

Dynamics of Combined Oral Contraceptive: A Study of Some Cytokines in Female Wistar Rats

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Abstract: 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. This research priority included efforts to discover the effect of combined oral contraceptives (COC) (DUOFEM) on some cytokines in female wistar rats. Eighty (80) female wistar rats aged 10-12 weeks weighing 180-250 g were used for the study. They were divided into four groups of 20 rats each comprising 10 treated and 10 control rats. The treated rats received 0.6mg/kg body weight of COC intragastrically for 36, 48, 60 and 72 days in five-day cycles (four-day treatment with one-day break). The COC was given intragastrically in 5-day cycles (4-day treatment with 1-day break). All controls were given fresh water *ad libitum* daily for the period of the experiment. The blood sample was drawn into plain tubes. An enzyme-linked immunosorbent assay (ELISA) was used for the quantitative determination of Erythropoietin (EPO), interleukin-6 (IL-6) and interleukin-11 (IL-11). There were significant decrease in interleukin-6 (IL-6) and interleukin-11 (IL-11), but no significant change in Erythropoietin. While further study is necessary, current evidence suggest that COC (DUOFEM) use provides contraceptives benefits with minimal potential adverse effects in healthy users.

Keywords: Combined Oral Contraceptives, Cytokines, Wistar rats.

I. INTRODUCTION

Global family planning programmes have been in existence in the developed world for several decades and are primarily designed to supply couples with the methods of family planning that best suit their needs. Birth control is a major factor in public health and welfare, preserving the general and reproductive health of women and allowing them to choose the movement of a planned pregnancy (WHO, 2009). The World Health organization (WHO) and other global organizations are seeking ways to increase the amount of information and access people have to contraception and other resources related to family planning all round the world.

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 (Echendu, *et al.*, 2011). Oral contraceptives had been reported 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 (Vessey, 1995).

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, 1999).

Despite the general acceptability and the obvious advantages that have been attributed to oral contraceptives 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 and venous thromboembolism risk (Margolis *et al.*, 2007).

Cytokines are peptides that have a fundamental role in communication within the immune system and in allowing the immune system and host tissues cells to exchange information. Cytokines are nonantibody proteins that can be made by a wide range of cell types. About 30 cytokines are recognized, including the interleukins (IL-1 to IL-24), tumor necrosis factors (TNFs), and transforming growth factors (TGF Beta 1-3). In general, cytokines are low molecular weight (200 amino acids) and have specific receptors. Collectively, cytokines mediate cellular intercommunications via autocrine, paracrine, or endocrine mechanisms. Cytokine

actions are the result of a complex network, often involving feedback loops and cascades, the overall response being dependent on the synergistic or antagonistic actions of its various components.

The haemopoietic growth factors and cytokines are the soluble regulators of blood cell production and are produced by several cell types in different sites in the body (Almedia *et al.* Haemopoietic growth factors regulate the proliferation, differentiation and maturation of haemopoietic precursor cells. These include IL-3, IL-6, IL-11, GM-CSF (Bernadette *et al.*, 2012).

Erythropoietin (EPO) is a cytokine and a glycoprotein hormone that is essential for the proliferation viability and terminal differentiation of erythroid progenitor through erythropoietin receptor (EPOR) expression (Wu *et al.*, 1995). Epo is produced mainly in the kidney and to some extent in the liver, brain and uterus. Epo is shown to act on non-haematopoietic cells such as central organs nervous system, the heart and the other organs and tissues resulting in tissue protection (Rei *et al.*, 2000). Epo has antiapoptotic, antioxidant and anti-inflammatory effects. The effects of COCs on several haemorrhological and haemostatic parameters has been reported (Akhigbe, *et al.*, 2008). Recently, EPO has emerged as a multifunctional growth factor that plays a significant role in the nervous.

II. MATERIALS AND METHODS

The combined oral contraceptive used is DUEFEM®. They were obtained from family clinic, Ahmadu Bello University Teaching Hospital, Shika-Zaria, and from the Society for Family Health (SFH) Abuja, Nigeria. COCs DUOFEM® tablets which combined ethinylestradiol and Norgestrel were manufactured by Wyeth Ayerst (USA) and packed and marketed by the Society for Family Health, Lagos, Nigeria. DUOFEM® is a child spacing pill containing ferrous fumarate tablets. Each DUOFEM cycle contains 28 pills; each white tablet contains 0.3mg Norgestrel and 0.03mg Ethinylestradiol and each brown tablet contains 75mg ferrous fumarate. DUOFEM® has a molecular weight of 312.4458g/mol (Chitturi *et al.*, 2003). Eighty (80) female wistar rats aged 10-12 weeks weighing 180-250 g were used for the study. They were divided into four groups of 20 rats each comprising 10 treated and 10 control rats. The treated rats received 0.6mg/kg body weight of COC intragastrically for 36, 48, 60 and 72 days in five-day cycles (four-days treatment with one-day break).

