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HYPOGLYCEMIC AND HYPOLIPIDEMIC ACTIVITY OF *SOLANUM NIGRUM* IN ALLOXAN INDUCED DIABETIC ALBINO RATS

Arumugam Sengottaiyan*[1 & 2], L. Praburaman [1], Koildhasan Manoharan [3], Rathika Rajinikanth [1], Muthusamy Govarthanan [4] and Thangaswamy Selvankumar [1]

Department of Biotechnology, Mahendra Arts & Science College [1], Kalipatti- 637501 Tamil Nadu, India
Department of Biotechnology, Vivekhanandha College of Arts and Science for Women [2], Namakkal, Tamil Nadu, India
Department of Botany, Sethupathy Govt. Arts College [3], Ramanathapuram, Tamil Nadu, India
Division of Biotechnology, College of Environmental and Bioresource Sciences, Chonbuk National University [4], Iksan-570 752, Republic of Korea

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Correspondence to Author:

Arumugam Sengottaiyan

Department of Biotechnology, Mahendra Arts & Science College, Kalipatti- 637501 Tamil Nadu, India

E- mail: sengottaibiotech@rediffmail.com

ABSTRACT

The objective of this study is to induce experimental diabetes mellitus using Alloxan monohydrate in normal adult albino rats and study the anti-diabetic activity of changes in body weight, consumption of food and water, volume of urine and levels of glucose between normal and diabetic rats. Diabetes mellitus (DM) is a common endocrine disorder. Hypoglycemic agents from natural and synthetic sources are available for treatment of diabetes. Indian medicinal plants have been found to be useful to successfully manage diabetes. The effect of methanolic and water extract of *Solanum nigrum* leaves was investigated in normal, glucose load conditions and Alloxan monohydrate -induced diabetic rats. Significant hypoglycemic activity was exhibited by the poly herbal formulation.

INTRODUCTION: Diabetes mellitus (DM) is common endocrine disorder affecting more than 150 million people worldwide and this number is likely to increase to 300million by the year 2025, [16] out of which more than one- fifth are Indians.

According to the International Diabetes Federation, India has been declared as the diabetes capital of the world**.** Medicinal plants have been used as sources of drugs for treatment of diabetes in developing countries where the cost of conventional medicines is a burden to the population [23]. Despite the introduction of hypoglycemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem.

Many indigenous Indian medicinal plants have been found to be useful to successfully manage diabetes. One of the great advantages of medicinal plants is that these are readily available and have no side effects.

World Health Organization [36] has suggested the evaluation of the potential of plants as effective therapeutic agents, especially in areas in which we lack safe modern drugs.

The objective of present study is to investigate the effect of methanolic and water extract of *Solanum nigrum* leaves was investigated in glucose load conditions in normal rats and alloxan monohydrate induced diabetic rats.

Diabetes mellitus is a metabolic disease as old as mankind and its incidence is considered to be high (4-5%) all over the world. In spite of the introduction of hypoglycemic agents, diabetes and related complications continue to be a major medical problem. Since time immemorial, patients with non-insulin requiring diabetes have been treated orally in folk medicine with a variety of plant extracts. In India a number of plants are mentioned in ancient literature (Ayurveda) for the cure of diabetic conditions known as 'madhumeha' and some of them have been experimentally evaluated and the active principles isolated [8, 9, 10, 26, 29].

Hyperglycaemia or diabetes mellitus is caused by inherited or acquired deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentration of glucose in the blood, which in turn damage many of the body systems in particular the blood vessels and nerves.

Chronic hyperglycaemia during diabetes causes glycation of body proteins that in turn lead to secondary complications effecting eyes, kidneys, nerves and arteries. A part from currently available therapeutic options many herbal medicines have been recommended for the treatment of diabetes medicinal plants have the advantage of having no side-effects. Some of them are be used in traditional systems of medicines from hundreds of the years in many countries of the world [31].

