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DANAZOL–INDUCED BIOCHEMICAL CHANGES IN THE RAT OVARY

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Abstract: Effect of varying doses of danazol, a synthetic steroid derivative of 17α–ethinyl testosterone has been observed on the biochemistry of the rat ovary. Biochemically total proteins decreased and total lipids increased with the danazol treatment. Triglycerides, the stored form of lipids formed the major components of lipids in the treated ovaries. The amount of phospholipids, glycolipids, cholesterol and free fatty acids decreased in the ovaries with increased danazol treatment. The functional significance of these changes have been discussed with ovarian physiology especially in relation to follicular growth and atresia.

Key words: danazol ovary proteins lipids steroids

INTRODUCTION

Steroid, steroid derivatives, gonadotropin antagonists, chlorinated pesticides, amines and metals have been shown to influence fertility rate in mammals (1). The effect of various chemosterilants on the rat ovary is effectively utilized for reproductive alterations (1, 2). Danazol is a synthetic steroid chemically related to 17 α–ethinyl testosterone (ethisterone). It suppresses the pituitary ovarian axis by inhibiting the output of gonadotropin from the pituitary gland (3). Danazol administration leads to suppression of gonadal growth, ovarian follicle selection and dominance and inhibition of antiandrogenic properties (4). Danazol is being used clinically in the management of endometeriosis (5), chronic cystic mastitis (6), precocious puberty (7), congenital angioneurotic edema and as a male (8) and female (9) antifertility agent. However, the mechanisms by which danazol exerts its therapeutic effects are poorly understood. Although a great deal of information has accumulated on the biological efects of danazol on the mammalian ovary, the exact mechanism of its action, especially at the subcellular level is not clear. Scant information is available regarding the mechanism by which danazol alters the biochemistry of the ovary. The present study will investigate the danazol induced changes in the levels of various macromolecules in the rat ovaries.

METHODS

Adult disease free female albino rats of about three months of age (wt. 100–150 gm)

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were procured from the small animal colony, Punjab Agricultural University, Ludhiana. They were housed in individual cages and maintained in standard hygienic conditions. The laboratory chow from Lipton Ltd., Calcutta and water were supplied *ad libitum*. After acclimatisation, their ovarian cyclicity was checked by daily examination of the vaginal smears. The rats showing regular ovarian cyclicity (4–5 days) were selected for the present investigation. The rats were divided into eight groups according to the treatment given to them such as:

Groups	Control	I	II	III	IV	V	VI	VII
Danazol (mg/kg body weight)	0	2	4	6	12	24	48	96

Each group consisted of at least five rats. The danazol suspended in propylene glycol was injected subcutaneously for 14 days continuously. Control animals received daily injections of propylene glycol. At the end of the experiment, the rats were anaesthesized with chloroform, their ovaries were excised and weighed and ovarian histomorphometric parameters were studied (10). A known amount of ovarian tissue after pooling was processed for the estimation of protein by the method of Lowry et al (11). Extraction and estimation of total lipids was done by the method of Folch et al (12). Total phospholipids were estimated by the method of Ames (13), glycolipids by modified method of Svennerholm (14), free fatty acids by method of Lowry and Tinsley (15), cholesterol by method of Zlatkins and Zak (16) and triglycerides by method of Vanhandal and Zilversmit (17). The results were statistically analysed using one way analysis of variance (18).

RESULTS

Ovarian cyclicity was disturbed in all the trated rats, where most of them remained in diestrous stage. The animals treated with danazol had smaller ovaries and maximum loss in ovarian weight was observed in rats that were given the 6 mg/kg dose of danazol whereas the ovarian weights of 2 mg/kg danazol treated rats did not differ significantly from those of control group ($P \leq 0.05$) (Table I). Histologic examination of ovarian tissue in these rats revealed that the rate of atresia was maximum in the case of the highest dose (96 mg/kg) and decreased in rats fed with the lower doses of danzol (Table I). Very few stage I follicle (with a diameter of about 80 µm) appeared to be normal in 96 mg/kg danazol treated rat ovaries. Large preantral and antral follicles were mostly affected in all the categories of the treated rats. Degenerating corpora lutea (diameter < 700 µm) with pyknotic granules were abundant in danazol treated rats. Interstitial gland tissue of thecal origin also occurred in abundance in treated rat ovaries.

