav Chetty, Department of Botany & Sri Venkateswara University, Tirupathi, India. The sample was kept in the University herbarium with voucher number 1256. The leaves of *Solanum seaforthianum* were collected and washed thoroughly, surface water was removed by air drying and leaves were dried under shade drying for 3 to 4 days till the colour of the leaf changes to buff/ pale yellow colour and the leaf becomes brittle. The leaves were powdered and passed through the sieve with mesh number 100-120 mesh to get uniform coarse fine powder. The coarse powder is better fit for the extraction process and this was the reason to make the coarse fine powder to get a good yield of extract.[17]

2.2 Preparation of extracts

Methanolic extract & Chloroform extract were prepared with powder by using the solvents Methanol & Chloroform. The powdered material of 100 g was macerated with 500 ml of Methanol for 48 hrs. at room temperature. The extract was filtered with sterile whatman No.1 filter paper into a clean conical flask. The marc in the whatman filter paper was again extracted with 300 ml of methanol and the procedure was repeated. Now both the extracts were pooled and transferred into a rotary flash evaporator for the evaporation of solvent. The evaporated extract was preserved at 4^0C in an airtight bottle until further use. The same procedure was followed for the preparation of chloroform extract also.[18]Male Albino rats (seven to eight weeks-old) weighing 180 g to 210 g were used for experimental study (approval no: ARTI/CPCSEA/2017/26/A). The animals were procured from National Institute of Nutrition (NIN), Hyderabad and the animals were acclimatized for a period of 07 days before the study. Standard temperature of (26 ± 2^0 C), relative humidity (45-55%) and 12 hrs dark/light cycle were maintained in the quarantine. All the animals were fed with a rodent pellet diet and water was allowed *ad-libitum* under strict hygienic conditions. Animals that are described as fasting were deprived of food for at least 16 hours but were allowed free access to drinking water. Ethical clearance (approval no: ARTI/CPCSEA/2017/26/A) for performing the experimental animals was obtained from the Institutional Animal Ethical Committee (IAEC). Acute toxicity studies were conducted as per the OECD (Organization for the Economic Co-operation and Development section 4. Test No.425) guidelines. A test dose of 2000 mg/kg body weight was administered orally to the rats as per the principle of the Limit Test of OECD.[19] Six male rats in each group were fasted overnight and the methanolic & chloroform extracts of *Solanum seaforthianum* were administered to each group of 06 animals. The rats were observed every 2 hours throughout a day to monitor its behavioural, neurological and autonomic profiles. Further rats were observed every 24 hours for 14 days for its lethality.

2.3 Induction of diabetes

The animals were kept fasting for 16 hrs and access of drinking water only before the induction of diabetes. Diabetes was induced in fasted animals by giving a single intra peritoneal injection of 120 mg/kg body weight of Alloxan monohydrate in sterile saline. After 5 days of alloxan injection, the hyperglycemic rats possessing glucose levels more than 250 mg/dl were separated and divided into 07 different groups of 06 animals in each group. The grouping of animals was as follows.[17]

Table 1. Grouping of animals

S. No.	Groups	Treatment
1.	Group I	Served as Normal control and did not receive any treatment
2.	Group II	Served as Diabetic control and received alloxan monohydrate and treatment with Vehicle (0.2 ml of 2% aqueous gum acacia)
3.	Group III	Alloxan monohydrate and treatment with Methanolic extract of *Solanum seaforthianum* (200 mg/kg body weight, p.o) Served as test group
4.	Group IV	Alloxan monohydrate and treatment with Methanolic extract of *Solanum seaforthianum* (400 mg/kg body weight, p.o) Served as test group
5.	Group V	Alloxan monohydrate and treatment with Chloroform extract of *Solanum seaforthianum* (200 mg/kg body weight, p.o) Served as test group
6.	Group VI	Alloxan monohydrate and treatment with Chloroform extract of *Solanum seaforthianum* (400 mg/kg body weight, p.o) Served as test group
7.	Group VII	Alloxan monohydrate and treatment with Glibenclamide- (10mg/kg body weight, p.o) served as the standard group.

The treatment was started on the same day (Day-0) and continued for 10 days with respective drugs/doses to all groups of animals. During this treatment period, animals in all groups had free access to standard diet and water. Blood glucose levels were estimated on 04th Day, 07th Day & 10th Day (Last day of treatment) of treatment. On the last day after the treatment the blood samples were collected by puncturing the retro orbital plexus of animals and centrifuged at 8,000 rpm for 20 minutes at 4^{0}C. The supernatant layer of serum was separated and used for the estimation of Cholesterol, Triglycerides, HDL, LDL, Serum Urea, Serum Creatinine & Total Protein and Blood glucose

3. STATISTICAL ANALYSIS

All the values of results were expressed as mean with Standard error mean. Statistical significance among the groups were tested using one-way analysis of Variance (ANOVA) followed by Tukey's multiple comparison.

