Research Paper

Volume : 4 | Issue : 9 | Sept 2015 • ISSN No 2277 - 8179

The Effect of Selected Steroidal and Non-Steroidal Contraceptive Pills on Changes in the Body and Organ Weights of Wistar Female Albino Rats.

Zoology

KEYWORDS : Steroidal and non-steroidal contraceptive oral pill, body, organ weight, female wistar albino rat

M.P.Chikhale

Department of Zoology, Rajarshee Shahu Science College ,Chandur Rly, Dist.Amravati , Maharashtra, India. 444904.

ABSTRACT

The effects of steroidal and non-steroidal contraceptive oral pill on changes in the body and organ weight were studied in wistar female albino rats for 30 days. The steroidal 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) . In steroidal pill fed rats almost all the reproductive organs and related glands exhibited moderate to highly significant decrease in the respective organ weights. However, uterus, adrenal and thyroid gland showed significant increase in their weights. Similarly in non-steroidal pill fed rats there was highly significant decrease in the weight of fallopian tubes and further the adrenal and thyroid weights were also found to be increased significantly ($p<0.01$).In the present investigation a dose of 0.14mg/ml/rat/day has resulted into 49.14% increases in uterine weight however, dose of 0.29mg/ml/day/rat has resulted into 20.76% decline in uterine weight. The rate of gain in the body weight was decreased in all the groups treated with norgestrel + ethinylestradiol as well as Centchroman indicating some adverse effects on the rats. More research is needed to calculate the perfect dose which will not lead to any adverse effect.

Introduction

Many women worry about birth control causing weight gain. It is not surprising that women often blame contraception for their weight gain. Hassan *et al*., 2003 studied the weight variation in a cohort of women using copper IUD for contraception and recorded non hormonal contraception (i.e., copper intrauterine device, barrier methods) has not been associated with a change in body weight. Combined hormonal contraception pill has not been associated with a change in body weight (Gallo et al., 2004). Rudel and Kincl (1971) in their review noted that progesterone was an increase in the body and liver weights of female rats receiving parenteral progesterone. The levonorgestrel-releasing intrauterine device, in long-term users, has been associated with a small increase in weight that is equivalent to the weight gain associated with increasing age (Ronnerdag and Odlind, 1999). Increase in uterine weight is usually associated with estrogenic activity. Whereas decrease in uterine is usually correlated with relevant antiestrogenic activity of a particular compound (Rudel *et al*., 1967). Dorfman et al., 1961 has been reported in the uterine weight of mature mice by the administration of 16mg of norethindrone , but increase has been found in the immature rat by administering norethindrone as well as depoprovera (McPherson, 1974).

Centchroman (ormeloxifene) is a novel non- steroidal contraceptive agent developed by the Central Research Institute (CDRI), Lucknow, India. It offers an unique combination of weak estrogenic and potent antiestrogenic properties and it is provided free of cost through Government sponsored Family Welfare Program. Today several steroidal and non-steroidal preparations in the form of oral pills are available in the market with varying results. 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 changes in the body and organ weights of female albino rats.

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 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 cyclicity of female rats was observed by daily examination of vaginal smear cytology. Only the female rats displaying at least two consecutive estrous cycle of 4-5 days duration were selected for the present study .Experimental animals were acclimatized fort night. After recording their initial body weights, they were divided into two main groups, 1) Control and2) 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 throught the experimental period.

Pills

The experimental female albino rats were given selected steroidal and non-steroidal contranceptive oral pills in calculated doses. Steroidal Contraceptive pill are 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 Steroida 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 up to 30 days DDW being used as vehicle.

Group II: Group of combined oral contraceptive pill. This group was again dividedinto 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 were 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 .