In control rats, normal follicles at all stages of folliculogenesis were observed: large antral follicles (diameter > 672 µm) were also observed. Current corpora lutea (diameter > 800 µm) of the present cycle, alongwith a few degenerating corpora lutea from previous cycles were also noticed (Table 1).

With 2 and 4 mg/kg body weight dose of danazol the proteins decreased significantly in the rat ovaries (Table II). Higher doses of danazol (6, 12 and 24 mg/kg body weight) resulted in an insignificant increase in

TABLE I : Effect of different concentrations of danazol on ovarian activity in rat.

Dose of danazol (mg/kg body weight	*Ovarian cyclicity*	*Ovarian weight mg (100 g^{-1} body wt) Mean±S.E.*	*Percentage Atresia*			*Corpus luteum per day*		*Interstial gland Tissue (IGT)*
			Small	*Medium*	*Large*	*Developed*	*Regressing*	
0	Normal	$27.97^{a} \pm 1.94$	12	20	60	5–7	4–6	Less
2	Normal	$26.72^{a} \pm 1.81$	20	25	56	2–4	3–5	More
4	Disturbed	$13.31^{bc} \pm 0.49$	32	40	60	1–3	3–8	Abundant
6	Disturbed	$11.01^{c} \pm 0.74$	40	46	70	1–3	4–9	Abundant
12	Disturbed	$13.37^{bc} \pm 1.64$	57	35	78	1–2	4—5	Abundant
24	Disturbed	$16.10^{b} \pm 0.40$	68	45	85	0–1	4–9	Abundant
48	Disturbed	$16.47^{b} \pm 0.39$	76	46	82	0–1	6–9	Abundant
96	Disturbed	$16.63^{b} \pm 0.89$	95	All Atretic		0–1	8–10	Maximum

($P \leq 0.05$)
Figures with different superscripts in a column differ significantly from each other.

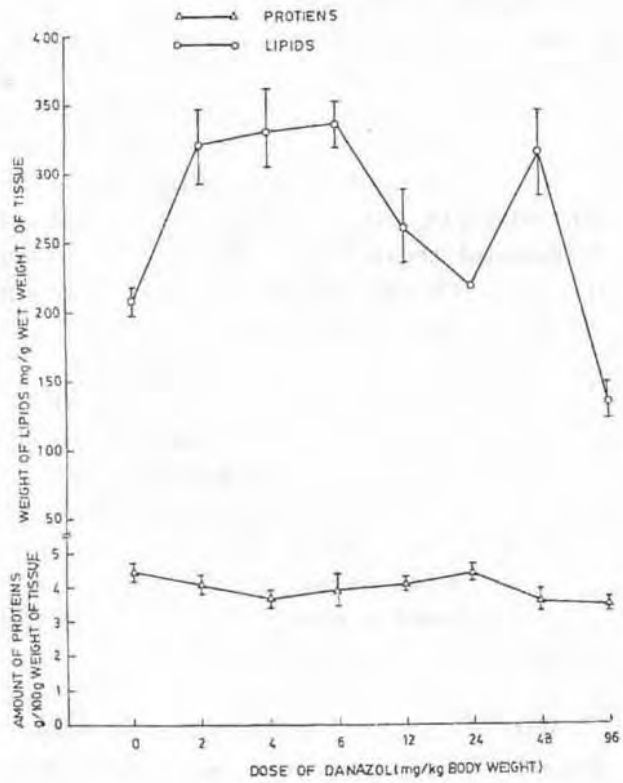

Fig. 1 : Amount of protiens and lipids in the ovary of control and danazol treated rats.

