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Effects of Oral Contraceptive Pills on Ovarian Tissues in Female Wistar Rats

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ABSTRACT: *The study was designed to investigate the effect of Oral Contraceptives Pills on Ovarian tissues in female Wistar rats. Thirty adult female Wister rats aged 10-12 weeks with about 150-200kg were used for this study. The rats were allotted to three groups of 10 rats each comprising 20 treated and 10 control group, the treated rats received 0.18mg/kg for each cycle received brown tablet 75mg/kg of microgynon and microlut received 0.03mg/kg orally for 72 days. group one serve as control group fed with vital feed and distilled water. While group (2) were treated with microgynon lenovorgestrel 150mg and Ethinylestradiol 130mg and brown tablet containing ferrows Fumarate, all contain 28 pills. Group three treated with microlut levonorgestrel 130mg contain 35 tables, all drugs were administered for 72days. At the end of the experiment, all animals in each group were sacrificed a day after the end of the last dosage administered of extract under chloroform anesthesia. Results from the experiment shows that there was an effects of the oral contraceptive pills used on the test subjects. In figure 1, which is the control group, all ovarian structures were present including the graffian follicle. But after the experiment, the two experimental groups observed showed absent of graffian follicle. This indicate the effects of the oral contraceptive pills used. The progesterone inhibit ovulation by acting on leutinizing hormone via a negative feedback mechanism.*

KEYWORDS: effects, oral contraceptive pills, ovarian tissues, female wistar rats

INTRODUCTION

Oral contraceptive pills are now used by more than 100 million women throughout the world. In many African countries, the pill is the most popular method of contraception (CHPE, 2014). Its effectiveness and relative safety have made the pill not only an accepted, but often a preferred method of contraception for many women. Also, eighty percent of all 35-year old women use or have used the pill. Initial oral contraceptive formulations contained very high levels of synthetic estrogen and progesterone, based on the assumption that these levels were necessary to prevent pregnancy (Skouby and Jesperson, 2012; Kaunitz, (2014). Henderson et al (2019) stated that the initially marketed formulations of OCs contained 150µg of the estrogen component mestranol and 9.85mg of the progestin component norethynodrel. The minor side effects produced by each of

these steroids, such as nausea, breast tenderness, weight gain, irregular menstrual bleeding as well as thrombosis were common and occasionally severe enough to cause discontinuation of use (Avonts et al, 2015; Henderson et al, 2016; Smith et al, 2018). Microgynon a combined pill (0.15mg levonorgestrel and 0.03mg ethinylestradiol) and Primolut- N a mini pill (5mg norethisterone) are among the most common drugs used in Nigeria for contraception and also for other non contraceptive benefits. The estrogen/progestin combination is the most effective type of OC formulation, because these preparations prevent ovulation mainly by interfering with release of gonadotropin-releasing Hormone (GnRH) from the hypothalamus. The combination OCs probably do have a direct inhibitory effect on the gonadotropin producing cells of the pituitary, in addition to affecting the hypothalamus. This effect occurs in about 80% of women ingesting combination OCs. It is unrelated to the age of the patient or the duration of OC use, but is related to the potency of the preparations.

Contraception is an important aspect of reproductive health and plays a major role in the prevention of unwanted pregnancy arising from rape for example. It is therefore a significant factor in reduction of induced abortion rate and improvement in maternal health care (Kebe, et al., 2019). Reports on the use of oral contraceptives had been found to have beneficial effects in reducing the incidence of pelvic inflammatory disease, decrease risk of ectopic pregnancy, benign breast lesions, ovarian and endometrial cancers, protection against osteoporosis and rheumatoid arthritis among the users (Cragg and Newman, 2013). Oral contraceptives are sometimes used to treat heavy or irregular menstruation and endometriosis. Oral contraceptive agents can also be used in hormonal replacement therapy, and in the emergency post-coital contraception. Oral contraceptive decreases the risk of ectopic pregnancy, benign breast lesions, ovarian and endometrial cancers, and offer protection against osteoporosis and rheumatoid arthritis (Rosing, 2019).

Despite the general acceptability and the obvious advantages that have been attributed to oral contraceptive use, some serious side effects have been reported in women taking them. Studies have indicated a relationship of oral contraceptives use and cardiovascular disease, altered levels of coagulation factors, thrombosis, platelet changes, atherosclerosis and multiple sclerosis. Estrogen has been known to have prothrombin effects and elevates cardiovascular .

