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Research Article

PHARMACOGNOSTICAL AND HYPOGLYCEMIC ACTIVITY OF DIFFERENT PARTS OF *SOLANUM NIGRUM* LINN PLANT

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ABSTRACT

Diabetes is a series of metabolic conditions associated with hyperglycemia and caused by defects in insulin secretion and/or insulin action[1, 2]. The rapidly increasing incidence of diabetes mellitus is becoming a serious threat to mankind health in all parts of the world[3, 4]. Moreover, during the past few years some of the new bioactive drugs isolated from plants showed antidiabetic activity with more efficacy than oral hypoglycemic agents used in clinical therapy. The traditional medicine performed a good clinical practice and is showing a bright future in the therapy of diabetes mellitus[5]. It has been attributed that the antihyperglycemic effect of these plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or facilitation of metabolites in insulin-dependent process. Hence, treatment with herbal drugs has an effect on protecting β-cells and smoothing out fluctuation in glucose levels[6, 7]. In the present study we have screened the aqueous and hydroalcoholic extracts of different parts of *Solanum nigrum* plant, viz leaf, fruit and stem for hypoglycemic activity in Sprague Dawley rats. Different doses of the extract 200, 400mg/kg body weight were employed to evaluate the Oral Glucose Tolerance with standard Metformin. Results indicated that aqueous extracts of Leaf and fruit possess significant hypoglycemic effect in dose dependent manner, followed by hydroalcoholic extracts. The stem extract of *S.nigrum* has no profound effects. The ash values were: leaf- 3.928, fruit - 6.723, stem - 11.90, crude fibre leaf - 8.42, fruit - 15.19, and stem - 14.73. Potassium and sodium were analyzed for all the parts which revealed that the leaves have the highest content of 2.6ug/mL & 0.75ug/mL respectively. Further phytochemical analysis was also performed for the different parts. The results suggest the validity of the clinical use of the plant in diabetes mellitus control, after further toxicological and in vivo antidiabetic studies.

Keywords: *Solanum nigrum,* Hypoglycemic activity, Oral glucose tolerance test, Pharmaconostical activity

INTRODUCTION

Type II diabetes mellitus is a metabolic disorder that is primarily characterized by insulin resistance, relative insulin deficiency and hyperglycemia. It is rapidly increasing in the developed countries and there is also evidence that this pattern will be followed in much of the rest of the parts of the world in coming years. Diabetes affects over 150 million people worldwide and this number is expected to double by 2025. It is associated with factors which directly contribute to cardiovascular disorders including resistance, dislipidemia, atherosclerosis, hypertension [8, 9], endothelial dysfunction and vascular inflammation[10, 11]. Obesity is another risk factor in the development of diabetes and CHD[12]. A medicinal plant *Galega offinalis* led to the discovery and synthesis of metformin[13], and is still used for new oral antidiabetic drugs without side effects. Plants are more potent healers because they promote the repair mechanisms in the natural way[14]. A multitude of herbs, spices and other plant materials have been described for the treatment of diabetes throughout the world [15, 16, 17]. The medicinal plants might provide a useful source of new oral hypoglycemic compounds for development of pharmaceutical entities or as a dietary adjunct to existing therapies[18]. Despite the presence of known antidiabetic medicines in the pharmaceutical market, screening for new antidiabetic sources from natural plants is still attractive because they contain substances that have an alternative and safe effect on diabetes mellitus[19].

Solanum nigrum Linn is a well known traditionally used medicinal plant. It is reported to possess antihelmintic, anti-inflammatory, antidiabetic antimicrobial, antihyperlipidemic, antitumour and neuro pharmacological properties[20]. Although literature on the hypoglycemic activity of the plant as a whole is abundant, the comparative efficacy of each part is not known. Hence this study will highlight the hypoglycemic activity and pharmacognostical evaluation of each part of the traditional medicinal plant.

