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Anti-diabetes and hypoglycaemic properties of *Hemionitis arifolia* (Burm.) Moore in rats

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Abstract

Hemionitis arifolia, a folklore anti-diabetes fern, was evaluated for its hypoglycaemic and anti-diabetic properties using rats. Glucose lowering effect and anti-diabetes activity were studied using glucose tolerance test in normal rats and alloxan diabetic rats, respectively. When different extracts were tested, the ethanol and, to some extent, the water extracts were found to lower the levels of blood glucose in glucose fed rats. The ethanol extract showed optimum activity at 200 mg/kg. The extract exhibited only marginal hypoglycaemic activity in overnight fasted normal rats and it was devoid of conspicuous toxic symptoms in sub-acute toxicity evaluation in mice. When the alcohol extract was fractionated by sequential solvent extraction, the activity was found in ethyl acetate fraction (50 mg/kg). This fraction containing steroids and coumarins showed anti-diabetes activity in alloxan diabetic rats as judged from serum glucose levels, liver glycogen content and body weight. This fraction is an attractive material for further research vis-à-vis drug development.

Keywords: *Hemionitis arifolia*; Alloxan diabetic rats; Anti-hyperglycaemic activity

1. Introduction

Diabetes mellitus (DM) is an important medical problem and it is on the raise (King et al., 1998). The available oral hypoglycaemic agents are insufficient and there is a need for discovering more effective and safe oral hypoglycaemic agents (Ramachandran, 2001). DM is known from ancient time onwards and numerous medicinal plants are used to control diabetes in traditional medicine. In south India, many medicinal plants are traditionally used to treat DM. Some of them were scientifically verified (Subramoniam and Babu, 2003). Previous works in this laboratory revealed the anti-diabetes properties of *Artemisia pallens*, *Geophila reniformis* and *Cassia klenii* (Subramoniam et al., 1996a; Subramoniam et al., 1998; Babu et al., 2002, 2003). Many anti-diabetes plants used in folk and tribal medicine in remote villages in Kerala state, India are not known to the main stream population. Tropical Botanical Garden and Research Institute (TBGRI) is engaged in ethno-medico-botanical studies to bring to light such medicinal plants. Search conducted by one of the authors revealed that sterile fronds of a fern, *Hemionitis arifolia* (Burm.) Moore [family: Hemionitidaceae] is used in folk medicinal practices to treat DM in certain remote villages of Trivandrum district, Kerala state. In ethno-medical practices, the same fern is also used to treat aches and burns (Dixit, 1984; Johnson, 1999). The present study was undertaken to scientifically verify the traditional claim using experimental (normal and diabetic) rats.

2. Materials and methods

2.1. Collection of plant materials

Hemionitis arifolia was collected from the forest area at Njaranneeli, Trivandrum district and identified by the taxonomists of TBGRI and a voucher specimen (specimen no. 41712) has been deposited in the herbarium of TBGRI.

2.2. Chemicals and reagents

All chemicals and reagents used were analytical grade and purchased from E. Merck India Ltd., Mumbai and SRL, India.

Abbreviations: DM, diabetes mellitus; IU, international unit; WBC, white blood cells; EA fraction, ethyl acetate fraction; GPT, glutamate puruvate transaminase; GOT, glutamate oxaloacetate transaminase; ALP, alkaline phosphatase; KAU, king amstrong unit

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2.3. Preparation of Hemionitis arifolia extracts

The whole plants were cleaned, dried and powdered. To prepare water extract, the powder was extracted with distilled water (5 g/100 ml) with constant stirring for 4 h and then filtered through a filter paper. Residue was again extracted as above with water. The combined filtrate was freeze-dried in a lyophilizer. The yield of the water extract was approximately 24% of the plant powder. (Since the heat sensitivity of the extract with reference to bio-activity is not known, the extraction was carried out at low temperature without using rigorous extraction procedures.) The alcohol extract of the plant powder was prepared similarly using ethyl alcohol instead of distilled water. However, in this case the combined filtrate was evaporated to dryness in a rotary evaporator under reduced pressure at 40 °C. The yield of the alcohol extract was approximately 15% of the plant powder. The hexane extract of the powder was prepared as above using *n*-hexane instead of alcohol. However, to ensure complete extraction, 2 g powder was extracted with 100 ml hexane and the process was repeated three times. The filtrates from the extractions were combined and dried in a rotary evaporator under reduced pressure at 40 °C. The yield of the hexane extract was approximately 2.8% of the plant powder.

