J MEDICINE 2010; 11 : 17-19

ANTI-HYPERGLYCEMIC EFFECT OF *STEPHANIA GLABRA* TUBERS IN ALLOXAN INDUCED DIABETIC MICE

DEEPAK KUMAR SEMWAL[1], USHA RAWAT[1], RUCHI BADONI[1], RAVINDRA SEMWAL[2], RANDHIR SINGH[3]

Abstract

Different doses of ethanolic extract of Stephania glabra *tuber were evaluated for anti-hyperglycemic activity in alloxan induced diabetic mice. The oral administration of 100, 200 and 500 mg/kg body weight showed significant hypoglycemic activity. Glibenclamide (oral hypoglycemic agent, 25 mg/kg,* p.o.*) has been used as standard.*

Key words: Stephania glabra*, Menispermaceae, hydroxypalmatine, alloxan, glibenclamide, hypoglycemic activity*

Introduction

In continuation of our work on phytochemistry and biological activity of *Stephania glabra* tuber, the anti-hyperglycemic activity of the ethanolic extract was evaluated in alloxan induced diabetic mice.[1,2]

Diabetes mellitus is a chronic, worldwide heterogeneous and life-threatening disease which is most common metabolic disorder, characterized by hyperglycemia, glycosuria, hyperlipemia, negative nitrogen balance and some times by ketonemia. The prevalence of diabetes will be 5.4% by the year 2025, with the global diabetic population reaching to 300 million. Among all the WHO regions, South East Asian region are highest affected with maximum global burden of the disease and by year 2025 there will be nearly 80 million diabetic in the region.[3,4] However, there are many plants present in nature which possess marked hypoglycemic activity. These plants can be used in the treatment of diabetes either as a crude extract or individual component isolated from such plants which is responsible for the antidiabetic activity.

Stephania glabra of family Menispermaceae is a large, climbing shrub, indigenous to lower Himalaya. The tubers of the plant used for the treatment of variety of disorders, including asthma, tuberculosis, dysentery and fever. It is also used as psycomedicine by natives of India.[5] In this paper, we report the potent hypoglycemic activity by oral administration of ethanolic extract from *Stephania glabra* tubers in comparison of standard drug.

Material and Methods

Collection of Plant material

The Tubers of Stephania glabra were collected from Chaka, nearby Chandravadani temple (Tehri Garhwal) during October 2006 and identified from Taxonomic Laboratory, Department of Botany H.N.B. Garhwal University, Srinagar. A voucher specimen (GUH- 17600) was kept in Departmental Herbarium.

Preparation of the extract

Coarsely powdered tubers of the plant were extracted three times with 95% ethanol at 50°C for 15 hours. The extraction mixture was filtered and concentrated upto dryness under reduced pressure for evaluation of antidiabetic activity.

Preparation of doses

The oral doses of *Stephania glabra* (SG) tubers at 100, 200 and 500 mg/kg, *p.o.*, body weight were prepared in distilled water for determination of

1. Department of Chemistry, University of Garhwal, Srinagar, U.K. 246174, India
2. Department of Pharmaceutics, K.N. Modi, Institute of Pharmaceutical Education and Research, Modi Nagar, India
3. Department of Pharmaceutical Sciences & Drug Research, Faculty of Medicine, Punjabi University, Patiala, P.B., 147002, India

Correspondence: Deepak Kumar Semwal, Department of Chemistry, University of Garhwal, Srinagar, India, E- mail: dr_dks.1983@yahoo.co.in

hypoglycemic effect whereas the oral doses of 100, 200, 500 and 1000 mg/kg, *p.o.*, were prepared for LD_{50} experiments. Glibenclamide (as standard) 5 mg/kg, *p.o.* was prepared with distilled water.

Study of test drug and positive control on experimental animals

Swiss albino mice of either sex (35-50 g body weight) were employed for present study. These animals were deprived to food for 16 h but allowed free access to water. They were housed in the departmental animal house and exposed to normal light. Experiments were performed according to the guide for the care and use of laboratory animals, from the CPCSEA, Ministry of Environment and Forest, Govt. of India (Reg. No.-107/1999/ CPCSEA). After deprived to food for 16 h, mice were divided into six groups (six animals each), (I-VI), namely normal control, diabetic control, diabetes + SG-100, diabetes + SG-200, diabetes + SG-500 mg/kg and positive control. Induction of diabetes was performed using a modification in the method described by Shan et al.[6] The diabetes was produced by an injection of alloxan (60 mg/kg, dissolved in saline) in the tail vein of mice. The diabetic state was assessed by blood glucose levels 36 h later of alloxan administration, the mice having blood glucose more than 150 mg/dL were only selected for the study. Animals which presented glucose levels lower than 150 mg/dL were rejected. The group of normal control (I) was not administered by alloxan and only received distilled water. Rest of the groups (II-VI) received alloxan and 36 h later were treated with distilled water (diabetic control), group III-V with 100, 200 and 500 mg/kg, *p.o.* respectively of SG extract and group VI was treated with glibenclamide 5 mg/kg, *p.o.* as standard. Blood samples of normal and alloxan induced diabetic mice were collected at 0, 1, 3, 6, 18 and 24 h during the treatment. In each case, 10 µL of serum sample was collected and estimated for glucose by GOD-POD method.[7]

LD_{50} Experiment

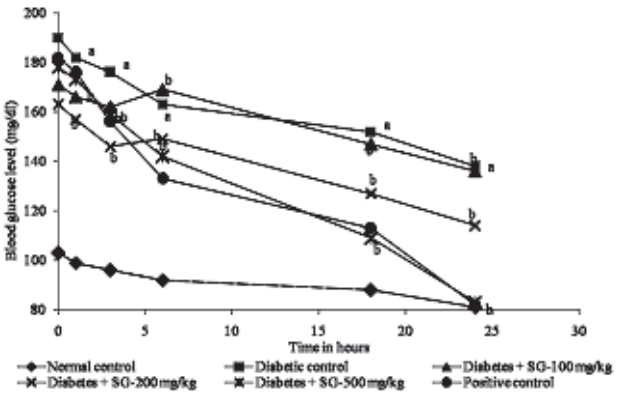
The mice were administered SG, orally at doses of 100, 200, 500 and 1000 mg/kg, *p.o.*, body weight and observed continuous for 1 h intermittently up to 24 h for any gross behavioral changes and deaths.