Body weight and organ weight study:
Changes in the body and organ weights of female albino rats from both control and experimental groups were noted regularly. The body weights of the rats were recorded every alternate day up to 30 days of treatment. After 30 days , control and experimental female albino rats were sacrificed with cervical dislocation and there remove the tissues like ovary, fallopian tube, uterus, kidney, adrenal gland, heart , liver and thyroid gland. The tissues were then taken out quickly blotted and weighed to the nearest of ± 0.1 mg with electronic balance.

Statistical Analysis:
Student's "t" test was used to test the significant level.

Observation and Results
Upon oral administration of steroidal (ethinyl estradiol + norgestrel) and non-steroidal (Centchroman) oral contraceptive pill seperately for 30 days, the female rats exhibited significant changes in their body weights.

The female rats of control group were given equal amount of vehicle for 30 days. The body weights of these rats were found to increase gradually during 30 days. The rise in body weight of rats of this group was 8.73 % after 10 days, 17.46% after 20 days, 25.39% after 30 days.

The rats of both the experimental groups also showed duration dependent increased body weight, however this rise in body weight was insignificant when compared with the rise in body weight of control rats (Table 1.1 and 1.2)

The experimental results of this study are given in table - 2.1. In steroidal pill fed rats almost all the reproductive organs and related glands exhibited moderate to highly significant decrease in the respective organ weights. However, uterus (fig.1) , adrenal and thyroid gland showed significant increase in their weights.

Similarly in non-steroidal pill fed rats there was highly significant decrease in the weight of fallopian tubes and further the adrenal (fig.2) and thyroid weights were also found to be increased significantly ($p<0.01$).

Discussion
The female albino rats of approximately same age and weight were fed with three different doses of steroidal pills containing ethinyl estradiol and norgestrel for 30 days and also three different doses of non-steroidal Centchroman contraceptive pills. The rate of gain in the body weight was decreased in all the groups treated with steroidal pills as well as Centchroman indicating some adverse effects on the rats (Table1.1). All the rats fed with equal and measured quantity of food and water was provided *ad-libitum*.Increase in the relative weight of adrenal gland (Fig.2) in the drug treated group particularly at higher doses (0.43mg/ml/rat/day of steroid and 0.87 mg/ml/rat/day of Centchroman) may be explained on the basis of marked reduction in the gain of body weight.

The present results show that graded doses of Centchroman caused a progressive decrease in the weight of the uterus. A maximum response in the uterine weight was attainted (Table 2.2). In the present investigation a dose of 0.14mg/ml/rat/day has resulted into 49.14% increases in uterine weight however, dose of 0.29mg/ml/day/rat has resulted into 20.76% decline in uterine weight. Dorfman *et al.*, 1961 has been reported in the uterine weight of mature mice by the administration of 16mg of norethindrone , but increase has been found in the immature rat by administering norethindrone as well as depoprovera (McPherson, 1974).

That is, 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

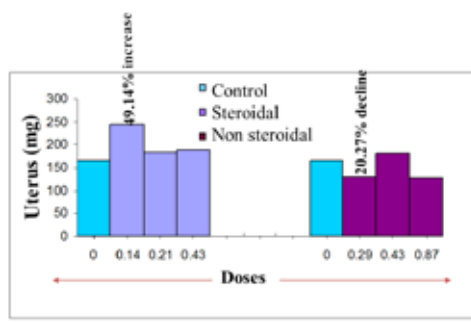

Fig 1 . Alterations in weight of uterus (mg) of the female albino rat fed with steroidal and non-steroidal contraceptive pills for 30 days.