2.4. Animals

Inbred Wistar rats (150–200 g weight) and Swiss albino mice (6–7 weeks old), reared in Tropical Botanic Garden and Research Institute (TBGRI) animal house were used. Animals were caged in uniform hygienic conditions and fed with standard pellet diet (Lipton, India Laboratories, Bangalore) and water ad libitum as per the guide lines of Institute Animal Ethics Committee.

2.5. Glucose tolerance test

This was done as described elsewhere (Babu et al., 2002). Rats were divided into indicated number of groups. Control group received the vehicle (2% gum acacia or 5% Tween 80; 1 ml, p.o.). The experimental groups received the herbal drug (water suspension or extracts or fractions) at indicated doses in an identical manner. In the screening study, a relatively high dose [500 mg (dry weight)/kg] of the water suspension of the plant powder was taken to detect activity, if any.

The rats of all the groups were loaded with 60% glucose (3 g/kg, p.o.) 30 min after herbal drug administration. Blood samples were collected by retro-orbital puncture, just 1 min prior to drug administration, and at 30, 90 and 150 min after glucose loading under mild ether anesthesia. Serum glucose levels were measured immediately. Six overnight fasted animals were used in each group.

2.6. Hypoglycaemic study in normal fasted rats

To investigate hypoglycaemic effect, if any, of the ethanol extract, the overnight fasted rats were divided into two groups of six each. Control group received 1 ml of 5% Tween 80 and the experimental group received 200 mg/kg alcohol extract. Blood samples were collected at 0, 120 and 180 min after the extract administration and glucose levels were measured as described above.

2.7. Alloxan-induced diabetic rats

Rats were injected with alloxan (60 mg/kg) through tail vein. Five days later, blood samples were drawn and glucose levels were determined to conform induction of diabetes. The diabetic rats showing blood glucose levels in the range of 400–450 mg/dl were selected for the efficacy evaluation of the herbal drug (Subramoniam et al., 1996a).

2.8. Isolation of an active fraction

The alcohol extract from the plant was suspended in water and sequentially extracted with hexane, chloroform, ethyl acetate and butanol; each fraction was tested for activity using glucose tolerance test. The active ethyl acetate fraction was subjected to chemical analysis to determine the classes of compounds present in it (Wagner et al., 1984). The fraction was tested for the presence of alkaloids (Dragendorff reagent and Mayer's reagent), coumarins (Borntrager reagent), flavonoids (Shinoda test), steroids (Liebermann–Buchard test), and terpenes (vanillin–sulphuric acid reagent). The yield of this fraction was 14% of the alcohol extract.

2.9. Determination of the efficacy of the active fraction in alloxan diabetic rats

The alloxan diabetic rats were divided into three groups of six each. The control group was given 1 ml of 5% Tween 80, p.o. daily. The test group was given daily dose of the active fraction (50 mg/kg). The third group received insulin (5 IU/kg, i.p.; Knoll Pharmaceuticals Ltd., India) daily. Weight and sex matched six normal rats were kept as a normal control group. The treatment was continued for 12 days. (Death started in the diabetic control group of rats on the 12th day.) Blood samples were collected on days 1, 4, 8 and 12. On day 12 animals were killed after blood collection and liver samples were removed for glycogen estimation.

2.10. Estimation of blood glucose and liver glycogen

Serum glucose was estimated spectrophotometrically using a commercial assay kit (Monozyme, India Ltd.). Liver glycogen was estimated by the method of Carroll et al. (1956).

2.11. Toxicity evaluation in mice

To study sub-acute (short term) toxicity, four groups of mice each containing six male mice (20–25 g body weight) were used. One group was kept as control and groups 2, 3 and 4 received 200, 400 and 800 mg/kg alcohol extract, respectively. The drug was administered daily for 30 days (p.o.). Control group received the vehicle in an identical manner.