EFFECT ON BIOCHEMICAL PARAMETERS AFTER ADMINISTRATION OF DIFFERENT DOSES OF ESTRADIOL IN MALE RATS

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Abstract: In the animal models prepared by us in whom the animals are hypophysectomised and treated with gonadotropin to maintain the spermatogenesis, the estradiol is delivered through sialistic capsules in hypophysectomised animals maintained on exogenous supply of gonadotropin. The present study has been designed to determine the dose specific effects of estradiol on the overall activity of the animal. In order to find out this, different doses of estradiol in sialistic capsules viz 1cm.2cm.4 cm were implanted in the animals. A comparison of biochemical parameters of proteins, cholesterol and sialic acid was done with the controls. Serum as well as tissues such as liver, reproductive organs and accessory organs was used. The results show that 1cm sialistic capsule though had some effect on these parameters but were not significant to have an effect on the overall metabolism of the animal.

Index Terms: Spermatogenesis, Estrogen, Sialistic Capsule.

I. INTRODUCTION

The role played by estrogens in the spermatogenic regulation yet remains unclear, but it has been depicted to suppress the male reproductive functions to a severe extent. [18]Estrogen hormone serves to play a dual role by acting as survival factor as well inhibitory agent by suppressing the gonadotropin production when given in excess.[17] It is widely known that estradiol inhibits spermatogenesis[19] and testosterone production[8]The present study has been designed to determine the dose specific effects of estradiol on the overall activity of the animal. In order to find out this, different doses of estradiol in sialistic capsules viz 1cm.2cm.4 cm were implanted in the intact animals. A comparison of biochemical parameters of proteins, cholesterol and sialic acid was done with the controls. Serum as well as tissues such as liver, reproductive organs and accessory organs were used. In the animal models prepared by us in which the animals are hypophysectomised and treated with gonadotropin to maintain the spermatogenesis, the estradiol is delivered through sialistic capsules in hypophysectomised animals maintained on exogenous supply of gonadotropin.

II. MATERIAL AND METHODS

Male rats in the body weight range of 200-250 gm were procured from the Central Animal House, Rajasthan University, Jaipur and were maintained at the animal house, Zoology department, JNVU. Animals were kept in a well ventilated room, exposing them to 12 hrs light and 12 hrs darkness and were given food pellets at lib. In another experiment, testicular tissue was obtained from hypophysectomised animals. Hypophysectomised animals were treated with 400IUhCG and 1IUFSH. In these animals sialistic capsules filled with estrogen were implanted subcutaneously in the abdominal skin.

III. EXPERIMENT DESIGN

3.1The animals were divided into two groups. Each group consisted of 5 animals.

Group I : This group consisted of 5 animals. Intact, male rats in the body weight range of 200-250 gm were treated as controls, whereas the experimental animals were implanted with 1cm,2 cm,& 4 cm long sialistic capsules filled with estradiol. The controls received empty sialistic capsules.

GroupII: Animals in this group were hypophysectomised and were maintained on 400 IU hCG and IU FSH. These animals were then implanted with 1 cm sialistic capsule filled with estrogen. The controls received empty capsules.

CHEMICALS: Estradiol were purchased from Sigma and Pharmacia respectively. Solvents of reagent grades were used. Chemicals for in situ labeling and vital staining were purchased from Organon and Oncor.

3.2SIALISTIC CAPSULES: They were prepared by methods described earlier and implanted.[20]

3.3SERUM BIOCHEMISTRY: Serum protein,cholesterol was estimated by routine methods

3.4TISSUE BIOCHEMISTRY: The following organs were excised and weighed: Testis, Epididymis, Seminal Vesicles, Vas deferens, Ventral Prostate. The testis was divided into two portions – one was used for histology and the other was used for Biochemical analysis. Following parameters were studied to make biochemical evaluation.

a. Protein
b. Cholesterol
c. Sialic acid

Frozen tissues were analyzed for quantitative estimation using following biochemical techniques. Each set of the experiment contained minimum two samples. Individual tissue samples were used for each parameter.Cholesterol was estimated in Testis and Liver, by method of Zaltkis,*et al.,* (1953). Protein was estimated in Testis, Cauda epididymis, Caput epididymis, Seminal Vesicle and Ventral prostate. (*Lowry,et al., 1951*) Sialic acid was estimated by the method of Warren (1959).