TABLE II : Total amount of proteins and lipids (g%) in the control and danazol treated rat ovaries.

Dose of danazol (mg/kg body weight)	*Proteins*	*Lipids*
0	4.45 ± 0.21^{ab}	22.88 ± 1.08^{c}
2	4.15 ± 0.25^{abc}	32.40 ± 3.03^{a}
4	3.66 ± 0.16^{bc}	32.97 ± 3.09^{a}
6	3.95 ± 0.52^{abc}	33.39 ± 1.40^{ab}
12	4.10 ± 0.08^{abc}	25.54 ± 2.17^{bc}
24	4.17 ± 0.21^{a}	24.34 ± 1.80^{c}
48	3.68 ± 0.32^{abc}	31.82 ± 2.50^{a}
96	3.47 ± 0.19^{c}	13.95 ± 0.74^{d}

Values are mean ± SE of 8 animals.

protein contents ($P < 0.05$) although they are still less than the control ovaries. Further 48 and 96 mg/kg body weight dose of danazol again resulted in a significant decrease in protein contents in the treated ovaries (Table II, Fig.1). Total lipids increased in the treated ovaries except for a slight variation with 12 and 24 mg/kg body weight of danazol (Table II, Fig. 1).

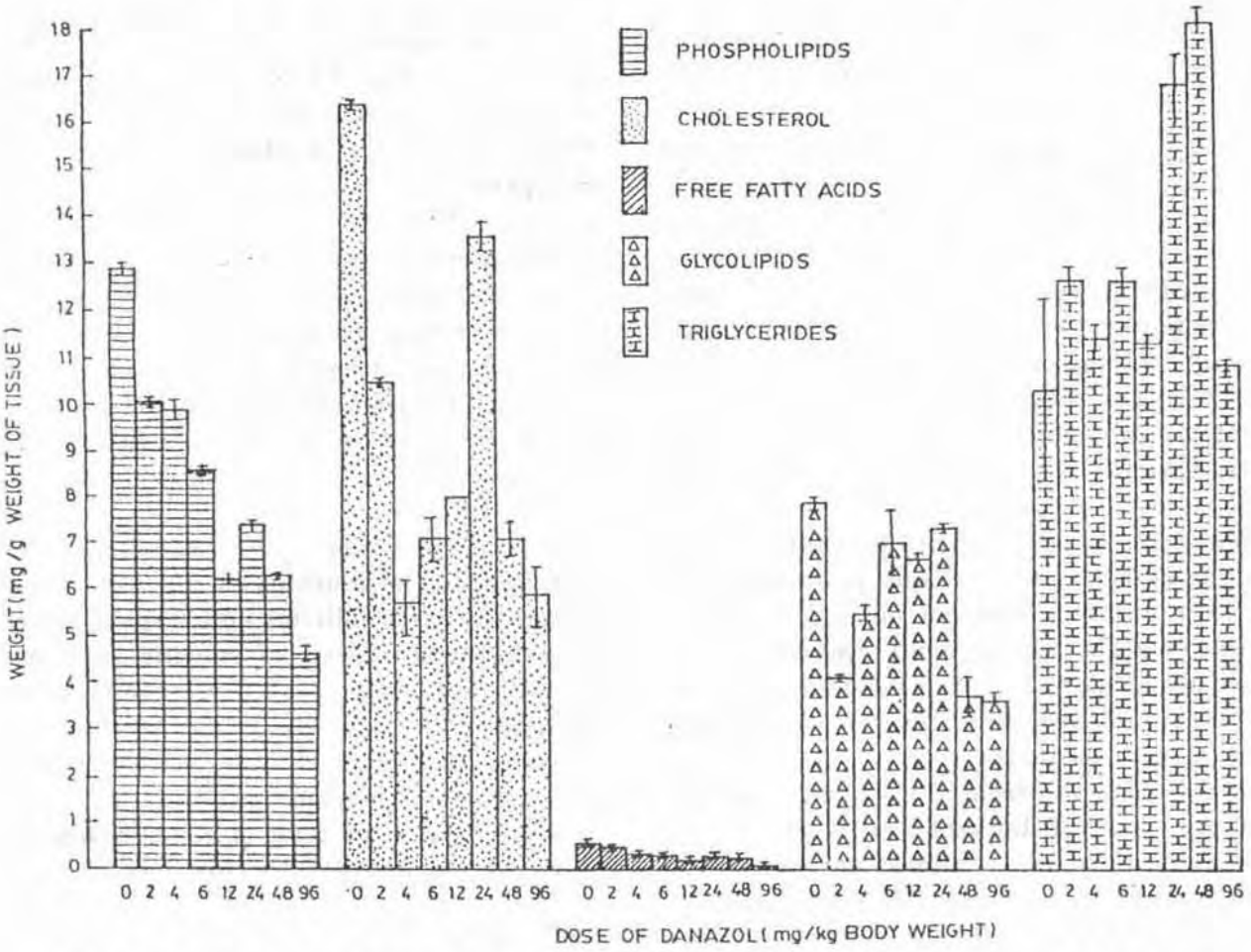

Fig. 2 : Quantitative distribution of various lipid fractions in control and danazol treated rat ovaries.

TABLE III : Quantitative distribution of various lipid fractions (mg/g wt. of tissue) in control and danazol treated rat ovaries.

Does of danazol (mg/kg, body wt.)	*Phospholipids*	*Cholesterol*	*Free fatty acids*	*Glycolipids*	*Triglycerides*
0	12.93 ± 0.03^{a}	16.46 ± 0.17^{a}	0.06 ± 0.05^{a}	7.92 ± 0.17^{a}	10.30 ± 1.98^{b}
2	10.06 ± 0.06^{b}	10.50 ± 0.23^{d}	0.51 ± 0.01^{b}	4.16 ± 0.08^{d}	12.66 ± 0.38^{b}
4	9.93 ± 0.32^{b}	5.70 ± 0.51^{g}	0.37 ± 0.02^{c}	5.50 ± 0.26^{c}	11.40 ± 0.34^{b}