Data and statistical analysis

Results are expressed as the mean ± S.E.M. of 6 independent experiments. The data were analyzed for statistical significance by one-way ANOVA test; P values < 0.05 were considered to be significant.

Results

The hypoglycemic effect of the different doses of ethanolic extract of SG tubers on alloxan induced diabetic mice is given in Figure 1. The fasting blood glucose levels of diabetic untreated mice (Group 2) were significantly higher than those of normal untreated mice (Group 1). The ethanolic extracts of SG tubers at a dosage of 500 mg/kg *p.o.*, produced the maximum fall of 53% whereas the mice treated with glibenclamide at a dose of 5 mg/kg *p.o.*, resulted in 54% fall in the blood glucose levels which is almost similar to that of 500 mg/kg dose. The doses of 100 and 200 mg/kg produced a slight increase in the blood glucose level from 3 to 6 h whereas a continuous decrease in the blood glucose level by 19% and 33%, respectively was observed upto 24 h of treatment. The extract was found most effective in high concentration i.e. 500 mg/kg which produced a continuous reduction in the blood glucose. The fasted normal mice (group 1) reduced their blood glucose by 21% whereas the alloxan induced diabetic group (group 2) reduced the blood glucose level by 26%. From the above discussion, it may conclude that the SG tuber extract in low concentration is either poor active or inactive since it reduced the blood glucose by 19% in comparison of diabetic control group (26%). The doses of 100, 200, 500 and 1000 mg/kg *p.o.,* were



Values are mean ± S.E.M. for six animals, standard deviation observed: ± 0.5-2.5.

Abbreviation: a = $p < 0.05$ Vs Normal control, b = $p < 0.05$ Vs Positive control; SG= Stephania glabra and Positive control= glibenclamide

Fig. 1. *Hypoglycemic effect of SG tubers extract in alloxan induced diabetic mice*

also taken for LD_{50} experiment in separate animals for determination of side effects but no lethality or deaths were observed upto 21 days of the experiments.

Discussion

In the present study, ethanolic extract of tubers of SG at a dose of 500 mg/kg could produce a significant fall in blood glucose levels by about 52% in diabetic mice, after 24 h of treatment. But in low concentration i.e. 100 mg/kg produced poor hypoglycemic effects. Hence, the extract in high concentration may be taken for hypoglycemic action without causing any side effect unlike insulin and other synthetic drugs. The potency of SG extract may be increased by the purification of the extract or isolation of active constituents from the extract. Alloxan produced significant increase in blood glucose level by damaging pancreatic â-cells, resulting decrease in endogenous insulin secretion, which decreases the utilization of glucose by the tissues and thus called an effective diabetes-induction agent.[8] In present case, alkaloids are the major constituents of the plant and these may perhaps responsible for the activity. The antidiabetic activity caused by positive control glibenclamide in alloxan-induced diabetic mice is an indication of the presence of some beta cells, as glibenclamide is known to stimulate insulin secretion from beta cells.[9]

From the present study we may conclude that SG extract, in totality, was effective in reducing the blood glucose level in dose dependent manner under our experiment conditions and the extract was found to be safe for further biological studies as no lethality was observed upto 1000 mg/kg per oral in mice. The SG tubers extract having long duration glucose lowering action because maximum effect was observed upto 24 h. However, further investigations are required to carry out the purification and identification of the antidiabetic components of SG extract and to elucidate the mechanism of hypoglycemic effect of the SG extract.

Acknowledgement

Our sincere thanks to CPCSEA, Ministry of Environment and Forest, Govt. of India for permission of experiments on animals.

References

1. Semwal, D.K., Rawat, U. Antimicrobial hasubanalactam alkaloid from *Stephania glabra*. Planta Med 2009a; 75: 378-380.
2. Semwal, D.K., Rawat, U. Gindarudine, a novel morphine alkaloid from *Stephania glabra*. Chin Chem Lett 2009b (in press).
3. Semwal, D.K., Bamola, A., Rawat, U. Chemical Constituents from Some Antidiabetic Plants. Univ J Phytochem Ayur Heig 2007; 2 (3): 40-48.
4. World Health Organization. Diabetes Mellitus: Report of a WHO Study Group. WHO Technical Report Series 727, Geneva, 1985.
5. Gaur RD. Flora of District Garhwal North West Himalaya (With ethno botanical notes), 1st ed. TransMedia, Srinagar Garhwal, India. 1999:76.
6. Shan, J.J., Yang, M., Ren, J.W. Anti-diabetic and hypolipidemic effects of aqueous-extract from the flower of *Inula japonica* in alloxan-induced diabetic mice. Biol Pharm Bull 2006; 29: 455-459.
7. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem 1969; 6: 24.
8. Ryle PR, Barker J, Gaines PA, et al. Alloxan-induced diabetes in rat- Protective action of (-) epicatechin. Life Sci 1984;34:591-595.
9. Rao BK, Sudarshan PR, Rajasekhar MD, et al. Antidiabetic activity of *Terminalia pallida* fruit in alloxan induced diabetic rats. J Ethnopharmacol 2003; 85: 169–172.