4. RESULTS

No mortality and significant behavioral changes were observed at the dose of 2000 mg/kg body weight *Solanum seaforthianum* of both methanolic & chloroform extracts and the doses were fixed as 200 mg/kg body weight as low dose and double of this dose 400 mg/kg body weight as higher dose for both the extracts in this study. The table- 2 results revealed that the diabetes was induced successfully in all the group of animals except normal control with the administration of Alloxan which was confirmed by the elevated blood glucose levels more than 250 mg/kg body weight after 05 days of alloxan administration. As the treatment progressed the glucose levels on Day-4, Day-7 and Day-10, the elevated Glucose levels were decreased consistently and brought towards normal by the last day of the drug treatment. The Methanolic extract of *Solanum seaforthianum* higher dose of 400 mg/kg body weight treated group rats has shown significant reduction of elevated blood glucose levels on 10th day of the treatment which was estimated as 188.5±2.078 mg/dL. Similarly the higher dose (400 mg/kg) of chloroform extract of *Solanum seaforthianum* treated group also shown significant reduction in Blood glucose levels on 10th day which was estimated as 166.16±3.655 along with the standard group of Glibenclamide 10 mg/kg body weight which was estimated as 141.16±2.167. Among the four tested groups the higher doses (400 mg/kg body weight) of methanolic & chloroform extracts of *Solanum seaforthianum* has shown the significant reduction of elevated blood glucose levels in the similar passion with group treated with standard Glibenclamide at the dose of 10mg/kg body weight. Among the higher doses of the two extract treated groups the higher dose of Chloroform extract treated group has shown the best results which were closer to the standard treated group. Table– 3 showing that the Cholesterol, Triglycerides, LDL, Urea & Creatinine levels were increased in Diabetic control group which is marked in Diabetic condition. But, treatment with *Solanum seaforthianum* methanolic & chloroform extracts and standard drugs have reduced the elevated parameters of Cholesterol, Triglycerides, LDL, Urea & creatinine. On the other hand, the HDL & Total Protein levels were significantly reduced in Diabetic control group and the decreased levels of HDL & total protein were reversed after the treatment with methanolic & chloroform extracts of *Solanum seaforthianum* and standard drug which was observed in Groups III, IV, V, VI & VII. Among the four testing groups the higher doses (400 mg/kg body weight) of methanolic & chloroform extracts has reversed the alter parameters of cholesterol, triglycerides, LDL, urea, creatinine, HDL and total proteins to normal after the treatment of 10 days. Among the two higher doses, chloroform extract of *Solanum seaforthianum* has shown significant reversal of the altered parameters and proven good results close to standard drug treated groups.

Table 2. Effect of Solanum seaforthianum (S.S) on glucose levels

S. No.	Treatment group	Glucose (mg/dl)			
		Day-1	Day-4	Day-7	Day-10
1.	Normal control	90.3 ± 1.5	90.5 ± 1.1	91.5 ± 1.4	89.3 ± 1.7
2.	Diabetic control	284.8 ± 3.0	286.3 ± 2.9^{+++}	279.8 ± 1.7^{+++}	273.1 ± 1.9^{+++}
3.	S.S Meth. extract (200 mg/kg)	282.0 ± 3.2	266.6 ± 2.6*	254.8 ± 1.9**	243.3 ± 2.2***
4.	S.S Meth. extract (400 mg/kg)	275.5 ± 1.7	258.0 ± 1.9**	217.5 ± 1.9***	188.5 ± 2.0***
5.	S.S $CHCl_3$ extract (200 mg/kg)	283.6 ± 4.0	264.1 ± 1.8**	245.6 ± 1.6***	233.5 ± 2.6***
6.	S.S $CHCl_3$ extract (400 mg/kg)	279.0 ± 4.4	255.0 ± 1.7**	210.0 ± 2.0***	166.1 ± 3.6***
7.	Glibenclamide- 10 mg/Kg	279.0 ± 3.8	213.3 ± 2.1***	184.3 ± 1.8***	141.1 ± 2.1***

Values are expressed as Mean ± SEM $^{+++}P < 0.001$, $^{++}P < 0.01$, $^{+}P < 0.05$ when compared to normal Control $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ when compared to Diabetic Control

Table 3. Effect of Solanum seaforthianum (S.S) on biochemical parameters

S. No.	Treatment group	Cholesterol (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	T.P (g/dl)
1.	Normal control	114.1± 2.4	69.6 ± 2.1	37.5 ± 0.9	40.1 ± 1.1	31.5 ± 0.7	0.5 ± 0.01	7.5 ± 0.2
2.	Diabetic control	196.1 ±2.5^{+++}	166.1 ± 1.9^{+++}	12.1 ± 0.7^{+++}	153.8 ± 2.1^{+++}	63.1± 1.3^{+++}	1.51 ± 0.04^{+++}	4.3 ±0.2^{+++}
3.	S.S Meth. extract (200 mg/kg)	169.8 ± 2.8***	142.6 ± 2.5**	17.0 ± 0.7***	125.5 ± 1.5***	51.1 ± 1.1*	1.2 ± 0.02**	4.5 ± 0.2***
4.	S.S Meth. extract (400 mg/kg)	152.6 ± 1.8***	105.6 ± 1.9***	23.8 ± 0.7***	81.6 ± 2.3***	37.5 ± 0.7***	0.7 ± 0.01***	5.5 ± 0.1***
5.	S.S $CHCl_3$ extract (200 mg/kg	172.8 ± 1.5**	143.8 ± 2.0**	17.0 ± 0.5***	126.0 ± 1.1***	44.1 ± 1.5**	1.1 ± 0.01***	5.9 ± 0.2***
6.	S.S $CHCl_3$ extract (400 mg/kg)	144.0 ± 2.3***	93.1 ± 2.4***	32.6 ± 0.6***	70.1 ± 1.4***	34.1 ± 0.6***	0.6 ± 0.01***	6.7 ± 0.1***
7.	Glibenclamide- 10 mg/Kg	123.6 ± 2.2***	73.3 ± 2.0***	34.6 ± 0.6***	42.3 ± 1.5***	32.5 ± 0.7***	0.6 ± 0.01***	7.5 ± 0.2***