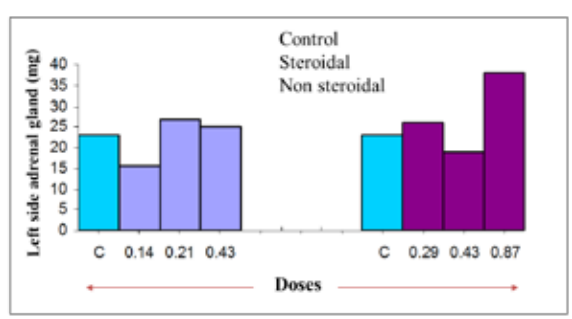

Fig 2 :- Alterations in weight of left side adrenal gland (mg) of the female albino rat fed with steroidal and non-steroidal contraceptive pills for 30 days.

in terms of the action of 0.14mg/ml/rat/day of estradiol + progesterone. Roy and Datta (1976**)** reported the antiestrogenic action of Centchroman as four times more potent than its estrogenic effect. Further the increased uterine and ovary weight in the steroid fed rats may be due to increased lipid synthesis in response to estradiol and progesterone.

Ethinyl estradiol + norgestrel caused a significant ovarian atrophy and reduction in the ovarian weight. This suggests that LH output from the pituitary gland was reduced. At the same time FSH was significantly increased. The altered secreations of the FSH, LH, progesterone and estrodiol in the present investiga-

tion also indicate some negative effect on the ovarian activities (Chikhale, 2015).

Conclusion :

The rate of gain in the body weight was decreased in all the groups treated with steroidal pills as well as Centchroman indicating some adverse effects on the rats. Various doses of Centchroman caused a progressive decrease in the weight of the uterus. The effect of Centchroman was only about one sixth of that produced by estradiol + progesterone indicating antiestrogen action of Centchroman as about six times more potent than its estrogenic effect. More research is needed to calculate the perfect dose which will not lead to any adverse effect on the ovary.

Table. 1.1 Alterations in body weights (grams) of female albino rats before and after feeding the steroidal pills for 30 days.

Sr. No.	Duration Of Treatment	Control	Steroidal Pill		
			0.14 mg/ ml/rat/ day	0.21 mg/ ml/rat/ day	0.43mg/ ml/rat/ day
1	Initial Weight (gm)	126 ± 1.62	124 ± 1.15	125 ± 0.78	123 ± 0.80
2	10 day (gm)	137 ± 1.80 (8.73 %)	122 ± 3.16 (-1.61)	131 ± 4.88 (+4.80)	129 ± 2.22 (+4.87)
3	20 day(gms)	148 ± 1.11 (17.46 %)	128 ± 3.20 (+3.22)	137 ± 3.56 (+9.60)	135 ± 4.40 (+9.75)
4	30 day(gms)	158 ± 2.15 (25.39 %)	129 ± 2.25 (+4.03)	141 ± 5.25 (+12.80)	149 ± 3.86 (+21.13)

* 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.

Table. 1.2 Alterations in body weights (gms) female albino rats before and after feeding the non-steroidal pills for 30 days

Sr. No.	Duration Of Treatment	Control	Non-steroidal Pill		
			0.29 mg/ ml/rat/ day	0.43 mg/ ml/rat/ day	0.87mg/ ml/rat/ day
1	Initial Weight (gm)	126 ± 1.62	124 ± 0.65	127 ± 1.55	125 ± 1.05
2	10 day (gm)	137 ± 1.80 (8.73 %)	128 ± 5.28 (+3.22)	129 ± 3.35 (+1.57)	132 ± 2.11 (+5.60)
3	20 day(gms)	148 ± 1.11 (17.46 %)	133 ± 3.55 (+7.25)	135 ± 2.88 (+6.29)	138 ± 4.62 (+10.40)
4	30 day(gms)	158 ± 2.15 (25.39 %)	140 ± 2.18 (+12.90)	135 ± 4.56 (+6.29)	142 ± 3.99 (+13.60)

* Non - steroidal Pill:- Centchroman (30 mg) (SAHELI)

* The Values are mean of 6 replicates ± SE

* Values in parenthesis indicate percent change over control

Table. 2.1 :-Alterations in organ weights (milligram's or grams) of female albino rats before and after feeding the steroidal pills for 30 days.

