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INFLUENCE OF STEROIDAL AND NON-STEROIDAL CONTRACEPTIVE PILLS ON HORMONAL ALTERATIONS IN WISTAR FEMALE ALBINO RATS.

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ABSTRACT

The effects of steroidal and non-steroidal contraceptive oral pills were studied in wistar female albino rats for 30 days. In steroidal pill fed rats FSH, estradiol and cortisol were found elevated at all the dose levels and LH, Progestesone and Testosterone were depleted significantly. In nonsteroidal contraceptive pill fed rats, no significant change was seen in FSH levels. However, there was a significant depletion in LH, Estradiol, Progesterone and Testosterone level. The steroidal and non-steroidal contraceptive oral pill in the present investigation indicates some negative effect on the ovarian activity.

KEY WORDS: Steroidal and non-steroidal contraceptive oral pill, hormonal alterations, female wistar albino rat.

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INTRODUCTION

Oral contraceptives allow effective and convenient family planning for women and couples worldwide and have revolutionized the reproductive lives of millions of female populations. The discovery of estrogen and progesterone and their potential contraceptive effects led to an enormous amount of research on fertility regulation in females. The estrogen component of combined oral contraception is either ethinyl estrodiol or mestranol and the progestagens used are cyproterone acetate, desogestral ethynodiol diacetate, gestodene, levonorgestrel, lynoestrenol, megestrol, norethisterone, norethisterone acetate, norethynodrel, norgestimate and norgestrel. Currently used estrogen is ethinylestradiol and commonly used progestogen is norethisterone. The different progestins have somewhat different physiological effects, interact differently with estrogens, possibly modifying the effects of both hormones [8,9]. The progesterone facilitates the action of estradiol in the LH surge prior to ovulation [7]; a contraceptive action of a progesterone antagonist was also contemplated.Centchroman (ormeloxifene) has been marketed in India since last 20 years as an alternative to steroidal Oral Contraceptive Pills (OCPs) and it is provided free of cost through Government sponsored Family Welfare Program [11]. Centchroman, a third generation Selective Estrogen Receptor Modulator (SERM) selectively acts on estrogen receptors as agonist and antagonist in different reproductive tissues [10].Now a days there are several steroidal and non-steroidal preparations in the form of oral pills are available in the market, however their results vary greatly. Some are known to produce some side effects which may be physiologically serious hence it is thought to study the effect of selected steroidal and non-steroidal contraceptive pills on hormonal alterations in the albino rat.

MATERIALS AND METHODS

Experimental Animal Models

The present study was carried out in wistar female albino rats weighing about 125g ± 2 g. The animals were procured from National Institute of Nutrition (NIN), Hyderabad. Animal experiments were conducted according to " INSA – Ethical guidelines for the use of animals for scientific research after getting permission from ethical committee".The animals were kept in vivarium throughout the period of experiment. They were regularly fed on standard pellet diet provided by National Institute of Nutrition, Hyderabad and water *ad-libitum*. The remaining food and waste matter was removed from the cages on the next day and proper care was taken to avoid any infection. Only healthy rats were used for the present experiments.Estrous cycle of female rats was observed by daily examination of vaginal smear. Only the female rats displaying at least two consecutive estrous cycle of 4-5 days duration was selected for the present study and animals were acclimatized fort night. After recording their initial body weights, they were divided into two main groups, 1) Control and 2) Experimental. The cages of rats of both the sexes were kept side by side to avoid Boot and Lee effect. However, the females were kept assorted through the experimental period.

Pills

The experimental female albino rats were selected for steroidal and non-steroidal contranceptive oral pills in calculated doses. Steroidal Contraceptive pill is combined oral contraceptive pill with Brand name was Choice .Each Tablet contains Norgestrel 30 mg and Ethinylestradiol 0.03 mg (Manufactured by : Hindustan latex limited). Non Steroidal oral contraceptive Pill with Brand name, Saheli. Each Tablet Contain Centchroman - 30 mg (Manufactured by :- Hindustan Latex Limited).

Doses

Dilutions of pills were made by using double distilled water (DDW). The combined oral contraceptive pill (norgestrel + ethinylestradiol) was diluted to 0.14mg/ ml (Low Dose) , 0.21 mg/ml (dose as per literature), and 0.43 mg/ ml (high dose).