MATERIAL AND METHODS

Plant

Solanum nigrum Linn (Solanaceae), fruits, leaves and stem were collected during June-July from Tirunelveli region of Tamilnadu State, India and were identified at C.C.R.A.S. Govt. of India, Tirunelveli, Tamilnadu.

Extraction

The different plant parts were shade dried and powdered coarsely. Aqueous and hydro alcoholic (methanol-water) extracts were prepared by cold maceration method and then filtered and concentrated in vacuo.

Animals

Sprague Dawley rats weighing 250-300g were used. The animals were acclimatized for 10-12 days and were fed with standard pellet diet and water *ad libitum*. They were housed in polypropylene cages sand maintained under standard environmental conditions. Six animals were used for each test sample. The experimental protocols were subjected to the scrutinization of the Institutional Animal Ethics Committee and were cleared by the same.

Oral glucose tolerance test

The animals were deprived of food for 14 hours before and during the experiment but were allowed free access of water. Seventy two animals were divided into 12 groups of 6 animals each as follows.

GROUP1- Standard metformin treated animals (500mg/kg body weight)

GROUP2- Normal animals

GROUP3- Solanum fruits aqueous extract (200mg/kg body weight) treated animals

(FA-LD)

GROUP4- Solanum fruits aqueous extract (400mg/kg body weight) treated animals

(FA-HD)

GROUP5- Solanum fruits hydroalcohol extract (200mg/kg body weight) treated animals

(FHOH - LD)

GROUP6- Solanum fruits hydroalcohol extract (400mg/kg body weight) treated animals

(FHOH - HD)

GROUP7- Solanum leaf aqueous extract (200mg/kg body weight) treated animals

(LA- LD)

GROUP8- Solanum leaf aqueous extract (400mg/kg body weight) treated animals

(LA -HD)

GROUP9- Solanum leaf hydroalcohol extract (200mg/kg body weight) treated animals

(LHOH -LD)

GROUP10- Solanum leaf hydroalcohol extract (400mg/kg body weight) treated animals

(LHOH- HD)

GROUP11- Solanum stem aqueous extract (400mg/kg body weight) treated animals

(SA -HD)

GROUP12- Solanum stem hydroalcohol extract (400mg/kg body weight) treated animals

(SHOH- HD)

After overnight fasting, an initial blood sample was taken from the tip of the tail of each rat of different groups under mild anesthesia and blood glucose was measured by strip method. Without delay glucose solution (2g/kg body weight) was administered by a gavage, the animals were kept under observation and blood was collected at 60, 120, 240 minutes respectively for blood glucose estimation.

Pharmacognostical Methods

Ash Values

Total Ash

About 2 g accurately weighed powdered drug from the three samples were incinerated in a silica crucible at a temperature not exceeding 450°C for 4 hours in a muffle furnace (Gallen Kamp hot box) until free from carbon. It was then cooled and weighed. The % w/w of ash with reference to the air-dried drug was calculated at 550°C for 8 hours[21].

Acid insoluble ash

Ash was prepared as above, using 25 mL dilute hydrochloric acid the ash from the dish was washed and boiled for five minutes, filtered, cooled and weighed.

Water soluble ash

It was determined in a similar way to acid insoluble ash using 25 mL of water in place of dilute hydrochloric acid

Determination of extractive value

Accurately weighed 5 g of air-dried powdered drug was macerated with 100 mL of 90% alcohol of the specified strength in a closed flask for 24 h, shaken frequently during first 6 h and allowed to stand for 18 h. It was then filtered rapidly, taking precautions against loss of the solvent and 25 mL of the filtrate were evaporated to dryness in a tared flat-bottomed shallow dish and dried at 100°C to constant weight. The % w/w of alcohol soluble extractive value was calculated with reference to the air-dried drug[22].

