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ANTI-HYPERGLYCEMIC ACTIVITY OF ETHANOLIC EXTRACT OF *SOLANUM SURATTENSE* ROOT

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ABSTRACT: The ethanol extract of *Solanum surratense*(EESS) (Family: Solanaceae) root was investigated for its anti-diabetic effect in Wistar Albino rats. Diabetes was induced in Albino rats by administration of single dose of Alloxan monohydrate (150mg/kg). The ethanol extract of *Solanum surratense* at the dose of 200mg/kg and 400mg/kg, p.o. was administered as single dose per day to diabetes induced rats for a period of 21 days. The effect of EESS root on body weight of the animals, blood glucose, serum lipid profile [cholesterol, triglycerides], serum enzymes[serum glutamate oxaloacetate transamines(SGOT), Serum glutamate pyruvate transaminases(SGPT), alkaline phosphatase(ALP)], total protein were measured in the diabetic rats. EESS root elicited significant ($p< 0.01$) reductions of blood glucose, lipid parameters and serum enzymes and significant ($p<0.01$) reductions of blood glucose. From the above result, it is concluded that EESS root possesses significant anti-diabetic effects in Alloxan induced diabetic rats.

INTRODUCTION: Diabetes mellitus (DM) is a group of metabolic disorder characterized by hyperglycemia, which is associated with abnormalities in carbohydrate, fat and protein metabolism result in chronic complications including micro vascular, macro vascular and neuropathic disorders [1]. Pancreas is an endocrine gland located posterior and slightly inferior to the stomach. The anatomy of pancreas was given **figure 1**. The 99% of pancreatic cells are arranged in clusters called 'acini', and are having 1-2 million tiny groups of endocrine tissues called, pancreatic islets of Langerhans. The pancreatic islets includes four types of hormone secreting cells,

- Alpha cells: Constitute about 20% of pancreatic islet cells, it secret glucagon which elevates blood glucose level.
- Beta cells: Constitute about 70% pancreatic islet cells, it secrete insulin which reduces the blood glucose level
- Delta cells: Constitute the 5% of pancreatic islet cells, it secrete somatostatin which inhibit insulin release.
- F cells: constitute the reminder of pancreatic islet cells, it secrete pancreatic polypeptide, which inhibit the secretion of somatostatin and pancreatic digestive enzymes.

Insulin and various types of hypoglycemic agents such as biguanides and sulphonylureas old and new are available for the treatment of diabetes [2].

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However, none of this medication is ideal due to toxic side-effects and in some cases diminution of response after prolonged use [3]. Medicinal plants and their bioactive components are used for the treatment of diabetes mellitus throughout the world, especially in countries where access to the conventional anti-DM agents is adequate [4]. Although several medicinal plants gained importance for the treatment of DM, many remain to be scientifically investigated.

Many plants reported useful for the treatment of DM in the ayurvedic system of medicine have been tested for their hypoglycemic activity. The medicinal plant *Solanum surratense* is a very popular plant belonging to the family Solanaceae was found to have anti-diabetic properties in leaf [5]. The present study was conducted to investigate the anti-diabetic activity of *Solanum surratense* roots in Alloxan induced diabetic rats.

MATERIALS AND METHODS:

Plant material: The plant *Solanum surattense* belonging to family Solanaceae is widely found to occur throughout India, often waste land on road side and in open scrublands. The plant was collected from ABS Botanical garden, Kaaripatti, Salem District, Tamil Nadu. And identified confirmed by Dr. A. Balasubramanian, Executive Director of A.B.S. Botanical Gardens, Kaaripatti, Salem, Tamil Nadu.

Preparation of extracts: The coarse powdered form of roots of *Solanum surratense* was taken and subjected to successive solvent extraction. The extraction was carried out with the following solvents in increasing order of polarity: Hexane, Ethanol and Aqueous. The solvent was then distilled, evaporated and vaccum dried.

Photochemical analysis: The various extracts of roots of *Solanum surratense* were subjected to the following test for the identification of its various active constituents by standard methods. Carbohydrates were identified by Molisch's test, Fehlings test and Benedict's test. Alkaloids by Dragendorff's test, Mayer's test and Hager's test. Glycosides by Legal Test, Baljet test, Keller Killiani test, Borntrager's test, Flavonoids by Shinoda's test and Sodium hydroxide test, Steroids by Libermann-Burchard test, Salkowski's test, Fixed oils and fats by Saponification [6].

Chemicals: All solvents and chemicals used were of Analytical grade. Nice Chemicals, Cochin. Ranbaxy Laboratories, New Delhi, Sigma Chemicals, Bangalore, SD Fine Chemicals, Mumbai, India.