IV. RESULTS & DISCUSSIONS

4.1 SERUM BIOCHEMISTRY

a. Protein: Intact rats treated with 4 cm capsule show an significant reduction **(***P ≤ 0.05) in* the serum protein levels whereas the changes in protein levels in serum whereas the animals treated with 1 cm,2cm capsules showed an insignificant decrease when compared with the mean values of the control animals. The reasons for this could be that normally only 10% of the total receptor side of the testicular cytosol can be occupied by endogenous estradiol.[16,4] On exogenous administration of estradiol rest of the sites is also occupied which are then trans located to the nucleus and binds with chromatin to exert effect upon gene expression. [3]It has been suggested that an excess amount of estrogen from Sertoli cells regulate the testosterone biosynthesis by Leydig cells [8]

b. Cholesterol: Administration of 4 cm capsules caused significant reduction **(***P ≤ 0.05) in* the serum cholesterol values when compared with that of the control values. (Fig A)

4.2 TISSUE BIOCHEMISTRY

Tissue protein: The results show insignificant decrease in protein concentration in all the tissues.

Tissue Cholesterol

a. Testis: A non-significant decrease in cholesterol content of testis was noticed in rats treated with 1 cm capsule. The animals treated with 2 cm capsule as well as 4 cm capsule showed significant reduction in the cholesterol content and attained significance (*(P ≤ 0.01)* in comparison with intact control group. Cholesterol is the precursor for the biosynthesis of steroid in the testis.[15]A fluctuation in the quantitative biochemical analysis of cholesterol in the testis indicates abnormalities in the testis. In the present study, we have found reduction in the cholesterol level after treatment with various doses of estradiol. This reduction was found to be insignificant statistically in the animals treated with 1 cm capsule but in case of the animals treated with 2 cm and 4 cm capsule there was a significant reduction in the cholesterol levels. However, these results are not in accordance with the result obtained by earlier workers.[6] They have observed accumulation of cholesterol in testis in Hypophysectomised rat and mice. Cholesterol has basically an important role in the maintenance of cell membrane of the cells in their various stages of development. A reduction in cholesterol concentration may be attributed to loss of these germinal cells and damage to the advance stages of spermatogenesis. (FIGB)

b.Liver*:* A significant decrease **(***P ≤0.05) in* cholesterol level was observed in the Liver of rats treated with 1cm capsules when compared to control group.with the increasing dosages of E2 (2cm and 4cm),significant decrease in the cholesterol contents was observed(FIGC)

Tissue Sialic Acid

a.Testis: The concentration of sialic acid reduced insignificantly in the animals treated with 1 cm capsule, whereas the animals administered with 2 cm capsule& 4cm capsule showed significant (*P ≤0.05) decrease* in the sialic acid concentrations. **(**Fig.D)

b.Caput Epididymis: Animals treated with 1 cm sialistic capsule filled with estrogen showed insignificant reduction in the sialic acid concentrations .The animals treated with 2 cm & 4cm capsules filled with estradiol also did not show any significant change in the sialic acid concentrations. **(**Fig.E)

c.Cauda Epididymis: There was a moderately significant reduction in the sialic acid concentrations in the Cauda epididymis of animals treated with 1 cm capsule whereas the animals administered with 2 cm and 4 cm capsules showed highly significant reduction in the concentration of sialic acids**. (**FigF)

d.Seminal Vesicle Though the concentrations of sialic acids reduced by estrogen treatment in all the groups but the reduction was not found to be significant.

e.Ventral Prostate: The sialic acid concentrations in the ventral prostate did not change in any of the groups and were found to be in the normal range.

In the present investigation there was moderately significant reduction in the Sialic acid concentration. The reduction of sialic acid establishes the fact that estrogen concentration is effected at late maturation stages of spermatids. The almost necrotic condition of the testis is due to reduction in sialic acid concentration.[1]These results are in accordance with the results obtained by others.[5,2] They have also observed reduction in sialic acid after treatment with the test substance.