The COC was given intragastrically in 5-day cycles (4-day treatment with 1-day break). All controls were given fresh water *ad libitum* daily for the period of the experiment. Animals were sacrificed after anaesthesia with chloroform and 5ml of blood was obtained via cardiac puncture and placed in plain bottles. Interleukin 11 and 6 (IL-11, IL-6), erythropoietin (EPO) and liver function tests were determined by ELISA method.

III. RESULTS

Erythropoietin (EPO) was not significantly increased in EPO in all the treated groups (A; 12.3±1.9, B; 14.4±1.5, C; 10.8±1.3 and D; 10.7±1.2), compared to the controls (P<0.431, P<0.324, P<0.057 and P<0.108). Interleukin 6 (IL-6) was significantly decreased in treated groups C; 34.7±8.0 and D; 26.1±1.5 compared to the controls (37.7±1.8 and 47.1±9.8). Interleukin 6 (IL-6) was significantly increased in treated group A; 30.9±7.2 and B; 30.7±8.7 compared to controls (20.8±1.2 and 26.0±1.6). Interleukin 11 (IL-11) was significantly decreased in treated groups C (37.9±1.3) and D (46.8±1.8) compared to the controls (P<0.005 & P<0.002). There was no significant increase in IL-11 in treated groups A and B compared to the controls (P<0.086 and P<0.987).

The effects of combined oral contraceptives on some cytokines in female wistar rats

Groups	IL-11 (ng/L)	IL-6 (ng/L)	EPO (iu/L)
A (36 Days)	45.2±7.6	30.9±7.2	12.3±1.9
Control	42.4±9.6	20.8±1.2	10.5±1.2
P-value	0.086	0.001	0.431
B (48 Days)	51.1±9.1	30.7±8.7	14.4±1.5
Control	50.1±9.1	26.0±1.6	11.9±1.1
P-value	0.987	0.005	0.057
C (60 Days)	37.9±1.3	34.7±8.0	10.8±1.3
Control	41.7±1.0	37.7±1.8	11.1±1.4
P-value	0.005	0.050	0.324
D (72 Days)	46.8±1.8	26.1±1.5	10.7±1.2
Control	49.0±2.2	47.1±9.8	12.4±1.2
P-value	0.002	0.005	0.108

IV. DISCUSSION

There was no significant change in serum erythropoietin level in all the controls. This finding is contrary to that of Prechile *et al.* (1972) who found that estradiol benzoate inhibited the production of erythropoietin (EPO). Erythropoietin promotes the survival, proliferation and differentiation of erythrocytic

progenitors. EPO deficiency is the primary cause of the anaemia in chronic kidney disease. It appears COC has little or no effect in EPO production. Decreased RBC count in this study may not be as a result of COC effect on EPO. There was a significant decrease in serum level of interleukin-6 (IL-6) and a slight decrease in serum interleukin-11 (IL-11). Estrogen is able to decrease IL-6 expression by blocking the osteoblast's synthesis of IL-6 receptors IL-11 and IL-6 are haemopoiesis-promoting factors capable of enhancing the growth of myeloid, erythroid and megakaryocytic progenitor cells. They are capable of mediating a complex array of pro- and anti-inflammatory effects. IL-6 produces C-Reactive Protein (CRP) which leads to cardiovascular risk. Experimental studies have shown strong correlations between the risk of cardiovascular diseases and inflammatory markers such as CRP and tissue neurosis factor- α (TNF α) (Subhadeep *et al.*, 2008). IL-6 is a pleiotropic cytokine which stimulates B-lymphocyte and T-lymphocyte differentiation, and activates macrophages and NK cells. The finding of reduced serum IL-6 is in agreement with the finding of Straub *et al.* (2000) who reported decrease serum level of IL-6 in menopausal women taking hormonal replacement therapy.

IL-6 plays important physiologic roles, deregulated overproduction of IL-6 causes various pathologic conditions, including autoimmune, inflammatory, and lymphoproliferative disorders. It has been shown that IL-6 is involved in immune-inflammatory diseases such as rheumatoid arthritis (RA), Castleman disease, juvenile idiopathic arthritis (JIA), and Crohn disease. Elevated serum IL-6 has been observed in patients with these diseases and the IL-6 levels correlate with disease activity (Uson *et al.*, 1997).

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