Animal used: Wistar Albino rats of either sex weighing between 180-250g were used. Animals were obtained from IRC Perundurai, Erode, Tamil Nadu. Animals were housed under standard conditions of temperature (24±2^{o}C) and relative humidity (30-70%) with a 12:12 (light: dark) cycle. The animals were given standard diet and water ad *libitum*. All procedures involving animals were carried out under the Institute ethics committee approval 688/02/C-CPCSEA) of Nandha College of Pharmacy, Erode.TN.

Toxicity studies: Toxicity studies of the *Solanum surratense* root extract were carried out in Swiss Albino mice of either sex weighing between 20 and 25 g [7]. The *Solanum surratense* root extract was found to be safe till 2000 mg/kg (p.o).

Experimental induction of diabetes: Animals were allowed to fast 18 h and were injected with freshly prepared aqueous solution of Alloxan monohydrate (150 mg/kg, i.p.). After the injection they had free access of food and 0.5% glucose solution overnight to prevent from hypoglycemic shock. The development of diabetes was confirmed after 48 hrs of Alloxan injection. Animals were maintained in the diabetic state over a period of 21 days. Serum glucose was measured by reported method. Rats showing fasting serum glucose level (> 200mg/dl) were selected for study [8].

Experimental grouping of animals: Different groups of rats were used to study the effect of ethanolic root extracts of *Solanum surattense*. The rats were divided into five groups each consisting of six rats:

Group I: The rats received Normal Saline. These animals serve as normal controls.

Group II: Received a Single dose of Alloxan monohydrate (150 mg/kg body weight) in normal saline intraperitoneally and served as negative control.

Group III: Received the root extract 200mg/kg for 21 days and served as Test I.

Group IV: Received the root extract 400mg/kg for 21 days and served as Test II.

Group V: Received Glibenclamide 5mg/kg for 21 days and served as positive control.

Biochemical Estimation: After the experimental regimen, the blood was collected through the retro-orbital puncture of eye of animals under mild diethyl ether anesthesia in Eppendorff's tube (1ml) containing 50µl of anticoagulant (10% trisodium citrate) and the serum was separated by centrifuging at 6000 rpm for 15min. The biochemical parameters, Cholesterol, Triglycerides, Total protein, SALP, SGOT, and SGPT were determined [9] by using the commercial kit available (Ecoline, Manufactured by Merck Specialties, Private limited, Ambernath).

Statistical analysis: The collected data were subjected to appropriate statistical test including one way ANOVA, followed by an appropriate Dunnett's t-test, P-value of less than 0.05, 0.01 and 0.001 were considered as less significant, significant and more significant respectively. The analysis was carried out using graph pad prism software.

RESULT AND DISCUSSION:

Phytochemical Analysis: Compounds of different polarity from dried root of Solanum surratense were extracted by sequential extraction process using different solvents such as hexane, ethanol and aqueous. These sequential extracts were subjected to preliminary phytochemical screening for the presence of different chemical constituents. Of all extracts tested, ethanol extract was found to contain the highest number of phytochemicals such as alkaloids, saponins, glycosides, carbohydrates, tannins, flavonoids and triterpenoids (**Table 1**).

Table 1: Data for qualitative phytochemical analysis of various extracts of *Solanum surattense*

Phytoconstituents	Hexane extract	Ethanolic extract	Aqueous extract
Alkaloids	-	+	-
Saponins	-	+	+
Glycosides	-	+	+
Carbohydrates	-	+	+
Tannins & Phenolic compounds	-	+	+
Flavonoids	-	+	+
Steroids	-	+	-
Proteins and Amino acids	-	-	-
Triterpenoids	+	-	-
Fixed Oils and Fats	+	-	-
Gums and Mucilage	-	+	+

(+) Present, (-) Absent

Body weight: Body weight increased significantly ($p< 0.05$ and $P< 0.01$) in all groups except Group I (**Table 2**). All animals ingested normal amounts of food and water during the study period.