Plants play a major role in the discovery of new therapeutic agents and have received much attention as sources of biologically active substances including antioxidants, hypoglycemic and hypo lipidemic agents [18]. Flavonoids and poly phenols are being used to treat diabetes and dyslipidemia [19]. This is based on the fact that, excessive oxidative stress is implicated in the pathology and complications of DM and polyphenols with antioxidant properties exert beneficial antidiabetic effect by correcting the disturbed oxidative milieu in diabetic conditions. [1, 34]

Medicinal plants contain both the organic and inorganic constituents. Abundant research work has been carried out on the organic constituents of the medicinal plants while little attention has been paid on the role of inorganic elements in the medicinal use of these plants. Most of these plants are found to be rich in one or more individual elements, thereby providing a possible link to the therapeutic action of the medicine [30, 32]. Trace elements play a very important role in the formation of the active chemical constituents present in medicinal plants and are therefore responsible for their medicinal as well as toxic properties [28].

Nowadays, Diabetes mellitus has become a real problem of public health in developing countries [20]. It is actually a chronic disorders related to abnormality of carbohydrate, fat and protein metabolism. This is due to defective or deficient insulin secretary response. This results into impaired glucose use, which is a characteristic feature of diabetes mellitus i.e. resultant hyperglycemia.

Over the years, various medicinal plants and their extracts have been reported to be effective in the treatment of diabetes [18]. Plants are rich sources of antidiabetic, antihyperlipedemic and antioxidant agents such as flavonoids, gallotannins, amino acids and other related polyphenols [22.]

Several drugs are used to control diabetes, however perfect glycemic control is rarely achieved [11]. From ancient times, plants have been used for treatment of Diabetes mellitus [12]. The use of Medicinal plants as alternative therapy is widely spread in the populations of underdeveloped countries, which have limited access to medical assistance. Diabetic women often use aqueous extracts of plants during pregnancy without any concern as to their possible outcomes. The effects of many of these plants have already been proven experimentally in animals and humans while others require further investigations [25].

Diabetes mellitus is now recognized as a serious global health problem [17]. Westernized cultures and populations experiencing rapid acculturation are showing a sharp rise in non-insulin-dependent diabetes mellitus [5, 37].

The prevalence of NIDDM is increasing exponentially [14]. It is estimated that more than 300 million people in the world will have diabetes by the year 2025.

Nutritional factors including antioxidants have great influence in the management of diabetes mellitus and its complications [2]; an imbalance between oxidative stress and antioxidative defense mechanisms in diabetics can result in cell and tissue damage and accelerate diabetic complications. Administration of appropriate antioxidants could prevent or retard diabetic complications to some extent [24].

Solanum nigrum is a species in the *Solanum* genus, native to Eurasia and introduced in the Americas, Australasia and South Africa. Parts of this plant can be highly toxic to livestock and humans, and it is considered a weed. Nonetheless, ripe berries and cooked leaves are used as food in some locales; and plant parts are used as a traditional medicine. There is a tendency in literature to collectively refer to many of the black nightshade species as *'Solanum nigrum'* [21].

MATERIALS ANDS METHODS

Plant Material: Leaves of, *Solanum nigrum* were collected from botanical garden of Kolli hills, Tamilnadu, India, and dried in shade till total moisture is removed from the plant.

Extraction: Leaves of *Solanum nigrum* were coarsely powdered and extracted. The extraction process was done with the help of Soxhlet apparatus. Methanol and water solvents were used. Extracts were kept in desiccators for the removal of remaining moisture.

Methanolic Extract: 15gm of dry powder was subjected to Soxhlet extraction with 300 ml methanol (95%) as solvent, extraction was carried out for 10 cycles and temperature was maintained at 65°C. Color of extract was dark green.

Water Extract: 15gm of dry powder was subjected to Soxhlet extraction with 300 ml water as solvent, extraction was carried out for 10 cycles and temperature was maintained at 100°C. Color of extract was dark green.

Animals: Male albino rats weighing about 125 to 150 gram (45-60 days old) maintained under standard experimental conditions (Temperature 27±2°C, relative humidity 60±5% and 12 hours light/dark cycle) were housed in standard environmental conditions.

