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Study of Antidiabetic activity of ethanolic extracts of aerial parts of *Smilax perfoliata* and *Flemingia wightiana*

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ABSTRACT

The present study was conducted to evaluate the antidiabetic activity of ethanolic extracts of *Smilax perfoliata and Flemingia wightiana* against alloxan induced diabetes in rats. The alcoholic extracts of *Smilax perfoliata and Flemingia wightiana (*100mg/kg &200mg/kg*)* were administered orally to the animals with diabetes induced by alloxan. The plant extracts were effective in decreasing blood glucose levels. The results were compared with the effects of standard drug glibenclamide (0.25 mg/kg p.o). The preliminary phytochemical screening of the extracts of the two plantns revealed the presence of flavoniods, tannins and alkaloids which may be responsible for the antidiabetic activity.

Keyword : *Smilax perfoliata Flemingia wightiana*, Alloxon induced, Antidiabetic study

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INTRODUCTION

Diabetes-mellitus is a chronic disease characterized by elevated blood glucose levels and disturbance in carbohydrate, fat and protein metabolism. These metabolic abnormalities result, in part, from a deficiency of the blood sugar-lowering hormone insulin. This deficiency in insulin results in type 1 diabetes or insulin dependent diabetes mellitus (IDDM). Type 2 diabetes or non-insulin dependent diabetes mellitus (NIDDM) is a result of hyperglycemia caused by overproduction of glucose at the hepatic level or because of abnormal cell function or insulin resistance at target cells [1,2]. Currently available synthetic antidiabetic agents produce serious side effects like hypoglycemic coma and hepatorenal disturbances. Moreover, they are not safe for use during pregnancy [3,4]. Hence, the search for safer and more effective hypoglycemic agents is continuing. Smilax perfoliata and Flemingia wightiana are used in folklore for many ailments for the past several years which are available in the hilly regions of southern Andhra Pradesh [5]. The present study focuses on the scientific study antidiabetic potential of these two plants

MATERIALS AND METHODS

Animals

Wistar albino rats weighing between 200-250 gm were employed for antidibetic activity. These animals were housed under standard environmental conditions (temperature of 22 $\pm$ 1°C with an alternating 12 hour light–dark cycle and relative humidity of 60 $\pm$ 5 %), one week before the start and also during the experiment as per the rules and regulations of the Institutional Animal Ethics committee. They were fed with standard laboratory diet and water was allowed *ad libitum* during the experiment [6].

Plant Material

Fresh plats were obtained from Tirumala hills of Andhra Pradesh identified and authenticated by the botany department of S V University, Tirupati. The plants were cleaned, shade dried and milled into coarse powder by mechanical grinder.

Preparation of Extract

The coarse powder of the plants was extracted using ethanol as solvent by soxhlet apparatus. The solvent was removed under pressure to a semi solid mass. Standard methods were used for screening the phytochemicals [7] and it was found that the plants contain alkaloids, flavonoids, tannins and sterols.

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Chemicals

All the chemicals, solvents and reagents used in this experiment were of analytical grade. Alloxan ((Loba chemie, Mumbai), glucose kits (Span Diagnostic Ltd., Surat, India). Glibenclamide (Daonil, Hoechst, India,) were used in this experiment.

Experimental Protocol

The animals were acclimatized to the laboratory for a period of one week prior to the experiment. About 70 animals as described above were selected for the experiment; six animals were kept separately as normal control group (Group 1). Remaining 64 animals were made diabetic by a single intraperitoneal injection of alloxan (150 mg/kg of body weight) dissolved in citrate buffer (P^H 4). Since alloxan may produce fatal hypoglycaemia due to massive pancreatic insulin release, rats were treated with 20% glucose solution (15 – 20 ml) intraperitoneally after 6 h. The rats were provided with 5% glucose solution bottles in their cages for the next 24 h to prevent hypoglycaemia [8]. After 15 days, rats that were exhibiting moderate form of diabetes (> 300 mg/dl) were selected for experiment.

The diabetic surviving animals (as described above) were grouped into six, containing six animals in each group where as the non-diabetic induced animals were used as Group 1.

Group-1: Normal control animals administered with 10 ml/kg of 2% v/v aq. Tween 80 solution per orally.

Group-2: Diabetic control animals administered with 10 ml/kg of 2% v/v aq. Tween 80 solution per orally.

Group-3: Diabetic surviving animals administered with EESP 100 mg/kg per orally once daily for 30 days

Group-4: Diabetic surviving animals administered with EESP 200 mg/kg per orally once daily for 30 days

Group-5: Diabetic surviving animals administered with EEFW 100 mg/kg per orally once daily for 30 days

Group-6: Diabetic surviving animals administered with EEFW 200 mg/kg per orally once daily for 30 days

Group-7: Diabetic surviving animals administered with Glibenclamide 0.25 mg/kg per orally once daily for 30 days

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Blood was collected from the inner canthus of the eye under light ether anaesthesia using capillary tubes in fresh vials containing heparin 5000 IU as anticoagulant agent. Serum was separated in an ultra centrifuge at 2000 rpm for 2 min [9].

Glucose levels were estimated by commercially available glucose kits based on glucose oxidase method.

All the values were expressed as Mean± S.D. The differences between control and treatment groups were tested for significance using ANOVA and P<0.05 were considered significant. The results are shown in the table and the bar diagram below.

