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Genetic Effect Of Hormonal Contraceptives On Mitotic Index Of Somatic Cells In Female White Rats/Iraq

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Abstract
The current study is designed to detect the effect of birth control pills on the effects of the Mitotic index (MI) in the bone marrow cells of female white rats, where two doses of the drug (50 and 100) mg/ml were tested. In addition to the negative control and positive control groups dosed with a genetically mutated drug, cyclophosphamide CP, these concentrations were given to the white rats by dosing method for a period of 15 days, and the average percentage Mitotic index (MI) for bone marrow cells was calculated. The study reached the following results:
1- The concentration of 50 mg/ml showed an increase in the Mitotic index of the bone marrow cells but it was slight and insignificant.
2. The concentration 50 100 mg/ml showed Significant superiority in raising the percentage in the Mitotic index of the bone marrow cells.
3- The decrease in the Mitotic index for positive control significantly, so that this decrease formed a significant difference when compared with negative control.
Keyword: *Hormonal Contraceptives. Genetic Effect. Somatic Cell. Mitotic index*

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I. Introduction

Voluntary control of fertility has taken a large part of the attention of recent researchers in order to find effective methods of birth control that have a key role in helping women who cannot get pregnant due to health problems such as high blood pressure during pregnancy, bleeding, severe anemia and cervical cancer ,and other serious diseases, as well as helping women who want to plan the family and choose the time and number of times of pregnancy . Bongaarts & Hodgson (2022) ; Berek *et al*, 1996).

Hence the importance of contraception in securing life and reducing morbidity rates, thus enhancing the quality value of life and preventing unwanted pregnancies (Jaffer, 2000).

Combination birth control pills consist of chemically synthesized steroids similar in physiological effect to those secreted by ovaries (Hart &Norman, 2000).

The importance of combined contraceptive pills comes not only because of their effectiveness in preventing pregnancy, but they are also used in the treatment of many pathological conditions, the most important of which is the regulation of menstrual disorders, such as the treatment of irregular bleeding associated with polycystic ovarian syndrome (Ferro & Jamie ,2005) .

Which if treated with estrogen is equivalent at a progestin dose results in an increase in the growth of the endometrium, and the treatment of discomfort that may occur before the menstrual cycle by inhibiting the occurrence of ovulation, especially when there are coagulation diseases, as the occurrence of ovulation naturally may lead to shock resulting from severe internal abdominal bleeding (Carr and Blackwell, 1998), and due to the lack of studies in this field, this study was designed for the purpose of identifying some changes that may occur in the coefficient of cell division due to the use of hormonal contraceptives. OCP Oral Contraceptive Pills (Michael & Lindberg ,2007).

Oral contraceptives (OCs) are oral contraceptives to prevent pregnancy., In the 1960s, oral contraceptives became available, the original indications for use in the United States were for the treatment of endometriosis and habitual abortion rather than contraception. Despite the fact that by 1980 DMPA was approved as contraceptive in more than 80 countries (Al-Shammari, 2001), it was not until 1992, after the World Health Organization (WHO) published the results of a 9-year study. A study showed that users of this inject able contraceptive method did not have a significant increase in the risk of breast malignancies and gynecological diseases. (WHO, 2004). Despite its side effects associated with its use, it is preferred by most

women for its high efficiency compared to other contraceptives (Ligniers and Silberstein, 2000), and that the failures in preventing pregnancy that occur in some women during its use are not due to inefficiency, but to irregular in the way it is used, and the cases of failure are estimated at about (1 per 100 women) (Taylor *et al*. 2006). There are currently two most common types of oral contraceptives, the first contains two synthetic female hormones (estrogen and progesterone) that resemble hormones produced naturally by the ovaries.The second type contains only progesterone.The main mechanism by which OCP works is to prevent the development of the follicle and thus inhibit ovulation (David *et al*., 2004) with its effect on the hypothalamus gland (Trussell & James, 2007) . In addition, the contraceptive pill increases the thickening of the uterine epithelium, which prevents the implantation process, and it also increases the viscosity of cervical mucus and thus hinders the movement of sperm transmission (Larimore & Stanford, 2000).
Nowadays, oral contraceptives are widely available and used for family planning (Carey, 2012).

II. Material And Methods

Laboratory animals:
The animals were divided into four groups:-
1-The first group: - The animals of this group were dosed with a solution of physiological phosphate (phosphate buffered saline PBS) (negative control).