Crude fibre content

Crude fibre was obtained from the loss in weight on ignition of dried residue remaining after digestion of fat-free samples with 1.25% each of sulphuric acid and sodium hydroxide solutions under specified conditions[23].

$$\% \text{ Crude fibre} = \frac{\text{Loss of weight of ignition}}{\text{Weight of sample used}} \times 100$$

Estimation of Sodium and Potassium

Sodium and Potassium content in different plant parts viz, leaves, fruits and stem were analyzed using Flame Photometer after acid digestion of samples.

Phytochemical Screening

The extracts obtained from the different parts were subjected to preliminary screening to identify the phytoconstituents using different phytochemical tests[23, 24].

Statistical Analysis

Data is presented as means + SEM. One way analysis of Variance (ANOVA) with Dunnett's significant difference post hoc test was used to compare differences among groups. Each sample treated groups were compared with vehicle treated groups. Data was statistically handled through SPSS software version 10. P value <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds[25]. The preliminary phytochemical screening of fractions of different plant parts of *S. nigrum* aqueous and hydroalcoholic extract revealed the presence of alkaloids and flavanoids, phenolics and macronutrients which may be responsible for the observed hypoglycemic effects of these fractions by possibly stimulating insulin release from pancreatic beta cells.

Total ash and fibre contents were higher in the leaves compared to the fruit and stem. The total ash content of the leaves is similar to the values reported for some commonly consumed leafy vegetables including *Ocimum graticinum, Hibiscus esculenta* and *Ipomea batata*. Ash content of the fruits obtained in the present study is 6.723. This value compares favourably with a reported value of 7.18% for *S.nigrum* from Congo Brazzaville [26]. The acid insoluble ash, water soluble ash and total ash of stem was found to be greater than that obtained from the fruit and leaves. The leaves contained the highest content of crude fibre and the aqueous extractive value was found to be high in fruits (table 2).

Table 1: The Phytochemical Components of Different Parts of *Solanum Nigrum*.

Components	Leaf		Fruit		Stem	
	Aqueous	Hydroalcohol	Aqueous	Hydroalcohol	Aqueous	Hydroalcohol
Alkaloids	+	+	+	+	+	+
Cardiac glycosides	-	-	-	-	-	-
Flavonoids	+ +	+ + +	+	+ + +	-	-
Carbohydrates	+ +	+	+ +	+	-	+
Phenolics	++	+ + +	+	+ +	+	+

Iron	++	+	+ +	+ +	+	+
Protein	-	-	-	-	-	-

+++, high concentration, ++, medium concentration, +, low concentration, - not detected

Table 2: Pharmacognostical Evaluation

Name	Extractive Value (%)		Total Ash (%)	Water Soluble (%)	Acid Insoluble (%)	Crude Fibre (%)	Sodium (Ug/Ml)	Potassium (Ug/Ml)
	Aqueous	Hydro Alcohol						
Leaf	21.04	19.22	3.928+0.824	2.316+0.24	1.154+0.15	8.42+0.22	0.75	2.6
Fruit	24.44	12.41	6.723 + 0.56	4.233+0.43	1.686+0.31	15.19+0.22	0.9	1.1
Stem	19.95	19.53	11.907+0.88	10.300+0.1	2.183+0.05	14.73+0.55	1.3	2.2

