ANTI-FERTILITY EFFECTS OF EMBELIN IN FEMALE SPRAGUE-DAWLEY RATS MAY BE DUE TO SUPPRESSION OF OVARIAN FUNCTION

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(Received: June 10, 2003; accepted: June 17, 2004)

Effects of embelin on oestrous cycle, plasma levels of progesterone and oestradiol, and *in vitro* production of oestradiol and progesterone by mixed ovarian cells was studied. Forty adult (4 months old) regularly cycling female Sprague-Dawley rats were divided into four groups of 10 rats each. Groups I and II (controls) were given 1 ml/kg body weight of physiological saline or corn oil (vehicle). Groups III and IV received 10 mg/kg and 20 mg/kg body weight embelin in corn oil, respectively. Emberlin disrupted the oestrous cycles in Groups III and IV animals, and there was a significant depression in plasma oestradiol ($p < 0.05$) and progesterone ($p < 0.02$) at both 10 and 20 mg/kg body weights, respectively. Isolated mixed ovarian cells from embelin treated rats produced significantly less progesterone and estradiol than controls *in vitro.* It is concluded that embelin probably interferes with reproductive functions in female rats by suppressing ovarian production of sex steroid hormones.

Keywords: Embelin – rat – ovaries – oestrogen – progesterone

INTRODUCTION

Embelin is a naturally occurring benzoquinone widely found in plants of the family Myrcinaceae. These plants are widely distributed in Africa and Asia, and include *Ardisia humilis, Embelia tsjersimucottam* and *Rapanea umbellata* [8]. When extracted, embelin (2,5-dihydroxy-3-undecyl-2, 5-cyclohexadiene-1, 4-benzoquinone) appears as a reddish brown, lipid soluble crystalline compound [10]. The substance has been used in many African and Asian countries as herbal medicine against a wide range of ailments including gastrointestinal and nervous disorders [3, 8]. It is also used to treat swollen breasts of mothers or in women having difficulty with birth [8].

In recent times a lot of interest has been generated by the findings that embelin may have anti-fertility, and possibly contraceptive properties in males and females [2, 4, 5, 13]. It has a strong anti-implantation activity in rats [14], and causes fetal resorption and a reduction in ovarian weights [2]. These effects are thought to be due

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0236-5383/$ 20.00

to its oestrogenic activities [13]. In male rats embelin disrupts testicular histology, reduces gametogenic counts and lowers accessory sex gland fructose levels [6]. Treatment of rats with embelin also results in a decreased level of testosterone. The effects in male rats, are reportedly reversible [11]. However, the mechanisms by which embelin causes the outlined effects on reproductive functions are not understood. This study was designed to look at the probable actions of embelin on the oestrous cycle and sex steroid hormone production in female rats.

MATERIALS AND METHODS

Chemicals and Animal Welfare

Embelin of more than 99% purity was used in this study [10]. Adult male rats (Sprague-Dawley, aged 4 months) were used in all experiments. All rats were caged individually in plastic bottomed cages with wire tops (45 cm × 28 cm × 21 cm). They had access to water and rat chow *ad libitum* (from Kenya Grain Growers Union, Nairobi). The animals were subjected to natural lighting conditions 12 : 12 h light/darkness, and average room temperatures of 23 °C. Their beddings consisted of sawdust which was changed everyday.

A total of 40 female rats were used for the study. They were randomly divided into four groups (Groups I, II, III and IV) consisting of 10 animals each. Groups I and II were controls and received subcutaneous injections of physiological saline or corn oil at 1 ml/kg body weight on the flanks every other day for 20 days. Groups III and IV were the test groups and received subcutaneous injections of embelin dissolved in corn oil at 10 mg/kg body weight and 20 mg/kg weight, respectively, in a maximum of 0.25 ml of corn oil [12], every other day for 20 days. Each animal received a total of 10 injections by the end of the experiment.

Effects of embelin on body weight and oestrous cycle patterns

Before the start of treatments, all animals were weighed and monitored daily for 10 days by examination of vaginal smears, to ascertain that they were cycling normally. They were thereafter left to rest for 4 days and then injected subcutaneously, on the flanks, with the appropriate preparation (i.e. saline, corn oil or embelin solution) every other day for 20 days. Each animal was weighed daily, and the stage of the cycle was determined by vaginal smear. On treatment days the rats were weighed and vaginal smears taken before injection with the appropriate compounds.