The non-steroidal oral contraceptive pill (Centchroman) was diluted to 0.29 mg/ ml (Low Dose) , 0.43 mg/ml (dose as per literature) and 0.87 mg/ml (high dose) . The doses of both drugs were calculated as per body weight of rats considering the human consumption and available literature.

Experimental set up

Experiments were carried out by dividing female albino rats into three groups:

Group I :- Control female albino rats administered orally with 1ml DDW/ rat / day upto 30 days DDW being used as vehicle .

Group II :- Group of combined oral contraceptive pill. This group was again divided into three sub-groups .

Sub-group I :- Experimental female albino rats administered orally with 1 ml norgestrel + ethinylestradiol/ rat / day upto 30 days. 1 ml dose contains 0.14 mg norgestrel + ethinylestradiol.

Subgroup II:- Experimental female albino rats who received 1ml norgestrel + ethinylestradiol / rat / day upto 30 days . 1 ml dose contains 0.21 mg norgestrel + ethinylestradiol.

Subgroup III :- Experimental female albino rats administered orally with 1 ml norgestrel +ethinylestradiol/rat/day upto 30 days. 1 ml dose contains 0.43 mg norgestrel+ ethinylestradiol.

Group III :- Group of rats was administered orally with Centchroman. This group was divided into three sub groups.

Subgroup I:-Experimental female albino rats administered orally with1ml Centchroman / rat / day upto 30 days . 1 ml dose contains 0.29 mg Centchroman.

Subgroup II:- Experimental female albino rats administered orally with1mlCentchroman / rat / day upto 30 days. 1 ml dose contains 0.43 mg Centchroman.

Subgroup III:-Experimental female albino rats administered orally with 1 ml Centchroman / rat / day upto 30 days. 1 ml dose contains 0.87 mg Centchroman .

Collection of Blood sample and Hormonal Assay

The venous blood was obtained from the caudal vein of the control and experimental female albino rats prior to sacrifice to study the hormonal assay. The blood was collected into vacutainer tubes (5 ml) and were kept for separation of serum. Then the separated serum was centrifuged at 3000 rpm in an ultracooling centrifuge for 30 minutes. The serum was stored at - 20^0 C until it was assayed for hormones.Known quantity of separated serum from control as well as experimental rats were used to study the changes in hormones like FSH, LH, estradiol, progesterone, cortisol and testosterone by using the Radio Immuno Assay (RIA) Techniques .

RESULTS

The female albino rats were fed with specific doses of steroidal and non-steroidal contraceptive pills for 30 days. During this experimental period no mortality was observed in all female albino rats administered orally with vehicle, steroidal combined oral pill (ethinylestradiol + norgestrel) and non-steroidal oral pill (Centchroman) seperately.In steroidal pill fed rats FSH, estradiol and cortisol were found to be significantly elevated at all the dose levels while LH, Progestesone and Testosterone were depleted significantly (Table-1). In nonsteroidal contraceptive pill fed rats no significant change was seen in FSH level (Table-2). However, there was a significant depletion in LH, Estradiol, Progesterone and Testosterone level. The only hormone whose blood level was elevated was cortisol.

Table 1

Alterations in hormonal profile of female albino rats fed with steroidal contraceptive pills for 30 days.

Hormonal Parameter	Control	Steroidal contraceptive pills		
		0.14 mg/ml/rat/ day	0.21 mg/ml/rat/da y	0.43mg/ ml/rat/day
FSH (MIU/ML)	0.73 ± 0.04	1.70±0.08 (+132.87)	1.75 ± 0.11 (+139.27)	1.90 ± 0.05 (+160.27)
LH (MIU/ML)	7.20 ± 0.08	5.10 ±0.16 (-29.16)	2.60 ± 0.24 (-63.88)	2.71 ± 0.08 (-62.36)
Estradiol (PG/ML)	74.90±0.12	113.00 ±0.56 (+50.86)	79.10 ±1.92 (+5.60)	78.30 ± 2.14 (+4.53)
Progesteron e (NG/ML)	21.00±0.16	5.00 ± 0.22 (-76.19)	6.60 ± 0.44 (-68.57)	5.40± 0.32 (-74.28)
Cortisol (MLG/DL)	0.70 ± 0.04	0.98 ± 0.01 (+40.00)	1.50 ± 0.16 (+114.28)	1.10 ± 0.01 (+57.14)
Testosterone (NG/ML)	0.41 ± 0.08	0.11± 0.01 (-73.17)	0.05± 0.01 (-87.80)	0.12 ± 0.01 (-70.13)

