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Effects of *Calotropis procera* on oestrous cycle and on oestrogenic functionality in rats☆

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Abstract

Effects of ethanolic and aqueous extracts of *Calotropis procera* (Ait.) R.Br. roots, have been studied on oestrous cycle and on some parameters of oestrogenic functionality in rats. Both extracts have been shown to interrupt the normal oestrous cycle in 60 and 80%, respectively, of rats treated. The rats exhibited prolonged dioestrous stage of the oestrous cycle with consequent temporary inhibition of ovulation. The contemporary administration of commercial oestro-progestinic preparation exhibited the same effects in 100% of rats treated. However, the extracts have not demonstrated to possess oestrogenic activity when tested in immature female bilaterally ovariectomized rats. © 2001 Éditions scientifiques et médicales Elsevier SAS

Keywords: *Calotropis procera*; Roots; Oestrous cycle; Antiovulatory activity; Ovariectomized rats

1. Introduction

Calotropis procera (Ait.) R. Br. (Asclepiadaceae) is a xerophytic, erect shrub growing widely throughout the tropics of Asia and Africa, commonly known as 'Arka' in India. In the traditional Indian medicinal system, different parts of the plant are used as a purgative and antihelmintic agent and for the treatment of leprosy, ulcers, tumors, piles, diseases of the spleen and liver [1]. The latex is used as an abortifacient [2].

Different parts of the plant have been reported to possess a number of biological activities such as proteolytic [3], antimicrobial [4], larvicidal [5], nematocidal [6], anticancer [7,8], antiinflammatory [9].

Phytochemically, the plant has been investigated for cardenolides from the latex and leaves [10,11], triterpenoids [12,13], anthocyanins from the flowers [14] and hydrocarbons [15].

According to effect of *C. procera* extracts on early and late pregnancy in albino rats, reported by Prakash et al. [16], an investigation has been under taken to study the effect of the root extracts of *C. procera* on oestrous cycle and on oestrogenic functionality in rats.

2. Materials and methods

2.1. Plant material

Authenticated roots of *Calotropis procera* were supplied by the traditional Medicine Department of the Ministry of Health of Malì. Voucher specimens are deposited at the Pharmaco-Biological Department at the University of Messina.

2.2. Extract preparation

The roots (25 g) dried and powdered were extracted in a Soxhlet extractor with 90% ethanol (250 ml) for 5 h. The extract was evaporated to dryness under reduced pressure at low temperature in a rotary evaporator and the quantity of residue obtained was 2%. For aqueous extract preparation the roots (100 g) dried and powdered was boiled in water (1000 g) for 30 min.

The decoction was lyophilized. The lyophilized was kept in sealed bottles under vacuum. The yield (dry weight) was 15%.

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Table 1
Effects of *C. procera* ethanolic extract (10%) on oestrous cycle in rat

Treatment (5 days)	Dose mg/kg/os dried drug	Animals showing inhibition of ovulation (%)	Prooestrus	Oestrus	Metaoestrus	Dioestrus
Control	–	0	+	+	+	+
Ethanolic extract	25	60	+	–	–	+
Ethanolic extract	50	60	+	–	–	+
Ethanolic extract	100	70	+	–	–	+
Milvane	[a]	100	+	–	–	+

[a] Ethynylestradiol 0.030 mg/kg, gestoden 0.050 mg/kg 1st and 2nd day; ethynylestradiol 0.040 mg/kg, gestoden 0.070 mg/kg 3rd, 4th and 5th day.

Table 2
Effects of *C. procera* decoction (10%) on oestrous cycle in rat

Treatment (5 days)	Dose mg/kg/os dried drug	Animals showing inhibition of ovulation (%)	Proestrus	Oestrus	Metaoestrus	Dioestrus
Control	–	0	+	+	+	+
Decoction	25	80	+	–	–	+
Decoction	50	75	+	–	–	+
Decoction	100	80	+	–	–	+
Milvane	[a]	100	+	–	–	+

[a] Ethynylestradiol 0.030 mg/kg, gestoden 0.050 mg/kg 1st and 2nd day; ethynylestradiol 0.040 mg/kg, gestoden 0.070 mg/kg 3rd, 4th and 5th day.

