



ANTIHYPERGLYCEMIC EFFECT OF METHANOLIC EXTRACT OF ARTEMISIA PALLENS WALL IN STREPTOZOTOCIN INDUCED DIABETES MELLITUS

Mrs. Anusha Murali, Research Scholar, SRK University, Bhopal, India.

Dr. Anusha Bhaskar, Senior Scientist, Sri Venkateshwara Research Centre, Thanjavur.

Abstract

Aim: The hypoglycemic effect of the methanolic extract of the aerial parts of *Artemisia pallens* (100, 200 and 500 mg/kg body weight) was examined in normal, glucose load conditions streptozotocin (STZ) - induced diabetic rats.

Materials and methods: The aerial parts of *Artemisia pallens* was extracted with methanol and its hypoglycemic activity was tested in normal and streptozotocin-diabetic animals at oral doses of 100, 200 and 500 mg/kg and compared with a group of animals treated with the reference drug tolbutamide.

Results: In the normal rats, the methanolic extract of *Artemisia pallens* (200 mg/kg) significantly reduced the blood glucose levels after an oral glucose load from 130.9 ± 5.5 to 83.6 ± 3.4 mg% 2h after oral administration of the extract. A significant reduction in blood glucose was also observed in STZ diabetic rats from 241.0 ± 6.6 to 97.7 ± 5.7 mg% after 21 days of daily oral administration of the extract ($p < 0.001$).

Conclusion: We observed that 200 mg/kg extract gave the best result, however the 100 mg/kg was also effective in reducing the blood glucose. However, when the dose was increased to 500 mg/kg the extract did not have the desired effect. These findings establish the fact that long term administration of the extract may have a good anti-hyperglycemic action and since only low dose are active it could be a good source of hypoglycemic compounds.

Conflict of Interest: The authors have no conflict of interest.

Keywords: *Artemisia pallens*; *Streptozotocin*; *Blood glucose levels*; *antihyperglycemic effect*.

Introduction

The term diabetes mellitus describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycaemia (high blood sugar) with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. In 2006, according to the World Health Organization, at least 171 million people worldwide suffer from diabetes. The incidence is increasing rapidly and it is estimated that by the year 2030, this number will double [1]. Diabetes is a common and very prevalent disease affecting the citizens of both developed and developing countries [2]. The greatest increase in prevalence is however, expected to occur in Asia and Africa, where more patients will likely be found by 2030. Statistical projections from India suggested that the number of diabetes will rise from 15 million in 1995 to 57 million in the year 2025, thus making India the country with the highest number of diabetics in the world. [3, 4].

The search for appropriate antihyperglycemic agents has been focused on plants after the WHO recommendation in 1980 [5]. The pharmaceutical drugs are expensive and also have side effects on prolonged use. For this reason a renewed attention to complementary medicine has grown popularity. Because of their perceived effectiveness, with minimal side effects in clinical experience and relatively low costs, herbal drugs are prescribed widely, even when their contents of the biologically active constituents are unknown [6].

Artemisia pallens Wall ex Dc (Asteraceae) is popularly known as Sage Brush or Wormwood; it is widely distributed in South Africa and South America and also found South in India. This genus is named in honor of Artemis the Greek goddess of chastity. This plant has been screened for the following pharmacological activities antimicrobial [7], antidiabetic [8] and antinociceptive [9] and would healing activity [10]. The objective of the present study is to test the antihyperglycemic effect of *Artemisia pallens* in streptozotocin induced diabetic rats.

Materials and methods

Plant material

The aerial parts of *Artemisia pallens* were collected from Pachamallai Hills, Thuraiyur district, Tamil Nadu. The plant species was identified by Dr. S. Kalavathy, Professor of Botany, Bishop Heber College, Trichy, Tamil Nadu, India. The plant materials were dried under shade and were coarsely powdered and stored in well closed container till further use.

Extraction

The coarsely powdered aerial part of *Artemisia pallens* (1 g) was extracted overnight with 1:1 methanol (10 ml for each extraction). The extracts were combined and concentrated under reduced pressure by rotary evaporation. The residue (1.25%) was preserved until study [11].

Animals

Male Wistar rats (weighing 150 – 200 g), obtained from Bharathidasan University were maintained under standard environmental conditions (12:12 h dark cycles) and fed with a standard diet (Hindustan Lever, India) and water ad libitum.