** Steroidal pill :-Norgestrel (0.30 mg) + Ethinylestradiol (0.03 mg) (CHOICE)*
** The Values are mean of 6 replicates ± SE*
** Values in parenthesis indicate percent change over control.*
** All values are significant at p < 0.01*
** NS :- Not significant*

Table 2

Alterations in hormonal profile of female albino rats fed with non-steroidal contraceptive pills for 30 days.

Hormonal Parameter	Control	Non - steroidal contraceptive Pills		
		0.29 mg/ml/rat/day	0.43 mg/ml/rat/day	0.87mg/ ml/rat/day
FSH (MIU/ML)	0.73 ± 0.04	0.71 ± 0.05^{NS} (-2.73)	0.66 ±0.08^{NS} (-9.58)	0.70 ±0.08^{NS} (-4.10)
LH (MIU/ML)	7.20 ± 0.08	3.70 ±0.16 (-48.61)	2.45 ± 0.66 (-65.97)	2.60 ± 0.11 (-63.88)
Estradiol (PG/ML)	74.90 ±0.12	69.20 ± 5.15 (- 7.61)	21.50 ± 2.14 (-71.29)	18.00± 1.06 (-75.96)
Progesterone (NG/ML)	21.00±0.16	14.50 ± 1.22^{NS} (-30.95)	19.00 ± 1.11^{NS} (-9.52)	11.60± 0.99^{NS} (-44.76)
Cortisol (MLG/DL)	0.70 ± 0.04	0.80± 0.02 (+14.28)	1.40 ± 0.09 (+100.00)	0.80 ± 0.58 (+14.28)
Testosterone (NG/ML)	0.41 ± 0.08	0.07± 0.01 (-82.92)	0.35± 0.01 (-14.63)	0.09 ± 0.01 (-78.04)

Non - steroidal Pill:-Centchroman (30 mg) (SAHELI)
** The Values are mean of 6 replicates ± SE*
** Values in parenthesis indicate percent change over control.*
** All values are significant at p < 0.01*
** NS :- Not significant*

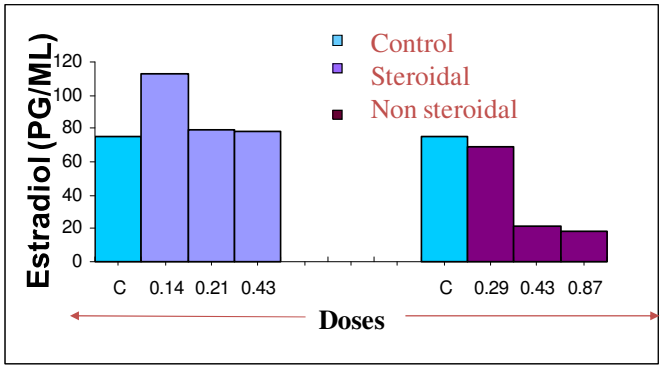

Fig. 1 :- Alterations in estradiol (PG/ML) in the female albino rat fed with steroidal and non -steroidal contraceptive pills for 30 days (Doses mg/ml/rat/day,control rats were administered equivalent amount of vehicle).

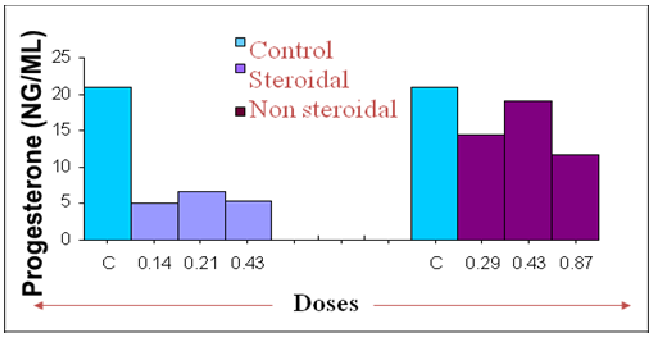

Fig. 2:- Alterations in progesterone (NG ML) in the female albino rat fed with steroidal and non-steroidal pills for 30 days (Doses are mg ml rat day, control rats were contraceptive administered equivalent amount of vehicle).