TABLE 2: THE EFFECT OF ETHANOLIC ROOT EXTRACT OF *SOLANUM SURATTENSE* ON BODY WEIGHT OF ANIMALS

Groups	Day 1	Day 7	Day 14	Day 21
Normal Saline	233.33±0.88	242.5±0.76	244.33±1.17	246.5±1.78
Alloxan (150 mg/kg)	243.16±2.27**	248±4	250.5±3.66	253.33±3.72*
Glibenclamide (5mg/kg)	246.66±1.30**	225±1.05**	228.66±1.14**	233±1.15**
Test-I (200mg/kg)	243.33±1.30**	250±1.62*	254.3±1.82**	257.5±2.26**
TEST-II (400mg/kg)	220±0.00**	247±1.13**	250.33±1.17*	254.33±1.85**

Data represents mean ± SEM (n=6). *$p< 0.05$, **$p< 0.01$ compared to normal control

Blood glucose level: The effects of root extract on fasting blood glucose level in alloxan induced diabetic rats were given in Table 3. The lower dose of root extract, 200gm/kg produced a slight decrease in fasting blood glucose level on day 21, when compared with diabetic controlled animal. The higher dose of root extract, 400 mg/kg produced a less significant reduction (P<0.05) from 14th day and a significant reduction (P<0.01) from 21st day, in fasting blood glucose level, when compared with diabetic control group. After 21th days treatment the root extract was found to be more potent at the higher dose, 400mg/kg and it brought down the elevated blood glucose level in alloxan induced diabetic rats nearer to the normal range.

TABLE 3: THE EFFECT OF ETHANOLIC ROOT EXTRACT OF *SOLANUM SURATTENSE* ON FASTING BLOOD GLUCOSE LEVEL (mgdl^{-1}) IN ALLOXAN INDUCED DIABETIC RATS

Treatment	1stday	7thday	14thday	21st day
Normal Saline	85.6±0.82	88.3±1.21	88.6±1.2 7	87.3±1.26
Alloxan (150 mg/kg)	217.3±4.61	232.6±3.11	245.3±3.026	275.6±2.75
Glibenclamide (5mg/kg)	224.6±3.13	196±0.82	133.0±1.52**	121.3±1.15**
Test-I (200mg/kg)	231.6±2.02	204.0±3.25	179.3±2.02 *	156.3±1.70 **
TEST-II (400mg/kg)	222.3±2.34	198.2±3.43	154.6±1.27 *	132.5±1.9**

Data represents mean ± SEM (n=6). *p< 0.05, **p< 0.01 compared to normal control

Biochemical Parameters: The higher dose of test drug produced significant reduction in triglyceride level when compared to the diabetic control group and less significant in cholesterol level and total protein level (**Table 4**). Both the lower and higher dose of the test drug produced a significant action (P< 0.01) in SALP and SGPT when compared to normal control (**Table 5**).

TABLE 4: EFFECT OF ETHANOLIC ROOT EXTRACT OF *SOLANUM SURATTENSE* ON CHOLESTEROL, TRIGLYCERIDES, TOTAL PROTEIN OF CONTROL AND EXPERIMENTAL RATS

Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Total protein (g/dl)
Normal control (Normal saline)	109.76±5.36	78.25±2.98	8.46±1.38*
Diabetic control (150 mg/kg Alloxan)	138.31±5.06	108.73±4.57	4.26±0.94**
Glbenclamide (5mg/kg)	113.83±0.6**	81.36**	7.26±1.91**
Test-I (200 mg/kg)	129.76±4.29*	98.46±5.26*	5.63±0.86*
Test-II (400 mg/kg)	115.83±3.30*	83.28±4.09**	6.93±1.22*

Data represents mean ± SEM (n=6). *p< 0.05, **p< 0.01 compared to normal control

TABLE 5: EFFECT OF ETHANOLIC ROOT EXTRACT OF *SOLANUM SURATTENSE* ON SALP, SGOT AND SGPT OF CONTROL AND EXPERIMENTAL RATS

Groups	Salp (IU/l)	sgot (IU/l)	sgpt (IU/l)
Normal control (Normal saline)	265.25 ± 1.62	39.57 ± 0.07	65.37 ± 1.84
Diabetic control (150 mg/kg Alloxan)	432.72 ± 1.61**	82.49 ± 1.42**	109.9 ± 2.52**
Test-I (200 mg/kg)	373± 5.9**	68.30 ± 1.93**	89.4 ± 2.32**
Test-II (400 mg/kg)	323.50 ±4**	51.8± 20*	76.8 ± 1..90**
Glibenclamide (5 mg/kg)	308.14 ± 1.82**	45.30 ±1.43**	73.57 ± 2.28**

Data represents mean ± SEM (n=6). *p< 0.05, **p< 0.01 compared to normal control.

CONCLUSION: The plant *Solanum surratense* (Linn) family Solanaceae is taken for the present study. Aerial parts were selected and they are extracted. Phytochemical tests were done and the plant contains alkaloids, flavonoids, carbohydrates, glycosides, tannins and fixed oils. The results suggest that the alcoholic extract of *Solanum surratense* root possesses significant facilitation of anti-diabetic activity.

Further investigation should be made to elucidate the mechanism of *Solanum surratense* and its role in anti-diabetic activity.

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