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RESEARCH ARTICLE

EFFECTS OF GRADED DOSES OF ESTROGEN BENZOATE ON THE MICROANATOMY OF TESTIS IN MALE ALBINO RATS

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ABSTRACT

The research was conducted in the department of Anatomy Government Medical College Srinagar. The aim of our study was to elucidate the effects of estrogen treatment on the microanatomy of Testis in male Albino rats with reference to the treatment regimen received. For this research we selected sixty male albino rats with an average weight of100gms. The animals were kept in the animal house of Government Medical College Srinagar and divided randomly into three groups. Group A served as control, while Group B and C received daily low and high doses of estrogen benzoate diluted in coconut oil respectively. From each group animals were sacrificed at intervals of one, three, six and twelve weeks. 5-6 micrometer thick histological sections of testis were cut, fixed on glass slides and stained with Haematoxyline & Eosin. Microscopic changes in the testis were recorded. It was observed that estrogen benzoate produces dose and duration dependent histopathological changes in the testis of male albino rats. Our studies revealed that estrogen treatment significantly decreased the diameter of the seminiferous tubules and induced fatty degeneration in the surrounding connective tissue. An increase in collagen fiber synthesis in the extracellular matrix (ECM) surrounding the seminiferous tubules was also induced. Spermatogenesis was impaired resulting in mainly spermatogonia being present. Both Sertoli and Leydig cells showed morphological alterations and glycoprotein accumulations. These results demonstrate that increased estrogen levels drastically impact the human testis.

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INTRODUCTION

Estrogen is thought to have a regulatory role in the testis because estrogen biosynthesis occurs in testicular cells and the absence of ERs caused adverse effects on spermatogenesis and steroid genesis. Moreover, several chemicals that are present in the environment, designated xenoestrogens because they have the ability to bind and activate ERs, are known to affect testicular gene expression. However, studies of estrogen action are confounded by a number of factors, including the inability to dissociate estrogen-induced activity in the hypothalamus and pituitary from action occurring directly in the testis and expression of more than one ER subtype in estrogen-sensitive tissues. Estrogens are widely prescribed steroids. Some food stuffs like soy, grapes and its extract like wine and some cosmetics of our daily use like skin creams and hair dyes contain variable amounts of estrogen. Alfalfa,animal flesh, Anise seed, Apples, Baker's yeast Bareley, Beets, Carrots, Cherries, Chickpeas, Clover, Cowpeas (blackeyedpeas). Cucumbers, Dairy foods, Dates, Eggs, Eggplant. Fennel, Flaxseeds, Garlic, Hops, Licorice, Oats, Olive oil, Olives, Papaya, Parsley, Peas, Peppers Plums Pomegranates, Potatoes, Pumpkin Red beans, Red clover, Rhubarb, Rice Sage. Sesame, Soybean, Sprouts, Soybeans, Split peas, Sunflower seeds, Tomatoes, Wheat, Yams, Soybeans, and soy derivatives have large amounts of natural estrogens. Flaxseed, pulses, citrus fruits, wheat and licorice also have fairly large amounts of natural estrogens. Estrogens are absorbed by oral, parenteral and transdermal routes. Liver plays a key role in its metabolism. It conjugates estrogen to form glucuronides and sulfates which are mainly excreted in urine. Estrogenexerts its effects on many tissues of body by acting on estrogenic receptors (Thiagaraj, 1987). Thus present research was under takento study the effect of graded doses of estrogen benzoate on the microanatomy of testis of male albino rats which shows testicular atrophy with markedly reduced spermatogenesis, vacuolation of Sertoli cells and reduction in Leydig cells, narrow seminiferous cords surrounded by an extensively thickened lamina propria and fatty degeneration of surrounding tissue.

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MATERIALS AND METHODS

Sixty male albino rats weighing on an average 100 grams were taken from animal house of Government Medical College Srinagar for the present study. These animals were divided into three groups.

Group A---12 rats (Control Group) were given normal diet and no drug.
Group B---24 rats were injected 0.05mg of estrogen benzoate daily.
Group C---24 rats were injected 0.2 mg of estrogen benzoate daily

All rats were kept under uniform husbandry conditions in 60 iron cages separately. They were fed with normal dietconsisting of Gram, vegetables and tap water. One milliliter of injection estrogen benzoate (5mg) was diluted with 4mlof coconut oil i.e., each ml of diluted oil contains 01 mg of estrogen benzoate. One ml of diluted drug was taken in subcutaneous (insulin) syringe containing 40 graduations i.e. 01graduation = 0.025mgs. Group B animals were injected2 graduations of drug (0.05mg) daily while as group C animals received eight graduations (0.2mg) daily.