Contraception is the intentional prevention of fertilization from taking place through the use of various devices, sexual practices, barrier methods and hormonal contraception. Contraception is as old as human existence. For centuries, humans have relied on their imagination to avoid pregnancy. Oral contraceptives are a simple form of contraception used by women worldwide. The oral contraceptive is one of the greatest and most influential developments of the twentieth century. It is regarded as the most reliable method of contraception, and one of the easiest. They are widely available in most pharmacies and chemist shops. Oral contraceptives are highly reliable, non-permanent means of contraception after the rhythm method when correctly applied. Proper medication can achieve a successful rate of contraception as high as 98% or above (Beral, 2017). Oral contraceptives prevent pregnancy primarily by inhibiting ovulation and venous thromboembolism risk (Margolis et al, 2017).

There is little or no data on the effects of Combined Oral Contraceptives on haematological parameters, especially the growth factors such as the cytokines or interlukin-11 and 6, and erythropoietin. Also, little or no data have been established in Liver Function Test, Serum Electrolytes, Fibrinogen, Antithrombin, Protein C and Protein S, using animal model. It is hoped that the study might throw some light on the need of monitoring the risks of taking COC (Duofem). The world population is now seven billion. Nigeria's population is estimated to be 176 million and will reach 400 million by the year 2050 (Carl, 2011). Rapid population growths would have a detrimental effect on socioeconomic development of Nigeria. There is a concern over population explosion in Nigeria and the drive to control it is leading to indiscriminate used of oral contraceptives. Oral contraceptives are used for preventing abortion, unplanned pregnancies resulting from rape and teenage pregnancies. The effective control of reproduction can be essential for child spacing and to allow the woman to achieve her individual goals and to contribute to her sense of wellbeing. Unintended pregnancy leads to induced abortion which is not legalized in Nigeria, except to save the woman`s life. The primary focus of this study is to investigate the possible effects of oral contraceptives, their possible mechanism of actions and any associated side effects. The knowledge obtained might provide useful intervention towards solving the problem(s). Thus, safety in the use of contraceptives and improvement in the health of the user are assured

Reproductive toxicity is increasingly becoming recognized as an important part of overall toxicology (Wang et al., 2018). The commonly used conventional combined oral pills are usually associated with many untoward adverse effects, necessitating newer and indigenous drugs. The use of plants for medicinal and mythological purposes, and for solving problems related to ill health has been practiced in the African and other societies for many years (Mohammed et al ., 2014).

MATERIALS AND METHOD

Experimental Animal

Thirty 30 adult female Wister rats aged 10-12 weeks weighting about 150-200kg were used for this research work and they were acclimatized in their various cages for a period of two weeks before the commencement of the treatment. The animals were housed under standard conditions with 12 hours light and 12 hours dark cycle throughout the duration of the experiment.

Experimental Design and Procedure

Thirty (30) animals were allotted to three groups consisting 10 rats each, animals in group one served as control group fed with vital feed and distilled water, while group two were treated received 0.18g/kg after each cycle with microgynon levonorgestrel 150mg and Ethinylestradiol 130mg and 1 brown tablet contain ferrows fumarate, all contain 28 pills. Group three treated with microlut levonorgestrel 30mg contain 35 tablets, all this drugs was used for 72 days.

Termination of Experiment

At the end of the experiment, all animals in each group were sacrificed a day after the end of the last administration of extract under chromophore anesthesia. The ovaries of these animals were

removed, and part of these tissues were preserved in labeled bottles container containing 10% of buffered formalin.

HISTOLOGICAL ANALYSIS

A buffered Formalin bottled containing 10% solutions were used to preserved the ovaries of the experimental Wistar rats. Each bottle was leballed up and allowed to stand for 72 hours this is in order to ensure maximum penetration. After that, there were placed in ascending grade for dehydration with ethanol solution. 70% of ethanol solution was used for an hour then subsequent 95% solution then alcohol solution for absolute dehydration. Tissue clearance took place in three subsequent changes of xylene with each lasting for 15 minutes. Tissue embedding with paraffin wax was carried out overnight to form blocks which were trimmed and sectioned at 5 micro thickness using a rotary microtome.

Warm water at 28°c bathed the tissue samples and dried up on an aluminum glass slides with haematoxylin and Eosin staining method which cleared up 95% of the xylene in alcohol and washed up in running tap water for 15 minutes. The differentiated section of turned blue after addition of 1% alcohol and Eosin for 1 minute. The stained section were viewed under a light microscope and photomicrograph of the stained tissues were taken.

RESULTS

The result findings showed that there was a significant impact of the oral contraceptive pills used during the experiment.

