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Antidiabetic Activity of *Curculigo orchioides* Root Tuber

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Abstract

Curculigo orchioides Gaertn. (Hypoxidaceae) is known as ''talamuli'' or ''musali'' in Ayurveda and as ''nilapanai'' in Siddha. The root tuber is used in the treatment of diabetes and several other diseases. Both alcohol and aqueous extracts were tested with alloxan-induced diabetic rats. Blood glucose levels were evaluated on the 7th, 14th, and 21st days. Doses of 500 and 1000 mg/kg body weight of both extracts produced significant ($p < 0.001$) hypoglycemic activity in alloxanized rats when compared with diabetic control.

Keywords: Antidiabetic activity, *Curculigo orchioides*.

Introduction

Diabetes mellitus is a chronic disease characterized by high blood glucose levels due to absolute or relative circulating insulin levels. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, there is a growing interest in herbal remedies, due to side effects associated with synthetic therapeutic agents (Holman & Turner, 1991; Kameswara Rao et al., 1997). The Indian traditional systems of medicine, especially Ayurveda and Siddha, have put forward a number of therapeutic claims for plant drugs. However, it is important to provide scientific proof and justify the various medicinal uses enunciated in traditional systems.

Curculigo orchioides Gaertn. (Hypoxidaceae) is known as ''musali'' or ''talamuli'' in Ayurveda and ''nilapanai'' in Siddha systems of medicine (Narayana Aiyer & Kolammal, 1963; Yoganarasimhan, 2000). The genus *Curculigo* Gaertn. consists of 10 species, of which three are found in India. These are *C. capitulata* Kuntze, *C. latifolia* Dryand, and *C. orchioides* Gaertner (Santapau & Henry, 1976; Anonymous, 1950).

Curculigo orchioides is an acaulescent herb found in the subtropical Himalayas from Kumaon eastward to Khasi Hills, Manipur, Bihar, Chota Nagpur, West Bengal, and Western Ghats (Sharma et al. 2002). The root tuber is used in the Siddha system of medicine for the treatment of pain, diabetes, and leucoderma, and as an aphrodisiac; in Ayurveda it is used for treatment of diseases such as sprue, piles, disorders of blood, and also as an aphrodisiac and rejuvenator. Further, they are also used for skin trouble, as a demulcent, diuretic, tonic, in diarrhea, piles, jaundice and asthma in combination with aromatics and bitters (Yoganarasimhan, 2000). It is also claimed to possess antioxidant properties (Venukumar & Lathanm, 2002; Wu et al., 2005), anti-inflammatory and hepatoprotective activities (Rao & Mishra, 1996a, 1996b). Curculigenin A and curculigol are reported to possess antihepatotoxic properties (Rao & Mishra, 1997). It is also reported to be used as Yang tonifying herb, which exhibited regulative effects on thyroid dysfunction in thyroidectomized rabbits (Min et al., 1998).

The phytochemical constituents reported in *Curculigo orchioides* are corchioside A, 25-hydroxy-33-methyl-pentatriconta-6-one, 21-hydroxy-tetracontan-20-one, 27-hydroxy-tricontan-6-one, 2-methoxy-4 acetyl-5-methyl tricontane, linoleic, linolenic, arachidic, and 4- methyl heptadecanoic acids, oleic, and palmitic acids, curculigol, curculigenin A, curculigosaponin A–F, cycloartenol, sitosterol and stigmasterol (Chatterjee & Pakrashi, 2001), orcinol-3-*O*-β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside, orcinolglucoside, orcinol-1-*O*-β-D-glucopyranosyl-(1-6)-β-D—glucopyranoside, curculigoside, curculigoside B, curculigoside C, 2,6-dimethoxyl benzoic acid, syringic acid (Wu et al., 2005), orchiosides A–B (Gupta et al., 2005), and esters (Kumari et al., 2004). The current study investigated the antidiabetic activity of *Curculigo orchioides* in rats.