Sialic acid is synthesized and secreted by the epithelial cells of the epididymis. It is believed to help in lubrication and sperm transport by reducing the friction between spermatozoa. Sialic acid also helps in maturation, capacitation and fertilization of sperms[11,13,14.] Lower concentration of sialic acid may cause deteriorating effect on the structural integrity of the spermatozoa[10] In the present investigation a significant reduction has been observed in the concentration of sialic acid. This reduction must have led to prevention of maturation of spermatozoa leading to impaired spermatogenesis [20]

A significant reduction in the cholesterol concentration has been observed in animals implanted with 2 cm and 4 cm capsules. Thus it may be reasonably safe to conclude that estrogen effects the levels of cholesterol and sialic acids in the testis and

epididymis which result in the reduction in sperm motility and sperm density which further effect the weight of the organ. In a similar study, significant inhibition of structural and functional integrity of accessory glands has been observed following estradiol treatment.[9, 12,18] In their studies and later on others have proposed that reduction in androgen level impedes the activity of these reproductive organs thereby rendering them hostile for their concerned function.[7]

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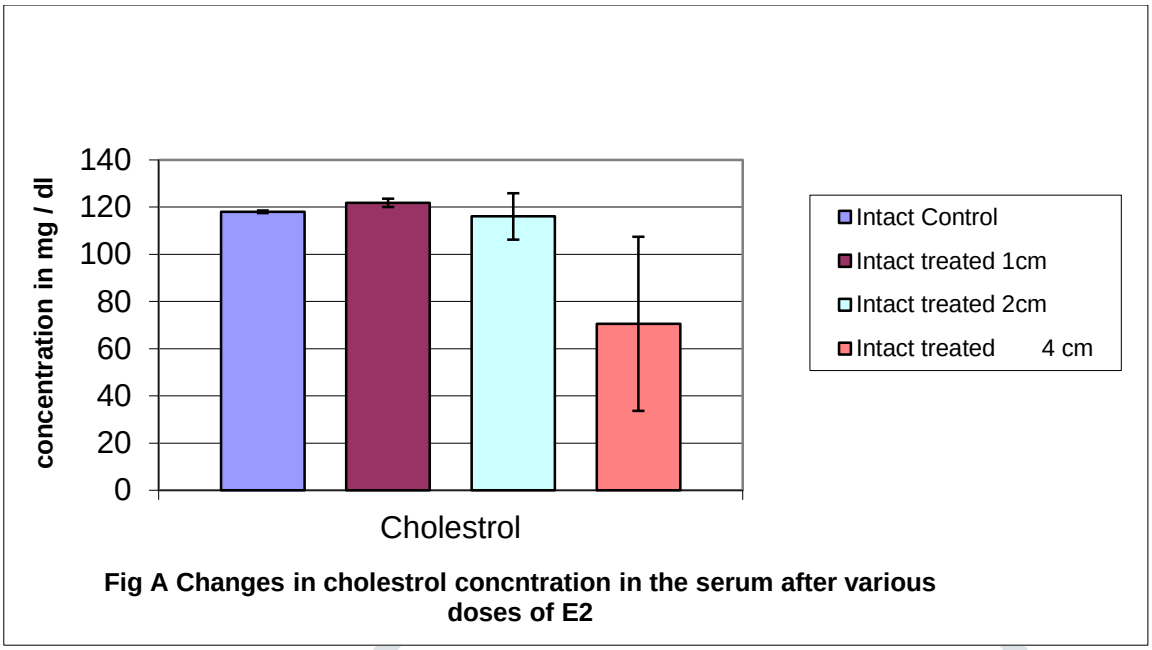