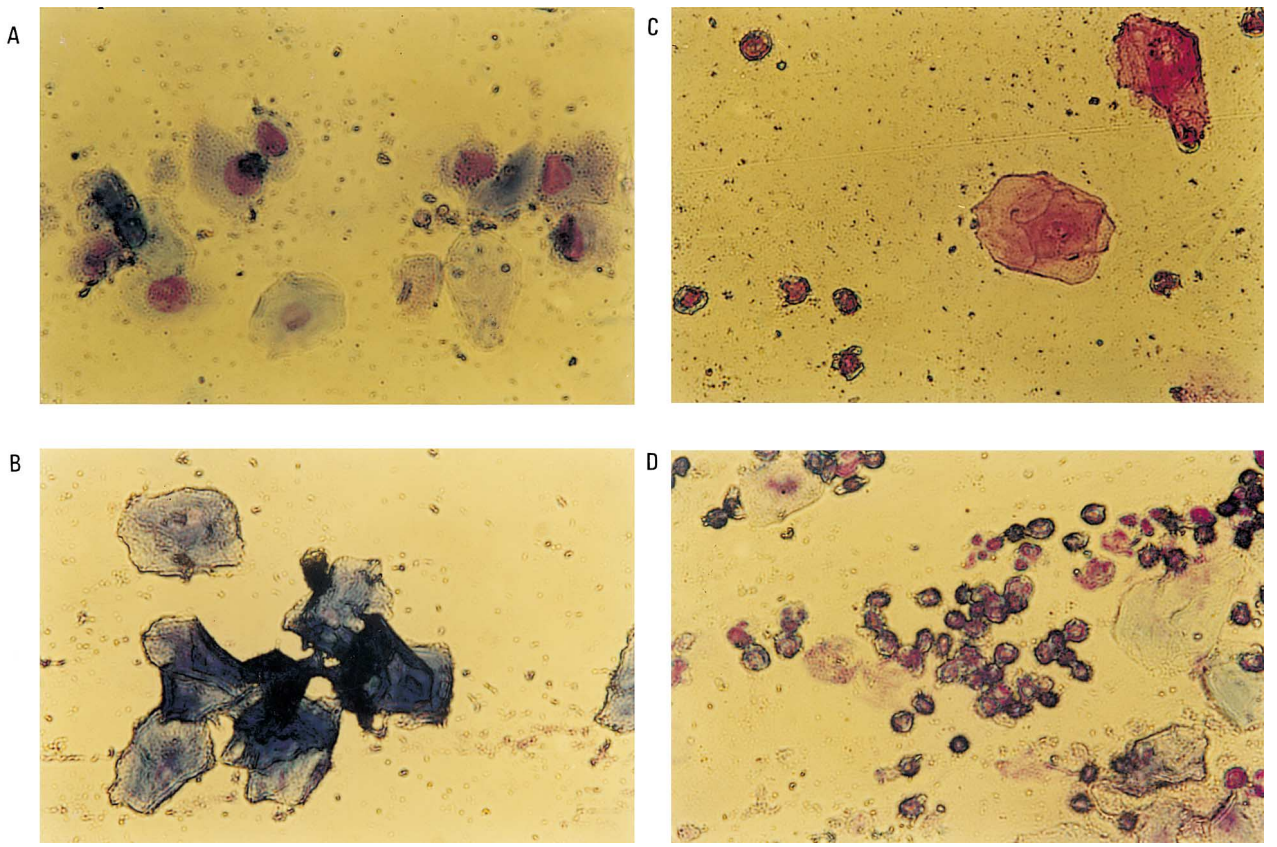

Fig. 1. Vaginal smears of rats on 4-day oestrous cycle. May Grunwal-Giemsa × 400. Controls: 1st day (A); 2nd day (B); 3rd day (C); 4th day (D).