Dissolve the following ingredients in (500 ml) of distilled water and then completethe volume to (1000 ml):
0.20 g potassium chloride (KCl)
8.00 g Sodium Chloride (NaCl)
1.15 g sodium monohydrogen phosphate (Na2HPO(4
0.20 g Dihydrogen Potassium Phosphate (KH2PO (4Sterilize and refrigerate 4°C (Hudson & Hay, .(1980)
2-The second group: - It is the group of animals dosed with a genetically mutated drug (Mutagenic), and this drug is cyclophosphamide (CP) (positive control).
3-The third group: - The animals of this group were dosed with a solution of birth control tablets at a concentration of 50 mg/ml.
4-The fourth group: - The animals of this group were dosed with a solution of contraceptive tablets at a concentration of 100 mg/ml.

All animals of these groups were dosed orally using a syringe daily for 15 days, where the animals were explained for the purpose of genetic testing at the end of the experiment and **5** animals were allocated to each group. the combination contraceptive tablets were of the type microgynon ED of German origin,one strip contains 21 tablets and each tablet consists of a low dose of ethinyl-estradiol (EE) of (0.03) mg equivalent to a higher dose of levonorgestrel of (0.15) mg two doses were used 50,100 mg/ml, and the dose was done for 15 days.

Mitotic Index

The test of the division coefficient was conducted according to the method (Allen et al.,1977) where each rat was injected with (0.1) ml of Cholchicin Solution through the peritoneum membrane (Dissolved 1 tablet of Cholchicin (1mg) in (0.5ml) of PBS) and after (2.5) hours ,the animal was sacrificed by the method of separating the spinal cord from the neck and explained directly for the purpose of obtaining somatic cells from the bone marrow as follows:
1-the animals were dissected by cutting the skin directly and extracting the organsfrom their sites.
2-Using a sterile syringe and (5 ml) of PBS solution the cells were extracted fromthe bone marrow.
3-the tubes were placed in a Centrifuge at speed of (2000 cycles / minute) for (5minutes).
4-Remove the floater and add to the precipitate (10 ml) of KCl (0.075) Hypotonic Solution(Dissolve 2.85 g of potassium chloride salt in 250 ml distilled water, then complete the volume to 500 ml of distilled water, sterilize with the locker and refrigerate 4º C.) and then incubate the tubes in a vibrating water bath at (37 º C) for (30 minutes).
5-the tubes were placed in a Centrifuge at a speed of (2000 r/min) and for a periodof (5 minutes).
6-Remove the floater and add to the precipitate (5 ml) of the stabilizer solution Sorenson's Buffer (Prepare the solution by dissolving (7.08 g) of (Na2HPO4) and (6.74 g) of (KH2PO4) in (50 ml) of distilled water, then complete the volume to (100 ml) with distilled water, sterilize the locker and store in the refrigerator (4 º C). prepared gradually in the form of drops that flow on the inner wall of the tube with continuous mixing, then complete the volume of the added stabilizer to reach (5 ml).
7-The tubes were placed at a temperature of (4 º C) for half an hour for the purpose of fixing the cells. The installation process has been repeated three times.
8-The tubes were spun in a centrifuge by quickly (2000 r/min) for a period of (5 minutes), then the floating solution was removed and the cells were suspended again in an appropriate volume (1-2 ml) of cold

stabilizer.

9-The pipes containing the installed cells were shaken and (8-6) drops of the contents of the tube were dropped on a clean glass slide vertically from a distance of about (3 feet) Mitotic index .MI%)) = number of divided cells / total number of cells (divided and non-divided) × 100.

Statistical Analysis

The data were analyzed according to the Complete Random Designer to study the effect of different coefficients, and the significant differences between the averages were compared with the least significant difference (L.S.D) Least Significant Difference and the values were fixed in the form of (rate± standard error).

II. Results & Discussion

1. **Mitotic index (MI (test**

Figure (5) shows that there are differences between the values of the cell division coefficient using concentrations of 50 and 100 mg/ml of contraceptive tablets on bone marrow cells, where the value was (9.0%) and (11.3%) respectively, where the difference was non-significant for the dose 50 mg/ml, while the difference was significant for the dose of 100 mg/ml when compared with the MI value of negative control of (8.3%) and thus formed a significant difference at the level of probability (P0.01) It is also noted that the values of the cell division coefficient of control The positive was low compared to the negative control (3.7%) so that the difference was high (P< 0.01).

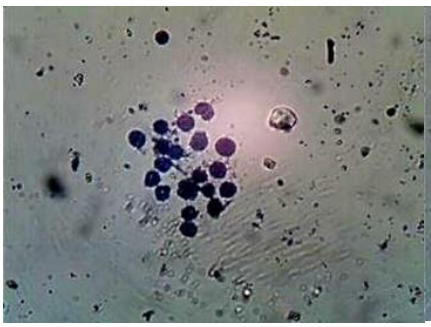

Fig (1) Undivided Nuclei Of Bone Marrow Cells White Rats Dosed With CP.