Fig A Changes in cholestrol concntration in the serum after various doses of E2

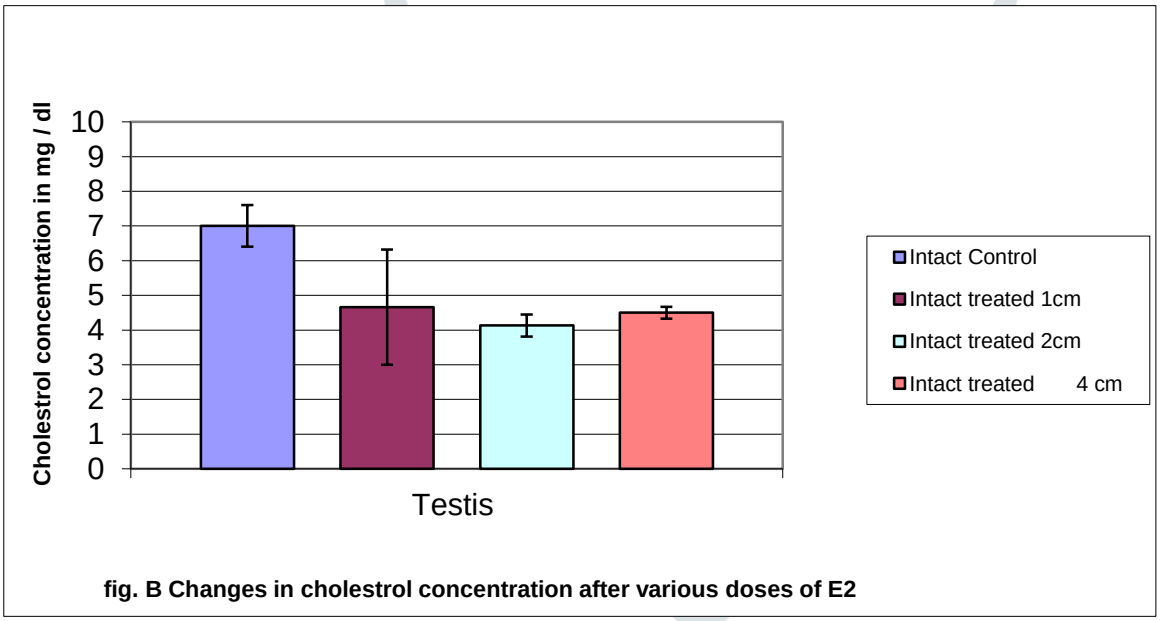

fig. B Changes in cholestrol concentration after various doses of E2

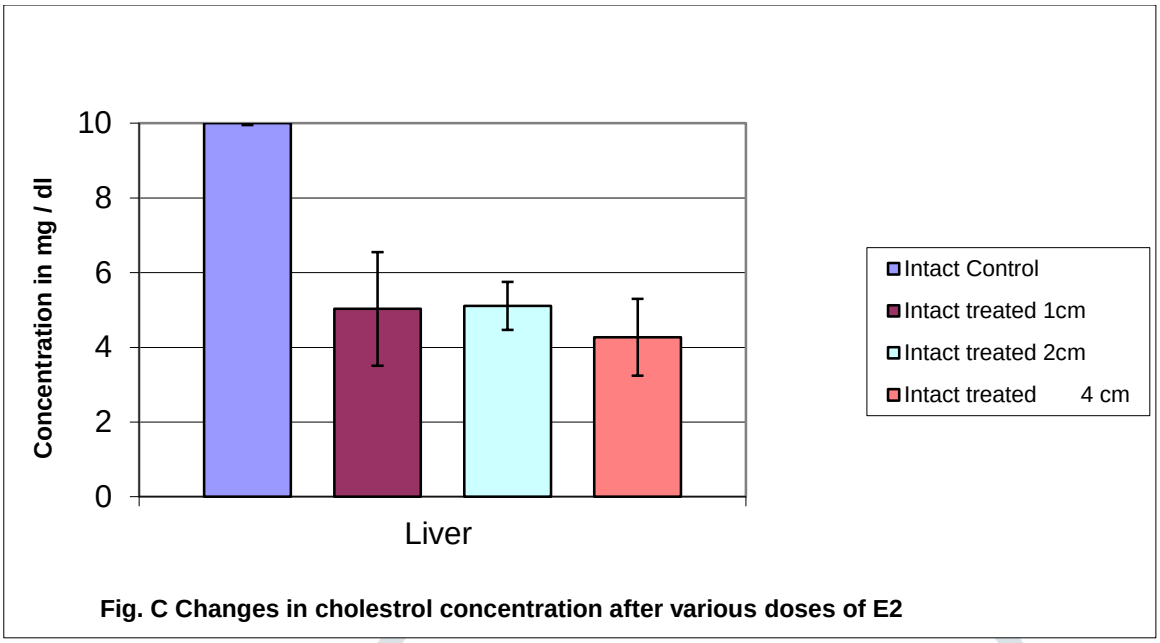

Fig. C Changes in cholestrol concentration after various doses of E2