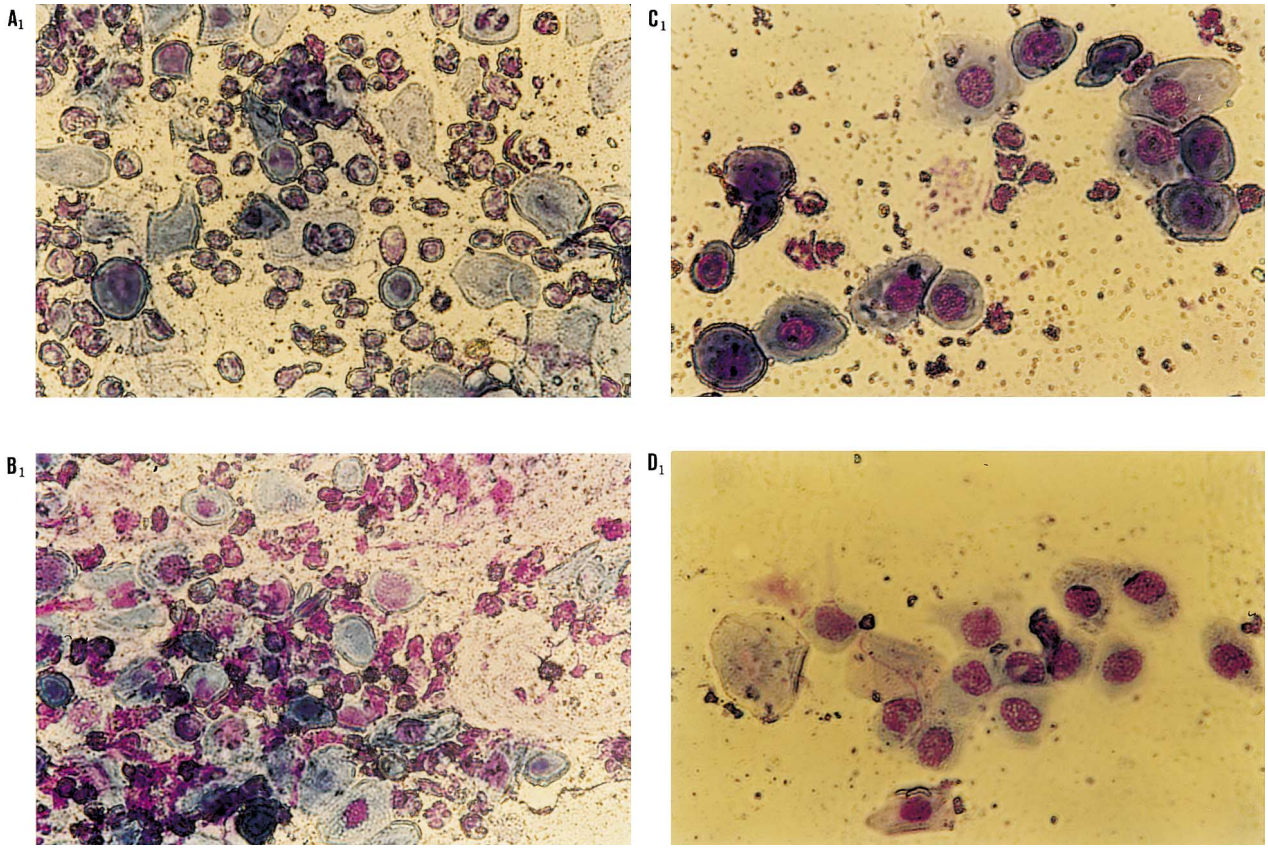

Fig. 2. Vaginal smears of rats on 4-day oestrous cycle. May Grunwal-Giemsa × 400. *C. procera* aqueous extract 100 mg dry root/kg: 1st day (A1); 2nd day (B1); 3rd day (C1); 4th day (D1).

2.3. Antiovulatory activity

Experiments were carried out in virgin female Wistar rats weighing 200 ± 21 g. All animals were housed under controlled conditions of temperature (23 ± 2°C), humidity (65%) and light (14 h light/10 h dark) with water and food ad libitum.

The vaginal smear of each rat was examined daily for 15 days to select animals showing regular cycles (4–5 days). The selected rats were randomized into groups of six animals each. The extracts were administered orally by an intragastric rubber catheter for 5 days (one complete cycle). Six groups of animals were treated. Extracts were given to each group at doses of 25, 50 and 100 mg/kg of dry roots. Each dose of extract was suspended in a volume of 5 ml/kg body weight. To the seventh group of animals a commercial oestro-progestinic preparation (Milvane) was administered orally for 5 days at doses of 0.030 mg/kg of ethinylestradiol and 0.050 mg/kg of gestoden, 1st and 2nd day; 0.040 mg/kg of ethinylestradiol and 0.070 mg/kg of gestoden 3rd, 4th and 5th day.

Control rats received an equal volume of distilled water or mixture water–ethanol (3:1) in a similar manner. Vaginal smear of each rat was examined daily for the 5 days of treatment and during the following 15 days in the morning.

2.4. Oestrogenic activity

Wistar immature female albino rats, 20–25 days old, weighing 35 ± 5 g were bilaterally ovariectomized under light ether anaesthesia and were used on the 6th day after operation. These rats were randomized into groups of five animals and oestrogenic activity of aqueous extract of *C. procera*, compared with estradiol benzoate (EB), was assayed according to Edgreen and Calhoun [17]. The root decoction was administered orally by an intragastric rubber catheter for 5 days at a dose of 100 mg/kg dry roots. EB was administered by subcutaneous injection for 5 days at a dose of 0.1 μg/rat. The controls received vehicle alone in a similar manner. The animals were weighted and sacrificed 24 h after the last dose.

