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Evaluation of estrogenic activity of alcoholic extract of rhizomes of *Curculigo orchioides*

K. Vijayanarayana [a,*], Rashmi S. Rodrigues [b], K.S. Chandrashekhar [a], E.V.S. Subrahmanyam [a]

[a] *N.G.S.M Institution of Pharmaceutical Sciences, Paneer, Deralakatte, Mangalore 574160, Karnataka, India*
[b] *Lotus Labs Private Limited, Bangalore 560052, Karnataka, India*

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Abstract

The rhizomes of *Curculigo orchioides* Gaertn. (Amaryllidacea) is an important Ayurvedic as well as Unani drug. It is present in several drug formulations used in the treatment of menorrhagia and other gynecological problems. In this study, we conducted a comparative study of estrogenic activity of alcoholic extract of *Curculigo orchioides* with diethylstilbestrol in bilaterally ovariectomized young albino rats. Bilaterally ovariectomized albino rats were divided into five groups ($n=9$) receiving different treatments, consisting of vehicle (0.6% w/v sodium carboxy methyl cellulose), ethanolic extract of rhizomes of *Curculigo orchioides* at three different doses (viz., 300, 600, 1200 mg/kg body weight) and standard drug diethylstilbestrol (DES) at a dose of 2 mg/kg body weight. All these were administered orally daily for 7 days. Estrogenic activity was assessed by taking percentage vaginal cornification, uterine wet weight, uterine glycogen content and uterine histology as parameters of assessment. Alcoholic extract of *Curculigo orchioides* showed a significant increase in percentage vaginal cornification, uterine wet weight ($P<0.001$), uterine glycogen content ($P<0.001$) and a proliferative changes in uterine endometrium compared to the control.

Keywords: *Curculigo orchioides*; Estrogenic activity; Ovariectomized rats; Vaginal cornification

1. Introduction

Curculigo orchioides Gaertn. (Amaryllidacea) is a small perennial herb, with an elongated tuberous rootstock, found wild in sandy regions of hotter parts of India and Srilanka. The rhizome is an important Ayurvedic as well as Unani drug. It is present in several drug formulations for a wide variety of ailments, especially as a general tonic, as an aphrodisiac and in the treatment of bone fracture (Nadkarni, 1976). Its use in the treatment of menorrhagia and other gynecological problems is mentioned in 'Dravyangunavignyana' (Gogate, 1982).

The rhizomes were known to contain resin, tannin, mucilage, fat and ash containing oxalate of calcium. Various flavonoid, phenolic and triterpenoid glycosides were isolated from the rhizomes of *Curculigo orchioides*. β-Sitosterol and crystalline needles of sapogenin have also been detected (Krishna Kumar and Vaidya, 1992; Mashelkar et al., 2000).

Formulations containing *Curculigo orchioides* are being promoted for use in conditions like irregular menses, menopause, breast cancer and infertility (Sharma et al., 1991; Prema, 2002). Thus the evaluation of the estrogenic activity of *Curculigo orchioides* was carried out to know whether its beneficial effect in various gynecological problems and breast cancer is due to its estrogenic activity.

2. Materials and methods

2.1. Material

Rhizomes of *Curculigo orchioides* were collected from the field areas of Manjeshwar in the month of December. The plant was identified and confirmed by Dr. Noeline J. Pinto, Head of the Department of Botany, St. Agnes College, Mangalore. A voucher specimen (V. no. NGSM 3561) has been deposited in the pharmacognosy department of N.G.S.M. Institute of

* Corresponding author. Tel.: +91 944 8255124; fax: +91 824 2203992.
E-mail address: vinpharmacol@yahoo.co.uk (K. Vijayanarayana).

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Table 1
Effect of alcoholic extract of *Curculigo orchioides* on uterine wet weight and uterine glycogen content in bilaterally ovariectomized albino rats

Group	Treatment (route)	Dose (mg/kg)	Uterine wet weight (mg)	Uterine glycogen content (μg/mg of uterine tissue)
1	Control 0.6% w/v Sod. CMC (p.o.)	–	98.7 ± 3.1	0.4387 ± 0.024
2	Standard DES (p.o.)	2	221.3 ± 8.3[b]	1.0270 ± 0.046[b]
3	*Curculigo orchioides* extract (p.o.)	300	162.55 ± 4.03[b]	0.7284 ± 0.036[a]
4		600	184.95 ± 6.34[b]	0.9254 ± 0.050[b]
5		1200	262.2 ± 7.2[b]	1.2169 ± 0.118[b]

Values are mean ± S.E.M. of nine animals in each group. Data were analysed by one-way ANOVA followed by Dunnett's *t*-test.
[a] $P < 0.01$ compared to control group.
[b] $P < 0.001$ compared to control group.