Effects of embelin on plasma oestrogen and progesterone

The stage of the cycle was determined by vaginal smears a day following the last injection, the rats killed and blood samples taken by cardiac puncture and separately put into test tubes containing EDTA. Blood samples corresponding to the four stages of the oestrous cycle (diestrus, proestrus, oestrus and metestrus) were separately grouped for control animals and for each embelin treatment groups. The samples were assayed for progesterone and oestrogen by radioimmunoassay [16] which had been validated for rat blood samples [12].

Effects of embelin on oestrogen and progesterone production in vitro

Ovarian cells were prepared as previously described by other workers [12]. Briefly, after sacrifice ovaries from each of the experimental groups (Groups I, II, III and IV) were dissected out, pooled and ovaries from each group processed together. Thus, in total each group comprised ovaries from 10 animals. Distinction in sampling the ovaries was made only with regards to treatment mode and not stage of the cycle. Ovaries were trimmed and placed in cold minimun essential media (MEM) containing 20 mM of sodium bicarbonate. The tissues were thoroughly minced and the mixture of ovarian cells isolated by filtration through a nylon mesh, and cell-containing filtrate pre-incubated in plastic flasks in MEM media, in an atmosphere containing 95% O_2/5% CO_2 in a shaking water bath at 34 °C for 30 min. The cell suspension was then centrifuged at 1000×*g*, and the pellet re-suspended in MEM media. Viability of the cells was determined by the dye exclusion method using Trypan Blue, and the cell suspension diluted to achieve a concentration of 2.5×10^3 viable cells per 300 µl.

Cell suspensions in MEM media (2.5×10^3 viable cells) were placed in LP3 tubes. Ten [10] microlitres of LH (1.7 mU/ml) and FSH (1.3 mU/ml) in phosphate buffered saline (pH 7.2) were added to some of the tubes, in triplicate. In other tubes appropriate volumes of gonadotropin-free phosphate buffered saline were added to the cell suspensions. The total volume of each of the incubates was made up to 500 µl with MEM media. The control and test samples were incubated in a shaking water bath in an atmosphere of 95% O_2/5% CO_2 at 34 °C for 3 h. At the end of the experiments the incubation media were harvested, and the amounts of hormone secreted in each tube determined by radioimmunoassay.

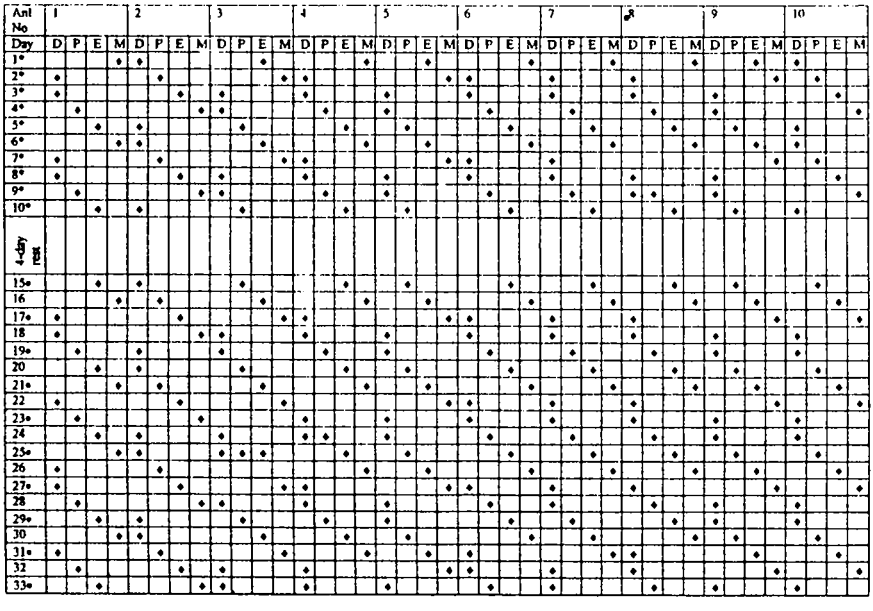
Statistical analysis

The data are reported as means ± SEM, where *n* is the number of rats or observations. Statistical differences were determined by an unpaired *t*-test.