The following parameters of oestrogenic activity were evaluated: vaginal opening, total weight, uterine weight, uterine luminal epithelium and endometrial cellular organization.

2.5. Acute toxicity

Ethanolic and aqueous extracts of the roots of *C. procera* were administered orally to groups of ten rats, each at doses of 1, 1.5 and 2 g/kg of dry roots. Each dose of extract was suspended in a volume of 10 ml/kg.

The rats were observed for 7 days for toxic effects.

2.6. Statistical analysis

The results are expressed as mean values ± SE and analysed by Student's *t*-test for paired or impaired data.

3. Results

3.1. Antiovulatory activity

Ethanolic and aqueous extracts of *C. procera*, at all doses assayed (25, 50 and 100 mg/kg of dry roots), provoked a significant modification of the vaginal smear in 60 and 80% of treated rats (Tables 1 and 2). The oestrous cycle so modified was characterized by prolonged dioestrous stage with consequent temporary inhibition of ovulation (Figs. 1–3).

A commercial oestro-progestinic preparation (Milvane), assayed in comparison, have been shown to interrupt the normal oestrous cycle in 100% of rats treated (Fig. 4). Duration and stages of oestrous cycle no was modified in controls. Aqueous extract was more active when compared to ethanolic extract.

No dose-dependent effect of extract was observed on the vaginal cycle. A gradual normalization of the cycle started 10 days after the end of the treatment; at the fourth cycle all the animals showed a normal cycle.

3.2. Oestrogenic activity

In comparison with controls, EB increased significantly ($P < 0.01$) body weight, uterine weight and uterine luminal epithelium of bilaterally ovariectomized immature rats (Table 3). Furthermore, it provoked vaginal opening in 100% of treated rats.

C. procera decoction at a dose of 100 mg/kg dry roots, did not alter body and uterine weight significantly in comparison with controls. Furthermore, administration of aqueous extract of *C. procera* did not modify the histoarchitecture of the uterus or the induced vaginal opening.

