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ORAL CONTRACEPTIVE PILLS "ANOVLAR 1" INDUCED CLASTOGENIC EFFECT IN HUMAN GENOME

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ABSTRACT

This work was planned to investigate the genetic effect of the oral contraceptive drug "Anovlar 1" on human chromosomes. In order to achieve such a purpose, Heparinized blood samples were collected from healthy adults. Lymphocyte cells were isolated and cultures were performed at 37 ºC for 72 hrs. The results obtained showed that the tested drug was proven to be a positive clastogen, since C-metaphases; stickiness; fragments and polyploidy were observed in a concentration-response relationship. The result, however, revealed that the tested drug has clastogenic activity upon human genome.

INTRODUCTION

Estrogen and progestron play a vital role in preparing the female reproductive tract for the reception of sperm and implantation of a fertilized ovum. Agents that mimic the effects of these hormones act as contraceptives (Gilman *et al., 1985).*

However, oral contraceptives have been available for general use only within the last three decades (Bishun *et al.,* 1976). In general, contraceptive pills were found to have many effects on the ovary, uterus, breast and liver functions (Khalifa, 1992). In addition, it might affect the metabolism of carbohydrates and lipids. Other effects including cardiovascular and skin manifestations were also reported (Gilman *et al.,* 1985). Katzung (1985) reported that contraceptive pills depress ovarian functions with subsequent decrease in the number of developed follicles and corpora lutea. Minimal stromal oedema with diminshed amount of endogenous estrogen and pregnandiol in urine were demonstrated while the cervix of the uterus might show some hypertrophy and polyp formation. After prolonged use cervical mucus becomes thick and less copious. Furthermore, the endomerium might show stromal deoiduation towards the end of the cycle. Lactation is also suppressed considerably in women using contraceptive pills. The incidence of vaginal, uterine and breast cancer was found to increase, together with increased benign hepatoma incidence with contraceptive usage (Greenwald *et al.,* 1971 ; and Huggins & Guintoli, 1979).

Oral contraceptives were shown to be capable in inducing sticky degeneration and improper spreading with clumping of the meiotic chromosomes in dogs (William *et al.,* 1968); dominant lethal mutations in female mice (Badr & Badr, 1974); an increase of aneuploid frequencies among oocytes of female mice (Rohrborn and Hansmann, 1974); the breakage of lymphocyte chromosomes (Littlefield *et al.,* 1975); mutagenic effect in Ames strains *(*Ahmed *et al.,* 1985); genotoxic effects in

Saccharomyces cerevisiae, Allium cepa, Mus musculus and human leucocyte cultures (Seehy *et al.*, 1989 and Seehy & Hafez, 1992).

This work aims at investigating the clastogenic activity of the contraceptive pills ‘‘Anovlar 1’’ upon human genome

MATERIALS AND METHODS

The oral contraceptive pills ‘‘Anovlar 1’’ used in this study are produced by Schering AG Company in the from of tablets, each one is composed of Ethinyl estradiol (0.05 mg) and Northestrone acetate (1mg).

The following concerntrations per liter medium were chosen and tested:

1- 0.42 □g ethinyl estradiol + 8.5 □g Norethisterone acetate.
2- 0.85 □g ethinyl estradiol + 17 □g Northisterone acetate.
3- 1.70 □g ethinyl estradiol + 34 □g Northisterone acetate.
4- 4.25 □g ethinyl estradiol + 85 □g Northisterone acetate.
5- 8.5□□g□ethinyl estradiol + 170 □g Northisterone acetate.

The above mentioned concentrations correspond to 0.5; 1; 2; 5; and 10 folds of the daily therapeutic dose.

Heparinized venous blood was collected from normal healthy adults. The standard leucocyte cultures used in this investigation consisted of Minimum Essential Medium (Eagle) with L-Glutamine (SIGMA) supplemented with penicillin-streptomycin (10000 units – 10000 □g / ml). For each 10 ml of this medium, 0.5 ml whole, blood, 0.25 ml phytohemaglutinin (SIGMA) were added. The cultures were incubated in tightly sealed tubes at 37 ؛C for 72 hours. The proper concentration of the drug was added to the culture. Four culture, for each tested concentration were employed. 24 hours after the inucubation.

Two hours prior to harvest 0.1 ml colcemid (SIGMA) was added to two out four cultures for each tested concentration and then the cultures were reincubated for 2 hours.

The method decribed by Schwazacher (1974) was used in order to prepare the metaphases Staining was carried out using 10% Giemsa (pH 6.8) for 5 min., slides were then air dried and chromosomes were examined for deletion, break, gap.. etc. (Brusick, 1986). For each concentration 100 scorable metaphase cells were examined for the different types of chromosomal aberrations.

In order to investigate the effect of the used concentration upon cell proliferation a metaphase index as well as mitotic index (from untreated cultures with colcemid) based on at least 1000 counted cells were recorded.

RESULTS

Mitotic index :

Cytological examination showed that the mitotic index (estimated in absence of colcemid) was found to be 14.80 in the control group. It ranged from 21.10 after treatment with a half the daily therapeutic dose to be 45 after treatment with 10 folds of the daily therapeutic dose. The results are given in Table (1) and illustrated in Figures (1-10).