Sr. No.	Organ	Control	Steroidal Pill		
			0.14 mg/ml/ rat/ day	0.21 mg/ml/ rat/day	0.43mg/ ml/ rat/day
1	Ovary (mg)	Right	79.30 ±3.55	65.80 ± 1.22 (-17.02)	66.9 ± 3.20 (-15.63))	60.00 ± 2.22 (-24.33)
		Left	46.20 ±2.18	45.40 ± 2.65 (-40.41)	50.00 ± 1.66 (-34.38)	69.00 ± 5.15 (-9.44)
2	Fallopian Tube (mg)	Right	163.20 ±3.15	95.60 ± 3.14 (-58.57)	125.4 ± 5.22 (-23.16)	146.00 ±3.80 (-10.53)
		Left	157.20 ± 2.86	167.30 ± 5.13 (+6.42)	114.6 ± 5.10 (-27.09)	135.00 ± 3.22 (-14.22)
3	Uterus (mg)		679.00 ± 3.10	576.60±5.16 (-15.67)	647.00±8.10 (-4.71)	637.40 ±6.66 (-6.12)
4	Kidney (mg)	Right	794.30 ± 2.85	724.00 ±8.16 (-8.85)	674.7 ± 6.22 (-15.05)	739.6±8.25 (-6.88)
		Left	788.20 ± 5.16	669.90± 5.25 (-15.00)	635.6± 8.80 (-19.36)	664.6±7.33 (-15.68)
5	Adrenal Gland (mg)	Right	24.10 ± 1.22	19.10 ± 3.25 (-20.74)	23.00 ± 1.65 (-4.56)	27.00 ± 2.85 (+12.03)
		Left	22.90 + 1.25	15.60 ± 2.13 (-31.87)	26.90 ± 3.15 (+17.46)	25.00 ± 1.88 (+9.17)
6	Thyroid Gland (mg)	Right	10.00 ± 0.90	9.60± 0.85 (-4.00)	10.4 ± 1.15 (+4.00)	10.1 ± 0.86 (+1.00)
		Left	9.45 ± 0.56	7.10 ± 0.66 (-24.87)	8.2 ± 0.52 (-13.22)	10.2 ± 1.03 (+7.93)
7	Heart (mg)		679.00 ± 3.10	576.60±5.16 (-15.67)	647.00±8.10 (-4.71)	637.40 ±6.66 (-6.12)
8	Pituitary Gland (mg)		9.30 ± 0.55	8.50 ± 0.62 (-8.60)	7.7 ± 0.50 (-17.20)	9.00 ± 0.45 (-3.22)
9	Liver (gm.)		7.22 ± 2.16	4.77 ± 0.45 (-34.07)	5.53 ± 0.92 (-23.40)	6.83 ± 0.33 (-5.40)

* 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.

Table. 2.2 :-Alterations in organ weights (milligram's or grams) of female albino rats before and after feeding the non-steroidal pills for 30 days.