RESULTS

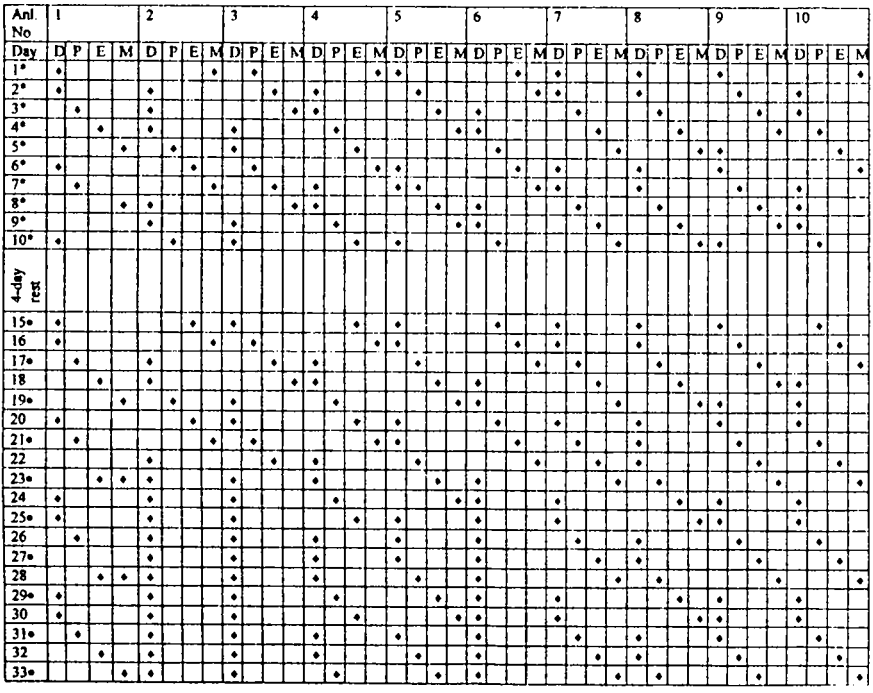
Effects of embelin on body weights and oestrous cycle pattern

There were no significant effects on body weights that could be attributed to embelin treatment. The control and treated animals showed similar body weights ranging from 243.5 ± 5.2 g at the start of the experiments to 251.7 ± 8.3 g at time of termination, for controls, and 236 ± 4.3 g to 269.1 ± 5.2 g for embelin treated animals. Regarding the patterns of oestruos cycles (Fig. 1a, b and c) all the control animals (Group I treated with physiological saline and Group II treated with corn oil) showed normal 4–5 day oestrous cycles during both the pre-treatment and treatment periods (Fig. 1a. Note that only the results for corn oil-treated controls have been given in this report as results for saline treated controls were very similar). In embelin treated animals (Groups III and IV) various degrees of disruption of the cycles were evident during the treatment period (Fig. 1b and c). The effects were more serious in Group IV, receiving 20 mg/kg body weight (Fig. 1c), than Group III receiving 10 mg/kg body weight (Fig. 1b). In the former, four animals persisted in the diestrus stage after the fifth injection of embelin (day 23 of the experiment) while in Group III only 3 rats exhibited the same (Fig. 1b and c).