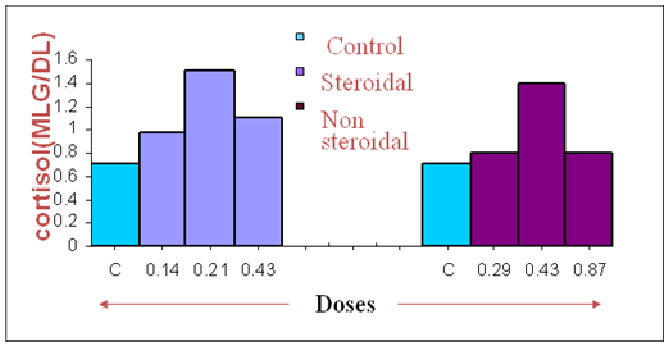

Fig. 3 :- Alterations in cortisol(MLG DL) in the female albino rat fed with steroidal and non-steroidal contraceptive pills for 30 days (Doses are mg ml rat day, control rats were administered equivalent amount of vehicle).

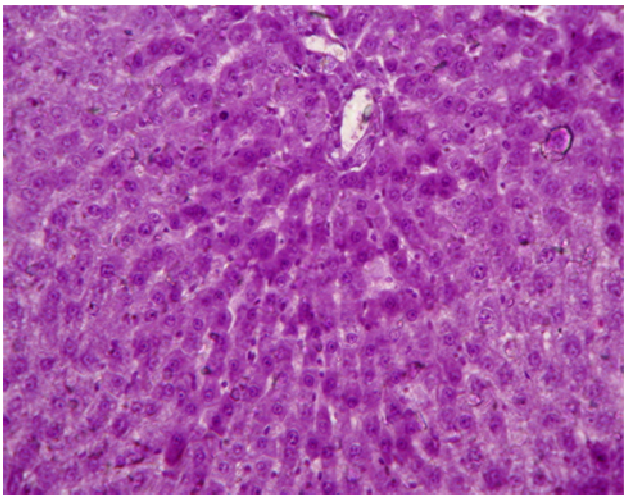

Fig. 4:- T. S. of liver showing hyperactive hepatic cells.

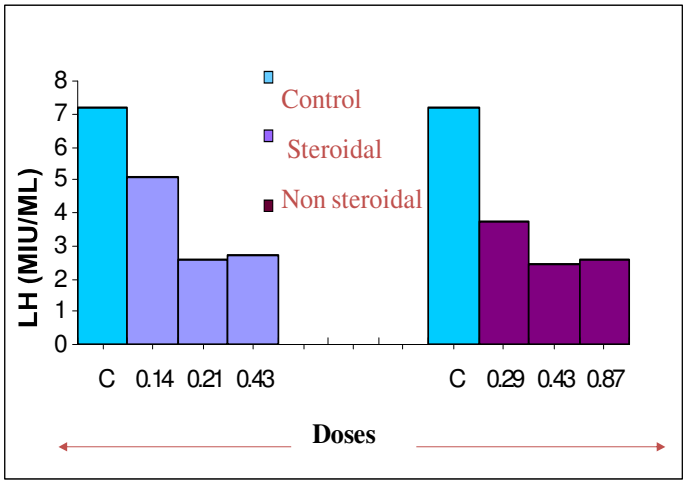

Fig. 5 : Alterations in LH (MIU/ML) in the female albino rat fed with steroidal and non-steroidal contraceptive pills for 30 days (Doses are mg/ml/rat/day control rats were administered equivalent amount of vehicle).

