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Research Article

HAEMATOLOGICAL CHANGES IN WISTAR FEMALE ALBINO RATS FED WITH STEROIDAL AND NON-STEROIDAL CONTRACEPTIVE PILLS

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ABSTRACT

The effects of steroidal and non-steroidal contraceptive oral pill on haematological parameters were studied in wistar female albino rats for 30 days. The steroidal combined oral contraceptive pill (norgestrel + ethinylestradiol) was diluted to 0.14 mg/ 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 result shows a reduction in Red blood cell (RBC) and White blood cell (WBC) counts in all the groups that were given steroidal combined oral pill (ethinylestradiol + norgestrel) and non-steroidal oral pill (Centchroman), compared to the controls in each group. The reduction of RBC, WBC and Lymphocytes was found in steroidal combined oral pill (ethinylestradiol + norgestrel)**.** In the present investigation a dose of 0.43 mg/ ml (high dose)and 0.87 mg/ml (high dose) has more reduction in Red blood cell (RBC) and White blood cell (WBC) counts as compare to other groups. The changes in haematological parameters 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.

Keywords: Steroidal and non-steroidal, Contraceptive oral pill, Haematological parameters, Female wistar albino.

INTRODUCTION

Oral contraceptive allows effective and convenient family planning for women and couple worldwide and has revolutionized the reproductive lives of millions of females. Oral pills are the most frequently used hormonal contraceptives and commonly contribute to increased blood pressure, blood clots, heart attack and stroke (VanHylckamaVlieg and Middeldorp, 2011). Administration of Oral Contraceptive (OC) steroids has been shown to increase plasma viscosity and haematocrit value (Lowe *et al*., 2000). Drife (1991) suggested that the frequency of cadiovascular, hemostatic and metabolic risks associated with oral contraceptive are directly related to the hormonal dose of oral contraceptives. The effect of Centchroman has been studied by Srivastava *et al.* (1984), on platelet aggregation.Micronized progesterone appears to achieve this effect without producing any adverse effects on hemostasis and blood pressure (Sitruk-Ware *et al*., 1987). The Chemical contents in contraceptive oral pills are known to produce large number of hormonal and biochemical alterations (Chikhale, 2015a). Centchroman offers a unique combination of weak estrogenic and potent antiestrogenic properties (Chikhale, 2015b). Therefore, we designed this study to observe the effects of steroidal combined oral pill (ethinylestradiol + norgestrel) and non-steroidal oral pill (Centchroman) contraceptives on haematological parameters in the female wistar rats.

MATERIALS AND METHODS

Experimental animal models

The present study was carried out in wistar female albino rats weighing about 125 g ± 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

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next day and proper care was taken to avoid any infection. Only healthy rats were used for the present experiments.

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) and non-steroidal oral contraceptive pill with Brand name, Saheli. Each tablet contains 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 0.14 mg/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 divided into three sub-groups.

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

Subgroup II: Experimental female albino rats who received 1 ml 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 up to 30 days. 1 ml dose contains 0.29 mg Centchroman.

Subgroup II: Experimental female albino rats administered orally with 1ml Centchroman/rat/day up to 30 days. 1 ml dose contains 0.43 mg Centchroman.

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

Haematological study

The venous blood was obtained from the caudal vein of the control and experimental female albino rats to study the haematological parameters like R.B.C. Count, T.L.C. and D.L.C using Neubauer's Chamber (Dacie and Lewis, 1982).

Statistical analysis

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

RESULTS AND DISCUSSION

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) separately. In both the sets of experiments erythrocytes as well as total leucocytes were depleted. The depletion of leucocytes in non-steroidal pill fed rats was more (Table 1). Polymorphs were decreased in number but at the same time lymphocytes were increased. In steroid pill fed rats polymosphs were increased and leucocytes were decreased (Table 1).

The result shows a reduction in Red blood cell (RBC) and White blood cell (WBC) counts in all the groups that were given steroidal combined oral pill (ethinylestradiol + norgestrel) and non-steroidal oral pill (Centchroman), compared to the controls in each group. The finding of lower RBC, WBC and Lymphocytes in steroidal combined oral pill (ethinylestradiol + norgestrel) agrees with the finding of Sajida *et al.* (2006). Abdalla *et al.* (2009), studied haematological parameters in Sudanese women and reported that no changes in full blood count in women on combined oral contraceptives (COC). The difference may be as a result of the use of different COC with different concentration of estrogen and progesterone and the duration of use. Estrogen therapy especially with high doses of synthetic estrogen in COCs has been shown to increase platelet count and platelet aggregation, to enhance cloth formation increasing the incidence of thromboembolic disease (Meade *et al.*, 1980).

Table 1. Alterations in Haematological parameters of female albino rats fed with steroidal and Non- steroidal contraceptive pills for 30 days.

Parameter	Control	Steroidal contraceptive pills			Non - steroidal contraceptive Pills		
		0.14 mg/ml/ rat/day	0.29 mg/ml/ Rat/ day	0.43 mg/ml/ rat/day	0.87 mg/ml/ rat/day	0.21 mg/ml/ rat/day	0.43 mg/ml/ rat/day
R.B.C. 10^6 cell/cu	5.95 ± 0.88	5.63 (-5.37)	5.51 (-7.39)	4.78 (-19.66)	5.51 (-7.39)	5.59 (-6.05)	4.08 (-31.42)
W.B.C. 10^3 cell/cu	5.90	5.90 (Nil)	4.85 (-17.79)	4.1 (-30.50)	2.45 (-58.47)	4.05 (-31.35)	3.30 (-44.06)
Polymorph %	20.10	27.11 (+35.00)	20.24 (Nil)	26.66 (+30.00)	16.24 (-20.00)	18.22 (-10.00)	13.40 (-35.00)
Lymphocytes	74.66	68.08 (-8.10)	71.28 (-4.05)	68.36 (-8.10)	79.24 (+6.75)	76.55 (+2.70)	76.62 (+2.70)
Monocytes	1.00	1.00 (Nil)	2.00 (+100.00)	1.00 (Nil)	--	2.00 (+100.00)	2.00 (+100.00)
Eosinophil	3.00	2.00 (-33.33)	4.00 (+33.33)	3.00 (Nil)	3.00 (Nil)	3.00 (Nil)	5.00 (+66.66)
Basophil	2.00	2.00 (Nil)	3.00 (+50.00)	2.00 (Nil)	2.00 (Nil)	1.00 (-50.00)	4.00 (+100.00)

* Steroidal pill-Norgestrel (0.30 mg) + Ethinylestradiol (0.03 mg) (Choice)

* 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

CONCLUSION

The erythrocytes as well as total leucocytes were depleted 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 depletion of leucocytes. 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 haematological parameters.

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