Statistics -Values are expressed as mean +SD

Table 3: Oral Glucose Tolerance Test

Groups	Treatment dose	Before treatment	60 min	120 min	240 min
GROUP I	METFORMIN500mg/kg body weight	75.333+3.748	84.00+3.983**	77.833+3.198**	77.833+1.833**
GROUP II	CONTROL	78.5+3.085	141.66+ 6.864	147.166+5.747**	123.33+4.978**
GROUP III	(FA-LD)200mg/kg body weight	76.0+1.065	128.67+9.29**	91.83+3.103**	84.66+3.518**
GROUP IV	(FA-HD)400mg/kg body weight	79.167+2.868	180.66+14.662. **	107.50+7.451**	92.833+4.445**
GROUP V	(FHOH - LD)200mg/kg body weight	84.00+3.386	138.666+7.953**	93.83+4.362**	75.00+5.304**
GROUP VI	(FHOH - HD)400mg/kg body weight	81.5+2.500	133.00+10.764 **	107.166+16.538**	77.33+4.828**
GROUP VII	(LA- LD)200mg/kg body weight	80.4+2.400	101.2+5.499**	90.6+2.694**	85+5.263**
GROUP VIII	(LA- HD)400mg/kg body weight	81.4+4.501	100.2+4.409**	80.2+4.532**	76.4+5.519**
GROUP IX	(LHOH -LD)200mg/kg body weight	85.6+7.979	85.6+7.979**	93.2+7.742**	96.6+3.172
GROUP X	(LHOH- HD)400mg/kg body weight	84+3.821	98.4+4.545**	82.2+9.041**	83.2+7.946**
GROUP XI	(SA -HD)400mg/kg body weight	80.8+4.499	96.4+3.326**	94.8+6.507**	106+12.802
GROUP XII	(SHOH- HD)400mg/kg body weight	89+8.240**	95.2+10.161**	92.6+6.933**	88.8+14.412*

Values are expressed as mean + SEM - Values are statistically significant at P< 0.05*, P< 0.01 **

As is the case with other diseases, medicinal plants have been used since ancient times to treat and manage diabetes mellitus in traditional medical systems of many cultures throughout the world[27,28,29]. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible have been traced to the occurrence of natural products with medicinal properties[30]. Currently, medicinal plants continue to play an important role in the management of diabetes mellitus, especially in developing countries, where many people do not have access to conventional antidiabetic therapies [31, 32]. The currently available drug regimens for management of diabetes mellitus have certain drawbacks and therefore there is a need to find safer and more effective antidiabetic drugs [33, 34]. This study was undertaken to evaluate the hypoglycemic activity of *Solanum nigrum* in normal rats. We found that the glucose levels were normal in healthy rats fed with different extracts of *Solanum nigrum* Linn plant and no mortality was observed indicating that there were no acute toxic effects of *S.nigrum* Linn feeding. The effect of aqueous and hydroalcoholic extracts of the three different parts of *S. nigrum* Linn i.e. fruit, leaf and stem (dosage- 200mg and 400mg/kg body weight) on Sprague Dawley rats are shown in the table 3. The supplementation with fruit and leaf extract improved the glucose tolerance in the fasted normal rats. The blood glucose levels of rats-treated with glucose (2g/kg body weight) peaked after 60 min and this was considered as the hyperglycemic state. For the metformin control there was a significant decrease in the BGLs of rats at 120 and 180 min. The hydroalcoholic and aqueous extract of stem did not show any significant change whereas the hydroalcoholic extract of fruit and aqueous extract of *S. nigrum* leaf showed significant hypoglycemic activity and almost similar to the metformin control, which is consonant with the study conducted by earlier researchers [35, 36]. Further it was noticed by other researchers that the chronic administration for longer duration leads to significant decrease in blood sugar compared to control [36]. Thus it can be concluded that *Solanum nigrum* also has the anti- diabetic property.

CONCLUSION

With the current trend on increasing awareness in traditional medicine, the plant derived agents have been attracting much interest as natural alternatives to synthetic compounds. Scientists are trying to tap the pharmaceutical and food values of these unidentified plants. It is postulated that these plants (traditional medicine) will be a major source of new chemicals and raw materials for the pharmaceutical industry. As many plants found are in the wild regions, there is a need to have them grow at the local level. Also more of these compounds should be subjected to animal and human studies to determine their effectiveness in whole organism systems. In addition, detailed investigations at molecular and cellular levels are necessary to elucidate antimicrobial and other biological activities. This may result in a new era of chemotherapeutic treatment of injection by using plant derived principles.

From this preliminary investigation it has been concluded that the leaves and fruit of *S.nigrum* have significant hypoglycemic activity, the flavanoids present in the plant might be an active component responsible for this activity. We progress on to isolate the active biocomponent responsible for the activity.

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