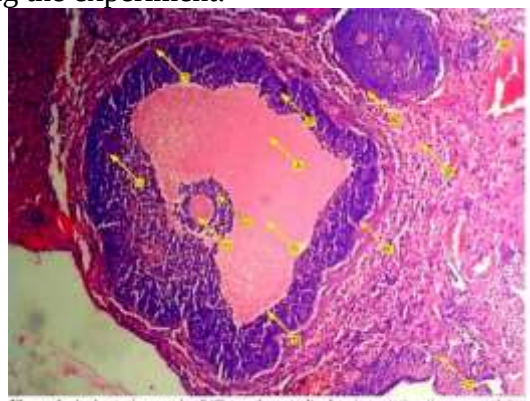

Figure 1: A photomicrograph of (Group 1 control), showing ovarian tissue containing Primary, secondary follicles and corpus leuteum OC Oocyte, A antrium, GF graffian Follicle, PF primary Follicle, SF secondary follicle, CR corona radiata, CL corpus liteum graffian follicles is also present **(H&E X40)**

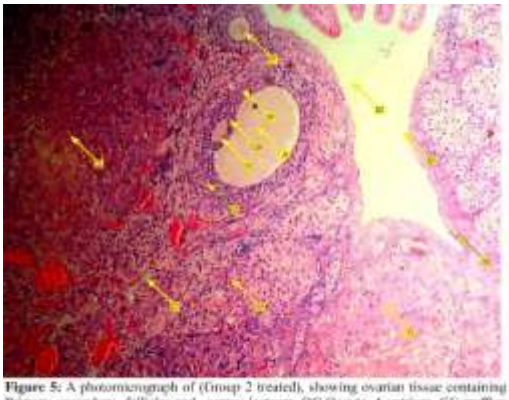

Figure 5: A photomicrograph of (Group 2 treated), showing ovarian tissue containing Primary, secondary follicles and corpus leuteum, OC Oocyte, A antrium, GF graffian Follicle, PF primary Follicle, SF secondary follicle, BC basophilic cells, CR corona radiata, CL corpus liteum. no graffian follicles **(H&E X40)**

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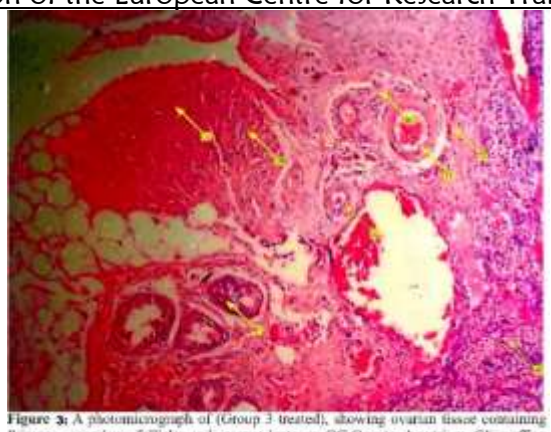

Figure 3: A photomicrograph of (Group 3 treated), showing ovarian tissue containing Primary, secondary follicles and corpus luteum, OC Oocyte, A antrum, GF graffian Follicle, PF primary Follicle, SF secondary follicle, CR corona radiata, CL corpus luteum, no graffian follicles **(H&E X40)**

DISCUSSION

In this study, the effects of oral contraceptive pills among female Wistar rats were investigated. The result findings were compared in three subsequent classes. Class one was the control group, class two and three were the experimental group. In the experimental groups, the following oral contraceptive pills were used: microgynon lenovorgestrel and Ethinylestradiol 130mg in group two and microlut levonorgestrel (Combined oral contraceptive pills) 130mg contain 35 tablets were used in group three respectively.

The graffian follicle is the matured ovum (oocyte) that is developed in the ovary with several significant structures. These structures include: Theca interna, theca externa, zona pellucida, antrum, germinal epithelium etc. These structures maintain the physiological action of the ovum. In this experiment, the control group (Figure 1) contained all the required structures and tissues of the ovaries with a cleared visible graffian follicle. While in the experimental group (figure 2 and 3) graffian follicle was absent. This is due to the effects of the oral contraceptive pills used.

The oral contraceptive pills used contains progesterone hormone. This is a steroid hormone that is synthesized in the ovary and release by corpus leutium. The progesterone stops ovulation. It thickens and decreases the amount of cervical mucus. This makes it difficult for spermatozoa to enter the uterus and fertilize the ovum. During contraception, the progesterone inhibits the effects of the leutinizing hormone (LH) to cause ovulation. This is achieved via a negative feedback mechanism.

CONCLUSIONS

The research work is carried out on female Wistar rats to investigate the effects of oral contraceptive pills in the department of human physiology, Bauchi State university Gadau. The test subjects were classified into two groups and a control group. Lenovorgestrel and Ethinylestradiol 130mg pills were administered in the subjects. After termination, tissue samples of the ovaries were taken and analyzed in the histology laboratory and haematology department of

Federal Medical Centre Azare. The result findings showed absence of graffian follicle in the test subjects which directly entails the effects of the oral contraceptive pills.

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