Acute Toxicity Studies: Healthy adult Wistar albino rats of either sex, starved overnight were divided into five groups and were orally fed with the methanol and aqueous extract of *Solanum nigrum* in increasing dose levels of 100 and 200, mg/kg body weight (Ghosh, 1984). The rats were observed continuously for 2 h for behavioral, neurological and autonomic profiles and after a period of 24 and 72 h for any lethality or death [35].

Induction of diabetics: Hyperglycemia was induced by injecting alloxan monohydrate at a dose of 120 mg/kg intraperitoneally. The animals were kept under observation and after 48 hrs were tested for hyperglycemia using glucometer.

Group I (control): This group was kept as normal control animals without any treatment.

Group II (Diabetic control): This group was taken as diabetic control with the injection of Alloxan monohydrate.

Group III: This group was taken as Standard control with the injection of Alloxan and the Standard glibenclamide.

Group IV: This group was treated with *Solanum nigrum* methanol extract was mixed with rat feed.

Group V: This group was treated with *Solanum nigrum* water extract was mixed with rat feed.

Oral Glucose Tolerance Test in normal rats (OGTT): Rats were divided into five groups and were administered normal saline and dose of 500 mg/kg oral of aqueous extract. Glucose solution 2 g/kg was administered 30 min after the administration of the extract. Blood samples were withdrawn from retro-orbital at intervals of 30, 60 and 120 min of glucose administration and the level of blood glucose was measured [4].

Experimental Design: Body weights and blood glucose level of individual animals were recorded before and the period of study in week interval. The end of the 21st day, the experimental animals were sacrificed along with their control group. The animals were fasted overnight, sacrificed by decapitation and blood was collected from individual animals and the serum was obtained from the clotted blood samples for

Biochemical studies. The Liver and other organs were immediately excised, after perfusing with physiological saline, the organs were blotted dried and it is used for further analysis.

Collection of Blood Sample: Blood samples were collected from the tail vein puncture of treated rats. The samples were taken in to tubes with anticoagulant. From these samples blood sugar was estimated by a standard method [3].

Estimation of Lipid Profile in Blood sample: Blood samples were collected by tail vein puncture in the morning hours before the mice were fed and watered and were immediately processed for the blood serum separation. Clear serum was separated and kept at -20°C till used for biochemical analysis.

A rise in blood creatinine level is observed only with marked damage to functioning nephrons. Therefore, this test is not suitable for detecting early-stage kidney disease. A better estimation of kidney function is given by the creatinine clearance (CrCl) test. Creatinine clearance can be accurately calculated using serum creatinine concentration and some or all of the following variables: sex, age, weight, and race, as suggested by the American Diabetes Association without a 24-hour urine collection.

Estimation of Electrolytes' in Blood sample: Electrolytes are positively and negatively charged molecules, called ions that are found within cells, in the bloodstream, and in other fluids throughout the body. Electrolytes with a positive charge include sodium, potassium, calcium, and magnesium; the negative ions are chloride, bicarbonate, and phosphate. The concentrations of these ions in the bloodstream remain fairly constant throughout the day. Changes in the concentration of one or more of these ions can occur during various acute and chronic disease states and can lead to serious consequences and especially it affects heart, kidney & liver.

Estimation of total protein in Blood sample: Serum globulin was calculated as the difference between total protein and albumin. The estimation of serum total cholesterol was done by Wxbenga and Pileggi's method by using cholesterol kit manufactured by M/s Mediprob Laboratories Pvt. Ltd., Hyderabad, India.

Liver Glycogen Test:

Extraction of glycogen from liver with Trichloroacetic acid: A 200 mg. sample of liver is weighed on a torsion balance and finely ground with 20 ml. of 5% TCA in a mortar or preferably in a homogenizer [27]. The precipitate of proteins is filtered off and the clear filtrate submitted to analysis.

Iodine reagent: 16.5 ml of Iodine reagent (Lugol's solution) is prepared by dissolving 1 gm of iodine and 2 gm of KI in 20 ml of water. It is added to 990 ml of an aqueous solution, containing 25% w/v) of KCI.