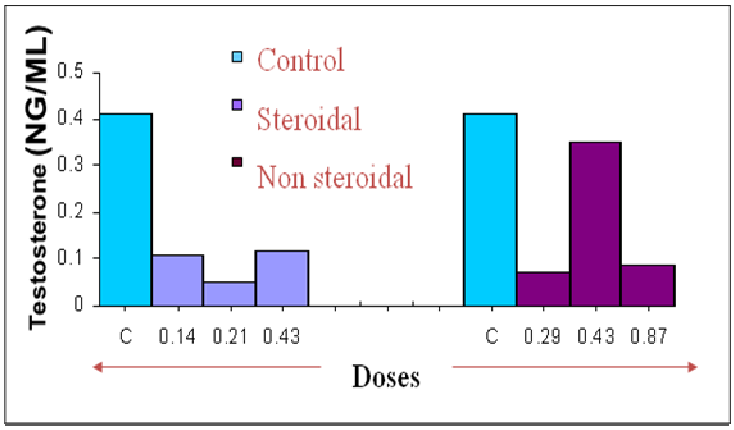

Fig. 6 :- Alterations in testosterone (NG/ML) in the female albino rat fed with steroidal and non-steroidal contraceptive pills for 30 days (Doses are mg/day, control rats were administered equivalent amount of vehicle).

DISCUSSION

In the present investigation lowered serum estrogen was seen in Centchroman fed rats (Fig.1). The degenerated corpora lutea and decreased serum progesterone (Fig.2) might have affected the progesterone-estrogen ratio which affects the implantation. This proves that Centchroman have anti-implantation activity. However, Luteotropihc activity in adult rats has been reported to increase after exogenous treatment of estrogen [4] . On the contrary, progesterone inhibits the post- coital ovulation in rabbit [1]. Though Centchroman is reported to be antiprogestation in its activity, no significant alterations were seen in the serum progesterone (Fig.2) level in the Centchroman treated rats which indicates no significant effect on the maturation of follicles. However, the estradiol level in serum was seen depleting at normal and higher dose of Centchroman. This may be because of excretion of estradiol through kidney as Centchroman acts as the antagonist of estrogen. Further the binding sites of estrogen receptors might have been occupied by Centchroman as it is antiestrogenic in nature [1] .At these specific dose levels, the effect of Centchroman was only about one sixth of that produced by estradiol + progesterone. Therefore, roughly speaking the antiestrogen action of Centchroman is about six times more potent than its estrogenic effect when both are expressed in terms of the action of 0.14mg/day of estradiol + progesterone. Roy and Datta (1976)[5] reported the antiestrogenic action of Centchroman as four times more potent than its estrogenic effect.Both steriodal and non- steroidal pill fed rats showed significant increase in total serum cortisol (Fig.3). The increase in total cortisol ranged between 40% to 114 % in case of steroidal pill consumption and 14.28% to 100% in the case of Centchroman fed rats. This may partly be stressed induced phenomenon. Metcalf and Beavan (1963)[2] and Williamson and Moody (1970)[3] also demonstrated increased serum cortisol level after estrogen therapy in human female volunteers. The increased serum cortisol implies an increased synthesis of transcortin by the liver. Liver cells were formed to be hyperactive in the present investigation in both the experimental sets of rats (Fig.4).The altered secretions of the FSH, LH, progesterone and estrodiol in the present investigation indicates some negative effect on the ovarian activities. The suppression of total serum testosterone by 70-80%, which has been observed during present experiments (Table1 and Table 2) might have been caused by both a reduction of gonadotropin release and a rapid direct inhibitory effect of sex steroids on ovarian and adrenal steroid synthesis. Since the serum testosterone (Fig.6) and LH (Fig.5) of female rats were found to be decreased after Centchroman and steroid treatments, it is possible that the reduced circulating level of androgens and gonadotrophins contribute to the alterations in androgen sensitive parameters in target organs or else their response to androgen might be affected. Centchroman, at the selected three doses, does not affect hyphothalamo- pituitary-gonadal axis as evidenced from unaltered serum concentration of FSH (Table 1.). In these rats serum LH level was decreased significantly and progesterone exhibited in significant change. These result indicate forcefully uncontrolled abnormal ovulation leading to formation of corpus luteum. But corpus luteum secreted less amount of progesterone due to deficiency of enough LH (Fig.5).

SUMMARY AND CONCLUSION

Ethinyl estradiol+norgestrel reduced LH output from the pituitary gland. At the same time FSH was found to be significantly increased. The altered secretion of FSH , LH, Progesterone and estradiol in the present investigation indicate some negative effect on the ovarian activity. Thus the present results indicate that the trials of Centchroman as a contraceptive pills should be given a second thought. No doubt, it has anti-estrogenic action , it prevents the ova to pass through fallopian tubes and anti-implantation activity however it also shows some adverse effect on the ovary. More research is needed to calculate the perfect dose which will not lead to any adverse effect on the ovary.

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