This process of drug administration was continued regularly for 12 weeks. The animals were sacrificed in four sittings i.e. after intervals of one, three, six and twelve weeks. In each sitting three rats from group A, six rats each from group B and Cwere taken out of their cages after anesthetizing with chloroform. The limbs of each rat were fixed on board with pins.A midline incision was given on anterior abdominal wall. Testis were identified, cleaned, dissected out and putin dishes containing formal saline. These tissues were processed manually for block making using standard histological techniques. Sections measuring 5-6 micrometers were cut and fixed on glass slides. The sections were stained with Haematoxyline & Eosin. The microscopic observations were recorded group wise using a Compound light microscope. Appropriate photographs were taken using photographic Microscope and labeled.

RESULTS

In all groups of animals. Following microscopic observations were recorded.

Control group: In this group cut section of testes showed the usual microscopic structure consisting of well-defined capsule, Deep to the capsule,, there is tunica vasculosa– which is a highly vascularised connective tissue. seminiferous tubule, sertoli cells, loose connective tissue \ interstitial tissue & blood vessels

Group B and Group C: The changes in the microscopic structure began to appear after second week of drug administration in the form of increase in thickness of the capsule and significantly decrease in the diameter of tubules. Estrogen treated patients showed fatty degeneration within the surrounding connective tissue (→, PAS) Figure A.

Rats w/o estradiol treatment showed no vacuoles in their connective tissue (PAS) Figure B.

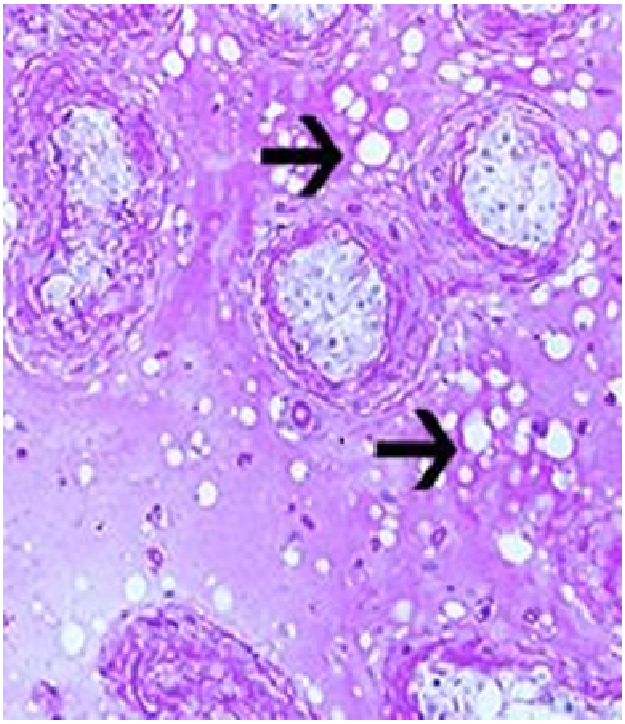

Figure A

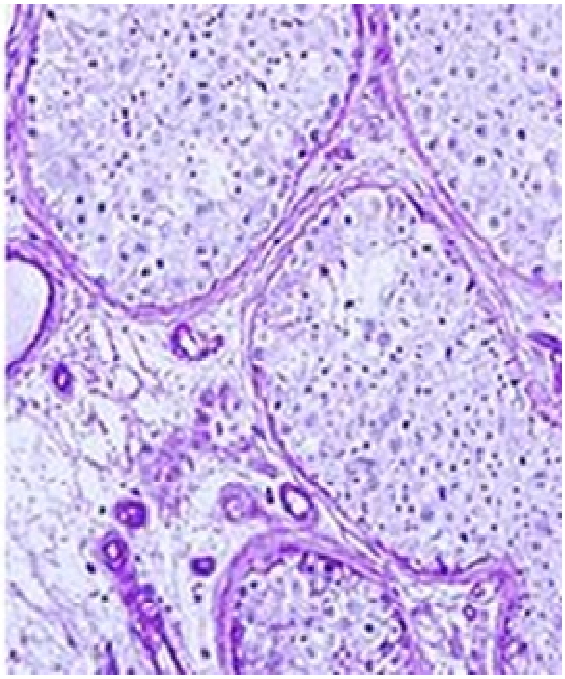

Figure B

Following estrogentreatment, rats showed a large number of vacuoles outside the seminiferous tubules within the surrounding connective tissue (Fig.A, arrows). Estradiol treatment caused an increase in thickness of the seminiferous tubule ECM

All rats show Sertoli cell vacuolation following treatment with estrogen. In addition, most Leydig cells appeared fewer in number, irregularly shaped and with degradation of the nucleus and cytoplasm occurring. Following estrogen treatment, all rats showed synthesis of glycoproteins both within the seminiferous tubules and in the connective tissue surrounding the tubules.