- **Procedure:** In a colorimeter tube 2 ml of a liver extract is added to 3 ml. of iodine reagent. After mixing, the optical density is read in a photometer at 650nm, against the blank. Blank is obtained by adding 2 ml of 5% TCA to 3 ml of reagent in the same way.

RESULTS & DISCUSSION: The effect of single oral administration of water and methanolic extracts of *Solanum nigrum* are shown in **(Table 1)**. An experimental study reveals that the Methonolic and water extracts from *Solanum nigrum* (100 and 200 mg/kg) orally administered produced a significant decrease in the blood glucose level in the model of alloxan-induced diabetes in rats. Methanol extract is effective even at a higher dose (200 mg/kg) in decreasing blood sugar level in alloxan treated rats. The plant extract almost brought down blood glucose level by 50 % in diabetic animals.

Thus, it may be effective like tolbutamide. This study reports for the first time, the anti hyperglycemic effect of *Solanum nigrum* suggests that the active principle from this plant could be effective in the treatment of diabetes.

The positive effect of methanol and water extracts was also studied in other biochemical parameters (TC, LDL, HDL, VLDL and TAG) (**Table 2).** Normally diabetic rats tend to lose weight but after treatment with the two extracts, the body weight did not decline significantly. It is therefore clear that *Solanum nigrum are* a good for reducing not only the overall physiological and biochemical effects due to diabetes but also physical effects like body weight food and water intake.

Study of urea and creatinine levels in diabetic induced rats have indicated that there was no significant increase in the serum creatinine level in the plant extracts treated groups. However, when compared to the diabetic control, the methanol extract treatment indicated a significant increase of 30% in the urea level **(Table 3).** The positive effect of the two extracts was also seen in other biochemical parameters such as sodium, potassium, chloride, Bicarbonate. In this analysis *Solanum nigrum* methanol extract was significant to role in diabetic control activity **(Table 4).**

Table 5 demonstrates that the effect of the methanol and water extract on total protein. Methanol extract was effective even at a higher dose (200 mg/kg) in decreasing level in alloxan treated rats [38].

TABLE 1: EFFECT OF *SOLANUM* PLANT EXTRACT ON BLOOD GLUCOSE IN MULTI-DOSE TREATED IN ALLOXAN INDUCED DIABETIC RATS IN ORAL ROUTE.

Groups	Treatment		Blood Glucose Level (mg/dl)			
			Initial	Day 7	Day 14	Day 21
I	Normal control		90.46±3.80	92.82±2.92	94.32±1.73	91.29±3.44
II	Diabetic control		293.8±5.27	286.91±5.05	291.8±5.41	289.41±9.75
III	Alloxan + Glibenclamide		285.86+6.92	205.25+7.06	183.18+6.15	178.13±9.75
IV	Alloxan + methanol extract	100 mg	284.48±5.32	277.76±5.65	265.76±2.60	253.75±0.19
		200 mg	287.48±5.32	262.98±7.08	234.90±32.0	198.70±60.99
V	Alloxan + water extract	100 mg	285.48±5.32	273.90±76.9	268.87±90.07	255.67±0.70
		200 mg	283.86±6.92	265.90±6.98	233.90±7.67	200.02±6.23

Note: Values are given as mean± S.E.M. from six rats in each group. *** P < 0.001 significant from normal and diabetic control animals

TABLE: 2 EFFECT OF *SOLANUM* PLANT EXTRACT ON SERUM LIPID PROFILE IN ALLOXANISED RATS ON 21TH DAY OF STUDY

Groups	Treatment	Total Cholesterol (mg/dl)	HDL (mg/dl)	Triglycerides (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
A	Normal control	45.65±5.90	24.60±1.47	33.40±3.45	22.5±6.86	16.33 ± 1.40
B	Diabetic control	32.20±2.49	25.80±0.98	30.20±1.90	23.60±1.9	45.06 ± 1.94
C	Alloxan + Glibenclamide	36.00±2.23	26.60±1.02	36.20±1.60	22.20±2.0	18.75 ± 1.84
D	Alloxan + Methanol extract	55.24±3.0	30.08±5.8	38.80±87.0	26.33±6.2	18.25±2.34
E	Alloxan + Water extract	63.90±7.0	34.79±5.07	50.20±56.0	29.58±2.8	21.25±2.42