Sr. No.	Organ		Control	Non-steroidal Pill		
				0.29 mg/ml/rat/ day	0.43 mg/ml/rat/ day	0.87 mg/ ml/rat/ day
1	Ovary (mg)	Right	79.30 ±3.55	55.10 ± 2.55 (-30.51)	60.80 ± 2.60 (-23.32)	72.90 ± 3.20 (-8.07)
		Left	46.20 ±2.18	49.50 ± 4.02 (-35.03)	59.3 ± 3.15 (-22.17)	66.7 ± 4.05 (-12.46)
2	Fallopian Tube (mg)	Right	163.20 ±3.15	172.30 ±5.65 (-18.93)	168.3 ± 4.10 (+3.12)	120.5±10.88 (-26.16)
		Left	157.20 ± 2.86	148.20±3.22 (-5.72)	159.10±2.66 (+1.20)	98.9 ± 8.62 (-37.08)
3	Uterus (mg)		679.00 ± 3.10	130.10±6.11 (-20.76)	181.6±3.15 (+10.59)	128.3±9.36 (-21.86)
4	Kidney (mg)	Right	794.30 ± 2.85	654.00±12.15 (-17.66)	712±6.88 (-10.36)	824±12.15 (+3.73)
		Left	788.20 ± 5.16	610.4±10.11 (-22.55)	627.6±10.15 (-20.37)	806.5±11.32 (+2.32)
5	Adrenal Gland (mg)	Right	24.10 ± 1.22	29.10 ± 2.30 (+20.74)	19.4 ± 1.05 (-19.50)	31.5 ± 4.33 (+30.70)
		Left	22.90 + 1.25	26.00 ± 1.66 (+13.53)	19.1 ± 2.06 (-16.59)	38.00 ± 2.66 -65.93
6	Thyroid Gland (mg)	Right	10.00 ± 0.90	10.90 ± 1.08 (+9.00)	11.3 ± 0.48 (+13.00)	16.30 ± 1.32 (+63.00)
		Left	9.45 ± 0.56	9.2 ± 0.32 (-2.64)	10.00 ± 0.66 (+5.82)	76.1 ± 1.55 (+70.37)
7	Heart (mg)		679.00 ± 3.10	689.00± 8.15 (+1.47)	589.00±16.10 (-13.25)	822.9±15.62 (+21.19)
8	Pituitary Gland (mg)		9.30 ± 0.55	9.70 ± 0.75 (+4.30)	8.29 ± 0.25 (-10.86)	8.9 ± 0.50 (-4.30)
9	Liver (gm.)		7.22 ± 2.16	8.05 ± 0.88 (+11.49)	5.57.± 0.99 (-22.85)	7.99 ± 0.92 (+10.66)

* Non - steroidal Pill :-Centchroman (30 mg) (SAHELI)

* The Values are mean of 6 replicates ± SE

* Values in parenthesis indicate percent change over control.

REFERENCE

Chikhale, M. P. (2015). Influence of steroidal and non-steroidal contraceptive pills on hormonal alterations in wistar female albino rats. International Journal of Pharma and Bio Sciences, 6(3): (B) , pp.521 – 528. | | Dorfman, R. I., Kincl, F. A. & Ringold, H. J. (1961) . Anti-estrogen assay of neutral steroids administered by subcutaneous injection. Endocrinology, 68 , pp.17. | . | Hassan, D. F., Petta, C.A., Aldrighi, J.M., Bahamondes, L. & Perrotti, M.(2003). Weight variation in a cohort of women using copper IUD for contraception . Contraception,68, pp. 27–30. | | Gallo, M., Grimes,D.A. Schulz, K., & Helmerhorst, F. (2004). Combination estrogen-progestin contraceptives and body weight: systematic review of randomized controlled trials. Obstet Gynecol,103, pp. 359–73. | | McPherson, J.C., Costoff, A., Eldridge, J.C. & Mahesh V.B. (1974). "Effect of Various Progestational Preparations on Gonadotropin Secretion in Ovariectomized Immature Female Rats . Fertil. Steril. 25:1063-1070, | | Ronnerdag, M. & Odlind, V. (1999). Health effects of long-term use of the intrauterine levonorgestrel-releasing system: a follow-up study over 12 years of continuous use. Acta Obstet Gynecol Scand, 78, pp.716–21. | | Roy, S. & Datta, J.K.(1976). Nature of estrogenic and antiestrogenic actions of Centchroman on rat uterus. Contraception, 13, pp. 597. | | Rudel, H.W., Lebberz, M., Maque-topete, M., Martinez- Manautou, J., & Blessler, S.J. (1967). Assay of the anti-oestrogenic effects of progestagens in women," J Reprod Fert , 13, pp. 199. | | Rudel, H.W. & Kincl, F.A. (1971). The toxicity of progesterone . In: Tausk M, ed. International Encyclopedia of Pharmacology and Therapeutics, New York, NY, Pergamon Press, 405-40, 1971.