Values are expressed as Mean ± SEM $^{+++}P < 0.001$, $^{++}P < 0.01$, $^{+}P < 0.05$ when compared to normal Control $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ when compared to Diabetic Control

5. DISCUSSION

Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted.[20] Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptozotocin. Alloxan has a destructive effect on the beta cells of the pancreas.[21, 22]Alloxan causes a massive reduction in insulin release by the destruction of b-cells of the islets of langerhans, thereby inducing hyperglycemia [23] Insulin deficiency leads to various metabolic alterations in the animals viz increased blood glucose, increased cholesterol, increased levels of alkaline phosphatase and transaminases. [24,25] The results of the present study indicate that *Solanum seaforthianum* leaf extract was found to reduce the glucose level in alloxan induced diabetic rats. Alloxan has been shown to induce free radical production and cause tissue injury. The pancreas is especially susceptible to the action of alloxan induced free radical damage. In the present investigation methanolic and chloroform extracts of *Solanum seaforthianum* leaves demonstrated significant anti-diabetic activity. The results from the present study also indicate that *Solanum seaforthianum* leaf extract can reduce the levels of serum urea, serum creatinine, serum cholesterol, triglycerides, low density lipoprotein and increase the serum protein, high density lipoprotein and confirms the possibility that the major function of the extract are on the protection of vital tissues (kidney and liver) including the pancreas, thereby reducing the causation of diabetes in the experimental animals. The mechanism to reduce the serum glucose lies in both the traditional and allopathic drugs might have[26]

✓ Stimulated the beta cells of pancreatic islets to release insulin.
✓ Resisted the hormones which increase the blood glucose levels.
✓ Increased the sensitivity of Insulin towards its receptors which is the key mechanism for entry of glucose into cells.
✓ Decreased the leading out of glycogen.
✓ Enhanced the use of glucose in all the tissues and organs of the body.

The reasons for altered lipid profile parameters might be:

Since sufficient glucose is not available for the cells for their normal activities, they depend on alternative lipids as a source of energy and insulin also plays a key role in controlling the lipid levels in the body. All the harmful Lipid parameters such as Cholesterol, Triglycerides & LDL levels were increased in diabetic animals due to disturbed lipid metabolism and in contrast to this the parameters of lipid like HDL levels were reduced in diabetic animals.[27] The

metabolites of urea & creatinine which are supposed to be cleared off from the body as a regular process. But, they were retained in the body due to excessive elimination of glucose in urine which occupies the carriers responsible for transport of metabolites from the body to the urine. This might be the mechanism of retaining of metabolites in the body which was observed as elevated levels of urea & creatinine in diabetic animals. All these mechanisms were proposed on the basis of previous literature and further research needs to be done to find out the molecular mechanism at cellular level.[28] The present study indicates that *Solanum seaforthianum* can partially inhibit alloxan renal toxicity as observed from serum urea and creatinine levels.[28] The literature reports reveal that flavonoids and tannins present in the plant extract are known to possess antidiabetic activity. In the present investigation the observed antidiabetic potential of test extract may be due to presence of similar phytoconstituents which was evident by preliminary phytochemical screening.

6. CONCLUSION

From the above results, it can be concluded that the methanolic and chloroform extracts of *Solanum seaforthianum* has significantly (*** $P < 0.001$) decreased elevated glucose, Triglycerides, Cholesterol and LDL levels and increased the decreased HDL levels in the alloxan induced diabetic rats thus improving the kidney functions. The higher doses (400 mg/kg body weight) of both the extracts has shown the results very close to the standard drug glibenclamide and among the two extracts the Chloroform extract has shown better antidiabetic activity compared with methanolic extract. Hence, *Solanum seaforthianum* is effective against diabetes mellitus and the possible mechanism might be increasing the sensitivity of insulin towards its receptors which was disturbed due to free radical damage done by alloxan. Further research needs to be done to find out the exact mechanism at molecular level.

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8. AUTHORS CONTRIBUTION STATEMENT

V S S S Gupta Atyam, designed and performed the experiments, derived the models and analysed the data. Nadendla Rama Rao, involved in planning and supervised the work processed the experimental data, drafted the manuscript and supervised the findings of this work.

9. CONFLICTS OF INTEREST

Conflict of interest declared none

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