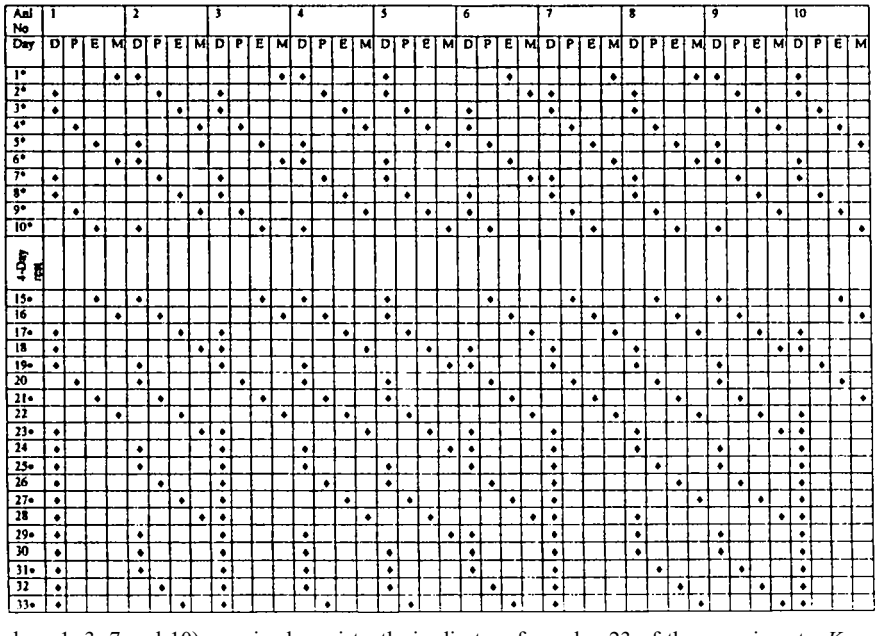


a)

Fig. 1. Oestrous cycle patterns in corn oil- and embelin-treated rats. In a) corn oil-treated control rats exhibited 4–5 day cycles throughout the experimental period. Control rats treated with physiological saline showed similar patterns to the above. In b) in rats treated with 10 mg/kg body weight of embelin three rats (numbers 2, 3 and 6) remained in diestrus after day 23 of the experiments while the rest showed varying degrees of cycle disruptions. In rats treated with 20 mg/kg body weight of embelin c) four rats



b)



c)

(numbers 1, 3, 7 and 10) remained persistently in diestrus from day 23 of the experiments. *Key:* D – diestrus; P – proestrus; E – oestrus; M – metestrus; * – days of injection; Anl No – animal number

Effects of embelin on plasma progesterone and oestradiol

Table 1 shows progesterone and oestradiol levels in corn oil-treated control and embelin treated rats. Plasma progesterone ranges at both doses of embelin (5.0 ± 0.8 nmol/l at oestrus and 19.4 ± 3.5 nmol/l at diestrus for Group III animals; 4.3 ± 0.7 oestrous and 16.3 ± 2.7 nmol/l at diestrus for Group IV rats) were significantly different ($p < 0.02$) from those of corn oil treated controls (6.0 ± 0.3 nmol/l at oestrus and 23.3 ± 0.1 nmol/l at diestrus) (Table 1). Oestradiol levels in corn oil controls and the two embelin treated groups are shown in Table 1. Oestradiol levels in controls animals were 586.0 ± 37.2 pmol/l at oestrus and 275.4 ± 22.0 pmol/l at diestrus. Plasma oestradiol levels in both the experimental groups (310.0 ± 40.3 pmol/l at oestrus and 166.4 ± 18.3 pmol/l at diestrus for Group III and 266.0 ± 17.1 pmol/l and 166.5 ± 13.3 pmol/l for Group IV) were significantly different ($p < 0.05$) from the control groups (Table 1).

Table 1
Plasma progesterone and oestradiol in corn oil-treated control rats and rats treated with 10 or 20 mg per kg bwt embelin

Category	Oestrus	Metestrus	Diestrus	Proestrus
		Corn oil treated controls		
Progesterone (nmol/L)	6.0 ± 0.3 (n = 3)	12.2 ± 0.7 (n = 2)	23.2 ± 0.1 (n = 2)	9.5 ± 0.7 (n = 3)
Oestrogen (pmol/L)	586.0 ± 37.2 (n = 3)	340.5 ± 14.3 (n = 2)	275.4 ± 22.0 (n = 2)	450.3 ± 20.2 (n = 3)
		Embelin 10 mg/kg bwt		
Progesterone (nmol/L)	5.0 ± 0.8 (n = 2)*	13.2 ± 0.7 (n = 3)	19.4 ± 3.5 (n = 3)*	8.5 ± 1.4 (n = 2)
Oestrogen (pmol/L)	310.8 ± 40.3 (n = 2)	220.3 ± 17.4 (n = 2)	166.4 ± 18.3 (n = 3)	222.3 ± 13.4 (n = 2)
		Embelin 20 mg/kg bwt		
Progesterone (nmol/L)	4.3 ± 0.7 (n = 2)	11.5 ± 2.2 (n = 2)	16.3 ± 2.7 (n = 4)	7.3 ± 0.9 (n = 2)
Oestrogen (pmol/L)	266.0 ± 17.1 (n = 2)	160.3 ± 12.1 (n = 2)	166.5 ± 13.3 (n = 4)	230.4 ± 20.1 (n = 2)

The values are mean ± SEM. Inter-assay and intra-assay coefficients of variation ranged between 8–12% in all assays.