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Materials and Methods

Plant material

Curculigo orchioides was collected from the Botanical Garden of the University of Agricultural Sciences, GKVK Campus, Bangalore, on June 20, 2005. The plant material was identified at the herbarium of Regional Research Institute, Bangalore (RRCBI), and authenticated by Dr. Yoganarasimhan, Department of Pharmacognosy, MSRCP, Bangalore. A voucher herbarium specimen (Richa Joshi 001) has been deposited in the herbarium of PG Department of Pharmacognosy, MSRCP, Bangalore. A voucher sample of the tested material for experimental studies is preserved at the crude drug museum of MSRCP, Bangalore. About 3 kg of fresh root tuber was collected from forests of Tirunelveli, Tamil Nadu, on November 9, 2004. The cleaned tubers were cut into small pieces of 1–2 cm, washed, dried at room temperature, powdered, and sieved through 60 mesh and stored in air tight containers.

Preparation of plant extracts

Alcohol extract

A weighed quantity (500 g) of the air-dried powdered drug was taken and extracted with ethanol (90%) in a Soxhlet extractor. The extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50°C (yield 12.5%w/w, dry weight basis). The ethanol extract was dissolved in distilled water containing 2% v/v Tween 80 (as a suspending agent).

Aqueous extract

A weighed quantity (500 g) of the air-dried powdered drug was taken and macerated with hot water at 80°C. The maceration process was carried out for 24 h. The macerate was filtered through Whatman no. 1 filter paper. The filtrate was concentrated in a rotary flash evaporator (yield 16.2% w/w, dry weight basis) and was dissolved in distilled water for experimental studies.

Animals

Swiss albino mice (20–30 g) and albino rats of the Wistar strain (180 ± 10 g) of either sex were maintained under controlled conditions of light (12 h) and temperature 25 ± 1°C in the animal house of M.S. Ramaiah College of Pharmacy, Bangalore, 2 weeks prior to the experiment for acclimatization. Animals had access to food and water *ad libitum*. Prior to the start of experiment, animals were fasted overnight, with free access to water. All the pharmacological work was carried out as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) norms after obtaining approval from the Institutional Animal Ethics Committee of M.S. Ramaiah College of Pharmacy, Bangalore.

Acute toxicity studies

Acute toxicity studies were carried out on Swiss albino mice by the method proposed by Ghosh (2005). Alcohol and aqueous extracts at doses of 30, 100, 300, 1000, and 3000 mg/kg body weight were administered to separate groups of mice ($n = 6$) after overnight fasting. Subsequent to administration of drug extracts, the animals were observed closely for the first 3 h for any toxic manifestations, such as increased motor activity, salivation, clonic convulsions, coma, and death, followed by observations at regular intervals for 24 h. The observations were continued for a further 7 days. At the end of the experimental period, the animals were observed for signs of toxicity, morphologic behavior, and mortality (Jainu & Shyamala Devi, 2004).

Induction of diabetes mellitus

Albino rats of the Wistar strain were used for the study. Six animals of group 1 were retained as the normal control group. Remaining animals were injected with 120 mg/kg of alloxan monohydrate (Spectrochem Pvt. Ltd., Mumbai, India) in sterile normal saline (Venkatesh et al., 2003). The animals were fed with normal pellet chow and water for 7 days. After 7 days, alloxan-injected rats were tested for induction of hyperglycemia following the method of Nelson and Somogyi (Banerjee & Sen, 1980; Varley, 2002) after 18 h fasting. Only those animals with blood glucose levels above 200 mg/dL were employed for the study and subsequently divided into groups 2 to 7.

Experimental design

Each group was composed of rats. Group 1 (normal control) animals and group 2 (positive control) alloxan-diabetic animals were fed with distilled water and lab diet for 21 days. Group 3 alloxan-diabetic animals were treated with glibenclamide (500 µg/kg, p.o.) (Bal Pharma, Bangalore, India) for 21 days. Groups 4 to 7 alloxan-diabetic animals were treated with alcohol extract 500 mg/kg, alcohol extract 1000 mg/kg, aqueous extract 500 mg/kg, and aqueous extract 1000 mg/kg, p.o., respectively, for 21 days.