6	8.63 ± 0.12^{c}	7.03 ± 0.57^{e}	0.36 ± 0.02^{c}	7.00 ± 0.76^{a}	12.66 ± 0.32^{b}
12	6.26 ± 0.12^{d}	8.02 ± 0.10^{e}	0.24 ± 0.01^{cd}	6.63 ± 0.17^{ab}	11.36 ± 0.28^{b}
24	7.43 ± 0.06^{e}	13.66 ± 0.34^{b}	0.31 ± 0.01^{cd}	7.30 ± 0.15^{a}	16.80 ± 0.71^{a}
48	6.36 ± 0.08^{e}	7.10 ± 0.46^{ef}	0.27 ± 0.01^{d}	3.70 ± 0.47^{d}	18.06 ± 0.54^{a}
96	4.56 ± 0.20^{f}	5.90 ± 0.60^{fg}	0.11 ± 0.003^{e}	3.60 ± 0.08^{d}	10.93 ± 0.01^{b}

Values are mean±SE of 8 animals.

96 mg/kg body weight of danazol resulted in a drastic decrease in ovarian lipids as compared to the control rat.

With the danazol treatment, the amount of phospholipids, glycolipids and free fatty acids decreased in the treated ovaries as compared to the control although an insignificant increase is observed at the dose of 24 mg/kg body weight (Table III, Fig. 2). Maximum amount of cholesterol is found in the control rats (16.46 ± 0.17 mg/kg wet weight of tissue). With the danazol treatment the amount of cholesterol decreases initially and then there is a significant increase in its amount at doses 6, 12, 24 mg/kg body weight of danazol. For higher doses of danazol 48 and 96 mg/kg body weight cholesterol amount again decreased significantly ($P < 0.05$) (Fig. 2). The amount of triglycerides is significantly ($P < 0.05$) less in the control rat ovaries as compared to the treated rats except for the highest dose of danazol i.e. 96 mg/kg body weight, where it is almost comparable to the control rats (Table III, Fig. 2).