All the studies were conducted in accordance with the National Institute of Health guide [12]. The work was carried out in CPCSEA approved (743/03/abc/CPCSEA dt. 3.3.03) Animal House of PRIST University, Thanjavur. (Ethical Committee details)

Chemicals

Streptozotocin (STZ) was obtained from Sigma Chemical C., St. Louis, MO, USA. All other chemicals were of analytical grade.

Preliminary tests

Phytochemical tests: This test was done to show the presence or absence of secondary metabolites according to the methods described by Harbourne [13].

Acute toxicity test

This test was to ascertain safety of the plant in mice and was done according to the method described by Lorke [14] with a little modification.

Assessment of hypoglycemic activity in normal healthy rats

The animals fasted overnight were divided into five groups of 6 animals each. Control rats (group I) were given vehicle (distilled water) only, while group II, III and IV received *A. pallens* methanolic extract suspended in distilled water orally at doses 100, 200 and 500 mg/kg, respectively. The fifth group received 200 mg/kg of tolbutamide, used as a reference standard drug. Blood glucose levels were estimated before and after 1,2,4,6 h of extract administration.

Assessment of anti-hyperglycemic activity in normal rats oral glucose tolerance test (OGTT) [15]

After overnight fasting, the rats were given the reference drug and test extracts orally and 30 min later glucose (10 g/kg) was administered orally. Blood samples were collected before the administration of the glucose and after 30, 60, and 120 min.

Assessment of plant extract on STZ diabetic rats

Rats were made diabetic by single intraperitoneal administration of streptozotocin (55 mg/kg) dissolved in 0.1 M citrate buffer, pH 4.5. Forty-eight hours later, blood samples were collected and glucose levels were determined to confirm the development of diabetes. Only those animals which showed hyperglycemia (blood glucose >240 mg/dl) were used in the experiment.

The diabetic rats were divided into six groups of six animals each. Group I received vehicle alone and served as control. Groups II, III and IV received *A. pallens* extract (100, 200 and 500 mg/kg/day) for 4 weeks suspended in vehicle. Group V received tolbutamide (200 mg/kg). Blood samples were drawn at 1, 2, 4 and 6 h after extract administration and then at weekly intervals till the end of the study.

Biochemical analysis

Blood samples were collected and centrifuged. Blood glucose level was measured by glucose oxidase method [16].

Statistical analysis

All experimental data were expressed as mean \pm S.D. The difference between test and controls were evaluated by Student's t-test.

Results and Discussion

The present study was conducted to evaluate the beneficial effect of *A. pallens* extract on blood glucose levels in STZ induced diabetic rats. Streptozotocin-induced hyperglycemia in rodents is considered to be a good model for the preliminary screening of agents active against type II diabetes [17] and is widely used and this causes damage to the pancreatic β cells by nitric oxide free radicals. In the present investigation *A. pallens* showed a significant antihyperglycemic activity. The maximum reduction in glucose levels was observed in groups receiving 200 mg/kg of the extract. It is very interesting to note that the drug exhibited an optimum antihyperglycemic activity at 200 mg/kg body weight and a further increase did not have any significant effect on blood glucose levels. This suggests that the drug at higher doses may not produce hypoglycemia. However blood glucose levels were not altered in normoglycemic rats further strengthening the antidiabetogenic potential of the extract. The main mechanism by which it brings about its antihyperglycemic activity may probably be by stimulating peripheral glucose consumption since streptozotocin causes complete destruction of the β cells of the pancreas. We suggest that maximum utilization is achieved with 200 mg/kg of the plant extract and therefore higher doses are less effective.

Assessment of hypoglycemic activity in normal rats

The effects of the treatment with *A. pallens* extract and tolbutamide on blood glucose concentration in normal fasted and diabetic rats after acute treatment are shown in Table 2.

Normal rats treated with 100 and 200 mg/kg showed a significant fall of 15 and 21.5% in blood glucose respectively, after 6h of oral administration. A dosage of 500 mg/kg body weight did not have the desired effect. While a 24% change was observed with tolbutamide.

Assessment of hypoglycemic activity by OGTT in normal rats

The hypoglycemic effect of the *A. pallens* extract (200 mg/kg) was comparable to that seen in the tolbutamide treated rats (36% vs 33.4%). Treatments with 100 and 200 mg/kg also showed significant reduction in blood sugar and higher dose of 500mg/kg did not show much antihyperglycemic activity (Table 3).

Assessment of hypoglycemic activity on STZ diabetes rats

Diabetic rats were treated with 100, 200 and 500 mg/kg of the *A. pallens* extract showed a reduction in blood glucose of about 8, 16 and 1.2% respectively, after 6 h of treatment (Table 4).