TABLE 3: EFFECT OF *SOLANUM* PLANT EXTRACT ON UREA & CREATININE IN ALLOXANISED RATS ON 21TH DAY OF STUDY

Groups	Treatment	Urea(mg/dl)	Creatinine(mg/dl)
A	Normal control	30.09±1.09	0.56±0.02
B	Diabetic control	32.93±1.10	1.47±0.04
C	Alloxan + Glibenclamide	33.97±1.34	0.64±0.01
D	Alloxan +*Solanum* Methanol extract	28.40±2.09	0.4±1.08
E	Alloxan +*Solanum* Water extract	26.60±3.87	0.09±0.72

TABLE 4: EFFECT OF *SOLANUM* PLANT EXTRACT ON ELECTROLITES IN ALLOXANISED RATS ON 21TH DAY OF STUDY.

Groups	Treatment	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Bicorbonate (mmol/L)
A	Normal control	143	4.5	102	24
B	Diabetic control	137	4.2	105	26
C	Alloxan + Glibenclamide	146	4.3	101	25
D	Alloxan +*Solanum* Methanol extract	140	4.2	99	25
E	Alloxan +*Solanum* Water extract	141	4.3	99	28

TABLE 5: EFFECT OF *SOLANUM* PLANT EXTRACT ON TOTAL PROTEIN IN ALLOXANISED RATS ON 21TH DAY OF STUDY

Groups	Treatment	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	HB (%)
A	Normal control	6.0	3.9	2.0	8.2
B	Diabetic control	6.2	4.1	2.2	9.2
C	Alloxan + Glibenclamide	6.4	3.4	3.0	8.8
D	Alloxan +*Solanum* Methanol extract	6.2	3.4	2.1	8.2
E	Alloxan +*Solanum* Water extract	6.4	3.7	2.2	8.0

In this study, we discuss about the hypoglycemic and antidiabetic effects of the *Solanum nigrum* on normal and Alloxan-induced-diabetic rats. Acute toxicity studies revealed the non-toxic nature of the *Solanum nigrum* plant extract.

There was no lethality or any toxic reactions found with the selected dose until the end of the study period. The dose of the test drug has been selected on the basis of dose calibration curve. The results of the study have shown that the methanol extract of S.N. at a dose of 200 mg/kg body weight has a marked hypoglycemic activity by improvement of the glucose tolerance test in normoglycemic rats and by lowering the blood glucose levels in Alloxan -induced-diabetic rats [38].

The results of the study have shown a significant difference between the initial and final fasting glucose levels of methanol extract *Solanum nigrum* and glibenclamide treated groups. Induction of diabetes by alloxan leads to loss of body weight due to the increased muscle wasting and loss of tissue proteins [33, 7]. The results obtained with the methanol extract treatment in chronic diabetic model further clarified the antidiabetic effect of the extract.

After 21 days of plant extract treatment, gain in the body weight was observed in diabetic rats and the results were comparable with that of the standard drug glibenclamide. Methanol extract of *Solanum nigrum* showed significant increase in serum insulin level.

A marked decrease in triglycerides, total cholesterol, LDL and VLDL was observed. The possible mechanism of antidiabetic action of methanolic extract of *Solanum nigrum* may be by increasing the pancreatic secretion of insulin from the existing beta cells, by its release from the bound form.

Animals treated with plant extract indicated a significant decrease in the glycosylated hemoglobin level which could be due to an improvement in insulin secretion.

In conclusion, it can be stated, that the methanol extract of *Solanum nigrum* has beneficial effects, in reducing the elevated blood glucose level and lipid profile of alloxan-induced-diabetic rats, but has no effect on normal rats.

From this preliminary investigation, it has been concluded that the leaves of *S. nigrum* have significant hypoglycemic activity.