Effects of embelin on oestradiol and progesterone production by ovarian cells in vitro

Effects of embelin on estrogen and progesterone production *in vitro* are shown in Table 2. Isolated ovarian cells from corn oil-treated controls and embelin-treated animals secreted both progesterone and oestrogen *in vitro.* However, cells from embelin treated animals produced significantly less of the two sex steroid hormones than cells from corn oil-treated animals (Table 2). The addition of LH and FSH to the incubations increased production of the two steroid hormones by control cells, but did not have any significant effects in incubations containing cells from embelin treated rats (Table 2).

Table 2
Production of progesterone and oestradiol by isolated mixed ovarian cells *in vitro*

Category	Corn oil-treated controls (n = 10)		Embelin 10 mg/kg bwt (n = 10)		Embelin 20 mg/kg bwt (n = 10)	
	gonadotropin free	with LH + FSH	gonadotropin free	with LH + FSH	gonadotropin free	with LH + FSH
Progesterone (nmol/l)	6.2 ± 1.5	8.3 ± 1.1	2.4 ± 0.7	3.6 ± 0.9	3.3 ± 0.5	3.6 ± 1.0
Oestrogen (pmol/l)	107.3 ± 14.3	143.3 ± 15.2	70.3 ± 8.3	84.6 ± 9.2	68.1 ± 6.3	83.6 ± 7.4

The values are mean ± SEM; $p < 0.05$.

DISCUSSION

This study set out to look at effects of embelin on some reproductive parameters in female rats. Although embelin has been reported to have various side-effects [7, 9], there were no adverse clinical effects reported during the course of this experiment, and the body weights of the animals were not affected by the treatments. This may be attributed to the lower dosages used in this study as compared to those in other studies [13].

The oestrous cycles of rats treated with embelin were disrupted to various degrees with a total of 7 rats (3 in Group III and 4 in Group IV) out of 20 rats (35%) remaining in diestrus after the 5th injection (day 23) up to the end of the experiments. The other embelin treated animals in both groups showed various degrees of disruption of cycles as compared to controls, thereby attesting to the ability of embelin to interfere with reproductive processes in rats.

The above observations were reinforced by the finding that embelin suppressed plasma oestradiol and progesterone levels. Normal plasma oestradiol values are important for the manifestation of the cyclical vaginal cellular changes that are used routinely in laboratories for determining stages of the oestrous cycle in rats. A sup-

pression of plasma oestradiol levels would be manifested by loss of cornification of vaginal epithelium, similar to what occurs during normal diestrus in rats [17]. Thus, the lowering of oestradiol levels would account for our observations regarding disruption of the cycles, especially the persistence of some of the rats in the diestrus stage.

The mechanisms by which embelin lowers plasma sex steroid levels still remain unclear. Possible points of action may include the hypothalamo-pituitary-gonadal axis [13], the liver and the kidneys. In the hypothalamus embelin may interfere with the synthesis and release of GnRH while at the pituitary level it may affects synthesis and release of gonadotropins [13]. However, our studies did not address the latter. At the ovarian level it is possible that the compound interferes with cell surface receptors resulting in reduced or abolished signal transduction for steroid synthesis. There could also be an interference with one or more of the ovarian steroidogenic enzymes. However, the above proposals still remain to be elucidated.

Low levels of progesterone and oestradiol may also be due to increased elimination of steroid hormones in the liver and kidneys. It has been reported that some of the herbal preparations used by humans for various ailments affect hepatic and renal functions [6, 8] and may speed up or lead to rapid elimination of various compounds in the body.

The results in this study compliment earlier reports [14] indicating that female rats treated with embelin exhibited a reduction in ovarian weights, and had pregnancy losses through fetal resorption. In rats ovarian progesterone is necessary for maintenance of pregnancy up to term [1, 15]. In this study both plasma progesterone levels and production of progesterone by isolated ovarian cells from embelin-treated animals were suppressed. However, more studies are still required to comprehensively chart out the mechanisms by which embelin suppresses sex steroid hormones and produces its anti-fertility effects.

ACKNOWLEDGEMENTS

I wish to thank Ms Christine M. Gichora and Mr. J. Okaka for technical assistance, and Ms Marietta N. Muthama for helping with the graphs and figures. This work was supported with grants and materials from the WHO Research in Human Reproduction (RHR).

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