DISCUSSION

Intraperitoneal administration of varying doses of danazol to female rats was consistently followed by significant reduction in ovarian weights and disturbed cyclicity. This reflects the inhibitory influence of danazol on synthesis or release of pituitary gonadotrophins or both which results in gonadal suppression, ultimately inhibiting the ovulatory functions (5, 19). Danazol treatment resulted in a dramatic increase in the percentage of follicular atresia. This may be due to the failure of optimum secretion of gonadotrophins and estradiol because of the antigonadotropic and antiandrogenic property of danazol (20). Increased amount of IGT in danazol treated rat ovaries indicates the transformation of atretic follicles in IGT which increases with treatment (21, 22).

The decrease in the amount of proteins in danazol treated ovaries as compared to the control can be attributed to increased incidence of atresia at all stages of follicular development. During the process of atresia the degeneration of follicles result in decrease in total protein content (2). Danazol due to its antigonadotropic property inhibits the secretion of gonadotrophins (19). FSH and LH stimulates the growth and differentiation of follicles and corpora lutea through the synthesis of specific proteins (21, 22) and their inhibition results in a decrease in the proteins in the treated rat ovaries. Maximum decrease in protein contents at 6 mg/kg body weight dose indicates that a plateau of responsiveness might have reached with this dose.

The total lipid contents in the rat ovaries increased with danazol treatment. The follicular kinetics have depicted that the rat ovaries treated with danazol possess a greater number of atretic follicles and interstitial gland tissue which along with degenerating corpora lutea store abundant lipoproteins and may be major source of lipids in the treated ovaries. During atresia steroidogenesis is low (restricting the mobilization of lipids) and thus results in lipid accumulation (21). Danazol through its inhibitory effect on 3βHSDH results in decrease in steroidogenesis (3, 19). The comparative low levels of total lipids with 96 mg/kg body weight doses of danazol may

be attributable to few atretic follicles and excessive amount of interstitial gland tissue which results in the mobilization of lipids after treatment (21).

The high amount of phospholipids and glycolipids are considered to be used for the construction of cellular membranes and the supply of energy rich substances to the growing follicles and oocyte in control ovaries (21–24). The decrease in their amount in danazol treated rat ovaries can be attributed to the degenerative changes in various organelles including smooth endoplasmic reticulum and mitochondria as a result of increased atresia (2, 23, 25). Free fatty acids are contributed by blood phospholipase activity (26). Since danazol because of its antigonadotropic property inhibits the secretion of gonadotropins (FSH and LH), the fall in the quantity of free fatty acids in the treated animals endorses the hormonal regulation of unsaturated fatty acid biosynthesis (27).

Cholesterol may be produced either by *de novo* synthesis in the granulosa and theca cells (28) or may be transported from the plasma into the follicle (29). In the present investigation cholesterol amount decreased initially in the treated ovaries. The decreased number of follicles in the treated ovaries may account for less synthesis and thus low levels of cholesterol. Moreover, it has been suggested that cholesterol utilization in regressing rat luteal tissue is not reduced (28). Excessive interstitial gland tissue in treated ovaries may also account for low cholesterol levels (21). The accumulation of triglycerides signifies the metabolic inactivity in relation to steroidogenesis in danazol treated rat ovary. The biochemical seqence for triglyceride storage is the hormonal milieu associated with luteolysis, perhaps $PGF_2\alpha$ (27). An important role for triglycerides enriched with unsaturated fatty acids may be to provide substrate for intraluteal prostaglandin synthesis which may participate in the process of regression (27).

It may thus be concluded that danazol induces biochemical transformation in the ovarian tissue possible through the hypothalamic pituitary ovarian axis, but further studies are required to elucidate the precise mode of action at the receptor/ subcellular level.

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