Liver Glycogen Test: The amount of glycogen present in the livers of the mice is graphically represented;

- X-axis Glucose equivalent (mg/ml)
- Y-axis Optical density at 650nm

The values for the relative rates of synthesis were very similar in all the diabetic groups, indicating that the rate of synthesis of the enzyme increased early in diabetes and is maintained at the increased rate throughout the time period studied. The values for rate of degradation and synthesis suggest that liver glycogen synthtase in diabetic animals turns over more rapidly than normal ones **(Figure 1 & 2)**.

Total percentage of glucose level of the diabetes induced rat at the time intervals after administration of *Solanum nigrum* extracts were tabulated. Upon analysis of protein, electrolytes, lipids, urea & creatinine there is a decrease in these values found when compared with the normal ones. The difference in blood glucose level of different mice in between control versus standard drug and the test is calculated.

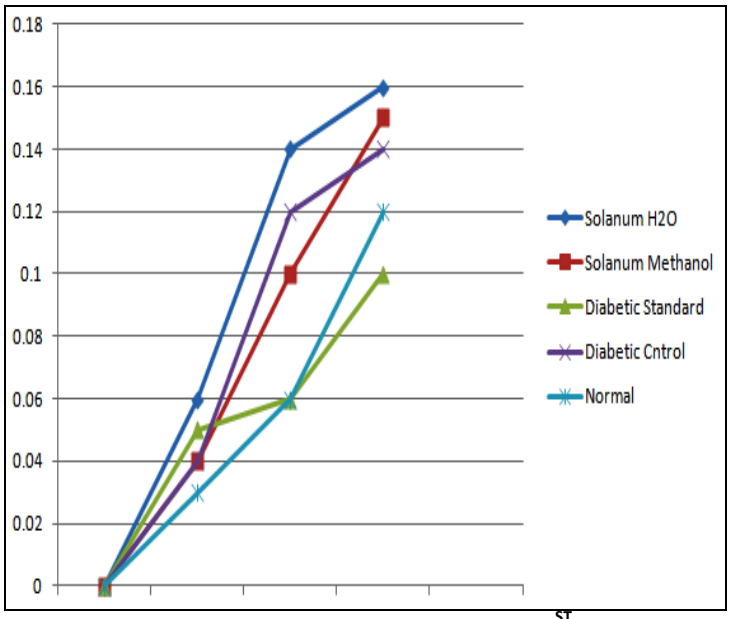

FIGURE 1: ESTIMATION OF GLYCOGEN ON 1ST DAY AFTER TREATMENT

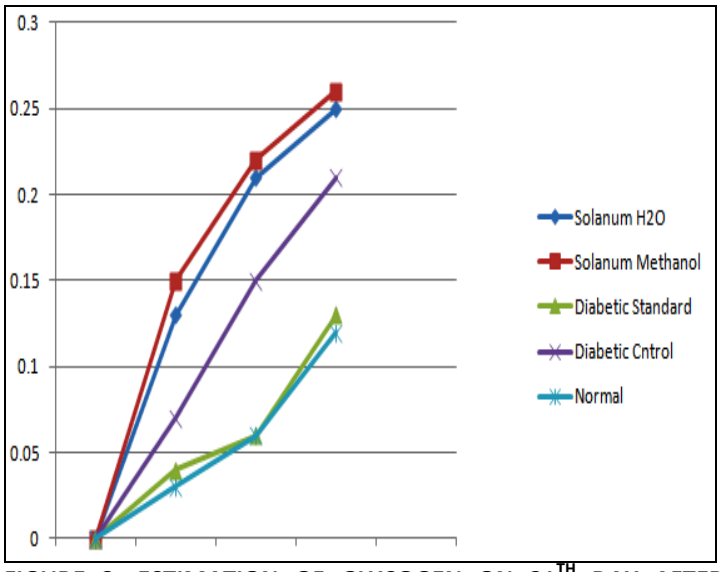

FIGURE 2: ESTIMATION OF GLYCOGEN ON 21TH DAY AFTER TREATMENT

CONCLUSION: From this preliminary study, it is being revealed that the plant extract of *S. nigrum* possess significant antidiabetic activity.

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