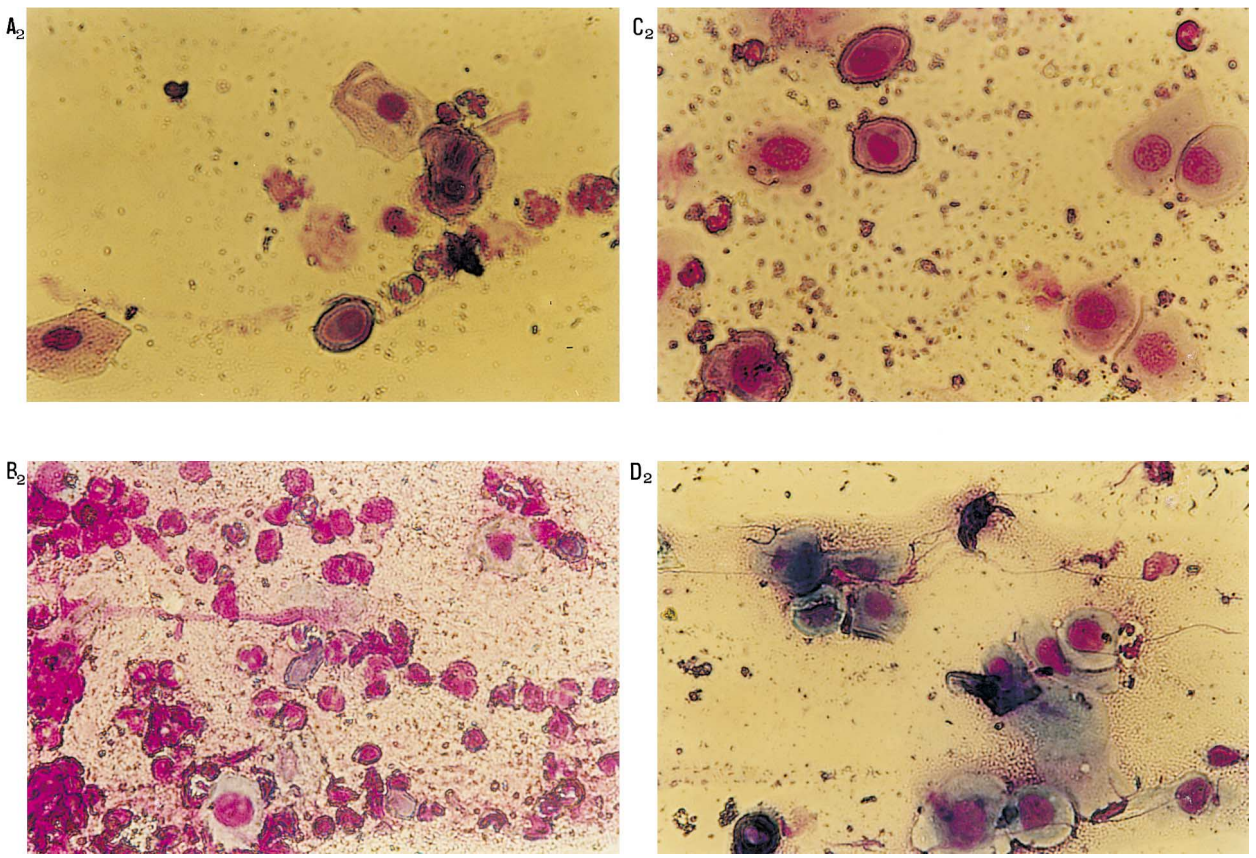

Fig. 3. Vaginal smears of rats on 4-day oestrous cycle. May Grunwal-Giemsa × 400. *C. procera* ethanolic extract 100 mg dry root/kg: 1st day (A2); 2nd day (B2); 3rd day (C2); 4th day (D2).