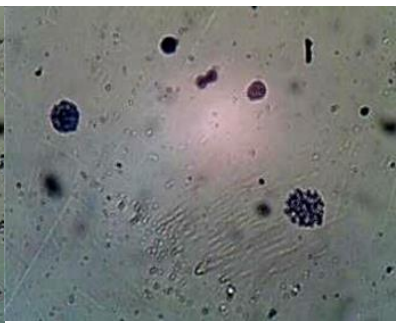

Fig (2) Divided And Non-Divided Nucleus Of In Bone Marrow Cells In White Rats.

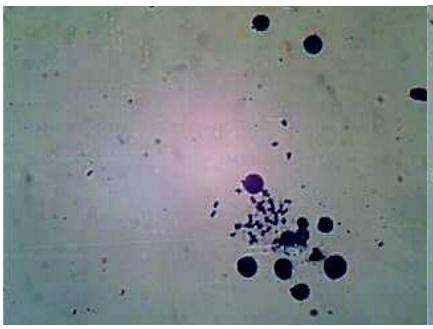

Fig (3) Divided And Non-Divided Nuclei In The Marrow

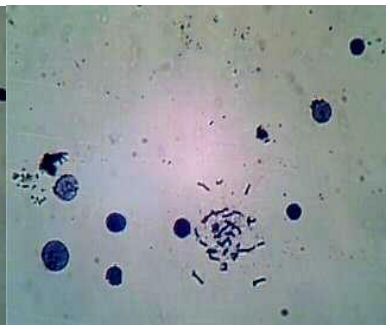

Fig (4) Shows The Chromosomes Of BoneBone Marrow Cells.

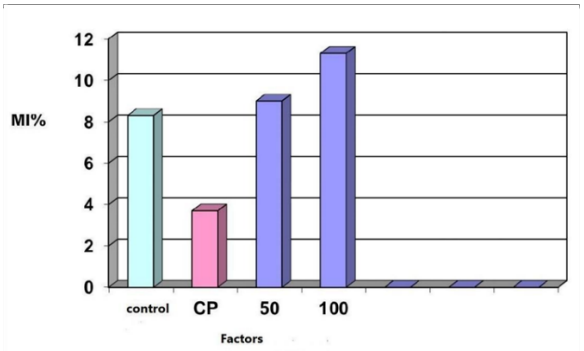

Fig (5) Effect Of Different Concentrations Of Contraceptive Tablets On The Mitotic Index Of The Bone Marrow Of Female White Rats.

Table (1) Effect of Treatment with Contraceptive Pills on Bone Marrow Female White rats.

Groups	Dose (mg/ml(	Mitotic index	
		Rate	Standard error
1	Control -	8.3a	±0.43
2	CP +	3.7b	±0.81
3	50mg/ml	9.0a	±0.21
4	100mg/ml	a11.3	±0.39

* Probability > 0.01 when compared with negative control.

* Different English letters within one column mean significant differences below the probability level (P< 0.01).

Through the results, it is found that the use of pills in liter keys 50 mg/ml had high mitotic index values compared to negative control, but they did not constitute a significant difference, and when the concentrations increased to 100 mg/ml, the value of MI rose so that it formed a significant difference when compared to negative control, and the reason for the high coefficient of cell division may be that these drugs have stimulated the ability of bone marrow stem cells to divide, as they may increase proteins. Involved in this process during the metaphase of the course of the division process, i.e. interferes with the genetic act controlling the process of cell division, making it a faster process than normal ,These results are similar to the findings of the study Greim *et a*l.,(2014).

When comparing these results with positive control, we clearly note the genetic toxic effects in the somatic cells of the mutagen (CP) through its ability to reduce the coefficient of cell division of bone marrow cells and increase the percentage of chromosomal changes in bone marrow cells, and this is consistent with the findings of a study (Al-Rubaie, 2000) These effects are due to the possession of metabolites of this drug the effectiveness of causing different effects of genetic material (DNA), as it is a factor (AlKalyting agent) while the metabolite (Acrolein) has the effectiveness of inhibiting cell division, as well as its ability to increase the percentage of chromosomal deviations higher than other metabolites of the drug sharba,(2009) ; Gimenez-Bastida *et al.*,(2018) .

III. Conclusions

1- Increased cell division coefficient using birth control pills.

2 – The increase in the mitotic index is related to the increase in concentration.

IV. Recommendations

1) Intensify studies on birth control pills to identify more properties they possess.
2) Conducting similar studies using other commonly used drugs.
3) Directing research centers and relevant authorities and studying all the advantages and characteristics of drugs used by humans for the purpose of determining their genetic risks.

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