After 3 weeks of daily treatment with *A. pallens* extract (100, 200 and 500 mg/kg) we observed a significant fall in blood sugar levels of 54, 59 and 9% respectively. At the same time tolbutamide caused a significant reduction of plasma glucose levels of 59 % comparable to that of 200 mg/kg dosage (Table 5). The higher dose of 500 mg/kg body weight did not have any profound effect on the blood glucose either after 6 h of treatment or even at the end of the 3 week study period.

The preliminary phytochemical analysis of *A. pallens* carried out in our lab (Table 1) showed presence of alkaloids, proteins, amino acids, carbohydrates, tannins, phenolic compounds, saponins and flavonoids. The antihyperglycemic activity of *A. pallens* may probably be due to the presence of several bioactive compounds; however, the active principle involved in the reduction of blood sugar is to be further studied. However, a good amount of polyphenols; gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, rutin, ferulic acid, quercetin and kaempferol are some of the compounds reported by Niranjana et al [11]. All the compounds found in a single plant may exert their action in a synergistic manner which may be responsible for the observed result.

Conflict of interest

The authors have no conflict of interest.

References

1. American Diabetes Association (2005) Standards of medical care in diabetes (Position Statement). *Diabetes Care* 28 (Suppl 1): S4 – S36.
2. King H, Aubert RE, Herman WH (1998) Global burden of diabetes 1995 – 2025 prevalence, numerical estimates and projections. *Diabetes Care* 21: 1414 – 1431.
3. Boyle JP, Honeycutt AA, Narayan KM, Hoerger TJ, Geiss LS, Chen H, Thompson TJ (2001) Projection of diabetes burden through 2050: impact of changing demography and disease prevalence in the US. *Diabetes Care* 24: 1936 – 1940.
4. Erasto P, Adebola PO, Grierson DS, Afolayan AJ (2005) An ethnobotanical

- study of plants used for the treatment of diabetes in the Eastern Cape Province, South Africa. *Afr J Biotechnol* 4: 1458 – 1460.
5. WHO Expert Committee on Diabetes mellitus 1980 Technical Report Series 646, Second Report, World Health Organization, Geneva.
 6. Valiathan MS (1998) Healing plants. *Curr Sci* 75: 1122 – 1126.
 7. Asolkar LV, Kakkar KK, Chakre OJ (1965 – 1981) Glossary of Indian medicinal plants with active principles. New Delhi: Publications and Information Directorate, CSIR: 92 – 97.
 8. Subramonium A, Pushpangadan P, Rajasekharan S, Evans DA, Latha PG, Valsaraj R (1996) Effects of *Artemisia pallens* Wall on blood glucose levels in normal and aloxan – induced diabetic rats. *J Ethnopharmacol* 50: 13 – 17.
 9. Karunanidhi N, Saravanan S, Kumar V, Ghosh S, Gupta JK (2003) Antinociceptive and sedative hypnotic effect of *Artemisia pallens* Wall. *Natural Product Science* 9: 91 – 92.
 10. Rasal, V. P., Kshirsagar, A., Bagali, R., Rai, S.K. (2005). Influence of *Artemisia pallens* Wall plant on experimental wounds in albino Rats. In: Prabhuji, S.K., Rao, G.P., Patil editors *Recent Advances in Medicinal Plant Research*. New Delhi: Satish Publication House; 393-401
 11. Niranjana A, Barthwal J, Lehri A, Singh DP, Govindrajana R, et al. (2009) Development and Validation of an HPLC – UV – MS – MS Methods for Identification and quantification of polyphenols in *Artemisia pallens* L. *Acta Chromatographica* 21: 105 – 116.

12. National Institute of Health Guide for the Care and Use of Laboratory animals. DHEW Publication (NIH), revised, Office of Science and Health Reports, DRR/NIH, Bethesda, USA 1985.
13. Harbourne JB Phytochemical methods: a guide to modern techniques of plant analysis, 2nd ed. London: Chapman and Hall 1984.
14. Lorke D (1983) A new approach to practical acute toxicity testing. Arch Toxicol 53: 275 – 289.
15. Du Vigneaud V, Karr VJ (1985) Carbohydrate utilization and disappearance. J Biol Chem 66: 281 – 300.
16. Triender P (1969) Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annu Clin Biochem 6: 24 – 27.
17. Ivorra Md, Paya M, Villar A (1989) A review of natural products and plants as potential antidiabetic drugs. J Ethnopharmacol 27: 243 – 275.