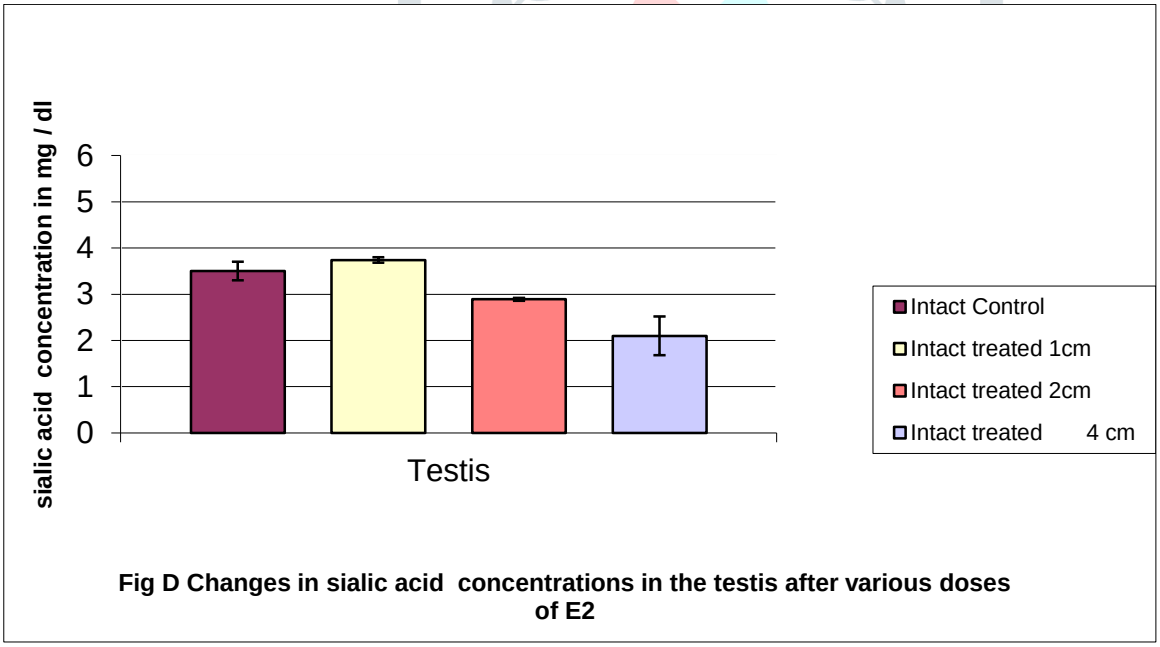

Fig D Changes in sialic acid concentrations in the testis after various doses of E2

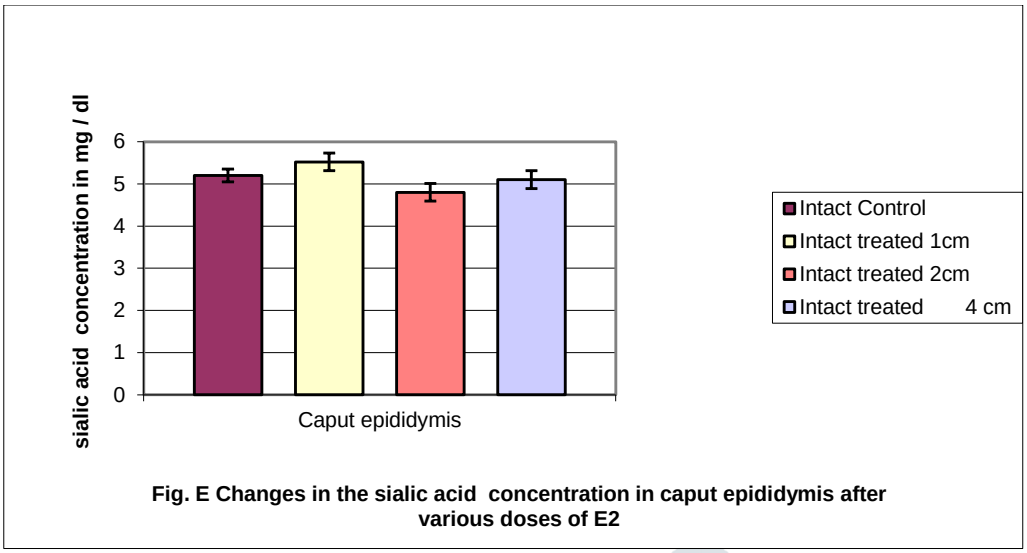

Fig. E Changes in the sialic acid concentration in caput epididymis after various doses of E2