Table (1) : Mitotic index after treatment with the tested concentrations of oral contraceptive "Anovlar 1"

Concentrations : correspond to the daily therapeutic dose.	Mitotic index
Control	14.80 ± 1.12
½ ** DTD	21.10 ± 2.04
1 DTD	26.20 ± 2.14
2 DTD	31.30 ± 2.44
5 DTD	40.20 ± 3.82
10 DTD	45.00 ± 3.94

*** Mitotic index = $\frac{\text{No. dividing cells}}{1000} \times 100$**

**** DTD : Daily Therapeutic Dose.**

Metaphase index (MI) :

Cytological examination showed that MI was found to be 11.2 in the control group. It was proven to be increased with the increasing of tested concentrations. It ranged from 11.8 to 28.7% after treatment with half and 10 folds of the daily therapeutic dose respectively (Table, 2) .

Chromosomal abnormalities :

The result obtained (Table, 3) showed that the aberrations induced were mainly stickiness; fragments and polyploidy. In addition, the cytological examination revealed that the affected metaphases contained frequently more than one type of aberrations (Figures 3-10).

Table (2) : Metaphase index after treatment with "Anovlar 1"

Concentrations : correspond to the daily herapeutic dose.	Metaphase index
Control	11.2 ± 1.22
½ ** DTD	11.8 ± 1.40
1 DTD	13.2 ± 1.82
2 DTD	16.5 ± 1.86
5 DTD	22.1 ± 2.21
10 DTD	28.7 ± 2.26

*** Metaphase index = $\frac{\text{No. metaphases}}{1000} \times 100$**

**** DTD : Daily Therapeutic Dose.**

Table (3) : Chromosome analysis of human leucocyte cultures after treatment with different concentrations of the oral contraceptive "Anovlar 1"

Concentration;	Percent of metaphases					Total percent of aberrant metaphases
	Stickiness	Gap	Fragment	Polyploidy	Deletion	
Control	1	2	-	-	1	4
½ ** DTD	2	1	2	-	5	10
1 DTD	4	3	6	2	1	16
2 DTD	8	4	5	4	-	21
5 DTD	16	-	8	7	2	33
10 DTD	23	-	11	13	-	47

*** DTD : Corresponds to the Daily therapeutic Dose.**

Fig1,2

3,4

5,6

7,8

9,10

DISCUSSION

The present investigation was carried out in order to study the clastogenic activity of the oral contraceptive drug "Anovlar 1" upon human chromosomes.

The analysis of mitotic as well as metaphase indices presented evidence that the tested drug was proven to be positive in inducing significant increases in mitotic phases (in absence of colcemid) as well as metaphase index. Increases in mitotic index were found to be higher than those in metaphase index. These differences may be caused by the colcemid treatment. However, this result presented an evidence that "Anovlar 1" enhances the cell proliferation.

The analysis of mitotic abnormalities gave an evidence for the capability of "Anovlar 1" in inducing stickiness; and fragments. Stickiness is a common type of chromosomal aberrations due to either alteration of the net charge of chromosomal proteins i.e., histones or non histones, or by induction of DNA cross-linking (Emery & Muller, 1998; Hafez, 1998; and Seehy, 2003). The result obtained, however, revealed that the tested drug was not only effective in inducing in changes chromosome structure but also in inducing spindle apparatus aberration, since polyploid cells were observed. Therefore, it could be suggested the "Anovlar 1" is a potent clastogen upon human genome. Such a conclusion agrees with that found by Khalifa (1992) on mice chromosomes; and with that reported by Ahmed *et al* (1985) on Salmonella test : and with that reported by Seehy & Hafez (1992) who investigated the clastogenic as well as the nutagenic effects of Nordette and Norminest on yeast, onion, bean, mice; and human lymphocytes. The present results, however, are in accordance with those found by Ali *et al* (1977), who investigated the mutagenic and carcinogenic effects of the contraceptive drug "Gyn-Anovlar 2 " in *S. pombe.*

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أقراص منع الحمل أنوفلار – ١ تحدث أثر تكسيرى في چينوم الإنسان

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أجريت هذه الدراسة على عقار منع الحمل أنوفلار – ١ بهدف معرفة قدرته على إحداث ضرر لكروموسومات الإنسان فى مزارع خلايا الدم الليمفاوية . تم جمع عينات دم من متطوعين أصحاء ثم فصلت الخلايا الليمفاوية وزرعت معمليا في بيئة خاصة على درجة حرارة ٣٧°م لمدة ٧٢ ساعة وقبل جمع الخلايا بساعتين أضيفت مادة الكولسميد لوقف الانقسام الميتوزى عند دور الوضع المتوسط والذى تم صبغه ثم فحص مجهرياً.

أظهرت نتائج الفحص المجهرى أن العقار – قيد الدراسة – ذو قدره تكسيرية موجبة للمادة الوراثية للإنسان فى مزارع خلايا الدم الليمفاوية حيث أستحث تكوين الشظايا واللزوجة الكروموسومية ونشاط الإنقسام الميتوزي علاوه على وقف تكوين خيوط المغزل بصورة معنوية . وتوافقت هذه النتائج مع نتائج آخرين استخدموا كائنات أخرى.