Pharmaceutical Sciences, Mangalore. They were dried under shade, powdered and subjected to Soxhlet extraction with 80% ethanol. The extract was concentrated to get a brownish sticky mass. The yield was 8.4%.

2.2. Animals and experimental set-up

Estrogenic activity of the alcoholic extract was assessed in bilaterally ovariectomized immature female Sprague–Dawley rats of 20–30 days old (weighing 50–60 g) using a standardized method with few modifications, taking percentage vaginal cornification, uterine wet weight, uterine glycogen content and uterine histology as parameters of assessment (Jonathan et al., 1995). The ovariectomized rats were divided into 5 groups each consisting of nine animals. Estrogenic activity of phytoestrogens ranges from 1/500 to 1/1000 to the activity of diethylstilbestrol (DES) (Cassidy, 1999). Based on this assumption a dose range between 300 and 1200 mg/kg of *Curculigo orchioides* extract was used:

- Group 1 (control): received 0.6% (w/v) Sod. CMC suspension at a dose of 10 ml/kg.
- Group 2 (standard): received aqueous suspension of diethylstilbestrol (NEMESTROL™) in 0.6% (w/v) Sod. CMC at a dose of 2 mg/kg.
- Group 3 (test): received aqueous suspension of alcoholic extract of *Curculigo orchioides* in 0.6% (w/v) Sod. CMC at a dose of 300 mg/kg.
- Group 4 (test): received aqueous suspension of alcoholic extract of *Curculigo orchioides* in 0.6% (w/v) Sod. CMC at a dose of 600 mg/kg.
- Group 5 (test): received aqueous suspension of alcoholic extract of *Curculigo orchioides* in 0.6%(w/v) Sod. CMC at a dose of 1200 mg/kg.

All these were administered orally daily for 7 days.

Vaginal cornification was examined daily. After 24 h of last treatment, hysterectomy was performed in all rats under pentobarbitone anaesthesia. Harvested uteri were cleaned carefully from adhering connective tissue and weighed. The three excised uteri from each group were fixed in Bouin's fluid and processed for histological preparations. Haematoxylin and eosin stained slides were examined under microscope for changes in cellular organization. The remaining uteri were used for glycogen estimation by anthrone method (Dayton et al., 1980). This study was conducted in accordance with the latest CPCSEA guidelines and the experimental protocol was approved by Institutional Animals Ethics Committee.

2.3. Statistical analysis

One-way analysis of variance (ANOVA) followed by Dunnett's *t*-test was used to analyze the difference in uterine wet weight, uterine glycogen content between different groups of treatments. Data of vaginal cornification were analyzed by two-way ANOVA followed by Bonferroni test.

3. Results

Assessment of estrogenic activity of alcoholic extract of *Curculigo orchioides* was done by taking percentage vaginal cornification, uterine wet weight, uterine glycogen content and uterine histology as parameters.

The alcoholic extract of *Curculigo orchioides* showed a dose dependent, statistically significant ($P < 0.001$) increase in uterine wet weight and uterine glycogen content compared to control (Table 1). The standard drug DES produced statistically significant ($P < 0.001$), 2.24-fold increase in uterine wet weight.

The alcoholic extract of *Curculigo orchioides*-induced proliferative changes in the uterine endometrium as evidenced by

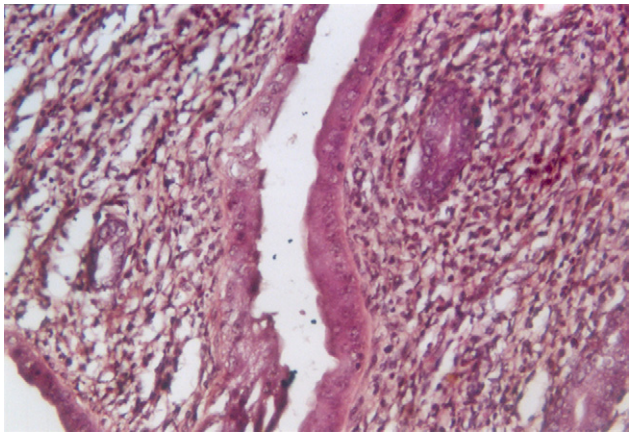

Fig. 1. Photomicrograph of haematoxylin and eosin stained transverse section of uterus of *Curculigo orchioides* extract (1200 mg/kg, p.o.) treated rat, showing proliferative stage (i.e., stimulated endometrium with loose stroma).