Effect of EESP and EEFW on glucose levels (mg/dl) in alloxan induced diabetic rats

	Basal	Day 0	Day 15	Day 30
Group 1	79.3±1.9*	78.29±2.3*	80.2±2.9*	79.1±2.1*
Group 2	78.6±3.6*	343.6±3.4	351.6±6.2	361.2±6.7
Group 3	78.2±3.1*	349.2±2.6*	311.3±4.2 (↓ 10.9%)	301.1±4.6 (↓ 13.8%)
Group 4	79.1±5.2*	347.9±4.8	302.6±3.9* (↓ 13.0%)	282.6±3.9 (↓ 18.8%)
Group 5	80.2±1.9*	351.2±1.9*	281.3±2.8* (↓ 19.9%)	231.5±4.2* (↓ 34.1%)
Group 6	76.1±2.7*	347.9±2.8*	264.1±4.5* (↓ 24.1%)	209.5±2.8* (↓ 39.8%)
Group 7	79.8±2.9*	346.7±3.7*	250.6±3.9* (↓ 27.7%)	181.4±1.9* (↓ 47.7%)

(N = 6), **Group 1** (2% v/v aq. Tween 80 solution, 10ml/kg): Normal control; **Group 2** (2% v/v aq. Tween 80 solution (10ml/kg): Diabetic control; **Group 3:** Ethanolic extract of *Smilax perfoliata* (100 mg/kg. p.o.)*;* **Group 4:** Ethanolic extract of *Smilax perfoliata* (200 mg/kg. p.o.)*;* **Group 5:** Ethanolic extract of *Flemingia wghtiana* (100 mg/kg. p.o.)*;* **Group 6:** Ethanolic extract of *Flemingia wightiana* (200 mg/kg. p.o.)*;* **Group 7:** (Glibenclamide, 0.25 mg/kg, p.o.). Values are given as mean ± SEM. Values in parenthesis indicates the percentage lowering of plasma glucose in comparison to the control. Diabetic control was compared with normal control, treated group was compared with basal values at 10th day. Values are statistically significant at *p<0.01.

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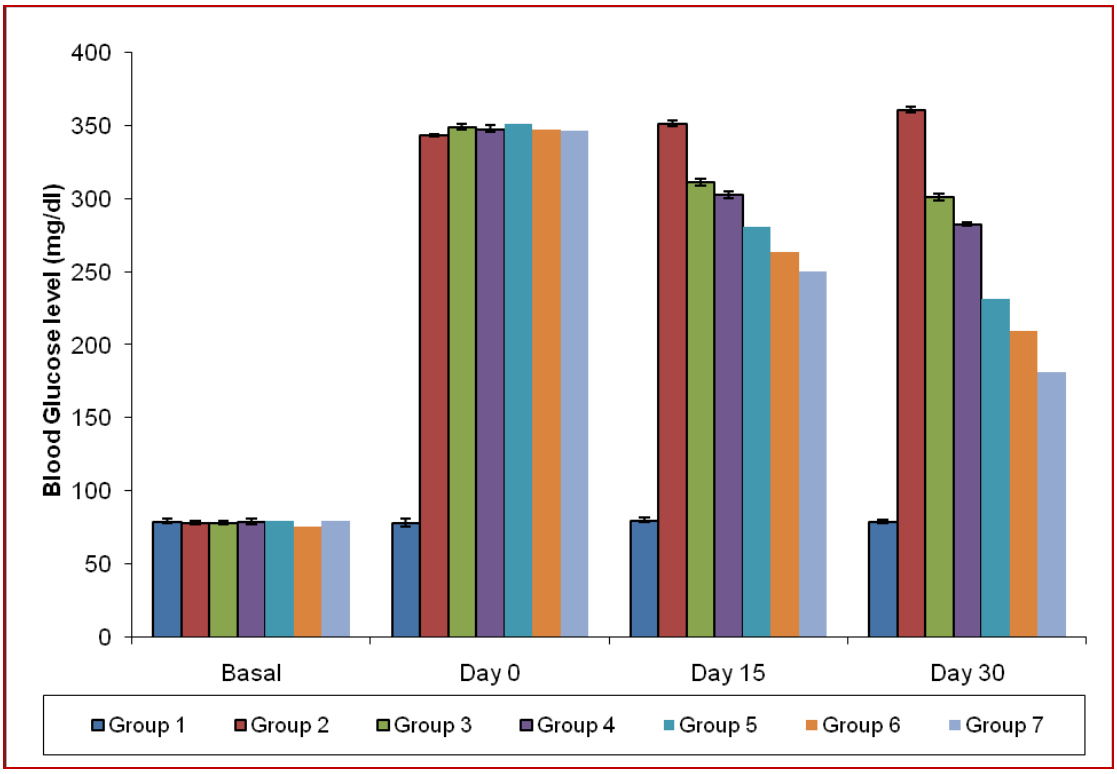

Effect of EESP and EEFW on glucose levels (mg/dl) in alloxan induced diabetic rats

RESULTS AND DISCUSSIONS

Plasma glucose level increased above 300-mg/dl forty-eight hours after administration of alloxan while non – diabetic control group remained unchanged. Oral administration of EEFW at doses of 100 and 200 mg/kg p.o. decreased the blood glucose levels by 19.9% and 24.1% on the 15th day and 24.1% and 39.8% on the 30th day of the experiment respectively. Under similar conditions, Glibenclamide at a dose of 0.25 mg/kg p.o. decreased the blood glucose level by 27.7% on the 15th day and 47.7% on the 30th day of the experiment. Since alloxan permanently destroys the pancreatic β cells, lowering of blood glucose level in alloxanised rats after administration of the extracts indicates that the extract possesses extra pancreatic effects [10]. From the phytochemical analysis it was found that the major chemical constituents of the extracts were alkalloids, flavanoids and tannins. As many as 150 plants having antidiabetic potential were found to posses flavanoids and tannins as active principles [11]. On the basis of the above evidences, the antidiabetic activity of both the extracts may be due to presence of flavanoids and tannins[12]. Further, EEFW was found to be superior to EESP in antidiabetic potential and this may plausibly due to the presence of substantial quantity of alkaloids.

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