EFFECTS OF β-SITOSTEROL ON THE OESTROUS CYCLE AND OVARIAN WEIGHT IN THE RAT

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BETA-sitosterol has been reported to increase the uterine weight of adult ovariectomized mice[1,2] and immature rabbits[3]. It also prevents implantation in rats when given postcoitally[4]. It manifests its contraceptive action at the uterine level due to its hormonal attributes such as estrogenic, gonadotrophic, antigonadotrophic and antiprogestational properties[5]. The present paper examines the effect of β-sitosterol on the oestrous cycle and ovarian weight in intact rats.

To evaluate the effect of β-sitosterol on oestrous cycle, inbred strain of adult female albino rats, 3-4 month-old and weighing 150–180 g body wt were selected. Vaginal smears were observed everyday and the different stages of the oestrous cycle were determined[6]. After three consecutive cycles, only rats showing normal 4–5 days oestrous cycle were selected for the study. After two normal (regular) cycles, each group received the following quantities of β-sitosterol/100 g body wt per day per rat subcutaneously (sc).

Group I: Control (received only the vehicle, sc); Group II: 50 μg; Group III: 150 μg; Group IV: 250 μg. All the animals were treated daily for 30 days to cover 6–7 cycles. Vaginal smear was examined daily between 9.00 and 10.00 a.m. and 3.00 and 4.00 p.m. to find out any alteration in the oestrous cycle. The duration and the different cell types found during the different stages of oestrous cycle were monitored by vaginal smear technique. All animals were sacrificed 24 h after the last injection by decapitation. Ovaries, uteri and pituitaries were dissected out, freed from surrounding tissues, blotted on a filter paper and weighed quickly to the nearest 0.1 mg on a single pan electric balance. The wet weights of organs were expressed as mg/100 g body weight (% wt.) and statistically evaluated.

Table 1 *Effect of β-sitosterol on oestrous cycle of intact rats*

Group	Percentage of animals in constant estrus (oestrous cycles*) 1	2	3	4	5	6
I	—	—	—	—	—	—
II	—	—	—	—	—	—
III	—	—	40	30	60	60
IV	—	60	100	100	100	100

*One cycle consisted 5 days.

The effect of β-sitosterol on oestrous cycle depended on the dose administered. While Group II animals showed no change in vaginal cyclicity, in Group III the oestrous cycle was disrupted in 60% of the animals and the cyclic changes of the vaginal smear became irregular. In Group IV animals, the incidence of persistent estrus was prolonged as long as the treatment continued (table 1).

During β-sitosterol treatment, there was no significant effect on the body weight with all the three doses 50, 150 and 250 μg, respectively.

Administration of β-sitosterol at doses of 50 μg and 150 μg failed to show any effect on uterine and pituitary weights but the ovarian weights increased significantly. On the other hand, 250 μg dose of the sterol induced a marked increase in ovarian, uterine and pituitary weights (table 2).

Thus, in the present investigation, β-sitosterol exerted differential effects on vaginal smear pattern. Of the three doses, 250 μg was the most effective as it induced prolonged cornified stage in 90% of rats. At 150 μg dose, though 60% of animals showed 'heat' period, the smear pattern was more erratic. According to Lednicer[7], every substance that is natural or

Table 2 *Effect of β-sitosterol on body weight and organ weights of intact female rats*

Dose (μg)	Body wt (g)	% Change	ovary (mg/100 g body wt)	% Change	Uterus (mg/100 g body wt.)	% Change	Pituitary (mg/100 g body wt)	% Change
0	150±6	100.00	32.3±2.6	100.00	112.7±8.7	100.00	3.9±0.31	100.00
50	156±7	104.00	41.8±3.6*	129.41	115.9±8.9	102.84	3.6±0.38	92.31
150	160±6	106.67	44.1±2.6*	136.53	135.0±9.7	119.79	4.1±0.27*	105.13
250	155±7	103.33	47.8±4.2*	147.99	178.2±9.6	158.10	5.0±0.30	128.21

Each value is the mean ± SEM of 10 animals; *t* test; * $P < 0.05$.

synthetic, steroid or non-steroid that causes vaginal cornification in rats or mice is designated as estrogen. Therefore in the present investigation, the extended cornified stage observed under β-sitosterol treatment suggests the probable estrogenicity of the compound. Similar findings have been corroborated[8] while observing the effect of *Embelia ribes* on oestrous cycle, wherein 70% of the rats exhibited prolonged cornified stage within 12 days of treatment. This has been attributed to the estrogenic nature of the plant principle.

It is generally accepted that sequential changes of vaginal smear in different phases of the oestrous cycle are closely associated with gonadal steroids which in turn are controlled by the secretion of pituitary gonadotrophins[9]. The observed increase in the pituitary, ovarian and uterine weights as studied by bioassay techniques and the uterotrophic response and cornification of vagina all confirm the estrogenic property of this phytosterol.

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HISTOCHEMICAL STUDY OF THE METABOLISM AND TOXICITY OF MERCURY

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MERCURY and its compounds are well known to be toxic but the precise mechanism of their toxic action is not clearly understood. This knowledge is essential in view of the increased environmental contamination of mercury. Since almost all the proteins and enzymes are potential targets of the mercurials[1], we have used a histochemical method for the demonstration of mercury or mercury-protein complexes in the tissues. Our aim was to study the intraorgan localization of injected mercury at different periods of time after the administration of a toxic dose of mercuric chloride. The information obtained was used to draw inferences about the metabolism, the site, and the mechanism of toxicity of mercury.

Male albino rats of Norway strain (*Rattus norvegicus*), weighing 160–200 g, were injected with mercuric chloride (5 mg/kg body weight) by intraperitoneal route. The control rats received 0.2 ml of 0.85% saline. The animals were then sacrificed in pairs under ether anesthesia after 1,2,3,4,5,6 and 24 h of receiving mercuric chloride. The liver and kidneys were removed, fixed, sections cut, stained for mercury by sulphide-silver method of Timm[2], and another section for histological examination was stained with hematoxylin-eosin.

The injected mercury was demonstrable as brownish-black deposits mainly in the kidney and to a lesser extent in the liver of experimental group rats. However, the distribution varied with time after mercury administration. During the first hour, mercury was found in the glomerular tufts, arterioles and interstitially between the cortical tubules. In the second hour, although mercury was diffusely present throughout the epithelia of cortical tubules, it was mainly concentrated in the glomeruli. By the third hour, mercury staining in the glomerulus persisted, and was also present diffusely in the proximal convoluted tubules, where it was more marked near the luminal margins of the tubular cells. This is in contrast to distal convoluted tubules which showed lighter staining and that too at the tubular epithelium margins. By the fourth hour,