DISCUSSION

Leavy M *et al* findings suggest that the patient receiving estradiol only displayed morphological features closest to the patient receiving estradiol and antiandrogens for the extensive period of 6 years. This suggests that long term administration of these drugs may result in decreased receptor responsiveness and a consequential increase in drug resistance. Another explanation might be an increased rate of elimination of the applied hormones within the body. We also came to conclusion that there is a significant decrease in the diameter of the seminiferous tubules in rats following estradiol treatment. This may explain the finding of Sapino *et al*.10.Who described a 20–70% decrease in weight of the testes after estradiol treatment 10. In light of the report by Gülkesen *et al*.2, who described a decrease in the diameter of the seminiferous tubules of hypospermatogenic men - a finding they noted had also been seen before in previous studies 11, 12,, this suggests that a reduced tubule diameter is correlated with impaired spermatogenesis2. This finding is clinically important as it suggests that success rates of sperm retrieval during testicular sperm extraction (TESE) could be increased by selection of testicular tissue revealing the physiological range of diameter

In the rats investigated, the inhibitory effect of estradiol on spermatogenesis might be due to the estradiol induced impairment of Sertoli cell function. Impaired Sertoli cell function and disturbed communication with germ cells results in the induction of germ cell death 13,14. This is supported by our results, which showed pronounced vacuolisation in the cytoplasm of the Sertoli cells after treatment with estradiol
Alongside alteration of Sertoli cell metabolism, estradiol treatment results in changes of the morphology of Leydig cells. Unlike their usual uniform shape and size, the Leydig cells of the estradiol treated patients appeared fewer in number and showed irregular form and size and signs of degeneration of the nucleus and cytoplasm. Leydig cells are responsible for production of testosterone which is essential for spermatogenesis to occur. It has been shown that excess estradiol exerts negative feedback on luteinizing hormone (LH), which in turn leads to reduced serum testosterone concentrations (Atanassova, 1999). Lack of testosterone prevents germ cells from passing the meiotic stage of division (Holdcraft, 2004; O'Donnell, 1994; O'Donnell, 1996). As excess levels of estradiol inhibit FSH and LH secretion, both the Sertoli and Leydig cell functions are compromised which inevitably results in impaired fertility in the patient 9. Besides its distinct effects on the cells involved in spermatogenesis, treatment with estradiol also induces strong alterations in the connective tissue surrounding the seminiferous tubules. Considerable fatty degeneration occurred in the interstitial tissue surrounding the tubules after estradiol treatment. It has been shown that increased adipose tissue in obese individuals leads to marked elevation of extra gonadal aromatase activity, which results in enhanced estradiol levels. In addition to the occurrence of fatty degeneration, collagen synthesis within the ECM of the seminiferous tubules was increased after estradiol treatment. This is of particular interest because an increase in thickness of the ECM has been correlated with male infertility 61. A correlation between hypospermatogenesis and ECM thickness has been demonstrated: testes from hypospermatogenic men showed an increase in thickness in the seminiferous tubule ECM alongside a decrease in diameter of the seminiferous tubules (Gülkesen *et al*., 2002). Similar effects are seen in the transsexual patients after estradiol treatment in this study, demonstrating a link between increased estradiol and enhanced synthesis of collagen fibers in the testicular tissue. Sertoli cells are involved in the deposition of ECM components such as collagen and laminin (Skinner *et al*., 1985; Borland, 1986). Thus, the increased collagen synthesis might also be a result of impaired Sertoli cell function. In summary, our study has shown that treatment with estradiol results in severely impaired spermatogenesis which is correlated with increased synthesis of glycoproteins in Sertoli cells and Leydig cells and increased collagen synthesis and fatty degeneration in the testicular connective tissue. The results of this study have added new insights by quantifying the significant decrease in diameter of seminiferous tubules after hormonal treatment, by linking the different effects seen to different treatment regimens and by assessing the effects of estradiol treatment on ERα expression in the human testis.

Ethical approval: The study protocol was approved by Government Medical college Ethics committee.

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