Determination of blood glucose levels in rats

Animals were fasted for 18 h with access to water, prior to the estimation of blood glucose. Samples of blood were collected on the 7th, 14th, and 21st days post-treatment by orbital sinus puncture under mild ether anesthesia for blood sugar estimation.

Table 1. Effect of different extracts of *C. orchioides* root tuber on blood glucose levels (mg/dL) of normal and diabetic rats (mean ± SEM).

Group	Treatment	Blood glucose levels (mg/dL) and % reduction in blood glucose			
		Day 0	Day 7	Day 14	Day 21
1	Normal untreated	110.47 ± 2.043	109.23 ± 1.355	110.56 ± 1.389	111.42 ± 1.649
2	Diabetic untreated	298.09 ± 2.410	302.71 ± 2.516	306.66 ± 1.593	309.04 ± 1.363
3	Diabetic rats treated with 500 µg/kg of glibenclamide	300.47 ± 2.600	245.23 ± 1.015*** (18.98%)	182.85 ± 0.6329*** (40.37%)	118.56 ± 0. 6810*** (61.63%)
4	Diabetic rats treated with 500 mg/kg of alcohol extract	300.47 ± 3.569	262.08 ± 5.729*** (13.41%)	230.29 ± 4.389*** (24.90%)	165.09 ± 2.512*** (46.57%)
5	Diabetic rats treated with 1000 mg/kg of alcohol extract	300.47 ± 2.600	255.23 ± 3.512*** (15.68%)	224.37 ± 4.142*** (26.83%)	142.40 ± 2.927*** (53.92%)
6	Diabetic rats treated with 500 mg/kg of aqueous extract	300.47 ± 4.069	284.28 ± 4.824* (6.08%)	280.47 ± 4.972*** (8.53%)	275.23 ± 4.762*** (10.94%)
7	Diabetic rats treated with 1000 mg/kg of aqueous extract (19%)	299.04 ± 2.410	278.61 ± 3.107** (7.95%)	276.10 ± 1.726*** (9.96%)	271.54 ± 0.8965*** (12.13%)

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. All values were expressed as mean ± SEM.

Results

Acute toxicity studies revealed that *C. orchioides* extracts were practically nontoxic when administered orally to mice; the LD_{50} value was higher than 3 g/kg. No lethality nor any toxic reactions were found up to the end of the study.

Alcohol and aqueous extracts showed significant hypoglycemic effects in diabetic rats. The alcohol extract showed significant hypoglycemic activity from the 7th day onwards ($p < 0.001$). The reduction in mean blood glucose level (BGL) was dose-dependent. The effects were comparable with the well-known antidiabetic drug, glibenclamide. The BGL of alloxanized rats on the 7th, 14th, and 21st days and percent reduction in blood glucose levels on the 7th, 14th, and 21st days are presented in Table 1.

Discussion

Although the precise mechanism of alloxan-induced diabetes remains unclear, there is increasing evidence that it involves the degeneration of islet β-cells by accumulation of cytotoxic free radicals. The pharmacological investigations on the alcohol and aqueous extracts of the root tuber of *C. orchioides* indicated that the plant extracts caused significant reduction in blood glucose levels in diabetic rats from day 14 onwards. Alloxan causes permanent destruction of the pancreatic β-cells (Zarrow et al., 1964). The observed hypoglycemic effect of the drug extracts in alloxan-diabetic rats points to pancreatic and extrapancreatic mechanisms of drug action. Hence, enhancement of peripheral utilization of glucose and/or increased insulin release may be cited as the possible mechanisms involved in the hypoglycemic action of *C. orchioides*. Further studies to identify the active hypoglycemic constituent(s) of root tuber of *C. orchioides* and their precise mechanism of action is required.

In conclusion, *C. orchioides* root tuber showed significant hypoglycemic effects, comparable with the antidiabetic drug glibenclamide, in diabetic rats. Thus, the claim made by the traditional Indian system of medicine regarding the use of root tuber of this plant in the treatment of diabetes is substantiated.

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