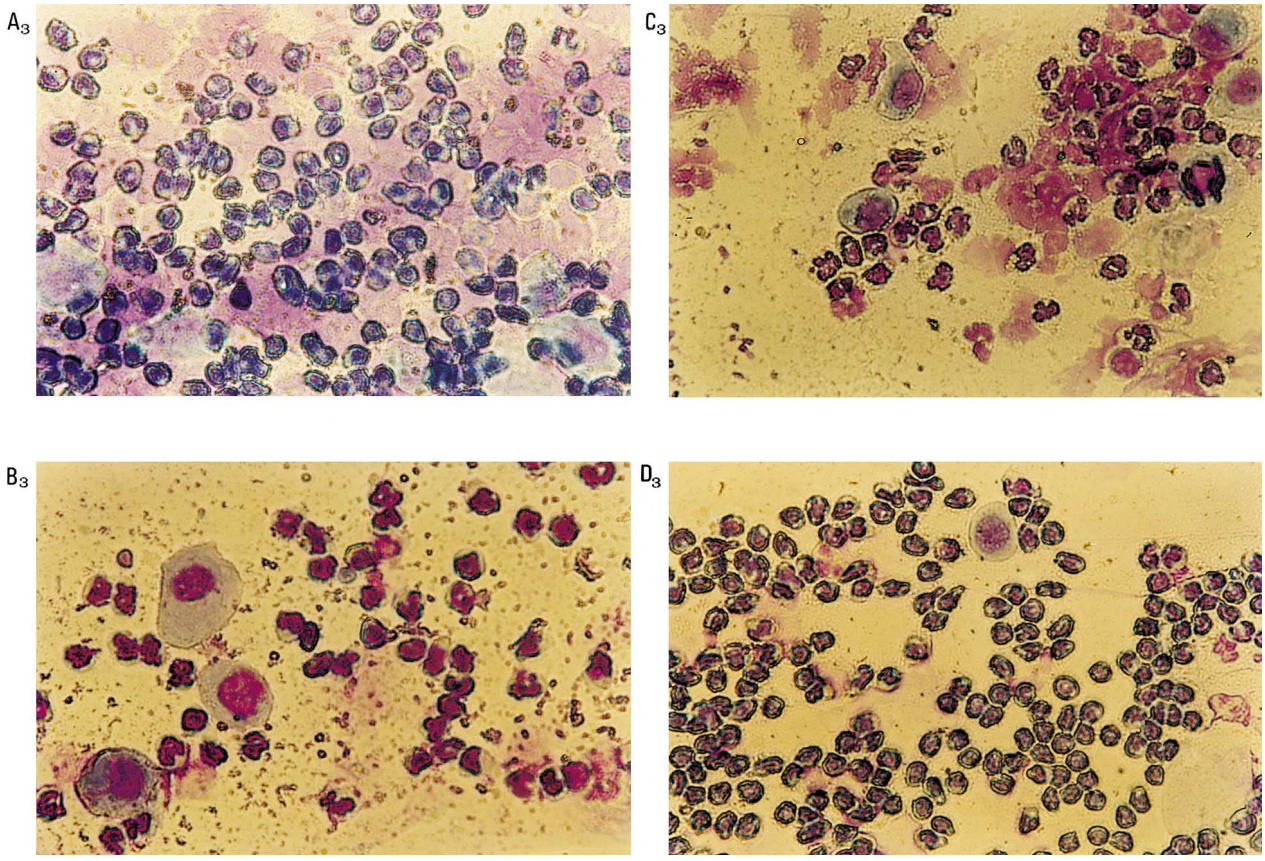

Fig. 4. Vaginal smears of rats on 4-day oestrous cycle. May Grunwal-Giemsa × 400. Ethinylestradiol 0.030 mg/kg; Gestoden 0.050 mg/kg: 1st day (A3); 2nd day (B3); 3rd day (C3); 4th day (D3).

Table 3
Effects of *C. procera* decoction (10%) and estradiol benzoate on some parameters of oestrogenic functionality in immature ovariectomized rats

Treatment (5 days)	Dose	Initial body weight (g)	Final body weight (g)	Uterus weight (mg/100 g)	Opening vaginal (% animals)
Control (vehicle)	–	38 ± 1.5	40 ± 1.2	13 ± 0.5	0
Estradiol benzoate	0.1 μg/rat s.c.	36 ± 1.2	52 ± 2.0 [a]	44 ± 1.0 [a]	100
Decoction	100 mg/kg os	37 ± 1.8	39 ± 1.7	14 ± 0.8	0

Mean ± S.E. of five animals for group.
[a] $P < 0.01$ versus control.

3.3. Toxicity studies

From Table 4 it is evident that the plant extracts were well tolerated orally in rats up to the dose of 1 g/kg with no mortality or side effects. However, doses of 1.5 and 2 g/kg of the extracts caused 20–40% mortality.

The symptoms observed included depression, anorexia and diarrhoea.

4. Discussion

The rat oestrous cycle is normally 4–5 days in duration. Three cell types can be observed, using a microscope, in the vaginal smear during a routine rat oestrous cycle (Table 5): (1) leukocytes; (2) round epithelial cells with easily distinguishable nuclei; and (3) cornified cells, in which nuclei are difficult to distinguish or are absent and the cell shape irregular.

The presence or absence of cell types and the relative proportion of each cell type determines the stage of the oestrous cycle of each rat.

Both extracts assayed produced on female Wistar rats temporary and reversible modification on oestrous cycle characterized by absence of oestrus and metaoestrus phases and dioestrus stage prolonged. Therefore, extracts provoked inhibition of ovulation

Table 4
Acute toxicity of *C. procera* roots extracts

Treatment	Dose g/kg/os	Incidence of symptoms (%)	Mortality (%)
Vehicle control	–	0	0
Aqueous extract	1	0	0
	1.5	30	20
	2	50	30
Ethanolic extract	1	0	0
	1.5	40	30
	2	70	40

Table 5
Stages of rat oestrous cycle

Day of cycle	N	C	L
Four-day cyclic rat			
Prooestrus [a]	+++	++	±
Oestrus [b]	±	+++	–
Metaoestrus	++	±	+++
Dioestrus	++	+	+++
Five-day cyclic rat			
Prooestrus [a]	++	+++	–
Oestrus [b]	±	+++	–
Metaoestrus	+++	±	+++
Dioestrus I	++	+	+++
Dioestrus II	+++	++	±

Cell types and the relative proportion of each cell type to determine the stage of the oestrous cycle of each rat. N, epithelial cells; C, cornified cells; L, leukocytic cells.

[a] Mating normally occurs on the night between the days of prooestrus and oestrus.

[b] Freshly ovulated ova are found in the oviducts on the morning of oestrus.

with consequent reduction of cyclicity. It is known that oestrous cycle and the shift in its various stages are primarily governed by synthesis of ovarian oestrogen which, in turn, is controlled by the secretion of pituitary gonadotropins and hypothalamic-relasing factors. Estrogen and progesterone are the hormones responsible for histologic and functional modifications of female genital tract. In fact, the exogenous administration of physiological doses of estrogen, in sexual immature rats, stimulated histoarctecture of huterus [18].

Aqueous extract, administered in immature rats, did not stimulate histoarctecture of uterus neither caused changes in the vagina.

The results obtained suggested that inhibitory effect on ovulation of roots *C. procera* is not of oestrogenic nature, other mechanisms should be investigated.

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